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Naturally occurring poly(dA-dT) sequences are upstream promoter elements for constitutive transcription in yeast

(gene expression/RNA polymerase II/chromatin/transcriptional enhancer/genome organization)

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ABSTRACT ***pet56, his3,* and *ded1* are adjacent but unrelated genes located on chromosome XV of the yeast *Saccharomyces cerevisiae*. *his3* and *pet56* are transcribed in opposite directions from initiation sites separated by ≈200 base pairs. Under normal growth conditions, both genes are transcribed at a similar basal level. Deletion analysis of the *his3* gene indicates that the upstream promoter element for constitutive expression is defined by a 17-base-pair region that contains 15 thymidine residues in the coding strand. Sequential deletions of the *pet56* gene indicate that this same region is required for wild-type transcription levels. Thus, this poly(dA-dT) sequence acts bidirectionally to activate transcription of two unrelated genes. Transcription of the *ded1* gene is initiated ≈300 base pairs downstream from the *his3* gene, and it occurs at a 5-fold higher level. This gene contains a 34-base-pair region containing 28 thymidine residues in the coding strand located upstream from the *ded1* TATA box. Deletion of this dA-dT stretch significantly reduces transcription below the wild-type level. Thus, for at least three different yeast genes, naturally occurring stretches of poly(dA-dT) serve as upstream promoter elements for constitutive expression. In addition, it appears that longer stretches of poly(dA-dT) are more effective upstream promoter elements. These transcriptional effects may be due to exclusion of nucleosomes from poly(dA-dT) regions.**

Yeast promoters are composed of at least two distinct elements, both of which are required for transcription (reviewed in refs. 1 and 2). The TATA element is a highly conserved sequence (consensus TATAAA) that has some general role in transcription. Upstream elements, which resemble mammalian enhancer sequences in that they act in both orientations and at long and variable distances from the initiation site, determine promoter specificity. Upstream elements for different classes of genes are defined by different DNA sequences and they require different *trans*-acting factors. Moreover, in all cases that have been examined, transcriptional regulation is mediated by the upstream sequences. In particular, promoter fusions between the upstream region of gene *A* and the TATA region of gene *B* are regulated similarly to the intact gene *A*. However, the fact that different upstream elements function when joined to a given TATA element strongly suggests that the mechanism of transcriptional activation is similar.

Although most yeast genes under study have been chosen because their expression is regulated in some interesting manner, it is likely that many, if not most, genes are transcribed at the same level under all conditions. General surveys indicate that ≈50% of the yeast genome is transcribed under normal growth conditions (3, 4). Analysis of thousands of individual yeast DNA segments indicates that most genes are transcribed at similar levels (5)—1–2 mRNA molecules per cell at the steady state (6). Moreover, in any particular regulatory situation, transcription and translation levels are altered for only a very low percentage of genes. Thus, it is possible that current studies have overlooked "typical" promoters.

If constitutive transcription of otherwise unrelated genes is achieved by a common mechanism, one might expect that the upstream elements of their promoters would contain similar DNA sequences. A common feature of many yeast promoter regions that has been noted by many investigators is the presence of poly(dA-dT) sequences (see *Discussion*).

A typical region of the yeast genome is represented by a 10.1 kilobase (kb) segment of chromosome XV that encodes the *ded1*, *his3*, and *pet56* genes, as well as three other genes of unknown function (6, 7). Although *ded1*, *his3*, and *pet56* are adjacent in the genome (Fig. 1), they encode unrelated functions. *his3* encodes the histidine biosynthetic enzyme IGP dehydratase, *pet56* is essential for mitochondrial function, and *ded1* is essential for cell growth (7). In normal growth conditions, all of these genes except *ded1* are transcribed at average rates; *ded1* RNA levels are about 5 times higher (6). Under conditions of amino acid starvation, *his3* transcription is increased ≈5-fold, whereas transcription of the other genes is not affected.

Here, I analyze sequential deletion mutants of the *his3*, *pet56*, and *ded1* promoters in order to determine the DNA sequences necessary for wild-type levels of transcription. The results indicate that constitutive transcription of all three genes depends on poly(dA-dT) sequences upstream of the TATA element. Moreover, it appears that the 17-base-pair poly(dA-dT) region between the *his3* and *pet56* genes serves as the upstream promoter element for both, and that the higher transcription level of the *ded1* gene is due to a longer poly(dA-dT) region.

