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EDITED BY

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Transposable Elements in Yeast

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I. Introduction

Transposable elements are DNA sequences that move to new genomic locations at a much higher rate than that of the bulk of the cellular DNA. Such mobile elements were first defined genetically as controlling elements in maize (McClintock, 1952, 1957) and have been studied on the molecular level in diverse organisms, such as bacteria, yeast, and fruit flies (Kleckner, 1977: Carlos and Miller, 1980; Green, 1980; Shapiro and Cordell, 1982). Certain general properties characterize these elements. Physically, these transposable DNAs have direct and/or inverted repeat of DNA sequence at each end. The ability to cause deletions or chromosomal rearrangements is characteristic of these elements, and many have been shown to affect the expression of chromosomal genes by inserting adjacent to or into these genes.

The first transposable element found in the yeast *Saccharomyces cerevisiae* was named Ty1 by Cameron *et al.* (1979). It was observed as a moderately repetitive DNA sequence, one copy of which was present adjacent to a tyrosine tRNA gene in one yeast strain but not in others. Analysis of the hybridization spectrum of this element to DNA from various laboratory yeast strains indicated that its locations in the yeast genome varied from strain to strain, a finding suggesting that it was transposable. Evidence for transposition was also obtained after prolonged growth at 37°C. Soon after, analysis of mutations in expression of the *HIS4* gene, which codes for activities required for histidine biosynthesis, revealed that two unstable His^- mutations were caused by insertion of DNA

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sequences homologous to Ty1 upstream from the structural gene (Roeder *et al.*, 1980). Other mutations, such as one that resulted in 20-fold overproduction of iso-2-cytochrome c (Errede *et al.*, 1980a,b) and seven different mutations that altered the expression of alcohol dehydrogenase (Ciriacy, 1976, 1979, Williamson *et al.*, 1981), were also found to be due to insertion of Ty1-like sequences into their regulatory regions. Other genetic phenomena such as deletions, translocations, and inversions were observed in connection with Ty1-like DNA sequences in yeast (Chaleff and Fink, 1980).

Similarities are apparent in the features of Ty1, *Drosophila* transposable elements such as *copia*, and integrated proviral forms of retroviruses in birds and mammals (see *Cold Spring Harbor Symp. Quant. Biol.* **45**, 1980). These elements are each composed of an internal DNA segment of several thousand base pairs flanked by a direct repeat of a DNA sequence that is several hundred base pairs long. They are bounded by the terminal dinucleotides 5′TG . . . CA3′ and are flanked by direct repeats 4–6 base pairs long, which appear to have been generated by duplication of a target DNA sequence upon integration of the element. All are transcribed into nearly full length polyadenylated RNA. There is evidence that *copia*, Ty, and proviruses can each alter the expression and regulation of adjacent genes (Bingham *et al.*, 1981: Hayward *et al.*, 1981; Payne *et al.*, 1982). Similarities between Ty, *copia*, and proviruses are a recurring theme in this article and point to the likelihood that these fascinating elements have a common origin.

Saccharomyces cerevisiae is very useful as a eukaryotic model system for molecular biologists. Features of the yeast system that make this organism particularly advantageous include ease of growth, small genome size, availability of selectable markers, ease of genetic manipulation, and availability of techniques for transformation with exogenous DNA (Beggs, 1978: Hinnen *et al.*, 1978). A number of useful shuttle vectors are available, and it is relatively easy to integrate DNA sequences into the yeast genome by homologous recombination. Using these techniques, investigators can, for example, replace a chromosomal DNA sequence with one that has been specifically altered *in vitro* (Scherer and Davis, 1979). Because of the advantages mentioned earlier, much has been and can be learned from the study of the properties of transposable elements in this organism.

