Formation of an Active Transcription Complex in the *Drosophila melanogaster* 5S RNA Gene Is Dependent on an Upstream Region

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We constructed deletion-substitution and linker-scanning mutations in the 5'-flanking region of the *Drosophila melanogaster* 5S RNA gene. In vitro transcription of these templates in *Drosophila* and HeLa cell extracts revealed the presence of an essential control region (–30 region) located between nucleotides –39 and –26 upstream of the transcription initiation site: deletion of sequences upstream of nucleotide position –39 had no detectable effect on the wild-type level of in vitro transcription, whereas mutations extending between positions –39 and 1 resulted in templates with decreased transcriptional levels; specifically, deletion and linker-scanning mutations in the –34 to –26 region (–30 region) resulted in loss of transcription. The –30 region is essential for transcription and therefore forms part of the *Drosophila* 5S RNA gene transcription promoter. Compared with the activity of the wild-type gene, mutant 5S DNAs exhibited no impairment in the ability to sequester limiting transcription factors in a template exclusion competition assay. While we do not know which transcription factor(s) interacts with the –30 region, the possible involvement of RNA polymerase III at this region is discussed.

Transcription of genes by RNA polymerase III is controlled by sequences within the region encoding the RNA. As first proposed by Sakonju et al. (17), these regions were defined as “control regions” rather than as “promoters,” because evidence was not available that RNA polymerase III recognizes and binds to these sequences. *Xenopus laevis* 5S RNA gene transcription requires three transcription factor activities (TFIIIA, TFIIIB, and TFIIIC) as well as RNA polymerase III (19). TFIIIA is a positive transcription factor that binds to the defined internal control region (5, 23). The interaction of TFIIIC, TFIIIA, and 5S DNA forms a stable complex, referred to as stable complex I, directed by the internal control region which is sequestered into the stable transcription complex by interaction with TFIIIB (1). The current model for class III gene transcription is that RNA polymerase III recognizes this stable complex and initiates transcription (1, 3, 20). Since the recognition sequences for transcription factors are downstream from the site of initiation, it is conceivable that RNA polymerase III itself interacts in the 5'-flanking region of 5S DNA.

Transposon-directed (Tn9) deletion mutants of *X. laevis* 5S DNA failed to reveal essential control sequences upstream of the 49-nucleotide nonrepetitive sequence immediately adjacent to the 5' terminus of the gene (6). Furthermore, extensive computer analysis of sequences preceding several 5S RNA genes revealed several common features (9) the deletion of which, while reducing transcription, did not result in loss of transcription (17). The transcription efficiency of *Bombyx mori* 5S DNA was, however, reduced to 5% of the level of wild-type transcription upon deletion of the sequence upstream to coordinate –17 (14). This critical region was shown to have a sequence similar to the 5'-flanking sequence necessary for *Bombyx* tRNA gene transcription (13). *B. mori* and *Drosophila melanogaster* tRNA gene transcription is similarly dependent on 5'-flanking se-

quences, and this raised the question whether the transcription of *Drosophila* 5S DNA also had 5'-flanking sequence requirements.

An earlier study on *D. melanogaster* 5S RNA gene transcription provided evidence that the 5S RNA gene was similar to the 5S gene of *Xenopus* sp. in containing an internal control region that directed its transcription (21). We constructed deletion-substitution (ds) and linker-scanning (LS) mutations in the 5'-flanking region of the *Drosophila* 5S RNA gene to investigate transcriptionally important sequences. The present study identifies a control sequence immediately 5' flanking the 5S RNA coding sequence that is essential for in vitro transcription. The mechanism of transcription control by the 5'-flanking region for *Drosophila* tRNA genes involves RNA polymerase III, and this is therefore a possible function for the 5S DNA 5'-flanking sequence (11).

MATERIALS AND METHODS

Plasmid DNA. The plasmid pDm5SI contains one copy of the *D. melanogaster* 5S rRNA gene (type I) inserted into the *EcoRV* site of pBR322 and for reference has a transcriptional orientation in the *Bam*HI-to-*Eco*RI direction. The construction of pDm5SI has previously been described (21).

