The Origins of Gene Instability in Yeast

G. Shirleen Roeder, Philip J. Farabaugh
Deborah T. Chaleff, Gerald R. Fink

Summary. Two unstable mutations at the *his*4 locus of yeast are due to the insertion of the transposable elements *Ty*912 and *Ty*917 into the *his*4 regulatory region. The two transposons are related, one being derived from the other by a substitution of 4000 base pairs of DNA. Element *Ty*912 includes identical terminal repeats, whereas the terminal repeats of *Ty*917 are not identical. Transposition of *Ty*912 or *Ty*917 generates 5-base-pair duplications of the target DNA at either end of the element. Expression and reversion of a *his*4 gene containing *Ty*912 or *Ty*917 is controlled by three unlinked regulatory genes. The properties of these regulatory genes are similar to those described for the controlling elements in maize.

Gene instability has intrigued scientists since the early days of genetics. Unstable mutations were described in the galactose region in *Escherichia coli* (*1, 2*), the white locus in *Drosophila* (*3-5*), and at many different loci in maize (*6, 7*). In each case a mutation in a normally stable gene resulted subsequently in frequent additional alterations in that gene. The molecular basis for the sudden occurrence of high mutability of a normally stable gene remained obscure until the advent of recombinant DNA techniques.

A combination of genetic and recombinant DNA techniques has identified transposable elements as the agents of gene instability in bacteria (*8, 9*). A transposable element is a segment of DNA capable of transposition intact from one position in the genome to another (*10, 11*). These transposition events involve recombination between nonhomologous DNA sequences and are independent of the generalized recombination functions of bacteria. In transposition, the original copy of the transposon is preserved; thus, transposition is conservative. In addition to promoting their own transposition, transposable elements can also promote deletion, inversion, and transposition of adjacent chromosomal DNA sequences (*12-14*). Many of the earlier reports of unstable genes in bacteria can now be explained by the presence of a transposable element adjacent to the mutant gene (*15-17*).

Sequence analysis of DNA has revealed important structural features of the bacterial transposons. Terminal repeated sequences are common to all transposable elements and are required for transposition to occur (*12*). These repeats can be in direct or inverted orientation with respect to each other. Moreover, at least two transposons, Tn3 (*18*) and Tn5 (*19*), encode transposase, a protein also required for transposition. A remarkable feature of all bacterial transposons is that, upon integration, they generate a short oligonucleotide duplication of the target DNA at either end of the element (*11*).

The molecular analysis of gene instability in eukaryotes requires the same technologies that were successful in the study of bacterial transposons. The cloned wild-type gene, together with strains containing unstable mutant forms of the gene, are the reagents necessary for elucidating the instability. The sophisticated genetic system of *Saccharomyces cerevisiae* makes this yeast an ideal eukaryote for studies of gene instability. Furthermore, yeast transformation technology facilitates the cloning of both wild-type and mutant genes (*20*). We have studied the *his*4 region of yeast because we have the cloned wild-type *HIS*4$^+$ gene and because we have isolated many thousands of *his*4 mutations (*21, 22*).

The wild-type *his*4 region of yeast DNA is extremely stable in standard laboratory strains. Analysis of independent interbreeding stocks of *S. cerevisiae* indicates that *his*4 is linked to *leu*2 and *MAT* (mating type) in the order *his*4-*leu*2-centromere-*MAT*. Moreover, Southern hybridization analysis (*23*) of the physical structure of the region around *his*4 shows that there are no gross rearrangements of DNA in these strains. The probe in this analysis was a 20-kilobase-pair (kbp) Bam HI restriction fragment containing the *his*4 region from S288C. This segment contains the *his*4 coding sequence as well as DNA corresponding to about 4 map units on either side of *his*4. The restriction patterns of DNA from *S. norbensis*, *S. carlsbergensis*, E1278b, and "yeast foam" (F. Lacroute) when Bam HI, Hind III, Pst I, and Eco RI were used were identical. These studies gave no evidence for an insertion element or gross rearrangement within the *his*4 Bam HI fragment of any of these strains.

Most of the *his*4 mutations made with conventional mutagens are relatively stable. More than a thousand mutagen-induced mutations of *his*4 have been subjected to reversion and genetic tests. All appear to be simple base pair changes at the *his*4 region on chromosome III. None leads to instability of *his*4. The behavior of these mutations contrasts dramatically with the behavior of two spontaneously derived *his*4 mutations, *his*4-912 and *his*4-917.

