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IS*10*/Tn*10* transposition efficiently accommodates diverse transposon end configurations

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Transposon Tn*10* and its component insertion sequence IS*10* move by non-replicative transposition. We have studied the array of reaction intermediates and products in a high efficiency *in vitro* IS*10*/Tn*10* transposition reaction. Synapsis of two transposon ends, followed by cleavage and strand transfer, can occur very efficiently irrespective of the relative locations and orientations of the two ends. The two participating ends can occur in inverted or direct orientation on the same molecule or, most importantly, on two different molecules. This behavior contrasts sharply with that of Mu, in which transposition is strongly biased in favor of inverted repeat synapsis. Mechanistically, the absence of discrimination amongst various end configurations implies that the architecture within the IS*10*/Tn*10* synaptic complex is relatively simple, i.e. lacking any significant intertwining of component DNA strands. Biologically these observations are important because they suggest that the IS*10* insertion sequence module has considerable flexibility in the types of DNA rearrangements that it can promote. Most importantly, it now seems highly probable that a single non-replicative IS*10* element can promote DNA rearrangements usually attributed to replicative transposition, i.e. adjacent deletions and cointegrates, by utilizing transposon ends on two sister chromosomes. Other events which probably also contribute to the diversity of IS*10*/Tn*10*-promoted rearrangements are discussed.

Keywords: chromosome/deletion/non-replicative/rearrangement/replicative

Introduction

Transposable elements are present in the genomes of all types of organisms where they promote not only their own transposition but also diverse other types of genome rearrangements. In a classical transposition reaction, interaction of transposase with inversely repeated sequences at or near the ends of the transposon results in insertion of the intervening transposon segment at a new target site. Transposons also promote other rearrangements in and around their genetic loci including deletions, inversions and replicon fusions.

Tn*10* is a composite bacterial transposon in which the genes for tetracycline resistance are flanked by two copies of an IS*10* insertion sequence module (Kleckner, 1989). IS*10*/Tn*10* transposition is a paradigmatic non-replicative reaction in which the transposing segment is completely excised from the donor site by double strand cleavages at the two transposon ends and then re-inserted intact at a new 'target' site (Kleckner *et al.*, 1996). *In vivo* and biochemical analyses have revealed several important features of the IS*10*/Tn*10* transposition reaction (e.g. Chalmers and Kleckner, 1994; Haniford and Kleckner, 1994; Bolland and Kleckner, 1995, 1996; Sakai *et al.*, 1995; Junop and Haniford, 1996; Kleckner *et al.*, 1996; J.Sakai and N.Kleckner in preparation; this work). For IS*10*/Tn*10*, as for other elements, the first major event of transposition is transposase-mediated synapsis of two transposon ends to form a stable synaptic complex. All of the chemical steps of the transposition reaction then occur within the context of this stable complex, sometimes called a 'transpososome'. Double strand cleavage at each transposon end occurs via an ordered nicking of the two strands; subsequent to and dependent upon double strand cleavage at both ends, the synaptic complex becomes committed to a particular target DNA molecule and, finally, the two 3′OH termini of the transposon undergo strand transfer to the two strands of a particular target site.

Tn*10* and IS*10* are also both associated with a variety of genome rearrangements. Some of these rearrangements can be explained in a straightforward way as simple extensions of the standard IS*10*/Tn*10* transposition reaction (Kleckner *et al.*, 1977; Ross *et al.*, 1979; Kleckner; 1989). Others, however, cannot be explained in this way. In the latter category, two prominent examples are adjacent deletions and cointegrates. In an adjacent deletion, a segment of material is deleted adjacent to one end of a Tn*10* or IS*10* element without affecting the integrity of the element itself. IS*10*-promoted adjacent deletions occur at ~2% the frequency of IS*10* transposition (Roberts *et al.*, 1991); Tn*10* also promotes this type of event, as shown in some of the earliest studies of this element (R.Chan, B.-K.Tye and D.Botstein, personal communication). IS*10*/Tn*10* also can appear in cointegrate structures at low frequency, at least in some situations (Harayama *et al.*, 1984; Weinert *et al.*, 1984; Ahmed, 1991; Eichenbaum and Livneh, 1995). In a cointegrate, a circular replicon lacking a copy of Tn*10* or IS*10* is integrated at the site of a resident element in a second replicon, with direct repeats of the resident element at the two fusion junctions.

Replicative transposition mechanisms account for these two types of rearrangements directly (Arthur and Sherratt, 1979; Shapiro, 1979), but it is not clear how they might arise with elements that undergo non-replicative transposition. Diverse explanations have been suggested from *in vivo* studies on both IS*10*/Tn*10* and its cousin IS*50*/Tn*5*. An attractive possibility is that such events might be promoted directly by the transposon via interactions between element copies on two sister chromatids (Lichens-Park and Syvanen, 1988; Roberts *et al.*, 1991). Other

models have also been suggested, many of which include abortive or significantly atypical action of transposase (Tomcsanyi *et al.*, 1990; Jilk *et al.*, 1993). Finally, a broad class of explanations involves transposition of an individual IS element followed by homologous recombination between copies at the old and new positions; in these models, the transposon serves as a portable region of homology (e.g. Kleckner *et al.*, 1979; Chumley and Roth, 1980; Kleckner, 1989; Eichenbaum and Livneh, 1995).

The availability of an active *in vitro* system for IS*10* transposition has made it possible to investigate the full range of transposase-promoted events in a defined way. The use of *in vitro* reactions eliminates two prominent complications of *in vivo* analysis: the possibility of homologous recombination and the requirement for physical integrity and biological stability of the resulting products. The results of such an investigation are presented below.

The major finding of this work is that the IS*10*/Tn*10* reaction is very flexible with respect to which configurations of transposon ends it can accept. In particular, synapsis of two IS*10*/Tn*10* ends, followed by cleavage and strand transfer, occurs efficiently irrespective of whether the two ends are present as inverted or direct repeats on the same molecule or on two different molecules, a configuration that we refer to as 'bimolecular synapsis'. This mechanistic flexibility implies that the architecture of the synaptic complex is relatively simple, that this modular insertion sequence has the capacity to directly promote diverse types of genome rearrangements other than canonical transposition and, most specifically, that events involving transposon ends on two sister chromatids are likely to be a prominent outcome of transposase-promoted reactions *in vivo*. These observations also raise the possibility that some IS elements previously thought to undergo both replicative and non-replicative transposition could, in fact, be exclusively non-replicative in nature. Finally, analogies between IS*10*/Tn*10* and maize *Ac*/*Ds* elements with regard to relaxed synapsis and sister chromatid events are discussed.

A second important finding is that biochemically aberrant transposition is relatively rare, i.e. almost every event which successfully achieves the first chemical step of the reaction goes on to completion, under standard conditions. However, we find that transposition occasionally stalls prior to completion: in a few percent of cases, double strand cleavage at two synapsed transposon ends is followed by strand transfer of a single end to a target site with concomitant cleavage of only one target DNA strand. Finally, we also present evidence that functional 'pseudo-end' sequences occur frequently in random DNA and that pseudo-end interactions and single end strand transfer events likely also contribute to the diversity of transposase-promoted rearrangements *in vivo*.

