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Sequence differences upstream of the promoters are involved in the differential expression of the *Xenopus* somatic and oocyte 5S RNA genes

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ABSTRACT

The Xenopus somatic and oocyte 5S RNA genes are differentially expressed in extracts of whole oocytes. In such extracts, sequence differences preceding the internal promoters significantly alter the relative activities of these genes. Following exchange of the sequences preceding the promoter, the activity of the somatic 5S gene decreased and that of the oocyte 5S gene increased. As a result, a 100 fold somatic transcriptional advantage was reduced to 5 fold. Analysis of deletion mutants showed that the relevant sequence differences are located between -34 and +37 relative to the initiation site. The observed transcriptional modulation is due both to sequence differences 5' to the initiation site and at positions 30 and 37 within the coding region.

INTRODUCTION

The two major classes of Xenopus 5S RNA genes are differentially expressed during development. Both the somatic and oocyte-type 5S genes are transcriptionally active during early oogenesis, but in gastrula-stage embryos and adult tissues, the oocyte 5S genes are virtually inactivated (1). The molecular basis for this differential expression is not well understood. One model suggests that decreasing concentrations of the transcription factor TFIIIA during embryonic development, coupled with early replication of the somatic 5S genes, result in progressively less factor bound to the oocyte 5S genes (2). Transcription complexes on the oocyte 5S genes may also be less stable in embryonic conditions (3). In extracts derived from whole oocytes, the transcriptional bias resembles that seen in embryos; the somatic 5S gene is 50-100 fold more active than the oocyte 5S gene (4,5). In contrast, in oocyte nuclear extracts, these genes have similar activities (6).

Fractionation of cellular extracts has indicated that at least three factors termed TFIIIA, B and C are required in addition to RNA polymerase III for accurate 5S transcription (7,8). TFIIIA is a 5S specific factor which binds the internal control region or promoter (9). The association of TFIIIA with the promoter is prerequisite for subsequent association by TFIIIC, followed by TFIIIB (10, 11). These factors together form a stable complex which remains associated throughout multiple rounds of transcription (12). There is no evidence that TFIIIB and IIIC interact with specific 5S DNA sequences, although TFIIIC binding is affected by sequences near the 5' promoter boundary (13). Deletion studies have shown that the promoter extends from position 50 to 83 within the coding region (14,15). Sequences preceding this promoter are not essential for transcription but their deletion does result in inaccurate initiation and a reduction in competition strength (6). In this study we have not examined the functional significance of all sequences preceding the promoter, but instead have focused on the biological relevance of the relatively few oocyte/somatic sequence differences within this region. Deletion studies do not address this question; deletion removes the conserved sequences as well as those which differ. To investigate the sequence differences, the regions preceding the 5S promoters have been exchanged such that conserved sequences are retained and the sequence differences are switched. As a result, any change in transcriptional activity is solely attributable to these base differences. In this report, we present evidence that the sequence differences preceding the promoter significantly affect the relative activities of the somatic and oocyte 5S genes in whole oocyte extracts.

MATERIALS AND METHODS

DNAs:

(GS): pX1s11 (16) contains a single repeat unit of *Xenopus laevis* somatic 5S DNA with 607 bp of 5' flanking sequence and 156 bp of 3' flanking sequence.

(AO): pX1o 3'+176 is a derivative of pX1o31 (17) containing

one copy of the X. laevis oocyte 5S gene with 356 bp of 5' flanking sequences and 56 bp of 3' flanking sequence.
(AS): pX1o/s+40 contains the 5' flank and 40 bp of the oocyte-5S gene joined at a Sau3A site at +40 to the somatic-5S gene and 156 bp of 3' flanking sequence.
(AS*): pX1o/s+8 has oocyte type sequences preceding the somatic 5S gene, joined at a Hae III site at +8.
(GO): pX1s/o+30 contains 549 bp of 5' flanking sequence and 30 bp of the somatic-5S gene joined at a Sau3A site at +30 to the oocyte-5S gene and 56 bp of 3' flank.
pX1s508 contains a 508bp Sau 3A fragment of somatic 5S DNA sequences upstream of position +30. pX1o386 contains a 386 bp Hind III-Sau 3A fragment containing oocyte 5S DNA sequences upstream of position +30. All of the above 5S DNAs are inserted into pUC18.