Unstable Alleles *his*4-912 and *his*4-917

Mutants *his*4-912 and *his*4-917 revert to His$^+$ at extremely high frequencies (10^{-5} and 10^{-4}, respectively). Recent studies show that the His$^+$ revertants derived from the *his*4-912 mutant carry a number of chromosomal aberrations including deletions, translocations, transpositions, and inversions (*24, 25*). Genetic analysis of these aberrations shows that the break points in the deletions, translocations, and inversions are in or close to the *his*4 region. The appearance of these unusual events only in the *his*4-912 mutant suggests that the *his*4-912

G. Shirleen Roeder and Philip J. Farabaugh are postdoctoral fellows, Deborah T. Chaleff is a graduate student, and Gerald R. Fink is professor of biochemistry, Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, New York 14853.

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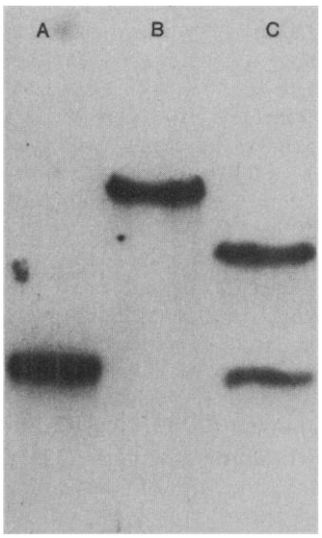

Fig. 1. Hybridization of *his*4 DNA to wild type, *his*4-912 and *his*4-917. DNA from the wild type (A), the *his*4-917 mutant (B), and the *his*4-912 mutant (C) was digested with Eco RI and analyzed by Southern hybridization, with the Sal I fragment of *his*4 DNA used as a probe (*23*).

ments sharing homology with the DNA from the *his*4 region were visualized by autoradiography. As shown in Fig. 1, the *his*4 regions in both the *his*4-912 and *his*4-917 mutants have restriction patterns different from that of the wild type. Similar results were obtained with many other restriction enzymes. The sizes of the restriction fragments found in the DNA of *his*4-912 and *his*4-917 indicate that each mutation results from the insertion of an element of approximately 6000 base pairs (bp). The restriction pattern of the insertion element in *his*4-912 is different from that in *his*4-917.

mutation is responsible for the genetic instability of the *his*4 region. Thus the *his*4-912 mutation is similar to bacterial mutations that result from the insertion of transposable elements.

Genetic analysis of *his*4-912 and *his*4-917 suggests that these mutations result from the insertion of foreign DNA into the *his*4 gene. This possibility was examined at the molecular level by Southern hybridization analysis. Total DNA from each of the mutants and their wild-type progenitor were digested with a number of restriction endonucleases and fractionated by electrophoresis through an agarose gel. After transfer of the separated restriction fragments to a nitrocellulose filter by the Southern blot procedure, the fragments were hybridized to a radioactive probe. The restriction fragment of the wild-type *his*4 gene in the Sal I site (Fig. 2). This Sal I restriction fragment carries one-fifth of the *his*4 coding sequence and includes the site of the *his*4-917 mutation. The YIp301 was introduced into a yeast strain that carries a deletion of the *ura*3 gene and the *his*4-917 mutation. Ura$^+$ His$^+$ transformants were selected. Since the yeast strain used as recipient carries a deletion that eliminates all of the *ura*3 DNA carried by the plasmid, integration of the plasmid into the yeast genome must occur by recombination between the *his*4 DNA of YIp301 and the *his*4 DNA on chromosome III. As shown in Fig. 2, Ura$^+$ His$^+$ transformants result from crossovers to the right of the *his*4-917 mutation, such that the resulting transformants carry a wild-type copy of the *his*4 gene. If this transformation is carried out with a plasmid that carries the Sal I fragment of *his*4 DNA but that lacks the *ura*3 gene, His$^+$ transformants resulting from integration of the plasmid into the *his*4 gene still occur. However the *his*4-917 mutation is unstable, making it difficult to distinguish these rare transformants from His$^+$ colonies resulting from reversion of the *his*4-917 mutation. Simultaneous selection for both His$^+$ and Ura$^+$ phenotypes permits the recovery of transformants only.

Ura$^+$ His$^+$ transformants carry a wild-type copy of the *his*4 gene, an integrated copy of the plasmid vector, and a duplication of the *his*4 Sal I restriction fragment. One copy of the latter carries the *his*4-917 mutation. As shown in Fig. 2, only one Eco RI fragment of the DNA of this strain can transform *E. coli* to ampicillin resistance; this fragment also carries the *his*4-917 insertion element. DNA from a Ura$^+$ His$^+$ yeast transformant was digested with Eco RI, ligated to form circular molecules, and used to transform *E. coli*. The *E. coli* transformants obtained yielded plasmid DNA with a size and restriction digestion pattern consistent with the results of the Southern analysis of the *his*4-917 strain. Furthermore, fragments from the *his*4-917 plasmid migrated with *his*4 DNA from the *his*4-917 strain when analyzed by Southern hybridization. Therefore the plasmid obtained contains the unrearranged *his*4-917 insertion element.

