15. D. Shortle, J. Pipas, S. Lazarowitz, D. DiMaio, D. Nathans, in *Genetic Engineering*, J. K. Setlow and S. Hollaender, Eds. (Plenum, New York, 1979), vol. 1, p. 73.
16. C. Green and C. Tibbets, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2455 (1980); D. Shortle, D. Koshland, G. M. Weinstock, D. Botstein, *ibid.*, in press.
17. M. Mandel and A. Higa, *J. Mol. Biol.* **53**, 159 (1970); P. C. Wensink, D. J. Finnegan, J. E. Donelson, D. S. Hogness, *Cell* **3**, 315 (1974).
18. L. Katz, D. T. Kingsbury, D. R. Helinski, *J. Bacteriol.* **114**, 577 (1973).
19. D. B. Clewell, *ibid.* **110**, 667 (1972).
20. J. H. McCutchan and J. S. Pagano, *J. Natl. Cancer Inst.* **41**, 351 (1968).
21. B. Hirt, *J. Mol. Biol.* **26**, 365 (1967).
22. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *ibid.* **113**, 237 (1977); T. Maniatis, A. Jeffrey, D. G. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184 (1975).
23. R. C. Parker, R. M. Watson, J. Vinograd, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 851 (1977).
24. R. Shapiro, B. Braverman, J. B. Louis, R. E. Servicis, *J. Biol. Chem.* **248**, 4060 (1973); H. Hayatsu, *Prog. Nucleic Acid Res. Mol. Biol.* **16**, 75 (1976).
25. V. M. Vogt, *Eur. J. Biochem.* **33**, 192 (1973); H. B. Gray, D. A. Ostrandev, J. L. Hodnett, R. J. Legerski, D. L. Robberson, *Nucleic Acids Res.* **2**, 1459 (1975); P. P. Lan and H. B. Gray, *ibid.* **6**, 331 (1979).
26. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
27. G. J. Todaro and H. Green, *ibid.* **23**, 117 (1964).
28. I. Macpherson and L. Montagnier, *ibid.*, p. 291.
29. We thank N. Haigwood for suggesting that Dpn I can be used to selectively digest DNA from *E. coli* HB101, P. Mounts for helpful discussions, nd L. English and T. Zeller for technical assistance. K.W.C.P. was supported by postdoctoral fellowships from the International Agency for Research on Cancer and from the Science Research Council of Great Britain. This work was supported by National Cancer Institute grant 5 POI CA 16519.

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Directed Deletion of a Yeast Transfer RNA Intervening Sequence

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Perhaps the most surprising discovery in the initial studies of eukaryotic gene structure has been that many genes contain interruptions in the coding sequences. The origin and the function of these intervening sequences (IVS or introns) are not yet well understood but are the subject of intense investigation. In this article we test whether or not one of these introns, located in a yeast tRNA suppressor gene, is essential for the expression of the gene.

Summary. Many eukaryotic genes contain intervening sequences, segments of DNA that interrupt the continuity of the gene. They are removed from RNA transcripts of the gene by a process known as splicing. The intervening sequence in a yeast tyrosine transfer RNA ($tRNA^{Tyr}$) suppressor gene was deleted in order to test its role in the expression of the gene. The altered gene and its parent were introduced into yeast by transformation. Both genes exhibited suppressor function, showing that the intervening sequence is not absolutely essential for the expression of this gene.

In *Saccharomyces cerevisiae* there are eight tyrosine transfer RNA ($tRNA^{Tyr}$) genes (*1*) and each can be converted to either ochre or amber suppressor, a dominant phenotype (*2*). Each of these genes contains an intervening sequence of 14 base pairs (bp) located 1 bp to the 3′ side of the anticodon (*3*). In the expression of the tRNA gene the entire gene is transcribed to form a tRNA precursor (Fig. 1). Then in the enzymatic process of splicing the intervening sequence is removed and the half molecules are rejoined to form the mature tRNA molecule (*4*).

It is not yet known whether splicing is an essential step in the synthesis of yeast $tRNA^{Tyr}$. Not all tRNA genes contain introns (in yeast we estimate that perhaps one-tenth of the tRNA genes contain intervening sequences), so splicing is not an obligatory step in the synthesis of all tRNA. The presence of an intervening sequence in a precursor gives that RNA molecule a distinctive structure so that one could invoke an essential role for the intervening sequence in processing, base modification, or transport from nucleus to cytoplasm. Alternatively, the intron could be part of the transcriptional recognition site. This latter possibility is raised from the results of Brown and his colleagues who have shown that transcriptional initiation of the *Xenopus 5S* gene by RNA polymerase III requires sequences in the middle of the gene. Eukaryotic tRNA genes are also transcribed by RNA polymerase III.

To test whether the intervening sequence is essential, we have engineered the precise deletion of the intron from the $tRNA^{Tyr}$ SUP6 ochre suppressor gene. The ochre suppressor gene can be introduced into yeast via transformation (*6*) so the phenotype of the altered gene could be compared with that of the parent.

