Action of a Transposable Element in Coding Sequence Fusions

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ABSTRACT

The original Casadaban technique for isolating fused cistrons encoding hybrid β-galactosidase proteins used a Mu*cts62* prophage to align the upstream coding sequence and *lacZ* prior to selection. Kinetic analysis of *araB-lacZ* fusion colony emergence indicated that the required DNA rearrangements were regulated and responsive to conditions on selection plates. This has been cited as an example of "directed mutation." Here we show genetically that the MuA and integration host factor (IHF) transposition functions are involved in the formation of hybrid *araB-lacZ* cistrons and propose a molecular model for how fusions can form from the initial strand-transfer complex. These results confirm earlier indications of direct Mu involvement in the fusion process. The proposed model explains how rearranged Mu sequences come to be found as interdomain linkers in certain hybrid cistrons and indicates that the fusion process involves a spatially and temporally coordinated sequence of biochemical reactions.

CASADABAN (1976) described a technique for fusing any *Escherichia coli* coding sequence to *lacZ*. The technique used a transposable Mu*cts62* prophage as portable genetic homology to align the desired *xyzA* coding sequence for protein X and the *lacZ* cistron (Figure 1). The theory of the method was that a subsequent spontaneously arising in-frame deletion would then create an *xyzA-lacZ* hybrid coding sequence directing the formation of an X-β-galactosidase fusion protein. The kinetics of appearance of colonies carrying fusions between the *E. coli araB* cistron and *lacZ* were unexpectedly complex and indicated that the underlying genetic process was regulated and responsive to conditions on the selective medium (SHAPIRO 1984). The cellular events underlying the kinetics of *araB-lacZ* fusion colony appearance have become an important issue in the "directed mutation" controversy (CAIRNS, OVERBAUGH and MILLER 1988; MITTLER and LENSKI 1990). In this paper, we present genetic evidence for a direct role of Mu transposition functions in the formation of hybrid *araB-lacZ* coding sequences and suggest a molecular model of how such functions might act in the fusion process.

Two previous results already suggested that Mu transposition functions played a direct role in the formation of *araB-lacZ* coding sequence fusions. One was the observation that enhanced repression of the Mu*cts62* prophage in the presence of a second Mu*c*$^+$*pAp1* prophage in the pre-fusion strain MCS2 also repressed the appearance of *araB-lacZ* fusion colonies (SHAPIRO 1984). Another indication of an active Mu role came from sequence analysis of fusions made to a variety of upstream coding sequences; these results showed that Mu*cts62* excision was often incomplete, leaving oligonucleotide segments derived from Mu termini as linkers between *lacZ* and its new 5′ sequence (summarized in SHAPIRO 1987). In some cases (discussed in more detail below), these linker regions were inverted. Concerted excision/inversion events may actually be rather common among transposable elements because their occurrence has also been inferred from sequence analysis of reversion events in maize and Antirrhinum (COEN *et al.* 1989).

MATERIALS AND METHODS

Bacterial strains: The basic pre-fusion strains MCS2 and its derivatives MCS1235 and MCS1237 have been described (SHAPIRO 1984). Strain MCS2 was derived from MC4143 (F$^-$ *araD139 araB*::+Mu*cts62* Δ[*lacIPOZYA, argF*]U169 *fla relA rpsL*) by homology-dependent lysogenization with λp1 (209, U118) as schematized in Figure 1 and described by CASADABAN (1976). These strains differ only in that MCS1235 and MCS1237 have the Mu*cts62pAp1* prophage (rather than Mu*cts62*) located between *araB* and the decapitated *lacZ* cistron. The Mu*A2098*::mini-Tn*10* mutation harboring a transposon insertion at coordinate 2 kb on the Mu map was first isolated in the MudII1681*cts62dlac* mini-Mu element of strain MS2098 (SHAPIRO and HIGGINS 1989). The mutation was recombined into the Mu*cts62* prophage of MCS2 by homologous replacement using two different transductional vectors. Strain MCS1330 was isolated by transducing MCS2 with a P1 lysate grown on strain MS2098 and selecting for a Tc^{r}Km^s clone which was no longer thermosensitive for growth and had lost the ability to produce phage. Screening for Km^s ensured that the transductant had not received the MudII1681*A2098*::mini-Tn*10* element. Strain MCS1366 was isolated by transducing MCS2 with a lysate induced from a derivative of MS2098 lysogenic for Mu*cts62pAp1* and selecting for a Tc^r transduc-