II. Yeast Transposable Element Ty

A. Physical Structure

Ty is defined as a family of disperse, repetitive DNA sequences, each of which is homologous to the original Ty1 sequence discovered by Cameron *et al.* (1979). Ty family members consist of an internal 5.3-kilobase (kb) fragment of DNA [epsilon (ϵ) DNA] bounded by copies of the direct repeat sequence delta

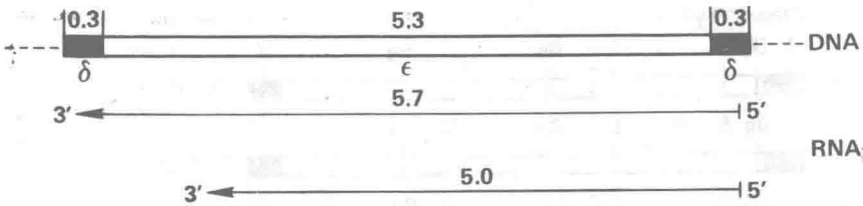

FIG. 1. General structure of Ty element DNA. Black bars represent the delta (δ) sequences, which are present in directly repeated orientation; white bar represents the epsilon (ϵ) region, and dashed lines represent chromosomal DNA sequence. A 5-base pair sequence of target DNA is duplicated upon transposition and one copy is present at each end after Ty integration. Arrows represent length and direction of the major RNA transcripts that have been characterized (Elder *et al.*, 1982). Numbers represent the lengths of the nucleic acid sequences shown in kilobases (kb).

(δ), which is about 0.3 kb long (Fig. 1). Members of this family have been observed as single units on several different chromosomes in yeast. Cameron *et al.* (1979) isolated from a yeast genomic library DNA clones that contain parts of two adjacent elements, a finding indicating that Ty copies also occur either tandemly or as free circles. Approximately 30 copies of the complete element per haploid genome are present in most laboratory strains of *S. cerevisiae*, although the number and distribution of the copies varies from strain to strain (Cameron *et al.*, 1979; Eibel *et al.*, 1980; Fink *et al.*, 1980). The number of Ty copies in wild-type isolates of *S. cerevisiae* is in general lower than in laboratory strains. Eibel *et al.* (1980) examined 21 natural isolates and found that the number of copies of Ty ranged from 4 to 20. *Saccharomyces norbensis*, which is closely related to standard laboratory strains of *S. cerevisiae* and produces viable spores when crossed with *S. cerevisiae* strains, appears to have no sequences that strongly hybridize to the epsilon region of Ty (Fink *et al.*, 1980). Its genome does, however, contain sequences that hybridize with a probe for the delta sequence (Roeder and Fink, 1982b).

Numerous Ty elements have been cloned, and many others have been studied indirectly by analyzing Southern hybridization profiles using adjacent DNA sequences as probes. The length of most of the Ty DNA sequences is about 5.9 kb. However, others may differ in length; for example, the cloned element Ty1-17 (Kingsman *et al.*, 1981) is somewhat longer because of an insertion near its right end. A comparison of the restriction enzyme cleavage maps of several cloned Ty elements is given in Fig. 2. Significant sequence heterogeneity, as reflected by restriction site polymorphism, exists among Ty elements. The restriction maps shown, as well as Southern analyses and heteroduplex analyses, allow division of these elements into two broad classes. The first class is exemplified by element Ty1(S13) (Fig. 2). Elements of this class generally contain one or two *Eco*RI sites. Those that have been tested cross-hybridize strongly to the original Ty1, which is adjacent to the Tyr tRNA-coding gene. Elements of the second

