Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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REGULATING Tn*10* AND IS*10* TRANSPOSITION

TRANSPOSABLE elements are ubiquitous inhabitants of both prokaryotic and eukaryotic genomes. They may be molecular parasites, surviving only because they replicate faster than their hosts; alternatively, they may survive because they confer a selective evolutionary advantage to an individual host, to a host population, or to a particular favorable gene such as antibiotic resistance (for interesting reading see CAMPBELL 1981a, b; ORGEL and CRICK 1980; SAPIENZA and DOOLITTLE 1981; SYVANEN 1984). For bacterial transposons, at least, this final possibility seems particularly simple and attractive. The ability of a gene to spread through a population is greatly enhanced if it can transpose onto and off of transmitting DNA molecules (viruses, plasmids or transforming DNA); transposition of genetic segments in the absence of any element is exceedingly rare. Furthermore, because the transposon remains tightly linked to the favorable gene, selection for and dissemination of that gene necessarily disseminates the transposable element as well.

Regardless of the evolutionary forces involved, stable coexistence of a transposable element with its host requires that a balance be struck between too little transposition and too much. If the element transposes too much, or in an inappropriate way, deleterious effects on the host will counterbalance the advantage conferred by transposition and may cause the host to evolve specific defenses. On the other hand, if the element does not transpose at some minimal level, it cannot maintain itself as an evolutionarily successful unit.

Tn*10* and IS*10*: Tn*10* is a bacterial tetracycline-resistance transposon. It is a composite element because its two ends are inverted repeats of an insertion sequence, in this case IS*10*. While either IS*10* element can transpose individually, IS*10*-Right is fully functional and IS*10*-Left is defective, relying primarily on transposition functions provided by IS*10*-Right. The two IS*10* elements can also cooperate to effect transposition of the entire Tn*10* unit or to generate certain Tn*10*-promoted rearrangements. All of these events are rare, approximately 1 per 10^3 cell generations for IS*10*, 1 per 10^7 for Tn*10*, and 1 per 10^5 for the rearrangements. All of these events require IS*10*-encoded transposase, which interacts with different pairs of outside and inside IS*10* ends to promote the various events. *In vitro*, Tn*10* and IS*10* transposition also requires a host protein which may be either IHF or HU and which plays an accessory role in the reaction, probably by altering DNA structure. IHF is likely to be the most important host factor *in vivo*.

Tn*10* and IS*10* transpose by a nonreplicative mechanism in which the element is excised from the donor site and inserted at a new target location without any replication of the element except for a small amount of repair synthesis at the very ends. The fate of the donor molecule is not firmly established, but is likely to involve chromosome loss and/or repair of the remaining double-strand gap against another copy of the donor region present on another molecule in the same cell. Loss of a chromosome need not be a lethal event, because bacteria contain more than a single chromosome under most growth conditions.

We describe below the mechanisms by which IS*10*-Right (hereafter called IS*10*) and Tn*10* modulate their transposition activities (Figure 1). Some of these mechanisms are intrinsic to the transposon or insertion sequence itself, while others involve interplay between the transposon and its host. They can all be rationalized as stratagems ensuring that transposition occurs at an appropriate rate and under appropriate circumstances. The existence of these many mechanisms, some of which are quite sophisticated, makes it clear that IS*10* and Tn*10* are evolutionarily successful creatures and that IS*10*, at least, has been with us for a long time. Many of these mechanisms are known or suspected to operate for other IS elements and similar general regulatory strategies are found in many types of transposons. The reader should consult *Mobile DNA* for descriptions of other elements and for a fuller review of Tn*10* and IS*10* with references to the primary literature (KLECKNER 1988).