In the following deletion mutants, the 5S DNA inserts are all inserted at the SmaI site or between the SmaI and EcoRI sites of pUC18 in the same orientation. (-GO) pX1s/o Δ5'-34 contains the insert from pX1s/o +30 extending from a Fnu4H site at -33 to an EcoRI site at +176. The vector residue at -34 matches the authentic 5S sequence. (-AS) pX1o/s Δ5' -6 contains the insert from pX1o/s+40 extending from nucleotides -6 to +142. (-AO) pX1o Δ5'-12 contains the insert from pX1o Δ3'+176 extending from a Fnu4H site at -11 to an EcoRI site at + 176. The vector residue at -12 matches the authentic 5S sequence. (-GS) pX1s Δ5'-34 contains the insert from pX1s11 extending from Fnu4H sites at -33 and +142 relative to the start site of the gene.

Transcription Assays:

Transcription assays were carried out in a Xenopus oocyte homogenate S100 as previously described (18). The extracts used in this study gave rise to 8-10 transcripts/gene/hour (TGH) for the somatic-5S and 15-25 times fewer transcripts for the oocyte-5S gene. Template DNAs (50-200 ng) were incubated in the presence of 400 ng pUC18 DNA in a 50 λ reaction at 22° C for 2 hrs. Reactions contained 45 mM Tris-HCl (pH 7.9), 60 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 0.6 mM ATP, CTP, and UTP

and 25 μM ($a^{32}P$)GTP (10 μCi). Reactions were stopped with 0.5% SDS and 20 mM EDTA and the RNA purified by phenol extraction and ethanol precipitation. The transcription products were analyzed by gel electrophoresis in 5% acrylamide-8 M urea sequencing gels or in separating gels according to the procedure of Wakefield and Gurdon (19). The gels were exposed to Kodak XAR-1 film and the bands quantitated by scanning laser densitometry (LKB Ultroscan). Alternatively, the radiolabeled RNAs were excised and quantitated by scintillation counting.

RESULTS

Construction of Hybrid 5S Genes

Sequences preceding the oocyte and somatic 5S genes are highly divergent except for regions of homology between positions -14 and -29 (16,17) (Fig. 1A). The sequences further upstream of the somatic 5S gene are GC-rich whereas those preceding the oocyte 5S gene are AT-rich. The ATsomatic hybrid 5S gene has oocyte-type sequences preceding the gene and at positions 30 and 37 (Fig 1B). The GCoocyte hybrid 5S gene has somatic-type sequences preceding the gene and at position 30. The terms upstream or flanking sequences will be used in this report to refer to sequences upstream or flanking the internal promoter, rather than the transcription initiation site, so as to include the internal base differences.

Independent Reactions

In vitro transcription reactions were carried out in S100 extracts of mature Xenopus oocytes. In independent reactions, the somatic 5S gene was approximately 20 times more active than the oocyte 5S gene (Fig. 2A, lanes 1 and 2). Following exchange of sequences preceding the promoter, the somatic transcriptional advantage decreased from 20 to 5 fold (lanes 3-4). Both genes were affected by the exchange; somatic 5S transcription decreased 3-4 fold whereas oocyte 5S transcription increased 2 fold, indicating that the somatic-type flanking sequence has a positive influence on transcription relative to the oocyte flank. This effect is also apparent in figure 2B. Transcription reactions were carried out for increasing periods of time ranging from 15 minutes to 2 hours. The somatic 5S gene was