MATERIALS AND METHODS

The starting DNA molecules used in this paper as well as the technical details for the experimental procedures have been described (8–10). The structures of the deletion mutant DNAs were verified by restriction endonuclease cleavage and by DNA sequencing (11, 12).

To analyze the *his3* deletions, mutant DNAs were cleaved with *Xba* I and introduced into yeast strain KY117 (relevant genotype, *ura3-52 his3-Δ200*) by selecting for Ura$^+$ colonies (8). The transforming DNA provides the only *his3* information in the resulting strains because the *his3-Δ200* allele removes the entire gene. Transformants containing a single copy of the introduced DNA integrated at the *his3* locus were identified by genomic hybridization methods (data not shown). To analyze *pet56* deletions, DNA molecules were cleaved with *Hpa* I and introduced into strain KY463 (relevant genotype a *ura3-52 his3*$^+$ *cyh2*^{r}) (10), and Ura$^+$ trans-

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Abbreviation: kb, kilobase(s).

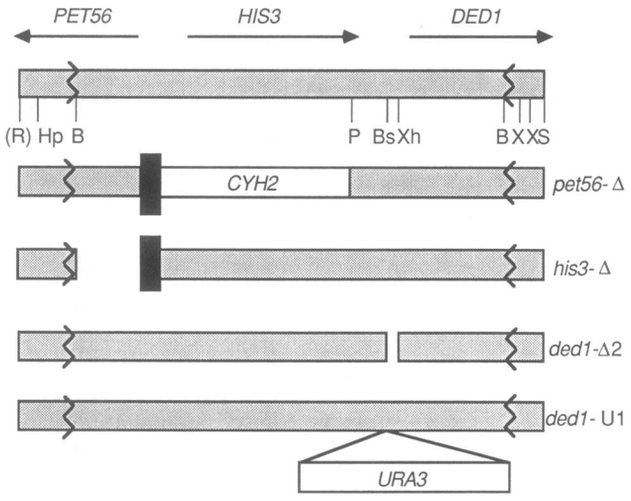

FIG. 1. Structure of the *pet56–his3–ded1* region in wild-type and mutant strains. The shaded bar at the top of the figure represents Sc2812, a 6.1-kb *Eco*RI/*Sal* I fragment (with a mutated *Eco*RI site) that contains the intact *pet56*, *his3*, and *ded1* genes (8). The location and orientation of the transcripts are indicated by arrows above the shaded bar, and restriction endonuclease sites are indicated as vertical lines below the bar. R, mutated *Eco*RI; Hp, *Hpa* I; B, *Bam*HI; P, *Pst* I; Bs, *Bss*HII; Xh, *Xho* I; X, *Xba* I; S, *Sal* I). The drawing is to scale for the 1765 base pairs between the *Bam*HI sites. The structures of various derivatives are shown below. The locations of the *pet56* and *his3* deletion end points (which occur at various positions in a 90-base-pair region) are indicated as solid boxes. All deletion mutants have an *Eco*RI linker at the joint between the shaded bar and the solid box. The inserted *cyh2* and *ura3* fragments (not to scale) are shown as open boxes.

formants due to single integration at the *his3* locus were selected. After growing these transformants in nonselective medium, Ura$^-$ segregants were selected by virtue of their resistance to 5-fluoroorotic acid (13). As expected from the structures of the *pet56* deletion DNAs, 50% of the segregants were His$^-$ and sensitive to 40 μM cycloheximide and thus represent gene replacement events (10); this was confirmed by genomic hybridization analysis (data not shown). To analyze the *ded1* derivatives, the 1.7-kb *Bam*HI fragments containing the intact *his3* structural gene were introduced into KY117; as expected, the His$^+$ colonies that arose were due to gene replacement (14). Because of the possibility that the mutant DNAs might be lethal because of inefficient expression of the *ded1* gene, the *Bam*HI fragments were also introduced into KY119, an isogenic **a**/α diploid. However, equal numbers of His$^+$ transformants were obtained with both host strains, and transformants of the haploid strain grew indistinguishably from the wild-type strain.