Features of IS*10* ensuring low levels of transposase expression: The frequency of IS*10* transposition is never very high because the level of transposase

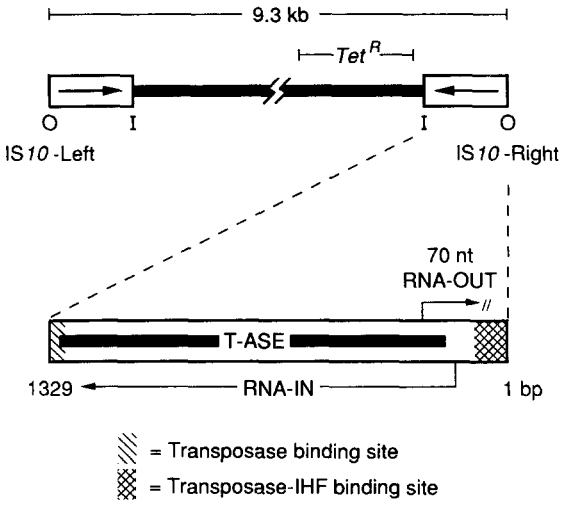

FIGURE 1.—The structure of Tn*10*.

protein is never very high (RALEIGH and KLECKNER 1986). A single copy of IS*10* in the bacterial chromosome generates fewer than 0.2 molecule per cell per generation as estimated from the level of β-galactosidase in a strain bearing a single-copy transposase/β-galactosidase fusion gene. So little protein is made that polypeptide chains can be detected in enzyme assays only if the cells are broken open so that unassociated fusion-protein monomers can reassemble into active tetramers *in vitro*.

This low level of protein is achieved by a combination of infrequent transcription (0.25 transcript per element per cell generation) and inefficient translation (fewer than 60% of transcripts being translated even once). Transcription is infrequent in part because the transposase gene promoter, pIN, is relatively weak (SIMONS *et al.* 1983), and in part because transcription is confined to a small fraction of the cell cycle (see below). Translation is inefficient primarily because there is no obvious Shine-Dalgarno ribosome binding site consensus sequence; most mutations that increase transposase gene translation bring the sequence closer to consensus (C. JAIN and N. KLECKNER, unpublished results).

Two subtler mechanisms may also contribute to inefficient translation by confining translation initiation on a nascent 5′ mRNA to the first few seconds after transcription is initiated. First, translation is reduced severalfold by fold-back inhibition, in which a region of transposase mRNA about 300 nucleotides (nt) from the 5′ end pairs with and sequesters the ribosome binding site near the start of the message (KITTLE and KLECKNER 1988; J. E. GONZALEZ and R. W. SIMONS, unpublished results). Second, preliminary experiments suggest that failure to initiate translation at the transposase AUG start codon may reduce the stability of the 5′ end of the mRNA (C. JAIN and N. KLECKNER, unpublished results).

Transposition of a single-copy element regulated by *dam* methylation: The most important regulatory mechanism for an IS*10* element present in single copy involves DNA adenine methylation at specific sites in IS*10* (ROBERTS *et al.* 1985; additional discussion in KLECKNER 1988). This mechanism not only reduces the basal level of transposition but also ensures that it occurs specifically in the most appropriate situations. *Escherichia coli* mutants exhibiting increased levels of Tn*10*-promoted rearrangements were isolated and subsequently shown to increase IS*10* transposition more than 100-fold. All of these mutations turn out to be alleles of the *dam* gene whose product, DNA adenine methylase, methylates the N-6 positions of the symmetrical adenines in the sequence 5′GATC. The effects of *dam* mutations are a direct consequence of the absence of methylation at two strategically located GATC sites in IS*10*, one of which overlaps the −10 region of the transposase promoter near the outside end of the element and the second of which occurs within the transposase binding site at the opposite (inside) end of IS*10*.

Because there is normally no fully unmethylated DNA in wild-type *E. coli* strains, and because hemimethylated DNA is generated only transiently upon replication of the transposon, we reasoned that the effects of *dam* mutations must reflect a normal process in which IS*10* is activated by hemimethylation. Additional analysis revealed this to be the case. Furthermore, passage of the replication fork generates two chemically distinguishable hemimethylated IS*10* species, and we found that only one of these is substantially activated for transposition. The ratios of approximate transposition rates for the fully methylated and the two hemimethylated species are 1:12:2,400 (minimum) to 1:12:60,000 (possible). These conclusions were reached largely by direct examination of IS*10* transposition from hemimethylated DNA *in vivo*. Hemimethylated elements were generated *in vivo* by transferring IS*10* from a Dam^+ host to a Dam^- host in an Hfr cross, where one specific strand of the transferred DNA is synthesized in the donor while the complementary strand is synthesized *de novo* in the recipient.

