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Position Effects in *Saccharomyces cerevisiae*

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Simple inversion of a DNA fragment simultaneously alters both *trp1* and *his3* expression in yeast even though the inversion break points map more than 300 base-pairs from either structural gene. In the inverted configuration, a *his3* null allele is abnormally expressed and the wild-type *trp1* allele is not expressed. These unexpected phenotypes are probably not caused directly by readthrough transcription from nearby promoters. Thus, it is important to consider potential position effects when interpreting the phenotypes of alleles produced by chromosomal rearrangement, as sequences located more than 300 base-pairs from the structural gene can play a major role.

1. Introduction

Classical genetic analysis has frequently involved strains in which the genomic topographies are rearranged by deletion, inversion, duplication or translocation. In general, gene expression does not depend upon genomic location. However, situations have been described in which the position of a particular allele affects its phenotype. These include position effects and transvection in *Drosophila melanogaster* (Lewis, 1950,1955), controlling elements in maize (McClintock, 1956), and mating type cassettes in yeast (Hicks *et al.*, 1977). The molecular bases for these phenomena are unknown. In *Escherichia coli* and *Salmonella typhimurium* there are instances formally analogous to position effects. These can occur by readthrough transcription from fused promoters (Jacob *et al.*, 1965) or by inhibition by convergent transcription (Levinthal & Nikaido, 1969; Ward & Murray, 1979).

The ability to introduce cloned, physically defined segments of DNA back into the organisms from which they were initially derived is essential for elucidating structure–function relationships of genetic elements. Derivatives of a particular gene can be obtained by a variety of *in vivo* or *in vitro* techniques. By determining the *in vivo* phenotypes of cloned derivatives, the elements necessary for proper expression and regulation can be dissected. However, these experiments necessitate constructing hybrid molecules consisting of DNA segments that are not usually adjacent, and in some cases originate from different organisms. Therefore, the interpretation of phenotypes following introduction of hybrid molecules containing

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mutated derivatives of a particular gene may be complicated by position effects. Here, I describe a situation in the yeast *Saccharomyces cerevisiae* in which inversion of a particular DNA fragment alters expression of two genes. A preliminary report of this work has been published (Struhl, 1979).

2. Materials and Methods

(a) *Phage, bacterial, and yeast strains*

The accompanying papers describe the *E. coli* strain *hisB*463, the yeast strain SC3, and the λ*his3* hybrid phages (λgt-Sc2619, λgt-Sc2639, λgt-Sc2666, λgt-Sc2667, λgt-Sc2669, λgt-Sc2670) and λgt-Sc2671 (Struhl, 1981; Struhl & Davis, 1981*b*). The yeast vectors YRp7 and YRp7′ have been described by Struhl *et al.* (1979) and Stinchcomb *et al.* (1979). These strains were propagated as described in the above references.

(b) *Construction of hybrid DNA molecules*

The appropriate vector and λ*his3* DNAs were cleaved with *Bam*HI endonuclease and then ligated with T4 DNA ligase. Hybrid molecules were introduced into the *E. coli* strain *hisB*463 and ampicillin-resistant transformants were selected. The resulting colonies were screened for sensitivity to tetracycline and for a His^+ phenotype in *E. coli*. As expected from results in an accompanying paper (Struhl, 1981), the relevant *Bam*HI fragments from the λ*his3* phages (except for λgt-Sc2619) confer a His^+ phenotype in either orientation in either vector. λgt-Sc2619 lacks the entire *his3* structural gene so the relevant YRp7 and YRp7′ hybrids were identified without using the His^+ complementation assay. The details for these procedures have been described (Struhl & Davis, 1980). The cloned *Bam*HI DNA fragments have been given new isolation numbers (see the legend to Fig. 1).

To determine the orientation of the *his3* fragment with respect to the vectors, DNAs were cleaved with *Eco*RI and *Xho*I. *Eco*RI cleaves 375 base-pairs from the site of the inserted *his3* fragments (Sutcliffe, 1978), and *Xho*I cleaves asymmetrically within the inserted *Bam*HI DNA fragments (Struhl & Davis, 1980).

(c) *Determining the phenotypes of hybrid molecules in yeast cells*

The transformation of yeast strain SC3 by hybrid DNA molecules was performed as described by Struhl *et al.* (1979). Following incubation with polyethylene glycol, portions of spheroplasts were immediately plated on medium lacking either histidine or tryptophan. His^+ or Trp^+ phenotypes were indicated by the presence of approximately 1000 colonies/μg after 3 days at 30°C.