To measure transcription levels, RNA was extracted from strains grown in yeast extract/peptone/dextrose (YPD) broth and hybridized to completion with an excess of single-stranded DNA probes, each labeled at their 5′ ends with ^{32}P (15, 16). After treatment of the hybridization mixtures with S1 nuclease, the resulting products were electrophoretically separated in a 6% acrylamide gel containing 7 M urea.

RESULTS

Deletion Analysis of the *his3* Gene. Previous deletion analysis of the *his3* gene defines an upstream promoter element located between nucleotides −113 and −158 (with respect to the mRNA start site) and a TATA element between −35 and −55 (9, 17). The upstream region contains two DNA sequences of interest (15) (Fig. 2); a 17-base-pair stretch containing 15 thymidine residues in the coding strand, and a sequence homologous to the regulatory site mediating the general control of amino acid biosynthetic genes (18–20).

To determine which of these regions is critical for the constitutive basal level of *his3* expression, a new series of sequential 5′ deletion mutants was generated with *BAL*-31 exonuclease and *Eco*RI linkers. Each mutant DNA contains the entire *his3* mRNA coding region, the TATA promoter element, and a variable number of base pairs upstream from the TATA region (Figs. 1 and 2). By joining these *his3* segments to a position within the *pet56* structural gene corresponding to *his3* nucleotide −447 (7), the resulting molecules are equivalent to the wild-type 6.1-kb *pet56–his3–ded1* chromosomal region except that sequences between −447 and a particular point near the *his3* promoter region are deleted. The position within the *pet56* structural gene was chosen in the hope that the region upstream of −447 would not contain upstream promoter sequences and hence would not interfere with measurements of *his3* transcription. The deletion mutant DNAs were integrated in single copy pre-

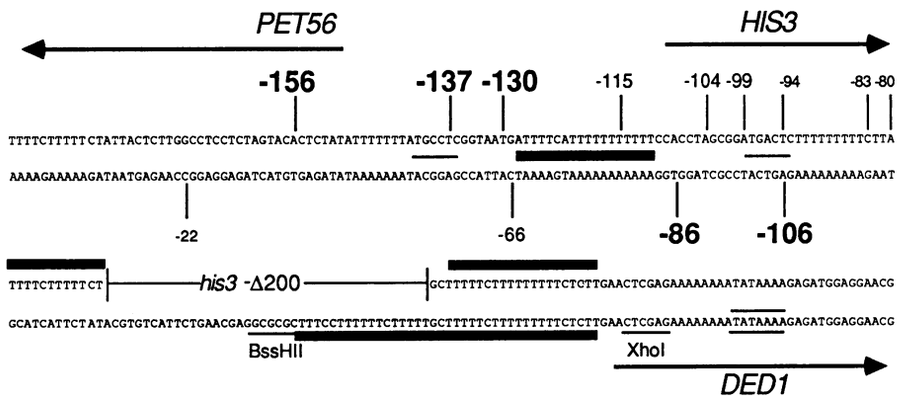

FIG. 2. DNA sequences of the *pet56*, *his3*, and *ded1* promoter regions and of deletion mutants. The top two lines show the nucleotide sequence of both strands of the divergent *his3–pet56* promoter region. Diagrammed between the strands are the poly(dA-dT) region important for the constitutive expression of *his3* and *pet56* (solid bar) and the TGACTC sequences important for induction of *his3* expression in response to amino acid starvation (thin lines). Each deletion mutant is indicated by a vertical line (*his3* deletions are shown above the two strands and *pet56* deletions are shown below), and the number associated with each line indicates the precise deletion end point with respect to the relevant RNA start site (defined as +1). A derivative with an end point of −X means that sequences upstream of −X are deleted. Derivatives that confer wild-type phenotypes are indicated by large bold numbers; derivatives conferring mutant phenotypes are indicated by smaller numbers. The bottom two lines show the DNA sequence of the relevant regions of *his3-Δ200* and of the wild-type *ded1* gene (top strands only). The locations of the poly(dA-dT) regions (solid bars), the *ded1* TATA sequence (thin lines above and below), and the *Bss*HII and *Xho* I sites are indicated. *his3-Δ200* represents a fusion between the *pet56* and *ded1* promoter regions.

cisely at the *his3* locus, and the resulting strains were assayed for the level of *his3* RNA (Fig. 3).