Technique for engineering specific deletions. The most precise and versatile way to introduce specific mutations in DNA is through the use of short synthetic oligonucleotides as site-specific mutagens. In this technique an oligonucleotide is synthesized which contains the desired mutation (point mutation, deletion or insertion). The oligonucleotide is used as a primer to direct the synthesis of DNA on a single-stranded circular DNA template. The oligonucleotide is thereby incorporated into the resulting circular DNA molecule forming a heteroduplex. Transformation into *Escherichia coli* followed by DNA replication resolves this heteroduplex into mutant and parental genes.

Until now this technique has only been used with ϕX174 DNA. Specific transition (*7*), transversion (*8*), and single-base deletion (*9*) mutations have been constructed.

We describe below a mutagenic strategy that is more general than previous strategies and differs from them in three ways:

1) The DNA to be mutagenized is double-stranded, allowing the use of recombinant plasmid DNA (in this case the yeast SUP6 gene cloned in the plasmid pBR322).

2) We demonstrate that large, specific deletions can be engineered. In this case

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we have directed the precise deletion of the 14-bp intervening sequence in the $tRNA^{Tyr}$ SUP6 gene.

3) The synthetic oligonucleotide used to create the mutation is also used in a hybridization assay to screen for clones containing the desired mutation. Thus, even though the mutation may be rare it can be easily and definitively recognized.

Construction of a plasmid containing the yeast $tRNA^{Tyr}$ SUP6 gene. Many of the yeast $tRNA^{Tyr}$ genes have been cloned as well as some of the suppressor alleles of these genes (*2*). A plasmid containing the ochre SUP6 gene, originally cloned as an Eco RI fragment, was made available to us (*10*). To aid in a general study of the expression of this gene we have recloned a subfragment containing the gene into the plasmid pBR322. This was done by cleaving the DNA with Alu I restriction endonuclease, ligating the fragment with Bam HI linkers, and digesting with Bam HI endonuclease to create cohesive ends (Fig. 1B). The smaller fragment was inserted into the Bam HI site of the plasmid pBR322. This clone is referred to as pYSUP6 Alu.

Directed deletion of the intervening sequence in the SUP6 gene. The strategy for constructing the IVS deletion is diagrammed in Fig. 2. The triester approach was used to synthesize the henicosamer 21-unit oligonucleotide TCTCAAGATT TAAAGTCTTGC (*11*). This oligonucleotide is complementary to residues 28 to 48 of the mature yeast ochre suppressor $tRNA^{Tyr}$. To prepare a single-stranded template, the supercoiled pYSUP6 Alu DNA was digested with Eco RI in the presence of ethidium bromide (150 μg/ml). This procedure produces a single nick in the DNA (*12*). This nicked DNA is a substrate for exonuclease III and digestion produces single-stranded circles.

The henicosamer was used as a primer to direct the synthesis of duplex DNA by the Klenow fragment of *E. coli* DNA polymerase I. The resulting double-stranded circular DNA, sealed by T4 DNA ligase, is a heteroduplex with 14 bases of the parental strand looped out. This DNA was used to transform *E. coli* χ1776, selecting for ampicillin resistance. One hundred ampicillin-resistant colonies were isolated. To test which of these colonies contained an altered suppressor gene, we used a hybridization assay. The henicosamer, which acted as the primer in the mutagenesis, was labeled with ^{32}P and used as a hybridization probe. Under the appropriate hybridization conditions (see below), this oligonucleotide will hybridize to the deleted gene, but not to the parent containing the IVS. Four colonies (isolates 22, 32, 35, and 36) showed positive hybridization in this assay. The failure to achieve complete mutagenic conversion may have been due to infectivity of residual single-stranded DNA molecules or to a low background of molecules incompletely digested by exonuclease III. DNA polymerase would repair these to reform parental molecules.

A demonstration of the selective hybridization is given in Fig. 3. In this experiment plasmid DNA's containing the deletion or the intact gene were digested with Bam HI, and the digestion products were separated by agarose gel electrophoresis. The DNA was denatured in situ and transferred to nitrocellulose paper by the method of Southern (*13*). The autoradiogram in Fig. 3 shows that the henicosamer only hybridizes to fragments containing the deletion.

Analysis of the deletion mutants. Plasmid DNA was prepared from the four χ1776 colonies isolated as described in Fig. 2. This DNA was used to transform the *E. coli* strain LS1. All of the transformants derived from plasmid preparations 22 and 32 hybridized with the ^{32}P-labeled henicosamer. By contrast, 50 percent of the transformants obtained from plasmid preparations 35 and 36 hybridized. This suggested that the primary mutant plasmids in χ1776 could result either from repair of the unpaired loop (isolates 22 and 32) or from replication of the heteroduplex to yield both mutant and parental plasmids within one cell (isolates 35 and 36).