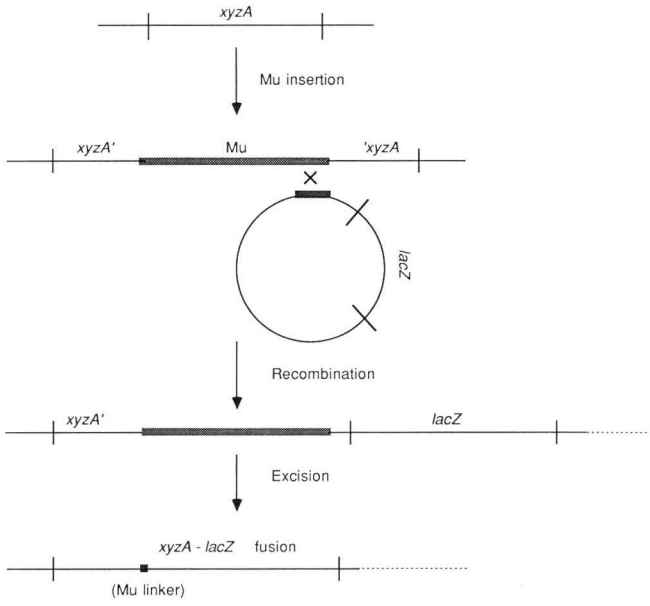

FIGURE 1.—The CASADABAN (1976) technique for fusing *lacZ* to any other coding sequence in the *E. coli* genome. Homologous recombination substrates for aligning the two coding sequences are the end of a Mu prophage inserted in the correct orientation into the chosen cistron (*xyzA*) and a terminal fragment of Mu located upstream of a decapitated *lacZ* cistron in a λ*plac* bacteriophage. The reciprocal recombination event depicted integrates the λ*plac* and positions *lacZ* downstream of *xyzA* to generate the prefusion structure. There is no promoter for *lacZ* transcription in the λ*plac*, and *lacZ* has an *ochre* triplet at codon 18, so that neither transcription nor translation can occur without a fusion to upstream sequences. An appropriate excision event removes all blocks to transcription and translation between the start of *xyzA* and the region that contains the sequence for the catalytically significant domain of β-galactosidase downstream of *lacZ* codon 18. A small number of Mu-derived nucleotides are frequently found in the hybrid coding sequence and constitute the "Mu linker" between the *xyzA* and *lacZ* domains.

tant that was also Km^sAp^s and phage-defective. In both MCS1330 and MCS1366, as mentioned below, the mini-Tn*10* marker was located in the Mu*cts62* prophage between *araB* and *lacZ* because it was deleted in the formation of *araB-lacZ* fusions. Dilysogenic derivatives of MCS1330 and MCS1366, such as MCS1380, were isolated by transducing the defective lysogens with P1 grown on an *arg*::Mu*cts62pAp1* strain, selecting for Ap^r, and screening for Tc^r, Arg^- and phage production phenotypes. Derivatives of MCS2, MCS1235 and MCS1237 carrying the *himA42*, *himAΔ82-Tc*,*hip115*,*hip157*,*hupA16*::*KAN* and *hupB11*::*CAM* mutations were isolated by P1 transduction, selection for linked antibiotic resistance markers, and screening (in the case of the *himA* and *hip* mutations) for loss of thermosensitivity and phage production.

Microbiological methods and scoring of fusion colonies: The basic procedures have been described (SHAPIRO 1984). Briefly, subclones of the various pre-fusion strains were grown overnight in TYE broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7) at room temperature, 10^7–10^8 bacteria of each culture were plated as confluent lawns on L-arabinose + lactose selective agar, and the plates were incubated at 32° with daily scoring for the emergence of fusion colonies. The exact titer of each culture was not determined because the kinetics of fusion colony appearance are independent of inoculum size over several orders of