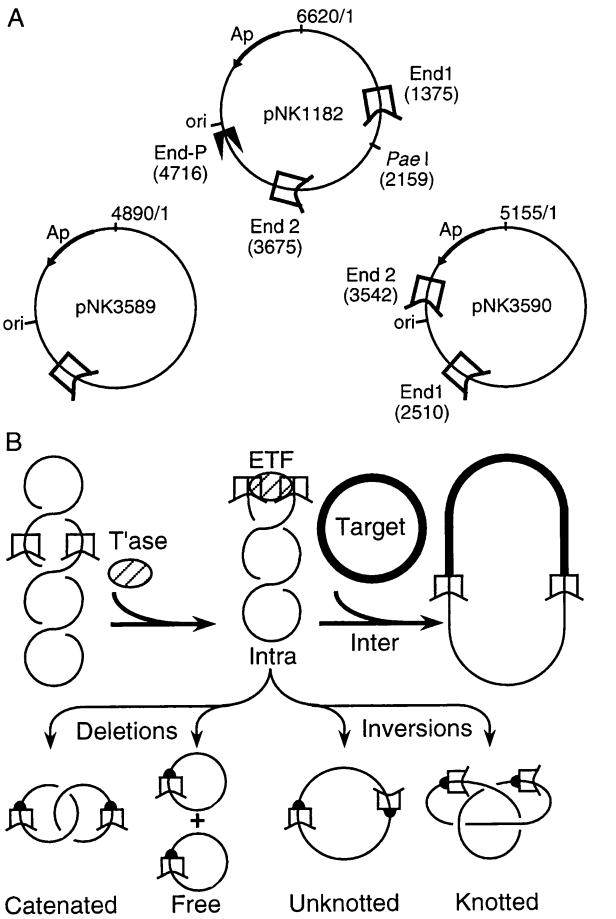

Fig. 1. Transposition substrates and reaction. (**A**) The structures of the transposition substrates are shown approximately to scale. Transposon ends (boxes) are the terminal 300 bp from the 'inside' end of IS*10*-Right. A sequence close to the origin of replication shares sufficient identity with the ends of IS*10* that it is recognized by transposase; we refer to this as a pseudo-end (End-P). *Pae*I is a unique restriction site located within the transposon. pNK3589 and pNK3590 have no strong pseudo-ends. ori, ColEI origin of replication; Ap, β-lactamase. (**B**) In the first step of the transposition reaction double strand cleavages precisely at the ends of the transposon produce an excised transposon fragment or 'ETF' (Benjamin and Kleckner, 1992). The ETF is linear but the ends are held together in a complex with transposase which prevents the supercoils from escaping (Haniford *et al.*, 1991). Transposition targets may be inter-molecular (Inter) or located within the transposon itself (Intra). In either case the 3′-hydroxyl groups at each end of the transposon are transferred to 5′-phosphate groups at the target site which overhang each other by 9 bp on each strand of the DNA and result in two 9 bp gaps in the product(s) (Benjamin and Kleckner, 1989). Strand transfer to an intra-molecular target site is expected to yield either inversion or deletion circles, depending on the orientation of the transposon ends with respect to the target site (Benjamin and Kleckner, 1989). If any supercoil writhe nodes are trapped between the transposon ends and the target site, the inversion and deletion circles are knotted and catenated, respectively. When predicting the topology of the knots and catenanes shown here we have assumed that only one twist node (which is of opposite sign to the writhe nodes) lies between the sites of strand transfer.

Results

Transposition in vitro with highly purified transposase

To examine the array of intermediates and products formed during Tn*10* transposition as well as the kinetics of the reaction, transposition was performed *in vitro* using a supercoiled plasmid substrate (pNK1182, Figure 1) containing two IS*10* ends in the usual inverted repeat configuration and highly purified, high specific activity, transposase (Figure 2A). To differentiate excision products from strand transfer products, parallel reactions were carried out using a mutant transposase proficient for excision but deficient in strand transfer (PS167; Haniford *et al.*, 1989). Strand transfer products are absent in PS167 reactions while excision products are present and accumulate.

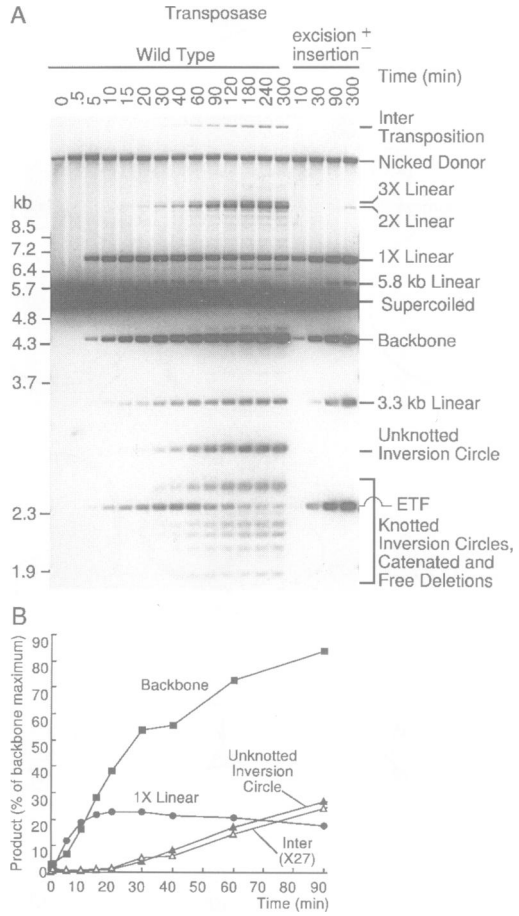

Fig. 2. Transposition reaction kinetics and novel products. (**A**) A time course of the standard transposition reaction with pNK1182 (Chalmers and Kleckner, 1994) is shown. Reactions with wild type or strand transfer defective transposase (PS167) were terminated at the times indicated. Bands corresponding to the substrate and known products are indicated. Novel products described in the text are labeled with their size. Products which are exactly one, two and three times the size of the linear substrate plasmid are referred to as 1X, 2X and 3X, respectively. (**B**) A PhosphoImager was used to quantify the Southern blot in (A) and data from the first 90 min of the wild type time course is plotted. The measurements were normalized with respect to the different sizes of the products so that the graphical points indicate the relative molar amounts of the different species. The amount of inter-molecular transposition product is multiplied by 27 so that it can appear on the same scale as the other products.

The normal Tn*10* transposition reaction produces two major excision products, 'Backbone' and 'Excised Transposon Fragment' (ETF), plus a variety of strand transfer products (Figure 2A). These strand transfer products include the large gapped circular species resulting from inter-molecular transposition of the transposon segment into an unreacted substrate molecule ('Inter', to be described elsewhere) and a constellation of small products that arise by strand transfer of the transposon ends into target sites within the transposon ('Intra'; described in Figure 1B). The most prominent of these intra-transposon products is an unknotted inversion circle.

All of these species exhibit appropriate kinetics. At early times, the products of excision predominate; at later times the ETF intermediate disappears as it is chased into strand transfer products, whereas the backbone species remains and accumulates to a high level. Inter-molecular and intra-transposon products appear with identical kinetics (Figure 2B). The ratio of inter- to intra-molecular products can be accounted for by the difference in the effective concentrations of the two types of target with respect to the transposon ends [estimated from the ring closure probabilities (Wang, 1986); data not shown]. These reactions also confirm that double strand cleavage at the two transposon ends is temporally distinct (Figure 2B), as observed previously both *in vivo* (Haniford and Kleckner, 1994) and in *in vitro* reactions using short linear fragments as transposon end substrates (Sakai *et al.*, 1995).

