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Insertion Sequences

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INTRODUCTION

General Scope

Since the publication in 1989 of the volume *Mobile DNA* (29), the field of transposition has experienced some striking advances. Not the least of these has been the development of various in vitro transposition systems for both prokaryotic and eukaryotic elements and an understanding of the transposition process at the chemical level for several of these (for reviews, see references 66, 179, 239, and 266). Another development has been the veritable explosion in the number of different transposable elements isolated and characterized at the nucleotide sequence level. In the case of bacterial insertion sequences (IS), only approximately 50 had been analysed at this level in 1989 (107), compared to over 500 today. This is equally true of eukaryotic "insertion sequences" such as *mariner*, derivatives of which have been found in over 240 insect species in addition to fungi, mammals, fish, and plants (295), and related elements such as Tc*1* (87, 274). This enormous diversity and distribution is astonishing. The number of different ISs has now become so great that there is a growing need for a framework to enable a systematic classification. We have attempted here to provide a classification of these elements by assigning them to various families or groups based on similarities and differences in structure, organization, and nucleotide and protein sequence relationships. We also include a brief description, where available, of the mechanism(s) involved in the mobility of individual ISs and of the structure-function relationships of the enzymes which mediate the transposition reactions, the recombinases/transposases (Tpases). Most of the elements of this framework are already available but scattered throughout the literature.

In assembling this review, we have limited our treatment to IS elements, which we loosely define as small (<2.5-kb), phenotypically cryptic segments of DNA with a simple genetic organization and capable of inserting at multiple sites in a target molecule. In this definition, we voluntarily eliminate several types of mobile genetic element. These include elements with RNA as intermediates such as the retroviruses, retrotransposons and retroposons (102, 315), DNA elements such as the conjugative transposons (306) which use a phage λ-type mechanism for their translocation, and elements such as bacteriophage Mu (239), Tn*7* (66), and transposons of the Tn*554* type (249), which are large and relatively complex. We have also eliminated an additional large and coherent group of elements, the type II transposons of the Tn*3* family (373). Although several are small and might qualify as insertion sequences [e.g., Tn*1000* (γδ) and IS*101* (324) and IS*1071* (86a, 251)], many are complicated in structure and include multiple antibiotic resistance genes carried by another type of transposable element, the integron (278).

Although they are not considered here in detail, many of these elements have significant functional similarities to ISs and have provided many of the important insights into transposition mechanism. Reference to these is included where they may prove useful to understanding the behavior of IS elements.

Nomenclature

Several systems of nomenclature are in operation. One, initiated in 1978 (201) and centralized by E. Lederberg (Stanford University), attributes a single number to an IS element (e.g., IS*1*). While adequate when only few ISs were known, this system does not include sufficient information and becomes less transparent with the large numbers of elements known today. A second system, which provides some information about the source of the element, includes the initials of the bacterial species from which it was isolated (e.g. ISRm*1* for *Rhizobium meliloti*). At present, we are confronted by both types of nomenclature in the literature. In addition, closely related elements with only few differences at the nucleotide sequence level are in some cases designated isoforms of the parent element and sometimes attributed a specific number. Finally, some elements have been baptized with names that fit none of these rules (e.g., RSalpha-9). Where appropriate, we have simplified certain of the more complicated assignments by adopting the nomenclature which includes the initials of the host bacterial species.

Sources of Bacterial Insertion Sequences

Bacterial insertion sequences were initially identified during studies of model genetic systems by their capacity to generate

mutations as a result of their translocation. Interest in antibiotic resistance and transmissible plasmids subsequently revealed an important role for these mobile elements in dissemination of resistance genes and in promotion of gene acquisition. In particular, it was observed that several different elements were often clustered in "islands" within plasmid genomes and served to promote plasmid integration and excision (46). In addition, two copies of certain ISs flanking a DNA segment were found to be able to act in concert, rendering the intervening region mobile. These structures were termed composite or compound transposons (29). The influence of ISs on bacterial pathogenicity and virulence could also have been anticipated at that time by the isolation of an IS*1*-based compound transposon specifying a heat-stable toxin (318). With the development of studies of the mechanisms of bacterial pathogenesis over recent years, the finding of association between ISs and many pathogenic and virulence functions has become increasingly frequent. Such associations have been observed in animal pathogens (e.g., *Bacillus* [225], *Bordetella*, *Brucella*, *Campylobacter*, *Clostridium* [45, 65], *Escherichia* [61, 109, 148, 318], *Haemophilus* [88], *Neisseria* [135], *Vibrio* [327], and *Yersinia* [99, 101, 122, 130]), plant pathogens (e.g., *Agrobacterium* [258], *Erwinia*, and *Pseudomonas*), and symbionts (e.g., *Rhizobium* [104]). Another area which has received increasing attention over recent years is that of bioremediation. Here, too, insertion sequences can be clearly associated with genes forming parts of degradative or catabolic pathways (49), an observation foreshadowed by the early identification of transposons carrying genes permitting citrate utilization (159). Association of ISs with these biological processes does not imply that the processes are in any way "special." It presumably simply reflects the (evolving) interests of the investigator. What these examples do underscore, however, is the importance of transposition mechanisms in assembling sets of "accessory" functions in bacteria.

It should not be forgotten, of course, that ISs are involved in phenomena other than the acquisition of accessory functions. Many form an integral part of the chromosomes of most bacterial species, where they have been shown, for example, to participate in chromosome rearrangements (see, e.g., references 128, 215, and 301) and in plasmid integration (reference 215a and references therein). In certain cases, the localization of different specific IS elements at defined places in the chromosome is sufficiently stable to allow them to be used as markers in restriction fragment length polymorphism studies for species typing and for epidemiological purposes (e.g., IS*6100* in *Mycobacterium tuberculosis* [316], IS*1296* in *Mycoplasma mycoides* [58], IS*200* in *Salmonella* [323], and IS*1004* in *Vibrio cholerae* [35]). One exception, which has as yet exhibited no ISs, is the common laboratory strain of *Bacillus subtilis* (372), whose entire genomic sequence has recently been determined (189).

GENERAL FEATURES AND PROPERTIES OF INSERTION SEQUENCE ELEMENTS

Organization

In addition to being small, insertion sequences are genetically compact (Fig. 1). They generally encode no functions other than those involved in their mobility. These include factors required in *cis*, in particular recombinationally active DNA sequences which define the ends of the element, together with an enzyme, the Tpase, which recognizes and processes these ends. The Tpase is generally encoded by one or perhaps two open reading frames and consumes nearly the entire length of the element.

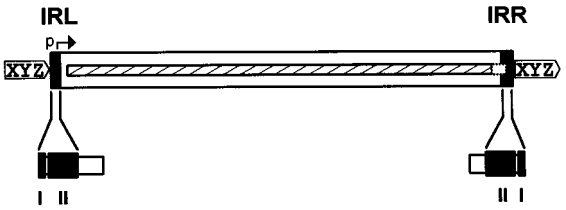

FIG. 1. Organization of a typical IS. The IS is represented as an open box in which the terminal IRs are shown as grey boxes labelled IRL (left inverted repeat) and IRR (right inverted repeat). A single open reading frame encoding the transposase is indicated as a hatched box stretching along the entire length of the IS and extending within the IRR sequence. XYZ enclosed in a pointed box flanking the IS represents short DR sequences generated in the target DNA as a consequence of insertion. The Tpase promoter, p, which is partially localized in IRL, is shown by a horizontal arrow. A typical domain structure (grey boxes) of the IRs is indicated beneath. Domain I represents the terminal base pairs at the very tip of the element whose recognition is required for Tpase-mediated cleavage. Domain II represents the base pairs necessary for sequence-specific recognition and binding by the Tpase.

Terminal inverted repeats. With several notable exceptions (the IS*91*, IS*110*, and IS*200*/*605* families [see Table 2]), the majority of ISs exhibit short terminal inverted-repeat sequences (IR) of between 10 and 40 bp. In cases examined experimentally, the IRs can be divided into two functional domains (Fig. 1). One (II) is positioned within the IR and is involved in Tpase binding. The other (I), which includes the terminal 2 or 3 bp, is involved in the cleavages and strand transfer reactions leading to transposition of the element (82, 83, 152, 166, 227, 378). A similar organization has also been proposed for the transposon Tn*3* (153). The simple single terminal Tpase binding sites of ISs are to be contrasted with the multiple and asymmetric protein binding sites of bacteriophage Mu (69) and transposons Tn*7* (67) and probably Tn*552* (242). Multiple protein binding sites are also a characteristic of the complex En/Spm and Ac elements of maize (114, 191). By accommodating different binding patterns at each end, such an arrangement can provide a functional distinction between the ends either in the assembly or in the activity of the synaptic complex. In addition, indigenous IS promoters are often located partially within the IR sequence upstream of the Tpase gene, by convention known as IRL. This arrangement may provide a mechanism for autoregulation of Tpase synthesis by Tpase binding. Binding sites for host-specified proteins are also often found within or close to the terminal IRs, and these proteins may play a role in modulating transposition activity or Tpase expression.

Domain structure of Tpases. A general pattern for the functional organization of Tpases appears to be emerging from the limited number which have been analyzed. The sequence-specific DNA binding activities of the proteins are generally located in the N-terminal region, while the catalytic domain is often localized toward the C-terminal end (IS*1* [221, 377], IS*30* [322], Mu [199], Tn*3* [224], IS*50* [365], and IS*911* [270]). One functional interpretation of this arrangement for prokaryotic elements is that it may permit the interaction of a nascent protein molecule with its target sequences on the IS, thus coupling expression and activity. This notion is reinforced by the observation that the presence of the C-terminal region of both the IS*50* and IS*10* Tpases appears to mask the DNA binding domain and reduce binding activity (162, 357). This arrangement would favor the activity of the protein in *cis*, a property shared by several Tpases (see "Activity in *cis*" below). Similar masking appears to occur with the IS*1* (374b) and the IS*911* (139) Tpases. In several cases, these domains are assem-

bled into a single protein from consecutive *orf*s by translational frameshifting (see "Programmed translational frameshifting" below).

An additional characteristic of some, if not all, Tpases is the capacity to generate multimeric forms essential for their activity (266). This is true of both prokaryotic elements such as bacteriophage Mu (52), IS*50* (357), and IS*911* (139) (but apparently not IS*10* [39]) and eukaryotic elements such as the retroviruses (171) and the *mariner*-like element, Mos*1* (213).

With the results of an increasing number of structural studies of these types of enzymes, it will be of great interest to compare the overall similarities of equivalent functional domains, as has recently become possible for the catalytic domains of retroviral integrases, Mu transposase, and other polynucleotidyl transferases such as the Holiday resolvase, RuvC, and RNase H (121, 289).

Direct target repeats. Another general feature of IS elements is that on insertion, most generate short directly repeated sequences (DRs) of the target DNA flanking the IS. Attack of each DNA strand at the target site by one of the two transposon ends in a staggered way during insertion provides an explanation for this observation. The length of the DR, between 2 and 14 bp, is characteristic for a given element, and a given element will generally generate a duplication of fixed length. Certain ISs have been shown to generate DRs of atypical length at a low frequency, presumably reflecting small variations in the geometry of the transposition complex (reference 107 and references therein). Although some notable exceptions exist in which there is a systematic absence of DRs (either within a given family or in several independent transposition events of a given element), care should be taken in interpreting the absence of DRs in isolated cases. A lack of DRs can simply result from homologous inter- or intramolecular recombination between two IS elements, each with a different DR. This would result in a hybrid element carrying one DR of each parent. It can also arise from the formation of adjacent deletions resulting from duplicative intramolecular transposition. In this case, a single copy of the DR is located on each of the reciprocal deletion products (see, for example, references 342 and 356).

Control of Neighboring-Gene Expression

Many IS elements have been shown to activate the expression of neighboring genes. A nonexhaustive list includes IS*1*, IS*2*, and IS*5* (107) and, more recently, elements such as IS*406* (305), IS*1186* (262), IS*481* (85), IS*928*B (214), ISSg*1* (78), IS*1490* (150), and ISVa*1* (336). Many other examples can be found in the literature.

It has been known for some time from experimental observation that elements such as IS*1*, IS*2*, and IS*5* possess outwardly directed −35 promoter hexamers located in the terminal IRs. When placed (by transposition) at the correct distance from a resident −10 hexamer, new promoters capable of driving the expression of neighboring genes can be created. Potential −35 hexamers were detected within the terminal IRs of many ISs (107). The list of elements which have been demonstrated experimentally to carry functional −35 hexamers is now extensive and includes IS*21* (283), IS*30* (70), IS*257* (203), IS*2* (330), IS*911* (338), and IS*982* in *Lactococcus lactis* (214).

It is interesting that in several cases, an inwardly directed −10 hexamer has also been detected in the IRL of several elements. When two ends of such an element are juxtaposed, by formation of head-to-tail dimers or of circular copies of the IS, the combination of the −10 hexamer with a −35 hexamer resident in the neighboring right end can generate relatively strong promoters (IS*21* [283], IS*30* [70], and IS*911* [338]). This arrangement can lead to high Tpase expression and consequent increases in transposition activity (see "**IS*3* family**," "**IS*21* family**," and "**IS*30* family**" below).

Other elements have been reported to influence the expression of neighboring genes by endogenous transcription "escaping" the IS and traversing the terminal IR (e.g., IS*3* [56], IS*10* [60], IS*481* [85], and IS*982* in *Escherichia coli* [214]).

An additional type of control of neighboring genes is illustrated by the (normally cryptic) *bgl* operon of *E. coli*. Activation of the operon can be accomplished in several ways, including insertion of either IS*1* or IS*5* upstream or downstream of the promoter (284, 285). Although a detailed explanation of the effect is not available, it has been suggested that activation involves changes in DNA structure (e.g., changes in curvature or topology), since mutations in the *cap*, *topI*, and *hns* genes have a similar activation effect. For IS*5*, activation is abolished by internal deletions, leaving only 25 bp of IRL and 32 bp of IRR, but is restored by providing an IS*5*-encoded gene product, Ins5A, necessary for transposition in *trans* (303). The implication of these results is that interaction of Ins5A with the IS*5* ends in some way changes the topology of the *bgl* promoter region. At present, no other examples of such control mechanisms are available.

Control of Transposition Activity

Transposition activity is generally maintained at a low level. An often cited reason for this is that high activities and the accompanying mutagenic effect of genome rearrangements would be detrimental to the host cell (89). Tpase promoters are generally weak, and many are partially located in the terminal IRs, enabling their autoregulation by Tpase binding.

Tpase expression and activity. While many of the classical mechanisms of controlling gene expression, such as the production of transcriptional repressors (IS*1* [95, 221, 377] and IS*2* [147]) or translational inhibitors (antisense RNA in IS*10* [179]), are known to operate in Tpase expression, several other mechanisms have also been uncovered.

(i) Sequestration of translation initiation signals. Protection of certain elements from activation by impinging transcription following insertion into highly expressed genes has been shown to operate at the level of translation initiation. In these elements, internal IR sequences are located close to the left ends and contain the ribosome binding site or translation initiation codon for the Tpase gene. Transcripts from the resident promoter include only the distal repeat unit, while transcripts from neighboring DNA include both repeats and would generate secondary structures in the mRNA which would sequester translation initiation signals (74, 182). This has been demonstrated experimentally for IS*10* and IS*50*, but several additional ISs carry such potential structures and might be expected to exhibit a similar mechanism (287).

(ii) Programmed translational frameshifting. A second mechanism acts at the level of translation elongation and involves programmed translational frameshifting between two consecutive open reading frames. Typically a −1 frameshift is observed in which the translating ribosome slides 1 base upstream and resumes in the alternative phase. This generally occurs at the position of so-called slippery codons in a heptanucleotide sequence of the type Y YYX XXZ in phase 0 (where the bases paired with the anticodon are underlined), which is read as YYY XXX Z in the shifted −1 phase (see, e.g., reference 55, 96, and 110). The sequence A AAA AAG is a common example of this type of heptanucleotide. Ribosomal shifting of this type is stimulated by structures in the mRNA

which tend to impede the progression of the ribosome, such as potential ribosome binding sites upstream or secondary structures (stem-loop structures and potential pseudoknots) downstream of the slippery codons.

Translational control of transposition by frameshifting has been demonstrated both for IS*1* (95, 217, 310) and for members of the IS*3* family (268; see also reference 55), but it may also occur in several other IS elements (see, for example, "IS*5* family" below). In these cases, the upstream frame appears to carry a DNA recognition domain whereas the downstream frame encodes the catalytic site. While the product of the upstream frame alone acts as a modulator of activity, presumably by binding to the IR sequences, frameshifting assembles both domains into a single protein, the Tpase, which directs the cleavages and strand transfer necessary for mobility of the element. The frameshifting frequency is thus critical in determining the overall transposition activity. This is treated in more detail in the sections describing the IS*1* and IS*3* families. Although it has yet to be explored in detail, frameshifting could be influenced by host physiology thus coupling transposition activity to the state of the host cell.

(iii) Translation termination. A third potential mechanism derives from the observation that the translation termination codon of Tpase genes of certain elements is located within their IRs. Although, to our knowledge, no extensive analysis of the significance of this arrangement has yet been undertaken, it seems possible that in some manner it couples translation termination, Tpase binding, and transposition activity.

The Tpase gene of several elements does not possess a termination codon. These include IS*240*C, a member of the IS*6* family (57a); two members of the IS*5* family, IS*427* (77) and ISMk*1* (228); and various members of the IS*630* family, including IS*870* and ISRf*1* (103). Instead, some of these elements insert into a relatively specific target sequence in which the target DR produced on insertion itself generates the Tpase termination codon (see "IS*630* family" below). The relevance of this as a control mechanism has yet to be explored.

(iv) Impinging transcription. Early studies of several elements demonstrated that impinging transcription from outside reduces transposition activity. Transposition of both IS*1* and IS*50* was shown to be sensitive, although other elements have, to our knowledge, not been examined (107). In bacteriophage Mu, transcription originating from within the element and impinging on the left end also reduces activity (116). It is possible that transcription disrupts the formation of intermediates including Tpase and one or both Mu ends, which normally lead to stable transposition complexes.

(v) Tpase stability. Tpase stability can also contribute to control of transposition activity. This has been demonstrated for IS*903*, where the Tpase is sensitive to the *E. coli* Lon protease (83). This sensitivity limits the activity of the Tpase both temporally and spatially and this may provide an explanation for the observation that several Tpases function preferentially in *cis* (see below). Indeed, mutant IS*903* Tpase derivatives have been isolated which exhibit an increased capacity to function in *trans*. These are more refractive to Lon degradation than is the wild-type protein (80). Some evidence that Lon may also be involved in regulating Tn*5* (IS*50*) transposition has also been presented (181). An observation which might also reflect Tpase instability is the temperature-sensitive nature of IS*1*-mediated adjacent deletions in vivo (279) and of IS*911* intramolecular recombination both in vivo and in vitro (138). For IS*911*, incubation of the Tpase at 42°C results in an irreversible loss in activity.

(vi) Activity in *cis*. Early studies on several transposable elements indicated that transposition activity was more efficient if the transposase was provided by the element itself or by a Tpase gene located close by on the same DNA molecule. This preferential activity in *cis* reduced the probability that Tpase expression from a given element would activate transposition of related copies elsewhere in the genome. The effect can be of several orders of magnitude and has been observed for a variety of elements including IS*1* (223, 271), IS*10* (245), IS*50* (156), and IS*903* (118, 120). This property presumably reflects a facility of the cognate Tpases to bind to transposon ends close to their point of synthesis and is likely to be the product of several phenomena.

For IS*903*, increased stability (83) and expression (80) have been shown to increase the capacity for Tpase activity in *trans*. Likewise, for IS*10*, mutations which increase translation of the Tpase also decrease the *cis* preference of the enzyme, and it has been suggested that the *cis* preference is strongly dependent on the half-life of the Tpase mRNA and the rate at which transcripts are released from their templates (162).

An additional consideration which may promote preferential activity in *cis* is reflected in the domain structure of known Tpases. In most of these cases, the DNA binding domain is located at the N-terminal end of the protein. This arrangement would permit preferential binding of nascent Tpase polypeptides to neighboring binding sites (see "Domain structure of Tpases" above). Moreover, in several cases it has been shown that the N-terminal portion of the protein exhibits a higher affinity for the ends than does the entire Tpase molecule, suggesting that the C-terminal end may in some way mask the DNA binding activity of the N-terminal portion. This is discussed in the sections below, which deal with the individual insertion sequences.

Host Factors

Transposition activity is frequently modulated by various host factors. These effects are generally specific for each element. A nonexhaustive list of such factors includes the DNA chaperones (or histone-like proteins) integration host factor (IHF), HU, HNS, and FIS; the replication initiator DnaA; the protein chaperone/proteases ClpX, ClpP, and ClpA; the SOS control protein LexA; and the Dam DNA methylase. In addition, proteins which govern DNA supercoiling in the cell might influence transposition.

The DNA chaperones may play roles in ensuring the correct three-dimensional architecture in the evolution of various nucleoprotein complexes necessary for productive transposition. They may also be involved in regulating Tpase expression. Several elements carry specific binding sites for IHF within or close to their terminal IRs. These can lie within or close to the Tpase promoter (e.g., IS*1* [108], IS*903* [118], and IS*10* [152]). IHF, HU, HNS, and FIS have all been variously implicated in the case of bacteriophage Mu, either in the control of Mu gene expression or directly in the transposition process itself (see reference 52 for a review). IHF appears to influence the nature of IS*10* transposition products by binding to a site 43 bp from one end (314). It also stimulates Tpase binding to the ends of the Tn*3* family member Tn*1000* (γδ) (362). Ironically, although IS*1* was the first element in which IHF sites were identified (one within each IR), conditions have not yet been found in which IHF shows a clear effect on transposition or gene expression (374b). For IS*50*, an element of the same family as IS*10*, both the protein Fis and the replication initiator protein DnaA have been reported to intervene in transposition (286).

Although their mode of action is at present unknown, several other host proteins with otherwise entirely different functions have been implicated in transposition. Acyl carrier pro-

tein was independently shown to stimulate 3′-end cleavage of Tn*3* by its cognate Tpase (224a) and, together with ribosomal protein L29, to greatly increase the binding of TnsD (a protein involved in Tn*7* target selection) to the chromosomal insertion site, attTn7 (313a). Moreover ACP and L29 moderately stimulate Tn*7* transposition in vitro, while L29 alone has a significant stimulatory effect in vivo (313a).

Certain factors involved in protein "management," such as ClpX, ClpP, and Lon, have been implicated in transposition. ClpX is essential for Mu growth (237), where it is required for disassembling the transpososome strand transfer complex and promoting installation of the phage replication machinery (184, 206). Recognition of Mu Tpase, pA, by ClpX requires the terminal 10 amino acids of pA (207). Together with ClpP, ClpX also plays a role in proteolysis of the Mu repressor (193, 360). As indicated above, the Lon protease is implicated in proteolysis of the IS*903* Tpase (80, 83). At present, the involvement of these proteins in the transposition of other elements has not been well documented.

The third class of host factor includes host cell systems which act to limit DNA damage and maintain chromosome integrity. Studies with IS*10* (179) and IS*1* (198) have demonstrated that high levels of Tpase in the presence of suitable terminal IRs lead to the induction of the host SOS system. Some controversy exists for Tn*5* (IS*50*). Reznikoff and colleagues have provided genetic evidence that transposition is inhibited by induction of the SOS system in a manner which does not require the proteolytic activity of RecA (358). On the other hand, Tessman and collaborators (185–187), using a different transposition assay, have found that constitutive SOS conditions actually enhance Tn*5* transposition. Moreover, using yet another assay system, Ahmed (6) has concluded that intermolecular transposition of Tn*5* is stimulated in the presence of RecA. Further investigation is clearly required to understand these apparently incompatible results.

Ahmed has also concluded that intermolecular transposition of the IS*1*-based transposon Tn*9* behaves in a similar way to that of Tn*5* with respect to the *recA* allele (6). In contrast, however, the frequency of adjacent deletions mediated by IS*1* was significantly increased in the absence of RecA. This has received some independent support in a physical assay, where it was shown that deletion products accumulate in a *recA* host but not in a wild-type host and, moreover, that like IS*1* induction of the SOS system, accumulation of such adjacent deletions was dependent on *recBC* (374a). It should be noted that the *recBC* genes have also been implicated in the behavior of other transposons such as Tn*10* and Tn*5* (216), where they affect precise and imprecise excision. However, this process is independent of transposition. It is more pronounced with composite transposons in which the component insertion sequences IS*10* and IS*50* are present in direct repeat and is stimulated when the transposon is carried by a transfer-proficient conjugative plasmid. It seems probable that such excisions occur by a process involving replication fork slippage (see reference 107 for further discussion).

Early studies implicated both DNA polymerase I (300, 329) and DNA gyrase (157, 325) in the transposition of Tn*5*. While the effect of gyrase may reflect a requirement for optimal levels of supercoiling, the role of DNA polymerase I remains a matter of speculation. It may be involved in DNA synthesis which is necessary to repair the single-strand gaps resulting from staggered cleavage of the target and which presumably gives rise to the direct target repeats. DNA gyrase has also been shown to be important in the transposition of bacteriophage Mu (259).

Another host function, the Dam DNA methylase, can be important in modulating both Tpase expression and activity. IS*10*, IS*50*, and IS*903* all carry methylation sites (GATC) in the Tpase promoter regions, and in each case, promoter activity is increased in a *dam* mutant host (292, 369). Additional evidence has been presented that the methylation status of GATC sites within the terminal IRs also modulates the activity of these ends (292). Similar sites have been previously observed in IS*3*, IS*4*, and IS*5*. A survey of the elements included in the database has shown that most groups or families (data not shown) contain members which have GATC sites within the first 50 bp of one or both extremities. The most numerous are members of the IS*3* (20 of 82 elements), IS*5* (32 of 68), and IS*256* (12 of 33) families. Except for IS*3* itself, where strong stimulation of transposition has been observed in a *dam* host (320), in most of these cases the biological relevance of these sites is unknown. Moreover, it should be pointed out that the probability that any 100-bp DNA sequence carries the GATC tetranucleotide is about 40%. The role of Dam methylation in IS*10* and IS*50* transposition is described in detail in the appropriate sections dealing with these elements.

Reaction Mechanisms

A detailed examination of the reaction mechanisms involved in transposition is outside the scope of this review and has been treated in depth elsewhere (238, 239). However, since such mechanisms are pertinent to an understanding of the various behaviors of the IS elements described below, we include here a brief and simplified description. The process can be divided into several defined steps, generally comprising binding of the recombinase to the ends; elaboration of a synaptic complex involving the recombinase, perhaps accessory proteins, and both transposon ends—this step involves either concomitant or subsequent (depending on the element) recruitment of the target DNA; cleavage and strand transfer of the transposon ends into the target; and processing of the strand transfer complex to a final product.

A rather surprising finding has been that the chemistry of cleavage and strand transfer is very similar, if not identical, in most of the limited collection of transposable elements analyzed in detail to date. These include retroviruses and the eukaryote Tc and *mariner* elements as well as bacteriophage Mu, IS*10*, Tn*7*, and IS*911*. The Tpase catalyzes cleavage at the 3′ ends of the element by an attacking nucleophile (generally H_2O) to expose a free 3′OH group (Fig. 2A and C, left panel). This hydroxyl in turn acts as a nucleophile in the attack of a 5′-phosphate group in the target DNA in a single-step transesterification reaction (Fig. 2C, right panel). A concerted transfer of both transposon ends to the target site while maintaining the correct strand polarity results in joining of each transposon strand to opposite target strands and leaves a 3′OH group on the cleaved target strand. Under certain conditions, the enzyme is also capable of "disintegrating" the transposon end by catalyzing the attack of the 3′ target OH group on the new transposon-target junction (Fig. 2C, right panel) (59, 270, 352). The reaction(s) does not require an external energy source and does not appear to involve a covalently linked enzyme-substrate intermediate, as do certain site-specific recombination reactions (134). Furthermore, it is worth underlining that since it is the donor strand itself which performs the cleavage-ligation step in the target DNA, no cleaved target molecule is detected in the absence of strand transfer.

Differences in the location of the target phosphodiester bond in the initial strand transfer reaction can lead to interesting variations in the overall transposition pathway. For the IS*3* family members IS*911* and IS*2*, a frequent product is a molecule in which only one transposon DNA strand is circu-

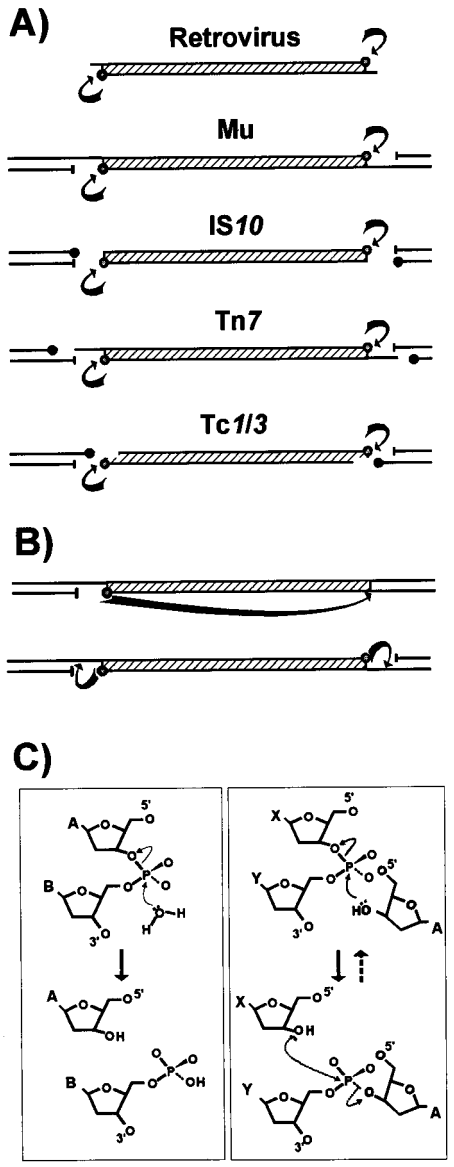

FIG. 2. Different types of Tpase-mediated cleavage at transposon ends. (A) Transposons are represented by hatched boxes, and flanking donor DNA is represented by black lines. The arrows indicate Tpase-mediated cleavages at the 3′ ends of each element which give rise to active 3′OH groups (open circles) and 5′-phosphate groups (⊣). Solid circles indicate 3′OH groups generated in flanking donor DNA. (B) Intramolecular strand transfer events which generate a single circularized transposon strand (top) or terminal hairpins (bottom). (C) Chemistry of the cleavage and strand transfer events. The left panel shows nucleophilic attack by a water molecule on the transposon phosphate backbone. The nucleotide shown as base A represents the terminal 3′ base of the transposon, and that marked B represent the neighboring 5′ nucleotide of the vector backbone DNA. Initial attack generates a 3′OH group on the transposon end. The right panel shows a strand transfer event. The 3′OH group at the transposon end acts as a nucleophile in the attack of the target phosphodiester backbone (bases X and Y), joining the 3′ transposon end to a 5′ target end and creating a 3′OH group on the neighboring target base (X). Also shown in this panel as dashed arrows is the disintegration reaction, in which the 3′OH of the target (X) attacks the newly created phosphodiester bond between the transposon (A) and target (Y) to regenerate the original phosphodiester bond between X and Y.

larized (Fig. 2B, top) (see "IS*3* family, IS*911*" below). This results from a free 3′OH group generated at one transposon end by the Tpase with the opposite end as a target. These molecules appear to be processed into transposon circles by "resolving" the complementary strand, and the circles can then undergo integration (see Fig. 8).

Another variation which could in principle occur in transposition reactions is one in which the exposed 3′OH group itself cleaves the complementary strand to generate a double-strand break. This would generate hairpin structures (Fig. 2B, bottom) and result in excision of the element from its donor site. This pathway has been adopted in V(D)J recombination (346), in which an intervening segment of DNA between two coding sequences must be eliminated. Deletion is accomplished by introduction of a single-strand nick at each boundary between coding and noncoding DNA to generate an exposed a 3′OH on the coding boundary which attacks the complementary strand. The two resulting hairpin structures are then joined and assembled into a new coding joint (277). Recently, this type of hairpin structure has been detected with IS*10* (175a).