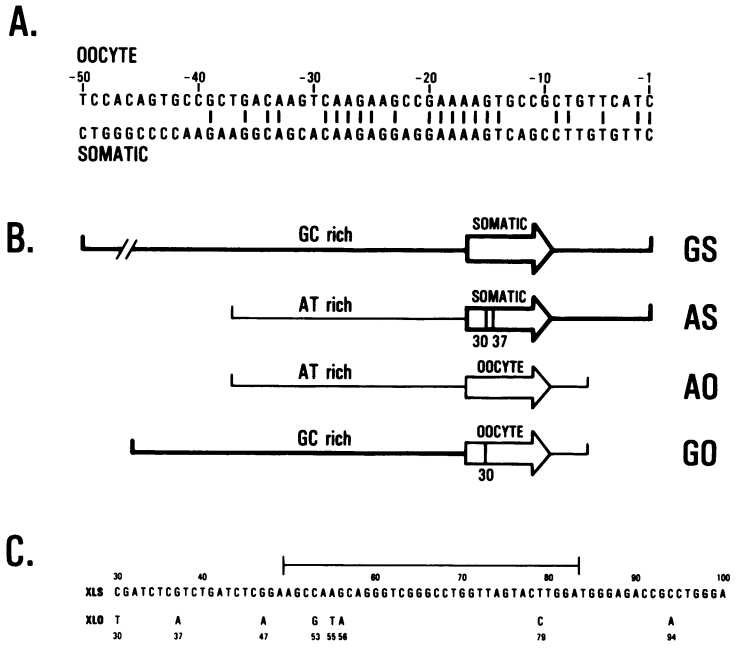

Figure 1. Native and Hybrid 5S DNA Sequences.
A. Comparison of sequences preceding the oocyte and somatic-5S genes. B. Construction of hybrid 5S genes. (GS); somatic 5S gene, (AS); ATsomatic having oocyte type sequences preceding the gene and at 30 and 37. (AO); oocyte-5S gene (GO): GCoocyte having somatic type sequences preceding the gene and at position 30. C. Internal sequence differences between the somatic and oocyte 5S genes. The position of the promoter is indicated.

several fold more active than ATsomatic and the oocyte 5S gene was two fold less active than GCoocyte. The relatively low oocyte 5S signal is not due to instability of transcripts (lanes marked C).

Mixed Template Reactions

Mixed template reactions were carried out at saturating DNA concentrations (Fig. 3). The native and hybrid 5S transcripts are distinguished on the basis of secondary structure by means of partially denaturing electrophoretic conditions (19). In a competition reaction at 5:1 oocyte/somatic DNA ratios, the somatic 5S gene was 100 fold more active than the oocyte 5S gene (lane 1). This transcriptional advantage is several fold greater than in independent reactions (Fig. 2A), indicating that