The *his*4-912 insertion element was cloned by a similar procedure, except that the plasmid used for transformation of the *his*4-912 strain was a pBR322 derivative that carries the Sal I fragment of *his*4 DNA but lacks the *ura*3 gene. His$^+$ transformants were distinguished from His$^+$ revertants by colony hybridization, with radioactively labeled pBR322 DNA used as a probe. The plasmid and the

Cloning the *his*4-912 and *his*4-917 Insertion Elements

To determine the structure and origin of the *his*4-912 and *his*4-917 insertion elements, it was necessary to isolate these elements from total yeast DNA. Therefore the *his*4-912 and *his*4-917 mutant genes were cloned by "integration and excision." In this technique, a plasmid vector is integrated into the mutant *his*4 gene by means of yeast transformation. The plasmid vector and the mutant gene are then excised as a single restriction fragment. The cloning of the *his*4-917 mutant gene is described in detail below and diagrammed in Fig. 2.

The YIp301 plasmid is pBR322 carrying the yeast *ura*3 inserted into the Ava I site and a Sal I restriction frag-

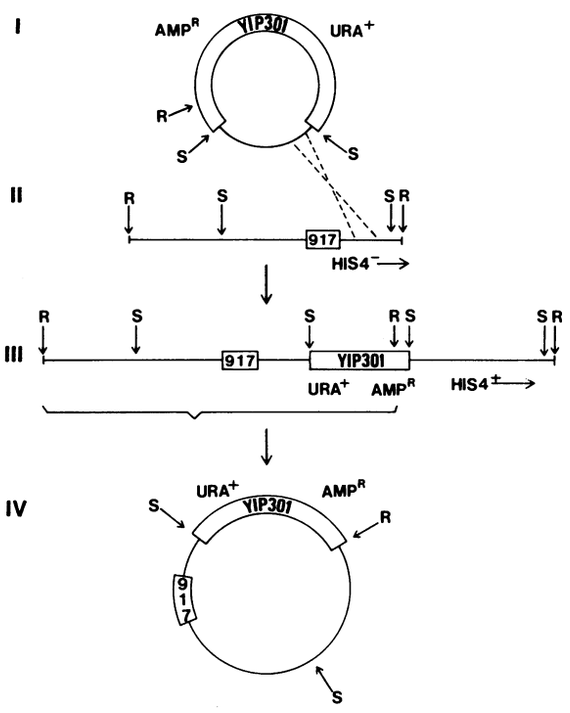

Fig. 2. Cloning the *his*4-917 mutation. (I) Plasmid YIp301 containing a Sal I fragment of the wild-type *his*4 gene. (II) The *his*4 region of DNA from a yeast strain carrying the *his*4-917 mutation. (III) The *his*4 region of DNA from a *his*4-917 yeast strain after integration of the plasmid shown in (I). (IV) The plasmid resulting from transformation of *E. coli* with an Eco RI digest of the DNA shown in (III). The solid lines indicate the *his*4 coding region and its flanking DNA. The *his*4-917 insertion element and the plasmid sequences are represented by the labeled segments. The dashed lines indicate the position of the crossover that gives rise to the integration of the plasmid into the yeast genome. The arrows indicate the sites of cleavage by Eco RI (*R*) and Sal I (*S*). *HIS*4 indicates the position of the *his*4 gene; the accompanying arrow, the direction of transcription.

*his*4-912 insertion element were excised as a Bam HI restriction fragment (*25*). Insertion elements *his*4-912 and *his*4-917 are 6200 and 6000 bp in length, respectively. The restriction maps of these elements are shown in Fig. 3.

Cloning by integration and excision permits the cloning of mutant genes for which biological complementation assays cannot be used. Furthermore, it eliminates the need for a clone bank from DNA of the mutant of interest. This approach can be adapted to cloning mutations in any gene for which a homologous DNA fragment is available.

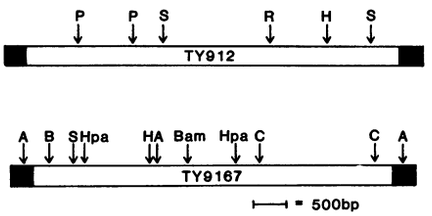

Fig. 3. Restriction maps of the *his*4-912 and *his*4-917 insertion elements. The closed bars represent the δ repeats. The open bars represent the internal regions of the elements. The arrows indicate cleavage sites for Pst I (*P*), Sal I (*S*), Eco RI (*R*), Hind III (*H*), Ava I (*A*), Bgl II (*B*), Hpa I (*Hpa*), Bam HI (*Bam*), and Cla I (*C*). The positions of the Ava I, Bgl II, Hpa I, and Cla I cleavage sites in the *his*4-912 insertion element were not determined.