Deletion mutants that remove *his3* sequences as far downstream as −130 are transcribed at levels indistinguishable from the wild-type gene. Thus, sequences further upstream from −130 are unnecessary for the constitutive expression of *his3*. However, mutants with more extensive deletions are transcribed much less efficiently. Mutants with deletion break points at −115, −104, and −99 are transcribed at ≈20% of the wild-type level. This indicates that a sequence between −129 and −115 is critical for basal levels of *his3* expression. Moreover, since deletion mutants described previously (9) define the downstream boundary at −113, the results here indicate that the upstream promoter element for constitutive *his3* expression maps between −113 and −130. This region contains 15 thymidine residues in the coding strand, including a stretch of 11 consecutive ones.

Mutants with end points at −94, −85, and −80 are even more defective. They are unable to grow in medium lacking histidine and, as expected, their *his3* levels are <10% of the wild-type level. The phenotypic difference between the −99 deletion and the −94 deletion indicates that the region between these two points has some functional role. Indeed, this region corresponds precisely with the TGACTC sequence that is critical for *his3* regulation as a function of amino acid starvation (18).

Deletion Analysis of the *pet56* Gene. *pet56*, a gene essential for mitochondrial function, is adjacent to *his3* in the normal genome, but it is transcribed in the opposite direction. As deduced from DNA sequence analysis, the *his3* and *pet56* protein-coding regions are separated by only 237 base pairs (7). Mapping the 5′ termini of pet56 RNA species (7) indicates that the primary initiation site is located only 192 ± 2 base pairs away from the *his3* initiation site at +1; several less-utilized initiation sites are observed both upstream and downstream from the major site. Thus, although *his3* and *pet56* encode unrelated cellular functions and are not coregulated, their promoter regions must be extremely close together or overlapping.

A series of deletion mutants that successively encroach on the *pet56* structural gene were created by the *BAL*-31–*Eco*RI linker method. The resulting DNA molecules are equivalent to the wild-type 6.1-kb *pet56–his3–ded1* fragment, except that the entire *his3* structural gene between the *Pst* I site and a particular point near the *pet56* promoter region is deleted and replaced by a 2.5-kb *cyh2* fragment (Figs. 1 and 2). To determine phenotypes, the wild-type *pet56–his3–ded1* chromosomal region was replaced by the various *pet56* deletion DNAs.

The following results indicate that a strain lacking all sequences upstream of −22 shows a classic petite phenotype and hence is defective for *pet56* expression. First, this strain forms small colonies on normal growth medium containing glucose. Second, the colonies are white even though these strains are genotypically *ade2*$^-$. Normally, *ade2* mutants accumulate an intermediate in adenine biosynthesis, which is oxidized and excreted as a red pigment. The pet mutants, however, are unable to oxidize this intermediate and hence remain white. Third, the cells fail to grow in glycerol medium, a condition that requires mitochondrial function.

A deletion that removes sequences upstream of −66 causes a mutant phenotype that is less extreme. On glucose medium, the colonies are somewhat larger and they are faintly pink. On glycerol medium, the cells grow, albeit extremely poorly as compared to wild-type cells. These results indicate that *pet56* expression is qualitatively observable but below the wild-type level. In contrast, deletions with end points at −86 or −100 behave indistinguishably from the wild-type allele in terms of colony size, colony color, and ability to grow in nonfermentable carbon sources.

The levels of *pet56* transcription in these deletion mutants were examined by quantitative S1 nuclease analysis (Fig. 4). The results confirm the predictions based on the qualitative phenotypes just described. Specifically, RNA levels in strains containing the −86 derivative are indistinguishable from those of the wild-type strain. On the other hand, strains containing the −66 derivative make considerably less RNA (roughly 10–20% of the wild-type level), whereas those containing the −22 derivative do not synthesize detectable levels of RNA (<10%).

These results indicate that the sequences necessary for wild-type levels of *pet56* transcription are located within the flanking 86 base pairs upstream from the initiation site.

