Autoregulation of *mariner* Transposase Activity by Overproduction and Dominant-Negative Complementation

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Genetic studies of the *mariner* transposable element *Mos1* have revealed two novel types of regulatory mechanisms. In one mechanism, overproduction of the wild-type transposase reduces the overall level of transposase activity as assayed by the excision of a nonautonomous *mariner* target element. This mechanism is termed overproduction inhibition (OPI). Another mechanism is observed in a class of hypomorphic missense mutations in the transposase. In the presence of wild-type Mos1 transposase, these mutations exhibit dominant-negative complementation (DNC) that antagonizes the activity of the wild-type transposase. We propose that these regulatory mechanisms act at the level of the transposase protein subunits by promoting the assembly of oligomeric forms, or of mixed-subunit oligomers, that have reduced activity. We suggest that these regulatory mechanisms may apply generally to *mariner*-like elements (MLEs). Overproduction inhibition may help explain why the MLE copy number reaches very different levels in different species. Dominant-negative complementation may help explain why most naturally occurring copies of MLEs have been mutationally inactivated.

Introduction

Soon after its initial discovery in *Drosophila mauritiana* and related species (Jacobson, Medhora, and Hartl 1986; Maruyama and Hartl 1991*a*), the transposable element *mariner* was shown to be present in a wide variety of arthropods (Lidholm, Gudmundsson, and Boman 1991; Maruyama and Hartl 1991*b*; Robertson 1993; Robertson and MacLeod 1993). The *mariner*-like elements (MLEs) can be classified into several distinct subfamilies, 40%–56% identical at the nucleotide level (Robertson and MacLeod 1993; Bigot et al. 1994). In a PCR survey of 404 insect species using primers for conserved regions of the MLEs in *D. mauritiana* and *Hyalophora cecropia,* 63 of the genomes of the species were found to contain MLEs (Robertson and MacLeod 1993). The species in which MLEs are present represent all major insect orders. MLEs are also found in invertebrates other than insects, including the nematode *Caenorhabditis elegans* and the planarian *Dugesia* (*Girardia*) *tigrina* (Garcia-Fernàndez et al. 1995). Apart from invertebrates, MLEs are present in the fungus *Fusarium oxysporum* (Langin, Capy, and Dabouss 1995) as well as in certain vertebrate species, including the human genome (Augegouillou et al. 1995). It is reasonable to suppose that the full phylogenetic distribution of MLEs is considerably wider than presently appreciated.

MLE sequences are typically about 1.3 kb in length and have short, approximately 30 bp, inverted repeats. Their target site is the dinucleotide TA, which is duplicated upon insertion of the transposon. The MLEs are members of a larger class of transposable elements whose transposase contains the so-called "D,D(35)E" motif of aspartic acid (D) residues and a glutamic acid residue (E) separated by approximately 35 amino acids (Kulkosky et al. 1992; Doak et al. 1994). The D,D(35)E motif is thought to be a binding domain for a divalent cation necessary for catalysis (Kulkosky et al. 1992). The motif is found in the *Tc1* family of transposable elements as well as in MLEs. The D,D(35)E motif is also present in bacteriophage Mu and in certain bacterial insertion sequences as well as in some vertebrate retroviruses (Craig 1995). Among all these proteins, the transposase of the MLEs is unique in having the D,D(35)E motif replaced with D,D(35)D.

The number of MLEs per genome varies widely from one species to the next. The numbers are modest in most *Drosophila* species (Maruyama and Hartl 1991*a*; Capy et al. 1991; Brunet et al. 1994) but not in all (Lohe et al. 1995). At the other extreme, the copy number is on the order of 1,000 per haploid genome in *H. cecropia* (Lidholm, Gudmundsson, and Boman 1991), 8,000 in *D. tigrina* (Garcia-Fernàndez et al. 1995), and 17,000 in the horn fly *Haematobia irritans* (Robertson and Lampe 1995). A given species can contain members of two or more subfamilies of MLEs, and it is not known whether the copy numbers of coexisting subfamilies are correlated. Paradoxically, the overwhelming majority of MLEs so far examined are nonfunctional owing to multiple frameshift mutations, small deletions, or translational termination mutations. Only one small category of active *mariner* elements, the *Mos1* class from *D. mauritiana* and related elements from *D. simulans,* has as yet been isolated (Medhora, MacPeek, and Hartl 1988; Capy et al. 1992).