DDE motif. Over the last few years, it has become clear that many of the enzymes involved in the reactions described above are related and, moreover, are part of a larger family of phosphoryltransferases which also includes RNase H and the RuvC Holliday resolvase. An acidic amino acid triad present in all these enzymes is intimately involved in catalysis, and its role is presumably in coordinating divalent metal cations (in particular Mg^{2+}) implicated in assisting the various nucleophilic attacking groups during the course of the reaction. For many ISs (the IS*3* and IS*6* families) and the retroviral integrases, this triad is known as the DD(35)E motif and is highly conserved, together with several additional residues (97, 172, 188) (Fig. 3) which include a K or R residue approximately 7 amino acids downstream from the E residue (87, 165, 266). In retroviruses, this motif interacts with the terminal base pairs of the element, presumably contributing to correct positioning of the transposon end in the active site (165). It is remarkable that such a motif can be found in many of the IS families defined here (Fig. 3). Although this conservation in the primary sequence is lower in certain of the other groups of elements and not all families have been explored in sufficient detail to ensure that the alignments shown in Fig. 3 are biologically relevant, mutagenic studies with some of these elements (e.g., the Mu, Tn*7*, IS*10*, and Tc*1* and Tc*3* Tpases) clearly underline the importance of these residues. Moreover, structural analysis has shown the presence of a related constellation of acidic amino acids arranged in a similar three-dimensional manner for retroviral integrases, bacteriophage Mu Tpase, RNase H, and RuvC (289, 290).

Other chemistries? Variations and exceptions to this unifying mechanism will certainly emerge. Not all ISs exhibit a well-defined DDE triad. For example, the Tpases of one group of elements, the IS*91* family, show significant similarities to enzymes associated with replicons which use a rolling-circle replication mechanism (see Fig. 15A). Indeed, evidence (232) suggests that IS*91* has adopted a rolling-circle transposition mechanism similar to that proposed by Galas and Chandler (106). In addition, members of the IS*110* family appear to encode a novel type of site-specific recombinase (205), while the IS*1* Tpase shows limited similarity to phage λ integrase (313), and active sites for the IS*66*, IS*200*/IS*605*, IS*1380*, ISAs*1*, and ISL*3* families (see Table 2) have yet to be defined.

Transposition Reactions and Different Types of Gene Rearrangement

While initiation of a transposition reaction proceeds via transfer of the 3′ end of the transposon, the outcome of the reaction is governed by cleavage of the 5′ end of the element (Fig. 2A). If cleavage at the 5′ end occurs concurrently with

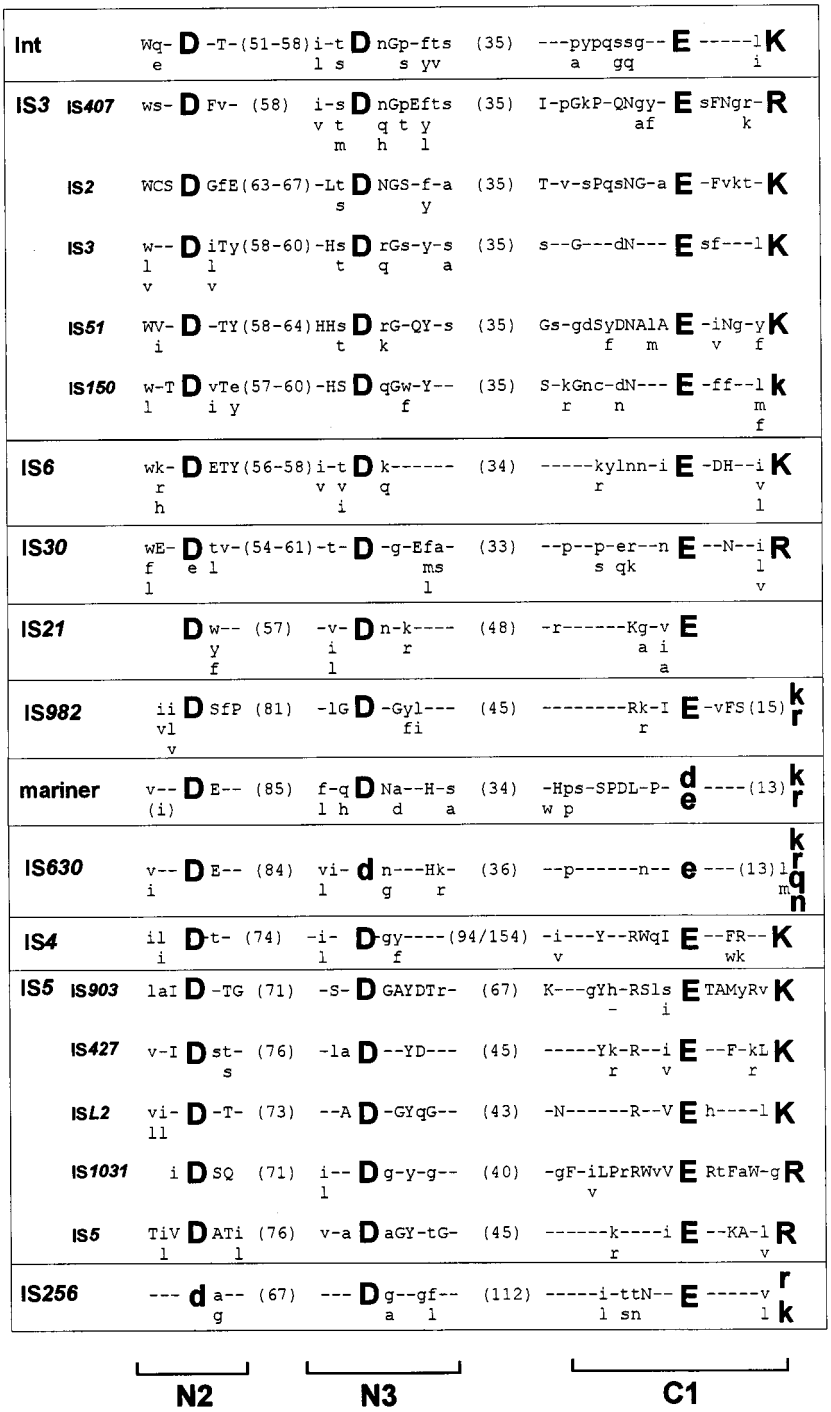

FIG. 3. DDE consensus of different families. The alignments are derived from the groups presented in Table 1. Amino acids forming part of the conserved motif are shown as large bold letters. Capital letters indicate conservation within a family, and lowercase letters indicate that the particular amino acid is predominant. The numbers in parentheses show the distance in amino acids between the amino acids of the conserved motif. The retroviral integrase alignment is based on reference 266. The IS*3* family is divided into the subgroups IS*407*, IS2, IS*3*, IS*51*, and IS*150*, as shown in Fig. 7B. The overall alignment (not shown) is essentially that obtained in reference 266. For IS*21*, see also reference 129; for *mariner*, see also references 87 and 295; for IS*630*, see also reference 87; for IS*4* and IS*5*, see also reference 288. The IS*5* family is divided into subgroups IS*903*, IS*427*, ISL*3*, IS*1031*, and IS*5*, as shown in Fig. 10. For IS*256*, see references 63 and 260. N2, N3, and C1 are regions defined in the IS*4* transposon family (288).

cleavage at the 3′ end, the transposon is physically separated from its donor molecule. Strand transfer to a target then results in direct insertion of the element (Fig. 4A, left). If 5′-strand cleavage occurs only after 3′-strand transfer, the donor and target molecules become covalently linked (Fig. 4A, right). Subsequent 5′ strand cleavage will separate the element from the donor backbone and will also result in a direct insertion. On the other hand, while 3′-strand transfer joins the transpo-

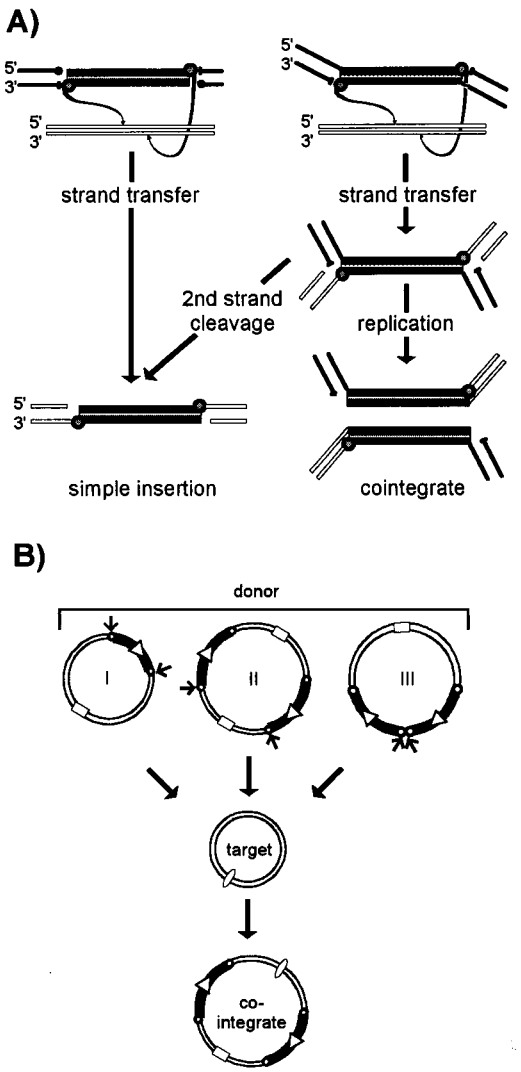

FIG. 4. Simple insertions and cointegrate formation. (A) Strand transfer and replication leading to simple insertions and cointegrates. The IS DNA is shown as a shaded cylinder. Liberated transposon 3′OH groups are shown as small shaded circles, and those of the donor backbone (bold lines) are shown as filled circles. The 5′ phosphates are indicated by bars. Strand polarity is indicated. Target DNA is shown as open boxes. The left panel shows an example of an IS which undergoes double-strand cleavage prior to strand transfer. The right panel shows an element which undergoes single-strand cleavage at its ends. After strand transfer, this can evolve into a cointegrate molecule by replication or a simple insertion by second-strand cleavage. (B) Replicative and nonreplicative transposition as mechanisms leading to cointegrates. Three "cointegrate" pathways are illustrated: (I) by replicative transposition, (II) by simple insertion from a dimeric form of the donor molecule, and (III) by simple insertion from a donor carrying tandem copies of the transposable element. Transposon DNA is indicated by a heavy line, and the terminal repeats are indicated by small open circles. The relative orientation is indicated by an open arrowhead. Square and oval symbols represent compatible origins of replication and are included to visually distinguish the different replicons. Arrows show which transposon ends are involved in each reaction.

son and target, it leaves a 3′OH in the target DNA at the junction. This can act as a primer for replication of the element and generate cointegrates where donor and target molecules are separated by a single transposon copy at each junction (Fig. 4A, right, and B, structure I). It is important to note that cointegrates identical to those produced by replicative transposition can be produced by a nonreplicative process either from a plasmid dimer (structure II) (26, 210) or from tandemly repeated copies of an IS element (structure III). The 5′ cleavage can vary from element to element (Fig. 2A). In retroviruses, only the 3′ cleavage occurs, removing 2 bp from the end of the double-strand DNA viral copy. Since no donor backbone is attached to the viral DNA, direct insertion can ensue. Bacteriophage Mu similarly undergoes only 3′ cleavage, the donor backbone remains attached, and cointegrate molecules result if replication occurs (Fig. 2A and 4A, right). For IS*10* and Tn*7*, both 3′ and 5′ cleavages occur and both elements undergo simple insertion. Double-strand cleavage at the ends of IS*10* is flush and is promoted by the single Tpase protein. Double-strand cleavage at the ends of Tn*7* leaves a 5′ 3-bp overhang and involves two proteins which cleave the 3′ (TnsB) and 5′ (TnsA) strands (67). Inactivation of the catalytic domain of TnsA prevents 5′-strand cleavage and results in the formation of branched-strand transfer intermediates in vitro and the production of cointegrates in vivo (231). Double-strand cleavage has also been demonstrated for the eukaryotic Tc*1*/*3* and P elements. However, whereas cleavage occurs precisely at the 3′ end, cleavage at the 5′ ends occurs 2 bases within the element in Tc*1* and Tc*3* (348) and 17 bases within the element in P (20).

The spectrum of possible DNA rearrangements is probably even larger. A suggestion that certain Tpases may be capable of generating synapses between two ends on different molecules was originally proposed based on the results of a genetic analysis of Tn*5* (210) and has more recently been demonstrated for IS*10* in vitro (54). Similar behavior as well as the capacity to act on directly repeated IS ends has recently been suggested for the IS*1* Tpase in vivo (198). These types of event obviously extend the spectrum of possible DNA rearrangements.

Transposition Immunity

One important property of some transposable elements is that of transposition immunity, in which a target molecule already carrying a copy of an element exhibits a significantly reduced affinity for insertion of a second copy. At present, this phenomenon appears to be limited to the more complex transposons, bacteriophage Mu and Tn*7*, as well as to members of the Tn*3* family. To our knowledge no insertion sequences have yet been clearly demonstrated to adopt this strategy although some evidence concerning IS*21* suggests that this element may show immunity (73, 128a). A priori, this behavior would be inappropriate for elements involved in the formation of compound transposons.

Although perhaps not immediately relevant to insertion sequences per se, immunity seems a sufficiently important phenomenon in the field of transposition to merit a short overview. For bacteriophage Mu, transposition immunity is displayed by target DNA carrying Mu end sequences and is transmitted by the MuB protein. MuB plays a key role in target capture and strand transfer by binding DNA in a nonspecific manner, providing a preferential target for the MuA Tpase complexed with Mu ends, and stimulating Tpase activity (199). MuB displays an ATPase activity which is stimulated both by DNA and MuA (5). ATP, but not ATP hydrolysis, is necessary for MuB binding and for strand transfer (3). Interaction of MuB with MuA (bound to the immune target) provokes ATP hydrolysis with subsequent release of MuB and consequent reduction in the attractiveness of the DNA molecule as a target (4). This mechanism serves to redistribute MuB preferentially to DNA molecules which do not contain a MuA binding site.

A similar mechanism has been proposed for transposon Tn*7* (17), where the presence of the right end of Tn*7* renders the

target immune (12). Here the Tpase is composed of two Tn*7* proteins, TnsA and TnsB. It acts in conjunction with TnsC which, like MuB, is a nonspecific DNA binding protein with ATPase activity (67).

Although transposition immunity of Tn*3* and the related Tn*1000* (γδ) is less well understood, it is known to require the presence of the 38-bp terminal IR on the immune target (15, 364). A major difference between Tn*3* and the phage Mu and Tn*7* systems is that only a single protein, the Tn*3* Tpase (TnpA), appears to be involved. As in these other two systems, immunity is mediated by Tpase binding to this end (9, 252, 364). Indeed, IHF, which stimulates Tpase binding to the IRs of Tn*1000* (362), also increases immunity (363).

Target Specificity

Where appropriate, insertion patterns of ISs are described in the sections dealing with the individual elements. Insertion specificity has also been treated in detail in a recent review (68). It is perhaps worthwhile, however, to summarize some of the more general issues concerning this aspect of transposition.

Target site selection differs significantly from element to element. Sequence-specific insertion is exhibited to some degree by several elements and varies considerably in stringency. It is strict in the case of one of the two Tn*7* transposition pathways, where insertion occurs exclusively with high efficiency into a unique chromosomal site (*att*Tn*7*) (67), and for IS*91*, which requires a GAAC/CAAG target sequence (233). Insertion sites are less strict but nevertheless are sequence specific for members of the IS*630* and *mariner*/Tc families, which both require a TA dinucleotide in the target; for IS*10*, which prefers (but is not restricted to) the symmetric 5′-NGCTNAGCN-3′ heptanucleotide; for IS*231*, which shows a preference for 5′-GGG(N)$_5$CCC-3′ (133); and for bacteriophage Mu, which shows a preference for 5′-NYG/CRN-3′ (240). For both IS*10* and the Tc*1*/*3* elements, sequences immediately adjacent to the consensus also influence the target choice (23, 261). A demonstration that IS*10* Tpase directly influences the target choice has been obtained by isolation of specific Tpase mutants which exhibit distinct alterations in target choice (22). Other elements exhibit regional preferences: for example GC- or AT-rich DNA segments (IS*186* [312] and IS*1* [105, 236, 375], respectively). Such regional specificity could reflect more global parameters such as local DNA structure. Indeed, the degree of supercoiling (IS*50* [212]), bent DNA (retroviruses [247] and IS*231* [133]), replication (Tn*7* [368] and IS*102* [30]), transcription (IS*102* [31] and Tn*5*/Tn*10* [50]), and protein-mediated targeting to or exclusion from transcriptional control regions (Mu [354] and yeast Ty1 [86]) have all been evoked as parameters which influence target choice. The nature of the target, e.g., whether it is a plasmid or chromosome, can also play a significant role (183). Target immunity can clearly be an additional factor.

Although much information on target specificity has been obtained by analyzing individual insertions, a more powerful approach is the use of population-based methods. Such methods provide a picture which is statistically more significant. They have been applied in the analysis of retroviral integration in vitro (247, 273), in the analysis of bacteriophage Mu insertion both in vitro (240) and in vivo (354), and in the investigation of IS*1*-mediated adjacent deletions in vivo (342). For retroviruses, this approach has revealed a preference for the exposed face of the nucleosome DNA helix and exclusion by DNA-bound regulatory proteins. For phage Mu, it has permitted definition of the target consensus in vitro and has allowed analysis of the effect of binding of various gene regulatory proteins on insertion in vivo.

Another phenomenon which may reflect insertion site specificity is the interdigitation of various intact or partial IS elements which has been noted repeatedly in the literature. Many of these observations are anecdotal and may reflect the scars of consecutive but isolated transposition events resulting from selection for acquisition (or loss) of accessory genes (see "Sources of bacterial insertion sequences" above). Some indication of the statistical significance of this is expected to emerge from the many bacterial genome-sequencing projects under way. On the other hand, several ISs exhibit a true preference for insertion into other elements. A preferred target for IS*231* is the terminal 38 bp of the transposon Tn*4430*, which includes both the sequence-specific and conformational components described above (133), while IS*21* has been reported to show a preference for insertion close to the end of a second copy of the element located in the target plasmid (280). In this latter case, the site-specific DNA binding properties of the Tpase are presumably implicated. At the mechanistic level, this phenomenon might be related to the capacity of IS*10* Tpase to form synaptic complexes with IS*10* ends located on separate DNA molecules (54).

Population Dynamics and Horizontal Transfer

The distribution of many insertion sequences within and between various bacterial species has often been investigated as part of the initial characterization of a new element, usually by simple Southern hybridization. Although useful in typing strains, much of the data remains purely descriptive. Few systematic attempts have been made to determine the dynamics of insertion sequences within bacterial populations in a controlled manner.

Hartl and colleagues (131, 302) have determined the distribution of IS*1*, IS*2*, IS*3*, IS*4*, IS*5*, IS*30*, and IS*103* in a heterogeneous collection of *E. coli* strains (ECOR collection). By fitting this data to a number of models, they concluded that these elements could be classified into three groups by the apparent strength of regulation: IS*1* and IS*5* (weakly regulated); IS*2*, IS*4*, and IS*30* (moderately regulated); and IS*3* (strongly regulated).

Based on an initial observation that bacteriophage P1 appeared to accumulate mutations due to insertion sequences when the host strain was stored in agar stabs (11), Arber and colleagues undertook a study of the changes in distribution of eight ISs (IS*1*, IS*2*, IS*3*, IS*4*, IS*5*, IS*30*, IS*150*, and IS*186*) from cultures of 118 individual clones isolated from a single 30-year-old stab of the well-characterized *E. coli* K-12 strain W3110 (250). The degree of variation in copy number was found to differ from element to element. When the number of each IS was counted, significant variation was noted in particular for IS*5* but also for IS*2*, IS*3*, and IS*30*. Lower variation was observed for IS*1*, IS*4*, IS*150*, and IS*186*. These variations in copy number were roughly correlated with the number of different patterns of hybridization obtained by extensive Southern blot analysis. For IS*30*, the data showed that copy number diversity increased in clones which had generated a particular restriction fragment carrying a tandem dimer of the element, a configuration which results in high transposition levels (see "IS*30* family" below).

Although given elements common to both these studies appear to display differences in their copy number diversity, it seems inherently unlikely that this could reflect a real difference in the behavior of a specific IS in the two sets of studies. Rather, it may occur because the *E. coli* W3110 strain used by

Naas et al. (250) was initially homogeneous whereas members of the ECOR collection (131, 302) have presumably undergone very different selective pressures.

Horizontal transfer of ISs in nature would not be surprising in view of the number and variety of autonomous extrachromosomal elements such as bacteriophages and plasmids which can serve as vectors, particularly promiscuous plasmids with wide host ranges. Several serendipitous observations, such as the isolation of identical IS*6* family members from *Mycobacterium fortuitum* and *Flavobacterium* (*Arthrobacter*) sp. (IS*6100* [170]), clearly support the idea that horizontal transfer occurs in nature.

Some information has been obtained concerning the evolution of certain insertion sequences within the enterobacteria. Analysis of the nucleotide sequences of IS*1*, IS*3*, and IS*30* from the ECOR collection and from other related enteric bacteria showed that each type of IS was highly conserved within *E. coli* (200). Since the degree of sequence divergence of several chromosomal genes within these clonal lineages was significantly higher, it was concluded that the ISs had a high turnover and rapid movement. Moreover, strains carrying one type of insertion element also tended to carry other types. This observation is consistent with the idea that multiple insertion sequences can be delivered by a single vector, for example a transmissible plasmid or phage (302). The homologs of these ISs carried by other species of enteric bacteria were divergent from the *E. coli* elements. This suggested a lower rate of transmission between species. Finally, the presence of mosaic variants of both IS*1* and of IS*3* in certain enteric species led to the conclusion that horizontal transmission (accompanied by recombination) had indeed occurred. Other studies have also compared the differences in the degree of nucleotide sequence variation of ISs with that of chromosomal genes. For IS*1* and IS*200* elements in natural populations of *E. coli* and *Salmonella typhimurium*, the results suggested that IS*200* has a significantly lower frequency of horizontal transfer than does IS*1* (37).

Data consistent with horizontal transfer are also emerging from studies with nonenteric bacteria. In one study, 17 isoforms of the ISS*1* sequence, isolated largely from bacteria which occupy another complex ecological niche, milk and cheese, were compared. They were determined to fall into three defined subgroups. Not only were nearly identical copies of these IS*6* family members isolated from distantly related *Streptococcus thermophilus* and *Lactococcus lactis* strains, but also mosaic copies were detected (40). Moreover, nearly identical IS*6* family members have also been found in *E. coli*, *Proteus vulgaris*, and *Pasteurella piscicida* (178).

For members of the IS*256* family, a phylogenetic tree of eight members was found to differ significantly from that of their host bacteria (123). Another study indicated that 10 members of the family isolated from actinomycetes formed a distinct group. While they exhibited a similar phylogenetic tree to their hosts (based on 16S RNA and superoxide dismutase genes) and most showed divergence similar to that of the 16S RNA and superoxide dismutase genes, IS*1512* and IS*1511* isolated from *Mycobacterium gordonae* showed significantly higher divergence (suggesting a higher mobility) and were more closely related to an element isolated from the *Rhodococcaceae* (260).

INSERTION SEQUENCE FAMILIES

Occurrence, Variety, and Systematics

Our current database contains 500 ISs isolated from 73 genera representing 159 bacterial species of both eubacteria and the archaea (Table 1). This list does not include ISs identified from the various genome-sequencing projects. It is important to note that the majority of these have not been tested for transposition activity and some may therefore carry mutations which render them inactive. In some instances, the alignments of translation products clearly indicated the occurrence of translation termination codons or the presence of one or more frame changes in the published sequence. It is possible that at least some of these are the result of sequencing errors. In many cases there is also some ambiguity concerning the exact tip of the IS and the number of directly repeated target base pairs generated. Where appropriate, these are noted in the text and the accompanying tables. In spite of the limitations inherent in the available data, we have been able to include 443 members of the collection in 17 families based on combinations of the following criteria: (i) similarities in genetic organization (arrangement of open reading frames); (ii) marked identities or similarities in their Tpases (common domains or motifs); (iii) similar features of their ends (terminal IRs); and (iv) fate of the nucleotide sequence of their target sites (generation of a direct target duplication of determined length). The general features of these families are shown in Table 2 and are presented in greater detail below. It should be noted that two families show some similarities to eukaryotic mobile elements: IS*630* is related to the widespread Tc/*mariner* group (87), whereas IS*256* is very distantly related to the plant transposon MuDR (91). We underline here that the classification scheme described below is not rigid and is provided only as a framework. Some families are more coherent and better established than others, and there are numerous uncertainties in several of the attributions of family status.

Sequences of the (putative) Tpases were aligned by PileUp software (Wisconsin package version 9.1; Genetics Computer Group, Madison, Wis.) to generate a table of pairwise distances (Distances software, Genetics Computer Group package). These data were converted into dendrograms of relatedness (relationships) by using Growtree software by the UPGMA method (unweighted pair group method using arithmetic averages). We emphasize that we have not explored these in detail and that they do not necessarily represent phylogenetic relationships between the different elements.

Figure 5 shows the distribution of insertion sequences between families. In this global analysis, we have distinguished IS isoforms, of which there are 158. We define these as elements which show a divergence of less than 5% in the amino acid sequence of their potential proteins. When reading frames are obviously interrupted by mutation or errors in sequence determination, a second rule adopted has been a divergence in the nucleic acid sequence of less than 10%. This rather arbitrary choice is for convenience only. The IS*3* (50 members excluding isoforms) and IS*5* (47 members) families are clearly the most preponderant, followed by IS*256* (29 members) and IS*4* (28 members). Together, these represent nearly 50% of the 337 distinct (non-isoform) database entries. The nucleotide sequences of 33 ISs were not available and a further 21 ISs did not fall into the established families.

The distribution of ISs among different bacterial genera and groups (146a) is presented in Tables 3 and 4 (including isoforms). What has become increasingly clear since the publication of *Mobile DNA* in 1989 (29) is that members of most individual families can be distributed across many different eubacterial and archaebacterial genera (Table 3). So far, two exceptions are IS*1*, which appears to be limited to the enterobacteria, and IS*66*, which is restricted to bacteria of the rhizosphere. Not unexpectedly, the groups of bacteria in which the largest number of elements have been documented are also

TABLE 1. Database used

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*1*A	IS*1*E, IS*1*K		IS*1*		*Escherichia coli* W3110	X52534	768	18/23	9
IS*1*B	IS*1*C	IS*1*A	IS*1*		*Escherichia coli* W3110	X17345	768	18/23	8, 9, 10, 14
IS*1*D		IS*1*A	IS*1*		*Escherichia coli* W3110	X52536	768	18/23	9
IS*1*F		IS*1*X1[d]	IS*1*		*Escherichia coli* W3110	X52538	768	20/23	ND[c]
IS*1*G		IS*1*R	IS*1*		*Escherichia coli* C600	J01730 [V][e]	768	17/23	8
IS*1*H			IS*1*		*Escherichia coli* ECOR50	U15127	764	21/23	ND
IS*1*N	NuXi		IS*1*		*Shigella dysenteriae*	J01737	766	20/23	ND
IS*1*R		IS*1*A	IS*1*		*Escherichia coli* (pR100)	J01730	768	18/23	ND
IS*1*S		IS*1*A	IS*1*		*Shigella sonnei*	M37615	768	18/23	ND
IS*1*SD		IS*1*A	IS*1*		*Shigella dysenteriae*	J01731	768	16/23	ND
IS*1*X1			IS*1*		*Shigella flexneri*	M37616	768	20/23	ND
IS*1*X2		IS*1*X1	IS*1*		*Escherichia vulneris* ATCC 29943	Z11605	768	18/23	ND
IS*1*X3			IS*1*		*Escherichia fergusonii* ATCC 35469	Z11603 [P][f]	694	ND	ND
IS*1*X4		IS*1*X2	IS*1*		*Escherichia hermannii* ATCC 33652	Z11604	768	20/23	ND
IS*2*			IS*3*	IS*2*	*Escherichia coli K-12*	M18426	1,331	32/41	5
IS*3*			IS*3*	IS*3*	*Escherichia coli K-12*	X02311	1,258	29/40	3, 4
IS*3*E	IS*3*	IS*3*	IS*3*	IS*3*	*Escherichia coli* ATCC 35382	Z11606	>1,181	ND	ND
IS*3*F	IS*3*	IS*3*	IS*3*	IS*3*	*Escherichia fergusonii* ATCC 35469	Z11607	>1,184	ND	ND
IS*3*G	IS*3*	IS*3*	IS*3*	IS*3*	*Escherichia fergusonii* ATCC 35471	Z11608	>1,184	ND	ND
IS*3*H	IS*3*	IS*3*	IS*3*	IS*3*	*Shigella dysenteriae* ATCC 13313	Z11609	>1,187	ND	ND
IS*4*			IS*4*		*Escherichia coli* K-12	J01733	1,426	16/18	11, 12, 13
IS*4*Sa			IS*4*		*Synechocystis* sp. strain PCC6803	U38915	1,303	15/19	ND
IS*5*			IS*5*	IS*5*	*Escherichia coli* K-12 (Lambda KH100)	J01735	1,195	15/16	4
IS*5*D	IS*5*-Delta	IS*5*	IS*5*	IS*5*	*Escherichia coli* DH5-alpha	X13668	1,283	15/16	4
IS*5*Sa			IS*5*	IS*1031*	*Synechocystis* sp. strain PCC6803	U38799	871	15/17	3
IS*5*Sb	IS*5*Sc	IS*5*Sa	IS*5*	IS*1031*	*Synechocystis* sp. strain PCC6803	U38915	871	15/17	3
IS*10*L		IS*10*R	IS*4*		*Salmonella typhimurium* (Tn*10*)	J01829 [V]	1,329	17/22	
IS*10*R	IS*10*		IS*4*		*Salmonella typhimurium* (Tn*10*)	J01829	1,329	17/22	9
IS*15*	IS*15*L, IS*15*R, IS*1522*	IS*26*	IS*6*		*Salmonella panama* LA46 (Tn*1525* from pIP112)	M12900	1,648	14	8
IS*15*DI	IS*15*-DeltaI	IS*26*	IS6		*Salmonella panama* LA46 (Tn*1525* from pIP112)	M12900	820	14	8
IS*15*DII	IS*15*-DeltaII	IS*26*	IS6		*Salmonella panama* LA46 (Tn*1525* from pIP112)	M12900 [V]	820	14	ND
IS*15*DIII		IS*26*	IS*6*		*Campylobacter* sp. strain BM2196	M12900 [V] [P]	>667	ND	ND
IS*15*DIV		IS*26*	IS*6*		*Salmonella typhimurium* (pBP11)	X13616	820	14	ND
IS*16*			IS*256*		*Enterococcus faecalis* BM4281(Tn*1547*)	U35366	1,466	28/42	8
IS*21*	IS*8*		IS*21*		*Pseudomonas aeruginosa* PAO25 (pR68.45)	X14793	2,131	30/42	4, (5)
IS*22*			ISNCY[g]		*Pseudomonas aeruginosa* PAO (pFP110)	ND	7,300	ND	ND
IS*26*	IS*6*, IS*26*L, IS*26*R, IS*46*, IS*140*, IS*160*, IS*176*		IS*6*		*Proteus vulgaris* UR-75 (Tn*2680* from pR*ts*1)	X00011	820	14	8
IS*30*	IS*121*, Tn*2700*, Tn*2702*		IS*30*		*Escherichia coli* K-12 (Tn*2671* from pNR1-Basel)	X00792	1,221	23/26	2
IS*30*D	IS*30*	IS*30*	IS*30*		*Escherichia coli* K-12	X62680	1,221	23/26	2
IS*30*H	IS*30*		IS*30*		*Escherichia hermannii* ATCC 33652	Z11753	1,221	23/26	ND
IS*50*L		IS*50*R	IS*4*		*Escherichia coli* (Tn*5*)	U15572	1,534	8/9	
IS*50*R	IS*50*		IS*4*		*Escherichia coli* DB729 (Tn*5* from pJR67)	U15573	1,534	8/9	8, 9, 10
IS*51*			IS*3*	IS*51*	*Pseudomonas syringae* pv. savastanoi TK2009-5 (pIAA1)	M14365	1,311	26	3
IS*52*			IS*5*	IS*5*	*Pseudomonas syringae* pv. savastanoi PB205-1L (pIAA2)	M14366	1,209	9/10	4
IS*53*			IS*21*		*Pseudomonas syringae* pv. savastanoi PB213 (pIAA2)	M83932	2,555	24/27	8
IS*53*K			IS*21*		*Pseudomonas oleovorans* TF4-1L (OCT)	J04618 [P]	>316	ND	ND
IS*60*			ISNCY		*Agrobacterium tumefaciens* LBA4060 (pAL108)	ND	1,200	ND	ND
IS*66*	IS*66t*, IS*66v2*		IS*66*		*Agrobacterium tumefaciens* A66 (pTiA66)	M10204	2,548	18/20	8
IS*66*-1	IS*66v1*	IS*66*	IS*66*		*Agrobacterium tumefaciens* (pTi15955)	ND	2,556	18/20	8
IS*70*			ISNCY		*Proteus mirabilis* (pR772)	ND	3,700	ND	ND
IS*71*			IS*66*		*Agrobacterium tumefaciens* (pTi15955)	ND	2,386	9/11	8
IS*91*			IS*91*		*Escherichia coli* EC185 (pSU233)	X17114	1,830	0	0
IS*91*B		IS*91*	IS*91*		*Escherichia coli* G7 (pRI8801)	X77671	>549	ND	ND
IS*92*L		IS*91*	IS*91*		*Escherichia coli* SU100 (pHly152)	ND	3,100	ND	ND
IS*92*R		IS*91*	IS*91*		*Escherichia coli* SU100 (pHly152)	ND	2,150	ND	ND
IS*100*			IS*21*		*Yersinia pestis* 106 Otten	Z32853	1,953	20/28	5
IS*100*kyp		IS*100*	IS*21*		*Yersinia pseudotuberculosis* (pKYP1)	U59875	1,954	20/28	ND
IS*100*L		IS*100*	IS*21*		*Yersinia pestis* EV76 (pLcr)	X78302	1,950	20/28	5

Continued on following page.

TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*100*X		IS*100*	IS*21*		*Yersinia pestis* EV7651F	L19030	1,924	20/28	ND
IS*102*		IS*903*	IS*5*	IS*903*	*Escherichia coli* (pSC101)	J01728	1,057	18	9
IS*103*		IS*150*	IS*3*	IS*150*	*Escherichia coli* K-12 $_{x}$342	X07037 [V]	1,443	22/31	4
IS*110*			IS*110*		*Streptomyces coelicolor* A3(2)	Y00434	1,558	0	(0)
IS*112*			IS*5*	ISL*2*	*Streptomyces albus* G J1147	X56644	883	16/20	2
IS*116*			IS*110*		*Streptomyces clavuligerus* NRRL3585	M31716	1,421	0	0
IS*117*	A3(2) minicircle		IS*110*		*Streptomyces coelicolor* A3(2) M130	X15942	2,527	0	0
IS*120*			IS*3*	IS*150*	*Clostridium thermocellum*	ND	1,447	10/12	3
IS*150*			IS*3*	IS*150*	*Escherichia coli* K-12 431	X07037	1,443	22/31	3
IS*161*			ISNCY		*Escherichia coli* (Tn*2424*)	ND	1,700	ND	ND
IS*186*A		IS*186*B	IS*4*		*Escherichia coli* RR1	M11300	1,341	23	10
IS*186*B	IS*186*-357, IS*186*-409		IS*4*		*Escherichia coli* RR1	X03123	1,338	23	(8), 10, 11
IS*199*			IS*3*	IS*150*	*Streptococcus mutans* V403	L23843	1,220	31/43	3
IS*200*			IS*605*	IS*200*	*Salmonella typhimurium* TR6238	X56834	707	0	0 (2)
IS*200*A	IS*200*	IS*200*	IS*605*	IS*200*	*Salmonella typhimurium* LT2	X56834 [V] [P]	700	0	0 (2)
IS*200*B	IS*200*		IS*605*	IS*200*	*Escherichia coli* ECOR8	L25844 [P]	>601	ND	ND
IS*200*C	IS*200*	IS*200*B	IS*605*	IS*200*	*Escherichia coli* ECOR51	L25845 [P]	>601	ND	ND
IS*200*D		IS*200*B	IS*605*	IS*200*	*Escherichia coli* ECOR63	L25846 [P]	>601	ND	ND
IS*200*E	IS*200*	IS*200*B	IS*605*	IS*200*	*Escherichia coli* ECOR72	L25847 [P]	>601	ND	ND
IS*200*F	IS*200*	IS*200*	IS*605*	IS*200*	*Salmonella typhimurium* SARA17	Z54217	709	0	ND
IS*200*G			IS*605*	IS*200*	*Yersinia pestis* 6/69M	U22457	707	0	ND
IS*200*H	IS200-SAO	IS*200*	IS*605*	IS*200*	*Salmonella abortusovis* SS44	Y08755	708	0	ND
IS*200*P	IS200-VP	IS*200*	IS*605*	IS*200*	*Salmonella typhimurium* LT2 (pSLT)	Y09564	708	0	1
IS*200*S			IS*605*	IS*200*	*Streptococcus pneumoniae* type 23	L20670 [P]	>651	0	ND
IS*204*			ISL*3*		*Nocardia asteroides* YP21	U10634	1,453	19/23	8
IS*222*			IS*3*	IS*3*	*Pseudomonas aeruginosa* (phage D3c)	U00100	1,232	20/23	ND
IS*231*A	IR1, IR1750′		IS*4*		*Bacillus thuringiensis* subsp. *thuringiensis* berliner 1715 (p65kb)	X03397	1,656	20	10, 11, 12
IS*231*B	IR1, IR1750*	IS*231*A	IS*4*		*Bacillus thuringensis* subsp. *thuringiensis* berliner 1715 (p65kb)	M16158	1,643	7/20	ND
IS*231*C	IR1, IR1750		IS*4*		*Bacillus thuringiensis* subsp. *thuringiensis* berliner 1715 (p65kb)	M16159	1,656	19/20	11
IS*231*D			IS*4*		*Bacillus thuringiensis* subsp. *finitimus*	X63383	1,657	17/20	ND
IS*231*E			IS*4*		*Bacillus thuringiensis* subsp. *finitimus*	X63384	1,614	0/20	ND
IS*231*F			IS*4*		*Bacillus thuringiensis* subsp. *israelensis* (p112kb)	X63385	1,655	19/20	12
IS*231*G			IS*4*		*Bacillus thuringiensis* subsp. *darmstadiensis* 73-E-10-2	M93054	1,649	20	ND
IS*231*H			IS*4*		*Bacillus thuringiensis* subsp. *darmstadiensis* 73-E-10-2	M93054 [P]	>817	ND	ND
IS*231*V			IS*4*		*Bacillus thuringiensis* subsp. *israelensis* (p112kb)	M86926 [V]	1,964	21/22	ND
IS*231*W		IS*231*V	IS*4*		*Bacillus thuringiensis* subsp. *israelensis* (p112kb)	M86926	1,964	21/22	ND
IS*232*A	IR2, IS*232*, IR2150		IS*21*		*Bacillus thuringiensis* subsp. *thuringiensis* berliner 1715 (p65kb)	M38370	2,184	48/67	ND
IS*232*B	IR2, IS*232*, IR2150	IS*232*A	IS*21*		*Bacillus thuringiensis* subsp. *kurstaki* HD73 (p75kb)	M77344 [P]	2,200	28/37	ND
IS*232*C	IR2, IS*232*, IR2150	IS*232*A	IS*21*		*Bacillus thuringiensis* subsp. *kurstaki* HD73 (p75kb)	ND	2,200	28/37	ND
IS*233*A			IS*982*		*Bacillus thuringiensis* subsp. galleriae T05001	ND	1,028	ND	8
IS*240*A	IRA		IS*6*		*Bacillus thuringiensis* subsp. *israelensis* (p112kb)	M23740	861	16/17	ND
IS*240*B	IRB	IS*240*A	IS*6*		*Bacillus thuringiensis* subsp. *israelensis* (p112kb)	M23741	861	16/17	ND
IS*240*C			IS*6*		*Bacillus cereus* CER484	ND	817	16/17	ND
IS*240*F			IS*6*		*Bacillus thuringiensis* subsp. *fukuokaensis* 84I113 (p197kb)	Y09946	806	16/17	ND
IS*256*	IS*256*E, IS*256*L, IS*256*R		IS*256*		*Staphylococcus aureus* (Tn*4001* from pSK1)	M18086	1,324	17/26	8
IS*257*-1	IS*257*/1	IS*257*R1	IS*6*		*Staphylococcus aureus* (pSH6)	X53952	791	21/26	ND
IS*257*-2	IS*257*/2	IS*257*R1	IS*6*		*Staphylococcus aureus* (pSH6)	X53951	790	20/26	ND
IS*257*-3	IS*257*/3	IS*257*R1	IS*6*		*Staphylococcus aureus* (pSH6)	X53951	789	23/27	ND
IS*257*R1			IS*6*		*Staphylococcus aureus* (Tn*4003* from pSK1)	X13290	790	18/20	8
IS*257*R2	IS*257*L	IS*257*R1	IS*6*		*Staphylococcus aureus* (Tn*4003* from pSK1)	X13290	789	23/27	8
IS*281*			ISNCY		*Streptomyces lividans* 803 (phage C43)	ND	1,400	ND	ND
IS*285*			IS*256*		*Yersinia pestis* 358 (pLcr)	X78303	1,318	22/29	8

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*292*			IS*66*		*Agrobacterium* sp. strain X88-292 (pTi292)	L29283	2,496	19/21	8
IS*298*			ISNCY		*Caulobacter crescentus* SC298	ND	800	12/16	4
IS*401*			IS*3*	IS*51*	*Burkholderia cepacia* ATCC 17616 (pTGL6)	L09108	1,316	23/26	3
IS*402*			IS*5*	IS*427*	*Burkholderia cepacia* ATCC 17616 (pTGL6)	M33173	914	16/17	3
IS*403*			ISNCY		*Burkholderia cepacia* 249	ND	800	ND	ND
IS*404*			ISNCY		*Burkholderia cepacia* 249	ND	1,100	ND	ND
IS*405*			ISNCY		*Burkholderia cepacia* ATCC 17616 (pTGL1)	ND	1,500	ND	ND
IS*406*			IS*256*		*Burkholderia cepacia* ATCC 17616	M83145	1,367	22/27	8
IS*407*			IS*3*	IS*407*	*Burkholderia cepacia* ATCC 17616	M82980	1,236	31/49	4
IS*408*			IS*21*		*Burkholderia cepacia* ATCC 17616 (pTGL1)	L09108 [P]	>2,530	40/48	8
IS*411*			ISNCY		*Burkholderia cepacia* ATCC 17616 (pTGL1)	ND	2,000	ND	ND
IS*415*			ISNCY		*Burkholderia cepacia* ATCC 17616	ND	2,100	ND	ND
IS*421*		IS*186*	IS*4*		*Escherichia coli* JA221	Y07501	1,342	23	11
IS*426*	IS*136*, IS<u>AT*1*</u>		IS*3*	IS*2*	*Agrobacterium tumefaciens* A208 (pTiT37)	X56562	1,319	24/33	5
IS*427*			IS*5*	IS*427*	*Agrobacterium tumefaciens* T37 (pTiT37)	M55562	1,271	13/16	2
IS*431*L		IS*257*R1	IS*6*		*Staphylococcus aureus* (pI524)	M18437	788	19/22	ND
IS*431*mec		IS*257*R1	IS*6*		*Staphylococcus aureus* BB270	X53818	790	18/20	ND
IS*431*R		IS*257*R1	IS*6*		*Staphylococcus aureus* (pI524)	M18437 [V]	790	17/20	ND
IS*466*			IS*6*		*Streptomyces coelicolor* K605 (pSCP1)	S70701, S70702 [P]	1,628	32/37	ND
IS*476*			IS*3*	IS*407*	*Xanthomonas campestris* pv. vesicatoria 81-23 race 2	M28557	1,226	22/27	4
IS*481*			IS*3*	IS*481*	*Bordetella pertussis* Tohama	M22031	1,043	22/23	6
IS<u>*481*G</u>		IS*481*	IS*3*	IS*481*	*Bordetella pertussis* NCTC 10908	M22031 [V]	1,039	22/23	6
IS<u>*481*P</u>	ISPARK	IS*481*	IS*3*	IS*481*	*Bordetella pertussis* 18-323	M28220 [V] [P]	>525	0/23	ND
IS*481*v1	RS1	IS*481*	IS*3*	IS*481*	*Bordetella pertussis* BPH30	M28220	1,043	20/23	6
IS*481*v2	RS2	IS*481*	IS*3*	IS*481*	*Bordetella pertussis* Wellcome28	M28220 [V]	1,043	20/23	6
IS*492*			IS*110*		*Pseudomonas atlantica*	M24471	1,202	0	5
IS*493*			IS*5*	ISL*2*	*Streptomyces lividans* CT2	M28508	1,643	21/24	3
IS*511*	IS*125*		IS*3*	IS*407*	*Caulobacter crescentus* CB15	U39501	1,266	34/41	4
IS*600*			IS*3*	IS*3*	*Shigella sonnei*	X05952	1,264	19/27	3
IS*602*L		IS*903*	IS*5*	IS*903*	*Capnocytophaga ochraceus* (Tn*602* from pGD10)	M20309 [P]	1,057	18	9
IS*602*R		IS*903*	IS*5*	IS*903*	*Capnocytophaga ochraceus* (Tn*602* from pGD10)	M20309 [P]	1,057	18	9
IS*605*	IShp, RS2		IS*605*		*Helicobacter pylori* NCTC 11638	ND	1,904	0	ND
IS*629*		IS*3411*	IS*3*	IS*51*	*Shigella sonnei*	X51586	1,310	23/25	ND
IS*630*	Tn*4730*, Tn*4731*, Tn*4733*		IS*630*		*Shigella sonnei*	X05955	1,153	21/29	2
IS*640*		IS*21*	IS*21*		*Shigella sonnei*	X05956	>1,085	ND	ND
IS*701*			IS*4*		*Calothrix* sp. strain PCC7601	X60383	1,389	22/25	4
IS*702*			IS*5*	ISL2	*Calothrix* sp. strain PCC7601	X60384	1,098	33/40	3
IS*711*			IS*5*	IS*427*	*Brucella ovis* ATCC25840	M94960	842	20/25	2
IS*801*	RS-I		IS*91*		*Pseudomonas syringae* pv. phaseolicola LR781 (pMMC7105)	X57269	1,512	0	0
IS<u>*802*</u>	RS-II		ISNCY		*Pseudomonas syringae* pv. phaseolicola LR781 (pMMC7105)	ND	<2,600	ND	ND
IS<u>*803*</u>	RS-III		ISNCY		*Pseudomonas syringae* pv. phaseolicola LR781 (pMMC7105)	ND	ND	ND	ND
IS*861*			IS*3*	IS*150*	*Streptococcus agalactiae* COH-I	M22449	1,442	22/26	3
IS*866*	IST-1		IS*66*		*Agrobacterium tumefaciens* (pTiTm4)	M25805	2,716	24/27	8
IS*867*	IS*867*a, IST-2, IS*867*b, IST-3		IS*66*		*Agrobacterium tumefaciens* (pTiTm4)	M63056 [P]	2,700	ND	ND
IS*868*			IS*3*	IS*51*	*Agrobacterium tumefaciens* AB3 (pTiAB3)	X55075	1,321	25/26	ND
IS*869*			IS*5*	IS*427*	*Agrobacterium tumefaciens* (pTiAB3)	X53945	849	14/16	2
IS*869*-1			IS*5*	IS*427*	*Agrobacterium tumefaciens* (pTi15955)	ND	849	4	2
IS*870*	IS*870.1*, IS*870.2*, IS*870.3*		IS*630*		*Agrobacterium vitis* 2657 (pTiAg57)	Z18270	1,146	10	2
IS*891*			IS*605*		*Anabaena* sp. strain M-131	M24855	1,352	0	0
IS*892*			ISNCY		*Anabaena* sp. strain PCC7120	M64297	1,675	21/24	8
IS*893*			ISNCY		*Anabaena* strain PCC7120	ND	1,200	ND	ND
IS*894*			ISNCY		*Anabaena* sp. strain PCC7120	ND	1,900	ND	ND
IS*895*	*mys*		IS*630*		*Anabaena* sp. strain PCC7120	M67475	1,192	26/28	2