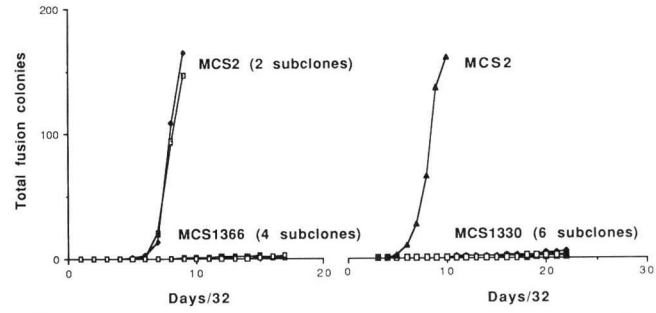

FIGURE 2.—The effect of a Mu*A* defect on fusion colony formation. The abscissa indicates the number of days of incubation at 32° on selective agar before scoring, and the ordinate indicates the cumulative total number of fusion colonies appearing at each scoring. Although not all the separate symbols can be seen in this computer-generated plot of the data, the results are for six platings of MCS1330 subclonal cultures and four platings of MCS1366 subclonal cultures, each harboring the Mu*A2098*::mini-Tn*10* mutation.

magnitude (SHAPIRO 1984). In all experiments, replicate cultures were plated and yielded similar results. The data are presented in the form of graphs showing the cumulative colony totals versus days of incubation at 32° (rather than as histograms of new colonies appearing each day) in order to facilitate comparisons between cultures of different genetic constitutions.

EXPERIMENTAL RESULTS

Defects in Mu transposition functions inhibit fusion colony formation: We took advantage of the recent isolation of a mini-Tn*10* insertion into the Mu*A* cistron of the MudII1681 element (SHAPIRO and HIGGINS 1989) to examine more directly the role of Mu transposition functions in coding sequence fusion. When this mutation was introduced into the pre-fusion strain MCS2, the appearance of fusion colonies was dramatically inhibited, confirming an active role for the Mu*A* transposition function in the formation of *araB-lacZ* fusions (Figure 2). In these experiments, no colonies were observed on any plates before the 4th day of incubation, indicating that no fusion events had occurred during growth in TYE broth prior to plating. The MCS2 control cultures produced colonies starting at day 5, and all three cultures had over 150 colonies by day 10. The Mu*A*-defective cultures MCS1330 and MCS1366 produced very few colonies after 22 and 18 days of incubation, respectively. At later times, more fusion colonies did appear on the MCS1330 and MCS1366 plates, but reliable quantitative data were difficult to obtain from these older plates because it was difficult to distinguish small fusion colonies from non-fusion papillae. As expected, the late-appearing fusion colonies derived from the Mu*A2098*::mini-Tn*10* strains had lost the transposon tetracycline-resistance marker together with the Mu*cts62*prophage.

We also tested pre-fusion strains carrying the *himA42*, *himAΔ82-Tc*, *hip115*, *hip157*, *hupA16*::*KAN*

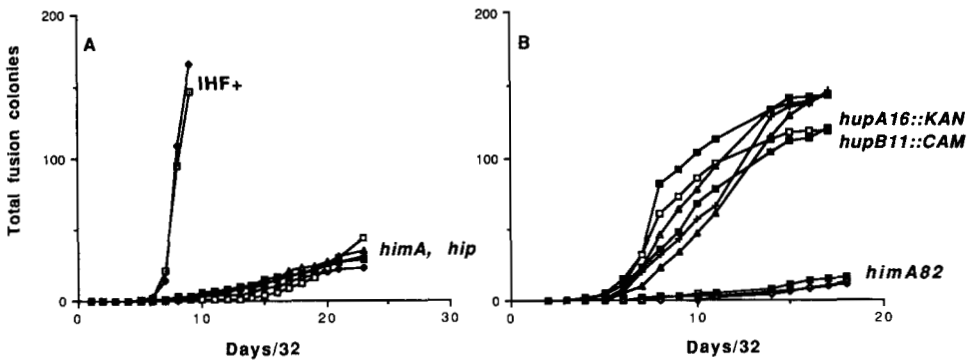

FIGURE 3.—The consequences of mutations affecting IHF and HU proteins for fusion colony formation. The abscissa and ordinate values are as described in the legend to Figure 2. (A) Two MCS2 cultures are marked IHF$^+$ and five IHF-defective cultures are marked *himA*, *hip*. These latter had the *hip115* (two cultures), *hip157* (two cultures) and *himA42* mutations. (B) Four different cultures had the *HimAΔ82-Tc* mutation, four cultures had the *hupA16*::*KAN* mutation, and two cultures had the *hupB11*::*CAM* mutation.