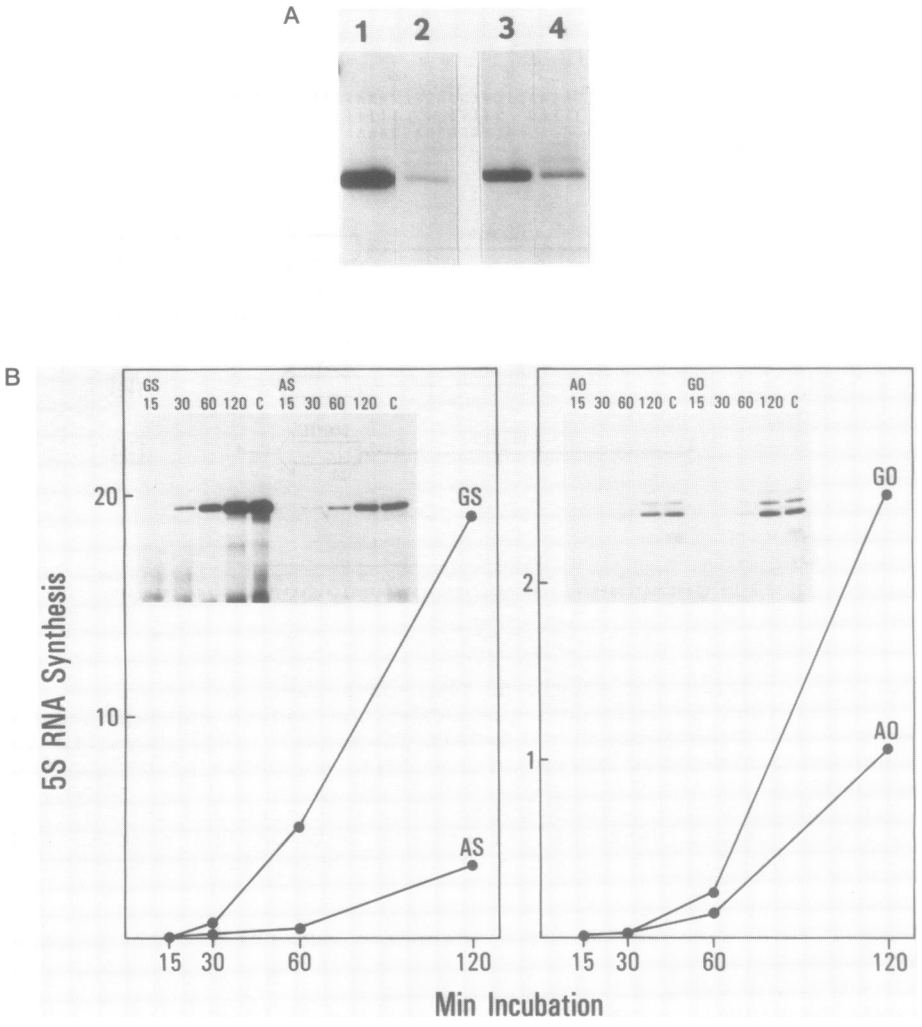

Figure 2. In Vitro Transcription of Native and Hybrid 5S DNAs. A. Transcription products of 5S DNAs in an oocyte homogenate S100 are shown. Independent reactions contained 50 ng of somatic (lane 1), oocyte (lane 2), ATsomatic (lane 3), or GCoocyte (lane 4) 5S DNAs.
B. Kinetics of 5S RNA Synthesis in S100. Transcription reactions were carried out for the indicated times. Exposure time for oocyte 5S (AO/GO) autoradiograph was appropriately 10 times greater than for somatic 5S (AS/GS). The relative amounts of RNA synthesis (vertical axis) were normalized against oocyte 5S (AO) levels. For lanes marked C, an excess of cold NTPs was added after 2 hours and the reaction incubated for an additional hour to assess RNA stability.