Insertion Elements *his*4-912 and *his*4-917 Are Repeated DNA Sequences

As described previously (*25*), the *his*4-912 insertion element shares extensive homology with *Ty*1, a family of repeated yeast DNA sequences (*26*). *Ty*1 is approximately 6000 bp in length and includes a 300-bp terminal repeat known as δ. When the *Ty*1-containing plasmid S13 is used to probe the *his*4-912 plasmid by Southern hybridization, all *his*4-912 restriction fragments that carry part of the insertion element hybridize extensively with the *Ty*1 probe. Furthermore, the *his*4-912 plasmid and the S13 plasmid hybridize to the same restriction fragments of total yeast DNA.

When the *his*4-917 plasmid is used to probe total yeast DNA, the pattern obtained is very similar to that obtained when the *his*4-912 and S13 plasmids are used as probes. However, Southern analysis of restriction fragments of the *his*4-917 plasmid (with the S13 plasmid used as probe) indicates that the *his*4-917 insertion element is not completely homologous to *Ty*1. A 3500-bp Ava I fragment that includes the middle and the right end of the *his*4-917 insertion element (band A in Fig. 4, a and b) shares much less homology with the *Ty*1 probe than a 2100-bp Ava I fragment derived from the left end of the element (band B in Fig. 4, a and b). In addition, a Cla I fragment (band C in Fig. 4, c and d) derived from the middle of the element shares no homology with *Ty*1. These results indicate that the leftmost third of the *his*4-917 insertion element shares extensive homology with *Ty*1 but that the rightmost two-thirds share very little. The simplest interpretation of this result is that a substitution of approximately 4000 bp of *Ty*1 DNA generated the *his*4-917 insertion element. This element will be referred to as *Ty*917.

A Cla I restriction fragment derived from the middle of the *his*4-917 insertion element was subcloned into pBR322 and used to probe the S13 plasmid as well as total yeast DNA. As expected, the plasmid containing the Cla I fragment did not hybridize to fragments of yeast DNA present in the S13 plasmid (bands D, E, and F in Fig. 4, e and f).

The Cla I fragment hybridizes intensely to approximately four different restriction fragments of total yeast DNA, and hybridizes very weakly to many more fragments (Fig. 4g). Therefore the Cla I fragment represents another class of repetitive DNA in yeast. It is unknown whether the DNA contained within the Cla I fragment is always part of a *Ty*917-like element or whether it can exist independently.

*Ty*912 and *Ty*917 Transposed into *his*4 Creating 5-bp Duplications

We examined the *his*4-912 and *his*4-917 mutations by DNA sequence analysis to determine the mechanism of insertion of transposable yeast elements and to identify the sites at which the insertions occurred. The sequences of the insertions demonstrate that *Ty*912 and *Ty*917 transpose by a mechanism similar to transposition in bacteria. In the *his*4-917 mutation there is insertion of a 6000-bp *Ty*917 element and duplication of *his*4 DNA flanking the insertion. The sequence 5′-CAATA-3′ (C, cytosine; A, adenine; T, thymine), which corresponds to nucleotides −71 to −67 of the *his*4 5′ noncoding region, appears at both ends of the inserted *Ty*917 element. The *his*4-912 insertion of *Ty*912 also created a 5-bp flanking duplication of nucleotides −167 to −157 (5′-TAAGA-3′) (G, guanine) in the process of transposition (*26*). Bacterial transposons also create flanking duplications during insertion (*11*), suggesting that the mechanism of transposition is similar between the prokaryotes and yeast. In order to demonstrate this congruence of mechanism, it is necessary to show that the yeast transposons, like the bacterial transposons, create a new 5-bp duplication at each subsequent insertion.

The sequences of the *his*4-912 (*27*) and *his*4-917 mutations show that *Ty*912 and *Ty*917 insert by a process of transposition. Since an approximately 330-bp sequence (δ) occurs as a direct repeat at the ends of each of these elements, the ends of the repeat define the ends of the element. Our sequence analysis of the points at which the element interrupted the *his*4 region shows that *his*4 DNA is joined directly to a δ element at each junction. Therefore only DNA from the transposable element inserted into the *his*4 region.