Construction of 5S RNA gene deletion mutations. 5S gene deletions in the 5'-flanking region were derived by treating linearized pDm5SI with *Bal* 31 exonuclease. The 5' deletions were generated from the *Bam*HI site of pDm5SI, and the 3' deletions were generated from the *Hind*III site of pDm5SI. After *Bal* 31 treatment, blunt ends were constructed with T4 DNA polymerase. The 5' and 3' deletion mutants were liberated from the vector with *Hind*III and *Sal*I, respectively. Both sets of deletion fragments were electrophoretically sized with 1% low-melting-point agarose gels. DNA fragments were purified by passing collected DNA-agarose solutions through Elutip DNA-binding columns (Schleicher & Schuell, Inc., Keene, N.H.) or by phenol extracting the

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agarose in a 400- μ l solution consisting of 400 mM LiCl, 50 mM Tris hydrochloride, and an equal volume of phenol warmed to 45°C. The recovered *Hind*III 5' deletion fragments and the *Sal*I 3' deletion fragments were ligated into pUC8 vector cut with *Sma*I-*Hind*III and *Sma*I-*Sal*I, respectively, and transformed into *Escherichia coli* HB101. Deletion endpoints were determined by DNA sequencing (12). During these studies the deletion methodology was changed by ligating the *Bal* 31-deleted fragments into M13mp9 instead of pUC8. Use of the M13 vector facilitated screening and subsequent deletion endpoint analysis by the dideoxy sequencing method (18). The deletion mutations are referred to as ds mutations and are named from their deletion endpoint.

Construction of 5S RNA gene LS mutations. The 5' and 3' *Bal* 31 deletion mutants were used to construct the 5S LS variants. These plasmids were constructed by joining *Eco*RI-*Hind*III 5' deletion fragments with *Eco*RI-*Sal*I 3' deletion fragments and inserting the reconstructed gene into *Hind*III-*Sal*I-cut pUC81 vector DNA (pUC81 was made from pUC8 by filling in the *Eco*RI site with Klenow fragment). The *Eco*RI linker of the LS mutations therefore was derived from the *Eco*RI site of the vector pUC8, which juxtaposed the *Sma*I cloning site of the deletion mutations. Recombinant plasmids were amplified and grown in *E. coli* HB101, screened, sequenced, and purified by two CsCl density gradient centrifugation steps.

In vitro transcription of 5S RNA gene. Transcription reactions were done in S-100 cell extracts prepared from *Drosophila* Schneider S3 cells or HeLa S3 cells. Unless otherwise specified plasmid DNA templates were transcribed in a total volume of 60 μ l of a mixture composed of the following: 15 μ l of S-100 cell extract, 30 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-KOH (pH 8.0), 80 mM KCl, 7 mM MgCl₂, 3 mM dithiothreitol, 500 μ M each ATP, CTP, UTP, 100 μ M [α -³²P]GTP (1 Ci/mmol), 3.6 U creatine phosphokinase, and 8.3 mM creatine phosphate; transcription reactions were incubated at 24°C for 2 h. After incubation of transcription reactions, a 50- μ l solution of 1 mg of proteinase K per ml, 0.1% (wt/vol) sodium dodecyl sulfate, and 30 mM HEPES-KOH (pH 8.0) was added, and incubation continued for 30 min at 37°C. Transcription samples were phenol-chloroform extracted and ethanol precipitated, suspended in 3 μ l of 80% (vol/vol) formamide-0.1% (wt/vol) bromophenol blue-0.1% (wt/vol) xylene cyanol FF-2 mM EDTA, and analyzed on 8% (20:1, acrylamide-bisacrylamide) polyacrylamide gels containing 8.3 M urea.

The reference template used in the template exclusion competition assays was constructed from ds5.93 and ds3.105 to form the LS maxigene LS105/93 which contains an additional 23 base pairs (bp) compared with the length of pDm5SI. For the competition assays, addition of the first template, the test template (wild-type, ds, or LS mutant DNAs), preceded the addition of LS105/93 by 20 or 40 min. A 100-ng sample of each template DNA was used, and the total amount of plasmid DNA in the reaction was maintained at 1 μ g by adding 800 ng of pUC81 with the test template. Transcription reactions were continued at 24°C for 90 min after the addition of LS105/93.