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*897*			ISNCY		*Anabaena* sp. strain PCC7120	ND	1,500	ND	ND
IS*898*			ISNCY		*Anabaena* sp. strain PCC7120	ND	1,000	ND	ND
IS*900*			IS*110*		*Mycobacterium paratuberculosis*	X16293	1,451	0	0
IS*901*			IS*110*		*Mycobacterium avium* FP8589	X59272	1,472	0	0
IS*902*		IS*901*	IS*110*		*Mycobacterium avium* subsp. *silvaticum* 0016	X58030	1,470	0	0
IS*903*	IS*903*L, IS*903*R, Tn*55*, Tn*601*		IS*5*	IS*903*	*Escherichia coli* (Tn*903* from pR6-5)	J01839	1,057	18	9
IS*903*B	IS*903*.B	IS*903*	IS*5*	IS*903*	*Escherichia coli* (Tn*2680* from pR*ts*1)	X02527	1,057	18	9
IS*904*		IS*1068*	IS*3*	IS*3*	*Lactococcus lactis* subsp. *lactis* FI5876	M27276	1,241	31/39	(4)
IS*905*A	IS*905*		IS*256*		*Lactococcus lactis* subsp. *cremoris* FI7304	L20851	1,313	20/28	8
IS*905*B	IS*905*	IS*905*A	IS*256*		*Lactococcus lactis* subsp. *cremoris* MG1614	L20851 [V]	1,313	20/28	8
IS*911*	ISSHO*1*		IS*3*	IS*3*	*Shigella dysenteriae* ATCC 11456	X17613	1,250	25/36	3, 4
IS*931*	RS1100, IS*933*		IS*256*		*Burkholderia cepacia* AC1100	M25495	1,477	38/39	8
IS*932*			ISNCY		*Burkholderia cepacia* AC1100	ND	3,400	ND	ND
IS*942*			IS*4*		*Bacteroides fragilis* TAL3636	J03326	1,598	13/15	8
IS*946*M	IS*946*	ISS*1*S	IS*6*		*Lactococcus lactis* subsp. *lactis* T-EK1 (pTR2030)	ND	800	ND	8
IS*946*V	IS*946*	ISS*1*S	IS*6*		*Lactococcus lactis* subsp. *lactis* T-EK1 (pTR2030)	M33868	808	18	8
IS*981*			IS*3*	IS*3*	*Lactococcus lactis* subsp. *lactis* LM0230	M33933	1,224	18/28	3
IS*982*	IS*982*A		IS*982*		*Lactococcus lactis* subsp. *cremoris* SK11 (pSK11L)	L34754	999	19/20	ND
IS*982*B	IS*982*	IS*982*	IS*982*		*Lactococcus lactis* bv. diacetylactis CRL264 (pCIT264)	S77101	999	19/20	ND
IS*982*C		IS*982*	IS*982*		*Lactococcus lactis* subsp. *cremoris* NIZOB40 (pNZ4000)	U93364	999	19/20	ND
IS*986*		IS*6110*	IS*3*	IS*51*	*Mycobacterium tuberculosis*	X52471	1,354	25/28	ND
IS*987*		IS*6110*	IS*3*	IS*51*	*Mycobacterium bovis* BCG44	X57835	1,355	25/28	3
IS*1000*A			IS*110*		*Thermus thermophilus* HB8	M33159	1,196	5/6	0
IS*1000*B		IS*1000*A	IS*110*		*Thermus thermophilus* HB8	M33159 [V]	1,196	5/6	0
IS*1001*	IS*1001*A, IS*1001*B, IS*1001*C		ISL*3*		*Bordetella parapertussis* B24	X66858	1,306	21/26	ND
IS*1004*			IS*605*	IS*200*	*Vibrio cholerae* O1	Z67733	628	ND	ND
IS*1016*C	ORF3/*frpC*, IS*1016*N		ISNCY		*Neisseria meningitidis* FAM20	L06299	713	19	ND
IS*1016*V1		IS*1016*V6	ISNCY		*Haemophilus influenzae* RM118	X58173	711	19	ND
IS*1016*V2		IS*1016*V6	ISNCY		*Haemophilus influenzae* RM153	X58174	711	19	ND
IS*1016*V3		IS*1016*V6	ISNCY		*Haemophilus influenzae* RM926	X58175	711	18/19	ND
IS*1016*V4		IS*1016*V6	ISNCY		*Haemophilus influenzae* RM926	X58176	673	19/0	ND
IS*1016*V5		IS*1016*V6	ISNCY		*Haemophilus influenzae* RM7004	X58177	242	19/0	ND
IS*1016*V6			ISNCY		*Haemophilus influenzae* bg. aegyptius BPF	X59756	712	19	ND
IS*1031*A	IS*1031*B		IS*5*	IS*1031*	*Acetobacter xylinum* ATCC 23769	M80805	930	22/24	3
IS*1031*C			IS*5*	IS*1031*	*Acetobacter xylinum* ATCC 23769	M98777	930	23/26	3
IS*1031*D		IS*1031*C	IS*5*	IS*1031*	*Acetobacter xylinum* ATCC 23769	M98778	930	23/26	3
IS*1032*			IS*5*	IS*1031*	*Acetobacter xylinum* ATCC 23770	M80805	916	9/15	3
IS*1051*			IS*5*	IS*5*	*Xanthomonas campestris* pv. dieffenbachiae 11044	X70380	1,158	13/15	ND
IS*1066*			IS*630*		*Pseudomonas* sp. strain P51 (Tn*5280* from pP51)	M61114	1,137	10	2
IS*1067*		IS*1066*	IS*630*		*Pseudomonas* sp. strain P51 (Tn*5280* from pP51)	M61114 [V]	1,137	10	2
IS*1068*	IS*904*N		IS*3*	IS*3*	*Lactococcus lactis* subsp. *lactis* NIZOR5 (Tn*5276*)	X52273	1,245	31/39	ND
IS*1069*		IS*1068*	IS*3*	IS*3*	*Lactococcus lactis* subsp. *lactis* NIZO22186	X78469	1,245	31/39	ND
IS*1070*			IS*30*		*Leuconostoc lactis* NZ6009 (pNZ63)	U17353	1,027	24/28	0 (?)
IS*1076*	IS*1076*L, IS*1076*R, Tn*5286*	IS*1068*	IS*3*	IS*3*	*Lactococcus lactis* subsp. *lactis* CNRZ270 (pUCL22)	X53013	1,296	31/39	3
IS*1081*			IS*256*		*Mycobacterium bovis* TMC410	X61270	1,435	19/26	8
IS*1086*			IS*30*		*Ralstonia eutropha* AE104	X58441	1,106	22/28	3
IS*1096*			ISL*3*		*Mycobacterium smegmatis* ATCC 607	M76495	2,259	24/26	8
IS*1106*			IS*5*	IS*5*	*Neisseria meningitidis* B15	Z11857	1,137	35/36	ND
IS*1110*			IS*110*		*Mycobacterium avium* LR541	Z23003	1,462	0	0
IS*1111*A			IS*110*		*Coxiella burnetii* NineMile7	M80806	1,450	7	ND
IS*1111*B		IS*1111*A	IS*110*		*Coxiella burnetii* NineMile7	M80806 [V]	1,458	7	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*1111*C		IS*1111*A	IS*110*		*Coxiella burnetii* NineMile7	M80806 [V]	1,452	7	ND
IS*1112*	ISX*oo1*		IS*30*		*Xanthomonas oryzae* pv. oryzae PXO86^{Rif}	ND	1,055	22/25	3
IS*1112*a	IS*203*a	IS*1112*	IS*30*		*Xanthomonas oryzae* pv. oryzae PXO96^{Rif}	ND	1,055	23/25	3
IS*1112*b	IS*203*b, IS*203*c	IS*1112*	IS*30*		*Xanthomonas oryzae* pv. oryzae PX112	ND	1,055	21/25	3
IS*1113*	TNX1		ISNCY		*Xanthomonas oryzae* pv. oryzae	ND	1,050	ND	ND
IS*1126*			IS*5*	IS*5*	*Porphyromonas gingivalis* W83	X77924	1,336	12	5
IS*1131*	ISC		IS*66*		*Agrobacterium tumefaciens* PO22 (pTi)	M82888	2,773	12	8
IS*1132*	DI-2		IS*256*		*Corynebacterium diphtheriae* Belfanti 1030	A07012	1,441	21/28	8
IS*1133*			IS*3*	IS*3*	*Erwinia amylovora* CA11 (Tn*5393* from pEa34)	Z12167	1,232	24/28	3
IS*1136*A			IS*605*		*Saccharopolyspora erythraea* NRRL 2338	L07626	1,406	7/13	(0)
IS*1136*B		IS*1136*A	IS*605*		*Saccharopolyspora erythraea* NRRL 2338	L07627, L07628 [P]	1,400	8/14	ND
IS*1136*C			IS*605*		*Saccharopolyspora erythraea* NRRL 2338	L07629, L07630 [P]	1,400	ND	ND
IS*1136*D			IS*605*		*Saccharopolyspora erythraea* NRRL 2338	L07631, L07632 [P]	1,400	ND	ND
IS*1137*			IS*3*	IS*51*	*Mycobacterium smegmatis*	X70913	1,361	22/24	3
IS*1138*			IS*3*	IS*150*	*Mycoplasma pulmonis* KD735-26	Z16416	1,288	18	3
IS*1138*B		IS*1138*	IS*3*	IS*150*	*Mycoplasma pulmonis* KD735-26	Z16416 [V]	1,288	18	3
IS*1139*		IS*1161*	IS*30*		*Streptococcus salivarius* ATCC 25975	Z17279	1,162	20/27	0 (?)
IS*1141*			IS*3*	IS*3*	*Mycobacterium intracellulare*	L10239	1,588	18/23	ND
IS*1151*			IS*4*		*Clostridium perfringens* NCTC 2062	Z18246	1,689	21	ND
IS*1161*			IS*30*		*Streptococcus salivarius* ATCC 25975	L07794	1,165	22/28	0 (?)
IS*1162*			IS*21*		*Pseudomonas fluorescens* ST (pEG)	X79443	2,634	37/50	ND
IS*1163*			IS*3*	IS*3*	*Lactobacillus sake* L45	X75164	1,180	32/48	3
IS*1164*			IS*256*		*Rhodococcus rhodochrous* J1	D67027	1,430	18/27	8
IS*1165*			ISL*3*		*Leuconostoc mesenteroides* subsp. *cremoris* DB1165 (p30kb)	X62617	1,553	20/39	ND
IS*1166*			IS*256*		*Rhodococcus* sp. strain IGTS8 (pSOX)	U08850	1,469	19/25	ND
IS*1167*			ISL*3*		*Streptococcus pneumoniae* CP1200	M36180	1,435	17/24	8 (?)
IS*1167*L		IS*1167*	ISL*3*		*Streptococcus pneumoniae* 13868	Z83335	1,484	18/24	ND
IS*1167*R		IS*1167*	ISL*3*		*Streptococcus pneumoniae* 13868	Z83335	1,464	20/24	ND
IS*1168*			IS*5*	IS*5*	*Bacteroides vulgatus* BV17 (pIP417)	X71444	1,320	13/17	ND
IS*1168*F	IS*1168*	IS*1168*	IS*5*	IS*5*	*Bacteroides fragilis* BF8	X71443 [P]	>777	ND	ND
IS*1169*			IS*5*	IS*5*	*Bacteroides fragilis* BF-F238 (pIP421)	X76949	1,317	16/20	ND
IS*1170*			IS*4*		*Bacteroides thetaiotaomicron* BT13 (pIP419)	X76948	1,604	12/15	ND
IS*1181*			ISL*3*		*Staphylococcus aureus* BM3121	L14544	1,512	17/23	8
IS*1182*			ISNCY	IS*1182*	*Staphylococcus aureus* BM3121	L43082	1,864	14/16	ND
IS*1186*		IS*1168*	IS*5*	IS*5*	*Bacteroides fragilis* BFr81R	X72301	1,300	13/17	4
IS*1191*		IS*905*A	IS*256*		*Streptococcus thermophilus* CNRZ368	X71808	1,313	20/28	8
IS*1193*D	IS*1193*		ISL*3*		*Streptococcus thermophilus* CNRZ368	Y13713	1,411	17/24	8
IS*1194*			IS*5*	IS*5*	*Streptococcus thermophilus* CNRZ368	Y13626	1,200	15/16	ND
IS*1201*			IS*256*		*Lactobacillus helveticus* CNRZ1094 (p34kb)	L26311	1,387	20/24	8
IS*1202*			ISNCY		*Streptococcus pneumoniae* SSZ serotype 19F	U04047	1,747	18/23	23 (?)
IS*1203*		IS*3411*	IS*3*	IS*51*	*Escherichia coli* O111:H$^-$ PH	U06468	1,310	22/25	ND
IS*1203*E		IS*3411*	IS*3*	IS*51*	*Escherichia coli* O157:H7 EDL933 (pO157)	X97542	1,032	ND	ND
IS*1206*	ISaB1		IS*3*	IS*3*	*Corynebacterium glutamicum Bl*15	X69104	1,290	19/26	3
IS*1207*	ISbB1	IS*31831*	ISL*3*		*Corynebacterium glutamicum Bl*15	ND	1,450	24	8
IS*1216*			IS*6*		*Enterococcus hirae* S185	X81654	809	19	ND
IS*1216*E		IS*1216*	IS*6*		*Enterococcus faecium* HM1073	U49512	808	24	ND
IS*1216*V		IS*1216*	IS*6*		*Enterococcus* sp. (pHKK701)	L38972	809	18	ND
IS*1221*A	G1135.2		IS*3*	IS*150*	*Mycoplasma hyorhinis* GDL-1	U01217	1,513	23/28	
IS*1121*B	IS1221	IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis* GDL-1	L33925	1,519	22/28	ND
IS*1221*C		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis* GDL-1	L33927	1,518	23/28	ND
IS*1221*D		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis* GDL-1	L33928	>813	ND	ND
IS*1221*E		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyopneumoniae* J	L33924	1,511	15/19	ND
IS*1221*F		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis*	L33926	1,509	22/28	ND
IS*1221*G		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis* SK76	X62936	1,527	16/26	4 (?)
IS*1221*H	ISJ*1251*	IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyopneumoniae* J	X17372 [V]	1,557	15/20	ND
IS*1221*I	ISJ*1252*	IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyopneumoniae* J	X17372	1,554	21/27	ND
IS*1221*J		IS*1221*A	IS*3*	IS*150*	*Mycoplasma hyorhinis* MM96	M37339 [P]	>773	ND	ND
IS*1222*			IS*3*	IS*407*	*Enterobacter agglomerans* 339 (pEA9)	X78052	1,221	22/25	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*1223*			IS*3*	IS*150*	*Lactobacillus johnsonii* ATCC 11506	U09558	1,492	21/26	3
IS*1236*			IS*3*	IS*3*	*Acinetobacter calcoaceticus* ADP1	U03772	1,237	30/39	3
IS*1237*			IS*5*	IS*427*	*Clavibacter xyli* subsp. *cynodontis*	X75973	899	19	3
IS*1239*			IS*30*		*Streptococcus pyogenes* MGAS1898 (M15)	U11799	1,080	7/15	ND
IS*1245*			IS*256*		*Mycobacterium avium*	L33879	1,414	31/40	ND
IS*1246*			IS*5*	IS*5*	*Pseudomonas putida* mt-2 (pWWO)	L11583	1,275	15/16	ND
IS*1247*			IS*1380*		*Xanthobacter autotrophicus* GJ10	X84038	1,672	12/16	4
IS*1248*A	IS*1248*		IS*5*	IS*427*	*Paracoccus denitrificans* PdX13	U08864	832	13/14	2
IS*1248*B	IS*1248*	IS*1248*A	IS*5*	IS*427*	*Paracoccus denitrificans* PdX22	U08856	832	13/14	2
IS*1249*			IS*256*		*Corynebacterium xerosis* M82B (pTP10)	U21300	1,385	27/31	8
IS*1251*			ISL*3*		*Enterococcus faecium* GUC	L34675	1,496	16/24	ND
IS*1252*			IS*30*		*Enterococcus* sp. (pHKK701)	L38972	1,065	21/28	ND
IS*1253*A	IS*1253*		IS*605*		*Dichelobacter nodosus* AC3577 (pJIR896)	U34772	1,689	0	ND
IS*1253*B	IS*1253*	IS*1253*A	IS*605*		*Dichelobacter nodosus* A198	U34771	1,698	0	ND
IS*1272*			ISNCY	IS*1182*	*Staphylococcus haemolyticus* Y176	U35635	1,935	15/16	ND
IS*1294*			IS*91*		*Escherichia coli* (pUB2380)	X82430	1,689	0	0
IS*1295*			IS*256*		*Rhodococcus* sp. strain IGTS8 (pSOX)	U08850	1,455	23/29	ND
IS*1296*			IS*3*	IS*150*	*Mycoplasma mycoides* subsp. *mycoides* L2	X84021	1,485	ND	ND
IS*1297*		ISS*1*N	IS*6*		*Leuconostoc mesenteroides* subsp. *dextranicum* NZDRI2218	U59101	808	17/18	ND
IS*1301*			IS*5*	IS*427*	*Neisseria meningitidis* B1940	Z49092	844	15/17	2
IS*1311*			IS*256*		*Mycobacterium avium* IMM147460	U16276	>1,317	15	ND
IS*1312*			IS*3*	IS*2*	*Agrobacterium tumefaciens* Bo542 (pTiBo542)	U19148	1,317	17/23	5
IS*1313*			IS*66*		*Agrobacterium tumefaciens* Bo542 (pTiBo542)	U19149	2,547	20/24	8
IS*1326*			IS*21*		*Pseudomonas aeruginosa* (In0 from pVS1)	U38187	2,470	22/26	ND
IS*1327*	IS*1327*L, IS*1327*R		IS*6*		*Erwinia herbicola* pv. gypsophilae Eh824-1 (pPATH)	X87144	810	15/16	ND
IS*1328*	RS.2		IS*110*		*Yersinia enterocolitica* WA-314	Z48244	1,355	0	0
IS*1341*			IS*605*		Thermophilic bacterium PS3	D38778	1,353	10/11	ND
IS*1353*			IS*3*	IS*150*	*Pseudomonas aeruginosa* Tn*21*::In2	U40482	1,613	20/24	ND
IS*1356*			IS*256*		*Burkholderia cepacia*	U44828	1,354	14/24	9
IS*1358*	VcIs1, *rfbQRS*		ISA*s1*		*Vibrio cholerae* O139 Bengal	U24571	1,326	16/17	ND
IS*1372*			IS*3*	IS*51*	*Streptomyces lividans* ZX7	U50076	1,304	22/27	3
IS*1373*			IS*5*	ISL*2*	*Streptomyces lividans* 66 1326.32	U05249	817	9/15	2
IS*1380*A			IS*1380*		*Acetobacter pasteurianus* NC11380	D90424	1,665	13/15	4
IS*1380*B		IS*1380*A	IS*1380*		*Acetobacter pasteurianus* NC11380	D90424 [V]	1,665	12/15	4
IS*1381*A			IS*5*	ISL*2*	*Streptococcus pneumoniae* R6	Z77725	846	18/20	ND
IS*1381*B		IS*1381*A	IS*5*	ISL*2*	*Streptococcus pneumoniae* R6	Z77726	865	18/20	ND
IS*1381*C		IS*1381*A	IS*5*	ISL*2*	*Streptococcus pneumoniae* R6	Z77727 [P]	>823	ND	ND
IS*1381*D			IS*5*	ISL*2*	*Streptococcus mitis* NCTC 12261	Z82003 [P]	>823	ND	ND
IS*1395*			IS*256*		*Mycobacterium xenopi*	U35051	>1,323	14/15	ND
IS*1397*			IS*3*	IS*150*	*Escherichia coli* EPEC25	X92970	1,432	20/25	3, 4
IS*1400*	RS.1		IS*3*	IS*407*	*Yersinia enterocolitica* Ye 8081	X94452	1,207	30/39	4
IS*1407*			IS*256*		*Mycobacterium celatum*	X97307	>1,399	ND	ND
IS*1408*			IS*256*		*Mycobacterium branderi*	U62766	>1,325	ND	ND
IS*1412*			IS*1380*		*Sphingomonas* sp. strain CF06	U50714	1,656	12/18	ND
IS*1413*			IS*256*		*Burkholderia cepacia* AC1100	U58191	1,429	24/32	9
IS*1415*			IS*21*		*Rhodococcus erythropolis* NI86/21	AF002247	2,580	24/36	5, 6
IS*1452*			IS*4*		*Acetobacter pasteurianus* NCI 1452	D63923	1,411	21	4
IS*1469*	IS*200*		IS*605*	IS*200*	*Clostridium perfringens* NCTC 8239	X71844	717	0	ND
IS*1470*			IS*30*		*Clostridium perfringens* NCTC 8239	X71844	1,212	22/27	ND
IS*1471*			IS*630*		*Burkholderia cepacia* 2a (pIJB1)	U67938	1,113	18/25	2
IS*1476*			ISL*3*		*Enterococcus faecium*	U63997	1,500	18/27	ND
IS*1490*			IS*256*		*Burkholderia cepacia* AC1100	U80795	1,433	19/25	8
IS*1500*A			IS*3*	IS*3*	*Leptospira interrogans* sv. icterohaemorrhagiae Verdun	U13012	1,235	24/31	ND
IS*1500*B		IS*1500*A	IS*3*	IS*3*	*Leptospira interrogans* sv. icterohaemorrhagiae RZ11	U13013	1,235	24/31	ND
IS*1511*			IS*256*		*Mycobacterium gordonae* ATCC 14470	U95315	1,142	ND	ND
IS*1512*			IS*256*		*Mycobacterium gordonae* 960592	U95314	1,428	18/24	ND
IS*1533*			IS*110*		*Leptospira borgpetersenii* sv. hardjo type hardjo-bovis RZ33	M82880	1,460	13/15	ND
IS*1533*W		IS*1533*	IS*110*		*Leptospira borgpetersenii* sv. hardjo type hardjo-bovis	M57713	1,452	13/15	ND
IS*1549*			IS*4*		*Mycobacterium smegmatis* LR222	ND	1,634	11	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
IS*1936*	IS*1936*L, IS*1936*R		IS*6*		*Salmonella wien* 20/70 (Tn*1935* from pZM3)	ND	800	ND	ND
IS*3411*	IS*3411*L, IS*3411*R	IS*629*	IS*3*	IS*51*	*Escherichia coli* (Tn*3411* from pOH3001)	M19532	1,310	24/27	3
IS*4321*L			IS*110*		*Enterobacter aerogenes* (pR751)	U67194	1,347	0	ND
IS*4321*R		IS*4321*L	IS*110*		*Enterobacter aerogenes* (pR751)	U67194	1,326	0	ND
IS*4351*	IS*4400*, IS4351_L, IS4351_R		IS*30*		*Bacteroides fragilis* V479-1 (Tn*4351* from pBF4)	M17124	1,155	20/25	3
IS*4502*	Tn*4502*		IS*256*		*Caedibacter taeniospiralis* 116 (pKAP)	U60645	1,412	22/26	ND
IS*4521*L	LTR	IS*4521*R	IS*3*	IS*2*	*Escherichia coli* DH1 (Tn*4521*)	M17617	722	ND	ND
IS*4521*R	RTR		IS*3*	IS*2*	*Escherichia coli* DH1 (Tn*4521*)	M17618	1,226	11	ND
IS*4811*	Tn*4811*, IS*456*		IS*5*	IS*1031*	*Streptomyces lividans* TK64	Z11519	5,396	9/11	3
IS*5376*			IS*21*		*Bacillus stearothermophilus* CU21	X67861	2,107	39/50	5
IS*5377*			IS*4*		*Bacillus stearothermophilus* CU21	X67862	1,249	18/20	9
IS*5469*	Tn*5469*		IS*6*		*Fremyella diplosiphon* Fd33R1	U33002	4,894	22/25	ND
IS*6100*	IS*6100*L, IS*6100*R, Tn*610*		IS*6*		*Mycobacterium fortuitum* FC1 (Tn*610*)	X53635	880	14	ND
IS*6110*			IS*3*	IS*51*	*Mycobacterium tuberculosis* H_{37}Rv	X17348	1,354	25/28	3, 4
IS*6120*			IS*256*		*Mycobacterium smegmatis* mc^2155	M69182	1,486	21/24	9
IS*6501*		IS*711*	IS*5*	IS*427*	*Brucella ovis* ATCC 25840	X71024	838	20/25	2
IS*6770*	IS*EF1*		IS*30*		*Enterococcus faecalis* CV34 (pLRM1)	L28754	1,065	22/28	ND
IS*8402*			IS*4*		*Synechocystis* sp. BO8402	L76080	1,322	19/22	8
IS*12528*			IS*5*	IS*1031*	*Gluconobacter suboxydans* IFO12528	D86632	905	22/26	3
IS*13869*	IS-*Bl*		ISL*3*		*Brevibacterium lactofermentum* ATCC 13869	Z66534	1,437	33/36	8
IS*31831*	IS-*Cg*		ISL*3*		*Corynebacterium glutamicum* ATCC 31831	D17429	1,453	19/24	8
ISAa*1*			IS*605*	IS*200*	*Actinobacillus* (*Haemophilus*) *actinomycetemcomitans* FDCY4	D64078	718	0	ND
ISAe*1*	ISAE1		ISL*3*		*Ralstonia eutropha* H1-4	M86608	1,313	18/24	8
ISAr*1*	Riat1		IS*630*		*Agrobacterium rhizogenes* HR1	K03313	1,146	11	2
ISAs*1*	ISAS*1*		ISAs*1*		*Aeromonas salmonicida* A450-ceg6	L27156	1,223	20/22	8
ISAs*2*	ISAS2		IS*30*		*Aeromonas salmonicida* A449-ceg4	L27157	1,084	24/29	3
ISAsp*1*			ISL*3*		*Anabaena* sp. strain PCC7120	U13767	1,419	19/22	ND
ISBf*1*			IS*21*		*Bacteroides fragilis* RBF103	U05888	2,787	6/8	ND
ISBf*2*			IS*21*		*Bacteroides fragilis* RBF49	U05886	>355	ND	ND
ISBj*1*	HRS1, HRS1a		IS*1380*		*Bradyrhizobium japonicum* serocluster 123 USDA424	L09226	2,071	6/7	4
ISBs*1*	RSBst-alpha		IS*982*		*Bacillus stearothermophilus* CU21	Z21626	996	18/21	ND
ISBs*2*	RSBst-beta		IS*630*		*Bacillus stearothermophilus* CU21	Z21626 [P]	>660	ND	ND
ISBt*1*	*orfX*		IS*3*	IS*150*	*Bacillus thuringiensis* subsp. *aizawai* HD229	L29100	999	15/17	ND
ISBt*2*			IS*3*	IS*150*	*Bacillus thuringiensis* YBT-226	S68409 [P]	>187	ND	ND
ISC*1217*			ISNCY		*Sulfolobus solfataricus* DSM1616	X70335	1,147	10/13	6
ISCm*1*	RS		ISNCY		*Clavibacter michiganense* subsp. *sepedonicum* Cs3R (pCS1)	ND	1,300	ND	ND
ISCp*1*	ORF2-4		IS*256*		*Clostridium perfringens* NCTC 2062	X60694 [P]	>1,128	ND	ND
ISCx*1*			ISL*3*		*Corynebacterium xerosis* M82B (pTP10)	U21300 [P]	>536	ND	ND
ISEc*1*	H-rptB, *RhsB*		ISAs*1*		*Escherichia coli* K-12	L02370	1,291	14/18	ND
ISEc*2*	H-rptC1, *RhsC*	ISEc*1*	ISAs*1*		*Escherichia coli* K-12	L02373	380	ND	ND
ISEc*3*	H-rptC2, *RhsC*	ISEc*1*	ISAs*1*		*Escherichia coli* K-12	L02373	1,290	16/18	ND
ISEc*4*	H-rptC3, *RhsC*	ISEc*1*	ISAs*1*		*Escherichia coli* K-12	L02373	954	ND	ND
ISEc5	H-rptE, *RhsE*	ISEc*1*	ISAs*1*		*Escherichia coli* K-12	L02372	1,291	14/19	ND
ISEc*6*	H-rpt, min.5	ISEc*1*	ISAs*1*		*Escherichia coli* K-12	D83536	291	ND	ND
ISEc*7*	H-rpt, *RhsF*	ISEc*1*	ISAs*1*		*Escherichia coli* ECOR 50	U15125, U15126 [P]	>276	18/25	ND
ISFn*1*			IS*91*		*Fusobacterium necrophorum* biotype AB FnS1	X68876 [P]	>712	ND	ND
ISH*1*			IS*5*	ISH*1*	*Halobacterium salinarium* S9	J01725	1,118	8/9	8
ISH*1-8*	ISH*1.8*		IS*605*		*Halobacterium salinarium* (phage H1)	X00805	1,887	0	(0)
ISH*2*			IS*4*		*Halobacterium salinarium* S9	J01726	521	19	10, 11, 12, 20
ISH*3*A	SH*3*	ISH*27-1*	IS*4*		*Halobacterium salinarium* R1	L19296 [P]	1,378	15/16	5
ISH*3*B		ISH*27-1*	IS*4*		*Halobacterium salinarium* NRC-1 (pNRC100)	ND	1,400	15/16	ND
ISH*4*		ISH*27-2*	IS*4*		*Halobacterium salinarium* SD104	ND	1,375	15/16	ND
ISH*6*		ISH*27-1*	IS*4*		*Halobacterium salinarium* NRC-1 (pNRC100)	ND	1,400	15/16	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
ISH*8*		ISH*26*	IS*4*		*Halobacterium salinarium* SD108	M58557 [P]	1,395	16/18	ND
ISH*11*			IS*5*	IS*427*	*Halobacterium salinarium* R1	X54752	1,068	15	(7)
ISH*23*		ISH*50*	ISNCY		*Halobacterium salinarium* PHH1 (pHH1)	ND	1,000	23/29	9
ISH*24*			ISNCY		*Halobacterium salinarium* PHH1 (pHH1)	ND	3,000	10	7
ISH*26*			IS*4*		*Halobacterium salinarium* PHH1 (pHH1)	X04832	1,384	19	11
ISH*26-1*		ISH*26*	IS*4*		*Halobacterium salinarium*	X04832	1,705	16	11
ISH*27-1*	ISH*61*		IS*4*		*Halobacterium salinarium* PHH1 (pHH1)	X54432	1,398	16	5
ISH*27-2*	ISH*61*		IS*4*		*Halobacterium salinarium* PHH1 (pHH1)	X54433	1,389	16	5
ISH*27-3*	ISH*61*	ISH*27-2*	IS*4*		*Halobacterium salinarium*	X54434	1,389	16	5
ISH*28*			IS*5*	ISH*1*	*Halobacterium salinarium* PHH1 (pHH1)	X59158	932	16	8
ISH*50*	ISH*4*		ISNCY		*Halobacterium salinarium* R1 (pHH1)	X01584	996	23/29	8
ISH*51-1*	ISH*51*L		IS*4*		*Haloferax volcanii*	X04389	1,370	15/16	5
ISH*51-2*	ISH*51*R		IS*4*		*Haloferax volcanii*	X04389	1,379	15/16	5
ISH*51-3*			IS*4*		*Haloferax volcanii* DS2	ND	1,400	ND	ND
ISH*s1*	ISHS*1*		ISNCY		*Halobacterium salinarium* S1	M38315	1,449	24/27	8
IS<u>IMb</u>*1*			IS*110*		*Moraxella bovis* EPP63	M32345	>968	ND	ND
IS<u>IMl</u>*1*		ISIMb*1*	IS*110*		*Moraxella lacunata* ATCC 17956	M34367	>969	ND	ND
ISJp*4*	ISJP*4*		IS*5*	IS*427*	*Ralstonia eutropha* JMP134 (pJP4)	U16782	915	18/19	3
ISL*1*			IS*3*	IS*3*	*Lactobacillus casei* S-1 (phage FSV-B)	X02734	1,257	23/40	3
ISL*2*			IS*5*	ISL*2*	*Lactobacillus helveticus* LH28	X77332	858	16	3
ISL<u>2A</u>	ISL*2*	ISL*2*	IS*5*	ISL*2*	*Lactobacillus helveticus* LH27	X77332 [V]	858	16	3
ISL*3*			ISL*3*		*Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842	X79114	1,494	27/38	8
IS<u>Ld</u>*1*	IS*RLd*TAL11451		ISNCY		*Leucaena diversifolia* (*Rhizobium* sp.) TAL1145	ND	2,500	ND	ND
IS<u>Lh</u>*1*			IS*982*		*Lactobacillus helveticus* ATCC 15009 (pLH3)	X81979	962	32/35	ND
IS<u>Ll</u>*1*		IS*982*	IS*982*		*Lactococcus lactis* subsp. *lactis* ME2 (pTN20)	M95956 [P]	>244	ND	ND
ISLtaq*1*			IS*3*	IS*150*	*Thermus aquaticus* ATCC 25104	D14009	1,187	7	ND
ISM*1*			ISNCY		*Methanobrevibacter smithii*	X02587	1,381	33/34	8
IS<u>Mi</u>*1*	IS<u>MINC</u>		IS*3*	IS*150*	*Mycoplasma incognitus*	M34841	1,405	22/29	3
IS<u>Mk</u>*1*			ISNCY		*Mycobacterium kansasii*	L11041	947	ND	ND
IS<u>Mt</u>*1*	IS<u>TUB</u>*1*		IS*5*	IS*427*	*Mycobacterium tuberculosis* $H_{37}Rv$	X65618	969	16/17	4
IS<u>Mt</u>*2*			IS*21*		*Mycobacterium tuberculosis* $H_{37}Rv$	Z77165	>2,200	ND	ND
IS<u>Mt</u>*3*			IS*21*		*Mycobacterium tuberculosis* $H_{37}Rv$	Z83858	2,213	40/50	5
IS<u>Ng</u>*1*	IS<u>NGB</u>		IS*3*	IS*150*	*Neisseria gonorrhoeae* 5019	M19676	1,446	5/6	ND
ISPa*1*	IS-PA-*1*		ISNCY		*Pseudomonas aeruginosa* PA103	M27175	1,035	0	5
ISPa*2*	IS-PA-*2*		ISNCY		*Pseudomonas aeruginosa* Ps388	M27186	785	0	5
ISPa*4*	IS-PA-*4*		ISNCY		*Pseudomonas aeruginosa* PAO-muc	U16785	2,564	0	ND
ISPa*5*	IS-PA-*5*		ISNCY		*Pseudomonas aeruginosa* PAO-muc	U16785	965	0	ND
ISPa*6*	IS-PA-*6*		ISNCY		*Pseudomonas aeruginosa* PAO-muc	U16784	1,329	0	ND
IS<u>Pg</u>*2*	PGIS*2*		ISAs1		*Porphyromonas gingivalis* A7436	U77885	1,207	18/19	10
IS<u>Pp</u>*1*			IS*30*		*Pedicococcus pentosaceus* PPE1.0	Z32771	1,039	21/23	ND
IS<u>Pp</u>*2*			ISL*3*		*Pseudomonas putida* ML2	U25434	>1,280	ND	ND
IS<u>pRm</u>*1*	ISpRm2011*1*, ISpRmUSDA1024-2	ISRm*1*	IS*3*	IS*2*	*Rhizobium meliloti* 2011	ND	1,400	ND	ND
IS<u>pRm</u>*3*	ISpRm22012-*1*, ISpRm22013-*1*		ISNCY		*Rhizobium meliloti* 220-12	ND	750	ND	ND
IS<u>Ps</u>*1*			IS*5*	IS*427*	*Pseudomonas syringae* pv. savastonoi	M11035 [P]	>203	ND	ND
IS<u>Psp</u>*1*			IS*5*	IS*5*	*Pseudomonas* sp. strain EST1001 (pEST1226)	M57500	1,188	9/12	(4)
IS<u>Psp</u>*2*	Tn*401*		ISL*3*		*Pseudomonas* sp. strain EST1001 (pEST1226)	M57500	1,416	23/24	ND
ISR*1*			IS*3*	IS*407*	*Rhizobium lupini* class IV (pRP4)	X06616	1,259	12/13	4
ISRf*1*			IS*630*		*Rhizobium fredii* USDA 257	M73698	1,136	10/17	2
IS<u>Rhf</u>*1*	IS-*Rf*		IS*3*	IS*3*	*Rhodococcus fascians* DSM 20131	ND	1,300	15/19	3
IS<u>Rj</u>*1*	RSalpha, RSRj-alpha-9		IS*630*		*Bradyrhizobium japonicum* USDA 110	X02581	1,122	6/8	2
IS<u>Rj</u>*2*	RSbeta, RSRj-beta		ISNCY		*Bradyrhizobium japonicum* USDA 110	ND	950	ND	ND
IS<u>Rj</u>*3*	RSgamma		ISNCY		*Bradyrhizobium japonicum* USDA 110	ND	1,000	ND	ND
IS<u>Rj</u>*4*	RSdelta		ISNCY		*Bradyrhizobium japonicum* USDA 110	ND	1,000	ND	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
ISRj5	RSepsilon		ISNCY		*Bradyrhizobium japonicum* USDA 110	ND	1,000	ND	ND
ISRj6	RSzêta		ISNCY		*Bradyrhizobium japonicum* USDA 110	ND	2,000	ND	ND
ISRl1			IS66		*Rhizobium leguminosarum* bv. viciae 897	L19650	2,495	14/15	ND
ISRl2	ISRlF7-2		IS5	IS1031	*Rhizobium leguminosarum* bv. viciae MSDJ4184	Z37965	932	14/17	3
ISRle39A			IS6		*Rhizobium leguminosarum* VF39SM	X99520	890	18/22	ND
ISRle39B		ISRle39A	IS6		*Rhizobium leguminosarum* VF39SM	X99520	890	17/22	ND
ISRm1			IS3	IS2	*Rhizobium meliloti* 1021 (pRmeSU47a)	X56563	1,319	25/32	5
ISRm2			IS66		*Rhizobium meliloti* 41 (pRme41a)	M21471, M21472, M15786, M15785 [P]	2,700	24/25	8
ISRm3	IS*Rm-3*		IS256		*Rhizobium meliloti* 102F70	M60971	1,298	30	8, 9
ISRm3G	ISRm3		IS256		*Rhizobium meliloti* GR4 (pRmeGR4b)	X63715	1,298	30	9
ISRm4			IS5	IS1031	*Rhizobium meliloti* GR4	X65471	933	12/17	3
ISRm5			IS256		*Rhizobium meliloti* IZ450	U08627	1,340	22/27	8
ISRm6			IS3	IS407	*Rhizobium meliloti* GR4 (pRmeGR4b)	X95567	1,261	21/26	4
ISRm7			IS3	IS407	*Rhizobium meliloti* (pRmJT5)	L76274	>912	ND	ND
ISRm8			IS66		*Rhizobium meliloti* 41 (pSym-a)	L08667	>1,235	ND	ND
ISRm2011-2	ISp-Rm2011-2		IS630		*Rhizobium meliloti* 2011	U22370	1,053	16/19	2
ISRo1			IS256		*Rhodococcus opacus* MR11 (pHG201)	U70364	1,474	15/23	8
ISRsp1			IS66		*Rhizobium* sp. strain NGR234 (pSym)	X74068	>2,200	ND	ND
ISRsp2			IS21		*Rhizobium* sp. strain NGR234 (pNGR234a)	AE000081	2,623	12/16	ND
ISS1A		ISS1S	IS6		*Lactococcus lactis* subsp. *lactis* IL964 (pIL105)	L35176	807	18	ND
ISS1B		ISS1S	IS6		*Lactococcus lactis* subsp. *lactis* bv. diacetylactic B-1 (pTL02)	D63820	808	18	ND
ISS1CH		ISS1N	IS6		*Lactococcus lactis* subsp. *lactis* CNRZ270	X60456	810	18	ND
ISS1D	ISS1/pTD1	ISS1N	IS6		*Lactococcus lactis* subsp. *lactis* W67 (pTD1)	X62737	808	17/18	ND
ISS1E		ISS1N	IS6		*Lactococcus lactis* MG1820	X60734	808	15/18	ND
ISS1M		ISS1N	IS6		*Lactococcus lactis* subsp. *cremoris* SK11 (pSK111)	X59946	808	16/18	ND
ISS1N	ORF-N1		IS6		*Lactococcus lactis* subsp. *cremoris* SK11 (pSK111)	M37395	808	18	ND
ISS1RS	RS1, RS2	ISS1S	IS6		*Lactococcus lactis* subsp. *lactis* CNRZ270 (pUCL22)	X60594	808	18	8
ISS1S	SK7		IS6		*Lactococcus lactis* subsp. *lactis* ML3 (pSK08)	M18294	808	18	8
ISS1T	SK13	ISS1S	IS6		*Lactococcus lactis* subsp. *lactis* ML3 (pSK08)	Z14022	808	18	8
ISS1W	ORF-W2		IS6		*Lactococcus lactis* subsp. *cremoris* Wg2 (pWV05)	M37396	809	17/18	ND
ISS1X		ISS1S	IS6		*Lactococcus lactis* subsp. lactis IL416	M77708	808	18	ND
ISS1Y		IS1216	IS6		*Enterococcus faecalis* CH19	ND	>448	ND	ND
ISS1Z		ISS1S	IS6		*Lactococcus lactis* subsp. *lactis* OZS1 (pOZS550)	M85192	808	18	ND
ISSc1			IS30		*Spiroplasma citri* (phage SpV1-R8A2B)	X51344	ND	25/31	ND
ISSd1	ISS1	IS600	IS3	IS3	*Shigella dysenteriae* 1-60R	M21947	1,266	19/27	ND
ISSe1	H-rpt		ISAs1		*Salmonella enterica* D2 sv. Strasbourg M388	U04165	1,284	5/9	ND
ISSf1			IS5	IS1031	*Shigella flexneri* (pR387)	X07848 [P]	>257	ND	ND
ISSg1			ISL3		*Streptococcus gordonii* M5	ND	1,186	17/24	ND
ISSm1		IS1247	IS1380		*Serratia marcescens* 82041944	M97172 [P]	>654	ND	ND
ISSs1			IS605		*Synechococcus* sp. PCC7002	M93569	>1,100	ND	ND
IST2			IS256		*Thiobacillus ferroxidans* ATCC 19859	J03859	1,408	22/28	9
IST3091	IS3091		IS30		*Thiobacillus ferroxidans* tfi-91 (pTF191)	U32113	1,077	>14/19	ND
ISTcSa			IS630		*Synechocystis* sp. strain PCC6803	U38915	947	20/24	2
ISV1L		ISVm5-2	IS5	IS903	*Vibrio parahaemoliticus* WP1	M64126	1,046	15/18	ND
ISV3L			IS5	IS903	*Vibrio parahaemoliticus* AQ3776	M64121	1,054	19	ND
ISV4R			IS5	IS903	*Vibrio parahaemoliticus* AQ3776	M64124	1,043	17/19	ND
ISV5L		ISV3L	IS5	IS903	*Vibrio parahaemoliticus* AQ3860	M64122	1,058	19	ND
ISV5R		ISV3L	IS5	IS903	*Vibrio parahaemoliticus* AQ3860	M64123	1,053	19	ND
ISVa1	ISV-A1		IS5	IS903	*Vibrio anguillarum* 775 (pJM1)	L40497	1,041	27/39	9
ISVa2	ISV-A2		IS5	IS903	*Vibrio anguillarum* 775 (pJM1)	L40498	1,054	21/25	9
ISVm2-4	ISVM2-4	ISVm5-2	IS5	IS903	*Vibrio mimicus* 6	M64131	964	18	ND
ISVm5-2	ISVM5-2		IS5	IS903	*Vibrio mimicus* 6	M64129	1,044	17/18	ND
ISVm7-2	ISVM7-2		IS5	IS903	*Vibrio mimicus* 6	M64130	748	18	ND
ISVmL	ISVML	ISVm5-2	IS5	IS903	*Vibrio mimicus* 6	M64127	1,031	17/18	ND

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TABLE 1—*Continued*

Name	Synonym(s)	Isoform	Family	Group	Origin	Accession no.	Length (bp)	IR[a]	DR[b]
ISVmR	ISVMR	ISVm*5-2*	IS*5*	IS*903*	*Vibrio mimicus* 6	M64128	1,031	15/18	ND
ISVNR		ISV*4*R	IS*5*	IS*903*	*Vibrio cholerae* 91	M64125	881	16/19	ND
ISWz*1*			IS*91*		*Weeksella zoohelcum*	U14952	>1,219	ND	ND
ISXa*1*			ISAs*1*		*Xanthobacter autotrophicus* GJ10	M26950 [P]	>947	ND	ND
ISXc*1*	ISXcB100-1		ISNCY		*Xanthomonas campestris* B100	ND	1,300	ND	ND
ISXc2	ISXcB100-2		ISNCY		*Xanthomonas campestris* B100	ND	1,700	ND	ND
ISX*c4*			ISNCY		*Xanthomonas campestris* pv. citri XW45 (pXW45N)	ND	5,500	ND	ND
ISX*c5*			ISNCY	ISX*c4*	*Xanthomonas campestris* pv. citri XW45 (pXW45J)	ND	6,900	ND	ND
ISYe			IS*3*	IS*150*	*Yersinia enterocolitica* 8081c	M29945 [P]	884	ND	ND

[a] IR indicates the length(s) of the terminal IR(s) in base pairs. A unique number refers to two IRs with the same length.
[b] DR indicates the number of target base pairs duplicated on insertion. Numbers in parentheses are still uncertain.
[c] ND, not determined.
[d] Elements which are underlined are those to which we have given a (new) name.
[e] [V] indicates a variant of the proposed accession number.
[f] [P] indicates a partial DNA sequence.
[g] ISNCY refers to unclassified IS elements.

those which have received the most attention (Table 4). This implies that a wealth of elements remain to be discovered in the less well characterized groups.

In the following, we describe first the essential features of each IS family and subsequently the current state of knowledge about the control of gene expression and transposition mechanism of the better-studied IS sequences within each family.

IS*1* Family

IS*1* was one of the first bacterial insertion sequences to be isolated and characterized (100, 144). The original examples were obtained from an F′*lac-pro*B plasmid (IS*1*K [167]) and the multiple drug resistance plasmid R100 (IS*1*R [254]). The nucleotide sequences of 17 variants of this IS from *Escherichia* and *Shigella* species have been determined (200, 343, 379). Of these, three have proved to be duplicates and one is only partially complete. Nine of the others exhibit sequence divergence of between 0.52 and 10% at the nucleic acid level. These have been called IS*1* isoforms. Two examples, IS*1*N and IS*1*H, are significantly different from the others (45 to 47% divergence in nucleotide sequence; 55 to 58% divergence at the protein level) but similar to each other (14 to 19% divergence at the protein level) and might be considered distinct members of the family. Except for IS*1*K(A) and IS*1*R(G), transposition of these elements has not been directly demonstrated experimentally in a controlled way but is implied from the isolation of mutants with spontaneous mutations in various genes. IS*1* is a component of several compound transposons such as Tn*9* (219) and Tn*1681* (318), where it is present in direct or inverted orientation flanking a chloramphenicol acetyltransferase and heat-stable toxin gene, respectively.

Integration of IS*1* is accompanied by duplication of generally 9 bp in the target DNA at the site of insertion (48, 117). Direct target repeats of 8, 10, and 14 bp (154, 220, 312) have also been observed. The frequency of their appearance is increased by mutations within the Tpase gene (220). The element exhibits a strong preference for insertion into AT-rich target regions (105, 236, 375).

IS*1* (Fig. 6) is one of the smallest "autonomous" bacterial insertion sequences isolated so far. It is 768 bp long, includes two approximately 23-bp imperfect inverted repeats (IRL and IRR) located at its ends (167, 254), and two partly overlapping open reading frames (*insA* and *insB′*) located in the 0 and −1 relative translational phases, respectively (Fig. 6A). The integrity of these frames is essential for transposition (164, 222).