The sequences of these two insertions suggest that insertion in yeast shows some sequence specificity. Both elements inserted into the noncoding region at the 5′

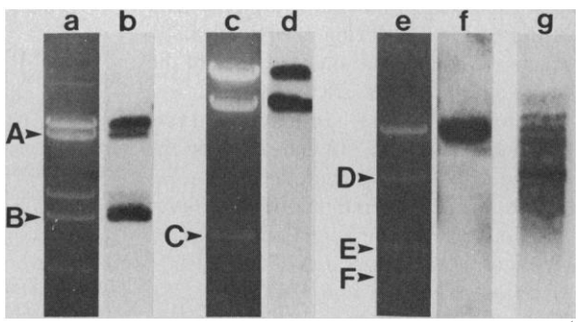

Fig. 4. Southern analysis of the *his*4-917 plasmid. (a) Ethidium bromide-stained gel of the *his*4-917 plasmid after digestion with Ava I. Fragment A has both end points in the *his*4-917 insertion element and includes the middle and most of the right end of the element (see Fig. 3). Fragment B has both end points in the insertion element and contains most of the leftmost third of the element (see Fig. 3). (b) Autoradiogram of the plasmid shown in (a) after hybridization to a radioactively labeled *Ty*1-containing plasmid (S13). (c) Ethidium bromide-stained gel of the *his*4-917 plasmid after digestion with Cla I. Fragment C is derived from the middle of the *his*4-917 insertion element (see Fig. 3). (d) Autoradiogram of the Cla I digest shown in (c) after hybridization to the radioactively labeled S13 plasmid. (e) Ethidium bromide-stained gel of fragments of the *Ty*1-containing plasmid S13 after digestion with Eco RI and Sal I. Fragments D, E, and F contain only yeast *Ty*1 DNA. (f) Autoradiogram of the S13 digest shown in (e) after hybridization to a radioactively labeled plasmid containing the Cla I fragment derived from the middle of the *his*4-917 insertion element. (g) Autoradiogram of a Sal I digest of total yeast DNA after hybridization to a radioactively labeled plasmid containing the Cla I fragment from the *his*4-917 insertion element.

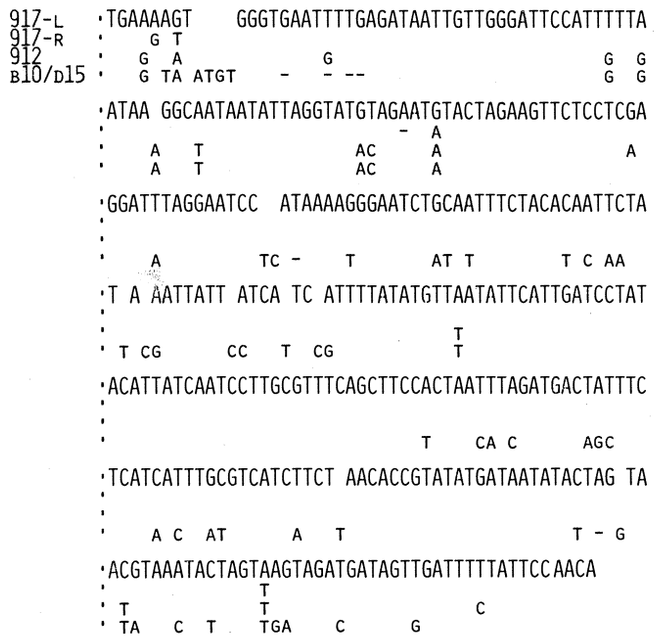

Fig. 5. Sequence divergence among the terminal repeats of yeast transposons. The left δ terminal repeat of *Ty*917, represented on the first line as a single strand, is compared with the right δ region of *Ty*917 and the δ regions of *Ty*912 and *Ty*1-B10/*Ty*1-D15. The 334-bp sequence of *Ty*917 is given in full. Where the sequences differ, the sequence present in the right δ of *Ty*917 is shown on line 2; that present in the δ of *Ty*912, on line 3; and that present in the δ of *Ty*1-B10/*Ty*1-D15, on line 4. A dash indicates the position of a 1-bp deletion of the sequence present in the left δ of *Ty*917. Spaces in the *Ty*917 left δ sequence indicate the insertion of bases in another δ sequence.

end of the *his*4 gene. This region has a high AT content (72 percent) compared with the average base composition for yeast DNA (approximately 60 percent AT). Two *Ty*1 insertions sequenced by Gafner and Philippsen (*28*) also occurred in sequences of high AT content (71 and 76 percent). The *E. coli* transposon *Tn*9 also shows this sort of regional specificity (*29*). Perhaps related to this specificity is the similarity in 5-bp duplications created at the four sites, 5′-TAAGA-3′, 5′-CAATA-3′, 5′-GAAAC-3′ (*28*), and 5′-ATTTT-3′ (*28*). No single nucleotide is invariant among these sequences, but the four are rich in AT and the composite sequence is very high in AT (80 percent). The specificity for regions of high AT content may reflect the need to unwind the DNA helix of the target site during insertion, favoring insertion at these sites because of the lower stability of AT-rich regions.