RESULTS

Construction of ds mutations. The plasmid pDm5SI contains the *D. melanogaster* 5S RNA coding region (135 bp) with 97 bp of 5'-flanking and 147 bp of 3'-flanking sequence

(21). Two series of ds mutations were constructed in pDm5SI. *Bal* 31 was used to delete *Drosophila* DNA sequences approaching the gene from both the 5' and 3' directions. The resulting deletion DNAs were recloned into plasmid or bacteriophage vector DNA. Since *Drosophila* sequences have been deleted and substituted with vector pUC8 sequences these 5S DNA variants are called ds mutations. The sequence of each ds mutation was determined, and the sequences of the 5' ds mutations relevant to this study are shown in Fig. 1.

Homologous in vitro transcription of 5' ds mutations. The 5'-flanking-region ds mutations which had deletion endpoints at nucleotides -94, -81, -80, -79, -62, and -51 directed levels of transcription equal to that of pDm5SI in *Drosophila* cell extracts (data not shown). Similarly, ds5.-45 and ds5.-39 directed transcription at a level equivalent to the wild-type transcriptional level of pDm5SI (Fig. 2), showing that the 5'-flanking region between coordinates -97 and -39 is not essential for in vitro transcription of the 5S RNA gene. The ds5.-39 mutation resulted in the disruption of a sequence of dyad symmetry. Since ds5.-39 transcription had the level of the wild-type gene, this symmetrical sequence does not appear to be necessary for in vitro transcription. The mutation ds5.-32 resulted in a 5S DNA template which had approximately 30% the activity of pDm5SI (Fig. 2). Further deletion resulted in ds 5S DNAs that did not transcribe or had a reduced transcriptional activity (Fig. 2). The size of the primary transcripts generated from mutant templates was identical to the size of the transcript of the wild-type gene. The transcript size and nearest neighbor nucleotide analysis (data not shown) demonstrated that the start site for transcription of the mutant templates remained the same as that for the wild-type gene. Deletions spanning positions -32 to 1 were transcriptionally deficient, and the 5' boundary of the essential sequence is between coordinates -39 and -32. The deletions ds5.-20 and ds5.-14, however, did support a low level of transcription (Fig. 2). Inspection of the substituted sequences of ds5.-20 and ds5.-14 revealed the introduction of wild-type sequences at many positions across the deleted region but in particular between -34 and -27 (Fig. 1). Since ds5.-32 maintained 30% of the pDm5SI transcription activity, we further reasoned that transcriptionally important sequences were located between positions -32 and -26 as well as between -39 and -32. While the precise nucleotide composition of the region that is essential cannot be derived from this series of mutations, comparing the common sequences of plasmids ds5.-32, ds5.-20, and ds5.-14 in these regions identified the sequence GAGT ATAA located at -34 to -27 which may have transcriptional importance (Fig. 1).

Construction and transcription of 5S DNA LS mutations. ds mutations revealed that a region in the 5'-flanking sequence within coordinates -39 and -26 was essential for transcription of 5S DNA. Since deletion to -26 removes this entire sequence, further deletion analysis was unable to determine whether other control regions were present in the 5S DNA. However, the transcriptional activity of mutations ds5.-20 and ds5.-14, although relatively low, implies that if a control region is located between coordinates -26 to -14, it is not essential. To test the presence of transcriptionally important regions in the 5'-flanking sequence, we constructed LS mutations by ligating appropriate 5' and 3' deletion mutations. The method used in the LS constructions resulted in replacing *Drosophila* sequence with the 10-bp *Eco*RI linker sequence 5'-GGGAATTC-3'. Several sets of LS mutations were constructed, some resulting in a

FIG. 1. Comparison of the 5'-flanking sequences of wild-type and partially deleted 5S RNA genes. The noncoding DNA strand is shown. SSI represents the sequence of the wild-type gene contained in pDm5SI, and nucleotide positions are numbered with respect to the transcription initiation site (position 1, boxed). *Drosophila* DNA sequences are shown in upper case, and the pUC8 vector sequence substituted in the construction of the deletion mutations is underlined. Deletion endpoints are shown on the left. The in vitro transcription efficiency (RTL, relative transcription level) and composition of the -30 region GAGTATAA sequence are shown on the right (see text and Fig. 2). Transcription quantitation was the average result of three separate experiments with a single extract and was relatively the same in four different preparations of Schneider S3 cell extracts.

deletion or insertion of one nucleotide compared with the length of the wild-type sequence in pDm5SI. A summary of the sequences of the 5'-flanking-region LS mutations is shown in Fig. 3. LS mutation 5S DNAs which have exactly the same size as the wild-type gene and therefore have a 10-nucleotide *Drosophila* sequence exactly replaced by the 10-nucleotide linker sequence are LS-37/-26, LS-22/-11, and LS-11/1.