The plasmid DNA from the four χ1776 isolates was further analyzed in a primer extension experiment. The primer used in this experiment was the decamer TCTCAAGATT (the 5′ end of the henicosamer). Each of the plasmid preparations described above was cleaved with Hha I. There is a Hha I site at position 27 in the tyrosine tRNA gene. Extensions of the ^{32}P-labeled primer on the Hha I cut template will be either 20 or 34 nucleotides long, depending on whether the gene contains the 14-base deletion or not (Fig. 4B). Lanes a and b show that the plasmids isolated from χ1776 can either be pure deletion (isolate 32, lane a) or a mixture of deletion and parent (isolate 35, lane b). Pure deletion plasmids can be isolated by recloning (lanes d and e). For all subsequent work pure deletion plasmids were isolated by retransformation and rescreening.

The exact nucleotide sequence of the deleted gene has been confirmed from DNA sequencing by the method of Max-

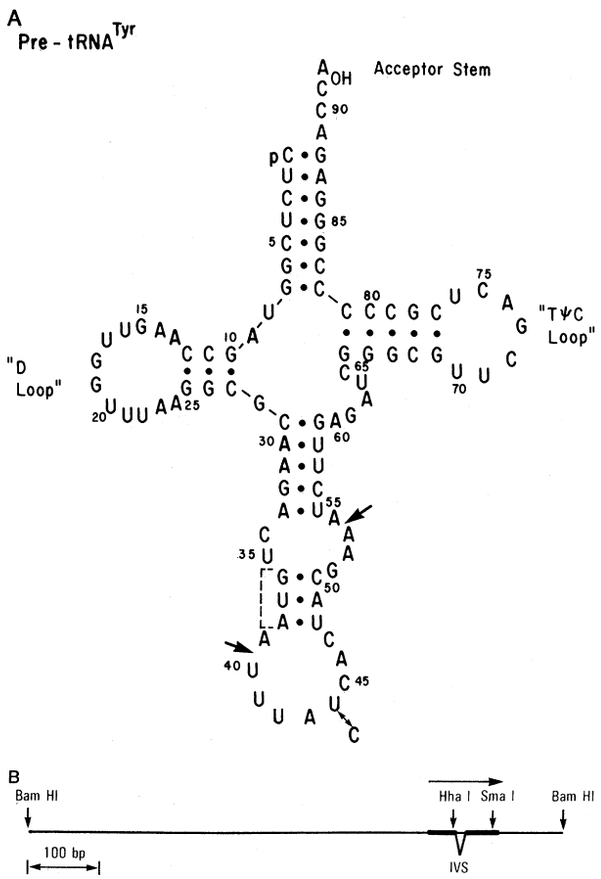

Fig. 1. (A) Nucleotide sequence and possible secondary structure of a $tRNA^{Tyr}$ precursor RNA containing the intervening sequence. The arrows denote the intervening sequence boundaries (*3*, *4*). The sequence shown is that of a wild-type RNA; the ochre suppressor RNA contains a G to U base change in the anticodon (position 36). (B) Map of the SUP6 gene region. The region shown is an Alu I restriction fragment with added synthetic Bam HI ends. The heavy lines represent the mature tRNA sequences. The arrow shows the direction of transcription of the tRNA gene.

am and Gilbert (data not shown) (*14*).

Transformation of the IVS deletion mutant into yeast. In order to determine whether the SUP6 gene lacking the intervening sequence is still functional we have introduced the altered gene into yeast via transformation. DNA fragments containing the parental SUP6 allele or containing the IVS deletion were ligated into the yeast transformation vector CV7 (*15*). This pBR322 derivative carries the wild-type yeast *leu2* gene and the 1.4-megadalton Eco RI fragment of the endogenous 2-μm yeast plasmid pScl and exists in yeast as an episomal element present at around ten copies per cell (*16*). The recipient yeast strain carries a double mutation at the Leu 2 locus and contains three ochre nonsense mutations (*lys1*-1, *trp5*-48, *ade2*-1). This strain was transformed with CV7 or CV7 containing either the parental SUP6 gene or the gene with the IVS deletion. Leu$^+$ transformants were selected and then tested for the simultaneous suppression of the three ochre loci (Table 1). As expected, Leu$^+$ transformants from the vector alone do not exhibit ochre suppression, whereas every transformant from CV7 containing the SUP6 allele