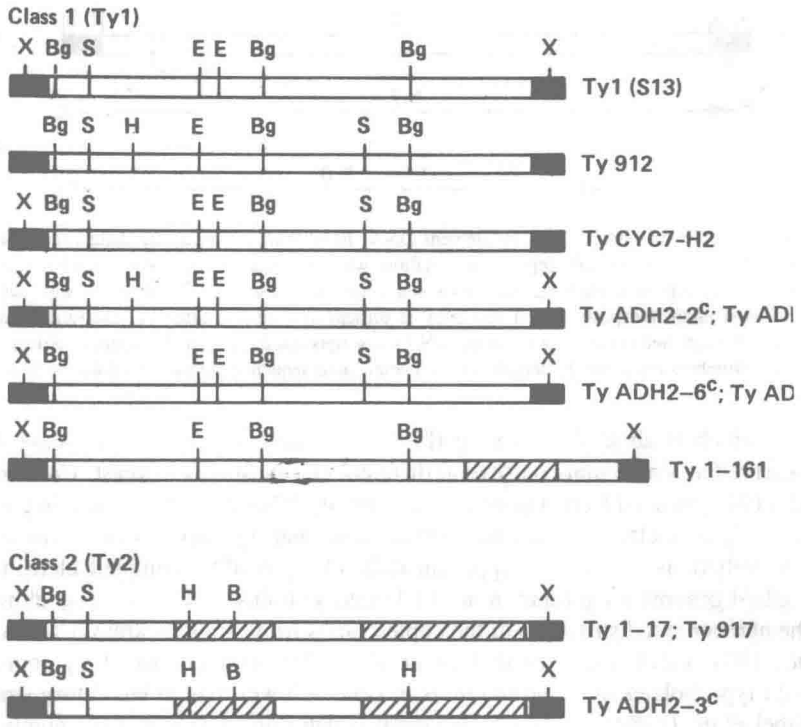

FIG. 2. Comparison of cloned Ty elements. Solid bars represent delta sequence; white bars represent epsilon regions. Crosshatched areas indicate the approximate locations of regions that are nonhomologous to Ty1 because of insertions (in the case of Ty1-161) or substitutions (for the Class 2 elements). Restriction enzyme cleavage sites are designated as follows: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII, S, *Sal*I; and X, *Xho*I. Only restriction enzyme cleavage sites that have been determined for all the elements shown are presented here. References for these restriction maps are Ty1(S13), Cameron *et al.*, 1979, and Eibel *et al.*, 1980; Ty912 and Ty917, Fink *et al.*, 1980, and Roeder and Fink, 1982b; TyCYC7-H2, Errede *et al.*, 1980a,b; Ty*ADH2-2c*, *-3c*, *-6c*, *-7c*, and *-8c*, Williamson *et al.*, 1983; and Ty1-161 and Ty1-17, Kingsman *et al.*, 1981.

class contain no *Eco*RI sites but contain a *Bam*HI restriction site in the corresponding region. Elements belonging to Class 1 or Class 2 are often referred to as Ty1 or Ty2, respectively. In cases where heteroduplex analysis has been done between Ty1 and Ty2 elements, two substitution loops are seen (Kingsman *et al.*, 1981; Williamson *et al.*, 1983). One substitution of about 1 kb begins about 2 kb from the left end of the Ty elements and encompasses the *Eco*RI sites of the Class 1 elements and the *Hin*dIII and *Bam*HI sites of the Class 2 elements. Closer examination indicates that there may be some homology within this substitution (V. M. Williamson, unpublished). The second substitution loop is about 2 kb

long and begins about 3 kb from the left end of the Ty element. Fink *et al.* (1980) have shown that a *Cla*I fragment about 1.6 kb long from this region of Ty917 does not cross-hybridize to Class 1 Ty elements. Heteroduplex and Southern hybridization studies have shown that this 2-kb region is conserved among Class 1 Ty elements: however, it has not been determined whether the corresponding region is conserved among Class 2 elements. The *Bgl*II restriction site shown near the left delta is conserved in all published examples. In fact, the left-most kilobase of DNA sequence appears to be conserved among all Ty elements. The five-base pair (bp) nucleotide sequence TACCA is present in direct repeat orientation at the ends of the epsilon region (i.e., at the delta–epsilon junctions) in both classes of Ty elements (Farabaugh and Fink, 1980; Gafner and Philippsen, 1980; Williamson *et al.*, 1983).

The proportion of elements in each of the two classes and of restriction site variants within each class varies considerably from strain to strain. For example, genomic blotting analysis by Eibel *et al.* (1980) indicates that in some yeast strains none of the DNA fragments that cross-hybridize with Ty1 appear to contain restriction sites for *Eco*RI. Two Australian yeast strains that they examined lack at least two of the three *Bgl*II restriction sites shown in Fig. 2 within their Ty elements. Similar experiments by Cameron *et al.* (1979) suggest that none of the Ty family members in strain S288C contain *Hin*dIII restriction sites and that the fraction of Ty elements containing one versus two *Eco*RI sites varies from strain to strain.

The 330-bp DNA sequence delta, which is found at the ends of all Ty elements, also occurs at other loci in the genome and is present in about 100 copies per genome (Cameron *et al.*, 1979). It is difficult to obtain a good estimate of the number of deltas in a genome because these repetitive DNA sequences occur both as single and clustered units. Cameron *et al.* (1979) found several delta sequences in a 12.5-kb DNA fragment containing a tyrosine tRNA gene (*SUP4*): sequence analysis has shown that five delta sequences are present as two pairs of inverted sequences and one single delta (Gafner and Philippsen, 1983; Rothstein and Helms, 1982). Another problem in estimating the number of delta sequences in a genome is that some solo delta sequences have diverged in sequence to such an extent that hybridization to a single prototype delta would not be observed (P. Philippsen, personal communication). Recombination between delta sequences at the ends of Ty elements is one potential mechanism for generation of solo delta sequences. In fact, several cases have been observed where a Ty element is lost from a particular place in the yeast genome, leaving behind a single (solo) delta sequence (Roeder *et al.*, 1980; Ciriacy and Williamson, 1981). It is possible that deltas themselves are transposable, but no evidence for this has been obtained.