Appropriate variations in the Hfr transfer experiment revealed the basis for these differences. Each of the two hemimethylated versions of the pIN promoter is activated relative to the fully methylated promoter. However, only one of the two hemimethylated inside ends is activated. This *cis* asymmetry ensures that only one of the two hemimethylated elements transposes. The magnitude of activation results in part from the independent effects of methylation on the two determinants and in part from a coupling mechanism: expression of transposase and activation of the inside end are temporally coupled, both GATC sites becom-

ing hemimethylated just as the replication fork passes. In the coupled situation, transposase protein is used more efficiently than it would be were the terminus and promoter activated at independent times.

One important prediction of *dam* regulation is that IS*10* transposition will occur only during a limited period of the cell cycle, immediately after the replication fork passes. Although the IS*10* GATC sites have not yet been examined, recent experiments suggest that a typical GATC site in the *E. coli* chromosome remains hemimethylated for 0.5–4 min, 1–10% of a generation (J. CAMPBELL and N. KLECKNER, unpublished results). Furthermore, during this interval, only one of the two sister chromosomes can generate an IS*10* transposition. This type of regulation should be specifically advantageous to an element like IS*10* that transposes by a nonreplicative mechanism and leaves a hole in the donor chromosome. *dam* regulation should ensure that transposition will never occur unless a second copy of the donor region is present, *i.e.*, on the sister chromatid. Thus, a cell will never die because a donor molecule is not repaired. Furthermore, the likelihood of repair may be increased because a homologous sister-chromatid region is always in the general vicinity of the donor molecule at the time of transposition.

Activation by hemimethylation should also mean that transposition will be transiently induced when IS*10* enters a new host by any mechanism that involves transfer of single-stranded DNA followed by synthesis of the complementary strand in the recipient cell (*i.e.*, conjugal transfer, some types of transformation, or infection by single-stranded DNA phages). This may be particularly important for expediting the dissemination of IS*10* in bacterial populations.

Mechanisms slowing the accumulation of multiple transposon copies: Although IS*10* transposes by a nonreplicative mechanism, the average number of transposons per cell in the population should increase with almost every cycle of transposition. If the gap left behind at the donor site is repaired by interaction with another chromosome, a second copy of the transposon is generated by the restoration process. If the gap is not repaired and the donor chromosome is lost, the average number of transposons per bacterial genome can still increase: if the transposon inserts into a chromosome other than the one that is lost, the number of transposons remains constant while the total number of chromosomes decreases. In either case, the transposon frequency further increases if the moving element inserts ahead of the replication fork in the target chromosome.

If transposition were left unchecked, the average number of transposons per cell would continue to accumulate, and at ever-increasing rates. With more copies, there would be more potential donor elements and, in addition, the transposase concentration would increase. The cell would eventually explode. For IS*10*, these risks are greatly reduced by diverse mechanisms.

IS10 transposase acts preferentially in cis: cis action is observable in complementation tests: the transposition frequency of an element making its own transposase is much greater than that of an equivalent element provided with the same level of transposase in *trans* (FOSTER *et al.* 1981; N. KLECKNER, unpublished results). As a consequence, increasing the numbers of IS*10* copies no longer increases the effective transposase concentration; each IS*10* element sees only its own transposase.

Formally, failure to observe complementation could result either from an intrinsic failure of the protein to move freely through the cell and/or from coupling of transposase action to transposase expression. In the first case, transposase made by one element would never reach a distant element. In the second case, transposase which reaches an element in *trans* could never act because silent transposons are poor substrates for transposase action. In fact, IS*10* uses both of these mechanisms.

Transposase is indeed intrinsically *cis*-acting. Even when all regulatory mechanisms are experimentally eliminated, transposase made in one location acts preferentially on transposon ends that are located nearby (MORISATO *et al.* 1983). The physical basis for this property is not understood. Perhaps some combination of high nonspecific DNA binding and a finite functional half-life prevents transposase from migrating very far from the site where it first contacts DNA. *cis* action of this type also requires that the transposase mRNA never diffuse very far from the template element. Because the mRNA cannot be separated from the template element until transcription is complete, mRNA localization might be facilitated by translational polarity and/or fold-back inhibition.