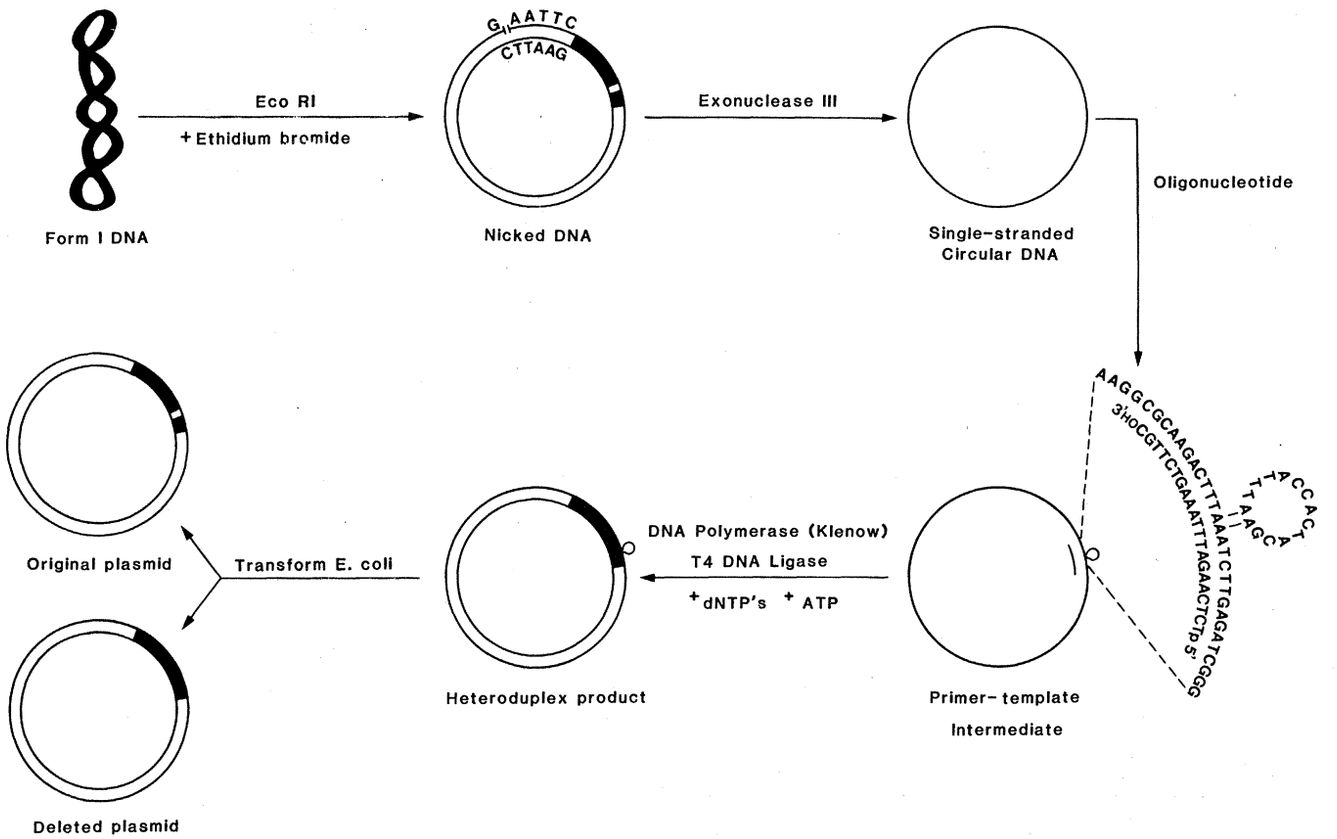

Fig. 2. Deletion of 14-bp intervening sequence from yeast SUP6 gene cloned in pBR322. Covalently closed circular plasmid DNA (pYSUP6 Alu, 10 μg) was nicked in one strand by digestion with the restriction endonuclease Eco RI (Boehringer Mannheim, 1000 units) in 1 ml of 100 m*M* tris-HCl, *p*H 7.2, 50 m*M* NaCl, 5 m*M* magnesium acetate, 0.01 percent NP-40 and ethidium bromide (150 μg/ml) (*12*) at 37°C for 1 hour. The reaction was then brought to 10 m*M* EDTA, extracted three times with ten volumes of water-saturated isobutanol to remove the ethidium bromide (100 μg/ml), once with a mixture of phenol and $CHCl_3$, twice with ether, and once with isobutanol to reduce the volume to 0.1 ml. The sample was desalted by centrifugation through a small (0.5 ml) Sephadex G-25 column and the DNA was recovered by precipitation with ethanol. Approximately 5 μg of the nicked DNA was incubated with 40 units of exonuclease III (Bethesda Research Laboratory) in 20 μl of 10 m*M* tris-HCl, *p*H 7.5, 2 m*M* $MgCl_2$, and 1 m*M* β-mercaptoethanol for 90 minutes at 37°C. The reaction was adjusted to 15 m*M* tris-HCl, *p*H 7.5, 7 m*M* NaCl, 7 m*M* $MgCl_2$, 7 m*M* DTT (dithiothreitol). Twenty units of bacterial alkaline phosphatase (Bethesda Research Laboratory) and 5 units of Hinf I (Bethesda Research Laboratory) were added, and digestion was continued for 30 minutes at 37°C. The sample was brought to 10 m*M* EDTA, extracted twice with a mixture of phenol and $CHCl_3$, once with ether, and desalted by centrifugation through a 0.5-ml Sephadex G-25 column equilibrated with water. Two-thirds of the single-stranded circular DNA preparation was combined with 60 pmole of 5′-phosphorylated oligonucleotide TCTCAAGATTTAAAGTCTTGC (henicosamer) in 38 μl of 200 m*M* NaCl, 13 m*M* tris-HCl, *p*H 7.5, 9 m*M* magnesium acetate, 20 m*M* β-mercaptoethanol, boiled for 3 minutes and immediately cooled to 0°C. To this was added 5 μl of a solution which contained the four deoxynucleoside triphosphates, at 4 m*M*, 0.5 μl of 100 m*M* adenosine triphosphate (ATP), 3 μl (3 units) of the Klenow fragment of DNA polymerase I (Boehringer Mannheim), and 4 μl (10 units) of T4 DNA ligase (gift of A. D. Riggs). After an overnight incubation at 12°C, portions of this reaction were used to transform χ1776 (provided by R. Curtiss), and the transformants were selected on agar medium containing (per milliliter) 100 μg of *dl*-α-ϵ-diaminopimelic acid (DAP), 50 μg of thymidine, and 20 μg of ampicillin. The resulting transformed colonies were replica-plated onto duplicate nitrocellulose filters (Millipore HATF090), grown overnight on the same agar medium, and then amplified for 5 hours on agar medium containing DAP (100 μg/ml), thymidine (50 μg/ml), and chloramphenicol (12.5 μg/ml). The filter was then prepared for hybridization (*21*) by incubating the filter in sixfold strength NET (NET consists of 150 m*M* NaCl, 15 m*M* tris-HCl, *p*H 7.5, 1 m*M* EDTA), fivefold