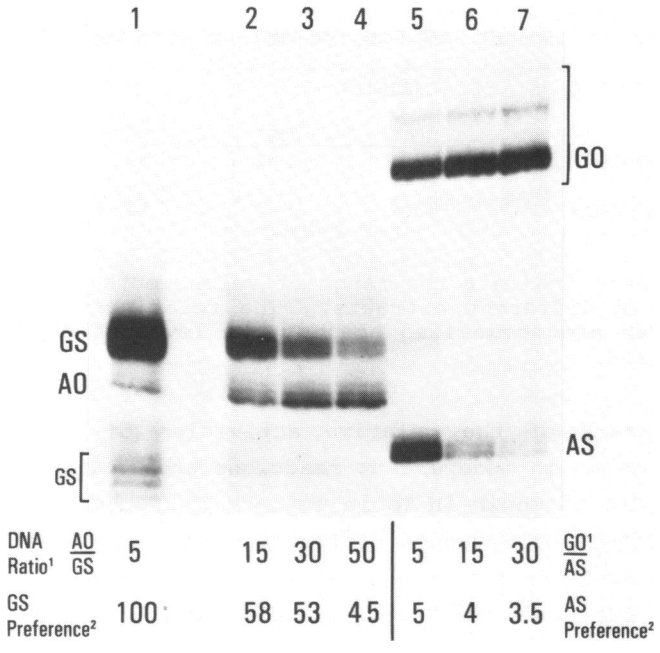

Figure 3. Mixed Template Reactions with Native and Hybrid 5S Genes.
All reactions contained a total of 100 ng of 5S DNA per 50 μl volume. In lanes 1-4, reactions contained somatic (GS) and oocyte (AO) 5S DNAs at the indicated O/S ratios. The level of somatic 5S transcription, relative to oocyte, is shown below. In lanes 5-7, reactions contained the hybrid 5S genes, GCoocyte (GO) and ATsomatic (AS), at the indicated O/S ratios. The level of ATsomatic transcription, relative to GCoocyte, is shown below.

the somatic 5S gene can compete more effectively for limiting factors in the extract. Following exchange of sequences preceding the promoter, the somatic 5S transcriptional advantage was reduced from 100 to 5 fold (lane 5); the activity of the somatic 5S gene decreased 5-8 fold in various experiments, whereas oocyte 5S activity increased 5-8 fold. At higher ratios of oocyte to somatic 5S DNAs, the transcriptional advantage of the somatic 5S gene decreased from 100 to approximately 50 fold (lanes 1-4). Similarly, the transcriptional advantage of ATsomatic over GCoocyte declined from 5 to 3 fold (lanes 5-7), This may result from nonproductive absorption of transcription factors onto oocyte and GCoocyte DNAs.

TABLE 1: Relative transcriptional efficiencies of 5S DNAs in independent and mixed template reactions with oocyte 5S DNA.

TRANSCRIPTIONAL EFFICIENCY*				
	AO	GO	AS	GS
INDEPENDENT	1	1-2	3-6	15-25
COMPETITION	1	5-8	12-30	50-100

*These numbers indicate the range of values obtained in a number of assays in different extracts. The relative transcriptional efficiencies are normalized against the level of oocyte-5S (AO) transcription.

A summary of the relative activities of the native and hybrid 5S genes in independent reactions and in competition with oocyte 5S DNA is shown in Table 1.

Relative Effect of Sequence Differences Preceding the Promoter

The somatic and GCoocyte 5S genes have different promoter sequences but identical somatic-type sequences preceding the promoter. When transcribed in a mixed reaction, the somatic 5S gene was 12 fold more active than GCoocyte (Fig. 4, lane 3). This 12 fold transcriptional advantage is attributable to the somatic 5S promoter and perhaps sequences 3' to the promoter. When both genes had oocyte type sequences preceding the promoter, the ATsomatic gene was similarly 10 fold more active than the oocyte 5S gene (lane 2). The somatic 5S gene was 70 fold more active than the oocyte 5S gene in this experiment (lane 1). Assuming the somatic 5S promoter provides 10-12 fold higher activity, the somatic-type sequences preceding the promoter must provide a 6-7 fold transcriptional enhancement to arrive at this 70 fold higher activity. Thus, 30% of the somatic 5S transcriptional advantage in the whole oocyte extract is attributable to sequence differences preceding the promoter.

Mapping the Sequence Differences Responsible for the Somatic Transcriptional Enhancement

The sequence differences which give rise to this transcriptional enhancement are near the start site of the gene. Deletion of the somatic type flanking sequence to -34 did not alter the activities of the somatic or GCoocyte 5S genes (fig. 4, lanes 3 and 6). Deletion of the oocyte type flank to -12 of