and *hupB11*::*CAM* mutations (Figure 3). The *himA* and *hip* loci encode subunits of the IHF protein which is necessary for normal Mu transcription (KRAUSE and HIGGINS 1986) and which can also play a direct role in the transposition process *in vitro* (SURETTE and CHACONAS 1989). The IHF-defective strains were also inhibited in fusion formation, although not as severely as the Mu*A* derivatives. Reversion of the *himA* and *hip* mutations did not accompany Mu excision because fusion derivatives of these strains all tested IHF$^-$ (determined by sensitivity to Mu*cts62pAp1* lysates), and the IHF$^-$ phenotype did not affect expression of *araB-lacZ* hybrid cistrons because *him A* and *hip* fusion strains grew normally on arabinose-lactose selective agar and required arabinose induction for growth. The *hupA* and *hupB* mutants were moderately retarded in fusion colony emergence, consistent with redundancy of these loci for HU protein function. No Hup$^+$Him$^+$ control is shown in Figure 3B because no parental pre-fusion strain was included in the particular series of platings from which these data are taken. However, other control platings gave higher results for Hup$^+$Him$^+$ strains as shown in Figures 2 and 3A. The level of the *hupA16*::*KAN* and *hupB11*::*CAM* effects on fusion formation can be estimated by comparing colony counts after 9 days of incubation from different experiments: for Hup$^+$Him$^+$, there was a mean of 154 (range 130–168, eight cultures); for *hupA16*::*KAN*, there was a mean of 47 (range of 33–63, four cultures); and for *hupB11*::*CAM*, there was a mean of 82 (range of 72–91, two cultures).

In order to confirm that the inhibition to fusion colony emergence in the Mu*A2098*::mini-Tn*10* strains was specific for the Mu*A* defect, we constructed strains with a Mu*A*$^+$ prophage elsewhere in the bacterial chromosome. This was accomplished by introducing a transposition-competent *arg*::Mu*cts62pAp1* prophage into the genome by P1 transduction, selecting for the ampicillin-resistance marker of the Mu derivative and confirming the arginine requirement. The resulting dilysogens showed complementation by producing many more fusion colonies than did their

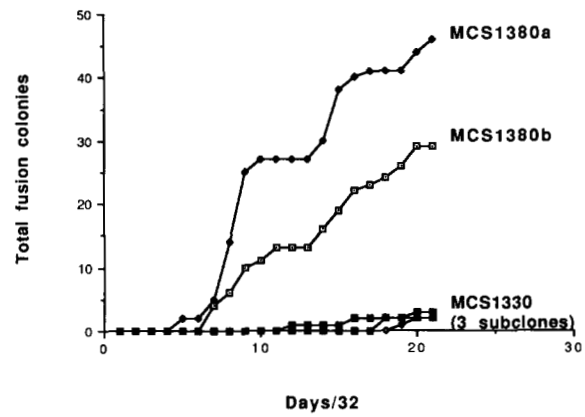

FIGURE 4.—Complementation of the Mu*A* fusion defect. The abscissa and ordinate values are as described in the legend to Figure 2. Strain MCS1380 was derived from the Mu*A2098*::miniTn*10* mutant MCS1330 by introduction of an *arg*::Mu*cts62pAp1* prophage as described in MATERIALS AND METHODS. The results for two independent dilysogenic cultures are shown.

Mu*A*-defective parents, although complementation resulted in the appearance of fewer fusion colonies than obtained from a Mu*A*$^+$ pre-fusion strain (Figure 4). The reduced yield of colonies from these dilysogens (as compared to the parental Mu*A*$^+$ pre-fusion strains) appeared to result from a lower level of prophage derepression when two copies of the Mu*cts62* repressor locus were present in the genome. We have previously reported enhanced repression of Mu*cts62* derivatives in other kinds of dilysogens. Addition of a second Mu*cts62pAp1* prophage into the parental MCS2 strain, which had Mu*A*$^+$ in *cis* to *araB* and *lacZ*, resulted in delayed and reduced fusion yields (SHAPIRO 1984). In addition, the MudII1681 *cts62dlac* fusion element was much less frequently derepressed for transposition and replication during colony development on glucose minimal agar when there were two copies in the genome than when there was only a single copy (SHAPIRO and HIGGINS 1988, 1989). Because the MudII1681 *cts62dlac* colony assay could detect derepression in cells that had lost their ability to reproduce (SHAPIRO and HIGGINS 1989), this last observation was inconsistent with a different explanation for reduced fusion yield from dilysogens,