A transcript is initiated from a promoter, pIRL, partially located in IRL (367) and is translated to give two products: InsA and InsAB′. The first and more abundant protein is encoded by the *insA* frame alone. The small, basic InsA protein binds specifically to the IRs (376, 377) and represses transcription from pIRL (221, 378). It also appears to inhibit transposition, probably by competing with the Tpase for binding to the ends of the element (95). The second protein, InsAB′, is the Tpase of the element. Its production results from a programmed translational frameshift between the *insA* and *insB′* frames which occurs at a frequency of approximately 1%. The site of frameshifting is an A_6C motif located at the 3′ end of the upstream *insA* frame (95, 310) (Fig. 6B). Natural transposition of IS*1* occurs at a relatively low frequency (approximately 10^{-7} in a standard mating assay). Insertion of an additional A residue within the A_6C motif to yield A_7C or replacement of the motif with GA_2GA_3C fuses the two reading frames, leading to constitutive production of the Tpase while eliminating the production of InsA. This results in levels of transposition of between 0.1 and 1% in vivo (94, 95). Indirect evidence has been presented suggesting that a translational restart within the *insA* frame gives rise to an InsAB′ protein with an N-terminal deletion. It has been proposed that this protein is the true Tpase (229, 230). No independent product of the downstream frame, *insB′*, alone has been detected. Transposition activity appears to depend on the InsAB′/InsA ratio (95). Since this is relatively insensitive to the intensity of transcription, this arrangement ensures that IS*1* is not activated by high levels of impinging transcription following insertion into highly expressed genes.

An additional control of Tpase expression may be exercised at the level of transcription termination. Early studies on the organization of IS*1* identified a region at the end of the *insA* gene which behaves as a Rho-dependent transcription terminator (151, 272). Premature transcription termination would therefore result in the production of an mRNA lacking the *insB* frame. The role of this sequence in the control of IS*1* transposition remains to be determined.

IS*1* generates both simple insertions and replicon fusions (cointegrates) which are composed of two copies of the insertion sequence in DR, one at each junction between the target and donor replicons. The occurrence of stable cointegrates as

TABLE 2. Major features of prokaryote IS families

Family	Group(s)	Size range (bp)[a]	DR (bp)[b]	ENDS[c]	IR[d]	No. of ORFs[e]	Comments[f]
IS*1*		770	9 (8–11)	GGT	Y	2	Phage λ integrase?
IS*3*	IS*2*	1,300–1,350	5	TGA	Y	2	DD(35)E
	IS*3*	1,200–1,300	3 (4)			2	
	IS*51*	1,300–1,400	3 (4)			2	
	IS*150*	1,400–1,550	3–5			2	
	IS*407*	1,200–1,250	4			2	
IS*4*		1,300–1,950	9–12	C(A)	Y	1	DDE
IS*5*	IS*5*	1,100–1,350	4	GG	Y	1	DDE
	IS*427*	800–1,000	2–3	Ga/g		2	
	IS*903*	1,000–1,100	9	GGC		1	
	IS*1031*	850–950	3	GAG		1	
	ISH*1*	900–1,150	8			1	
	ISL*2*	800–1,100	2–3			1	
IS*6*		750–900	8	GG	Y	1	DD(34)E
IS*21*		1,950–2,500	4 (5, 8)	TG	Y	2	DDE
IS*30*		1,000–1,250	2–3		Y	1	DD(33)E
IS*66*		2,500–2,700	8	GTA	Y	>3	
IS*91*		1,500–1,850	0		N	1	ssDNA Rep
IS*110*		1,200–1,550	0		N	1	Site-specific recombinase
IS*200*/IS*605*		700–2,000	0		N	1 (2)	Complex organization
IS*256*		1,300–1,500	8–9	Gg/a	Y	1	DDE, eukaryotic relatives
IS*630*		1,100–1,200	2		Y	1	DDE, eukaryotic relatives
IS*982*		1,000	ND[g]	AC	Y	1	DDE
IS*1380*		1,650	4	Cc/g	Y	1	
ISAs*1*		1,200–1,350	8	C	Y	1	
ISL*3*		1,300–1,550	8	GG	Y	1	

[a] Size range represents the typical range of each group.
[b] Length of direct target repeats. Less frequently observed lengths are included in parentheses.
[c] Conserved terminal base pairs. Capital leters (and capital letters within parentheses) refer to mostly (and often) conserved bases. Lowercase letters separated by slashes indicate alternative conservation at that position.
[d] Presence (Y) or absence (N) of terminal inverted repeats.
[e] ORF, open reading frame. Number in parentheses indicates the possible involvement of a second ORF in the transposition process.
[f] DDE represents the common acidic triad presumed to be part of the active site of the transposase. ssDNA, single-stranded DNA.
[g] ND, not determined.

transposition end products led to the suggestion that transposition of IS*1* can proceed in a replicative manner (106) while simple insertions may occur without replication (34). Thus, IS*1* may be capable of both replicative and conservative transposition. More convincing evidence in support of a duplicative transposition pathway has recently been obtained by analyzing the products of intramolecular transposition (342). In addition, these and other studies (307) detected excised circular copies of the IS*1*-derived transposon, and it was suggested that, as in the case of IS*911* (see "IS*3* family" below), such forms may integrate into a target molecule to give rise to simple insertions.

High levels of InsAB′ in the presence of suitable IS*1* ends induce the host SOS response, possibly reflecting endonucleolytic activity of the IS*1* Tpase (198). By using this in vivo assay system, originally developed for screening mutants of the IS*10* Tpase (see "IS*4* family" below), it was possible to show that for relatively short artificial derivatives of IS*1*, the level of response depends in a periodic manner on the distance between the ends. The periodicity was found to be about 10 to 11 bp and was also reflected in the transposition activity, suggesting a requirement for correct helical positioning of both ends. Two directly repeated ends were also capable of eliciting the SOS response, although they were not capable of giving productive transposition.

Inspection of the amino acid sequence of InsAB′ indicates the presence of an amino acid triad, H200, R203, and Y231, reminiscent of the catalytic site of integrases of the phage λ family (2, 13) in the C-terminal end of the protein. Derivatives with the H200L, R203L, or Y231F mutation are inactive in

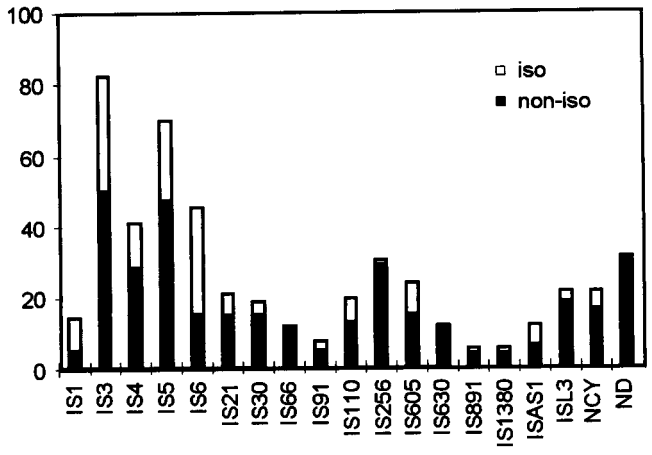


FIG. 5. IS distribution among different families. The figure shows the number distribution of the entire IS database into the various IS families. The numbers of isoforms are indicated as the open boxes, and the distinct individual members are shown as shaded boxes. NCY, not classified. ND, nucleotide sequence not determined.

several assays whereas an H200Q mutation retains a low level of activity. The relative activities of these mutants parallel those of similar mutants with mutations in the Flp recombinase (313). At present it is difficult to assess the relevance of these similarities to the transposition mechanism. It should be noted that in IS*1*N (253) and IS*1*H (379) the H200 residue is replaced with R but neither of these IS*1* derivatives has been demonstrated to be capable of autonomous transposition.

IS*3* Family

General features. Over 80 members of the IS*3* family have been found so far in 40 bacterial species. Of these, 32 proved to be isoforms or duplicate isolates and 11 were only partial sequences. The G+C content of members of the family ranges from 70% in the mycobacterial examples to 25% in those isolated from *Mycoplasma* species. In spite of this enormous variation, they are strikingly similar in many respects and form an extremely coherent and highly related family. Most show a similar G+C content to their host organism. Several family members are part of compound transposons. These include IS*3411* flanking genes for citrate utilization (158); IS*4521*, which flanks a heat-stable enterotoxin gene in enterotoxinogenic *E. coli* (148); and IS*1706* (149), which flanks genes of the Clp protease/chaperone family.

Members are characterized by lengths of between 1,200 and 1,550 bp, with one exception (IS*481* [1,045 bp]), and inverted terminal repeats in the range of 20 to 40 bp. These repeats are variable but clearly related (Fig. 7C). The majority of the elements terminate with 5′-TG-----CA-3′ and present an internal block of G and C residues of variable length. IS*3* family members generally have two consecutive and partially overlapping reading frames, *orfA* and *orfB*, in the relative translational reading phases 0 and −1, respectively (Fig. 7A). It has been demonstrated in at least three cases (IS*150* [351], IS*3* [308], and IS*911* [267]) that in addition to the product of the upstream frame, OrfA, a fusion protein, OrfAB, is generated by programmed translational frameshifting (55). However, in contrast to IS*1*, the product of the downstream frame, OrfB, is also detected. The frequency of frameshifting varies from element to element. It is approximately 50% for IS*150* (351) and only 15% for IS*911* (267).

Several members exhibit an organization which does not apparently conform to the generic IS*3* member. In IS*120*, for example, the relationship between the reading phases of the upstream and downstream open reading frames appears to be +1 rather than −1, while in ISNg*1* and ISYe*1* the characteristic motifs of OrfB (see below) are distributed between reading phases. Other members, such as IS*1076*, IS*1138*, IS*1221*, and IS*1141*, exhibit only one long open reading frame. Although these may be true variants, it cannot at present be ruled out that the variations are simply due to errors in sequence determination. IS*481* is significantly shorter (1,045 bp) than the others and carries only one open reading frame. Several individual examples of this element have been isolated. It is included in this family since it shows strong similarities in its putative Tpase sequence.

Family members from *Mycoplasma* species merit special attention. Not only does the host use a nonuniversal genetic code in which the opal termination codon TGA directs the insertion of tryptophan (257), but also their genomes are among the smallest bacterial genomes known and are extremely AT rich. To date, four different IS*3* family members have been observed in *Mycoplasma* and the nucleotide sequence of 14 individual examples has been determined. Of these, only IS*1138* and IS*1138*b have been directly demonstrated to undergo autonomous transposition (33). All exhibit similarly high A+T contents, and this unusual base composition could lead to difficulties in sequence determination. It is remarkable that typical IS*3* family characters have been maintained in such an "extreme" genetic environment. Nine of these are closely related and form a group of isoelements which have been called IS*1221*. As indicated above, one of these carries a single long reading frame (representing *orfA* plus *orfB*) instead of two consecutive overlapping frames. The others each carry insertions or deletions which destroy the equivalent of *orfA*, *orfB*, or both. Expression studies with *E. coli* indicate that a protein, equivalent to OrfAB, is indeed produced from the long open reading frame of IS*1221*. Interestingly, it appears that a second truncated protein, equivalent to OrfA, may be generated from *orfAB* by translational frameshifting, representing an inverted expression pattern to the majority of the family members (382). Although this appears not to be a general rule for IS*3* family members originating from *Mycoplasma* hosts, the presence of a similar single-frame arrangement in a second member, IS*1138*, indicates that it might not be rare. Because of the extremely high A+T content of these elements, many potential frameshift windows of the A_6(G/C) or A_7 type are expected to occur. Only direct experiments will therefore be able to determine which, if any, of these sequences are used to generate the Tpase or, conversely, an OrfA-like protein.

Extensive alignment studies of the predicted OrfA and OrfB amino acid sequences between themselves and with those of other transposable elements (87, 97, 172, 176, 288) have provided insights into the structure-function relationships of the proteins. The predicted primary amino acid sequences of the various OrfA proteins from different members of the family exhibit a relatively strong α-helix–turn–α-helix motif (Fig. 7A), suggesting that they might provide sequence-specific binding to the terminal IRs of their particular IS (304). The OrfB products, on the other hand, carry a DD(35)E motif and have additional identities to retroviral integrases and various other Tpases (Fig. 3) (266). These include two amino acids located 4 and 7 residues downstream from the glutamate residue. Interestingly, many members carry a putative leucine zipper located at the end of OrfA (and sometimes extending into the OrfB region of the OrfAB protein) (41, 344, 382). Studies with IS*911*

TABLE 3. IS family distribution among bacterial genera

Genus	IS*1*	IS*3*	IS*4*	IS*5*	IS*6*	IS*21*	IS*30*	IS*66*	IS*91*	IS*110*	IS*256*	IS*605*	IS*630*	IS*982*	IS*1380*	ISAs*1*	ISL*3*	NCY[a]	ND[a]	Total
Acetobacter			1	4											2					7
Acinetobacter		1																		1
Actinobacillus												1								1
Aeromonas							1									1				2
Agrobacterium		3		3				8					2						1	17
Anabaena												1	1				1	1	4	8
Bacillus		2	11		4	4							1	2						24
Bacteroides			2	4		2	1													9
Bordetella		5															1			6
Bradyrhizobium													1		1				5	7
Brevibacterium																	1			1
Brucella				2																2
Burkholderia		2		1		1					5		1						6	16
Caedibacter											1									1
Calothrix			1	1																2
Campylobacter					1															1
Capnocytophaga				2																2
Caulobacter		1																	1	2
Clavibacter				1															1	2
Clostridium		1	1				1				1	1								5
Corynebacterium		1									2						3			6
Coxiella										3										3
Dichelobacter												2								2
Enterobacter		1								2										3
Enterococcus					4		2				1						2			9
Erwinia		1			1															2
Escherichia	10	13	6	5			3		5			4				7			1	54
Fremyella					1															1
Fusobacterium									1											1
Gluconobacter				1																1
Haemophilus																		6		6
Halobacterium			11	3								1						2	2	19
Haloferax			3																	3
Helicobacter												1								1
Lactobacillus		3		2							1			1			1			8
Lactococcus		5			15						2			4						26
Leptospira		2								2										4
Leucaena																			1	1
Leuconostoc					1		1										1			3
Methanobrevibacter																		1		1
Moraxella										2										2
Mycobacterium		5	1	1	1	2				4	9						1	1		25
Mycoplasma		14																		14
Neisseria		1		2														1		4
Nocardia																	1			1
Paracoccus				2																2
Pediococcus							1													1
Porphyromonas				1												1				2
Proteus					1														1	2
Pseudomonas		3		4		5			1	1			2				2	5	3	26
Ralstonia				1			1										1			3
Rhizobium		5		2	2	1		4			3		2						1	20
Rhodococcus		1				1					4									6
Saccharopolyspora												4								4
Salmonella			2		5							5				1				13
Serratia															1					1
Shigella	4	5		1		1							1							12
Sphingomonas															1					1
Spiroplasma							1													1
Staphylococcus					8						1						1	2		12
Streptococcus		2		5			3				1	1					5	1		18
Streptomyces		1		4	1					3									1	10
Sulfolobus																		1		1
Synechococcus												1								1
Synechocystis			2	2									1							5
Thermophilic												1								1
Thermus		1								2										3
Thiobacillus							1				1									2
Vibrio				13								1				1				15
Weeksella									1											1
Xanthobacter															1	1				2
Xanthomonas		1		1			3												5	10
Yersinia		2				4				1	1	1								9
Total	14	82	41	68	45	21	19	12	8	20	33	25	12	7	6	12	21	21	33	500

[a] NCY, not classified; ND, sequence not determined.

TABLE 4. IS distribution among bacterial groups

Bacterial group	IS*1*	IS*3*	IS*4*	IS*5*	IS*6*	IS*21*	IS*30*	IS*66*	IS*91*	IS*110*	IS*256*	IS*605*	IS*630*	IS*982*	IS*1380*	ISAs*1*	ISL*3*	NCY[a]	ND[a]	Total
Spirochetes		2								2										4
Vibrioid gram negative					1							1								2
Curved gram negative																				0
Gram-negative rods and cocci		22	1	23	2	7	4	12	2	5	9		8		5	1	4	6	22	133
Facultatively anaerobic gram negative	14	22	8	19	7	5	4		5	3	1	12	1		1	10		6	2	120
Anaerobic gram negative			2	5		2	1		1			2				1				14
SO_4 or S reducing																				0
Anaerobic gram-negative cocci																				0
Rickettsiae and Chlamydiae										3										3
Anoxygenic phototrophs																				0
Oxygenic phototrophs			3	3	1							2	2				1	1	4	17
Aerobic chemolithotrophs							1				1									2
Budding bacteria		1																	1	2
Sheathed bacteria																				0
Gliding bacteria				2																2
Myxobacteria																				0
Gram-positive cocci		7		5	28		7				5	1		4			9	3		69
Sporeforming gram positive		3	12		4	4	1				1	1	1	2						29
Regular nonsporeforming gram positive		3		2							1			1			1			8
Irregular nonsporeforming Gram positive		1		1							2						4		1	9
Mycobacteria		5	1	1	1	2				4	9						1	1		25
Nocardioform actinomycetes		1				1					4	4					1			11
Multilocular actinomycetes																				0
Actinoplanetes																				0
Streptomyces		1		4	1					3									1	10
Maduromycetes																				0
Thermonosporas																				0
Thermoactinomycetes																				0
Other actinomycetes																				0
Mycoplasmas		14					1													15
Methanogens																		1		1
Archeal SO_4 reducers																				0
Halobacteria			14	3								1						2	2	22
Cell-wall-less archaebacteria																				0
Hyperthermophilic												1						1		2
Total	14	82	41	68	45	21	19	12	8	20	33	25	12	7	6	12	21	21	33	500

[a] NCY, not classified; ND, sequence not determined.

and IS*2* strongly suggest that this represents one domain of multimerization of the proteins (137a, 138, 139, 204).

The IS*3* family can be divided into several subgroups (Table 2) defined by deep branching in the alignment of the various OrfB sequences (Fig. 7B). We have designated these the IS*2* and IS*407* subgroups (which appear closely related) and the IS*3*, IS*51*, and IS*150* groups. One feature which lends biological credence to these subgroups is that they also clearly appear clustered (with some exceptions) in the results of the alignments with the upstream OrfA protein. Moreover, there is a strong correlation between the members of each group and the number of base pairs of target DNA duplicated on insertion: for elements in the IS*2* subgroup, insertion invariably leads to a 5-bp direct target repeat; for the IS*407* subgroup, a 4-bp repeat is observed; while for the other groups, a 3-bp repeat is obtained. In the latter cases, some of the elements, e.g., IS*911*, occasionally generate 4-bp repeats. This clustering is also exhibited to some extent in the nucleotide sequence of the terminal IRs (Fig. 7C) and is particularly marked in the IS*2*, IS*51*, and IS*407* subgroups. It can also be observed in the primary sequence details of the putative leucine zipper (data not shown). IS*481* appears to be relatively separated from the major groups. This is also reflected by the fact that it is the only known example which appears to generate a 6-bp direct target repeat (consensus NCTAGN) (325a).

Several members carry GATC methylation sites within 50 bp of their ends, which have been shown in one case, IS*3*, to modulate transposition activity (320). However, this is not a general characteristic of the family, nor is it restricted to any particular subgroup.

Little is known about the insertion specificity of members of the family. IS*2* exhibits a preference for a region of bacteriophage P1, but the basis of this preference is at present unknown (311). Both IS*911* (269) and IS*150* (361) have been found next to sequences which resemble their IRs, and IS*1397* is invariably located within intergenic repeated sequences in *E. coli* (bacterial interspersed mosaic elements [16]).

Finally, an element isolated from the ECOR collection of *E. coli* and closely related to IS*3411* carries a group II intron (98). The implication of this for the regulation transposition of this element has not been investigated.

IS*911*. One of the best-characterized members of the IS*3* family is IS*911*. OrfA and OrfAB bind specifically to the terminal IRs (139, 264). Constitutive production of OrfAB from a contiguous *orfAB* frame (generated by mutation and eliminating production of OrfA) leads to only a modest increase in

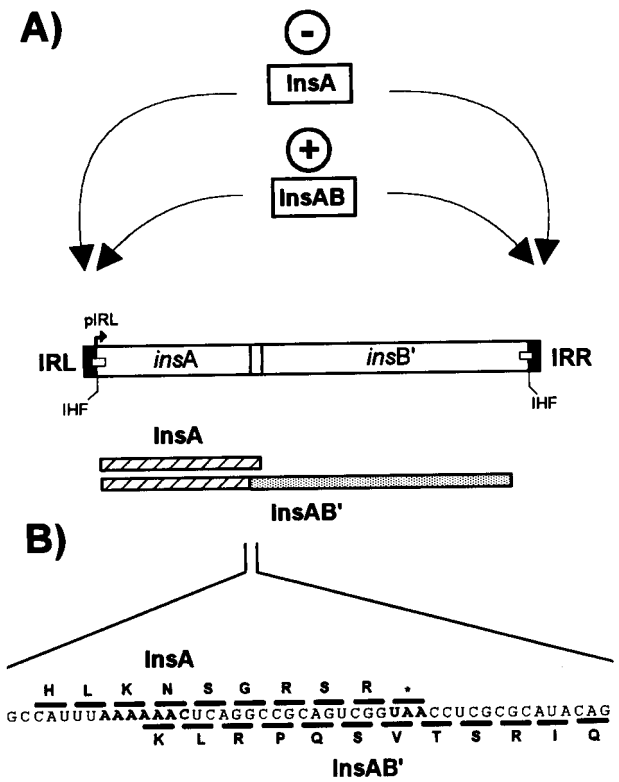


FIG. 6. Organization of IS*1*. (A) Structure of IS*1*. Left (IRL) and right (IRR) terminal IRs are shown as solid boxes. The relative positions of the *insA* and *insB′* reading frames, together with their overlap region, are shown within the open box representing IS*1*. The IS*1* promoter pIRL, partially located in IRL, is indicated by a small arrow. IHF binding sites, located partially within each terminal IR, are shown as small open boxes. The InsA protein is represented as a hatched box beneath. The InsA and InsB′ components of the InsAB′ frameshift product are shown as hatched and stippled boxes, respectively. Arrows indicate the probable region of action of InsA and InsAB′ proteins. The effect of InsA and InsAB′ on transposition is shown above. (B) RNA and protein sequence in the crossover region between the two open reading frames. Codons shown above the RNA sequence show the product of direct translational readout. Those below show the product of a −1 translational frameshift. The heptanucleotide A_6C frameshift sequence involved in production of InsAB′ from the wild-type IS*1* coding sequence is indicated in boldface type, as is the UAA termination codon for InsA.

intermolecular transposition activity. In contrast, high production of OrfAB in this way either in *cis* or in *trans* stimulates the production of excised circular transposon copies, whose formation is best explained by a single-ended attack by one IS extremity on the other (268). This is thought to occur in a two-step process (Fig. 8) in which one end of the transposon is cleaved to generate a free 3′OH, which, in turn, is used as a nucleophile to attack the opposite end. This results in the circularization of a single transposon strand visualized as a figure-eight molecule in which the transposon ends are joined by a single-strand bridge (Fig. 8) (265). It leaves a 3′OH group on the vector backbone at the point of insertion. Kinetic studies suggest that the figure-eight species is processed into transposon circles.

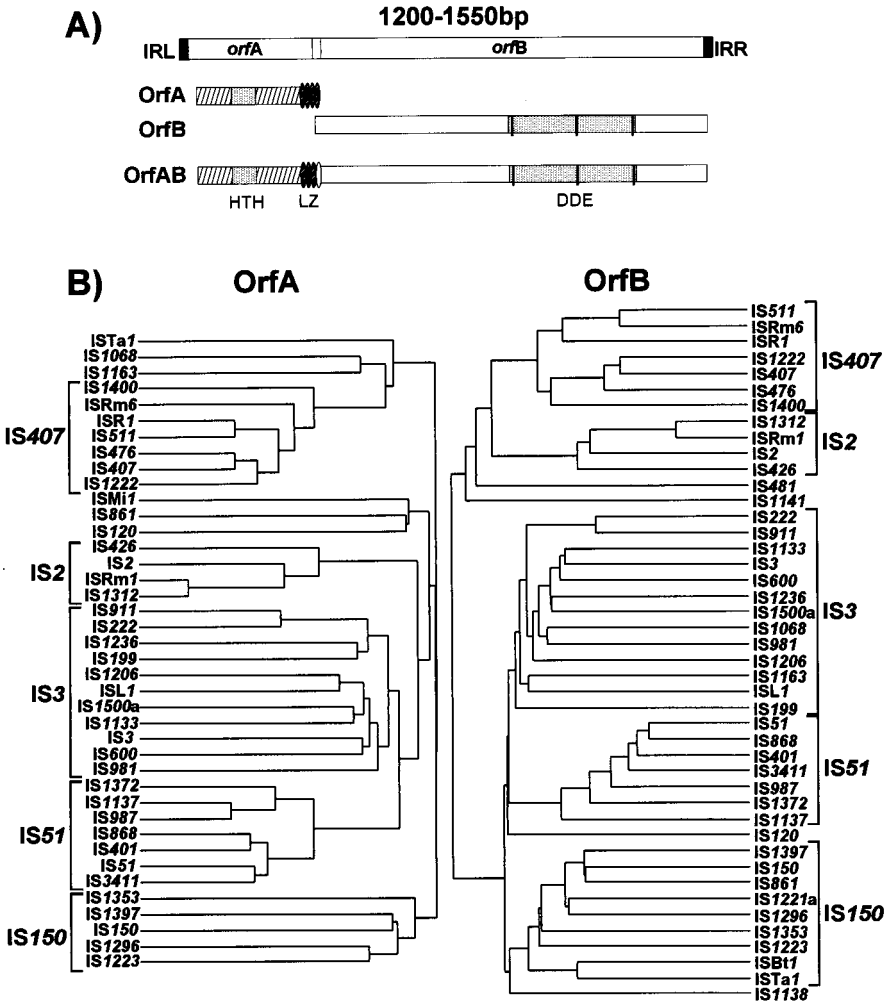
The figure-eight recombination reaction has been reproduced by using a cell-free system, but no transposon circles, excised linear transposon species, or indeed any product resulting from double-strand cleavage at the transposon ends was detected in the in vitro system. It is not known how the figure-eight form might be processed into transposon circles, although host factors which promote either replication or second-strand recombination are thought to be involved (270). By using a purified figure-eight substrate, the cell-free system was found to support a reaction equivalent to the disintegration characteristic of retroviral integrases (59) (see "Reaction mechanisms" above). Moreover, this activity is also exhibited by the OrfB region of the protein alone, demonstrating that the DD(35)E domain carries out catalysis (270).

Although it is not yet clear whether transposon circles are "natural" transposition intermediates, they are efficient Tpase substrates for intermolecular transposition in vivo (338; also see reference 269). Simultaneous high-level expression of OrfA with the OrfAB protein greatly reduces or eliminates the formation of excised transposon circles (and the figure-eight species) and stimulates intermolecular transposition. It also stimulates intramolecular transposition of a plasmid carrying a cloned circle junction. The development of an intermolecular transposition system in vitro (339) has demonstrated highly efficient integration of transposon circles in a reaction which requires both OrfAB and OrfA. Integration does not require a supercoiled donor molecule and is optimal when the abutted IRs are separated by 3 bp, as occurs in the circle junction. More recently, linear derivatives of IS*911* have been observed in vivo. They appear to be derived from the transposon circle rather than resulting from direct excision from the donor plasmid molecule. Moreover, while they undergo integration in vitro, the efficiency of this reaction is significantly reduced compared to that of the transposon circles (337a).

One striking feature of transposon circularization is that it creates a strong promoter at the circle junction in which IRR contributes a −35 hexamer and IRL contributes a −10 hexamer (294). This has suggested a novel mechanism for autoregulation of transposition (Fig. 8, right-hand pathway). Transposon circles are proposed to be generated at low frequencies by a combination of low Tpase levels (from the weak endogenous promoter) and host functions (to ensure processing of the second strand). Once formed, the junction promoter ensures high levels of Tpase which is capable of binding to the abutted ends, introducing two single-strand breaks (one at each end) generating an opened transposon molecule. Transfer of both ends to a suitable target completes the transposition cycle. Insertion results in the destruction of the efficient junction promoter. This model does not require double-strand cleavage and therefore takes into account the observation that the only activity detected for the OrfAB protein is cleavage and transfer of a single DNA strand. It also ensures that a suitable substrate is present before high levels of Tpase are produced. The results do not rule out alternative pathways involving simple excision (Fig. 8, left-hand pathway).

Other members. Several other members of this family are also being analyzed in detail. These include IS*2*, IS*3*, and IS*150*. All three have been shown to generate circles when supplied with high levels of the fused-frame Tpase (208, 308, 361).

IS*3* also generates adjacent deletions (308) but, unlike IS*911*, appears to undergo excision from the donor molecule as a linear form following a staggered double-strand break at each end. These forms have a 3-base 5′ overhang and may be an alternative type of transposition intermediate (309) (Fig. 8, left-hand pathway). Such forms may be equivalent to the linear IS*911* species derived from transposon circles. In addition, IS*3*-derivative transposons in which two abutted ends have been engineered undergo high levels of transposition (320). Insertion of IS*3* creates generally 3-bp and sometimes 4-bp direct target repeats. It is significant that plasmids in which the IRs are separated by 4 bp are more active than those in which they are separated by 8 bp. In these studies the authors were



C)

IS*407* TGACCTGCCCCCTGCAAACAGGGCCAGCCGGAGTCTAGTAAAGTTCGTTT
TGACCTGCCCCCATCAATAGGGCCAATGGGCTTCTAGCAAAGTCCCTTTA
ISRm*6* TGACCTGCCACGGGTAATTTCCTCCAGTTTGGATTAGAGTCCGGCCTATT
TGACCTGCCACTGAACTTTCATCCAGCGGCGATTAGAGCCTCGGCGTTTT

IS*2* TAGACTGGCCCCCTGAATCTCCAGACAACCAATATCACTTAAATAAGTGA
TGGATTTGCCCCTATATTTCCAGACATCTGTTATCACTTAACCCATTAC
ISRm*1* TGAATTAGCCCCCGAAAGGTCCGGACGTGCTCCCCCACTTAGCTAAGTGG
TGGAGTTGCCCCCAAAAGACCGGACATGCTCAGGTTTGAGATTGCGAAC

IS*3* TGATCTTACCCAGCAATAGTGGACACGCGGCTAAGTGAGTAAACTCTCAG
TGATCCTACCCACGTAATATGGACACAGGCCTAAGCGAGGTTCTTGTTTT
IS*911* TGAAGTGGCACACTGAATTTGGCCACCTGAACAGAGGTGATATGCTCACC
TGAAGTGGTCAACAAAAACTGGCCACCGAGTTAGAGTTTTTCCAGTATC

IS*51* TGAACCGCCCCGGGTTTCTCGGAGACCTTTTGTTTGAGTCATGCCACCTG
TGAACCGCCCCGGGTTTCTCGGAGACTCCAACCTTTGAGAGGATGGAGCG
IS*3411* TGAACCGCCCCGGGAATCCTGGAGACTAAACTTCCTGAGAAAGAGGTAAA
TGAACCGCCCCGGGTTTCCTGGAGAGTGTTTTATCTGTGAACTCAGGCTG

IS*150* TGTACTGCACCCATTTTGTTGGACGATGAAATGGAATAGCCCCTAATATG
TGTACTGACCCCAAAAAGTTGGACAGTTAAACACGAGGCATATAGGTCTG
IS*861* TGAACTGCACCCCAAAAGTTAGACAAAAAATCTAACGATTGGGGTGCTTT
TGTACTGACCCCCAAAAGTTGGACAATTTATTTTAAGTGAAGGATTTAGT

ISBt*1* TGAAAAAATATAAAGAATTTAGTGGAATTACAGATACTTGTGGGAAAAAT
TGAAAAAATACAAAAAACGTAACAAATTTTATAAAATATCAATATATGCC
IS*1138* TAAACTGGGACAAAAAAATAAAAAACACTTTTTAAAATATACTTTAAATA
TAAACTAGGACAAAAAAAGTGACTATTTAAAAAACACTTAGAACATCTCA
IS*1353* TGGGGTGCGGACAAAATCTTGGACTACTTTAGGAGTAGTTCATGTATTCG
TGGGGGTGCGGACGATTTCTTGGACGGTTTATACGGACATCAATCCGACC
IS*481* TGTGAAGATTCAATAGGTTGTATGCATGGTTCATCCGAACCGGATTTGAG
TGTGAAGATTCAATAGGTTGTATTCGTCCAGGTTGAGTCTGGAGATGGGG

FIG. 7. IS*3* family. (A) General organization of IS*3* family members. The solid boxes indicate the left (IRL) and right (IRR) terminal IRs. Transcription probably occurs from a weak promoter located partially in IRL. The two consecutive overlapping open reading frames are indicated (*orfA* and *orfB*) and are arranged in reading phases 0 and −1 respectively. The products of these frames are shown below. OrfA and OrfB are shown as hatched and open boxes, respectively. The position of a potential helix-turn-helix motif (HTH) is shown as a stippled box in OrfA, and the DDE catalytic domain is shown as a stippled box in OrfB. A potential leucine zipper (LZ) at the C-terminal end of OrfA and extending into OrfAB is also indicated. Each leucine heptad is indicated by an oval. Those present in the OrfA domain are cross-hatched, whereas that deriving from the frameshifted product is open. (B) Dendrogram based on the alignment of the amino acid sequences of predicted OrfA proteins from 40 different elements (left) and 44 predicted OrfB frames (right). The major groups are indicated by brackets. (C) Nucleotide sequences of the terminal IRs of two representative elements of each subgroup, together with some of the elements which do not clearly form part of these groups.

unable to engineer derivatives with two complete tandem IS*3* elements. This may be the result of the formation of a strong hybrid promoter which, as described for IS*911* and other ISs (see above), drives high levels of Tpase expression. This configuration of ends is equivalent to that found at the circle junction and suggests that abutted ends of IS*3* are also efficient substrates in transposition.