In addition, transposase should be effectively *cis*-acting because its action is coupled to its transcription by *dam* regulation. IS*10* elements at different locations in the genome become hemimethylated at different times. Any particular element will be immune to transposase that is made by elements elsewhere in the genome because its inside end will be fully methylated when those transposases are made.

IS10 multicopy inhibition: IS*10* has evolved an entire regulatory process whose sole function is to reduce transposition when the element is present in more than a single copy. IS*10* encodes a *trans*-acting negative regulator of transposase expression whose effectiveness increases with increasing concentration, *i.e.*, with increasing transposon copy number (SIMONS and KLECKNER 1983). The level of this regulator is adjusted so that transposase expression from an element present in single copy is barely affected (SIMONS and

KLECKNER 1983); however, the presence of even two copies results in a threefold inhibition, and 30 copies can inhibit as much as 50-fold (J. MATSUNAGA and R. W. SIMONS, unpublished results). Because transposase is *cis*-acting, any decrease in the level made per copy reduces the frequency of transposition of each copy. Thus, the combination of a *trans*-acting negative regulator and a *cis*-acting transposase means that as the number of IS*10* copies increases, the frequency of transposition per copy decreases. In fact, the overall rate of IS*10* transposition by the ensemble of copies in the cell probably remains essentially constant regardless of the copy number.

This IS*10*-encoded *trans*-acting negative regulator is a 70-nt antisense RNA, RNA-OUT. Its existence was discovered in the course of complementation experiments in which multicopy IS*10* plasmids were tested against transposase-defective Tn*10* derivatives introduced on λ phages. Mysteriously, control experiments demonstrated that wild-type Tn*10* transposed less frequently into cells containing a multicopy IS*10* plasmid than into cells lacking any plasmid. Deletion analysis then demonstrated that this multicopy inhibition was not due to transposase itself. The RNA nature of the inhibitor was revealed by genetic analysis coupled with *in vitro* identification of the responsible promoter, pOUT, located internal to and opposing the transposase gene promoter pIN. The template sequences for RNA-OUT and the transposase mRNA (RNA-IN) overlap for 36 bp. The key evidence that inhibition results from pairing of the two RNAs was the genetic observation that the inhibitory effect conferred by a wild-type plasmid could be titrated by an additional, truncated transposase gene, present in *cis* or in *trans*, expressing the 5′ end of RNA-IN at high levels.

IS*10* antisense control works in a simple and direct way. The region of RNA-IN that is complementary to RNA-OUT includes the ribosome binding site for the transposase gene, and RNA-OUT exerts its effect by preventing ribosome binding to RNA-IN (C. MA and R. W. SIMONS, unpublished results). The paired species is also cleaved by the double-strand-specific RNaseIII, but this cleavage is not required for antisense control (C. C. CASE, E. SIMONS and R. W. SIMONS, unpublished results; see SIMONS and KLECKNER 1988).

Specific features of the antisense system are important for its function. RNA-OUT forms a stem-loop structure, and the 5′ end of the transposase message, RNA-IN, is complementary to the top of the loop. Pairing initiates by interaction at the 5′ terminal sequence GCG, extends through the rest of the loop, and then proceeds by displacement of one strand of the RNA-OUT stem throughout the remaining region of complementarity to the 3′ end of RNA-OUT (KITTLE *et al.* 1989). The three initial G · C pairs are probably important for nucleation of stable pairing, and initiation at the terminus of RNA-IN permits free rotation of the nascent duplex around the RNA-OUT chain, thus permitting pairing over more than a single turn of the helix. Also, the stem domain of RNA-OUT has several base-pair mismatches while the RNA-IN/RNA-OUT hybrid has perfect complementarity; replacing imperfect matches with perfect ones during pairing may help to drive the strand-displacement reaction in the forward direction.

Biologically, the stem-loop structure of RNA-OUT allows presentation of an exposed single-stranded region (the loop) for pairing initiation in a structure where it is protected from nucleolytic degradation by the stem domain. Mutations in the loop domain alter the rate of antisense pairing *in vitro* but have little effect on stability *in vivo*. In contrast, mutations that reduce intramolecular pairing in the stem domain have little effect on the rate of pairing but drastically reduce the half-life of RNA-OUT inside the cell and are suppressed by compensatory mutations that restore stem pairing (CASE *et al.* 1989). The stem region probably acts to block the progress of exonucleolytic single-strand RNases, which are a major source of single-strand nucleolytic activity in *E. coli*. However, the duplex stem domain is also specifically arranged so as not to be a substrate for RNaseIII: a single-base mutation in RNA-OUT that extends the stem domain pairing up into the loop domain renders the molecule sensitive to that RNase.