strength Denhardt's solution (*22*), 0.5 percent SDS, 100 μg/ml denatured *E. coli* DNA (5 ml per filter) for 2 hours at 65°C. Hybridization was carried out with [5′-^{32}P]-labeled henicosamer (*21*), in a solution (2.5 ml per filter) containing sixfold strength NET, fivefold strength Denhardt's solution (*25*), 0.5 percent SDS, 10 percent Dextran sulfate (Pharmacia) and labeled henicosamer (2 ng/ml). Hybridization was carried out at 50°C overnight, the filters were washed at 0°C in sixfold strength SSC and exposed overnight to Kodak XR x-ray film with one Cronex Lightning Plus intensifier screen (DuPont). Colonies that appeared to hybridize to the labeled probe were picked, streaked onto a nitrocellulose filter, grown, amplified, and hybridized with probe. This procedure yielded four deletion mutants designated pYSUP6 Δ22, pYSUP6 Δ32, pYSUP6 Δ35, and pYSUP6 Δ36. The black area indicates the Alu fragment (Fig. 1) inserted at the Bam HI site of pBR322. This loop, which is not base-paired, and oligonucleotide are exaggerated for clarity.

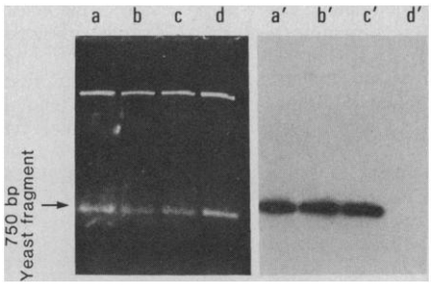

Fig. 3. Specificity of hybridization of the ^{32}P-labeled henicosamer to deletion plasmid DNA. Plasmid DNA was isolated from the deletion mutants described in Fig. 2. Approximately 0.5 μg of deletion plasmid DNA and 1 μg of pYSUP6 Alu DNA were digested with Bam HI (Boehringer Mannheim, 5 units) in 10 μl of 7 m*M* tris-HCl, *p*H 7.5, 7 m*M* NaCl, 7 m*M* $MgCl_2$, 7 m*M* dithiothreitol (DTT) at 37°C for 30 minutes. This DNA was then subjected to electrophoresis on a 1 percent agarose gel (*20*). The gel was stained with ethidium bromide (0.25 μg/ml) for 15 minutes and photographed under ultraviolet light. (Lane a) pYSUP6 Δ35; (lane b) pYSUP6 Δ32; (lane c) pYSUP6 Δ22; (lane d) pYSUP6 Alu. The DNA was then transferred to nitrocellulose (Schleicher and Schuell) as described by Southern (*13*) and hybridized with the ^{32}P-labeled henicosamer (2 ng/ml) in sixfold NET, fivefold Denhardt's, 0.5 percent sodium dodecyl sulfate, at 50°C, overnight. The filter was then washed at 0°C in sixfold SSC and autoradiographed (lanes a' to d').

shows the suppressor character. Moreover, it can be seen that cells harboring the SUP6 IVS deletion plasmids also suppress ochre mutations. This observation suggests that the intervening sequence is not essential for the expression of the SUP6 gene.

To demonstrate that the suppressor gene introduced on CV7 was in fact linked to *leu2*, a Leu$^+$ transformant (derived from CV7 SUP6Δ32 transformation) was grown for 12 to 15 generations without selection and then screened for reversion to Leu$^-$. Approximately 50 percent of the colonies had reverted. We examined 48 revertants for the coincident loss of suppressor phenotype, and found that 46 had lost the ability to suppress ochre mutations. Thus, the high frequency of reversion and simultaneous loss of both alleles suggest that the SUP6 deleted gene is located on an autonomously replicating plasmid.