In addition to the standard reaction intermediates and products just described, a number of other abundant products are observed in our *in vitro* reactions. Prominent among these are molecules whose molecular weights correspond to exactly twice and three times the length of a linearized substrate molecule, 2X and 3X linear, respectively. These products are linear in nature as determined by two-dimensional gel analysis (see last section of Results). Both of these species are strand transfer products, as they are absent in PS167 reactions. Also prominent are two other linear products, 3.3 kb and 5.8 kb in length, both of which are excision products as defined by their presence in PS167 reactions. The next two sections describes the further analysis of these 'non-canonical' products.

Bimolecular synapses

The appearance of a strand transfer product that is linear but three times the molecular weight of a linearized substrate molecule suggested to us the possibility of a trimolecular event in which transposon ends on two different substrate molecules synapse, undergo cleavage and then undergo strand transfer to a third, unreacted, substrate molecule (shown for substrates containing a single transposon end in Figure 3A).

This scenario also predicts the occurrence of additional non-canonical products (Figure 3A). If strand transfer following bimolecular synapsis is intra-molecular, the result is either a 2X linear molecule or, alternatively, a complementary pair of products, one of which is a circle smaller than the substrate (<1X) and the other of which is a linear species containing the remaining material (>1X<2X). The precise sizes of the complementary pair of products depends upon the position of the strand transfer target site. Species can be seen in Figure 2A at appropriate positions in the gel for these types of products.

The occurrence of such bimolecular synapses was confirmed by further analysis. To simplify the spectrum of reaction products and see bimolecular synapsis more clearly, we carried out a standard transposition reaction using a substrate (pNK3589; Figure 1A) containing a single active transposon end and no active pseudo-end sequences (see below). The substrate yields the forms predicted above (Figure 3C, lane 6): a prominent 3X linear product, a prominent 2X linear product, a cluster of species of somewhat less than 2X in length, and a number of products that migrate faster than 1X linear. For the latter two groups, the occurrence of discrete bands reflects preferential use of certain preferred 'hot spots' as target sites (Halling and Kleckner, 1982). This single end substrate also yielded 1X linear products at high levels; this species is expected from events in which bimolecular

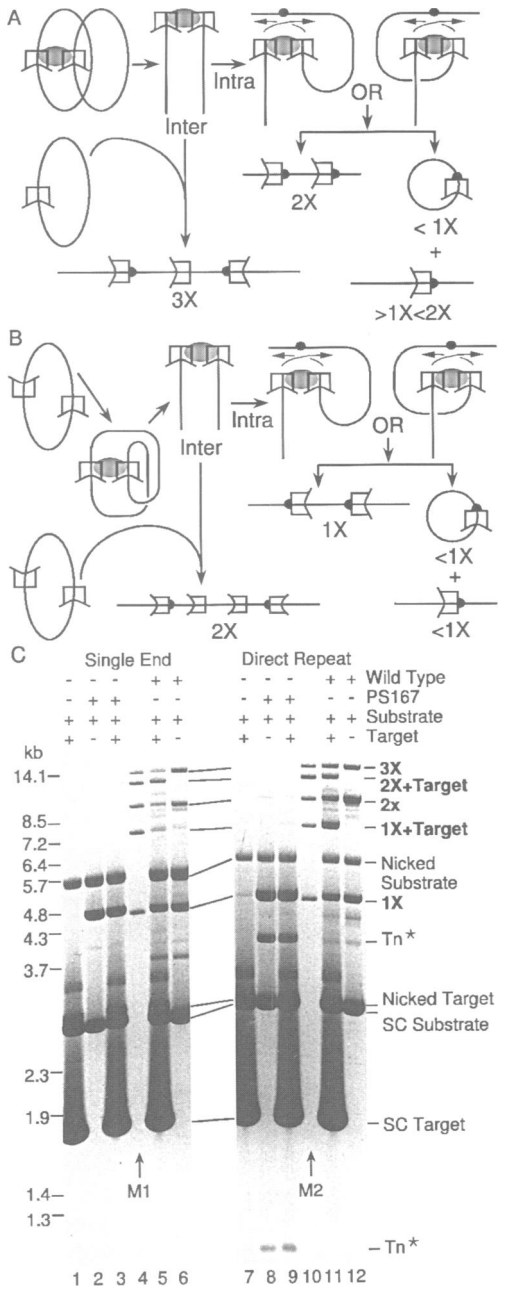

Fig. 3. Bimolecular and direct repeat synapsis. (**A** and **B**) Following synapsis and cleavage of transposon ends, two linear DNA molecules are held together in a complex with transposase in a structure that is analogous to the standard ETF. The products expected for subsequent strand transfer into inter- and intra-molecular target sites are shown. The location of the transposon ends illustrated in the products is arbitrary because target sites are largely not specific (Halling and Kleckner, 1982). (**C**) Standard transposition reactions with single end and direct repeat substrates (pNK3589 and pNK3590, Figure 1A) were carried out with wild type and strand transfer defective transposase (PS167). A reverse contrast photograph of an ethidium bromide stained agarose gel is shown. Special DNA molecular weight markers of the same size as the transposition products are used (see Material and methods); all of the markers are linear in structure and differ in size from the corresponding product by less than 1%. 1X, 2X and 3X are multiples of the sizes of the respective substrates; 2X+Target is the size of two substrate molecules joined to one target; 1X+Target is the size of one substrate molecule joined to one target. SC, supercoiled; M1 and M2, special molecular weight markers described above; Tn*, direct repeat synapsis followed by cleavage breaks the substrate into these two fragments.

synapsis and cleavage occur without subsequent strand transfer.

All of the linear strand transfer products resulting from bimolecular synapsis also have a characteristic feature at their ends: the sequences at the termini are specifically those which previously were adjacent to the transposon ends utilized (Figure 3A). It follows that in no case should the terminus of such a product occur at bp 1 of the transposon end, as all such transposon ends have undergone strand transfer. These features have been confirmed for the 3X and 2X linear species in a standard pNK1182 reaction using two dimensional gel electrophoresis with restriction digestion between the dimensions (data not shown, but see below for a similar example of the technique).

As a final demonstration of bimolecular synapsis, we examined a reaction mixture containing both the single ended substrate and a second, heterologous, plasmid which serves as a target molecule. Bimolecular synapsis followed by inter-molecular transposition into the heterologous target would yield specifically a linear product the size of two substrates joined to that target (2X+Target). A product of this size is detected and it is dependent upon the presence of wild type transposase and the second target plasmid (Figure 3C, lanes 3, 5 and 6).