As a consequence of these features, RNA-OUT is exceptionally stable; its half-life is more than 40 min as compared with a typical bacterial mRNA half-life of 1–2 min (CASE *et al.* 1989). This stability allows the cell to achieve a high steady-state level of the RNA with a promoter that is only moderately active. Such a mechanism does not permit the level of RNA-OUT to change rapidly, but rapid changes are not necessary for a regulatory process whose role is to reduce risks whose consequences are manifested on an evolutionary time scale. For a contrasting case, read about Col*E1* in SIMONS and KLECKNER (1988).

Mechanisms protecting IS*10* from fortuitous activation by external promoters: An additional set of mechanisms prevents fortuitous activation of the transposase gene by transcripts initiated outside of the element. Because IS*10* inserts randomly in DNA, the element runs a risk of positioning itself immediately adjacent to a strong chromosomal promoter that could direct transcription across the end of the element and through the transposase gene. Such read-through transcription would disrupt both regulation by *dam* methylation, which depends upon specific activation of pIN, and inhibition by antisense RNA-OUT, which will pair effectively only with target

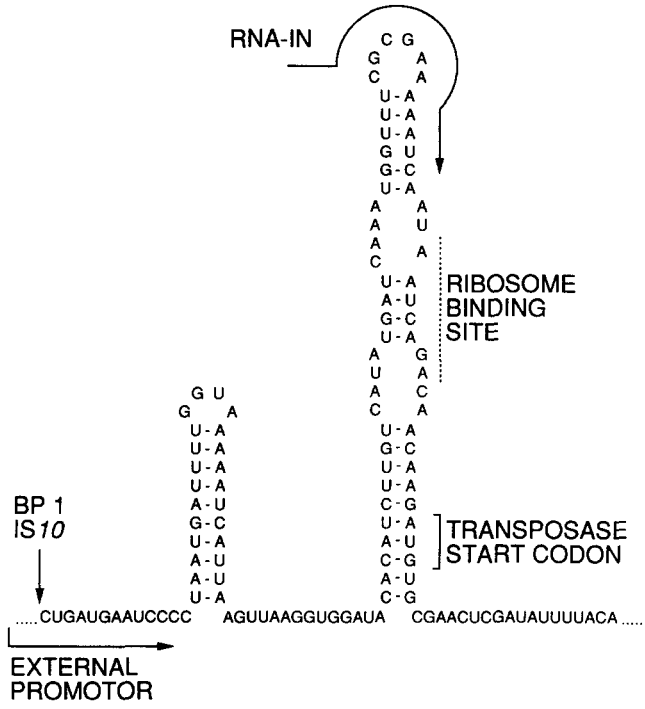

FIGURE 2.—IS*10* transposase expression from readthrough transcripts is blocked by sequestration of translation start signals in a region of secondary structure.

molecules having the precise 5′ end found in wild-type RNA-IN.

The most important IS*10* protection mechanism acts at the level of transposase translation: externally initiated transcripts yield less than 1% as much transposase protein per transcript as do transcripts initiated from pIN (DAVIS, SIMONS and KLECKNER 1985). A portion of this effect is probably due to premature termination of readthrough transcripts before they transverse the transposase gene. However, most inhibition occurs post-transcriptionally. The sequence between the end of IS*10* and the transposase ribosome binding site is such that readthrough transcripts form a strong stem-loop structure, essentially the complement to the RNA-OUT stem-loop, that sequesters the translation-initiation signals (Figure 2). The 5′ end of RNA-IN is at a position corresponding to the top of this loop, and RNA-1N therefore lacks the inhibitory structure.

A second protection mechanism is provided by the fact that transcription initiated outside of the element and across the outside terminus of IS*10* inhibits transposition, even when transposase is provided in *trans* (DAVIS, SIMONS and KLECKNER 1985). This direct inhibition in *cis* is severalfold when transcription is promoted by a fully induced *lac* promoter and is more than sufficient to counteract any small increase in the level of transposase from readthrough transcripts that escape other protection mechanisms.