Biological implications. The results of this experiment indicate that the intervening sequence in the tRNA^{Tyr} SUP6 gene is not essential for suppressor function. It remains to be seen whether or not the gene is functioning optimally although it should be straightforward to test the level of gene expression. Since all yeast genes do not contain intervening sequences, it is not surprising that processing pathways exist allowing the correct maturation of the suppressor. We do not yet know whether the suppressor tRNA produced by the deleted gene contains the normal nucleoside modifications.

Experiments similar to this have already been performed with genes from SV40 and with the mouse β-globin gene (*17*). Here the results are different from what we have observed. For synthesis of stable mRNA (messenger RNA) or transport from nucleus to cytoplasm (or both) an intervening sequence must be present in the transcriptional unit. It would appear from our results that splicing is not required for the transport of suppressor tRNA from nucleus to cytoplasm. This is only a tentative conclusion, however, because solid evidence concerning the intracellular location of the 2-μm yeast plasmid is not available. This plasmid is thought to be located in the nucleus, and nuclear functions are required for its replication (*16*). However, until the cellular location of the plasmid is firmly established, it is difficult to make any interpretation of the role of the intervening sequence in transport of the tRNA from nucleus to cytoplasm. Again we note that most tRNA genes do not contain intervening sequences so there must be a pathway for exit of tRNA from nucleus to cytoplasm which does not require excision of an intervening sequence.

With regard to transcription, it is possible that transcription is initiated else-