Four observations suggest that the ability to carry out efficient bimolecular synapsis is a normal, intrinsic feature of the IS*10*/Tn*10* transposition reaction. (i) Even with the standard substrate molecule containing the normal two-ended minitransposon, the frequency of 3X linear molecules is only slightly less than the frequency of canonical inter-molecular transposition products. (Compare '3X Linear' and 'Inter Transposition' species in Figure 2A; similarly, in two-dimensional gel analysis presented below, compare '3X' species on the arc of linears with the 2.3 plus 3.3 kb 'Inter' products on the arc of gapped circles.) (ii) These same relative frequencies are also observed not only under optimal reaction conditions as shown here, but also under stringent conditions (i.e. in reactions containing only substrate, transposase, buffer, magnesium and lacking glycerol; see Chalmers and Kleckner, 1994). (iii) Bimolecular synapses are efficient irrespective of whether the transposon end involved is from the 'inside' or 'outside' of IS*10*. The results presented above all utilize 'inside' ends of IS*10*, but the same results are obtained with single end substrates carrying a single 'outside' end. Furthermore, a reaction containing a mixture of the two substrates (which are of slightly different sizes) yields both of the original pairs of products plus products of the sizes expected for bimolecular synapsis between the substrates having one inside end and one outside end (not shown). (iv) A preliminary experiment was carried out *in vivo* to assess the sensitivity of various plasmids to the induction of high levels of transposase (data not shown). An hour after induction of transposase a vector without any strong pseudo-ends was stable, whereas a derivative carrying a single transposon end was largely destroyed.

Direct repeat synapses

IS*10* transposase also promotes synapsis, cleavage and strand transfer of a pair of transposon ends located on the same substrate molecule but in direct repeat orientation.

The occurrence of such events was first indicated by

analysis of the 5.8 kb excision product observed in transposition reactions with the standard substrate pNK1182 (Figure 2A). High resolution restriction mapping, using relevant sequencing ladders as markers, revealed that this species is produced by double strand cleavages precisely at one wild type transposon end and a sequence which closely resembles a wild type end (a 'pseudo-end') which is present in the direct repeat orientation (Figure 1A, End 2/End-P; discussed further below). Cleavage at these two ends breaks the substrate into two fragments, the 5.8 kb species and a 0.8 kb partner which is found below the region of the gel shown in Figure 2A.

In transposition reactions with PS167 transposase, the 5.8 kb excision species accumulates (Figure 2A), but with wild type transposase, this species appears and disappears in parallel with the normal ETF, as does its 0.8 kb partner (data not shown) suggesting that these species are intermediates which are further converted to strand transfer products. Further analysis confirms this to be the case. In order to simplify the reaction products we carried out transposition reactions using a substrate containing only two highly active transposon ends present in direct repeat (pNK3590; Figure 1A). The products predicted for direct repeat synapsis are shown in Figure 3B: a 2X linear for inter-molecular reactions, and 1X linear and a pair of complementary products (circle <1X + linear <1X) for the intra-molecular case. Such products are detected in reactions with the direct repeat substrate (Figure 3C, lane 12), and their respective linear and circular natures were also determined by two dimensional gel analysis (data not shown, but see last section of the Results for an example of the technique). If a second, heterologous, 'target' plasmid is included in the reaction, the expected product is the size of the substrate plus the target (1X+Target).

The most definitive demonstration of direct repeat transposition is provided in the latter case. In the appropriate reaction, the intermolecular product of direct repeat synapsis is detected in great abundance (Figure 3C, lane 11). The diagnostic band with exactly the expected mobility (1X+Target) is present at a high level, and is not produced in reactions with either the strand transfer defective transposase (lane 9), nor in the absence of the target plasmid (lane 12).

In reactions containing only the direct repeat substrate (Figure 3C) and in standard reactions with pNK1182 (Figure 2A) many additional products are present. These products include not only those from direct repeat synapsis but those from bimolecular synapsis also, which are expected from any substrate with one or more transposon ends. The presence of bimolecular synapsis is specifically indicated by the presence of 3X linears, which do not arise via direct repeat synapsis. In contrast, some other types of species are produced by both direct repeat and bimolecular synapsis; for example, 2X linears are produced in both cases (Figure 3A and B). Similarly, linears <1X are formed as cleavage products of both reactions and 1X linear is produced not only via direct repeat synapses but also by any reaction which stalls after cleavage at a single end, irrespective of the type of synapsis in which it was involved.

Direct repeat synapsis also appears to be a normal feature of the transposition reaction. The products of direct repeat synapsis are abundant under stringent reaction conditions (as defined above). Furthermore, the direct-repeat-transposition reaction is almost as efficient as normal transposition, even in pNK1182 where there is competition with the normal reaction and one of the ends involved is the pseudo-end. This can be seen clearly by comparing the relative frequencies of excision products in reactions with strand-transfer defective transposase (Figure 2A, right side): the 3.3 kb excision product, which involves End-P and End 1 in inverted orientation and the 5.8 kb excision product which involves End-P and End 2 in direct orientation occur at very similar levels.

Pseudo-end transposons

The analysis above documents the presence of a strong pseudo transposon end on the standard substrate molecule pNK1182. A more general type of analysis confirms the presence of this end and also reveals several other, weaker, pseudo-ends. Synapsis between any particular pair of ends in inverted orientation, followed by intra-transposon strand transfer, yields a family of inversion circles which are all of the same molecular weight (i.e. that of the corresponding transposon) but comprise a continuous series of species of increasing topological complexity due to the presence of different numbers of knot nodes. These nodes are created during strand transfer by trapping of superhelical nodes present in the ETF (Figure 1B). The simplest topological form of the inversion circle is unknotted, and forms of increasing complexity contain increasing odd numbers of knot nodes.

Such a series of products can be detected using a particular type of two-dimensional gel electrophoresis (Figure 4A and B). Reaction products are separated in the first dimension by a simple agarose gel, according to size and structure: members of a topological series of inversion circles migrate with increasing mobility according to the number of knot nodes present. Restriction digestion *in situ* with an enzyme that cleaves once within the transposon, linearizes all forms of the inversion circles. The separation of different topological forms in the first dimension, followed by identical mobility in the second, produces an easily distinguishable pattern: a row of horizontal spots whose position in the second dimension is at the molecular weight of the corresponding linear transposon segment.

A standard pNK1182 transposition reaction (Figure 2A) subjected to this type of analysis reveals not only the series of spots expected for the normal transposon at the position of 2.3 kb, but also several other analogous but less abundant series (Figure 4A and B). The most abundant secondary series corresponds to a 3.3 kb 'pseudo-transposon' formed by End 1 in combination with a pseudo-end. The pseudo-end in this element has been mapped and found to be identical to the pseudo-end involved in direct repeat synapsis with the wild type End 2 (above). Two other series of spots correspond to pseudo-transposons of approximately 4.5 kb and 5.6 kb for which the relevant ends have not been mapped. At longer exposures, additional such series can also be seen.