3. Results and Discussion

(a) *Inversion of one fragment generates two position effects*

The structures of the relevant hybrid DNA molecules are illustrated in Figure 1. Each molecule contains two segments of yeast DNA. One is an *Eco*RI-generated fragment that contains the intact *trp1* gene and *ars1*, a genetic element that allows hybrid DNA molecules to replicate autonomously in yeast cells (Struhl *et al.*, 1979; Stinchcomb *et al.*, 1979). The second set of segments are generated by *Bam*HI cleavage: these contain the entire *his3* structural gene, 600 base-pairs normally adjacent to the 3′-end of the gene, and a variable number of base-pairs normally adjacent to the 5′-end of the structural gene sequences fused to 240 base-pairs of DNA from the attachment site of bacteriophage λ. The intersections between λ and yeast DNA represent deletion break points within the wild-type *his3* sequence. The

TABLE 1

Phenotypes of hybrid molecules

his3 inserted fragment	Vector YRp7	Vector YRp7′
Sc2715	T	T
Sc2734	T	T
Sc2735	T	T
Sc2737	T	T
Sc2738	T	T
Sc2712	T	T
Sc2716	TH	TH
Sc2715′	"H"	T"H"
Sc2734′	"H"	T"H"
Sc2735′	"H"	T"H"
Sc2737′	"H"	T"H"
Sc2738′	"H"	T"H"
Sc2712′	—	T
Sc2716′	H	TH
Sc2791′	T"H"	ND

his3 fragments from various deleted derivatives were cloned into the *Bam*HI site of YRp7 and YRp7′. The orientation of the *trp1* and *his3* DNA fragments are indicated in Fig. 1. Wild-type *trp1* and *his3* phenotypes are indicated, respectively, by T and H. Null phenotypes (absence of any detectable growth) are not listed. Entries marked with an "H" phenotype indicate that the appropriate transformants grow at a reduced rate in the absence of histidine and do not grow in the presence of aminotriazole (see text). ND, not determined.

trp1 and *his3*-containing DNA fragments were cloned, respectively, into the *Eco*RI and *Bam*HI sites of pBR322 each in either of the two possible orientations. The distance between the cloned fragments is 375 base-pairs (Sutcliffe, 1978).

The YRp7-*his3* hybrid molecules were introduced into SC3 (*ura3*-52 *trp1*-289 *his3*-*Δ*1) (Scherer & Davis, 1979); transformants were selected by their growth in the absence of histidine or tryptophan. As described in the previous paper (Struhl & Davis, 1981*b*), molecules with structures such as YRp7-Sc2715 (Fig. 1) confer a $Trp^+ His^-$ phenotype. Such molecules confer a His^- phenotype because the normal *his3* promoter is severed from the structural gene. However, when the same yeast strain is transformed by molecules such as YRp7-Sc2715′ (with *his3* inverted with respect to *trp1*) the resulting transformants are $Trp^- His^+$. In other words, simple inversion of a DNA fragment simultaneously alters the phenotypes of two alleles. Therefore, *his3* and *trp1* are both subject to position effects in yeast cells.

(b) *Position effect on* his3

The position effect on *his3* depends upon the particular alleles used. Derivatives Sc2715, Sc2734, Sc2735, Sc2737 and Sc2738 have deletion break points less than 100 base-pairs from the 5′-end of the structural gene, and they show the effect. An analogous derivative (Sc2716) having its deletion break point 300 base-pairs outside the 5′-end does not.