The DNA sequences of several Ty-associated delta elements have been determined; these are compared in Fig. 3. The deltas at opposite ends of each Ty are

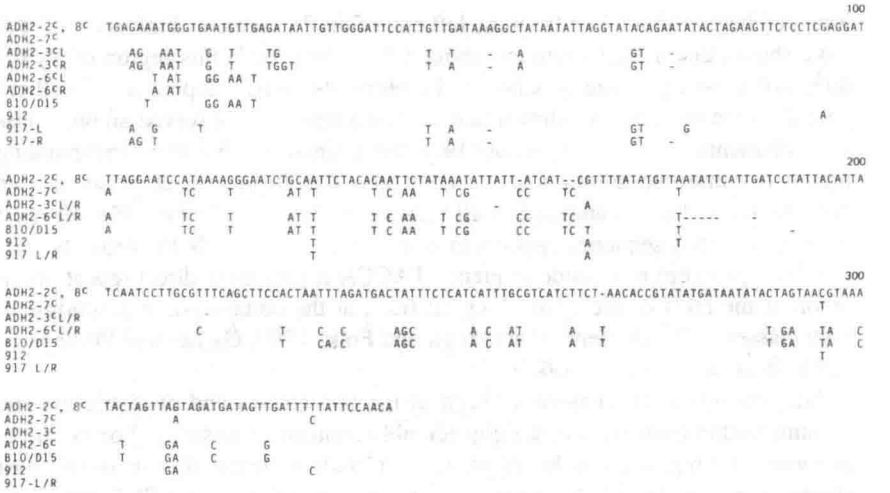

FIG. 3. Comparison of the DNA sequence of deltas at the ends of Ty elements. The complete sequence of the Ty*ADH2-2*^{c} delta is shown, and nucleotides that differ from this in the other delta sequences that have been determined are indicated. Ty elements are identified on the left margin, and the sequence is presented such that the 5′ to 3′ strand (left to right) of Fig. 2 is shown. Where the left (L) and right (R) deltas of a particular Ty element differ, both are presented. A dash (–) indicates that a nucleotide is not present in the delta sequence shown. References for these sequences are as follows: *ADH2-2*^{c}, *-3*^{c}, *-6*^{c}, *-7*^{c}, *-8*^{c}, Williamson *et al.*, 1983; B10/D15, Gafner and Philipsen, 1980, Ty912 and Ty917, Roeder and Fink, 1982b.

often identical, but in some cases differ by a few nucleotides. Delta sequences are AT rich (about 70%) and vary in length from 333 to 337 base pairs. Regions of patchwork homology between delta sequences occur. For example, the element *ADH2-7*^{c} delta is identical to the *ADH2-2*^{c} delta for the first 100 nucleotides but resembles the *ADH2-6*^{c} delta much more strongly for the following 100 nucleotides (Fig. 3). Some regions of DNA sequence are identical for all Ty-associated deltas. Sequences that are three or more nucleotides long and that are identical for the 18 delta elements diagramed in Fig. 3 are underlined in Fig. 4. Completely conserved sequences of 10 nucleotides or greater are boxed. The possibility that these conserved regions are important for regulation and initiation of transcription, for initiation of translation, and/or for transposition is discussed later. Solo delta sequences are less conserved than Ty-associated deltas, and several changes within the conserved regions are seen (Rothstein and Helms, 1982; P. Philippsen, personal communication). Not only are some regions of delta sequence highly conserved in yeast, but DNA sequence homology exists between delta sequences and the direct repeats at the ends of *Drosophila copia*

elements and retrovirus long terminal repeats (LTRs). The delta sequences are bounded by the nucleotides 5′ TG . . . TATTCCAACA 3′. These exact nucleotides also bound the *copia* element of *Drosophila* (Eibel *et al.*, 1980; Levis *et al.*, 1980). Retroviruses and *copia* elements, but not Ty elements, contain somewhat longer and often imperfect inverted repeats at their ends. This homology at the ends and additional sequence homology within the terminal repeats (Ju and Skalka, 1980) has led several scientists to propose a common origin for Ty elements, *copia* elements, and retroviruses. *Copia* element direct repeats differ from delta sequences in that they do not occur as solo sequences in the *Drosophila* genome (Levis *et al.*, 1980).