The pseudo-end is located close to the ColEI origin of replication in the pBR-derived substrate plasmid (Figure 1A). The sequence of this end is very similar to that of wild type IS*10* outside and inside ends (Figure 4C) but does differ from those ends at two positions thought to

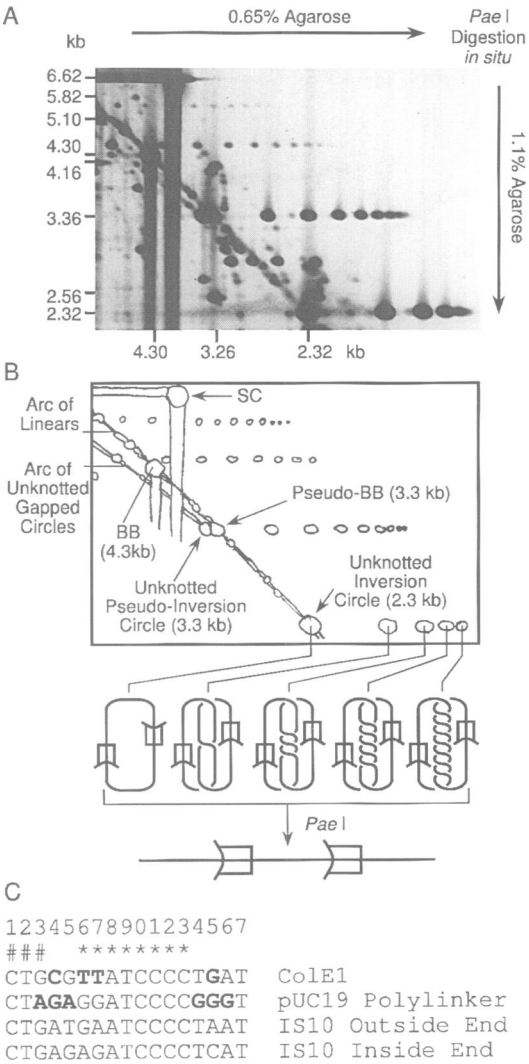

Fig. 4. Wild type and pseudo-transposon intra-molecular products. (**A**) A standard transposition reaction with pNK1182 was analyzed by two dimensional gel electrophoresis and Southern blotting. Between the dimensions the DNA was digested at a unique restriction site within the transposon. (**B**) A drawing of the gel in (A) indicating the identities of different features. Two arcs are present: the arc of linears contains linear products which were not cut by *Pae*I and which therefore maintained the same relative mobility in both dimensions of the gel; the arc of unknotted gapped circles contains products which had an unknotted, gapped circular structure in the first dimension but which were subsequently linearized by *Pae*I digestion between dimensions. To the right of the pseudo-backbone both of these arcs are coincident because of the resolution of the gel. Below the drawing of the gel are illustrated the predicted structures for the topological series of inversion circles before they were converted to linear form by *Pae*I digestion. As before, the location of the transposon ends illustrated is arbitrary because the location of the target sites is not specific. SC, supercoiled; BB, backbone. (**C**) The sequences of the ends of IS*10* are compared to those of the ColE1 pseudo-end and another found in the polylinker of pUC19. Bases shown in bold are different from those found in the ends of IS*10*. *, bases important for steps in the reaction before the first cleavage (i.e. probably transposase binding); #, bases important for steps of the reaction after cleavage.

be important in transposase recognition (bp 6 + 7; Huisman *et al.*, 1989). Notably, however, the pseudo-end retains the wild type sequence at bp 1, 2 and 3 which encode information that is especially critical for successful strand transfer (Huisman *et al.*, 1989; Haniford and Kleckner, 1994; Sakai *et al.*, 1995). A pseudo-end sequence competent for cleavage but disabled for strand transfer has also been identified in the vector pUC19 (Figure 4C). And, appropriately, this end has a base pair difference, relative to the wild type ends, at bp 3.

Unlike bimolecular and direct repeat synapsis, which occur even under stringent reaction conditions, efficient utilization of some pseudo-ends appears to be strongly dependent upon the presence of glycerol in the transposition reaction mixture. The activity of the ColEI pseudo-end is reduced greatly if glycerol is absent, for example. On the other hand, the activity of the pUC19 pseudo-end is not strongly affected. This behavior correlates well with the phenotypes of transposon end mutants (see above; Figure 4C). Taken together, this pattern of results suggests that the major effect of glycerol occurs at a step in the reaction before the first cleavage, perhaps by restoring transposase binding to defective transposon ends. Apparently, glycerol has little effect on the reaction with respect to wild type or topologically relaxed modes of synapsis, or subsequent chemical steps, or with mutant ends defective in post-cleavage steps (e.g. pUC19 pseudo-end). Similar observations on the effects of reaction components have also been made in other recombination systems, e.g. bacteriophage Mu (Mizuuchi and Mizuuchi, 1989).

The ColEI and the pUC19 pseudo-ends have also been analyzed for activity *in vivo* and the observed behaviors correlate well with their behaviors in reactions lacking glycerol. In these experiments (data not shown) transposase was expressed in cells containing a plasmid-bourne mini-transposon. DNA was recovered by standard plasmid 'mini-prep' procedures and examined for transposition products. Compared with the wild type 'inside' end, the ColEI pseudo-end is ~100-fold down at the cleavage step of the reaction, whereas the pUC19 pseudo-end is almost normal for cleavage but severely disabled for strand transfer.

Single end strand transfer is accompanied by single strand nicking at the target site

The availability of an efficient *in vitro* system for transposition made it possible to ask whether strand transfer ever occurs at a single transposon end. For this purpose, a standard transposition reaction with pNK1182 was analyzed by a second type of two dimensional gel analysis (Figure 5A and B). Electrophoresis in the first dimension at low agarose concentration separates the diverse species according to their mass and to a lesser extent their topological structure. In the second dimension at high agarose concentration nicked or gapped circles migrate much more slowly than linears, thus separating the two types of molecules into two distinct arcs. In such an analysis, single end strand transfer events are revealed as two types of signals that extend vertically upward from the arc of linears (Figure 5B and C).

Type I signals. One type of vertical signal extends all the way from the arc of linears to the arc of gapped circles (Figure 5B, arcs P, Q and R). Each such signal reflects a particular type of intra-transposon single end strand transfer event that yields a continuous series of lariat structures. In such a series, each of the structures is of the same molecular weight and the circular and linear portions of the lariat vary inversely in relative size depending upon