namely that coincident derepression of the active and defective prophages was lethal to potential fusion progenitors. In order to assess whether fusions arose in bacteria that had undergone some general process of Mu excision, 26 *araB-lacZ* fusion derivatives of the *arg*::Mu*cts62pAp1* strains were purified and tested. Because they all retained an active Mu*cts62pAp1* prophage, no such general excision process had occurred. The multiphasic nature of fusion colony appearance in these complemented Mu*A2098*::mini-Tn*10*/Mu*cts62pAp1* strains was intriguing and merits further investigation because it may shed light on the physiological events which lead to Mu activation and consequent fusion formation. A similar multiphasic pattern was reported for strain MCS2 plated on selective agar enriched with low levels of glucose (SHAPIRO 1984).

We do not know the basis for late fusion colony appearance in lawns of the Mu*A2098*::mini-Tn*10* bacteria. Although it is possible that an alternative, Mu-independent fusion pathway was involved, it should be kept in mind that mini-Tn*10* insertions are subject to precise excision. Thus, fusion colonies could have arisen from bacteria in the MCS1330 and MCS1366 lawns carrying MuA^+ revertant prophages. We were, however, unable to detect any tetracycline-sensitive MuA^+ bacteria in old lawns of MCS1330 and MCS1366 on minimal arabinose-lactose agar by replica-plating of isolated colonies; thus, there was no evidence for a high frequency excision event under selection conditions similar to the excision of *bgl*::IS103 on salicin-containing plates reported by HALL (1988).

MOLECULAR MODEL AND DISCUSSION

The results just presented are strong evidence that Mu transposition functions play an active role in the emergence of *araB-lacZ* fusions. Because the transposon insertion in the Mu*A* cistron did not produce an absolute block to *araB-lacZ* fusions, the possibility remains that other kinds of biochemical activities may have come into play and produced fusions after prolonged incubation. Nonetheless, the initial fusion waves seen on plates seeded with MCS2 must have involved Mu*A* activity because they were eliminated by the Mu*A2098*::mini-Tn*10* mutation (Figure 2). The IHF protein involved in expression of Mu transposition/replication functions was also seen to be involved in fusion events, although *himA* and *hip* mutations did not have as strong an effect as the Mu*A* block.

Coding sequence fusion as a complex biochemical event: Sequence analysis had provided one of the initial clues to the role of Mu transposition functions in cistron fusions. A number of hybrid β-galactosidase coding sequences prepared by the Casadaban technique contained nucleotides from the S (or *attR*) end

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Mu S terminus:
TGAAGCGGCG CACGAAAAAC GCGAAAGCGT TCACGATAAA TGCGAAAACT TTAGCTTTCG
ACTTCGCCGC GTGCTTTTTG CGCTTTCGCA AGTGCTATTT ACGCTTTTGA AATCGAAAGC
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malF fusion 6-3:
asp ala leu ser arg phe ser cys ala ala ser thr gln leu
GAC GCG CTT TCG CGT TTT TCG TGC GCC GCT TCA ACC CAA CTT
CTG CGC GAA AGC GCA AAA AGC ACG CGG CGA AGT TGG GTT GAA
FFF F-- --- --- --- --- --- --- --- --- --> ZZZ ZZZ ZZZ
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FIGURE 5.—Sequence of a *malF-lacZ* fusion isolated by the CASADABAN technique (FROSHAUER and BECKWITH 1984). The top sequence is the *S* terminus of the Mu*cts62* prophage which abutted *malF* nucleotides in the pre-fusion strain. The bottom sequence is the junction between *malF* and *lacZ* connected by a Mu-derived linker in the hybrid *malF-lacZ* cistron. Base-pairs derived from *malF* are indicated by F, base-pairs from *lacZ* by Z, and base-pairs from Mu by dashes and (for the terminal base-pair) an arrowhead.