IS*2* generates direct target duplications of 5 bp on insertion (111), although transposon circles generated with this element carry only a single base pair separating IRL and IRR (208). Moreover, while IS*2* carries a conserved terminal 5′-CA-3′ at

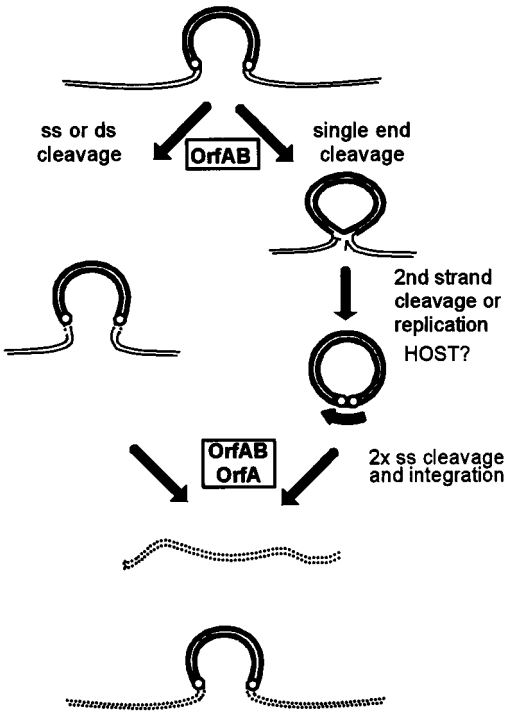


FIG. 8. Transposition pathways. Two possible pathways for transposition of IS*3* family members are shown. Transposon DNA is represented by heavy double lines, donor backbone DNA is represented by fine double lines, and target DNA is represented by a double dotted line. The ends of the transposon are represented by small open circles. The left-hand pathway represents transposon excision as a linear molecule by double-strand cleavage at each end followed by strand transfer into the target molecule. It does not entail the formation of an active junction. The right-hand pathway shows passage via a single circularized strand (figure-eight) mediated by OrfAB. Formation of a circularized transposon from this intermediate is thought to require a host factor. Insertion requires both OrfAB and OrfA. The 3′OH revealed on the donor backbone is shown as a half arrow. The heavy curved arrow indicates the strong p_{junc} promoter created by the abutted terminal IRs on circularization.

its right end, the left end terminates with 5′-TG-3′. Further analysis of IS*2* circles has demonstrated that the atypical IRL does not act as a strand donor but uniquely as a target in the circularization reaction. Functional studies indicate that the product of the upstream *orfA* may inhibit transposition (147). It has been shown to bind specifically to IRL at a sequence which overlaps the −10 hexamer of the resident Tpase promoter and to repress expression of OrfA. It does not appear to bind IRR (note that in the original article the authors reverse the standard definition of IRL and IRR [147]). Several other elements also exhibit small IR sequences which flank the −10 hexamer of the putative resident Tpase promoter. IS*2*-derivative transposons in which two abutted ends have been engineered also undergo high levels of transposition (208, 330), and, like IS*911*, the circle junction of IS2 also constitutes a strong promoter capable of driving Tpase expression. Several (but not all) IS*3* family elements may also carry similarly located potential −35 and −10 sequences within their IRs.

IS*4* Family

General features. Two subgroups were originally included in the IS*4* family (288). However, we have decided to separate the IS*4* and IS*5* subgroups into families in their own right. The IS*4* family is quite heterogeneous. There are 41 members, including 13 isoforms. It is difficult to justify division of this family into well-defined subgroups based on the relationships between their Tpases, although one major grouping (IS*231*A through IS*4* [Fig. 9A]) shows relatively closely related IRs (Fig. 9B) and tends to generate target repeats of between 10 and 13 bp (Table 2). Many, such as IS*10* and IS*50*, are involved in compound transposons (29). A single open reading frame spans most of the element. A previous alignment of these Tpases (288) revealed several regions of amino acid conservation: N1, N2, N3, and C1 (226), which encompass the D (N2), D (N3), and E (C1) regions of typical DDE motifs, respectively (Fig. 3). The distance between N3 and C1 in members of the IS*4* family is about 100 amino acids, compared with 33 to 40 amino acids for Tpases with the canonical DDE motif. In the case of IS*10* (see below), the importance of these regions has received experimental support. Several members carry GATC methylation sites, which, for both IS*10* and IS*50*, have been shown to play a modulating role in transposition activity (see below).

IS*10* and IS*50* (Fig. 9C) are certainly the best-characterized members of this group. Both transpose by a "cut-and-paste" mechanism, as does IS*231*A (205a). The mechanism of transposition of other members of this group is at present unknown.

IS*10*. IS*10* (Fig. 9C) forms part of the composite tetracycline resistance transposon Tn*10*. Since it has been recently described in a comprehensive review (179), only a brief summary is included here. Insertion results in a 9-bp target duplication and occurs in a relatively well defined target sequence with the symmetric 6-bp consensus: 5′-NGCTNAGCN-3′. It carries 22-bp terminal IRs, called outside (OE = IRL) and inside (IE = IRR) ends, describing their relative position in Tn*10*, and exhibits an elaborate ensemble of mechanisms to control its activity. The Tpase of IS*10* is expressed from a single long reading frame. Expression is protected from activation by external transcription by an IR sequence located close to the left end (see "Sequestration of translation initiation signals" above). The activity of the Tpase promoter (pIN) is modulated by the presence of a site for the Dam methylase located within the −10 hexamer. Methylation reduces pIN activity, but after replication, this site becomes transiently hemimethylated and the promoter strength is concomitantly increased. A second level of control is exercised by a small antisense RNA molecule transcribed from a second, outwardly directed, promoter located proximal to IRL (pOUT). This RNA is complementary to the region of Tpase mRNA carrying the ribosome binding site, acts in *trans*, and interacts with the mRNA to inhibit ribosome binding.

Dam methylation also exerts control at another level. In addition to the Dam site located in IRL, another site is localized in IRR. When these sites are methylated, the activity of the ends in transposition is reduced, whereas when they are hemimethylated, the transposition activity is increased. With this arrangement, both Tpase expression and transposition activity are coupled to replication of the donor molecule. Since transposition of IS*10* is nonreplicative, this ensures that passive replication of the IS occurs before transposition takes place. A binding site for the host IHF protein is also located at the left end of IS*10*, outside IRL. IHF plays a subtle role in IS*10* transposition in vivo by influencing the nature of transposition products (314).

Overproduction of Tpase in vivo in the presence of a suitable IS*10* derivative transposon leads to the appearance of a variety of transposon forms. Among these are excised transposon circles (243). These are not equivalent to those observed for IS*3* family members (see "IS*3* family" above) and have proved to be rearranged circular products of transposition

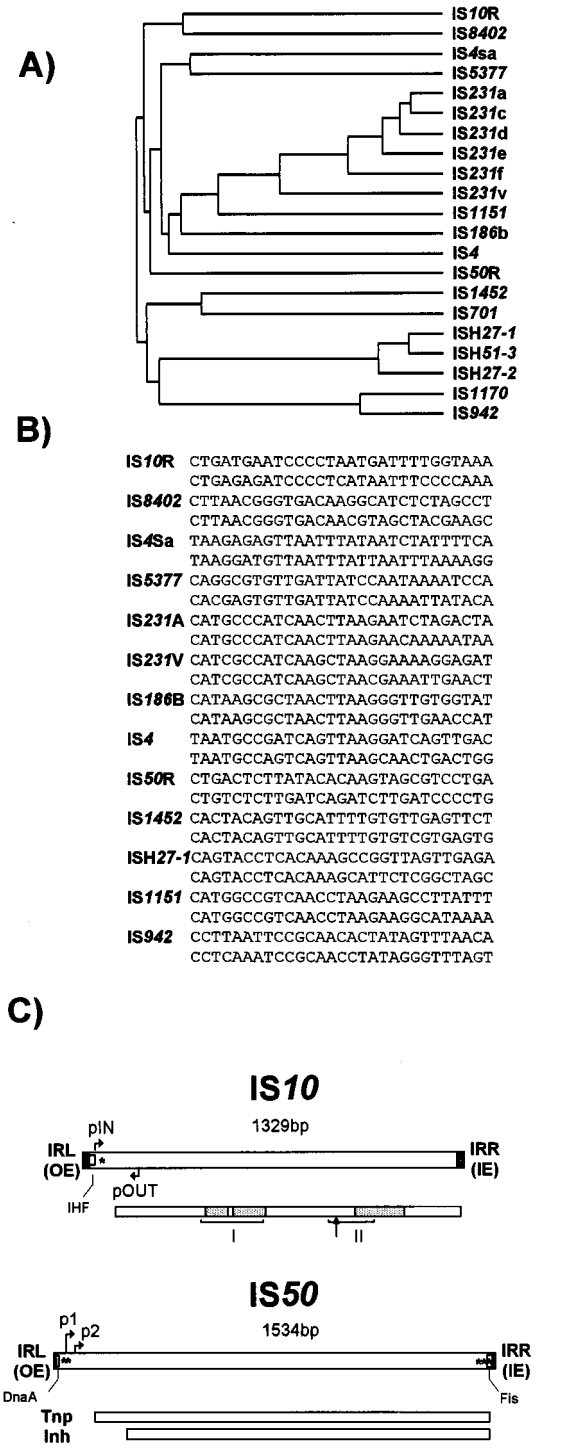


FIG. 9. IS*4* family. (A) Dendrogram based on alignments of the putative Tpases. (B) Terminal IRs of selected members. (C) Schematic representation of IS*10* and IS*50*. The terminal IRL (OE) and IRR (IE) are shown as solid boxes. Dam methylation sites (*) are also shown. For IS*10*, the Tpase promoter, pIN, and the antiRNA promoter, pOUT, are indicated as horizontal arrows. A mechanistically important IHF site is indicated by an open box next to IRL. The Tpase is represented underneath. Stippled boxes indicate the positions of the consensus sequence within members of the IS*4* family (from positions 93 to 132, 157 to 187, and 266 to 326). I and II indicate patch I and patch II, respectively, as defined by mutagenesis. The vertical arrow indicates a protease-sensitive site. For IS*50*, the promoters for Tpase and inhibitor protein, p1 and p2, respectively, are indicated as horizontal arrows. DnaA and Fis binding sites, located close to the left and right ends, respectively, are indicated by open boxes.

(179, 244). In addition, linear transposon species which are retained in a circular configuration by Tpase bound at the ends are also observed. IS*10* transposition involves double-strand cleavage at the ends of the IS, thus separating it from the donor backbone. An in vitro IS*10* transposition reaction has been developed by using a circular plasmid donor substrate (53). This generates an array of intermediates and products, some of which are identical to those found in vivo. However, additional species can be detected. These include the results of single-ended transpositions (lariat structures), reactions involving synapsis of directly repeated IRs, and even reactions between IRs on separate molecules (54). A simpler assay employs a linear double-stranded oligonucleotide substrate including the "outside" end of IS*10*, which carries the IHF binding site. This assay has allowed the isolation and analysis of a synaptic complex between two copies of the substrate DNA fragment, Tpase, and IHF. IHF is essential for Tpase binding and for the formation of synaptic complexes (299). By using this system, the two single-strand cleavages which take place at each of the transposon ends have been shown to occur in a specific order: the transferred strand is the first to be cleaved (38). More recently, data have been obtained suggesting that cleavage of the second strand at each end is promoted by nucleophilic attack with the liberated 3′OH generated in cleavage of the first strand, giving rise to hairpin structures at both ends (175a).

As indicated above (see "Terminal inverted repeats"), the ends of IS*10* can be divided into an internal Tpase binding domain and a terminal domain intimately involved in cleavage and strand transfer. Tpase function has also been probed by the isolation of Tpase mutants. Several ingenious screening procedures have been devised. One of these was a scheme for isolating derivatives with altered target specificity (ATS). A second exploited the capacity of IS*10* Tpase to induce the host SOS response in the presence of suitably oriented IS*10* ends. Mutants presumably defective in strand transfer but retaining the ability to generate an SOS response have been obtained. Most fall within two regions, called patch I and patch II (Fig. 9C) (137), corresponding to the N3 and C1 regions of the IS*4* group Tpases (Fig. 3) (179, 226, 288). Moreover, intragenic suppressors of the SOS and ATS mutations, derivatives which suppress the effect of mutations within the IS*10* ends, and mutations giving a hypernicking phenotype have also been localized in these regions (see reference 179 for a summary). Partial proteolysis of the Tpase generates an N-terminal domain containing patch I and a C-terminal domain containing part of patch II, which are connected in the complete protein by a short proteolytically sensitive linker region. This region also carries part of patch II. Interestingly, Tpase activity can be reconstituted with a mixture of the two proteolytic fragments (192). Recently, evidence has been presented suggesting that a single active site in the enzyme is used for 3′ and 5′ cleavage and for strand transfer (39).

IS*50*. IS*50* forms part of transposon Tn*5* and, like IS*10*, has been extensively studied (reviewed in references 27 and 286). It differs significantly from IS*10* in its organization and control (Fig. 9C). It inserts without notable target sequence specificity and carries small imperfect terminal IRs. The terminal 19-bp sequence is critical for transposition. Like IS*10*, IS*50* has adopted an elaborate series of mechanisms to control its activity. It encodes two proteins, the Tpase, called Tnp, expressed from a single long reading frame, and an inhibitor protein, Inh, translated in the same frame as Tnp but using an alternative initiation codon and lacking the N-terminal 55 amino acids. These proteins appear to be expressed from separate (and possibly competing) promoters, P1 and P2, respectively (Fig.

9C), which are located downstream from the terminal IR (182). As in the case of IS*10*, Tpase expression is protected from activation by external transcription by an inverted repeat sequence located close to the left end. Moreover, the presence of Dam methylation sites within the Tpase promoter, T1, renders Tnp expression sensitive to methylation while having little effect on the activity of the Inh promoter T2.

The ends of IS*50*, known as OE (=IRL) and IE (=IRR), carry a series of sites which modulate Tnp expression and transposition activity. Extensive mutagenesis has shown that these ends conform to the general two-domain organization with a protein binding domain and a terminal domain (166). Tnp specifically binds both 19-bp end sequences. OE includes a binding site for the host DnaA protein, and transposition activity is reduced in a *dna*A host (371). IE is somewhat more complex. It carries a binding site for the host protein, Fis, and three consecutive Dam methylation sites. Transposition activity is reduced by the presence of the Fis site (359) and by methylation of IE (369). The effect of methylation has been directly attributed to interference with Tnp binding (286).

Some analysis of Tpase organization and function has been undertaken. While Tnp specifically binds OE and IE, Inh does not (75). Moreover, the introduction of missense mutations or deletion of the 11 N-terminal amino acids destroys DNA binding activity (357). This has suggested that the DNA binding domain lies at least partially within the N-terminal 55 amino acids of Tnp. Interestingly, deletion of the C-terminal 100 amino acids increases the specific affinity of the protein for OE, suggesting that binding activity may be masked by the C-terminal part of the protein.

It is thought that the inhibitory action of Inh involves the formation of (inactive) heteromultimers between Inh and Tnp. Inh appears to be able to act in *trans*, whereas, like many Tpases, Tnp shows preferential action in *cis*.

More recently, an in vitro transposition system has been developed. This has clearly demonstrated that, like IS*10* Tpase, IS*50* Tpase is capable of introducing double-strand cuts at the ends of the element separating it from the donor plasmid backbone (116a).

IS*231*. IS*231*A was isolated from *Bacillus thuringiensis*, in which it flanked a δ-endotoxin crystal protein gene (225), and it has been proposed that it forms part of a composite transposon. IS*231*A is active in *E. coli*. Ten other isoforms have been isolated. Sequence analysis of the terminal IRs of these elements suggests that they conform to the two-domain structure (Fig. 1) with conservation of bp 1 to 3 and 6 to 12. Like IS*10* and IS*50*, a potential ribosome binding site for the Tpase gene would be sequestered in a secondary structure in transcripts originating outside the element. Although most examples carry a single open reading frame, Tpase expression from two elements (IS*231*V and IS*231*W) may occur by a +1 and +2 frameshift, respectively, but this has yet to be confirmed. Little is known about the transposition mechanism of this element, although it exhibits strong target specificity and recent evidence suggests that it transposes by a nonreplicative cut-and-paste mechanism (205a).

IS*5* Family

General features. The IS*5* family is a relatively heterogeneous group composed of 47 distinct members and 21 isoelements. They include sequences from both eubacteria and the archaea. The majority carry only a single open reading frame. Their lengths range from 850 bp (IS*869*) to 1,643 bp (IS*493*). The latter carries a second open reading frame upstream of the "Tpase" frame which is inessential for transposition (18). Similarly, IS*4811* (Tn*4811* [57]), which is greater than 5 kb, clearly contains other, as yet uncharacterized genes. The major feature which defines this group is the similarity between their putative Tpases (288). In particular, this includes the N2, N3, and C1 domains carried by the IS*4* group (Fig. 3). However, Tpases of the IS*5* family exhibit a spacing between the N3 and C1 domains of approximately 40 residues, a distance more consistent with the canonical DDE motif. This group can be divided into six subgroups according to the depth of branching between them (Fig. 10A). The division is supported by similarities in the terminal IRs (Fig. 10B), by correlation with the length of the target duplication, and, to a lesser extent, by the typical length of the entire IS (Table 2). It is important to note that most members of the IS*427* subgroup exhibit two partially overlapping open reading frames in a configuration in which the downstream frame is in phase −1 compared to the upstream frame and that most have a potential −1 frameshift window. Several members of the family are associated with compound transposons. These include IS*903* and IS*602*, which form part of the kanamycin resistance transposons Tn*903* (118) and Tn*602* (326), respectively, and ISVa*1*/ISVa*2*, which form part of a transposon carrying iron transport genes (336).

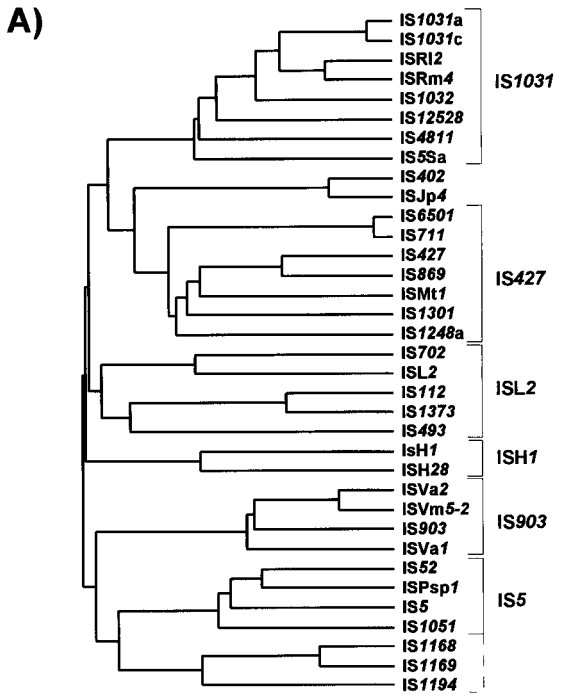
Several members exhibit GATC sites within their terminal 50 bp. This includes all members of the IS*903* subgroup and about 50% of the members of the IS*1031* and IS*427* subgroups. IS*903* transposition activity is modulated by Dam in vivo (cited in reference 292).

For two subgroups, IS*5* and IS*427*, and for two members of the ISL*2* group (IS*112* and IS*1373*), a preferred target sequence, YTAR (often CTAG), is observed in which either all four base pairs or the central TA is duplicated on insertion. It is important to underline that in many cases, the sequence of the original target site before insertion is not available. This can introduce ambiguities not only in estimating the number of duplicated target base pairs but also in defining the IRs. It is particularly important in several cases where the target repeat is symmetrical (e.g., CTAG) and where it is impossible to distinguish whether the element duplicates 2 or 4 bp and therefore to determine the exact ends of the element. Alignment of the ends of these elements in subgroups has permitted a number of ambiguities to be resolved. Members of the ISL*2* group which generate 3-bp DRs exhibit a preference for ANT, while those from the IS*1031* group (which generate exclusively a 3-bp DR) exhibit a preference for insertion sites with the sequence TNA. Neither the small ISH*1* group (8-bp DRs) nor the IS*903* group (9-bp DRs) exhibits marked target specificity.

Only two of these elements, IS*5* and IS*903*, have received significant attention.

IS*5*. In spite of the importance of IS*5* in generating mutations, the published work concerning this element is largely directed to an understanding of its coding capacity and expression properties. IS*5* carries one large open reading frame *ins5A*, which spans the entire element and is essential for transposition (303), and two small open reading frames, *ins5B* and *ins5C*, whose relevance to transposition remains to be demonstrated. Nothing is known about the transposition mechanism of this element.

IS*903*. The ends of IS*903* carry IRs of 18 bp which exhibit the typical two-domain organization (Fig. 1) (82). Transposase has been shown to bind specifically to the ends by using a region located in the amino-terminal portion of the protein (79, 332). In addition, recent results have revealed a region possibly involved in the formation of higher-order multimers and has pinpointed residues probably involved in catalysis (332). Little is known about its transposition mechanism, al-



B)

IS*427*	GGCCGTGAACCCATAAACTGGGTACCGGATTACTGAT
	GGCCGTAGACTCATAAGCCCGCAGCCACAGGCGCATT
IS*711*	GGGCTTGTCTGCATTCAAGGATTCCCTTTTGTACGAA
	GGGCGTGTCTGCATTCAACGTAACCAGATCATAGCGC
ISRm*4*	GAGGCTGACCGAAAAAGAGTTGAGTGAAATCAGTGAG
	GAGTCGGACTCGAAAAGTGATCAACGATAGCAATATC
IS*1031*	GAGCCTGATCCGAAAGTTTTTGAACAATACCAGCCTG
	GAGCCCGATCCGAAAGTTTTTCAAGCTTGACAATGCC
ISL*2*	AGAGTTACTGCGAAACATCTGCTATAATCTTAAATTA
	AGAGTTACTGCGAAACTAGATAGCCAGTTCTTGGTTG
IS702	AGAACTCTTGCAAAAGTCTTTTGTGGTATAATTAAGG
	AGAACTCTTGCATAAATATTTTTTGATAAAATAAAGG
IS*903*	GGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAA
	GGCTTTGTTGAATAAATCAGATTTCGGGTAAGTCTCC
ISVa*2*	GGCTTTGTTGCTTAAATCGATGGAACTAAATCTAGAA
	GGCTTTGTTGCTTAATTCGGTTGTAAGACATGGCAGC
IS*5*	GGAAGGTGCGAATAAGCGGGGAAATTCTTCTCGGCTG
	GGAAGGTGCGAACAAGTCCCTGATATGAGATCATGTT
IS*52*	GGAGCCGCTGCAAAAATAGCCAACAGCCCGTAGCCTT
	GGAGACGCTGAACAATTAACTCCGTTCGCACCGCCCC
ISH*11*	GAGGGTGTCATAGAACGAGTAGCACACCAAAGAGCAG
	GAGGGTGTCATAGAAGTCTTGTCACACCGTGATGATC
IS*402*	GAGACGGTTTCAAAAAGGCCCCTGGGGGCTGTTAACT
	GAGACGGTTTCATAAAGACTGATCGGCCCGCCGAAGG
IS*1106*	CTTTCGTGGGAATGACGAAAAGTGGCGGGAATGACGG
	CTTTCGCGGGAATGACGAAAAGTGGCGGGAATGACGA
ISH*1*	TGCCTTGTTTTGCCACCGATTGAGGGAAGTTTCAGAC
	TGCCTCGTTGAATCACAAGACAGCGTCGGGGTAGGGA
ISH*28*	GGCTCTGGTGAATCGCTGGACAGCGCCGGCTTCGACC
	GGCTCTGGTGAATCGCCAGAAGGCGTTTGATTTCACG
IS*1168*	GGGGACTTCTATAAATTAAATCCCTGATTATAAGTTG
	GGTGGGTTCTATCAATTCCATTTTTTATTTCTTGCAT

FIG. 10. IS*5* family. (A) Dendrogram based on Tpase alignments, showing the division of the family into various subgroups. (B) Terminal IRs of two members of each subgroup, together with those of several elements which fall outside these groups.

though an elegant genetic analysis provided strong evidence that IS*903* is not only capable of undergoing direct insertion but can also generate adjacent deletions in a duplicative manner (356).

IS*6* Family

The IS*6* family was named after the directly repeated insertion sequences in transposon Tn*6* (28). Historically, several of these elements were given individual IS numbers and have subsequently been determined to be identical. Many have been found as part of compound transposons. The IS*6* family of elements is composed of 45 members, 30 of which are isocopies. Several distinct family members have been shown to transpose. The putative Tpases are very closely related and show identity levels ranging from 40 to 94%. They generally range in length from 789 bp (IS*257*) to 880 bp (IS*6100*). However, one isolate, IS*15*, corresponds to an insertion of one iso-IS*6* (IS*15*Δ) into another (341). Although it has been included in the family based on similarities of its terminal IRs (Fig. 11B), IS*466* from *Streptomyces coelicolor* is 1,628 bp long and the Tpase open reading frame has yet to be determined. All carry short related (15 to 20 bp) terminal IRs and generally create 8-bp direct target repeats. No marked target selectivity has been observed (241). Interestingly, sequences identical to the *Mycobacterium fortuitum* IS*6100* have recently been identified as part of a plasmid-associated catabolic transposon carrying genes for nylon degradation in *Arthrobacter* sp. (170), from the *Pseudomonas aeruginosa* R1003 plasmid (132), and within the *Xanthomonas campestris* transposon Tn*5393*b (328). This suggests a relatively recent horizontal dissemination.

In IS*26*, an open reading frame is transcribed from a promoter located within the first 82 bp of the left end. It stretches across almost the entire IS and is required for transposition activity (241), and the predicted amino acid sequence of the corresponding protein exhibits a strong DDE motif (Fig. 3). Translation products of this frame have been demonstrated for IS*240* (76) and IS*26* (350a). Little is known about the control of Tpase expression, although recent evidence obtained with IS*6100* in *Streptomyces lividans* (317) and IS*26* in *E. coli* (350a) indicates that the transposition activity is significantly increased when the element is placed downstream from a strong promoter.

Where analyzed, members of the IS*6* family give rise exclusively to replicon fusions (cointegrates) in which the donor and target replicons are separated by two directly repeated IS copies (e.g., IS*15*Δ, IS*26*, IS*257*, and IS*1936*). Transposition of these elements is therefore presumably accompanied by replication. Following cointegration, a resolution step is required to separate donor and target replicons and to transfer the transposon to the target replicon. Recombination between directly repeated ISs necessary for this separation occurs by homologous recombination and requires a recombination-proficient host (Fig. 11C).

An interesting similarity has been observed to a 4,904-bp element isolated from the filamentous cyanobacterium *Fremyella diplosiphon* (Tn*5469* [IS*5469*] [168]). This relatively long element carries three consecutive open reading frames. The protein encoded by the first and longest frame, TnpA, is 909 residues long, compared to the IS*6* family Tpases of between 220 and 260 residues. While TnpA contains an N-terminal region which exhibits significant conservation with respect to the IS*6* Tpases (27 of 80 conserved residues over approximately 220 residues) (Fig. 11A), this region does not carry the DDE motif. On the other hand, a clear DDE motif resembling the Tpases of Tn*5090* (*Klebsiella aerogenes* plasmid R751 [276]) and of the relatively complex transposon Tn*552* (*Staphylococcus aureus* [298]) is located in the C-terminal region. Moreover, the ends of Tn*5469* exhibit a strong similarity to those of the IS*6* family members. This raises the possibility that recog-

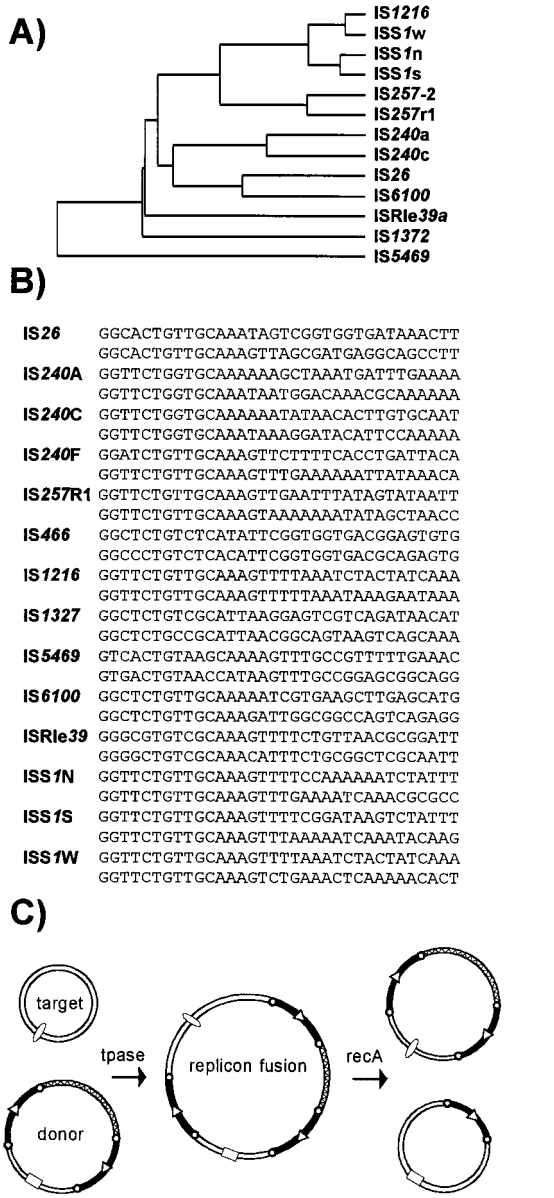


FIG. 11. IS*6* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs. (C) Transposition mechanism. A target plasmid is distinguished by an open oval representing the origin of replication. The transposon carried by the donor plasmid is composed of two copies of the IS (heavy double lines terminated by small circles) in direct relative orientation (indicated by the open arrowhead) flanking an interstitial DNA segment (shown as a zigzag). The donor plasmid is distinguished by an open rectangle representing its origin of replication. Tpase-mediated replicon fusion of the two molecules generates a third copy of the IS in the same orientation as the original pair (open arrowhead). Homologous recombination, using the *recA* system, between any two copies can in principle occur. This will either regenerate the donor plasmid, leaving a single IS copy in the target, delete the transposon, or transfer the transposon to the target (as shown), leaving a single copy of the IS in the donor molecule.

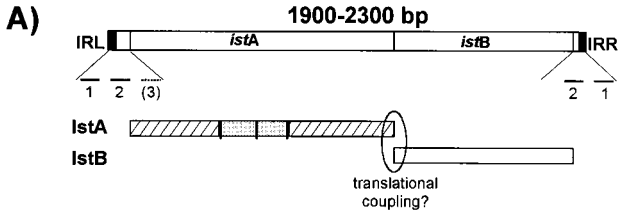
nition of the ends is determined by the IS*6*-like N-terminal region while catalysis is governed by a Tn*552*-related recombinase.