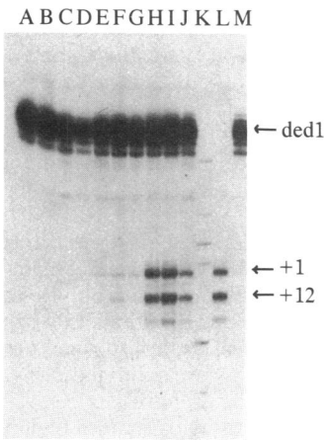

FIG. 3. Transcription in *his3* deletion mutants. RNA (50 μg) from strains containing the following *his3* alleles (listed by their deletion end point) were hybridized simultaneously to an excess of *his3* and *ded1* single-stranded probes and treated with S1 nuclease. Lanes: A, Δ200; B, −80; C, −83; D, −94; E, −99; F, −104; G, −115; H, −130; I, −137; J, −156. Lane K contains *Msp* I-cleaved pBR322 DNA 5′-end labeled with ^{32}P, and lanes L and M contain 50 μg of RNA from KY114 (wild type) hybridized separately to the probes. The positions corresponding to the *ded1* transcripts and the *his3* transcripts initiating from +1 and +12 are indicated (10, 15, 16).

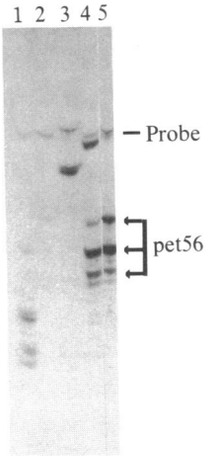

FIG. 4. Transcription in *pet56* deletion mutants. RNA (50 μg) from strains containing the following *pet56* alleles (listed by their deletion end point) were hybridized to an excess of *pet56* single-stranded probe and treated with S1 nuclease. Lanes: 1, Δ200; 2, −22; 3, −66; 4, −86; 5, wild type. The positions corresponding to the various *pet56* transcripts as well as the trace of renatured hybridization probe are indicated (7). The other bands observed in lanes 2–4 are due to read-through transcription from the *cyh2* region; their mobilities reflect the number of nucleotides from the 5′ end of the probe to the deletion break points.

Moreover, they indicate that the region between −66 and −86 is critical for *pet56* promoter function. This region coincides with the poly(dA-dT) stretch that defines the *his3* upstream element. In addition, the phenotypic distinction between the −22 and −66 derivatives is probably due to a TATA sequence located between the deletion end points (see Fig. 2). In this light, it is worth noting that *his3* promoter deletions that contain only the TATA element are expressed poorly, while those lacking both the TATA and upstream element are not expressed (17).

Deletion Analysis of the *ded1* Gene. The *ded1* gene, which is adjacent and downstream from *his3*, is essential for cell viability; deletion mutants that remove *ded1* structural sequences are unconditionally lethal (7). *ded1* encodes a 2.3-kb RNA species that is ≈5 times more abundant than *his3* or *pet56* RNAs (6). 5′ mapping of *ded1* RNA indicates that there are two predominant initiation sites, which are defined as the +1 and +10 transcripts (7). The nucleotide sequence upstream from these initiation sites has two features of interest. First, there is a TATAAA sequence located between nucleotides −60 and −65. This is the only region whose nucleotide sequence even remotely resembles the canonical TATA element. Second, a 34-base-pair region containing 28 thymidine residues in the coding strand is located between nucleotides −121 and −88. Since a poly(dA-dT) stretch is implicated as the upstream element for *his3* and *pet56* transcription, it seemed likely that this longer region would be important for *ded1* transcription.

Two *ded1* promoter variants were analyzed to determine whether this poly(dA-dT) region is important for transcription (Figs. 1 and 2). First, the region was deleted by removing DNA sequences between the fortuitously located *Bss*H2 and *Xho* I sites (*ded1-Δ2*). Second, to determine whether sequences upstream from this poly(dA-dT) region are unnecessary for wild-type *ded1* RNA levels, a 1.1-kb *ura3* DNA fragment (21) was inserted at the *Bss*H2 site (*ded1-U1*).

Although the *ded1-Δ2* allele confers sufficient *ded1* expression for cell viability, *ded1* transcription is reduced to ≈20% of the wild-type level (Fig. 5). In terms of mRNA molecules per cell, this level approximates the wild-type levels for the *his3* and *pet56* genes. On the other hand, the *ded1-U1* allele promotes transcription at the wild-type level. In both cases, the *ded1* transcripts are initiated from the correct sites. These results indicate that the poly(dA-dT) sequence is necessary for wild-type levels of *ded1* transcription and that sequences upstream of this region are relatively unimportant.