A feature of the sequence that we noted for the *his*4-912 mutation is the creation of a hyphenated, 24-bp, inverted repeat centered on one *Ty*912-*his*4 junction (*26*). There is a similar inverted repeat at the same junction of the *Ty*1-D15 insertion of Gafner and Philippsen (*28*). However, neither their other insertion (*Ty*1-B10) nor *his*4-917 creates an inverted repeat of any significance. The absence of an inverted repeat in two of the four insertions does not mean that this structure is irrelevant to the transpositional process; the creation of an inverted repeat at one junction of the transposon could result from insertion at preferred sites. "Hotspots" for the *E. coli* transposon Tn3 involve an inverted repeat between the end of the element and DNA at the target site (*30*). Many more insertions must be sequenced to determine whether this sort of site selection occurs with yeast transposons.

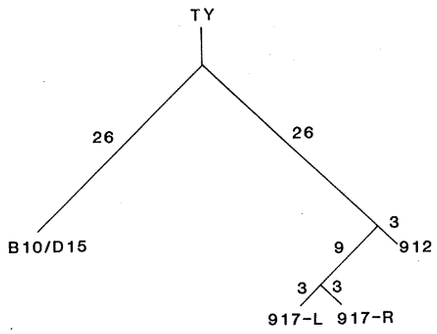

Fig. 6. A genealogical tree of the δ repeats of four yeast transposons. Sequence similarity between the δ's of *Ty*917, *Ty*912, *Ty*1-B10, and *Ty*1-D15 indicates that they all derive from a single sequence (*Ty*). The divergence among these sequences is consistent with the genealogical tree shown. The number of mutations that have accumulated in a δ since a branch point is proportional to the distance along the branches and is indicated next to the branch. Since no common ancestor of the two groups (*Ty*917/*Ty*912 and *Ty*1-B10/*Ty*1-D15) exists, the mutations that have occurred since their divergence cannot be attributed to either branch and therefore are divided equally between them. Although only five nucleotide differences exist between the *Ty*917 δ's, three mutations must have occurred in each δ. Nucleotide 7 has undergone an A → G transition in *Ty*917-L and an A → T transversion in *Ty*917-R.

The evidence from the *his*4-912 and *his*4-917 mutations clearly shows that *Ty*912 and *Ty*917 are transposable elements. This is the only case in eukaryotes in which transposition has been rigorously demonstrated. However, there are elements in *Drosophila* which are presumed to be transposable and which are flanked by oligonucleotide duplications of 5 bp [412, *copia*, and 297 of *Drosophila* (*31*)]. Moloney murine leukemia virus, a retrovirus, has a structure similar to that of bacterial transposons and creates a 4-bp flanking duplication upon integration into the mouse genome (*32*). Transposons are dispersed among both prokaryotes and eukaryotes, suggesting that the process of transposition is a universally important genetic phenomenon.

Sequence Divergence Among Yeast Transposons

*Ty*1 is a family of related elements originally described by Cameron *et al.* (*26*). The restriction maps of members of this family show a variety of differences (*25, 26*). The differences in restriction cleavage sites among the elements were presumed to reflect even greater sequence divergence at the nucleotide level. The existence of the *Ty*917 element, composed of a region homologous to *Ty*1 and a large nonhomologous region, emphasizes the potential for sequence divergence.

The *Ty* elements previously sequenced consisted of an approximatley 6-kbp internal segment flanked by a direct repeat of identical δ elements. However, the δ's from *Ty*917 are not identical. The sequences of the left δ and the right δ differ by substitutions of 4 bp and the deletion of 1 bp. Figure 5 compares the left δ and the right δ of *Ty*917 and those of the *his*4-912 *Ty*1 (*Ty*912), *Ty*1-B10, and *Ty*1-D15. (Since *Ty*1-B10 and *Ty*1-D15 are identical, only one comparison is shown.) *Ty*912 and *Ty*917 show very little sequence divergence. Only 15 differences (4 percent) occur between the δ regions. Within a region of 1085 bp which has been sequenced in both elements (including the two δ elements and approximately 420 bp from the internal region of the elements), there are only 36 base differences (3 percent). The extent of homology between these two transposons is striking, since *Ty*917 includes an approximately 4000-bp substitution of DNA with little or no homology to sequences in *Ty*912. The amount of se-

quence divergence between these two elements and *Ty*1-B10 is much greater. The δ regions of *Ty*1-B10 differ by 64 bp from those of *Ty*917 (19 percent) and 54 bp from those of *Ty*912 (16 percent).