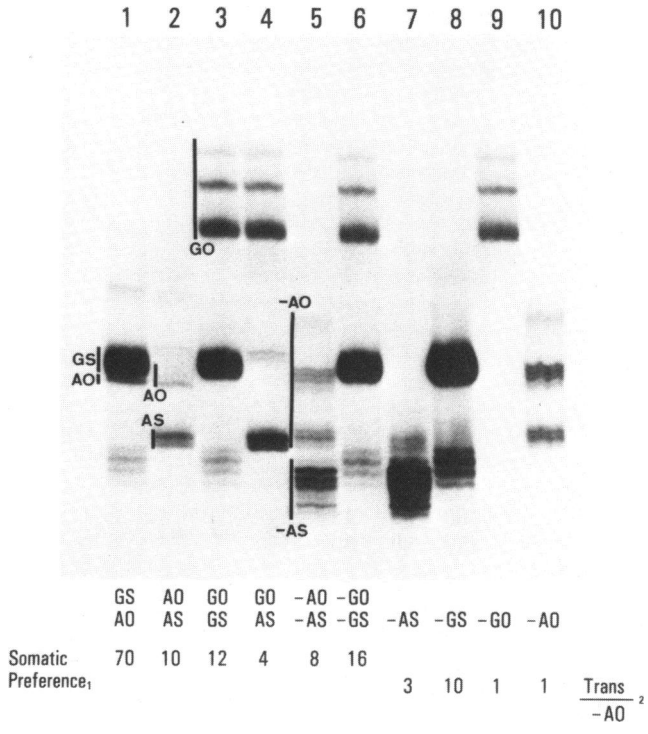

Figure 4. Effect of Deleting Sequences 5' to Native and Hybrid 5S Genes.
In lanes 1-6, reactions contained a total of 100 ng of 5S DNAs at a 5:1 oocyte/somatic ratio. Within some lanes, a vertical line identifies the major transcription products of a given DNA. Below, the competing DNAs are listed in the order in which the transcripts appear in the gel. The ratio of somatic to oocyte transcription is shown below (corrected for the DNA ratio). (-AS); ATsomatic 5S gene deleted to -6, (-AO); oocyte 5S gene deleted to -12, (-GO and -GS); GCoocyte and somatic 5S genes deleted to -34. In lanes 7-10, the 5' deletion mutants (50 ng) were transcribed in separate reactions. The level of activity relative to that of oocyte 5'Δ-12 (-AO) is shown below each lane.

the oocyte 5S gene or -6 of ATsomatic resulted in aberrant initiation but no significant change in the relative activities (lanes 2 and 5).

The somatic-type sequence differences at 30 and 37, as well as those preceding the initiation site, increase gene activity, as shown in figure 5. In this experiment, the somatic 5S gene was 8 fold more active than ATsomatic. A hybrid oocyte/somatic

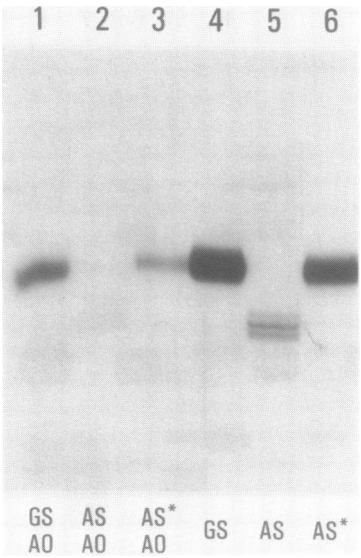

Figure 5. Effect of Internal Sequence Differences at Positions +30 and +37. Reactions contained 83 ng of oocyte 5S DNA and either 17 ng of somatic (lane 1), ATsomatic +40 with oocyte-type sequences preceding the gene and at +30 and +37 (lane 2), or ATsomatic* +8 with oocyte type sequences only preceding the gene (lane 3). Oocyte 5S transcripts are not visible. Independent assays contained 100 ng of somatic (lane 4), ATsomatic +40 (lane 5), or ATsomatic* +8 (lane 6).

5S gene (ATsomatic*), having oocyte-type sequences preceding the gene but not at 30 or 37, was intermediate in activity. The sequence difference at position 30 within the oocyte 5S gene in pXloΔ3'+176 is typical of the dominant oocyte-type sequence in Xenopus leavis, whereas the base change at position 37 is not typical. Therefore, the base difference at position 30 is likely to be more significant with regard to gene expression.