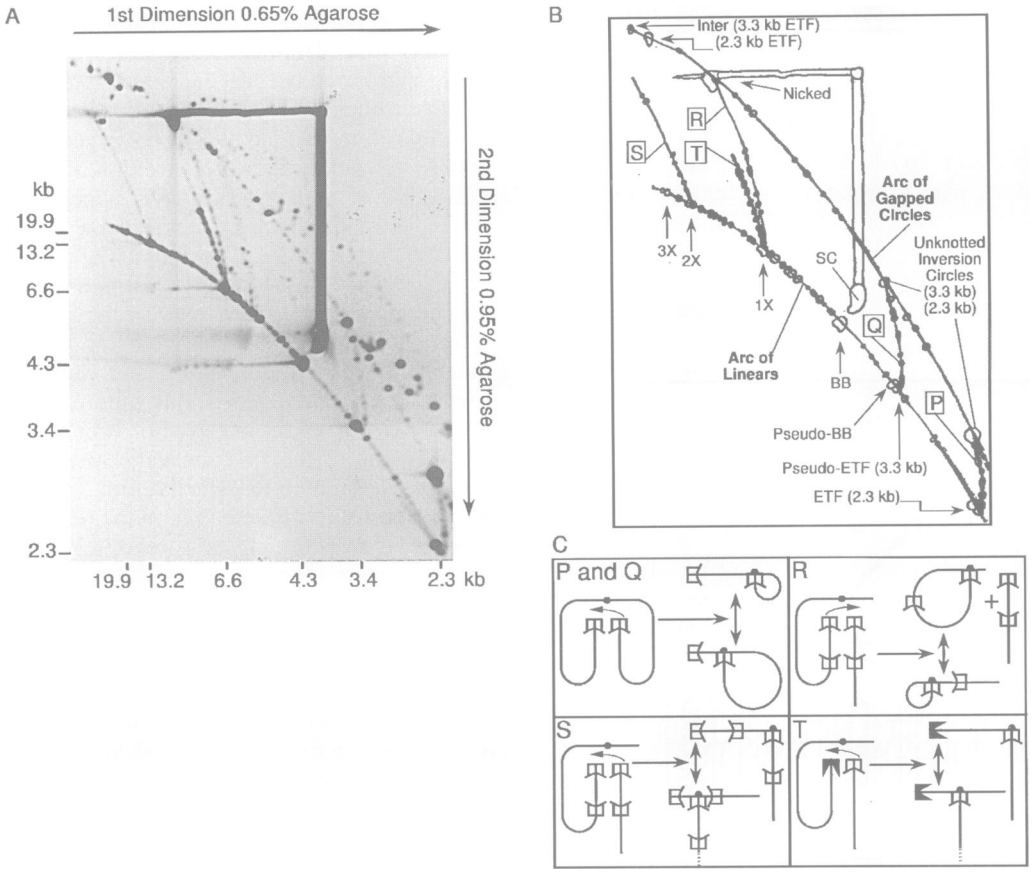

Fig. 5. Single end strand transfers. (**A**) A standard transposition reaction with pNK1182 was analyzed by two dimensional gel electrophoresis and Southern blotting. The two dimensions of the gel had different concentrations of agarose. (**B**) A drawing of the gel in (A) indicating the identities of different features. Assignments were made as follows: most species were identified by their relative position and intensities in a duplicate of the first dimension of the gel. Nicked and supercoiled substrate are present before the start of the reaction. 1X, 2X and 3X linears were identified by their size and restriction mapping (data not shown and Results). Substrate backbone, ETF and unknotted inversion circle were characterized previously (Benjamin and Kleckner, 1989, 1992; Haniford *et al.*, 1989, 1991). The pseudo-ETF and corresponding unknotted inversion circle were identified by restriction digestion of material recovered after gel electrophoresis (data not shown) and also by the analysis in Figure 4. The intermolecular product of the 2.3 kb ETF was identified by restriction digestion of material recovered after gel electrophoresis and also of individual representatives which were cloned by transformation into *Escherichia coli* (not shown). The identities of the arcs and spikes were deduced from their positions in the gel relative to known species and in some cases also from the pattern produced in the type of two dimensional gel used in Figure 4 (not shown). The prominent right angled smear is due to a relatively large amount of unreacted substrate; the smear traces the path of the supercoiled substrate (SC) through each of the two dimensions and probably represents molecules that have become nicked during electrophoresis and undergone a large decrease in mobility. Minor arcs to the right of the arc of gapped circles are omitted from the drawing; these have been identified as series of knotted inversion circles and catenated deletions corresponding to wild type and pseudo-transposons (unpublished data). (**C**) Each panel shows examples of the strand transfer events and target site locations which give rise to the products which form the secondary arcs P, Q, R, S and T. The arbitrary distribution of the target sites, and the concomitant differences in the structures of the products, is illustrated by examples at the two extremes; target sites either close to or far away from the untransferred end. Single end strand transfer is not expected to break both DNA strands at the target site because the reaction probably has a single step trans-esterification mechanism (see Discussion).

the position of the target site; at one extreme the structure extrapolates to that of a linear species while at the other extreme it extrapolates to that of a nicked/gapped circle (Figure 5C, arcs P, Q and R).

Type II signals. Another type of vertical signal has the form of a spike that extends upward from the arc of linears but never reaches the arc of gapped circles (Figure 5B, arcs S and T). Such signals represent a continuous series of 'T' structures in which the position of the junction varies depending on the position of the target site. These are analogous to lariat structures except that the 'transposon' was previously cleaved in two because of non-canonical synapsis. At one extreme the structure extrapolates to that of a linear species of the same total molecular weight. At the other extreme when the junction is close to the middle, the structure is most asymmetric and has the slowest mobility (Figure 5C, arcs S and T).

Arcs P and Q arise following normal synapsis and cleavage of transposon ends arranged as inverted repeats; strand transfer of either of the transposon ends to the target site produces a lariat structure (Figure 5C). Arc P is from the wild type transposon and arc Q is from the larger pseudo-transposon; each signal lies between the position of the respective ETF and unknotted inversion circle.

Arcs R and S arise following bimolecular synapsis and cleavage; strand transfer produces either a lariat or a 'T' depending upon whether the target site is on the same or the opposite molecule to the transferred end (Figure 5C). The 'T' structures contain two substrate molecules and

the series originates at the position in the gel of 2X linear. The lariats which comprise a single substrate molecule run between the positions of linear and nicked substrate. In principle, the lariat arc R could also arise from a normal intra-molecular synapsis followed by cleavage and strand transfer at only one end with the other end remaining chemically intact. This is not a likely scenario, however, because effective interaction with target DNA is strongly dependent upon cleavage at both transposon ends (J.Sakai and N.Kleckner, manuscript in preparation).

Arc T arises from direct repeat synapsis and cleavage between any wild type and any pseudo-end; strand transfer of one end produces a 'T' structure as already described. The 'T' structures all comprise a single substrate molecule and the series originates at the position of linear substrate. All direct repeat synapsis between any combination of wild type or pseudo-ends are expected to contribute to this signal, irrespective of the relative locations of the ends involved. Furthermore, many pseudo-ends can be expected to produce abundant single end strand transfer because of mutations in bp 1–3 which are known by genetic analysis to be critical for strand transfer (Huisman *et al.*, 1989; Haniford and Kleckner, 1994).

The detection of 'T' structures and lariats demonstrates that double strand cleavage at two synapsed transposon ends can be followed by strand transfer at only one of those two ends, with concomitant cleavage of only one of the two DNA strands at the target site. Such events are relatively rare, however: the levels of products in these signals amount to a few percent of levels of the corresponding double end strand transfer products.

Discussion

The IS10/Tn10 reaction efficiently accommodates diverse modes of transposon end synapsis

The observations presented demonstrate that IS*10* transposase efficiently synapses, cleaves and promotes strand transfer of two transposon ends irrespective of whether they occur in inverted orientation on the same molecule, as for a standard transposition event, or in direct orientation on the same molecule or, even, occur on two different molecules. Furthermore, this same flexibility is observed irrespective of whether the ends involved are those from the outside or inside of IS*10* or even when one end is a fortuitous pseudo-end. A variety of considerations suggest that tolerance of diverse synapsis modes is an intrinsic feature of the Tn*10* transposition reaction and not an *in vitro* artifact resulting from over-optimization or absence of a critical control element.

Mechanistic implications of synapsis flexibility

A number of recombination reactions exhibit strong biases in favor of particular modes of synapsis between a pair of interacting sites (Gellert and Nash, 1987; Mizuuchi, 1992a). In each of these cases, one specific configuration of interacting sites involving an intertwined synaptic architecture is highly favored (Nash, 1996). The relative flexibility of transposon end synapsis during IS*10*/Tn*10* transposition under stringent reaction conditions suggests that such geometric constraints do not play a major role. We thus infer that the overall configuration of the two ends within the IS*10*/Tn*10* synaptic complex is likely to be a relatively simple arrangement with little or no intertwining between the participating DNA duplexes.