of Mu as a linker between the amino terminal domain of the hybrid cistron and the *lacZ* sequences (summarized in SHAPIRO 1987). These Mu nucleotides were frequently rearranged in a particular way: they were inverted from their position in the prefusion strain, and the terminal sequences previously adjacent to the amino terminal domain of the upstream coding sequence now abutted the *lacZ* portion of the hybrid cistron. The sequence of *malF-lacZ* fusion 6-3 (FROSHAUER and BECKWITH 1984) illustrates this structure (Figure 5). These inverted Mu nucleotides could not have come from a simple Mu-*lacZ* deletion, and the fact that one of the inversion breakpoints was located precisely at a Mu terminus indicated a role for Mu*A* activity, which is known to cleave the phosphodiester bond between the last Mu nucleotide and the adjacent chromosomal nucleotide (CRAIGIE and MIZUUCHI 1987).

Molecular model for the role of Mu transposition functions in coding sequence fusions: Rearrangements like the ones reported by FROSHAUER and BECKWITH can be explained as the derivatives of a normal Mu*AB*-mediated strand-transfer product in which the Mu termini had ligated to a sequence in *lacZ* (Figure 6). The formation of the plectonemic complex and strand-transfer reactions in this model would be standard (CRAIGIE and MIZUUCHI 1987). This kind of strand-transfer product would be a precursor of an adjacent inversion product in the event that Mu replication were to be completed (SHAPIRO 1979). When unfolded, the strand-transfer product would have a branched structure that could permit the ligation of sequences upstream of the Mu*c* (*attL*) terminus (I′ in Figure 6*B*) to the free 3′ hydroxyl group of the cleaved *lacZ* strand (VI′ in Figure 6*B*). The ligation of upstream and *lacZ* sequences could occur before or after chain elongation. In the latter case, some nucleotides from the Mu S terminus would be included in an inverted orientation adjacent to *lacZ* sequence. The consequence of this ligation would be to generate a continuous DNA strand which, theoretically, might serve as a template for RNA polymerase from the