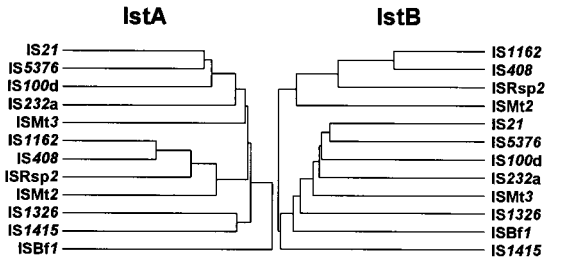
IS*21* Family

General features. There are 15 distinct members of the IS*21* family (but only 11 for which the entire nucleotide sequence has been established [Fig. 12B]) together with 6 iso-ISs. They carry related terminal IRs whose lengths may vary between 11 bp (IS*21*) and 50 bp (IS*5376*) and generally terminate in the dinucleotide 5′-CA-3′. Several members, but not IS*21* itself, carry multiple repeated sequences at their ends which include part of the terminal IRs (Fig. 12A and C) and which may represent Tpase binding sites. Insertion of these elements results in a direct target repeat of 4 bp or, more frequently, 5 bp, while two members (IS*53* and IS*408*) may generate 8 bp. They exhibit two consecutive open reading frames: a long upstream frame designated *istA* and a shorter downstream frame designated *istB* (Fig. 12A). The putative IstA and IstB proteins carry several blocks of highly conserved residues. Overall identities range from 10 to 59% for IstA and from 25 to 67% for IstB. The *istB* frame may be located in a relative reading phase of −1 (e.g., IS*21* and IS*5376*) or +1 (e.g., IS*232* and IS*1326*) compared to *istA*. It can be slightly separated from *istA* (17 bp for IS*408*) or can overlap for 1 bp (IS*21*) or for several base pairs (IS*232*, IS*5376*, and IS*1326*); it is generally preceded by a potential ribosome binding site. The arrangement of the two reading frames suggests that translational coupling may occur (Fig. 12A). The *istA* reading frame carries a motif related to the widespread integrase DDE motif but lacking the conserved K/R residue (Fig. 3) and a potential helix-turn-helix motif, while the *istB* frame carries a relatively well conserved potential nucleoside triphosphate binding domain (319). Sequence alignments of IstA and of IstB gives very similar relationships (Fig. 12B).

A partial sequence lacking the right end is available for one member, IS*408*. For another member, IS*53*, 45% identity of the potential *istA* frame with that of IS*1326* can be obtained by the introduction of several frameshifts. An additional sequence, IS*640* from *Shigella sonnei*, is a truncated iso-IS*21*. It carries an *istA* reading frame which is nearly identical to that of IS*21* but lacks *istB* and the right terminal IR. The "intact" members have lengths of between 2 and 2.5 kb and are therefore among the largest bacterial IS elements.

IS*21*. IS*21* has been studied in some detail. It was discovered in *Pseudomonas aeruginosa* as a constituent of R68. This conjugative plasmid carries a single copy of IS*21* and is inefficient in chromosome transfer. A derivative of R68, R68.45, exhibiting a marked increase in the transfer of chromosomal genes, was isolated (291) and proved to carry two directly repeated copies of IS*21* separated by 3 bp (283). Tandem duplication is presumably favored by the marked tendency of IS*21* to insert in, or close to, an IS*21* end but may also occur by unequal crossing over during replication of the donor plasmid or, as suggested for IS*30*, whose transposition mechanism shows some similarity to that of IS*21* (see "IS*30* family" below), by dimerization of the donor plasmid (Fig. 4B, structure II) and deletion of DNA between the two IS copies (structure III). The tandemly repeated copies of IS*21* promote insertion of the entire plasmid in a transposition event involving the abutted terminal IRs. Integration results in loss of the 3 bp located between the two ends in the parental R68.45 plasmid. The resulting replicon fusion thus has the appearance of a true cointegrate (i.e., with a single directly repeated copy of the IS at each junction), although transposition does not involve duplication of the insertion sequence during the insertion event. Both IstA and IstB are required for integration (281), and production of a second IstA derivative (from an alternative translational initiation codon eight codons downstream of that of IstA itself) greatly increases the efficiency of replicon fusions (282) for reasons that are at present unknown. Like retroviruses and several other transposable elements, IS*21* terminates





C)

IS*21* NONE

IS*53*

L1: GCTGACCGAAAACTGACCCAG (9-28)
L2: GCCGACTGAAAACTGACCCAG (42-62)
R1: GCTGATTGAAAACTGACCCAC (2528-2548) CS
R2: GCCGATTGAAAATTGACCCAG (2504-2524) CS

IS*232*A

L1: TAAATATGTACATTAACGCTTG (16-37)
L2: TAAATATGTACATTTTCAATTGGATTCT (40-67)
L3: TTAGTATGTATATTAAGCTAGATATTCA (88-115)
R1: TAAACATGTACATTTTCGCTTG (2148-2169) CS
R2: TAAGCATGTACAAAATCAATCATTTTCT (2118-2145) CS

IS*408*

L1: TTCCGGCGAACGTGATCACGCAT (7-29)
L2: TTCGGCGGATCGTGATCATGGAT (30-52)
L3: TTCGGTTGATCGTGATCGCTGAT (76-98)
R1: TTTCGGTGATGGTGATCAGGCGT (7-29) CS
R2: TTCGGCGAACGTGATCAGTTTG (30-51) CS

IS*5376*

L1: TAAAGCCGATGATAAAATCCCCA (4-26)
L2: TATAGCCGGAATAAAATTCCCCA (28-52)
R1: CAAGGCCGATTATTTTTTCCCCA (2082-2104) CS
R2: AATCGCCGGTTTAAAATTCCCCA (2058-2080) CS

IS*1162*

L1: CGTGACCGGCTGTTTCG (18-34)
L2: CGTGACCGGACATTTCG (41-57)
L3: CGTGACCGCTCATTTCG (64-80)
L4: CGTGACCG---ATTTC (88-100)
L5: CGTGACCGCCTGTTTCG (111-127) CS
L6: CGTGACCGACGATTTCG (134-150) CS
R1: CGTGACCGCTCATTTCG (2602-2618) CS
R2: CGTGACCGGTGGCTTCA (2578-2594) CS

IS*1326*

L1: GAGTTGCATCTAAAATTGACCC (5-26)
L2: GATTTGCGTCGAAATTTGACCC (39-60)
L3: GATATTGAGCGCAATTCGACGC (122-143)
R1: GATTTGCATTGAATTTTGACCC (2421-2442) CS
R2: GATTTGCACCCAAATTTGACCC (2445-2466) CS

IS*1415*

L1: GTAAGCTCCCCACTGGCGGCCA (15-36)
L2: GGTTTCTCCCCGCGTACGGCCA (60-81)
R1: AAGTTCTCCCCGTAGGCGGCCA (2545-2566) CS
R2: -AGTTCTCCCCGCTGGTGGCCA (2521-2542) CS
R3: G-GTTCTCCCCGGTGGCGGCCA (2498-2519) CS

FIG. 12. IS*21* family. (A) General organization. The terminal IRL and IRR are shown as solid boxes. The position of the *istA* and *istB* reading frames is also shown. The horizontal lines below show the relative positions of the multiply repeated elements whose sequences are presented in panel C. IstA (hatched box) together with the potential DDE motif (stippled box) and IstB (open box) are indicated below. The possibility of translational coupling between the two reading frames is indicated. (B) Dendrogram derived from alignment of the IstA and IstB gene products. (C) Nucleotide sequences of the multiple terminal repeats, together with their coordinates. CS, complementary strand. L1, L2, and L3, and R1 and R2, indicate internal repeated sequences at the left and right ends, respectively.

with 5′-CA-3′. Mutation of the terminal A abolishes replicon fusion (129).

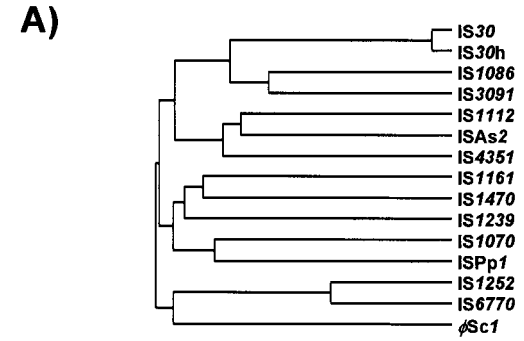
The duplication of IS*21* in R68.45 not only creates an efficient integrative donor structure by providing two closely spaced and correctly oriented IRs but also generates a strong promoter in which a −35 box located in the IRR of the upstream element is combined with a −10 box located in the abutted IRL of the downstream copy. This directs high levels of production of the IS*21* transposition proteins (283) in a similar manner to the junction promoter created by circularization of IS*911* and IS*2*. Insertion of IS*21* downstream of a strong promoter results in the efficient expression of transposition proteins. Thus, IS*21* appears not to have adopted a mechanism which "protects" it against high levels of impinging transcription. It is presumably the necessity for a transposition substrate carrying two abutted ends and the low frequency of formation of such molecules which acts as a control on transposition activity. More recently, a low level of circular IS*21* copies has been detected in preparations of IS*21*-carrying plasmid DNA (302a), raising the possibility that IS*21*, like at least some of the IS*3* family members, also transposes via a circular intermediate.

It has been demonstrated in vitro, using *E. coli* cell extracts enriched for IstA, that this protein is responsible for the 3′-end cleavage of IS*21* (281). A substrate carrying two abutted IS*21* ends separated by 3 bp is cleaved specifically at both 3′ ends in an IstA-dependent way.

IS*30* Family

General features. The IS*30* family comprises 15 distinct complete members whose nucleotide sequence has been determined and 4 isoelements (Fig. 13A). Their lengths vary between 1,027 bp (IS*1070*) and 1,221 bp (IS*30*) with a single open reading frame of between 293 and 383 codons, which spans almost the entire length. The termination codon is generally very close to the right end. Alignment of the putative Tpases reveals a well-conserved DD(33)E motif. An open reading frame from a *Spiroplasma citri* phage (ϕSc*1*) is distantly related to the family. The terminal IRs are in the range of 20 to 30 bp and exhibit significant homologies (Fig. 13B). It should be noted that the tips of the elements, as defined by the various authors, show significant sequence variation. Where it has been determined, 2- or 3-bp DRs are generated on insertion.

IS*30*. Of the different members of this family, IS*30* is the best characterized. It shows pronounced insertion specificity and generates DRs of 2 bp (51). It terminates with the dinucleotide 5′-CA-3′ (72), although many other members of the family do not. The long open reading frame is preceded by a relatively weak promoter (p30A) capable of driving Tpase expression with its −35 hexamer within IRL (71) and contains a weak internal transcription terminator. Premature termination of transcription at this site could lead to the formation of Tpase molecules truncated at their C-terminal end. Since the specific DNA binding domain which recognizes the terminal IRs is located in the N-terminal one-third of the protein (322), the potential truncated derivatives may lack the catalytic domain and regulate transposition activity by competition or interaction with a full-length Tpase. This arrangement may play the same role at the transcriptional level as does frameshifting at the translational level for certain other elements. A second weak promoter (p30C) located on the opposite strand upstream of a short open reading frame has been detected (71), and it has recently been shown that a small (150-nucleotide) transcript driven from this promoter and contained entirely



B)

IS*30*	TGTAGATTCAATTGGTCAACGCAACAGTTA
	TGTAGATTCAATCTGTCAATGCAACACCCC
IS*1086*	GGCGGCCTCAAATCTGAAGTGCAACACCTT
	GGCGGTTTCAAGTGCGAAGCGCAACACCCC
IS*1112*	GGCGGCTTCAACTCTGGATCGCAACACCAA
	GGCGAATTCAACTCTGATTCGCAACGCTTT
ISAs*2*	CGGGAATTCAGATAATAAGTGCGACACTCA
	CGCGAATTCAACTTCGAAGTGCGACACTCG
IS*4351*	CTTGAGTTCAACTTATAAATGCAACTTTTT
	GCTGAATTCAACTTGCAAATGCAACAGAAT
IS*1161*	GCCCTATTGTAAAATGAAGTGCAACACAAA
	CCCTAATTGTCAAGTCAAGTGCAACAAAGA
IS*1470*	GCTTAATTGTAAAATGAAATTGAACTAAAT
	TCTAAATTGCAATATCAAATTGAACTAGTC
IS*1239*	GAAAAATTCTAAAATGAACTTAGCCACTTT
	TATCTCTTTGCAAATTTCAAGTTGAAGTTA
IS*1070*	GGTAGATTGTAAAATTAAACCGAACACTTG
	GGTAGATTGCAAGAATTAACCGAACACTGT
ISPp*1*	GGTAGATTGTAAAATTAATCCGACGCTGTT
	GGTAGATTGCAAATTTAATCCGAATTTTTG
IS*1252*	CGCCGATTGTAAAATTAAGCTAGACAAATA
	TTTGATTTGTCAAATTAAGCTAGACAAAAT
IS*6770*	CGCGAATTGTAAAATTAAGTTAGAAAAATA
	TATGATTTGTCAAATTAAGTTAGAATAAAA
ϕSc*1*	TGCATCCTGTAATCGAAGTGCAACAAATCA
	TGAATTATGTAAAATGAAGTGCAACAAATC

FIG. 13. IS*30* family. (A) Dendrogram based on Tpase alignments. ϕSc1 is a homologous reading frame detected in a *Spiroplasma citri* bacteriophage. (B) Terminal IRs.

within the Tpase transcript acts as an antisense control by reducing translation of the Tpase mRNA (14).

As for IS*21* and IS*911*, the assembly of abutted IRL and IRR copies creates a strong promoter. In a tandem dimer of the element, this may result in increased expression of the Tpase from the downstream copy (70). The IRL-IRR junction is also recombinationally unstable. It promotes transposition reactions at high frequency in vivo (255), presumably in a similar way to the equivalent junction of IS*21* described above. The activity of the junction appears maximal when the spacer between the abutted ends is 2 bp (the length of target duplications introduced when IS*30* inserts). Lower activities are obtained with 1- and 3-bp spacers, while little or no activity is observed for 0 or >3 bp (249a).

IS*66* Family

The IS*66* family is composed of 12 members limited to agrobacteria and rhizobia. These include one isoelement and several elements for which only partial sequence data have been obtained. Members of this family are between 2.49 and 2.73 kb long and have very similar terminal IRs of between 15 and 27 bp (Fig. 14B). These elements are flanked by 8-bp direct target repeats. Each contains a number of open reading frames which varies among elements due to slight variations in the nucleotide sequences (either real or due to sequencing

B)

IS*1131*	GTGAGCGTCCGGTGAGCTGTTTTTGCT
	GTGAGCGTCCGGCAAACGTTTTCGGGT
IS*66*	GTAAGCCCACGGTGAAGGCCGTCTTGC
	GTAAGCCTCCGGTGAAGGCCACAGGTC
IS*866*	GTATGCGCCGCCTCCATCCCATTGATT
	GTATGCGGCGTCTCCGTCCCATTGATT
ISRm*2*	GTAAGCGCTCATTTCCATGCGCGTTGA
	GTAAGCGCTCATTTCCATGCGCCTTTT
IS*292*	GTAAGCGGCAAGCTGACGCCGTTCAGG
	GTAAGCGGCAAGCTGACCCCATCTGGC
ISRI*1*	GTAAGCGACAAGCCGGGGCCGTTCAGG
	GTAAGCGACAAGCTGACCCCATCTGGC
IS*1313*	GTAAGCGCGAATTTCTGTGCACCTTCT
	GTAAGCGCGATTTTCGATGCGCCTAGA

FIG. 14. IS*66* family. (A) Organization of IS*866*. A "best-guess" diagram of the open reading frames is shown. All are transcribed from left to right. The difference in shading is simply to facilitate their distinction. (B) Terminal IRs.

errors). We have used IS*866* as a reference since it appears to carry the most representative set of open reading frames (Fig. 14A). However, we underline the possibility that errors in this interpretation may well exist. We have defined here five open reading frames common to IS*66*, IS*292*, and IS*866*. While having the expected length and terminal IRs, IS*1313* carries only a single reading frame with similarity to those of IS*66* family members (open reading frame 2). An interesting observation concerning this family is the presence of a group II intron signature in open reading frame 5 (180), although its significance is at present unknown. No studies have addressed the transposition of IS*66* family members.

IS*91* Family

General features. The IS*91* family is composed of eight members, of which one, ISFn*1*, has been only partially determined (145) and three are isoforms. Two additional members, IS*92*L and R form part of a composite transposon. They exhibit homology to IS*91* as judged by Southern hybridization but appear to contain various large deletions and substitutions (374). Another member, IS*1294*, was discovered on plasmid pUB2380 (64). Comparison with other members of the family suggests that its Tpase could be extended to 389 amino acids rather than the 312 amino acids. This modification would increase to 62% the identity to the Tpase of IS*801*, another member of the family. The putative IS*91* Tpase exhibits 36% identity to that of IS*801*. One notable feature of several members is that while their 3′ ends are relatively constant, there is significant variation in their lengths at the 5′ end, excluding a well-conserved terminal 20 to 25 bp. This may be related to their particular transposition mechanism, discussed below.

IS*91* carries short (7- to 8-bp) imperfect terminal IRs, inserts into a rather specific tetranucleotide sequence (GAAC and CAAG), and does not generate direct target repeats on insertion (233). An alignment of the ends of IS*91* with those of IS*801* and IS*1294* shows highly conserved regions at both the left and right ends (Fig. 15B) and suggests that they also use the same target sequence (235). They carry a long open reading frame whose potential products have significant similarities and are related to a family of replication proteins of bacterio-

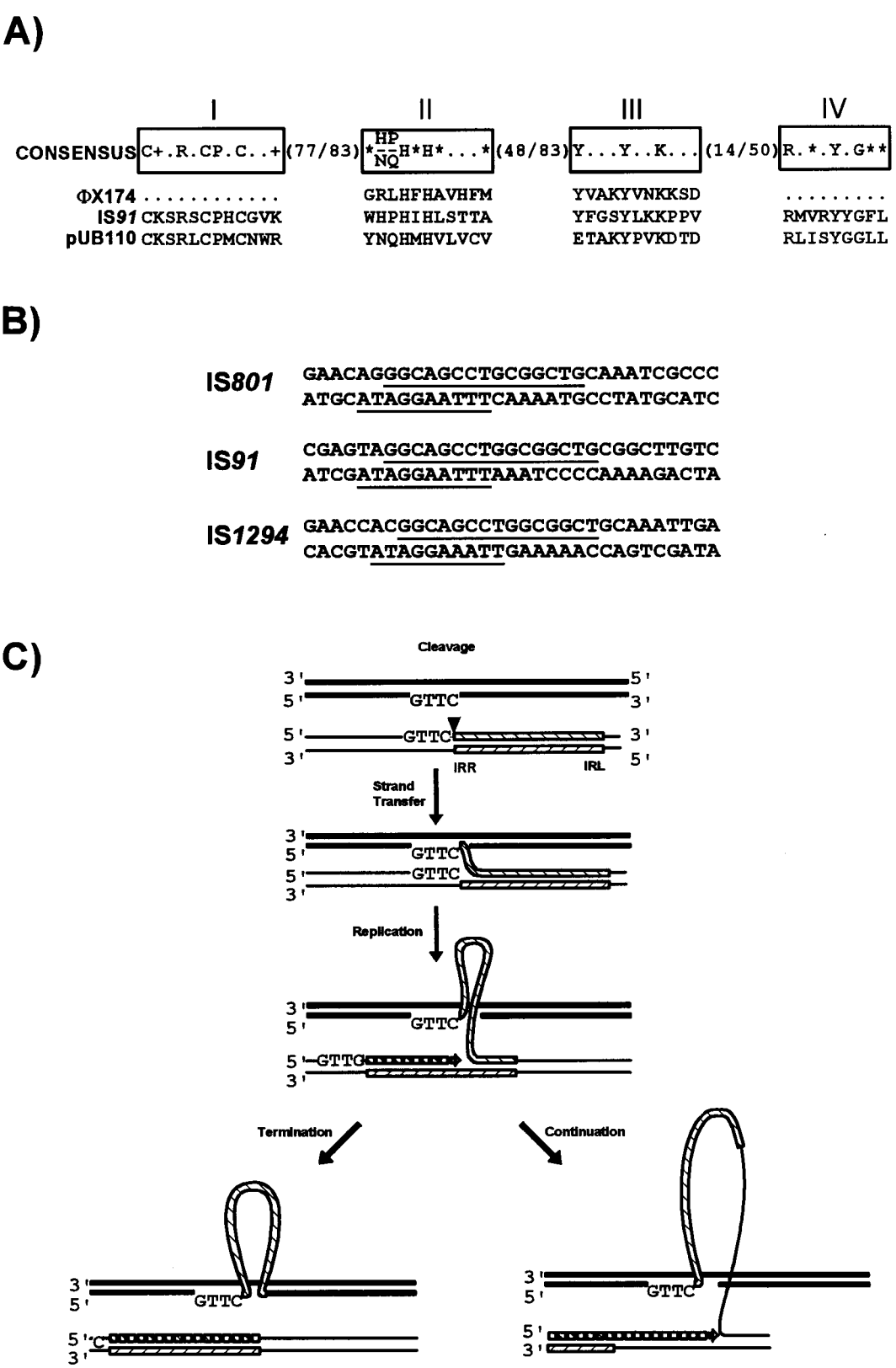


FIG. 15. IS*91* family. (A) Comparison of the primary Tpase sequence with related single-strand replicases. The four conserved regions are boxed and labelled I to IV. They are separated by various numbers of nonconserved amino acids as indicated. In addition to the standard one-letter amino acid code, + and * represent basic and hydrophobic amino acids, respectively. IS*91* is compared to bacteriophage ϕX174 and plasmid pUB110 replication proteins. (B) Transposon ends. Highly conserved sequences within the termini are underlined. The upper sequence in each pair represents the left end, and the lower sequence represents the right end. (C) Proposed rolling-circle mechanism for IS*91* transposition. IS*91* is shown as a hatched box with left and right termini, vector DNA is shown as a fine line, and target DNA is shown as a heavy line. Initial cleavage (vertical arrowhead) occurs at IRR and is followed by strand transfer to the conserved target sequence. Replication of the displaced strand in the donor DNA then takes place with priming from the liberated 3′ donor end. The left-hand pathway shows the result of correct cleavage and termination at the right extremity of the element. The right-hand pathway shows the result of progression through the termination signal and continuation into neighboring DNA of the donor molecule.

phages and small gram-positive plasmids which propagate by a rolling-circle replication mechanism (155, 234). The similarity is localised to a consensus of four motifs (Fig. 15A). These Tpases are more closely related to the ϕX174 gpA protein than to the plasmid Rep protein family, since they carry two tyrosine residues in motif III. In ϕX174, they are directly involved in catalyzing strand cleavage and transfer (136).

IS*91*. In addition to suggestions derived from protein homologies, experimental evidence has been obtained which strongly suggests that IS*91* transposition occurs by a polarized rolling-circle replicative mechanism (Fig. 15C) (232) similar to that proposed by Galas and Chandler (106). This conclusion is based on the observations that 81 bp at the right end of the element, including the terminal IRR, is sufficient for transposition and that the terminal IRL is dispensable. The target sequence GAAC or CAAG abutting IRR is also essential for further transposition. The products of "one-ended" transposition (in the absence of IRL) occur at high frequency, and all carry a constant end defined by IRR and a variable end defined by a copy of the target consensus located in the donor plasmid. Moreover, there is a high level of multiple tandem insertions, as though the "transposition-replication" apparatus recognizes the donor "target" consensus inefficiently and continues a second round. In the model proposed by de la Cruz and collaborators (232) and illustrated in Fig. 15C, donor strand cleavage results in a covalent complex between the 5′ IRR end and Tpase. This is followed by single-strand transfer into the target DNA at a site containing a consensus tetranucleotide. The attached single strand of the IS is displaced by replication in the donor molecule. Termination is triggered when the complex reaches either the 3′ IRL end or a tetranucleotide consensus sequence in the donor. The model does not directly address second-strand synthesis of the transferred IS, which could occur simply by replication of the recipient replicon. A second member of this family, IS*1294*, appears to promote natural one-ended transposition (64).

IS*110* Family

The IS*110* family is somewhat disparate. It contains 20 members (of which 7 are isoforms) (Fig. 16) varying between 1,136 and 1,558 bp, with most clustered in the 1,450-bp range. All family members have no or very small (<7-bp) IRs and do not generally create direct target repeats (IS*492* might generate 5-bp repeats [19]). Little overall similarity can be detected between the ends. A single long, relatively well conserved reading frame is present and shows some groups of identity within the N- and C-terminal portions. These elements share a highly conserved tetrad motif with reverse transcriptase (142), whose significance is at present unknown. Unlike IS*91*-related elements, no obvious rolling-circle replication signature is present in the predicted protein. The inclusion in this family of two additional elements, the *Streptomyces coelicolor* minicircle IS*117* (140) and a *Moraxella bovis* (and *M. lacunata*) DNA inversion system determining pilus synthesis (Piv), is based on significant similarities in their putative recombinase proteins (205). It should be noted that this family of recombinases is not related to the site-specific recombinases of the λ integrase or resolvase/invertase families.

Little information is available concerning the mechanism of transposition of these elements. However, *S. coelicolor* IS*117* was initially demonstrated in a circular form and integrates at a frequency 2 orders of magnitude higher than when cloned as a linear copy (141). In recent studies with IS*492* (259a) and IS*900* (231a), DNA fragments carrying abutted IS ends have been detected in vivo. Their appearance is dependent on an intact Tpase gene, and their nucleotide sequence is consistent with the formation of a circular form of the element (although it is also consistent with the presence of a head-to-tail dimer). Moreover, the junction of IS*492*, like several other ISs, appears to form a strong promoter in *E. coli* (89b). For both IS*117* (141) and IS*900* (231a), the target sequences exhibit some similarities to the circle junction, suggesting that insertion may occur by a site-specific recombination.

Finally, although little data is available concerning enzymatic activities of the putative Tpases of this family of elements, IS*900* Tpase has been detected by immunological methods in the *Mycobacterium paratuberculosis* host (335) and IS*492* Tpase has recently been purified and exhibits DNA cleavage activity specific for the ends of the element (259a).

IS*200*/IS*605* Group

Although IS*200* has been described as an autonomous element, it is often associated with other open reading frames in the form of a composite. This was first observed with the IS*605* element from *Helicobacter pylori*. In an attempt to group these elements to facilitate searches in the database, we have elected to refer the ensemble as the IS*605* group or family.

General features of IS*200*. IS*200* was originally detected in *Salmonella typhimurium* (194), but related elements have since been found in a variety of bacterial species including certain *Shigella* (112), *Clostridium* (44), and *Streptococcus pneumoniae* (60a). To date, 16 members of the family and 9 isoelements have been found. The most recent sequence determination (32) indicates that it is 707 bp long (and not 708 bp as previously described [113]), with a single long open reading frame preceded by several sequences capable of forming hairpin structures (Fig. 17A). One of these, which is located within 20 bp of the end, is thought to act as a transcriptional terminator and to prevent impinging transcription from activating the element (195), while another may sequester the ribosome binding site of the Tpase gene (32). The predicted product of its single open reading frame exhibits some similarities to that of open reading frame 1 (*orf1*) of *Halobacterium* ISH*1-8*. IS*200* does not carry terminal IRs. Despite much effort, few cases of IS*200* transposition or other forms of rearrangement have been documented (127).

IS*200* as a complex: the IS*605* family? In the few examples of IS*200* insertions in the *S. typhimurium* genome where the sequence of the unoccupied target site is also known, it seems clear that transposition resulted from a simple insertion of IS*200* alone (32, 47, 256). On the other hand, in a significant number of IS*200* isolates from other species, most of which were defined during sequence analysis of virulence determinants in the respective hosts, the Tpase open reading frame (*orf1* in Fig. 17B) has been found close to open reading frames associated with other presumed IS elements (*orf2* in Fig. 17B). This may simply reflect insertion preferences of one or another of the ISs. However, in *Helicobacter pylori*, an IS*200*-like Tpase, *orf1*, is located next to (and is transcribed in a divergent manner from) a second reading frame, *orf2*, which is related to the putative Tpase of IS*1341*. This unit, called IS*605*, is found repeated several times in the genome, and the two reading frames are always associated (25a, 337). A similar arrangement is found in IS*1253*A from *Dichelobacter nodosus* (36), where *orf2* is related to the putative Tpase of IS*1136*. In the *Halobacterium* element ISH*1-8*, *orf2* is related to IS*891*, although in this case both open reading frames are transcribed in the same direction (Fig. 17C). In most isolates describing the individual IS elements (IS*891*, IS*1136*, etc.), as well as for the majority of "individual" IS*200* derivatives identified, insufficient flanking-

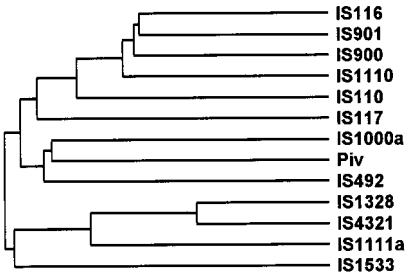


FIG. 16. IS*110* family. Only the dendrogram based on Tpase alignments is shown.

nucleotide sequence information is available to determine whether similar associations of open reading frames occur. It should be noted that for convenience, the entire complex is referred to as the IS*605* family in Table 1.

Another interesting taxonomic characteristic is that the different *orf2* frames have N-terminal and C-terminal domains which are interrelated, albeit distantly (Fig. 17D) (36), and could themselves be considered a group. As illustrated in Fig. 17D, the N-terminal portions of the single open reading frames of ISSs*1*, IS*1136*A, and IS*1341* and the N-terminal ends of *orf2* of IS*605* and IS*1253*A (as well as an entire open reading frame, *orfE*, located on a *Salmonella* virulence plasmid, [125]) have similarities, as do all the C-terminal domains (including that of IS*891*) and the entire *orf2* of ISH*1-8*. These relationships require further exploration.

IS*256* Family

A total of 33 members of the IS*256* family, including 2 isoforms, have been identified from a wide range of organisms. They are 1,298 bp (ISRm*3*) to 1,486 bp (IS*6120*) long. IS*256* was originally isolated as a component of the compound transposon Tn*4001* (218). Members carry related terminal IRs of between 24 and 41 bp (Fig. 18B), and most generate 8-bp direct target repeats but some 9-bp duplications have been observed. Interestingly, they also appear to carry internal IRs close to the ends (123, 260). A single long open reading frame carrying a potential DDE motif with a spacing of 112 residues between the second D and E residues together with a correctly placed K/R residue (Fig. 3) is present in most members, although the closely related IST2 (*Thiobacillus ferrooxidans*) and IS*6120* (*Mycobacterium smegmatis*) appear to encode two open reading frames and may represent members of a distinct subgroup (124) (Fig. 18A). However, the intervening putative amino acid sequence contains residues conserved in the single open reading of other members of the family. For example, in IST*2*, a single change of phase extends the N-terminal region of the longer open reading frame by 140 amino acids, which contains additional identities to other members of the family. It has been suggested that translational frameshifting may occur to generate a single transframe Tpase (124). This subgroup also includes most cases of 9-bp target repeats. For IS*285*, which has similarities to ISRm*3*, optimization of the alignment between the two Tpases leads us to propose the presence of a single open reading frame. Three frame changes are sufficient to generate the single reading frame, which then shows extensive identity (44%) to ISRm*3*. The actual configuration of the open reading frames in these cases requires experimental clarification. The overall similarity between the majority of members of the family (Fig. 18) is between 22 and 48%. IS*931* forms an outgroup, while IS*1249* shows similarity in a subdomain of

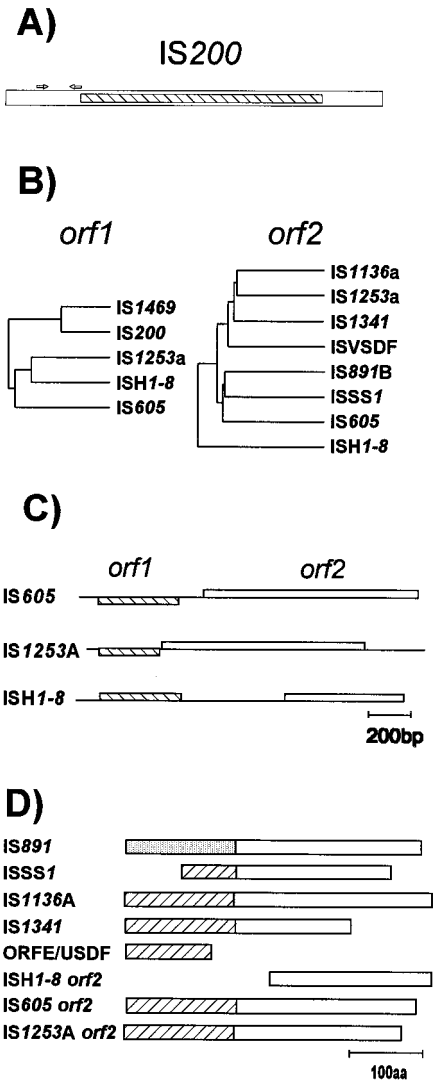


FIG. 17. IS*200* complex. (A) Organization of IS*200*. Short IRs (open arrows) are shown at the left end, and the relative position of the potential open reading frame (hatched box) is indicated. (B) Dendrogram of IS*200* family Tpases, *orf1* (left) and the associated *orf2* reading frames (right). (C) Relative localization of *orf1* and *orf2* in selected examples. The convention for the orientation of each reading frame is that frames shown above the line are transcribed to the right while those below the line are transcribed to the left. (D) Relationship between various examples of *orf2* and other IS elements. aa, amino acids.

the putative Tpase and also has somewhat related terminal IRs. The other members form three deep branching groups, each showing between 30 and 40% similarity. No striking statistical conservation in target sequences can be deduced from the limited number of insertions analyzed, and little is known about the mechanism of transposition of this family.