DISCUSSION

Somatic Type Sequence Differences Preceding the Promoter Increase Transcriptional Activity

A current model of 5S gene regulation suggests that the differential expression of the somatic and oocyte 5S genes results from preferential binding of limiting quantities of TFIIIA to the somatic 5S promoter (2,3). The results presented

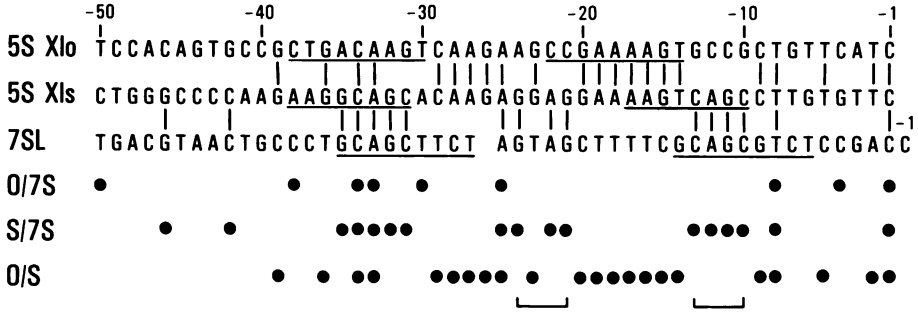

Figure 6. Sequence Homology Between the 5' Flanking Regions of Xenopus Somatic 5S and Human 7SL RNA Genes. Imperfect direct repeats are underlined. Below the sequences is a comparison of the homology between the oocyte-5S and human 7SL (O/7S), somatic-5S and 7SL (S/7S), and somatic and oocyte-5S DNAs (O/S). The two regions indicated by brackets are conserved between human 7SL and somatic-5S, but not by oocyte-5S DNA.

here suggest the mechanism may be more complex in that sequence differences preceding the promoters can alter the relative activities of these genes. Specifically, somatic type sequence differences between -34 and +37 result in increased activity. This region includes 14 base differences preceding the initiation site as well as positions +30 and 37 within the gene. These findings suggest that regulatory factors preferentially interact with the somatic 5S gene as a result of these sequence differences. It has been previously demonstrated that TFIIIA binding is not affected by sequences preceding the promoter (24), however these sequences might influence interaction by factors B or C. A point mutation at position 51, near the promoter boundary, was shown to be detrimental to interaction by TFIIIC (20). Alternatively, a factor other than IIIA, B, or C could mediate this effect. The somatic-type sequence differences preceding the gene and the internal base differences at 30 and 37 might have independent effects on transcription. The sequence differences preceding the gene may affect RNA polymerase III interaction; the region between -34 and -6 is clearly important for positioning the initiation site (Fig. 4).

Transcriptional Modulation by Sequences Preceding Class III Genes

The sequences preceding the human 7SL RNA gene are known to

increase gene activity (21). Within this region are short blocks of homology to the sequences preceding the somatic 5S gene. Two of these blocks flank the conserved GAAAAG element which is positioned upstream of a number of class III genes (22) and is inverted in the case of the 7SL gene (Fig. 6). These homologies are not present in the sequences preceding the oocyte 5S gene. Therefore these sequences may represent one source of the somatic 5S transcriptional advantage.

There are many other examples in which 5'flanking regions have positive or negative effects on class III gene activity. The regions upstream of 5S RNA genes of D. melanogaster, B. mori, and N. crassa have been shown to be essential, along with the internal control regions, for transcription in homologous extracts (23,24,25). Deletion studies have previously shown that the sequences preceding the Xenopus 5S promoters are not essential but do have a small effect on gene activity in nuclear extracts (6). The findings presented here show that these sequences have a greater effect on gene activity in whole oocyte extracts. This is the first evidence that sequence differences preceding the somatic and oocyte 5S promoters are important for the differential expression of these genes.

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