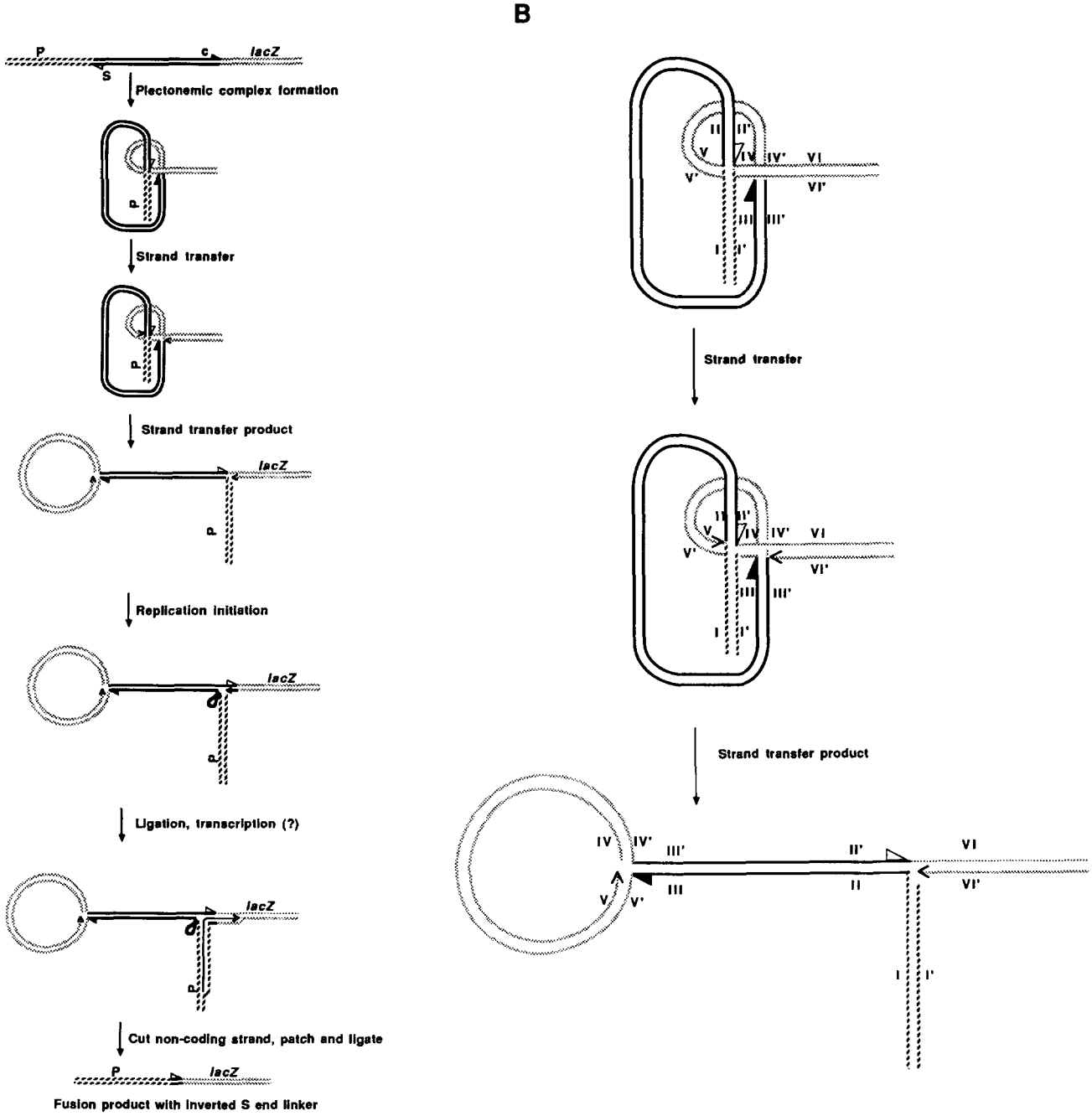

FIGURE 6.—A molecular model for Mu*A* activity in genetic fusions. (A) The top line schematizes the region of a pre-fusion strain with the Mu*cts62* prophage located between an upstream transcription unit (**P** = promoter) and a decapitated *lacZ* cistron. The two ends of the Mu prophage are indicated **c** (closest to the repressor cistron, solid arrowhead) and **S** (closest to the *S* cistron, open arrowhead). The first step in the fusion process is the same as the first step in Mu prophage replication and involves the formation of a plectonemic complex bringing together the two Mu termini and the target sequence, which in this case is located in the 5′ region of the *lacZ* cistron, in the appropriate geometry. The strand transfer reaction results in the ligation of 3′ hydroxyl groups from each Mu extremity (here indicated by the open and solid arrowheads) to 5′ phosphate groups spaced 5 base pairs apart at the target sequence; strand transfer also leaves two exposed 3′ hydroxyl groups in the flanking target DNA which may serve as primers for leading strand chain elongation (indicated by arrowheads) as well as two 5′ phosphate groups which were formerly attached to the Mu extremities. These two steps require Mu A and B and the *E. coli* HU proteins (CRAIGIE and MIZUUCHI 1987). The strand transfer product can be redrawn as a branched molecule with upstream and *lacZ* sequences in close proximity. If the 3′ hydroxyl group on the bottom strand of the *lacZ* sequence is used for chain elongation, strand displacement will result as indicated (**Replication Initiation**), and the newly replicated Mu **S** extremity may then be ligated to the free 5′ phosphate group on the bottom strand of the upstream sequence. This ligation will produce a continuous strand which may serve as a template for transcription from the promoter (**P**) in the upstream sequence. Either with or without such transcription, the displaced Mu DNA sequences may be endonucleolytically cleaved, leading to exonucleolytic resection and removal of the stalked structure. Polymerase patching using the intact coding strand as a template and religation would then generate a complete fusion product containing the rearranged Mu **S** terminal sequences. (B) The easiest way to follow the strand transfers is by labeling segments of the duplexes in the plectonemic complex and comparing them with the opened strand transfer product. In this cartoon, the arrangement of the plectonemic complex is depicted with the segments carrying Mu*cts62* termini passing above (I–I′ to II–II′) and below (III–III′ to IV–IV′) the segment carrying the *lacZ* target sequence (V–V′ to VI–VI′). Note where segments of previously complementary strands are joined at the two 3′ extremities of the Mu*cts62* prophage.

upstream promoter. In this way, a hybrid β-galactosidase could be synthesized from a cell which did not yet contain a stable fusion structure. Removal of the noncoding strand from the transcribed region (perhaps facilitated by R-loop formation), polymerase I patching, and ligation would complete formation of the stable fusion.