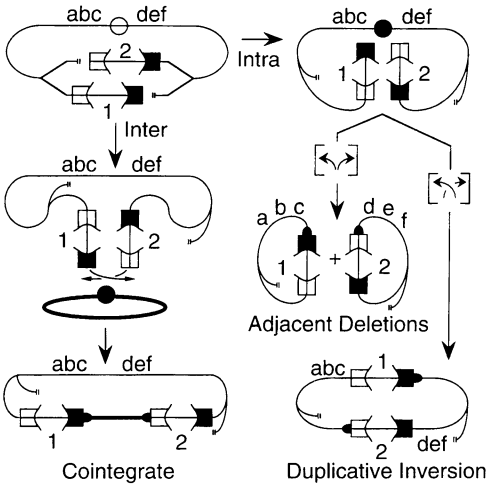

Fig. 6. Rearrangements promoted by inter-sister synapsis. The normal steps of the non-replicative transposition reaction give products characteristic of replicative transposition if synapsis is between the appropriate pair of ends on replicating sister chromatids. Intermolecular transposition produces a cointegrate, whereas intramolecular events give either a duplicative inversion or an adjacent deletion depending upon the orientation of the target site with respect to the transposon ends.

Biological implications of flexible synapsis

The ability of IS*10*/Tn*10* transposition to tolerate non-inverted-repeat synapsis opens up the possibility that IS*10* may promote a number of non-canonical transposition-related rearrangements *in vivo* at reasonable efficiency.

We are particularly intrigued by the possibilities provided by bimolecular synapsis. By utilizing two copies of IS*10* present on sister chromatids, a genetically unique copy of IS*10* could promote three events usually considered to be the hallmarks of replicative transposons: cointegrates, duplicative inversions and adjacent deletions (Figure 6). This possibility has been suggested previously on genetic grounds (Lichens-Park and Syvanen, 1988; Roberts *et al.*, 1991). This idea could explain the fact that IS*10*, and its relative IS*50*, appear to promote formation of adjacent deletions and cointegrates at low levels (Harayama *et al.*, 1984; Lichens-Park and Syvanen, 1988; Ahmed, 1991; Roberts *et al.*, 1991) even though genetic and biochemical evidence strongly suggest that IS*10* transposition is intrinsically and exclusively non-replicative (Bender and Kleckner, 1986; Bolland and Kleckner, 1995, 1996; J.Sakai and N.Kleckner, manuscript in preparation).

The possibility that IS*10* promotes inter-sister events is particularly attractive because of the way in which IS*10* transposition is modulated by N^6-adenine methylation of GATC sites (Roberts *et al.*, 1985; Kleckner, 1989). Specifically, when GATC sites near the ends of the element are fully methylated, IS*10* is inactive. Passage of a replication fork generates two hemi-methylated forms, both of which make increased levels of transposase and one of which undergoes activation of its previously inactive inside end; the inside end on the sister IS*10* element remains inactive. Since IS*10* outside ends are unaffected by methylation, this situation not only activates transposi-

tion at the appropriate time, i.e. when a sister chromosome is present, but also provides for a limited range of potential events: a single active IS*10* inside end may interact either with its outside end partner *in cis* or else with the outside end on the presumptively nearby sister chromatid, the combination required for biologically effective inter-sister synapsis (Roberts *et al.*, 1985, 1991).

The ability of non-replicative IS elements to promote replicative-like events in a single step should be evolutionarily advantageous. Cointegrate formation integrates one (circular) replicon into another (circular or linear) replicon, and cointegrate formation involving bacterial plasmids is likely important for disseminating specialized genetic determinants among different types of bacteria. Duplicative inversions are also important because they represent a single step mechanism for creating new composite transposons; and adjacent deletions should also contribute by bringing a resident IS element into close juxtaposition to a particular determinant of selective value during evolution of a composite transposon.

Other scenarios can be envisaged by which 'prototypically replicative' transposition products (including adjacent deletions and duplicative inversions) can be promoted by a non-replicative element. But all of these scenarios involve a combination of one transposon-promoted step and one or more host-promoted recombination and/or DNA repair steps (e.g. Berg, 1983; Biel and Berg, 1984; Tomcsanyi *et al.*, 1990). Indeed, the present work suggests a specific additional example of this type of event. Single end strand transfer following bimolecular synapsis can produce a lariat structure containing all of the sequences of the substrate (arc R in Figure 5C). If this structure were repaired by removal of the linear portion of the molecule, it would have the structure of an adjacent deletion. The current work does not exclude contributions from these alternative mechanisms but it does suggest that non-replicative elements have at their disposal a mechanism for promoting these important events that is frequent and does not depend upon host-promoted processes.

Possible generality of bimolecular synapsis and non-replicative transposition among IS elements

IS elements IS*1* and IS*903* are generally thought to promote both replicative and non-replicative transposition events (Biel and Berg, 1984; Weinert *et al.*, 1983, 1984). But evidence for replicative transposition in both of these cases rests on analysis of substrate molecules which were themselves replicating during the time that the events of interest were occurring. Thus, the current observations raise the possibility that these and other IS elements could also be mechanistically non-replicative, with apparent replicative transposition products arising via an inter-sister mechanism.

One observation supports the possibility that IS*1*, which readily forms cointegrates, is likely capable of relaxed modes of synapsis. A substrate containing a single IS*1* terminus is capable of inducing an SOS response *in vivo* in the presence of high levels of transposase (Lane *et al.*, 1994). This effect could be a consequence of excision that follows either direct repeat synapsis on dimeric substrate plasmids, as proposed, and/or bimolecular synapsis as shown here for IS*10*/Tn*10*.

Different IS elements appear to give non-replicative and apparently replicative products at varying relative frequencies (references above). Similarly, IS*10* appears to give cointegrates in some situations and not others (Harayama *et al.*, 1984; Weinert *et al.*, 1984; Ahmed, 1991). Perhaps these differences reflect differences in the efficiency of inter-sister synapsis and the processing and/or repair of transposition products.

Analogies between IS10/Tn10 and Ac/Ds

Transposition by the *Ac/Ds* elements in maize is non-replicative and appears to be regulated by methylation in a manner analogous to IS*10* (Fedoroff, 1989; English *et al.*, 1993, 1995; Kunze, 1996). For *Ac/Ds*, bimolecular synapsis appears to be quite common. Physical and genetic analysis of transposition products has shown that transposon ends may be present as direct or inverted repeats, and that McClintock's 'breakage–fusion–bridge' cycle is a direct consequence of the normal transposition reaction following synapsis of ends located on different sister chromatids (English *et al.*, 1993, 1995). Furthermore, in a half double *Ds*, synapsis appears to be constrained to ends on sister chromatids by the pattern of hemi-methylation produced by replication in a way analogous to that suggested above for IS*10* (Fedoroff, 1989; English *et al.*, 1995; Kunze, 1996).