What regulates the number of MLE copies per genome? Overshadowed by studies of the phylogenetic distribution of the elements and the search for evidence for horizontal transmission, this issue has been given little consideration. One possibility is that there is no regulation. Once in a genome, MLEs might transpose as often as possible until the increase in number is offset by natural selection resulting from detrimental effects in the host. In such a case, the equilibrium copy number should be positively correlated with genome size, as a greater amount of "junk" DNA will tolerate more insertions, and negatively correlated with effective population size, as insertions with detrimental effects on fit-

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ness are eliminated more effectively in large populations.

On the other hand, there may be mechanisms intrinsic to the molecular genetics of MLEs and to their interactions with the host that result in self-regulation of transposition. In this paper, we report two kinds of genetic evidence for the existence of such regulatory mechanisms. The evidence comes from studies of the *Mos1* element from *D. mauritiana* when present in the genome of the sibling species *D. melanogaster.* In one mechanism, overproduction of the wild-type transposase reduces the overall transposase activity; in the other mechanism, the production of transposase molecules with certain amino acid replacements interferes with the activity of wild-type transposase molecules present in the same cell.

Materials and Methods

Drosophila Strains

The w^{pch} "allele" refers to a recombinant DNA construct in which a *Bam*HI fragment containing a *mariner* element in the upstream region of the *white* gene of *D. mauritiana* was used to replace the homologous *Bam*HI fragment spanning coordinates 1.0 through 4.0 in the *white* gene of *D. melanogaster* (coordinates as in Bingham, Levis, and Rubin 1981). This chimeric gene was introduced into the *X* chromosome in the germline of *D. melanogaster* by *P*-element transformation (Garza et al. 1991). The *mariner* element in the w^{pch} allele, called the *peach* element, produces a nonfunctional transposase. In the presence of functional transposase from another source, the *peach* element is excised from the *white* gene in some somatic lineages. Excision in pigment cells of the eye restores the red pigment, and excision events result in mosaic eye color. Excision in germline cells results in progeny with w^+ eyes.

The symbol *h70–182* refers to a *P*-element construct, *P*[ry^+, *hsp70:Mos1*]*-182,* inserted by germline transformation in polytene chromosome band 33C6 in chromosome *2*. In this construct, the Mos1 transposase is under the control of a dual promoter consisting of the *hsp70* heat shock promoter joined to the endogenous *Mos1* promoter. The *h70–56* insertion is a similar *P*[ry^+, *hsp70:Mos1*] construct inserted in polytene chromosome section 61 of chromosome *3*. The designation *h26–67* refers to a chromosome *2* insertion of *P*[ry^+, *hsp26-Sgs3:Mos1*]*-67*; in this construct, the Mos1 transposase is driven by dual promoters consisting of the *Sgs3* promoter juxtaposed with the germline-specific enhancer *hsp26* (Frank, Cheung, and Cohen 1992) and joined to the endogenous *Mos1* promoter. For more detail on these constructs and strains containing them, see Lohe, Lidholm, and Hartl (1995).

EMS Mutagenesis

To identify single amino acid residues that are essential for Mos1 transposase function, ethylmethane sulfonate (EMS) mutagenesis was carried out in a stock homozygous for *h70–182* and a w^- mutation. The protocol for EMS mutagenesis was kindly provided by J. Belote and is described briefly. Newly emerged males were aged 3–5 days, and about 100 males were placed in a bottle containing a filter paper soaked in a solution of 20 mM EMS in 1% sucrose. The males were permitted to feed on the solution for 24 h and then placed in a bottle with fresh food to recover for a further 24 h. Sixty crosses were set up in bottles, each with 5–6 males and an excess of w^{pch} females. The flies were transferred onto fresh food 2 days, 5 days, and 8 days after EMS treatment. The F_1 progeny from the second and third broods were scored for reduced or absent eye-color mosaicism. Nineteen mutants that showed a clear reduction in the amount of w^{pch} mosaicism were recovered. The phenotypes of different mutants ranged from an absence of mosaicism to a low level of mosaicism (1–5 spots per eye) to a higher level of mosaicism with approximately 100 spots per eye. The normal level of mosaicism in the w^{pch}; *h70–182/+* genotype is at least 500 spots per eye, even in the absence of heat shock.