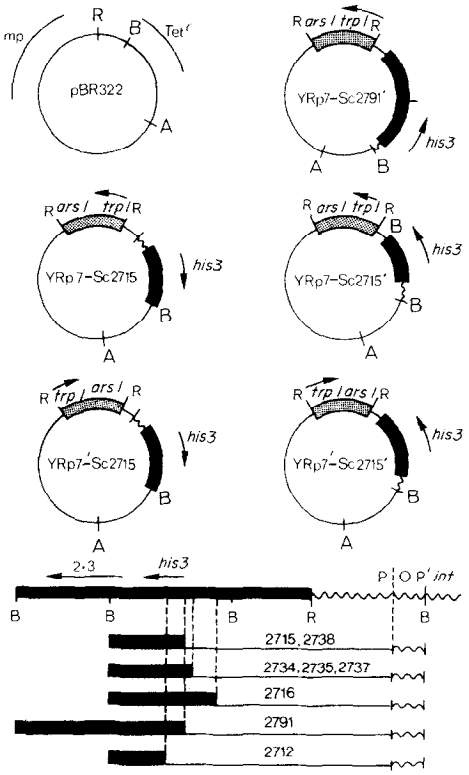

FIG. 1. Structure of hybrid molecules. The structures of several prototypic molecules are shown in the top part of the Figure (restriction sites for *Eco*RI (R), *Bam*HI (B), and *Ava*I (A) are indicated). pBR322 DNA sequences are indicated by a solid line; *ars1*,*trp1* by a shaded bar; *his3* sequences by a solid bar. The direction of transcription is shown for the *trp1*, *his3* and 2·3 kb mRNA genes (Struhl & Davis, 1981*a*). The lower part of the Figure is a physical map of the *his3* derivatives used in this study. The cloned *Bam*HI DNA fragments originate from λ*his3* deletion phages described previously (Struhl & Davis, 1980,1981*b*). These are Sc2715 (from λgt-Sc2639), Sc2734 (from λgt-Sc2666), Sc2735 (from λgt-Sc2667), Sc2737 (from λgt-Sc2669), Sc2738 (from λgt-Sc2670), Sc2712 (from λgt-Sc2619) and Sc2716 (from λgt-Sc2671). All include an intact *his3* structural gene (except for Sc2712) fused to λ attachment site sequences (Struhl, 1981; Struhl & Davis, 1981*b*). Sc2691 probably resulted from partial *Bam*HI cleavage of λgt-Sc2639 DNA. *Bam*HI DNA fragments from all these derivatives were cloned in both possible orientations in either YRp7 or in YRp7'.

The *his3* expression seen from YRp7-Sc2715' is unusual for two reasons. First, this allele confers a His^- phenotype when cloned as different DNA fragments and inserted into different vectors (Struhl, 1979; Scherer & Davis, 1980; Struhl & Davis, 1981*b*). Second, the His^+ transformants grow at a slower rate than those transformed by wild-type *his3* alleles, and they are extremely sensitive to aminotriazole, a competitive inhibitor of the *his3* gene product.

What DNA sequences are responsible for this *his3* position effect? The orientation dependence indicates that sequences present on the cloned *his3* fragment are unlikely to be involved. Rather, the important sequences appear to be located in the adjacent vector DNA. Insertion of the yeast *ura3* gene at the *Ava*I site of an analogous hybrid molecule does not affect this unexpected *his3* expression

(Scherer & Davis, 1980). Therefore, the sequences responsible are likely to be located between the *Bam*HI and the *Ava*I sites of pBR322 DNA.

How may the *his3* position effect be explained? In *E. coli*, a directional effect that suppresses the normal phenotype of promoter down mutations is likely to be explained by fusion of a new promoter to the structural sequences. However, this is unlikely to be the explanation for the aberrant *his3* expression. First, the hypothetical *his3* fusion messenger RNA would have a leader sequence of at least 300 nucleotides. In yeast, the evidence strongly suggests that translation is initiated at the 5′-proximal AUG codon (Stewart *et al.*, 1971; Sherman *et al.*, 1980). The fusion RNA would have at least eight AUG codons before the normal *his3* translation start site and read-through translation is precluded by in frame termination codons immediately before the normally translated sequences (Struhl & Davis, 1981*b*). Second, preliminary results indicate that the aberrant expression is accompanied by production of a normal sized *his3* mRNA; these alleles generally produce no detectable RNA (Struhl & Davis, 1981*b*). Another possibility is that there is readthrough transcription from a promoter in pBR322 DNA, but that it is processed into a normal *his3* mRNA. However, there is no evidence for processing of *his3* transcripts (Struhl & Davis, 1981*a*,*b*). A third possibility is that the important pBR322 sequences affect chromatin structure in some way that allows for aberrant expression; such an effect would have to be propagated over a distance of at least 300 base-pairs.

(c) *Position effect on* trp1

The 1·4 kb† yeast DNA fragment containing *trp1* is likely to contain the entire gene. When cloned in both possible orientations in a variety of plasmid or λ vectors, the fragment complements yeast *trp1* and *E. coli trpC* mutations (Stinchcomb *et al.*, 1979; my unpublished results). Thus, the position effect seen in YRp7-Sc2715′ and related derivatives is likely to result from inactivation of wild-type *trp1* expression.