A third level of protection is provided once again by *dam* methylation. Readthrough transcripts are expressed throughout the cell cycle. However, any residual transposase made from such transcripts will be effective only during that small fraction of the cell cycle when the inside end is activated. The importance of *dam* methylation in this regard is directly observable in IS*10* mutants where the contribution of readthrough transcription is elevated due to reduced translational protection (HUISMAN *et al.* 1989).

A fourth level of protection comes in the form of prevention: insertion of Tn*10* (and thus presumably of IS*10*) is inhibited by transcription of the target region. Transposition into the *E. coli* lactose operon assayed in *Salmonella typhimurium* is tenfold more frequent in the absence of inducer than in its presence; similar effects have been observed in the Salmonella histidine operon (CASADESUS and ROTH 1989). Thus, the probability of risk from external transcription is reduced by the transposon's choice of target sites. This situation should also reduce the probability that Tn*10* or IS*10* will insert into absolutely essential genes or genes that are required for growth at the time of transposition, because both kinds of genes are likely to be actively transcribed.

Tn*10*-specific regulation: Tn*10* poses the same threats to the cell as does IS*10*, although its much lower transposition frequency reduces their extent. The low frequency of transposition is a consequence of the length of the transposon; deletion analysis shows that the rate of Tn*10* transposition increases approximately 40% for every kilobase decrease in transposon length (MORISATO *et al.* 1983). The mechanism for transposition length dependence is not known, but is generally presumed to reflect some aspect of the way in which transposon ends find one another. For example, a complex of transposase protomers might initially bind at one end of the element and then initiate a one-dimensional search for the other end; in this case, length dependence might arise because the complex has a significant chance of getting stuck or otherwise decaying during the search (*e.g.*, WAY and KLECKNER 1985). It could be argued that this transposition length dependence is itself a device for reducing the deleterious effects of the transposon. Perhaps the level of transposition required for Tn*10* to be an evolutionarily successful creature is lower than that required by IS*10*: Tn*10* is presumably maintained because of its linkage to tetracycline resistance, whereas IS*10* maintenance may require the capacity to efficiently generate new types of composite transposable elements by transposition to new locations.

Tn*10* is subject to the same modulation mechanisms as IS*10* except that *dam* methylation operates in an attenuated form (ROBERTS *et al.* 1985). Although transposase expression is *dam*-regulated, the two termini of Tn*10* are both IS*10* outside ends and not *dam*-sensitive inside ends. Preferential transposition of

Tn*10* when the replication fork passes or upon single-stranded entry into a new host should be less dramatic than for IS*10*; furthermore, Tn*10* ends are not protected from outlaw transposase molecules generated by readthrough transcription or by other IS*10* elements.

Questions for the future: Additional features of IS*10* and Tn*10* remain to be discovered. The most important involve the roles of transposition host factors IHF and HU. The bacterial host may use these proteins to communicate with IS*10* regarding the desirability of transposition (HUISMAN *et al.* 1989 and unpublished results; J. KRULL-SUSSMAN and R. W. SIMONS, unpublished results). The outstanding question is: what is the cell trying to say?

A second question is: how does IS*10* manage to make enough transposase molecules to carry out a transposition? Analogies with other systems suggest that numerous molecules (as many as 12) might be needed (GRINDLEY *et al.* 1982; ABDEL-MEGUID *et al.* 1984). Perhaps many cells make some transposase but only a few make enough to produce a transposition. Alternatively, specific mechanisms might ensure that transposase is made in small bursts. In this case, most cells would make no transposase protein, but occasionally a cell would make all of the necessary molecules and would have a very high probability of undergoing transposition. Bursts would be economical and might also minimize potentially damaging abortive events.

Third, is there regulation at the level of transposase itself? IS*10* transposase appears to be rather stable, but is its effective level reduced, or its action modified, by virtue of some functional instability? Furthermore, how are the DNA cleavage activities of the transposon controlled? Interaction with the target site involves cleavage of relatively nonspecific sequences by a pair of staggered nicks located 9 bp apart; what features of the transposition reaction ensure that transposase does not act as a restriction enzyme?

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