The plasmid LS-37/-26, which disrupts the sequence GAGTATAA positioned at -36 to -27, was transcriptionally inactive (Fig. 3 and 4). LS-22/-11 (transcription shown in Fig. 6) and LS-11/1 both were transcriptionally active, however, at a level of 30 and 50%, respectively, of that of the wild-type gene (Fig. 4). While the present results indicate that the 5'-flanking region between -34 and -26 is essential for the in vitro transcription of *Drosophila* 5S DNA, the 5'-flanking sequence located between coordinates -26 and 1 is also important for wild-type levels of transcriptional efficiency.

Transcription of LS-11/1 demonstrated that the sequence immediately 5' to the 5S RNA-coding region is not essential for transcription in so far as an introduced *Eco*RI linker sequence did not result in a loss of transcription. The mutation LS-12/1 contains a 1-bp deletion and the same linker placement as LS-11/1 (Fig. 4). LS-12/1 has an

activity approximately equal to that of LS-11/1; however, LS-10/1, which has a 1-bp insertion and the same linker placement as LS-11/1, was transcriptionally inactive (Fig. 4).

FIG. 2. In vitro transcription of wild-type and ds *Drosophila* 5S RNA genes. Plasmid DNAs having deletion endpoints as indicated were transcribed in vitro with a *Drosophila* Schneider S3 cell extract. *Drosophila* 5S RNA is synthesized as a 135-nucleotide precursor (pre-5S) which is 3' end trimmed to form pre'5S and the 120-nucleotide mature-sized 5S RNA (5S) (21). Transcription products were resolved with denaturing polyacrylamide gels and detected by autoradiography.

FIG. 3. Comparison of the 5'-flanking DNA sequences of the wild-type and LS mutation-containing 5S RNA genes. The noncoding sequence is shown, and the nucleotide positions are shown relative to the transcription initiation site. The linker mutation is outlined, and nucleotides corresponding to wild-type sequence are in upper case. Nomenclature of the LS mutation is such that the 3' deletion mutation forms the 5' component and the 5' deletion mutation forms the 3' component of the gene unit; the numbers refer to the deletion endpoints. LS mutations that hold the dimensions of the wild-type 5S1 gene exactly are indicated in boxes. The -30 region is bold underlined. Transcription efficiency values (RTL, relative transcription level) are the average of five experiments.

Transcription of *Drosophila* 5S RNA gene templates in HeLa cell extract. We were interested in determining whether the control of 5S RNA gene transcription by the 5'-flanking sequence was a homologous reaction. Since *Drosophila* 5S DNA is transcriptionally active in HeLa cell extracts (21), we programmed HeLa extracts using several *Drosophila* 5S DNA 5' mutant templates. The transcription of these DNAs in HeLa extract is shown in Fig. 5. The results show that the *Drosophila* 5S DNA maintains the transcriptional requirement for the -39 to -26 region even with a heterologous transcription system. However, removing this region (LS-37/-26) does not cause as drastic a loss of transcription in HeLa extract as in *Drosophila* extract. Similarly, ds5.-26 resulted in a complete loss of transcription in the *Drosophila* extract but gave a minor transcription response in the HeLa extract. It appears therefore that the 5'-flanking sequence requirement is not unique to the use of the *Drosophila* transcription apparatus but rather appears to be intrinsic to the 5S DNA itself.