An interesting feature of this family is that it may also contain eukaryote homologs. The putative MurA gene product of the autonomous mutator element of *Zea mays*, MuDR (see "MuDR" below) exhibits some weak similarities to the Tpases of this group (91). MuDR generates 9-bp target repeats.

IS*630* Family

The IS*630* family is composed of 12 members, of which 1 is an isoform and 1 has been only partially sequenced. They are

interesting from several points of view, not the least being significant similarities to eukaryote "insertion sequences," such as Tc*1* and Tc*3* of the nematode *Caenorhabditis elegans* and the *mariner* family (366) (Fig. 3). They are between 1,100 and 1,200 bp long and show a high target specificity. Since the conserved target sequence appears to be duplicated at each end of the element, it is formally possible that one or other copy is an integral part of a terminal IR of the IS. This arrangement therefore introduces an ambiguity not only in determining the exact tips of the IS but also in assessing the number of duplicated target base pairs (see "IS*5* family" above).

Detailed studies concerning the target DNA sequence have been carried out for IS*630* (333). The target sequence was determined before and after insertion, and the results suggested that insertion generated a duplication of an invariant target TA dinucleotide. That this dinucleotide does not form an integral part of the IS was investigated by site-specific mutagenesis of the transposon donor to eliminate the terminal TA. Transposition of the IS from the mutated donor molecule resulted in insertions which all exhibited a flanking TA DR. This clearly demonstrates that insertion results in the duplication of the central TA dinucleotide (333). Further analysis demonstrated that IS*630* exhibits a strong preference for a 5′-CTAG-3′ target sequence (334). Moreover, point mutation of the CTAG target sites reduced or eliminated their attractiveness as insertion hot spots.

None of the other insertion sequences of this family have been analyzed in sufficient detail to determine unambiguously the exact tips of the element and the number of target base pairs duplicated. All known insertions of these elements are consistent with a TA duplication (Fig. 19B). For two insertions of the *Agrobacterium vitis* element IS*870*, it was possible to determine the target sequence prior to insertion. This sequence was found to carry a single CTAG copy (103), an observation which remains consistent with a simple TA dinucleotide target duplication. For coherence, we have therefore assumed that all members of this family generate an identical (TA) target duplication and have therefore defined the tips of the elements accordingly (Fig. 19B).

While IS*895* appears to contain two consecutive open reading frames (7), the other members of the family all carry a single long reading frame. Three elements (IS*870*, ISAr*1*, and ISRf*1*) show more than 70% identity, two (IS*1066* and ISRj*1*) show about 50% identity to each other and 40% to the other members, and two show less than 20% identity to each other and to the other members. It is interesting that the three elements most closely related on the basis of Tpase similarities (IS*870*, ISRf*1*, and ISAr*1*) appear not to carry translation termination codons for the Tpase gene. However, insertion into the specific target site, CTAG, with concomitant duplication (of either 2 or 4 bp) generates a TAG termination codon in phase with the Tpase gene. The influence of this arrangement on the transposition of the IS elements has yet to be determined.

Very little is known about the transposition properties of these elements. In IS*630*, while direct insertions can be observed at reasonable frequencies, no cointegrates could be detected (333).

IS*982* Family

The IS*982* family is composed of four members and two isoelements, for which the entire nucleotide sequence is known (Fig. 20A). It is restricted at present to gram-positive bacteria. The family members are between 962 and 1,026 bp long and carry similar terminal IRs of between 18 and 35 bp (Fig. 20B).

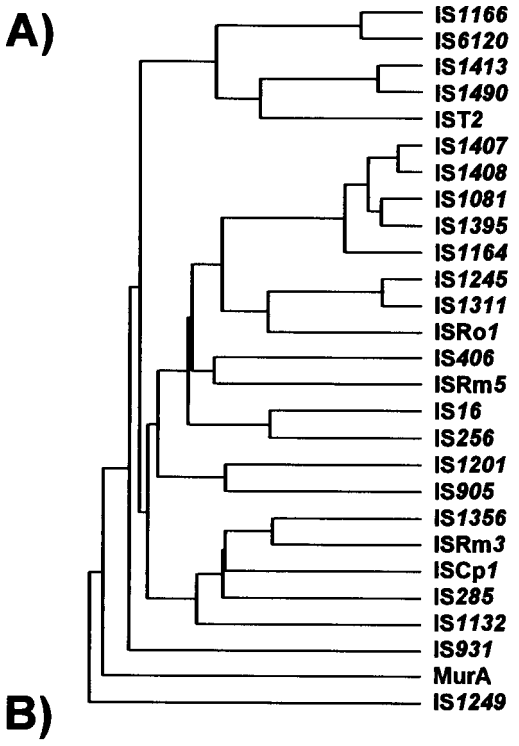


FIG. 18. IS*256* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

FIG. 19. IS*630* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

Their termini are closely related and have been defined by comparison of different individual copies (Fig. 20). As for several other IS elements, IS*982*B from *Lactococcus lactis* has been shown to provide a −35 hexamer in its right terminal IR which is capable of forming a hybrid promoter with a resident −10 sequence located at the target site (214). Family members contain a single open reading frame capable of encoding a protein of between 271 and 296 amino acids; the proteins exhibit between 33 and 44% amino acid identity among the family members. A possible DDE motif is also apparent (Fig. 3) but lacks the conserved K/R residue. Little is known about the transposition of these elements.

IS*1380* Family

Six members of the IS*1380* family (345) have been identified, of which two are isoforms (Fig. 21A). They are between 1,665 bp (IS*1380*) and 2,071 bp (ISBj*1*) long, and each carries a single long open reading frame. IS*1380* is present in extremely high copy number (approximately 100) in its host, *Acetobacter pasteurianus* (331). For ISBj*1*, a simple phase change around coordinate 1365 results in the extension of the potential Tpase

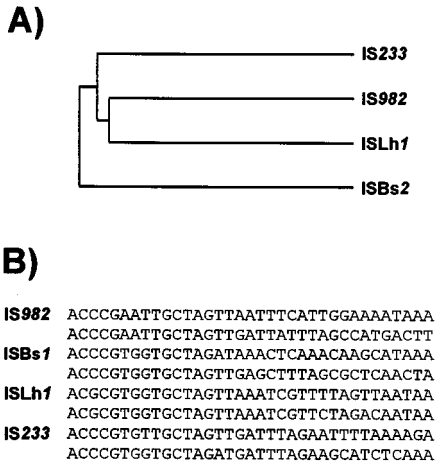


FIG. 20. IS*982* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

open reading frame from 328 to 454 amino acids (similar to that of the other group members) and significantly increases the quality of the alignment. The family members exhibit between 30 and 50% amino acid identity in the potential Tpase. In addition, ISBj*1* carries a 112-bp duplication in its right end. The terminal IRs are related (Fig. 21B) and are approximately 15 bp long. Insertion appears to generate a target duplication of 4 bp. Note that the external sequence of ISBj*1* is also consistent with a 4-bp duplication.

ISAs*1* Family

The ISAs*1* family is composed of 12 members, of which 6 are isoforms, restricted at present to gram-negative bacteria. They are 1,207 bp (ISPg*1*) to 1,326 bp (IS*1358*) long and generally carry terminal IRs of between 14 and 22 bp (Fig. 22) (although several of the IRs remain to be defined). A single open reading frame of between 294 and 376 amino acids occupies almost the entire length and shows between 26 and 50% identity. The putative Tpase of IS*1358* has been visualized with a phage T7 promoter-driven derivative (327), and that of ISAs*1* has been detected in *E. coli* minicells (126).

The family also includes the "H-repeats." These form part of several so-called Rhs (rearrangement hot spot) elements (143, 379). *E. coli* K-12 contains five large repetitions of this type (RhsA to RhsE, scattered around the chromosome with lengths of between 3.7 and 9.6 kb). They represent nearly 1% of the chromosome and provide homology for RecA-dependent rearrangements. These elements are present in many but not all wild-type isolates of *E. coli*. The most prominent Rhs component is a giant core open reading frame, whose features are suggestive of a cell wall surface ligand binding protein. Each Rhs element also contains another repeated sequence, the H-rpt element (*Hin*c repeat), displaying features of typical insertion sequences, although no transposition activity has yet been detected. For the sake of clarity, the H-rpt DNA sequences B (RhsB), C1 to C3 (RhsC), E (RhsE), and min.5 (381), as well as H-rptF (379), have been renamed ISEc*1* to ISEc*7*, respectively (Table 1). It is interesting that other members of the family (IS*1358*, ISAs*1*, and ISSe*1*) have been found associated with cell surface component genes in their respective hosts. Little is known about the transposition properties of this family of elements. However, recent experiments with the *Vibrio cholerae* element IS*1358* have demonstrated that inser-

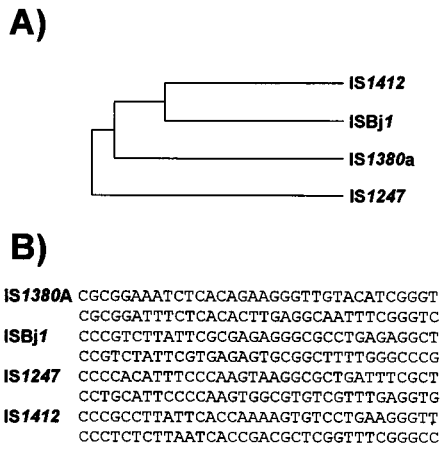


FIG. 21. IS*1380* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

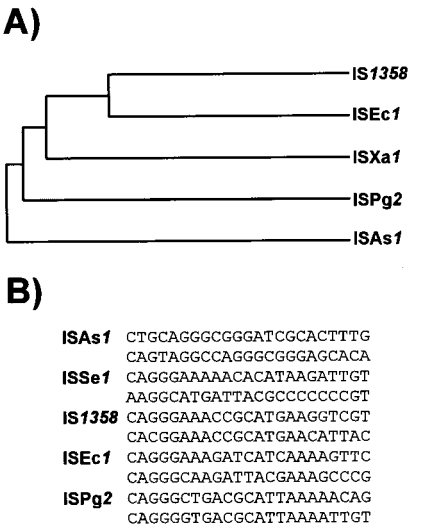


FIG. 22. ISAs*1* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

tion generates 10-bp DRs and that in *E. coli*, it undergoes simple insertion into a target plasmid, pOX38 (89a).

ISL*3* Family

The ISL*3* family is presently composed of 21 known members, of which 3 are isoforms. Except for ISCx*1*, for which only a partial sequence is available, and IS*1069*, which is significantly larger, they range in size from 1,186 bp (ISSg*1*) to 1,553 bp (IS*1165*). They carry IRs of between 15 and 39 bp, which are closely related (Fig. 23) and generate a direct target repeat of 8 bp. One long open reading frame is present which encodes potential proteins of roughly 400 to 440 amino acids, showing good alignment, particularly in the C-terminal half. IS*1096* harbors two open reading frames. The upstream open reading frame exhibits similarities to the ISL*3* family Tpases. The second, *tnpR*, is related to open reading frames from *Agrobacterium rhizogenes* and *Rhizobium* plasmids. In IS*1167*, the single reading frame appears to be distributed between two consecutive open reading frames with a potential for translational coupling suggested by overlapping initiation and termination codons (ATGA). For analysis purposes, we have considered only one fused frame. The elements fall into three deeply branching groups with about 35, 30, and 25 to 60% identities, respectively (Fig. 23). Small sequences (130 to 340 bp) related to the IS*1167* IRs have been detected in *Streptococcus sanguis*, *S. pneumoniae*, and *S. agalactiae* (8, 383). No obvious target sequence specificity has yet been observed, although there is some suggestion that there may be a preference for AT-rich regions.

Transposition of most of these elements has been demonstrated, but no analysis of their transposition mechanism has appeared. In one case, IS*31831*, derivatives of which have been used in mutagenesis, the majority of insertions were found to be simple and only rare clones carried accompanying vector DNA (350).

Unclassified Elements

Of the 500 ISs in the database, 54 remain to be classified. These are either elements whose nucleotide sequence is entirely unknown or with limited sequence information insufficient for allocation (33 examples) or elements whose entire nucleotide sequence is known but which show no marked relationships with more than one other element (21 ISs of which 5 are isoforms).

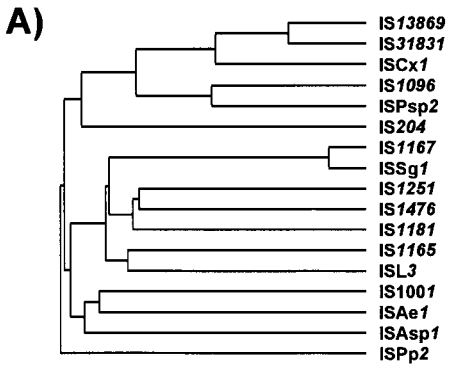
EUKARYOTIC INSERTION SEQUENCES

In addition to the large number of bacterial IS elements, a significant number of eukaryotic elements have been documented. The largest class described, and arguably the best characterized, is the *mariner*/Tc family, originally observed in *Drosophila mauritiana* (161) and *C. elegans* (92). These elements are structurally the closest to bacterial ISs. A second type of element which has also received extensive attention is the P element of *D. melanogaster*. A distinguishing character of this element is that Tpase binds to a subterminal region rather than to the terminal IRs. Binding of Tpase to subterminal motifs rather than to the terminal IRs is also a characteristic of two other groups of eukaryotic elements whose lengths exceed 4 kb: the *CACTA* family (restricted at present to plants), and the Ac family (with members in both plants and insects). Two characteristics of several eukaryotic elements which were thought to distinguish them from elements in eubacteria and archaebacteria are the presence of large numbers of defective copies and the facility with which an intact copy in the same genome can provide functions in *trans* for their mobilization (for reviews, see references 25, 93, and 190). However, studies of certain archaebacterial elements (146) and the accumulating data from bacterial genome projects suggest that multiple copies of inactive elements may not be limited to eukaryotes.

mariner/Tc Family

The presence of such elements has been extensively examined primarily in insect species (294), although they are found in many phyla including vertebrates, arthropods, ciliates, and fungi (reference 261 and references therein). The elements have been detected by using a specific PCR probe which exploits the presence of a highly conserved Tpase sequence.

The *mariner* family includes the Tc elements originally detected in *C. elegans* (92). These have been the subject of extensive analysis. Alignment of their Tpases (87) and their target specificity suggest that they are related to the IS*630* family of bacterial elements. Tc*1*, Tc*3*, and *mariner* show significant similarities (Fig. 3). Moreover, the insertion specificity of both Tc*1* and Tc*3* is strict. Analysis of 204 Tc*1* and 166 Tc*3* insertions indicated that all occurred into a TA dinucleotide which



B)

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IS13869 GGCTCTTCCGTTTTTAGAGTGCATTGTGGGTGTGGCAGA
        GGCTCTTCCGTTTTTAGAGTGCATTGATTTTGTGGGTGA
IS31831 GGCCCTTCCGGTTTTGGGGTACATCACAGAACCTGGGCT
        GGCTCTTCCTGTTTTAGAGTGCATTGATCTTATGGACCA
IS1096  GGCTCTTCGCAGTTGAGGGTGTAGAGGTCGTCGGCGCGT
        GGCTCTTCGCACTTGACGGTGTAGAGACGATCAGCTGCT
ISPsp2  GGCTCTTCGCATTTAAGGGTGTAGAAAAAATCTGACCGG
        GGCTCTTCGCATTTAAGGGTGTAGCTGATTTCTAAACCC
IS204   GGCTCTTCGCACTTGGTAGGGGCTGGTGCCGATCGACCC
        GGCTCTTCGCAGTTAGCAGTGGCATTGGCGCCGGCTGGG
IS1167  GGTTCTATAATATTTGTAGTGGGTAAATCCCCTATGGAT
        GGCTCTTTGTCAACTGTAGTGGGTTGAAAAAAAGCTAAG
ISSg1   GGCTCTGTAATATTTGTAGTGGGTAAATCCCCTATAGAT
        GGCTCATTGTCAACTGTAGTGGGTTGAAGAAAAGCTAAG
IS1251  GGCTCTATACTTTTTCGATGTTGGTGGATGACACCAGCA
        GGCTCTTTGTCAAATGATGTTGGTAAAGAGAAATCTTAG
IS1476  GGTTCTATAATATTTTGTGTTGGTGGAAAATCGAAGGCG
        GGCTCTTCGTCAAATCGTGTTGGTGAAGACAAATTTGAA
IS1181  GGTTCTTCATCTTTTATGGTGGGAAGGTAAAACTTCCTG
        GGTTCTCCACCAAATGTGGTGGGTATATAATTTAAAGAA
IS1165  GGGTCTAGAATTTTTGGTGTTGGAAAGTATTTCCATTCC
        GGAAGTTTGTCAAATGGTGTTGAAGGATAGTTTCTATCC
ISL3    GGCTCTATAATTTTTTTTACTGATGGATGTCAAGTAATA
        GGCTCTTTGTCAAATCTTACTGATGCTTGTCAAGAAATC
IS1001  GGTTCATCGCGCAATAACGTGGAGGGGTTTGGCAATTTT
        GGTTCATCGCGCATTGCCGGGGAAGGCGGCACGGATTTT
ISAe1   GCCTCTTTTGAATTTCAAGTGGGTGGTATCGTGGCGGGT
        GGCTCTTTTGAAAAATGAGTGGGTTATGCGGCATGCGGG
ISAsp1  GGTTCTTCCGGTAGTTTTATGGTGTTTATTAGAATTGAC
        GGTTCTTCAGTTAATTTTATGGAGAATTAATAGTAAAAT
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FIG. 23. ISL*3* family. (A) Dendrogram based on Tpase alignments. (B) Terminal IRs.

was duplicated during the insertion event (349), a target choice which is identical to that of IS*630*.

Both Tc*1* and the related Tc*3* element carry a single transposase gene, *tc1*A and *tc3*A, respectively, each interrupted by one intron at a different position. Tc*1* is 1,610 bp long and carries terminal IR sequences of 54 bp, while Tc*3* is 2,335 bp long with 462-bp IRs. The reason for this large difference in IR length is unknown. The IR sequences are recognized and bound by their cognate Tpases in vitro (62, 353). This is consistent with the limited conservation of nucleotides between the IRs of each element. Moreover, it has been shown that Tpase induction in vivo from a heat shock promoter leads to high levels of transposition of the cognate element (347, 353). Tc*1*A and Tc*3*A also carry the DD(35)E motif (87) (Fig. 3). Point mutations in these residues render the Tc*3* enzyme inactive in vivo (348). As in several bacterial Tpases, a sequence-specific DNA binding domain is located at the N-terminal end of both Tpases. It is followed by a region which overlaps the DD(35)E region and binds DNA nonspecifically. Interestingly, the N-terminal domain of Tc*1*A shows similarities and identities over a 40-amino-acid stretch to the N-terminal region of the IS*30* Tpase, which is itself sufficient for binding to IS*30* IRs. Also in the case of Tc*1*A, band shift assays, footprinting studies, and methylation interference studies have demonstrated a binding site between nucleotides 5 and 26 of the IR. Nucleotides 1 to 4 are not necessary for binding (353). This observation is consistent with the notion that the Tc*1* IR sequences are organized into a two-domain structure similar to that of several bacterial elements (Fig. 1). The binding site can be further subdivided into two distinct regions: one closer to the tip, which is contacted by the specific N-terminal Tpase domain, and one which is contacted by the nonspecific DNA binding domain (352). A leucine zipper motif has also been detected in the Tpases of certain members of this group (160). As in the IS*3* family, this may represent a multimerization domain, although recent structural studies with a truncated transposase derivative indicated that this region did not assume a coiled-coil motif (90).

Purified Tc*1*A has been demonstrated to have both endonuclease and phosphoryltransferase activities in vitro (Fig. 24). The major endonucleolytic activity detected was at the 5′ ends rather than, as might have been expected based on the activities of other Tpases (352), at the 3′ ends of the element. Moreover, cleavage was shown to occur specifically at the phosphodiester bond between the second and third nucleotide within the transposon. Phosphotransferase (strand transfer) activity was also observed in a "disintegration" assay.

Transposon circles have been detected in vivo (92, 275, 296), but their role as possible intermediates in transposition is not entirely clear. Tc*1* and Tc*3* appear to transpose by a cut-and-paste mechanism. Induction of Tc*3*A in vivo results in excision of Tc*3* as a linear form. One interesting characteristic of these molecules is that while the cleavage at the 3′ ends of the element occurs precisely between the transposon and its flanking sequences, 5′ cleavage occurs 2 nucleotides within the transposon in a manner similar to that observed in vitro (348). The result of these cleavages is that 2 nucleotides of each transposon end remain behind in the donor site, leaving a "footprint": TACATA (where the flanking TA dinucleotide is that generated by duplication during the original integration) (Fig. 24). It has been noted by Plasterk (261) that a related footprint observed for Mos*1* (TACCATA) suggests that excision of these elements occurs with a 3-nucleotide stagger.

Recently, systems which support Tc*1* and *mariner* transposition in vitro have been described (196, 353). In both cases, it has been demonstrated that the Tpase is the only protein required for transposition. For Tc*1*, double-strand cleavage was observed not only at each end of an element carried on a supercoiled plasmid donor molecule but also on molecules carrying a single end. Excision, but not cleavage of a single end, appeared somewhat reduced when the donor plasmid was linearized suggesting that supercoiling stimulates the two-ended reactions. The cleaved ends were shown to carry a 2-base 3′ overhang, as shown in Fig. 24. Intermolecular transposition was demonstrated by using a genetic assay, and the resulting products showed a similar target specificity to those analyzed in vivo. For *mariner* (*Himar1* from the hornfly, *Haematobia irritans*), the purified Tpase was demonstrated to generate a footprint in the terminal IR and to promote double-strand cleavage similar to that found with Tc*1* but with a 3-base 3′ overhang (196).

P Element

The P element of *D. melanogaster* was discovered by its role in the syndrome known as hybrid dysgenesis (see, for example, reference 177). This syndrome is characterized by a loss of

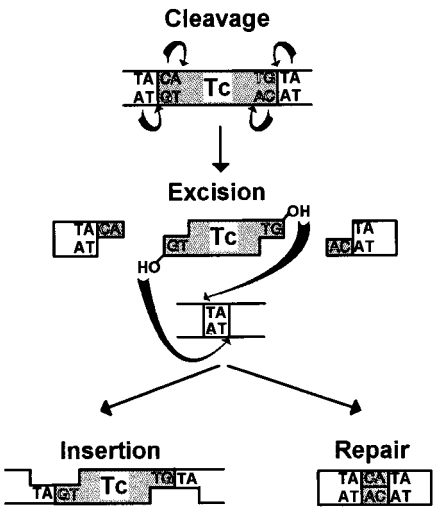

FIG. 24. Tc and *mariner* elements. Tpase-mediated cleavage at the terminal 3′ phosphodiester bond and at 2 nucleotides within the 5′ ends is indicated by curved arrows. Excision of the element (grey box) leaves two 2-nucleotide 3′ extensions in the vector backbone (open flanking box). Insertion is thought to occur by simple nucleophilic attack by the free 3′OH groups of a specific TA target dinucleotide, as shown by the long curved arrows. Repair of the donor backbone will leave a 2-bp insertion (footprint) compared to the original target sequence. This must proceed via the formation of a mismatch joint presumably followed by repair or replication to resolve this mismatch.

fertility in a cross between males which carry an autonomous P element and females which do not; it is due to high level P transposition in germ line cells. Tpase expression requires correct splicing of an mRNA which contains several introns. In particular, removal of one intron (intron 2-3) occurs only in the germ line, limiting transposition activity to these cells (see reference 93 for review). The element has been used extensively in mutagenesis of its host organism by using specifically tailored P derivatives. Like Tc*1*, Tc*3*, and *mariner*, it transposes by a cut-and-paste mechanism, and together with these, it is one of the best-characterized eukaryotic elements. It is 2.9 kb long, generates 8-bp target repeats on insertion, and specifies an 87-kDa Tpase produced from a single open reading frame which carries three introns. Transposition requires approximately 150 bp at each end, including 31-bp terminal IRs (248). Tpase does not bind to these, however, but instead recognizes neighboring internal sites (173). The terminal IRs are bound by a host protein which has been reported to show homology to KU (174), a protein also implicated in double-strand break repair and V(D)J recombination (297). Transposase binding to the "left" end represses Tpase expression (174). In addition, two types of repressor molecule have been detected, presumably encoded by defective (nonautonomous) elements (115). The first is an approximately 66-kDa derivative truncated at the C-terminal end of the Tpase. The second, KP, is a 207-amino-acid protein produced from an internally deleted element. KP forms dimers and, unlike the full-length Tpase, binds to multiple sites at the ends of P (202). A DNA binding domain has been localized to the N-terminal 98 amino acids. A leucine zipper motif has also been detected and has been shown to be important for KP activity in vivo (10) and to be necessary for dimerization in vitro (202).

In addition to Tpase binding, two other characteristics distinguish P from the elements discussed above. Studies with an in vitro system have shown that the Tpase requires GTP as a cofactor, although nonhydrolyzable analogs are also effective (175). Interestingly, a single-amino-acid change has recently been shown to switch cofactor specificity from GTP to XTP in vivo (246). The second characteristic concerns the nature of the double-strand Tpase-induced cleavages at each end. While 3′ cleavage occurs at the terminal nucleotide, 5′ cleavage occurs 17 bp within the element (20). This position, within the terminal IRs, is directly adjacent to the host factor binding site. The in vitro system has also revealed that both the left and right ends are required for cleavage activity, suggesting that prior formation of a synaptic complex is required.

Other Families

***CACTA* family.** The *CACTA* family (114) is named according to the nucleotide sequence of the highly conserved terminal IRs of its members. This group is currently restricted to plants and includes En/Spm and some Tam elements (Tam*1*, Tam*2*, Tam*4*, Tam*7*, Tam*8*, Tam*9*). Full-length members are large (>4 kb), carry nearly identical terminal IRs of 13 bp, and generate target duplications of 3 bp. Transposition requires both the left ~180 bp and the right ~300 bp. The En element produces two proteins by relatively complex differential splicing: TNPA (67 kDa) and TNPD (131 kDa). Both are required for transposition. TNPA binds a reiterated subterminal 12-bp motif but not the terminal IRs. The equivalents of TNPA from different members of this group do not appear to exhibit significant identities in primary sequence. This would be consistent with a role in binding to the element-specific reiterative repeats. Both DNA binding and multimerization domains have been localized for TNPA of En (340). On the other hand, the second and larger protein species shows significant identity (45% between the TNP2 proteins of Tam*1* and TNPD), and for this reason it has been proposed that it binds the related terminal IRs of the elements and catalyzes cleavage.

Ac. The organization of the Ac element (190) of maize is similar to that of Tam *3*, P, and hobo. It is 4,565 bp long and carries 11-bp imperfect terminal IRs and numerous reiterated subterminal 6-bp motifs. Insertion results in an 8-bp target duplication. A single open reading frame with four introns specifies a 112-kDa Tpase. Approximately 240 bp is required at each end for efficient transposition, and the frequency decreases as this is reduced to ~100 bp. Both left and right ends are required. Like those of P and En, the Tpase binds to the subterminal repeats, probably in a cooperative manner using a domain located in the C-terminal end of the protein. It binds with significantly lower affinity to the terminal IRs by using a domain located in the N-terminal end (21). The C-terminal 600-amino-acid sequence shows homology to the putative Tpases of Tam*3* and hobo, family members from plant and insect species, respectively.

MuDR. The MuDR transposon (25), the mutator element of maize, is highly active and has been used extensively to tag various maize mutants. The autonomous element is approximately 5 kb long, carries ~200-bp terminal IRs, and generates 9-bp target repeats on insertion. It contains two open reading frames transcribed in a convergent manner from promoters located in the terminal IRs and capable of specifying proteins of 207 (MURB) and 823 (MURA) amino acids. MURA is required for transposition and shows some similarities to the Tpases of the IS*256* family of elements (91). It is also capable of binding specifically to the terminal IRs of MuDR (24). Little is known about transposition of this family of elements at the molecular level.

CONCLUSIONS AND PERSPECTIVES

One of the major objectives of this review was to assemble available information concerning bacterial insertion sequences into a coherent framework. This has proved a daunting task. The various families defined here provide a starting point for the classification of these relatively simple transposable elements. It is clear, however, that this scheme will have to be updated and extended as additional ISs are isolated and characterized. Further examples are beginning to accumulate at increasing rates as a result of efforts in small-genome sequencing. The recently published nucleotide sequence of the *Rhizobium* sp. strain NGR234 megaplasmid, pNGR234a (536 kbp) (104), for example, alone includes between 30 and 40 potential ISs, and a large number have been identified in other genomes such as that of *Mycobacterium tuberculosis* (60b). The amount of information which is presented here represents only a fraction of that included in our IS database. The entire database is available at http://pc4.sisc.ucl.ac.be/is.html with appropriate links to the international sequence databases.

Although we have provided dendrograms for many of the families described here, these do not imply any particular phylogenetic relationship. The collection of sequences in this database will provide a useful starting point for phylogenetic analyses.

Structuring the known ISs into 17 families underscores the immense diversity of these elements. However, one characteristic which encompasses many of the IS families, including the majority of known IS elements, is a real or potential DDE motif. This has been demonstrated to be mechanistically important in retroviral, phage Mu, Tn*7*, IS*10*, and Tc*1* and Tc*3* transposition. It will therefore be of considerable interest to confirm the relevance of this catalytic triad in at least some additional families and to determine the spatial relationship of the residues within the three-dimensional structure. On the other hand, although the DDE motif appears to be a common theme in many of the elements described here, implying a shared chemistry, the detailed ways in which elements of each family assemble the individual cleavage and strand transfer reactions can vary and result in important differences in overall mechanism. Thus, certain elements with DDE chemistry generate replicon fusions exclusively (Mu and IS*6*) while others are excised from their donor molecule before forming a synaptic complex with the target molecule (IS*10*, IS*50*, and IS*911*) and yet others appear to require the presence of a target site prior to donor strand cleavage (Tn*7*). Details of second-strand cleavage also vary. Further studies of selected members of the DDE superfamily will undoubtedly uncover additional combinations of reaction steps and extend the known range of DNA transactions which contribute to genome plasticity.

Finally, while members of the DDE family represent the majority of known IS elements, a significant fraction do not. These families include IS*1*, IS*91*, and IS*110* (which are at present being actively analyzed) and IS*1380* and ISAs*1* (which to our knowledge are not). From a mechanistic and evolutionary point of view, it is important to understand how these elements may be related to bacteriophage λ integration (IS*1*), single-strand phage and plasmid replication (IS*91*), and site-specific inversion systems (IS*110*).

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