Each of 19 mutations mapped to chromosome *2*, suggesting that they were all located in the *h70–182* transposase gene. (Although the mutation screen might also have recovered mutations in host genes that decrease the rate of w^{pch} excision, such mutations would not necessarily map to chromosome *2*). Stocks were made by crossing putative mutants to a w^{pch}; *CyO/Sco* balancer stock. The mutant females tended to have twice as many spots per eye as males, probably because they carry two copies of the target w^{pch} allele on the *X* chromosome. Complementation crosses were set up among the mutants and also between the mutants and the transposase sources *h70–182* and *h70–56.*

Heat Shock Regime

Heat shock was carried out at 37°C for 1 h at 4–6, and 8 days after hatching.

Results

Genetic studies of the Mos1 transposase suggest two distinct types of regulatory mechanism. The first we call overproduction inhibition (OPI), in which excessive quantities of the wild-type transposase significantly reduce the overall frequency of excision of a target *mariner* element. The second mechanism results from dominant-negative complementation (DNC), which is observed in a significant proportion of hypomorphic mutations in the transposase.

Regulation by Overproduction Inhibition

The data in table 1 support the overproduction mechanism. The symbol *h70–182* refers to a *P*-element construct inserted into chromosome *2* in which transcription of the *Mos1* open reading frame is driven by a chimeric *heat-shock-protein-70* promoter juxtaposed with the endogenous *Mos1* promoter. The *h70–56* construct is identical with *h70–182* except that the insertion is in chromosome *3.*

In table 1, the assay of transposase function is the frequency of germline excision of a target *mariner* element present in the 5′ regulatory region in the *white-peach* (w^{pch}) allele. The *mariner* element present in w^{pch},

Table 1
Effects of Gene Dosage and Heat Shock (HS) on Transposase-induced *mariner* Excision from the w^{pch} Allele

Condition	Genotype	Growth Regime (°C)	Total Progeny	Percent Excision ± SE
A	*h70-182/+;+/+*	25	255	16.9 ± 2.3
B	*+/+;h70-56/+*	25	514	11.1 ± 1.4
C	*h70-182/+;h70-56/+*	25	2,377	9.8 ± 0.6
D	*h70-182/+;+/+*	HS	514	12.8 ± 1.5
E	*+/+;h70-56/+*	HS	1,608	10.8 ± 0.8
F	*h70-182/+;h70-56/+*	HS	2,347	6.2 ± 0.5

Comparison		χ^2	*P* value[a]
Single dose versus double dose, 25°C	(A + B versus C)	6.5	≈0.01
Single dose versus double dose, HS	(D + E versus F)	35.2	≪0.01
Single dose, 25°C versus HS	(A + B versus D + E)	1.6	≈0.22 (NS)
Double dose, 25°C versus HS	(C versus F)	19.1	≪0.01

[a] NS = not significant.

which is called the *peach* element, does not produce an active transposase, but *peach* can be excised by the Mos1 transposase (Maruyama, Schoor, and Hartl 1991). Excision of the *peach* element results in reversion to a wild-type phenotype.

The entries in table 1 show the effects of increased transposase resulting either from additional copies of a transposase source or from heat shock of the *hsp70:Mos1* constructs. Overproduction of transposase by either means results in a smaller frequency of excision. The levels of statistical significance are given at the bottom of the table. The inhibitory effect of excess transposase is highly significant in all cases except for the singe dose with heat shock (A + B versus D + E). In the latter case, there is a reduction in excision rate from 13.0% to 11.3%, but the lack of statistical significance suggests that the effect of heat shock on the chimeric *hsp70-Mos1* promoter is to produce a smaller increment of protein than that resulting from an additional dose of *hsp70:Mos1* at 25°C.

Regulation by Dominant-Negative Complementation

The dominant-negative complementation (DNC) type of *mariner* regulation results from certain hypomorphic mutant alleles of the transposase. To obtain such mutations, adult males of genotype *h70–182* were treated with EMS and crossed with w^{pch} females. Male progeny were examined for those in which the level of eye-color mosaicism was noticeably reduced. Putative hypomorphic mutations were reassayed by additional crosses and mapped. All mapped to chromosome 2, consistent with their having one or more nucleotide substitutions in the *h70–182* transposase gene. The molecular basis of each putative transposase mutation was confirmed by PCR and DNA sequencing (data not shown).