Transcriptional effect of 5'-flanking sequence is not related

FIG. 4. In vitro transcription of wild-type and LS mutations shown in Fig. 3, using *Drosophila* Schneider S3 cell-free transcription system. Designations are defined in the legend to Fig. 2.

to ability to function in a template exclusion competition assay. To test the abilities of the 5S RNA gene and the mutant ds and LS templates derived from it, to sequester transcription factors, we performed assays in which the reference template LS105/93 was added to transcription reactions after a 40-min preincubation of each gene in the transcription reaction. Each of the 5S DNAs reduced maxigene transcription (competed) to a level similar to, or lower than, the level of competition by the wild-type gene (Fig. 6). This result indicates that each mutant template is unimpaired in the

FIG. 5. Heterologous in vitro transcription. A HeLa cell extract was used to test transcription of the templates indicated. Transcription of *Xenopus* 5S DNA contained on the plasmid pX108 used as a HeLa transcription control results in the production of a major 120-nucleotide transcript. The *Drosophila* 5S DNA primary transcript is 135 nucleotides. Transcription products were fractionated on a denaturing polyacrylamide gel. The origin of the labeled products represented by X is unknown, and these products were not observed in all experiments.

ability to bind a limiting transcription factor(s). There was no difference in these results whether preincubation times of 20 or 40 min were used or whether deletion mutants were used (data not shown). Therefore, mutations in the 5'-flanking sequences, while leading to loss of transcription, do not appear to affect directly the sequences necessary for the binding of the limiting transcription factor(s) in these cell extracts.

DISCUSSION

Transcription analyses of ds mutations and LS mutations identified an essential control region in the 5'-flanking sequence of the *D. melanogaster* 5S RNA gene. The essential region of the 5'-flanking sequence is located between nucleotides -39 and -26 and referred to as the -30 region. It contains the sequence GAGTATAA which is positioned at nucleotide coordinates -34 to -27. The -30 region appears to be as important as the internal control region (21) for the in vitro transcription activity of the *Drosophila* 5S RNA gene.

Morton and Sprague (14) have demonstrated the importance of the 5'-flanking region for *Bombyx* 5S RNA gene transcription. For *Bombyx* 5S DNA deleting the region located between nucleotide positions -30 and -17, which contains a TATA sequence motif, resulted in a reduction of transcription activity; however, transcription activity was lost only after subsequent deletion of the sequences between coordinates -21 and -6 (14). Similarly the *Neurospora crassa* 5S RNA gene has a complex 5'-flanking transcription control region which contains a TATA motif (24). In *N. crassa* the TATA box is located at coordinate -29, and this sequence appears to fix the start point of transcription (24). For all the mutant *Drosophila* 5S DNAs that displayed transcription activity, the transcription start site remained the same as for the wild-type gene.

The LS mutations in the present study provide evidence that for *Drosophila* the -30 region is essential for transcription; however, these mutations do not discern the sequence requirement of this region. Therefore we have no formal proof as to the importance per se of the TATA-containing sequence motif. Also, the transcriptionally important sequences appear to extend further than the 8-bp motif. Nevertheless, the sequence and positioning of this motif is reminiscent of the TATA motif for RNA polymerase II-directed transcription (15), and whether the TATA motif is a functional component of the -30 region therefore deserves discussion.

The GAGTATAA sequence functions when an apparent five of eight nucleotides are present (Fig. 1). The importance of each of the eight nucleotide positions for transcription activity does not appear to be equally weighted since by comparing the transcription activities of ds5.-32, ds5.-20, and ds5.-14 it appears that the sequence GANT (-34 to -31) provides more activity than the TATAA component of the motif. The mutations ds5.-15, ds5.-14, and ds5.-13 provide a series of deletion mutations such that the five-of-eight motif, active in ds5.-14, is 1 nucleotide further away in ds5.-15 and 1 bp closer, relative to the gene, in ds5.-13. Since ds5.-15 and ds5.-13 did not direct transcription in vitro, this result suggests either that a five-of-eight base-pair motif can only function in a wild-type position or that the reason for transcription of ds5.-14 is alternate to the presence of such a five-of-eight motif. A further consideration in whether a five-of-eight base-pair motif is functional is the finding that ds5.-8 and ds5.1, each of which has a four-of-

FIG. 6. Template exclusion competition assay. DNA I indicated above each lane was preincubated in transcription reactions for 40 min before the addition of DNA II (LS105/93 [maxigene]), after which transcription was allowed to continue for an additional 90 min. For each assay maxigene transcription was quantitated by densitometer scanning of autoradiographs. Relative competition strength values are the average of four experiments.

eight base-pair fit, are not transcribed. It is not obvious why a five-of-eight motif would function, albeit reduced, and a four-of-eight motif would display a complete lack of transcriptional activity. One explanation could be that there are two regions in the 5'-flanking sequence that are transcriptionally important: the -30 region and a secondary region perhaps centered at position -15. The template LS-22/-11, and to a lesser extent LS-11/1, show that this -15 region is functionally sensitive to mutation. Since in ds5.-8 and ds5.1 the -15 region is removed, perhaps the four-of-eight base-pair -30 region is therefore insufficient to direct transcription. Unfortunately, the present series of 5' deletion mutations did not yield a template having a four-of-eight base-pair -30 region and an intact -15 region.