B. Transcription

Ty elements are transcribed into two abundant poly(A) RNA species of about 5.0 and 5.7 kb (Fig. 1). The 5′- and 3′-ends of the 5.7-kb transcripts have been determined (Elder *et al.*, 1983) and are indicated in Figs. 1 and 4. As is the case for other eukaryotic transposable elements and for retroviruses, the terminal direct repeats are involved in initiation and termination, respectively, of transcription (Varmus, 1982). The Ty transcripts are initiated within one delta sequence 93–97 bp from the epsilon region (labeled 3 in Fig. 4), then proceed through the epsilon region and terminate about 295 bases into the other delta (4 in Fig. 4), beyond the

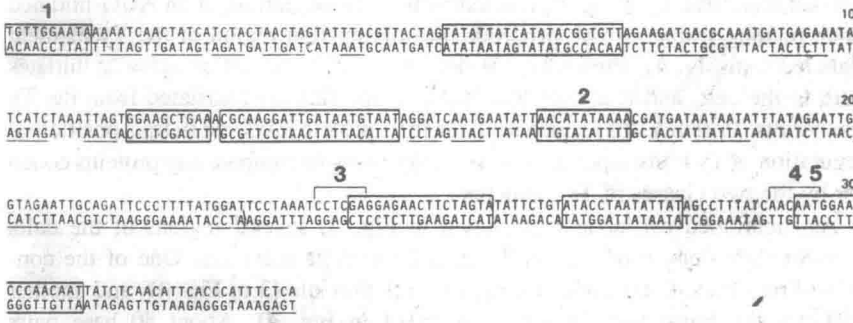

FIG. 4. Conserved and functionally important regions of delta sequence. DNA sequence of both strands of delta from Ty*ADH2-2*^{c} is shown. Note that the top strand is the complement of the sequence shown in Fig. 3. Sequences of three base pairs or longer that are conserved for all 18 Ty-associated delta sequences represented in Fig. 3 are underlined, and completely conserved regions of 10 bp or longer are boxed. Region 1 in conserved in all Ty-associated deltas, in *Drosophila copia* elements, in spleen necrosis virus long terminal repeats, and in yeast sigma elements. Region 2 contains a TATA sequence that is present upstream from several highly expressed yeast genes. Regions 3 and 4 contain transcription initiation and termination sites, respectively. Number 5 indicates the start (ATG) of an open reading frame that proceeds into the Ty element.

sequence corresponding to the transcription initiation site. Because delta sequences of a Ty element are usually identical, transcription of this RNA would require read-through of termination signals. A model involving RNA secondary structure to explain this has been proposed for proviral RNA synthesis (Benz *et al.*, 1980). Because the transcription termination site in the 3′ delta is past the transcription initiation site in the 5′ delta, the longer Ty RNA species contains direct repeats at its ends (corresponding to the region between 3 and 4 of Fig. 4), as does retroviral genomic RNA. Sequence heterogeneity at the 3′-end of the Ty RNA indicates that many different Ty elements are transcribed (Elder *et al.*, 1983). The size of the transcript and results of R-loop analysis make the presence of large intervening sequences unlikely. The shorter species of RNA appears to be initiated at the same position as the 5.7-kb species, but it terminates within the epsilon region of the Ty element. These transcripts together account for several percent of the total poly(A) RNA in logarithmically growing or stationary haploid yeast cells (Elder *et al.*, 1980). The level of RNA varies with the mating capability of the cells. Cells that can mate (for example, *MAT a* or MAT α haploid cells) have high levels of Ty RNA in both log phase and stationary phase. In cells that cannot mate but can sporulate (for example, *MAT a*/*MAT* α diploid cells), the amount of Ty transcript in log-phase cells is about 1/5 that in haploid cells and, in stationary cells, is 1/20 that in haploid cells. The level of 5.0-kb RNA is also lower in nonmating cell types than in mating cell types and is lower in stationary phase than in log phase for both cell types (Elder *et al.*, 1980). About 40 nucleotides downstream from the transcription start site is the beginning of an AUG-initiated open reading frame, which proceeds into the epsilon region of the Ty element (labeled 5 in Fig. 4). Presently it is not certain that translation actually initiates here in the cell, and it is not clear that any proteins are translated from the Ty transcripts. Possibly this sequence codes for a protein involved in transposition or regulation of Ty transcription. It will be interesting to compare any proteins coded for by the two classes of Ty elements.