In carrying out crosses with the transposase mutations, we noted that some of them showed strong dominant-negative complementation with the nonmutant *h70–182* transposase source. The data are shown in table 2. The EMS mutants are designated *EMS-73, -1043,*

Table 2
Effects of Dominant-Negative Transposase Mutations on Transposase-induced *mariner* Excision from the w^{pch} Allele

Genotype	Growth Regime (°C)[a]	Total Progeny	Percent Excision ± SE	χ^2	*P* value[b]
h70-182/+	25	2,269	14.7 ± 0.7		
h70-56/+	25	952	12.9 ± 1.1		
h26-67/+	25	708	12.0 ± 1.2		
EMS-73/+	25	355	4.8 ± 1.1	26.1	≪0.01
EMS-1043/+	25	325	1.5 ± 0.7	58.4	≪0.01
EMS-1034/+	25	389	0.8 ± 0.4	43.5	≪0.01
EMS-113/+	25	326	0.0 ± 0.0	55.1	≪0.01
EMS-73/h70-182	25	987	7.4 ± 0.8	33.7	≪0.01
EMS-1043/h70-182	25	1,022	5.9 ± 0.7	42.3	≪0.01
EMS-1034/h70-182	25	1,150	9.6 ± 0.9	18.0	≪0.01
EMS-113/h70-182	25	2,919	2.5 ± 0.3	261.6	≪0.01
EMS-113/+;h70-56/+	25	1,215	2.3 ± 0.4	92.8	≪0.01
EMS-113/h26-67	25	740	1.8 ± 0.5	60.2	≪0.01
EMS-113/h70-182	HS	772	2.2 ± 0.5	24.1	≪0.01
EMS-113/+;h70-56/+	HS	986	1.3 ± 0.4	2.9	≈0.09 (NS)
EMS-113/h26-67	HS	863	0.0 ± 0.0	15.3	≪0.01

[a] HS = heat shock.
[b] NS = not significant.

-1034, and *-113.* The designation *h26–67* refers to a *P*-element construct inserted in chromosome 2 in which transcription of the *Mos1* open reading frame is driven by a chimeric *Sgs3* glue protein promoter and *heat-shock-protein-26* enhancer along with the endogenous *Mos1* promoter. The *hsp26-Sgs3:Mos1* construct is efficiently transcribed in the germline but is not responsive to heat shock (Frank, Cheung, and Cohen 1992; Lohe, Lidholm, and Hartl 1995).

The upper part of table 2 shows the frequency of w^{pch} excision in the nonmutant *h70* and *h26* constructs along with those of the EMS mutations. It is clear that the mutations have profound effects in the germline as well as in the soma. For each mutant, the χ^2 value is the contingency χ^2 for the EMS mutation compared with the nonmutant *h70–182.*

The effect of dominant-negative complementation is shown by the second group of genotypes, which are heterozygous *EMS-i/h70–182.* The χ^2 values compare the germline excision frequency in *EMS-i/h70–182* with that in *+/h70–182.* In all cases, the heteroallelic combination has a significantly lower excision rate than the *+/h70–182* control. That the inhibitory effect applies to transposase sources other than *h70–182* is evident in the third set of genotypes in table 2, in which *EMS-113* is combined with *h70–56* or with *h26–67.* In each case, the χ^2 value is for the comparison of the specified genotype against the *h70–56/+* or *h26–67/+* control.

The bottom part of table 2 shows the combined effects of OPI and DNC. For each genotype, the frequency of excision with heat shock has been compared with a 25°C control. Heat shock results in a significant reduction in *EMS-113/h70–182.* There is also a reduction in excision frequency in *EMS-113/+; h70–56/+,* but it is of borderline statistical significance. In both these genotypes, heat shock induces both the *EMS-113* and the *h70:Mos1* promoters. However, the largest reduction is observed in *EMS-113/h26–67,* very likely because, in this case, the heat shock induces *EMS-113* but not *h26–67,* yielding a relatively greater DNC than in the other genotypes.

Discussion

The genetic evidence presented here implies the existence of two quite distinct mechanisms that regulate *mariner* activity as assayed by excision of the *peach* element from the w^{pch} allele. The first is by overproduction of wild-type transposase and the second is by dominant-negative complementation. We suggest that these mechanisms may play a role in the regulation of *mariner* transposition in natural populations. Other regulatory mechanisms may also play a role; for example, titration of active transposase by the intact ends of nonautonomous elements.