The entire 5'-flanking sequence between positions -39 and 1 is sensitive to mutations by LS and ds mutations. It is likely that there exist critical positive- or negative-acting base pairs (or both) and positions within the 5'-flanking sequence in the -30 region and perhaps the -15 region that confer the function of the 5'-flanking region. We contend that this mechanism would allow a variety of functional sequences within these two regions. The mutational approaches of deletion and linker scanning do not necessarily detect the important nucleotides because the full effect of the substituting nucleotides cannot be taken into account.

The universality among 5S RNA genes of the *D. melanogaster* 5S DNA -30 region control mechanism is not known. The -30 region sequence motif is conserved in sequence and position in four dipteran 5S RNA genes (16). A similar six-of-eight sequence is present in a transcriptionally active mouse 5S RNA gene at position -42, but not in an inactive human 5S RNA gene (4). Inspection of the DNA sequence of a Chinese hamster 5S RNA gene which is transcriptionally active in extracts prepared from HeLa or *X. laevis* (7) does not reveal a TATA motif in the 5'-flanking

region. The hamster 5S DNA 5'-flanking region, however, contains the sequence GAGCGCAG positioned at coordinate -45 and the sequence GCGAGCA at position -43 (7). Whether these sequences, which have some homology to the *Drosophila* sequence at the -30 region, are necessary for hamster 5S DNA transcription is not known. As already noted, upstream regions are also important for *Bombyx* (14) and *Neurospora* (24) 5S RNA gene transcription. Interestingly, the *Drosophila* 5S RNA gene is transcriptionally active in HeLa cell extracts (21), and the HeLa RNA polymerase III transcriptional apparatus functions more efficiently when the *Drosophila* -30 region is present, a result which supports the notion for a generic -30 region sequence.

In addition to RNA polymerase III, tRNA and 5S RNA gene transcription in human and *Xenopus* extracts requires TFIIB and TFIIC (2, 19, 22). 5S RNA gene transcription also requires the positive transcription factor TFIIA (10). The specific interaction of the transcription factors with the gene forms a transcription complex which is stable through many rounds of transcription initiation. The general model developed for class III gene transcription is that while RNA polymerase III has DNA-binding properties (8), specific polymerase function is brought about by RNA polymerase III recognition of the stable complex (1, 20). Sequestering of transcription factors by a class III gene can be measured by a template exclusion competition assay. In the competition assay, the templates containing the mutations LS-37/-26 and LS-22/-11 resulted in a level of maxigene transcription lower than when maxigene transcription was challenged with the wild-type template (Fig. 6). We had recently observed this extra competitive effect in different tRNA_{5^{Asn}} genes (11) and had concluded that the genes sequester transcription factors to form the stable complex and then sequester a limited concentration of RNA polymerase III activity to form a preinitiation complex. The 5'-flanking region of 5S DNA may function similarly to tRNA genes. Therefore, as for tRNA genes, the -30 region of 5S DNA may be the essential 5'-flanking region directing active transcription complex formation. The mechanism by which the -30 region controls active transcription complex formation and subsequent transcription initiation could involve a direct interaction of the -30 region with RNA polymerase III, or the -30 region could interplay in the interaction of the polymerase with one or more of the transcription factors in the stable complex.

The lack of observable 5'-flanking sequence conservation between *Drosophila* tRNA genes and between tRNA and 5S RNA genes makes it hard to discern whether RNA polymerase III has a specific recognition sequence. Because of the lack of observable sequence homology, the mechanism of positive- or negative-acting 5'-flanking sequences remains enigmatic. However, at least in vitro, the *Drosophila* tRNA_{5^{Asn}} and 5S RNA genes appear to have this transcription mechanism in common.