The DNA sequences of the δ repeats from the four *Ty* elements suggest a phylogenetic relation among the transposons (Fig. 6). According to this view, the *Ty*1-B10, *Ty*1-D15, *Ty*912, and *Ty*917 elements are all derived from a single ancestral transposon. The *Ty*917 δ's are more closely related to each other than to the *Ty*912 δ's. The *Ty*1-B10 and *Ty*1-D15 δ's are identical to each other but are only distantly related to the other elements. Nucleotides in the δ elements which are identical in *Ty*912 and *Ty*917 but different in *Ty*1-B10 and *Ty*1-D15 are presumed to be the result of mutations that occurred in the progenitor δ sequence after the divergence of the two groups. Differences between the *Ty*912 δ and those of both *Ty*917 and *Ty*1-B10/*Ty*1-D15 are due to mutations that occurred in the *Ty*912 sequence since it diverged from *Ty*917. Nine of the differences between the *Ty*917 δ's and those of *Ty*912 occurred before the divergence of the *Ty*917 δ's from each other. A *Ty*917 element containing nonidentical δ's could have resulted from mutations in the δ's of an intact *Ty* element or from a recombination event between elements containing highly related δ's.

The fact that two highly related elements transposed independently into *his*4 suggests that yeast transposons are restricted either by sequence-directed site specificity or by compartmentalization within the genome (that is, restriction of these elements to a single chromosome). Another possible explanation is that the elements of the *Ty*912/*Ty*917 type are capable of transposition but those of the *Ty*1-B10/*Ty*1-D15 type have lost this ability. In this case, the de novo transposed elements would belong to a restricted subpopulation. Further experimentation is necessary to resolve this issue.

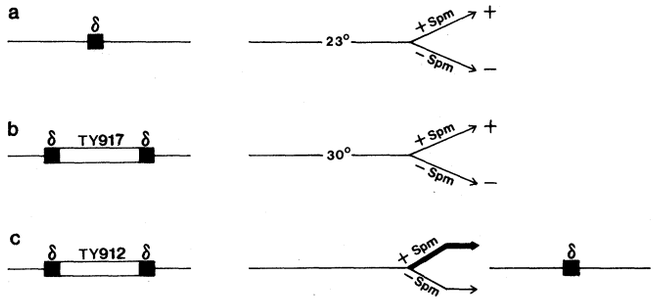

Fig. 7. Properties of suppressor - mutators. The effects of the Spm2 and Spm3 regulatory elements on insertion mutations at the *his*4 locus are represented diagrammatically. (a) The effect of Spm elements on the *his*4-912 revertant, which carries a single copy of δ. This revertant is capable of growth at 23°C in the absence of histidine only when the strain carries an active Spm element. (b) The effect of Spm elements on the *his*4-917 mutant. The *his*4-917 mutant is capable of growth in the absence of histidine only when the strain carries an Spm element. (c) The effect of Spm elements on the *his*4-912 mutant. Excision of the *his*4-912 insertion element, leaving behind a single copy of δ, occurs 100 to 1000 times more frequently in the presence of the Spm regulatory element.

Suppressor-Mutator Elements in Yeast

In general, the behavior of insertion mutations in maize, *Drosophilia*, and yeast parallels that of insertion mutations in prokaryotes. Integration of an element into a gene or the adjacent regulatory region leads to a mutant phenotype. Precise or imprecise excision of the element, or deletion, inversion, or translocation of chromosomal DNA adjacent to the element can lead to a complete or partial restoration of wild-type function or to a new mutant phenotype. Unlike prokaryotic insertion elements, the maize controlling elements most often exist as two-component systems. Of several two-component systems reported, the Spm system has been investigated most extensively (*33*–*37*).

One component of the Spm system, the receptor, inserts into or near a gene, causing a mutant phenotype. When the genetically unlinked Spm regulatory element is present, the suppressor activity of the Spm element can lead to a phenotypic suppression or enhancement of expression of the gene at the receptor locus. In the absence of the Spm regulatory element, the gene at the receptor locus is stable. However, when the Spm regulatory element is present, the mutator activity of this element promotes a high frequency of reversion and mutation of the gene at the receptor locus.

The insertion of *Ty*912 and *Ty*917 into the regulatory region of the *his*4 gene destroys the normal regulation of the gene. Expression of the *his*4 gene is now controlled by unlinked regulatory elements that act via the inserted sequence adjacent to the gene. These unlinked regulatory elements are similar to the maize Spm elements in their ability to promote both suppression and reversion of the gene at the receptor locus.

One class of yeast Spm elements suppresses the His$^-$ phenotype of the *his*4-917 mutation. Strains that carry the *his*4-917 mutation and either the *spm*2$^-$ or *spm*3$^-$ mutation are phenotypically His$^+$. Spm2 and Spm3 do not suppress the *his*4-912 mutation, but they do lead to a 100- to 1000-fold increase in the frequency with which this mutation reverts to His$^+$. These His$^+$ revertants result from recombination between homologous δ sequences to excise the internal region of the insertion element and leave behind a single copy of δ (*27*). Strains that carry a single copy of δ at the *his*4-912 site are His$^+$ at 37°C; however, they are His$^-$ at 23°C in *Spm*$^+$ strains but His$^+$ at 23°C in *spm*2$^-$ or *spm*3$^-$ strains. Thus the *spm*2$^-$ and *spm*3$^-$ mutations both promote reversion of the *his*4-912 mutation and suppress the cold sensitivity of the resulting revertants. The properties of the Spm2 and Spm3 elements are summarized in Fig. 7.