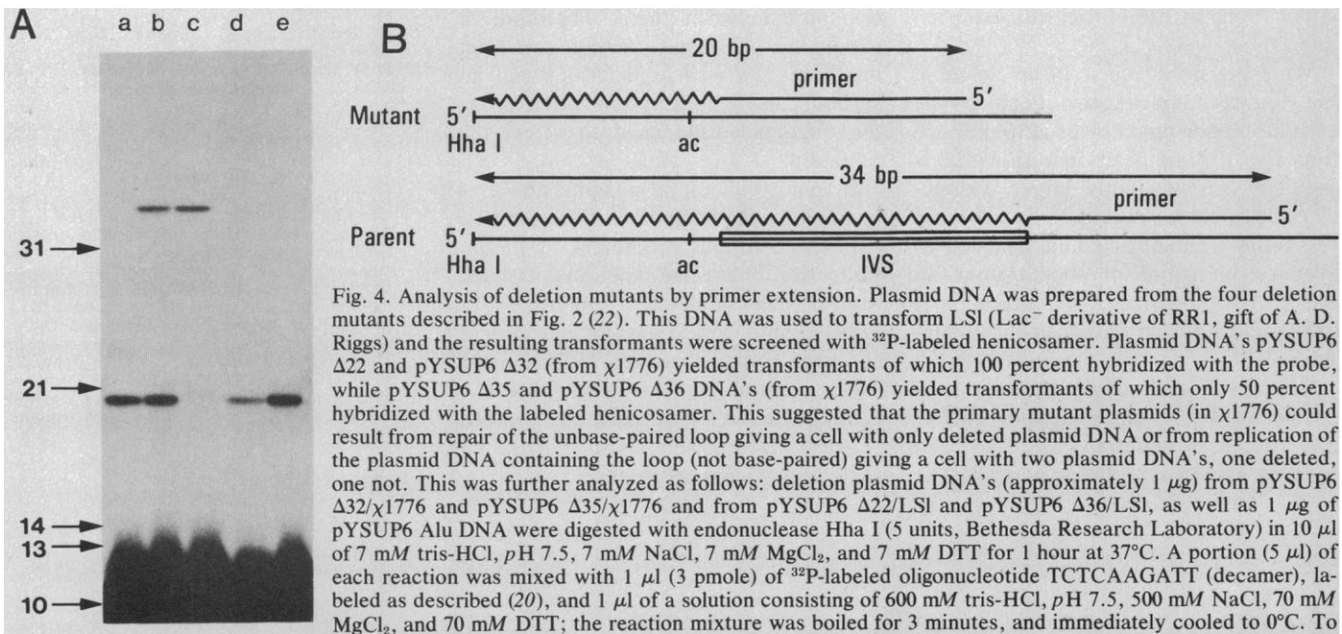

Fig. 4. Analysis of deletion mutants by primer extension. Plasmid DNA was prepared from the four deletion mutants described in Fig. 2 (*22*). This DNA was used to transform LSl (Lac$^-$ derivative of RR1, gift of A. D. Riggs) and the resulting transformants were screened with ^{32}P-labeled henicosamer. Plasmid DNA's pYSUP6 Δ22 and pYSUP6 Δ32 (from χ1776) yielded transformants of which 100 percent hybridized with the probe, while pYSUP6 Δ35 and pYSUP6 Δ36 DNA's (from χ1776) yielded transformants of which only 50 percent hybridized with the labeled henicosamer. This suggested that the primary mutant plasmids (in χ1776) could result from repair of the unbase-paired loop giving a cell with only deleted plasmid DNA or from replication of the plasmid DNA containing the loop (not base-paired) giving a cell with two plasmid DNA's, one deleted, one not. This was further analyzed as follows: deletion plasmid DNA's (approximately 1 μg) from pYSUP6 Δ32/χ1776 and pYSUP6 Δ35/χ1776 and from pYSUP6 Δ22/LSl and pYSUP6 Δ36/LSl, as well as 1 μg of pYSUP6 Alu DNA were digested with endonuclease Hha I (5 units, Bethesda Research Laboratory) in 10 μl of 7 m*M* tris-HCl, *p*H 7.5, 7 m*M* NaCl, 7 m*M* $MgCl_2$, and 7 m*M* DTT for 1 hour at 37°C. A portion (5 μl) of each reaction was mixed with 1 μl (3 pmole) of ^{32}P-labeled oligonucleotide TCTCAAGATT (decamer), labeled as described (*20*), and 1 μl of a solution consisting of 600 m*M* tris-HCl, *p*H 7.5, 500 m*M* NaCl, 70 m*M* $MgCl_2$, and 70 m*M* DTT; the reaction mixture was boiled for 3 minutes, and immediately cooled to 0°C. To each portion was added 2 μl of a solution which was 150 μ*M* in each of four deoxynucleoside triphosphates and 1 μl (0.2 unit) of the Klenow fragment of DNA polymerase I (Boehringer Mannheim). Each reaction was incubated for 15 minutes at room temperature and stopped by the addition of 10 μl of 96 percent formamide, 10 m*M* EDTA, 0.3 percent xylene cyanol FF, and 0.3 percent bromophenol blue. (A) Portions were subjected to electrophoresis on a 12 percent acrylamide, 0.6 percent bis-acrylamide, 7*M* urea gel in a tris-borate-EDTA buffer system (*23*). ^{32}P-labeled synthetic oligonucleotides of defined length were used as markers in parallel lanes and their position and size is indicated to the left of the figure. (Lane a) pYSUP6 Δ32/χ1776; (lane b) pYSUP6 Δ35/χ1776; (lane c) pYSUP6 Alu; (lane d) pYSUP6 Δ22/LS1; and (lane e), pYSUP6 Δ36/LS1. (B) For clarity a schematic representation of the results of the primer extension for mutant and parental DNA is shown (*ac*, anticodon).

where in the CV7 vector and that changes which would otherwise affect normal RNA polymerase III transcription of this gene are masked by "read-through" transcription. In previously published experiments we have altered the yeast $tRNA_3^{Leu}$ gene by introduction of the 21-bp *lac* operator into the middle of the intervening sequence (*18*). The altered gene is transcribed in *Xenopus laevis* germinal vesicle extracts to give a precursor that is 21 bases longer than the normal precursor. Thus, a drastic alteration of the intervening sequence in the $tRNA_3^{Leu}$ gene does not significantly affect its transcription in vitro. It does not appear, therefore, that specificity in transcription of these tRNA genes is determined by intron sequences.

In yeast it is possible to introduce an altered gene into a recipient cell in such a way that the altered gene cleanly replaces the parental gene with no other addition, deletion or rearrangement of genetic information (*19*). In light of the arguments presented above, a more rigorous test of the expression of the mutant gene would be to replace the *sup6*$^+$ gene in yeast with the deletion mutant.

Technical perspectives. The strategy used here in directing the deletion of the 14-bp intervening sequence is an important extension of the previous technology. The use of exonuclease III to produce single-strand circles allows the directed mutagenesis of genes carried on plasmids. Previously this technique had only been applied to ϕX174 DNA. Most applications in the future will use plasmid DNA.

We have produced a 14-bp deletion, but the technique seems general—the same approach might be used to produce a precise 1000-bp deletion (although it is possible that a slightly larger oligonucleotide might be required). In addition, the same techniques could be used to produce insertions or substitutions although here the size of the alteration is dependent on what it is feasible to synthesize.

A powerful part of the strategy here has been that the oligonucleotide used to promote these mutations has also been used to screen for clones carrying the mutation. In a previous study of the hybridization behavior of oligonucleotides to ϕX174 DNA (*20*) we showed that duplexes with single-base mismatches were sufficiently less stable than perfectly matched duplexes so that hybridization conditions could be found to discriminate between them. Therefore, it should be possible to screen for point mutations, as well as deletions and insertions, using as a hybridization probe the oligonucleotide used to direct the mutation.

It is not difficult to imagine how this technology will be applied. Genes can be hooked up to new promoters by the introduction of precise deletions. Defined segments can be deleted, substituted, or inserted into genes for the purpose of changing protein structure. Hybrid genes can be created. . . . The list goes on. The common feature is, of course, that the changes are precisely those that the investigator wishes to produce.