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LITERATURE CITED

1. Bieker, J. L., P. L. Martin, and R. G. Roeder. 1985. Formation of a rate-limiting intermediate in 5S RNA gene transcription. *Cell* 40:119-127.
2. Burke, D. J., and D. Soll. 1985. Functional analysis of fractionated *Drosophila* Kc cell tRNA gene transcription components. *J. Biol. Chem.* 260:816-823.
3. Carey, M. F., S. P. Gerrard, and N. R. Cozzarelli. 1986. Analysis of RNA polymerase III transcription complexes by gel filtration. *J. Biol. Chem.* 261:4309-4317.
4. Emerson, B. M., and R. G. Roeder. 1984. DNA sequences and transcription factor interactions of active and inactive forms of mammalian 5S RNA genes. *J. Biol. Chem.* 259:7926-7935.
5. Engelke, D. R., S. Y. Ng, B. S. Shastri, and R. G. Roeder. 1980. Specific interaction of a purified transcription factor with an internal control region of 5S genes. *Cell* 19:717-728.
6. Federoff, N. V. 1979. Deletion mutants of *Xenopus laevis* 5S ribosomal DNA. *Cell* 16:551-563.
7. Hart, R., and W. Folk. 1982. Structure and organization of a mammalian 5S gene cluster. *J. Biol. Chem.* 257:11706-11711.
8. Huet, J., M. Riva, M. Sentenac, and P. Fromageot. 1985. Yeast RNA polymerase C and its subunits. *J. Biol. Chem.* 260:15304-15310.
9. Korn, L. J., and D. D. Brown. 1978. Nucleotide sequence of *Xenopus borealis* oocyte 5S DNA: comparison of sequences that flank several related eucaryotic genes. *Cell* 15:1145-1156.
10. Lassar, A., P. Martin, and R. Roeder. 1983. Transcription of class III genes: formation of pre-initiation complexes. *Science* 222:740-748.
11. Lofquist, A., and S. Sharp. 1986. The 5'-flanking sequences of *Drosophila melanogaster* tRNA_{5^{Asn}} genes differentially arrest RNA polymerase III. *J. Biol. Chem.* 261:14600-14606.
12. Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65:499-560.
13. Morton, D. G., and K. U. Sprague. 1982. Silkworm 5S RNA and alanine tRNA genes share a highly conserved 5' flanking and coding sequence. *Mol. Cell. Biol.* 2:1524-1531.
14. Morton, D. G., and K. U. Sprague. 1984. *In vitro* transcription of a silkworm 5S RNA gene requires an upstream signal. *Proc. Natl. Acad. Sci. USA* 81:5519-5522.
15. Parker, C. S., and J. Topol. 1984. A *Drosophila* RNA polymerase II transcription factor contains a promoter-region-specific DNA-binding activity. *Cell* 36:357-369.
16. Rubacha, A., W. Sumner III, L. Richter, and K. Beckingham. 1984. Conserved 5' flank homologies in dipteran 5S RNA genes that would function on 'A' form DNA. *Nucleic Acids Res.* 12:8193-8207.
17. Sakonju, S., D. F. Bogenhagen, and D. D. Brown. 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. I. The 5' border of the region. *Cell* 19:13-25.
18. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
19. Segall, J., T. Matsui, and R. G. Roeder. 1980. Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. *J. Biol. Chem.* 255:11986-11991.
20. Setzer, D. R., and D. D. Brown. 1985. Formation and stability of the 5S RNA transcription complex. *J. Biol. Chem.* 260:2483-2492.
21. Sharp, S., A. Garcia, L. Cooley, and D. Soll. 1984. Transcriptionally active and inactive gene repeats within the *D. melanogaster* 5S RNA gene cluster. *Nucleic Acids Res.* 12:7617-7632.
22. Shastri, B. S., S. Y. Ng, and R. G. Roeder. 1982. Multiple factors involved in the transcription of class III genes in *Xenopus laevis*. *J. Biol. Chem.* 257:12979-12986.
23. Smith, D. R., I. J. Jackson, and D. D. Brown. 1984. Domains of the positive transcription factor specific for the *Xenopus* 5S RNA gene. *Cell* 37:645-652.
24. Tyler, B. M. 1986. Transcription of cloned *Neurospora* genes *in vitro*. *J. Cell. Biochem. Suppl.* 10D:162.