Overproduction Inhibition

Overproduction inhibition results from a dosage effect and may be mediated through any of a number of molecular mechanisms. One possibility that can be rejected is reduced transcription by means of transvection along the somatically synapsed homologous chromosomes. Although transvection can explain the reduced transposase activity in the genotype *h70–182/h70–182* relative to *h70–182/+,* and in the genotype *h70–56/h70–56,* relative to *h70–56/+,* transvection cannot account for the same effect being observed in *h70–182/+; h70–56/+.*

It therefore seems likely that OPI results from post-translational interactions between wild-type protein subunits. Suppose, for example, that the transposase can exist as two distinct types of oligomers—say, a dimer or a tetramer. Then overproduction of the wild-type transposase may shift the equilibrium toward formation of the higher order oligomer. It has been reported that the *Krüppel* transcription factor in *Drosophila* can function as either a monomer or a dimer; the monomer is a transcriptional activator, the homodimer a repressor (Sauer and Jäckle 1993). This example opens the interesting possibility that OPI could act at the level of transcription through autorepression of the *Mos1* promoter. On the other hand, OPI may act at the post-translational level if an excess of wild-type subunits promotes the formation of catalytically less active or inactive oligomers. For example, if the oligomer with the greater number of subunits is less active or inactive, then overproduction of the wild-type transposase may shift the equilibrium toward the less active oligomer, and the overall transposase activity will decrease. Alternatively, an excess of wild-type subunits may promote formation of an inactive oligomer of the same composition as the active transposase. For example, the phage Mu transposition reaction takes place within a higher order DNA-protein complex called a transpososome, at the core of which is a tetramer of Mu transposase (MuA) bound to the ends of the Mu genome (Savilahti, Rice, and Mizuuchi 1995). The Mu tetramer is formed in contact with the DNA. In the case of OPI, it is possible that excess transposase subunits promote oligomerization in the absence of DNA, hence inactivating the participating monomers.

The implication of OPI for *mariner* regulation is that each genome will have an optimal number of copies at which the rate of transposition per element is maximized. As the number of active copies grows above the optimum, overall transposase activity decreases. Because OPI does not completely repress transposition, the number of copies will continue to increase, though at a reduced rate. If, because of OPI, each additional copy results in a net reduction in the rate of transposition per element, then the rate of increase in copy number must decrease over time. However, even with a low rate of transposition, the copy number can increase because of Mendelian segregation and recombination among the copies already present. What is the optimal number of copies per genome? It will depend on the effectiveness of transcriptional activation and translation, which is determined by the type of MLE and by the genome of the organism in question. The higher the level of expression of an MLE, the smaller the number of copies needed for OPI. Differing levels of expression in different genomes may help explain why some species have only a few

copies of an MLE whereas others have thousands of copies.

Dominant-Negative Complementation

Dominant-negative complementation may have a number of possible molecular mechanisms, but the most likely is the formation of oligomers of reduced activity that contain both wild-type and defective subunits. In *Drosophila,* certain missense mutations in the gene for the dimeric Cu/Zn superoxide dismutase show dominant-negative complementation in which the enzyme activity of the *wildtype/mutant* heterozygote is 13%–37% of that observed in *wildtype/deletion* heterozygotes (Phillips et al. 1995). The mutant subunits are "poison subunits" inasmuch as they reduce the activity of wild-type subunits with which they form oligomers. In the case of the *Mos1* transposase, the mixed oligomers may have reduced transposase activity but may also have the effect of hindering the accessibility of the target *peach* element by DNA binding. In this manner, the effects of DNC may be at the protein level, at the target DNA level, or both.

Does DNC play a role in regulation in nature? In this context, a highly relevant observation is that the vast majority of MLEs from natural populations do not produce an active transposase even though some, such as the *peach* element, have a conserved open reading frame (Maruyama, Schoor, and Hartl 1991; Capy et al. 1992). It is also instructive to consider the time required for mutation pressure alone to reduce the number of active copies of a transposable element by half. The "half-life" of the elements in a genome equals approximately $0.7/\mu$, where μ is the probability of mutation per element per generation from an active to an inactive form. Approximately five half-lives, or $3.5/\mu$ generations, are required to mutationally inactivate 97% of the copies. If $\mu = 10^{-5}$, for example, then $3.5/\mu = 350{,}000$ generations. Thus, many of the defective elements in natural populations may have been inactivated purely by mutation pressure. On the other hand, some mutant elements may be positively selected for their repressing effect through DNC, and even copies with nonsense or frameshift mutations may play a role in regulation through titration of the transposase. The role of DNC and transposase titration in nature needs to be addressed experimentally. For example, one testable implication of DNC is that genomes containing multiple inactive MLEs should be resistant to invasion by closely related functional elements owing to the residual production of the DNC subunits that repress transposition.