Another Spm element, *spm*1$^-$, suppresses the His$^-$ phenotype of both the *his*4-912 and *his*4-917 mutations. In addition, the *spm*1$^-$ mutation suppresses the cold sensitivity of the His$^+$ revertants carrying a single copy of δ. Since δ is present in all three *his*4 mutants suppressed by *spm*1$^-$, it is likely that Spm1 acts at the common δ regions.

The existence of two-component systems of controlling elements in yeast offers the opportunity to analyze these systems at the molecular level. The yeast transformation technology permits the cloning of yeast genes based on their expression and complementation in yeast. Thus the Spm elements can be cloned on the basis of their ability to suppress the His$^-$ phenotype of the *his*4 insertion mutations. Using the cloned elements, it will be possible to determine the relation of the Spm regulatory elements to their receptors and to the yeast genome.

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37. The yeast regulatory elements are referred to as Spm because of their ability to both suppress (Sp) and mutate (m) the mutant *his4* genes. The similarity in nomenclature between the maize controlling elements and the yeast regulatory elements is not meant to imply that the two kinds of elements are mechanistically similar.
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Recombination of Dispersed Repeated DNA Sequences in Yeast

Stewart Scherer and Ronald W. Davis

Dispersed, repeated gene families have been found to be a general feature of eukaryotic genomes. Some, such as the actin genes of *Drosophila* (*1*) or the yeast histone genes (*2*), are multiple copies of structural genes, while others are organized like the transposable elements of bacteria (*3*). This latter class, including the *copia* genes of *Drosophila* (*4*) and the *Ty*1 family in yeast (*5*), seems to be capable of relatively rapid movement throughout the genome of its host. The integrated copies of the RNA tumor viruses share many structural similarities with these mobile dispersed families (*6*).

Summary. Yeast transformation can be used to insert new sequence arrangements into a variety of chromosomal locations by homologous recombination. These newly inserted sequences can recombine with similar sequences located on other chromosomes. In these events, information is duplicated without being lost at the site from which it is derived. Similar mechanisms might be utilized by cells to provide new functions during development or differentiation.

Recombination among the members of tandemly repeated sequence families has been proposed as a major mechanism for their evolution (*7*). However, the mechanisms that have been proposed for maintenance of sequence homogeneity among the members of the tandemly repeated families are not suited to the problem of the dispersed family. It has been suggested that reciprocal recombination between the members of a dispersed repeated sequence family may play a role in the inversions, translocations, and deletions of eukaryotic chromosomes.

Analysis of multigene families at the molecular level is complicated by the duplication of genetic information and by the hybridization between the DNA sequences coding for the various members of the family. A mutation in a single member of a large family may have no scorable phenotype. It is also difficult to assess the relative levels of transcription of each member of the family, as the transcripts are often indistinguishable except at the level of nucleotide sequence.

Transformation of yeast with DNA containing new sequence arrangements provides a mechanism for addressing many questions involving dispersed, repeated gene families. One can construct molecules that have selectable markers within or adjacent to a cloned, dispersed repeated element. This permits identification of a particular member of such a family. Also, one can introduce additional copies of sequences of known function to construct new dispersed gene families.

We have found that *Ty*1 sequences can recombine homologously during transformation experiments. Since *Ty*1 elements are dispersed throughout the yeast genome, the inclusion of *Ty*1 sequences in the transforming DNA allows one to introduce a particular sequence into a variety of chromosomal locations. Unusual genetic behavior by these novel sequence arrangements may provide approaches for such problems as position effects and the influence of transposable elements on the expression of neighboring genes.

Here we show that sequences introduced at several chromosomal locations can recombine with homologous sequences on a different chromosome. The events we observed are asymmetric (gene conversion); information is duplicated without being lost from its original location. The recombinational events occur when one copy of the sequences contains a deletion or a rearrangement. This suggests that the generalized recombination systems of the host can be utilized to transfer sequence information from one chromosome to another. These results also show that the recombination of dispersed repeated sequences is not limited to those organized as transposable elements. In many organisms there are examples of similar unlinked copies of structural genes coding for a wide variety of enzymes. Recombination of dispersed, related coding sequences might be an additional mechanism for providing new functions for a cell during development or in evolutionary time.

Stewart Scherer is a graduate student and Ronald W. Davis is professor in the Department of Biochemistry, the Stanford University School of Medicine, Stanford, California 94305.

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