As mentioned earlier and as shown in Fig. 4, several regions of the delta sequence are conserved among Ty-associated delta elements. One of the conserved regions follows the transcription initiation site (3 in Fig. 4), and another includes the translation initiation codon (4 in Fig. 4). About 80 base pairs upstream from the transcription initiation site is another conserved sequence, TATAAA (2 in Fig. 4). This exact sequence is present 80 to 100 base pairs upstream from the transcription initiation sites of a number of other highly transcribed yeast genes, a finding suggesting that this sequence is important for the high level of transcription of yeast genes (Dobson *et al.*, 1982; Russell *et al.*, 1983). A rather long stretch of conserved sequence occurs just upstream from here in the delta sequence, and a function for these sequences in Ty transcription promotion or regulation seems likely. Other conserved regions of delta sequence precede the transcription termination site of the 5.7-kb transcript (Elder *et al.*,

1983); and the sequence TAGT, which may be important for transcription termination in yeast (Zaret and Sherman, 1982), is present in one of these conserved regions.

C. Transposition

As do most other transposable elements, Ty generates duplications of target DNA at the site of its integration. Investigators that work with bacterial transposable elements propose that this short repeat is generated by the integration process (Grindley and Sherratt, 1979; Shapiro, 1979). One mechanism often proposed is that a staggered, double-stranded cut is made in the DNA, the ends of a linear form of a transposable element are attached to the protruding end of each strand, and the single-stranded regions are then filled in to produce a short repeat of target DNA at each end of the element. The length of the repeat generated in the target DNA varies for different transposable elements but is characteristic for each particular type of element. In prokaryotes, the length of this repeat ranges from 3 to 12 bp (Calos and Miller, 1980). A 5-bp duplication of target DNA resides at each end of the inserted Ty element. The same size duplication is generated at the site of *copia* and SNV (spleen necrosis virus) provirus insertion. These three elements each contain the sequence CAACA at the end of the direct repeat sequence, and it has been suggested that this sequence may be common to eukaryotic transposable elements that generate 5-bp repeats (Levis *et al.*, 1980).

When a Ty1 element is transposed, a copy appears to remain at the original location (Cameron *et al.*, 1979). This implies that, as is true for bacterial transposable elements, replication is involved in the transposition process (Calos and Miller, 1980). The structural similarity between the nearly full length RNA species transcribed from Ty elements and the retrovirus RNA genomes suggests that the Ty RNA species may act as an intermediate in transposition by mechanisms similar to proviral integration. The steps in this pathway have been studied extensively in retroviruses (reviewed by Varmus, 1982). Retroviral RNA genomes are reverse-transcribed into double-stranded DNA molecules before integration into the host genome. The primer for the reverse-transcription process is a tRNA species that is complementary to a sequence of DNA adjacent to the long terminal repeat of the retrovirus. There is, in fact, homology of the 3′-end of the yeast fMet tRNA with the right epsilon–delta junction of Ty (Eibel *et al.*, 1980). There is also evidence that a free, supercoiled, circular form of the Ty element exists at about one copy per 3×10^4 yeast cells (P. Ballario, P. Filetici, N. Junakovic, and F. Pedone, personal communication). Extrachromosomal circular copies of the eukaryotic transposable element *copia* are also found in cultured *Drosophilia* cells (Flavell and Ish-Horowicz, 1981), but it is not clear in either case that these are involved in transposition. The possibility remains that these

transposition events involve direct replicative transfer of DNA from the donor to the target site, as appears to occur for prokaryotic transposition (Shapiro, 1979; Harshey *et al.*, 1982).