The model in Figure 6 provides a straightforward role for Mu*A* activity, consistent with the protein's known biochemical properties, in coding sequence joining by the CASADABAN technique. It also explains the structures of complex fusion events with inverted Mu linkers that are difficult to understand on other kinds of break-and-join models. Several additional fusion structures have been described (SHAPIRO 1987). These include fusions with no Mu linkers, fusions with Mu*S* terminus linkers in their original orientations, and at least one fusion with internal rearrangement of the Mu linker (FROSHAUER and BECKWITH 1984). We have no detailed explanation for the last type of fusion. The model in Figure 6 can readily explain the fusions without a Mu linker by postulating that no chain elongation occurs before ligation and patching. To explain the fusions with Mu*S* terminus linkers in their original orientation requires additional assumptions. (They are not explained by reversing the orientation of Mu termini with respect to the target sequence in the plectonemic complex because that leads to fusions containing Mu*c* terminus linkers.) Since Mu*A* appears to be required for most (if not all) fusions, we may assume that an incomplete strand transfer reaction can lead to exonucleolytic degradation in both directions from a cleavage in the Mu*c* terminus-*lacZ* region and that subsequent ligation and patching will produce the fusions without disrupting the linkage between the Mu*S* terminus and the upstream coding domain. The model in Figure 6 has one further feature which may prove important in understanding the regulation of fusion events and the emergence of fusion colonies: a hybrid transcription template can be formed before all DNA rearrangements have been completed. It is possible that the hybrid RNA molecules could serve as templates for guiding the fusion process, possibly by reverse transcription (as suggested by CAIRNS, OVERBAUGH and MILLER 1988) or by other molecular mechanisms that remain to be defined.

The observation that Mu transposition functions play an active role in coding sequence joining is consistent with the recent results of MITTLER and LENSKI (1990) and helps to clarify the kinetics of fusion colony appearance. One important step in the DNA rearrangements needed to generate a fused *araB-lacZ* coding sequence is the activation of Mu transposition functions, and this activation could occur either during incubation on the selection medium (SHAPIRO 1984) or during prolonged aeration in glucose-minimal medium (MITTLER and LENSKI 1990). Such activation would be independent of the presence of arabinose and lactose and probably would involve processes similar to those which lead to the periodic derepression of a related MudII1681 *cts62dlac* element in colonies growing on glucose-minimal agar (SHAPIRO and HIGGINS 1989). Once Mu transposition functions are present in the pre-fusion strain, the events leading to the DNA rearrangements cartooned in Figure 6 would require the construction of a multicomponent nucleoprotein complex and the accurate execution of a coordinated series of biochemical reactions. There could be many possibilities for regulation and specificity at this stage of the fusion process, as suggested by CAIRNS, OVERBAUGH and MILLER (1988), and the results of MITTLER and LENSKI (1990) do not exclude a role for substrate-directed events in steps such as the choice of *lacZ* target sequences. Only further research will decide whether the strong form of the directed mutation hypothesis (*i.e.* that substrate plays a direct informational role in guiding adaptively useful DNA rearrangements) is correct for the formation of *araB-lacZ* fusions. The weak form of the hypothesis (*i.e.*, that selective conditions can stimulate the occurrence of DNA rearrangements needed for proliferation) has been confirmed in this system by all investigators.

Generality of mutational systems involving transposable elements: In discussing the relevance of the *araB-lacZ* fusion system to general theories of mutation, it has been argued that the presence of a Mu*cts62* prophage constitutes an artificial or exceptional element. Such arguments have been raised in evolutionary discussions ever since the first report of transposable elements and the proposal that they are major agents of genetic change (MCCLINTOCK 1950). Nowadays we know that transposable elements are not exceptional but are ubiquitous in the genomes of all organisms that have been studied (BERG and HOWE 1989). In Drosophila, moreover, the large majority of spontaneous mutations involve transposable elements (GREEN 1988), and there are certain naturally occurring situations, like hybrid dysgenesis (ENGELS 1989), where transposable elements can bring about major changes in genome structure. Thus, it is not realistic to exclude cases involving transposable elements from general discussions of genetic mutability. One of the salient features of all transposable elements studied is that their DNA rearrangement activities are subject to multiple levels of regulation (BERG and HOWE 1989). In our opinion, the real resolution to the directed mutation controversy will come when we have a much deeper understanding of this regulation and its connections to the control networks which govern all aspects of genomic functioning.

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