Significance of pseudo-end transposons

Genetic observations have demonstrated that a wild type transposon end, lacking a partner, can utilize a fortuitously occurring pseudo-end sequence as a partner (e.g. Polard *et al.*, 1992, 1994; Jilk *et al.*, 1993). The current work demonstrates that a wild type IS*10* end utilizes a pseudo-end partner at a significant frequency even in the presence of a second wild type end. Pseudo-transposons presumably represent one type of intermediate by which a new transposon with a new transposase/transposon end specificity might evolve.

The frequent occurrence of pseudo-end sequences in DNA provides additional opportunities for non-canonical transposon-promoted rearrangements. For example, an additional mechanism for the formation of adjacent deletions could involve synapsis of one IS*10* end with a pseudo-end sequence in the adjacent flanking DNA followed by strand transfer to an appropriately oriented target site located beyond the pseudo-end sequence. The product of strand transfer of the wild type end is the adjacent deletion; the pseudo-end strand transfer product is lost.

Single end strand transfer events

The current results demonstrate that the IS*10*/Tn*10* transposition reaction sometimes carries out double strand cleavage at two synapsed transposon ends followed by strand transfer of one end to a target DNA. In the products observed, the target DNA has been cleaved on only the strand involved in strand transfer. This is the expected consequence of strand transfer via direct nucleophilic attack by a 3′OH transposon terminus: in this mechanism, target strand cleavage and strand transfer are the two products of the same simple chemical reaction (e.g. Mizuuchi, 1992b).

It is possible to imagine that in an aberrant reaction, single end strand transfer might be accompanied by double

strand cleavage of the target DNA if water is able to act as a suitable nucleophile and take the place of the 3′OH terminus in cleavage of the second strand. For normal synapsis between two ends in inverted repeat, such an event would yield linear molecules and gapped-circular molecules lacking any linear tail, depending upon the location and orientation of the target site relative to the ends. More specialized analysis would be required to determine whether the arcs of linear and nicked/gapped circular products include species that arose in this way.

Materials and methods

Materials were from commercial sources and standard techniques for molecular biology were taken from Sambrook *et al.* (1989) or product manufacturer's instructions.

Proteins and DNA

Transposase was purified as described by Chalmers and Kleckner (1994). Wild type and strand transfer defective PS167 mutant transposases were expressed from pNK3291 and pNK3293, respectively. All other proteins were from New England Biolabs. Plasmid DNA was purified by the CsCl method; supercoiled monomers of all of the transposition substrates were further gel purified as described by Chalmers and Kleckner (1994).

The standard transposition substrate pNK1182 (6620 bp) has the terminal 300 bp from the inside end of IS*10*-Right arranged as an inverted repeat on either side of the kanamycin phosphotransferase gene from IS*903* (Morisato and Kleckner, 1987).

The pseudo transposon end in the ColE1 origin was located precisely by restriction digestion of the relevant cleavage products and comparison of the fragment size with the appropriate DNA sequencing ladder. The pseudo-end in the polylinker of pUC19M (Clonetech) was located by high resolution restriction mapping of cleavage products and comparison of the sequences of the ends of IS*10* with that of the plasmid; the location was confirmed by deletion of the pseudo-end.

For construction of substrates lacking strong pseudo-ends the vector pNK3587 (2492 bp) was constructed from pUC19M: the polylinker was removed between the *Acc*I and *Nar*I sites and a C to T point mutation was introduced at bp 788, which is bp 12 of the pseudo transposon end found at the ColE1 origin of replication (see Figure 4C). During the site directed mutagenesis the *Nde*I site was converted to *Nco*I. The single ended substrate pNK3589 (4890 bp) is pNK3587 with one 300 bp transposon inside end along with the kanamycin resistance gene from pNK1182 inserted at the *Nco*I site. The direct repeat substrate pNK3590 (5155 bp) is pNK3589 with a second identical transposon inside end inserted at the *Xmn*I site. The intermolecular transposition target was Bluescript SK+ (2961 bp).

Each of the 10 custom molecular weight markers used in Figure 3 was generated by cleavage of bacteriophage λ DNA with a different restriction enzyme. In each of the 10 cases the species corresponding to the molecular weight of interest was purified. Purified species were combined to give each set of five markers used for single end and direct repeat transposition reactions, respectively. In order of decreasing molecular weight (size in bp/restriction enzyme): 15606/*Ssp*I; 14677/*Ava*I; 13286/*Bgl*II; 12712/*Pvu*I; 10298/*Eco*NI; 9790/*Sph*I; 8113/*Sac*II; 7834/*Dra*I; 5109/*Aat*II; 4878/*Eco*RI.

Transposition reactions and gel electrophoresis

In vitro transposition reactions (Chalmers and Kleckner, 1994) were all carried out with 1 μg of gel purified monomer of the plasmid substrates, unless stated otherwise. This corresponds to 0.23, 0.31 and 0.29 pmol in the cases of pNK1182, pNK3589 and pNK3590, respectively. Approximately 1.2 pmol of transposase was added per μg of substrate. Transposase was always the last component added to reaction mixtures without a target, but when present the target was added last.

Standard agarose gels, Southern blotting and product quantification were as described by Chalmers and Kleckner (1994) unless stated otherwise. Agarose was either Ultrapure (BRL) or SeaKemp Gold (FMC). All gels were in TBE and Southern blots were probed with the complete sequence of the DNA present. The kinetics of transposition was analyzed by Southern blotting a 1.1% agarose (BRL) gel which had 50 ng of DNA from a standard transposition reaction per lane. Reactions with single end and direct-repeat end substrates were analyzed by ethidium bromide staining of a 0.65% agarose (FMC) gel. There was 0.5 μg of substrate per reaction and, when present, 5 μg of target.

Both of the different two dimensional gels were prepared on the same day from a single gel that constituted the first dimension. The gel (13×26 cm) for the first dimension was 250 ml of 0.65% agarose (FMC). Wells were 1 mm wide and were loaded with 50 ng of DNA from a standard transposition reaction in a volume of 2.5 μl. Electrophoresis was for 17 h at 1.7 V/cm. Two strips 4 mm wide, spanning individual lanes, were cut from the gel and trimmed by 2.5 cm at the top and 2.0 cm at the bottom. One strip was set into the gel for the second dimension without further treatment. The other strip, for the gel with a restriction digestion between the two dimensions, was incubated with gentle agitation for 12 h at 4°C in five changes of 300 ml of *Pae*I digestion buffer (New England Biolabs) in which the magnesium salt was replaced by 1 mM EDTA. After equilibration, digestion was with gentle agitation for 15 h at 37°C in 50 ml of *Pae*I digestion buffer, including magnesium, 100 μg BSA/ml and 6000 units of *Pae*I. Digestion was terminated by equilibrating the strip of gel with a large volume of TBE. For electrophoresis in the second dimension both strips were placed in gel molds, perpendicular to the direction of electrophoresis, and agarose at 60°C was poured in and allowed to cool. For the restriction enzyme digested strip, the gel was 1.1% agarose and electrophoresis was for 21 h at 1.7 V/cm. For the undigested strip, the gel was 0.95% agarose and electrophoresis was for 18.5 h at 2.9 V/cm. The gels (19.5×26 cm) were ~400 ml of agarose (BRL) and both the gels and the tank buffer contained 0.5 μg ethidium bromide/ml.

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