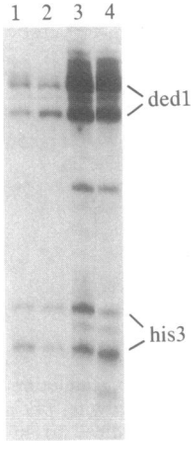

FIG. 5. Transcription in *ded1* derivatives. RNA (50 μg) from strains containing *ded1-Δ2* (lanes 1 and 2), *ded1*$^+$ (lane 3), and *ded1-U1* (lane 4) were hybridized simultaneously to an excess of *ded1* and *his3* single-stranded probes and treated with S1 nuclease. The positions corresponding to the *ded1* +1 and +10 transcripts and the *his3* +1 and +12 transcripts are indicated (7, 16).

The Case of *his3-Δ200*. *his3-Δ200* is an allele that deletes ≈1 kb of DNA, which includes the entire *his3* gene (8). DNA sequence analysis indicates that *his3-Δ200* represents a fusion between the initiation region of the *pet56* gene (end point, −10) and the upstream promoter element of the *ded1* gene (end point, −104) (Fig. 2). Nevertheless, *his3-Δ200* does not confer a petite phenotype even though it is a more extensive promoter deletion than the −22 derivative, which confers an extreme petite phenotype. As expected from the apparent wild-type phenotype of *his3-Δ200* cells, *pet56* RNA is easily detected, although the level is only ≈20% of that observed in wild-type strains (Fig. 5). However, most of the transcription begins at minor sites that are downstream from the major site. This phenomenon has been observed previously in deletions that remove the TATA elements of the *matα* genes (22). In this regard, it is worth noting that the promoter fusion in *his3-Δ200* lacks a functional TATA element.

With respect to the *ded1* gene, the *his3-Δ200* mutation removes part of the poly(dA-dT) region implicated as the upstream promoter element necessary for constitutive transcription. However, of the original 34-base-pair region, 17 base pairs are still present in this deletion, and they are directly fused to 12 base pairs containing 10 thymidine residues from the *pet56* region. As might be expected from the resulting 29-base-pair region that contains 24 thymidine residues, *ded1* transcription in *his3-Δ200* cells is indistinguishable from the wild type in terms of level and initiation sites (Fig. 3, lane A). Thus, it appears that in *his3-Δ200*, the *ded1* poly(dA-dT) sequence functions bidirectionally to activate ded1 and pet56 transcription. The observation that pet56 RNA levels are only ≈5% of *ded1* levels is probably due to the absence of a *pet56* TATA element.

DISCUSSION

Poly(dA-dT) Sequences Act Bidirectionally as Upstream Promoter Elements for Constitutive Transcription. Upstream promoter elements for a number of yeast genes have been defined primarily by analyzing the phenotypes of a set of deletion mutants that successively remove DNA sequences upstream from the structural gene. The generally observed result is that deletions up to a certain point confer transcriptional phenotypes that are indistinguishable from the wild-type gene, whereas deletions beyond that point significantly reduce transcription below the normal level. Although such analysis does not precisely delimit the upstream promoter element, it defines both the minimum contiguous region as well as critical DNA sequences necessary for wild-type transcription levels.

The sequential 5′ deletion analyses presented here indicate that the upstream elements of the *his3*, *pet56*, and *ded1* genes are defined by naturally occurring poly(dA-dT) sequences. For all three genes, deletion mutants that retain a particular stretch of dA-dT residues are transcribed equally efficiently as the wild-type gene, whereas related derivatives that lack this region are transcribed poorly.

In the best defined example, the *his3* region between −115 and −129 is critical for the basal transcription rate, whereas sequences >129 base upstream from the *his3* mRNA coding region are not necessary. Moreover, the phenotype of many other deletion mutants indicates that besides the TATA element, sequences downstream from −113 are not important for the basal level (10, 18). This means that the region between −113 and −129, which coincides with the poly(dA-dT) stretch, is necessary and sufficient to define the upstream promoter element for constitutive expression. This poly(dA-dT) stretch is distinct from the regulatory sequences that

induce *his3* expression in response to amino acid starvation (18). Thus, it appears that constitutive and induced *his3* expression is mediated by different upstream elements.