The 5-bp duplicated sequence of target DNA, as well as the surrounding DNA in the region of Ty insertion, has been determined for several Ty elements. In some cases short stretches of homology of target DNA with sequences within the delta have been noted: and in one case a Ty element was found to insert within a solo delta in the opposite orientation (Gafner and Philippsen, 1980). However, for five cases in which the Ty element was found upstream from the *ADH2* gene, no extensive homology was seen between delta and the target DNA region (Williamson *et al.*, 1983). It may be that although delta homology can promote Ty integration, homology is not required for integration. One characteristic of all Ty integration sites studied so far is that they are AT rich. It is also significant that all the Ty insertion sites determined so far have been between yeast structural genes rather than within coding regions. Because many of the insertion sequences were identified because of an alteration of adjacent gene expression by Ty insertion, one could argue that the insertion sites observed are biased for intergenic insertion. However, when Eibel and Philippsen (1982) examined cleaved genomic DNA from 200 spontaneous *lys2*$^-$ mutants, in no case was there a Ty insertion into the coding sequence, which is 4200 bp long. In two cases, Ty insertion had occurred into the promotor region. Ty elements and δ sequences are also found in the neighborhood of tRNA genes (Cameron *et al.*, 1979: Gafner *et al.*, 1982; Eigel *et al.*, 1982). Thus one can postulate that certain features of intergenic DNA promote Ty insertion, that coding region DNAs inhibit insertion, or that Ty insertion into a coding region is detrimental to the cell beyond inactivation of that gene.

D. Effects of Ty1 Insertion on Gene Expression

Several mutants in *S. cerevisiae* are altered in gene expression as a result of insertion of a Ty element upstream from a structural gene. In some cases this insertion has resulted in loss of expression of the adjacent gene. Two mutations, *his4-912* and *his4-917*, have been described where transposition of Ty to a location upstream from the *HIS4* gene has produced a His$^-$ phenotype (Roeder *et al.*, 1980). In one case, the element Ty912 (see Fig. 2) is oriented so that the direction of transcription of the Ty element is the same as that of the affected gene. In the other case, transcription of the Ty element Ty917 is away from the gene. For mutations *his4-912* and *his4-917*, the sites of insertion are 98 and 8 base pairs upstream from the transcription start sites, respectively (Fink *et al.*, 1980). Three cases where insertion of a Ty element into the promotor of the *LYS2* gene has resulted in reduced expression of *LYS2* have also been observed (Eibel

and Philippsen, 1982). Many cases have been reported where increased production or altered regulation of the adjacent gene product has resulted from insertion of a Ty element into the 5′-flanking region of a gene. For example, two mutations, *CYC7-H2* and *CYP3-4*, have been described where insertion of Ty results in overproduction of iso-2-cytochrome c (Errede *et al.*, 1980a; Clavilier *et al.*, 1976; Montgomery *et al.*, 1982). Seven different mutations (*ADH2-1^c*, *-2^c*, *-3^c*, *-6^c*, *-7^c*, *-8^c*, *-9^c*)[1] have been identified in which insertion of a Ty element results in constitutive expression of the glucose-repressible isozyme of alcohol dehydrogenase (ADHII) (Williamson *et al.*, 1981). Six revertants of a *his 3* promotor deletion were due to insertion of Ty (Scherer *et al.*, 1982).

For all characterized Ty-associated mutations except *his4-912*, the Ty element is inserted so that the direction of transcription of Ty is away from that of the affected structural gene. In most cases the expression of the associated gene is affected by the mating competence of the yeast strain. Specifically, the 20-fold overproduction of cytochrome c associated with mutation *CYC7-H2* is seen in cells with the ability to mate (for example, *MAT a* or *MAT* α haploid cells) but only a 1- to 4-fold increase is seen in cells incapable of conjugation (i.e., in *MAT a/MAT* α diploid cells or in haploid cells carrying certain sterile mutations: *ste7*, *ste11*, and *ste12*, but not *ste5*) (Rothstein and Sherman, 1980; Errede *et al.*, 1980a,b). Cis-dominant overproducing mutations at loci involved in arginine catabolism and urea utilization (*CAR1*, *CAR2*, and *DUR1,2;* mutations *cargA$^+$O^h*, *cargB$^+$O^h*, and *durO^h*, respectively) are affected by the mating condition of the cells in a similar manner (Lemoine *et al.*, 1978; Deschamps and Wiame, 1979). Errede *et al.* (1980a) proposed the term *ROAM* (*r*egulated *o*verproducing *a*lleles responding to *m*ating signals) to categorize such mutations. Since then, the two mutations, *cargA$^+$O^h-1* and *cargA$^+$O^h-2*, have each been shown to contain a Ty element upstream from the arginase structural gene (Jauniaux *et al.*, 1981, 1982). The mating effect has also been shown to occur for all of the positively affected Ty mutations at the *ADH2* locus. However, for *ADH2*, the ROAM effect is clearly evident only when the cells are grown on a nonfermentable carbon source such as glycerol (Young *et al.*, 1982).