Comparison with *P*-Element Regulation

Because the *P* element was one of the first transposable elements discovered in *Drosophila* (Bingham, Kidwell, and Rubin 1982), there is a tendency to use *P* as a standard of reference against which every other transposable element in *Drosophila* must be compared. However, *P*-element regulation exhibits a number of features that appear not to be typical of other transposable elements (see Lozovskaya, Hartl, and Petrov 1995 for a recent review). For example, the *P* element is expressed only in the germline and regulated at the level of RNA splicing (Laski, Rio, and Rubin 1986; Roche, Schiff, and Rio 1995). Furthermore, the *P*-element induces a cytoplasmically inherited "*P* cytotype" that represses transposition. The repressing effect of *P* cytotype produces the characteristic difference between reciprocal crosses observed in the syndrome of P-M hybrid dysgenesis (Kidwell, Kidwell, and Sved 1977; Engels 1989). Cytotype determination is still poorly understood. It depends not only on the presence of a certain type of deleted *P* element, called the *KP* element, in the genome (Andrews and Gloor 1995), but it also depends on the positions of the *KP* elements (Misra et al. 1993). It has been suggested that some *KP*-like elements may repress owing to antisense RNA whose transcription is driven by read-through from external promoters (Rasmusson, Raymond, and Simmons 1993).

The *mariner*-like elements differ from *P* in many ways. MLEs have an extraordinarily wide host range and very high copy numbers are possible. The elements are expressed in somatic cells as well as in the germline. Although most naturally occurring MLEs have been mutationally inactivated, they are nevertheless quite uniform in size. Although there are many relatively small deletions/insertions, large deletions of the type typically observed in naturally occurring *P* elements are not found. Most importantly, nothing analogous to cytotype determination has been reported for *mariner*-like elements or, as far as we are aware, for any other elements in the *Tc1/mariner* superfamily. Likewise, no syndrome of hybrid dysgenesis mobilizing a *mariner*-like element has been reported (but see Petrov et al. 1995 for a possible case implicating a *Drosophila Tc1*-like element).

There are, however, two features of *P*-element regulation that warrant additional discussion. The first is the nature of the *KP* elements. These elements produce a truncated transposase protein whose effect is to inhibit the overall rate of transposition. It has recently been demonstrated that certain mutations in the leucine-zipper motif implicated in protein–protein interactions eliminate the repressing effect of *KP* (Andrews and Gloor 1995). It is not clear whether the KP protein interacts with wild-type transposase subunits or with host factors required for transposition.

The "multimer poisoning" model of *KP* repression (Andrews and Gloor 1995) is analogous in broad outline to the dominant-negative complementation observed with *Mos1* but substantially different in the details. The *KP* element is a unique type of deletion that may have arisen only once and spread throughout the species. In the case of *mariner,* dominant-negative complementation may characterize a significant proportion of hypomorphic EMS-induced transposase mutations; each of the first three tested showed this phenomenon (table 2).

The second feature of *P*-element regulation that may be similar in *P* and *mariner* is that of transposase titration by nonautonomous elements with intact DNA-binding sites. A possible role for titration in *P* cytotype determination has been suggested (Simmons and Bucholz 1985), and we have suggested a possible role for titration in the maintenance of nonautonomous copies of

mariner. The titration presumably acts in the same manner in both cases by creating an excess of DNA-binding sites relative to transposase subunits.

Diversification of MLEs: Escape from Repression?

In pairwise comparisons, members of different major subfamilies of MLEs differ from each other at 40%–56% of their nucleotide sites; there is also considerable sequence divergence among elements within subfamilies (Robertson and MacLeod 1993). Some of the diversity undoubtedly reflects the consequence of random mutation, lineage expansion, and lineage extinction, which inevitably results in clusters of related sequences. On the other hand, the processes of repression that we have described may provide a mechanism for positive selection for diversification. In the presence of either OPI or DNC, a mutant element can escape the inhibition if it possesses an oligomerization domain so altered that the subunits can form oligomers only with themselves. Such an element is free to undergo amplification as well as to hitchhike on whatever potential vectors for horizontal transmission may be available. There may also be positive selection for transposase mutants that recognize altered DNA-binding domains, because such an enzyme would not be so susceptible to titration. Considered from the standpoint of selfish DNA, therefore, part of the impetus for MLE diversification may derive from selection pressure to evade regulatory mechanisms of the type described here.

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