Similar analysis for the *pet56* promoter indicates that sequences between −66 and −86 are critical for the upstream element. This region coincides with the *his3* upstream element, which indicates that the same poly(dA-dT) region is essential for the constitutive transcription levels of two unrelated genes. This strongly suggests that poly(dA-dT) sequences activate transcription in a bidirectional manner, analogous to yeast enhancer-like sequences (8, 23).

In the case of *ded1*, deletion of a 34-base-pair region that contains 28 thymidine residues significantly reduces transcription below the wild-type level. In addition, the phenotype of *ded1-U1* suggests that sequences upstream of this region are relatively unimportant, although it should be noted that the end of the *ura3* fragment contains poly(dA-dT) stretches that may influence transcription. Therefore, because constitutive *ded1* levels are ≈5 times higher than those of *his3* or *pet56*, it seems likely that longer stretches of poly(dA-dT) are more effective upstream promoter elements.

The influence of long poly(dA-dT) sequences was first observed in rare constitutive *adr2* up-promoter mutants (24). In two of these mutants, the expansion of a normal 20-base-pair dA-dT sequence to a 54- or 55-base-pair homopolymer stretch presumably causes high levels of constitutive expression. The results presented here suggest that these *adr2* mutants do not represent a bizzarre artifact, but rather reflect functions of poly(dA-dT) tracts that are actually used in wild-type genes.

Possible Molecular Mechanisms. There are two mechanisms by which poly(dA-dT) sequences might activate transcription. One possibility is that, by analogy to proposed transcription factors that recognize different upstream elements, a specific protein interacts with poly(dA-dT) regions. For example, the *Drosophila melanogaster* D1 protein recognizes 6-base-pair A+T-rich regions, although the sequence of dA-dT residues is not important (25). A second possibility, which I personally favor, is that the RNA polymerase II transcription apparatus recognizes the unusual structure of poly(dA-dT) sequences. Poly(dA-dT) is unique among DNA sequences in that it has a helix repeat of 10.0 base pairs instead of the normal 10.6 (26, 27). It is also associated with kinks in DNA (28), although it should be noted that imperfect poly(dA-dT) regions are more "kinky" than pure homopolymer sequences.

Perhaps the most interesting observation is that poly(dA-dT) regions within natural DNA sequences prevent nucleosome formation *in vitro* (29, 30); 80-base-pair regions completely exclude nucleosomes, wherease 20-base-pair stretches disfavor nucleosome formation. Since the DNA within nucleosomes is far more resistant than purified DNA to the action of nucleases, a reasonable view of chromatin structure is that it represents a transcriptionally inert template. In this view, the basic structure must be disrupted before transcription can occur. The poly(dA-dT) regions that are important for *his3*, *pet56*, and *ded1* expression may be large enough to alter the basic chromatin structure such that general transcription factors can access the template. An attractive aspect of this hypothesis is that poly(dA-dT) sequences behave as constitutive upstream elements because they do not require specific transcription factors for their activity.

General Significance of Poly(dA-dT) Sequences. The yeast genome is composed of $\approx 10^7$ base pairs with an average AT composition of 60%. On a random basis, a *his3–pet56* sequence (15 of 17) should occur at a frequency of 10^{-5}, or once per 100 genes that are 1 kb long. Nevertheless, a computer search of the DNA sequences of all the yeast gene regions in GEN.bank indicates that 25% of them contain poly(dA-dT) tracts of comparable size, and in every instance, such sequences are always found outside the mRNA coding regions. In addition, 8-base-pair dA-dT stretches, which should occur at a frequency of 10^{-4} (once per 10 genes), are found in 80% of the yeast DNA sequences, usually several times per sequence, and with a single exception, are located in noncoding regions. These observations, when combined with the results in this paper, suggest that poly(dA-dT) sequences are used as upstream promoter elements for the constitutive transcription of many (but obviously not all) yeast genes. In addition, they suggest that poly(dA-dT) sequences are not simply "spacers" between genes, but rather can serve as functional genetic elements.

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