Ty-associated mutations have many interesting characteristics that have facilitated investigation of the Ty elements themselves. For example, many of these mutations are unstable, and further changes in gene expression occur spontaneously. These changes in expression are most commonly accompanied by loss of the Ty element with retention of a single delta sequence in front of the gene.

[1]Designations for alcohol dehydrogenase genes have been changed recently (Michael Ciriacy, personal communication). Therefore, designations here may differ from those presented in earlier publications. The structural gene coding for ADHII has been changed from *ADR2* to *ADH2*. Mutations that overproduce ADHII because of insertion of Ty adjacent to the structural gene *ADH2* have been changed from *ADR3-2^c*, *ADR3-3^c*, etc. to *ADH2-2^c*, *ADH2-3^c*, etc.

Other genomic DNA changes such as translocations, inversions, and transpositions have also been associated with alterations in expression of these genes. For example, the Ty-associated His$^-$ mutations *his4-912* and *his4-917* regain a His$^+$ phenotype at high frequencies (10^{-5} and 10^{-4}, respectively) (Roeder *et al.*, 1980). These phenotypically His$^+$ yeast strains do not revert to true wild-type expression. They are cold sensitive and recessive for *HIS4* expression and have retained a single delta sequence in place of Ty (Chaleff and Fink, 1980; Roeder and Fink, 1980). More complex rearrangements such as translocations, inversions, transpositions, and deletions were associated with the phenotypic reversion when revertants were isolated from a diploid strain homozygous at *his4-912* (Chaleff and Fink, 1980; Roeder and Fink, 1980). These will be discussed further in Section II,F. The seven mutants that overproduce ADHII as a result of Ty insertion are also unstable, and the frequency of spontaneous ADHII$^-$ derivatives varies considerably (from 10^{-3} to 10^{-7}), depending on the particular Ty element and on yeast strain background differences (Ciriacy and Williamson, 1981). Most of these ADHII$^-$ derivatives retain a single delta sequence in place of the Ty element and differ from wild type in that they no longer express high levels of ADHII activity under derepression conditions. However, some do express a small amount of ADHII when derepressed. Other ADHII$^-$ mutations appear to contain more complex DNA rearrangements similar to those seen with *his4-912*.

Unlinked loci that are involved in Ty-associated gene expression have been identified. Mutations that occur at three different loci and that result in a His$^+$ phenotype associated with Ty-insertion mutations *his4-912* and *his4-917* have been described (Roeder *et al.*, 1980). These are called *spm* for supressor-mutator, after the phenotypically similar system in maize. Mutation *spm1* suppresses the His$^-$ phenotype of both *his4-912* and *his4-917*. Mutations *spm2* and *spm3* suppress the *his4-917* mutation but not the *his4-912* mutation (Roeder and Fink, 1982b). All three *spm* mutations suppress the cold sensitivity of the His$^+$ revertants, which carry a single copy of δ. Unlinked reversions of a *his3*$^-$ mutation that has a Ty element 5′ to the *HIS3* structural gene have also been observed to occur at a high frequency (Scherer and Davis, 1980b). Mutations that occur at four different loci (called *tye1–tye4* for Ty effectors) and that reduce expression of Ty-associated ADHII mutations have been reported (Ciriacy and Williamson, 1981). Dubois *et al.* (1982) have isolated unlinked recessive mutations (*roc* for *ROAM* mutation control) in two complementation groups (*roc1* and *roc2*); these mutations reduce overproduction in all tested *ROAM* mutations (*cargA*$^+$*O*^{h}; *cargB*$^+$*O*^{h}, *durO*^{h}, and *CYC7-H2*). These *roc* mutations have no affect on the mating ability of the yeast in which they exist. The *roc1*, *roc2*, and *ste7* mutations also result in reduced Ty RNA levels. Certain mutations conferring sterility, *ste7*, *ste11*, and *ste12*, both affect expression of *ROAM* mutations and alter Ty-associated gene expression. Errede *et al.* (1980a,b) have proposed that *ROAM*