The position effect on *trp1* depends upon the orientation of both the *trp1* and the *his3*-containing DNA fragments. In three out of four possible orientations, *trp1* function is normal. Inactivation of *trp1* expression occurs only in the orientation indicated by YRp7-Sc2715′. The inactivation does not depend upon the *his3* allele or upon *his3* expression because derivatives YRp7-Sc2712′ (lacking part of the *his3* structural gene) and YRp7-Sc2716′ (containing the entire *his3* locus) have the same effect. Inhibition of *trp1* expression can be prevented by inserting another *Bam*HI-generated DNA fragment between the *his3* and the *trp1*-containing fragments. In YRp7-Sc2791′, the inserted *Bam*HI DNA fragment is the one normally adjacent to the *his3*-containing fragment in genomic yeast DNA. Taken together, these results indicate that something present on the *his3*-containing DNA fragment confers a directional position effect on *trp1* gene expression. The effect must be propagated over at least 375 base-pairs (the distance between the cloned fragments), but it can be eliminated by insertion of DNA between the relevant sequences.

† Abbreviation used: kb, 10^3 bases.

There are several possibilities for the *trp1* position effect. In *E. coli*, the usual explanation for a directional position effect that inactivates a wild-type allele is inhibition by convergent transcription (Levinthal & Nikaido, 1969; Ward & Murray, 1979). Yeast cells normally produce a 2·3 kb mRNA species adjacent to the 3′-end of the *his3* mRNA coding sequences (Struhl & Davis, 1981*a*). This transcript is initiated within the *his3*-containing *Bam*HI DNA fragments used here, but is normally terminated about 2 kb past the end of the cloned fragment. Therefore, under conditions when *trp1* expression is not observed, transcription initiated at the normal site for the 2·3 kb mRNA species should read through into the *trp1* region. However, the *trp1* gene in YRp7-Sc2715′ is transcribed in the same direction as the *his3* gene and the 2·3 kb mRNA (Tschumper & Carbon, 1980). Thus, it is unlikely that the *trp1* gene is inactivated by a convergent transcription mechanism. An interesting possibility is that chromatin structure effects act at a distance from the site of transcriptional initiation. Such a model could also account for the position effect on *his3*. However, there is no evidence for this or any other model.

(d) *Position effects and the interpretation of phenotypes*

Though molecular mechanisms have yet to be determined, it is clear that yeast cells are subject to various kinds of position effects. The simple inversion of a particular DNA fragment can simultaneously alter the expression of two structural genes mapping at least 300 base-pairs from the inversion break points. In *Drosophila*, because the molecular nature of mutations is difficult to determine, clear examples of position effect are noted only when the chromosomal rearrangements are visible by light microscopy (Lewis, 1950,1955). The position effects described here are associated with relatively small rearrangements. However, this does not mean that position effects in *Drosophila* are mechanistically unlike those in yeast.

The position effects described here may not represent an uninteresting, artifactual situation. In *S. cerevisiae*, there are three copies of the mating type genes. The copy at the mating type locus determines the mating type; the other two copies are silent (Hicks *et al.*, 1977). Recent structural analysis indicates that the three copies are identical in DNA sequence, and that the region encoding the 5′-ends of the mRNA transcripts is in the middle of the locus. This indicates that sequences located at least 700 to 1400 base-pairs away affect transcriptional initiation (Nasmyth *et al.*, 1981; Klar *et al.*, 1981).

The existence of effects that act at a distance should exert caution on interpreting the results of genetic experiments. This is particularly pertinent for those using recombinant DNA molecules with structures bearing little resemblance to naturally occurring DNA. How is one to assess the "real" phenotype of a particular allele if its expression can vary with position? Regrettably, in the absence of other kinds of supporting data, the best way is to clone a given allele in a variety of genetic environments. The assumption is that position effects represent an unusual situation. The position effects on *his3* and *trp1* occur only in particular molecules; thus it seems quite likely in these cases that the "real" phenotypes of the alleles used are $Trp^+ His^-$.

Interpreting phenotypes of chromosomal rearrangements produced in the absence of recombinant DNA technology may have similar complexities. For example, most mutations in the *bithorax* region of *D. melanogaster* are chromosomal aberrations, and their complementation patterns are complex (Lewis, 1978). In these and other cases, it may be difficult to distinguish whether a mutation defines the gene itself or results in a position effect on expression of the gene.

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