Table 1. Fraction of Leu2$^+$ transformants which show ochre suppression. The yeast transformation vector CV7 (kindly provided by J. Hicks) is a pBR322 plasmid carrying both the 1.4 megadalton Eco RI restriction fragment of the yeast plasmid pScI and a Sal I-Xho I restriction fragment containing the yeast Leu2$^+$ allele (*16*). Fragments containing the parental or deleted SUP6 genes were inserted into the Bam HI site of CV7. The recipient yeast strain DH484 (*ade2*-1, *leu-2*-3, *leu2*-112, *can*^{r}, *trp5*-48, *ura4*-11, *lys1*-1 *a* ψ^-, J. Hicks) was transformed with plasmid DNA according to the method of Hsiao and Carbon (*24*). The transformation efficiency was approximately 100 transformants per microgram of plasmid DNA for all four plasmids. Leu$^+$ transformants were selected, and the colonies were tested for suppression of the *ade2*-1, *trp5*-48 and *lys1*-1 ochre mutations. We have not yet determined why one of the CV7SUPΔ32 transformants does not show the suppressor phenotype.

Plasmid	Fraction of Leu2$^+$ transformants showing ochre suppression
CV7	0/25
CV7SUP6 Alu	25/25
CV7SUP6 Δ22	15/15
CV7SUP6 Δ32	24/25

References and Notes

1. M. V. Olson, D. L. Montgomery, A. K. Hopper, G. S. Page, F. Horodyski, B. D. Hall, *Nature (London)* **267**, 639 (1977).
2. R. A. Gilmore, J. W. Stewart, F. Sherman, *J. Mol. Biol.* **61**, 157 (1971).
3. H. M. Goodman, M. V. Olson, B. D. Hall, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5453 (1977).
4. R. C. Ogden, G. Knapp, C. L. Peebles, H. S. Kang, J. S. Beckmann, P. F. Johnson, S. A. Fuhrman, J. N. Abelson, in *Transfer RNA: Biological Aspects*, D. Söll, J. N. Abelson, P. R. Schimmel, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1980), pp. 173–190.
5. D. F. Bogenhagen, S. Sakonju, D. D. Brown, *Cell* **19**, 27 (1980); S. Sakonju, D. F. Bogenhagen, D. D. Brown, *ibid.*, p. 13.
6. A. Hinnen, J. Hicks, G. R. Fink, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1929 (1978).
7. A. Razin, T. Hirose, K. Itakura, A. D. Riggs, *ibid.*, p. 4268; C. A. Hutchison III, S. Phillips, M. H. Edgell, S. Gillam, P. Jahnke, M. Smith, *J. Biol. Chem.* **253**, 6551 (1978); S. Gillam and M. Smith, *Gene* **8**, 81 and 99 (1979).
8. S. Gillam, P. Jahnke, C. Astell, S. Phillips, C. A. Hutchison III, M. Smith, *Nucleic Acids Res.* **6**, 2973 (1979).
9. M. Smith, personal communication.
10. The ochre SUP6 gene was given to us by P. Philippsen and R. Davis.
11. T. Hirose, R. Crea, K. Itakura, *Tetrahedron Lett.* (1978), p. 2449.
12. D. Shortle and D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2170 (1978); R. C. Parker, R. M. Watson, J. Vinograd, *ibid.* **74**, 851 (1977).
13. E. M. Southern, *J. Mol. Biol.* **94**, 51 (1975); *ibid.* **43**, 1305 (1978).
14. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
15. K. Struhl, D. T. Stinchcomb, S. Scherer, R. W. Davis, *ibid.* **76**, 1035 (1979).
16. T. D. Petes, *Annu. Rev. Biochem.* **49**, 845 (1980).
17. P. Gruss, C.-J. Lai, R. Dhar, G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4317 (1979); D. H. Hamer, K. O. Smith, S. H. Boyer, P. Leder, *Cell* **17**, 725 (1979); S. P. Goff and P. Berg, *J. Mol. Biol.* **133**, 359 (1979); R. C. Mulligan, B. H. Howard, P. Berg, *Nature (London)* **277**, 108 (1979); D. H. Hamer and P. Leder, *Cell* **18**, 1299 (1979); C.-J. Lai and G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 71 (1979).
18. J. D. Johnson, R. Ogden, P. Johnson, J. Abelson, P. Dembeck, K. Itakura, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2564 (1980).
19. S. Scherer and R. Davis, *ibid.* **76**, 4951 (1979).
20. R. B. Wallace, J. Shaffer, R. F. Murphy, J. Bonner, T. Hirose, K. Itakura, *Nucleic Acids Res.* **6**, 3543 (1979).
21. D. Hanahan and M. Meselson, *Gene* **10**, 63 (1980).
22. R. W. Davis, M. Thomas, J. Cameron, T. P. St. John, S. Scherer, R. A. Padgett, *Methods Enzymol.* **65**, 404 (1980).
23. A. C. Peacock and C. W. Dingman, *Biochemistry* **6**, 1818 (1967).
24. C.-L. Hsiao and J. Carbon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3829 (1979).
25. D. T. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641 (1966).

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