MOLECULAR AND CELLULAR BIOLOGY, Nov. 1988, p. 5056–5058
0270-7306/88/115056-03$02.00/0

Vol. 8, No. 11

# Effect of Sequence Differences between Somatic and Oocyte 5S RNA Genes on Transcriptional Efficiency in an Oocyte S150 Extract

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Received 21 April 1988/Accepted 18 August 1988

**The differential expression of the *Xenopus laevis* somatic and oocyte 5S RNA genes is partially, but not solely, due to several base differences near the 5′ boundary of the internal control region. A hybrid oocyte 5S gene with somatic-type base changes at +47, +53, +55, and +56 had intermediate transcriptional activity in oocyte S150 extracts. These base substitutions also resulted in increased affinity for a factor(s), other than TFIIIA, which forms a stable complex with the 5S gene.**

The *Xenopus laevis* somatic and oocyte 5S RNA genes are both expressed during the early stages of oogenesis; however, during embryogenesis, the oocyte-type 5S genes are selectively inactivated (23). The mechanism governing this developmental switch is not fully understood but appears to involve the differential stability of factor-DNA complexes (20, 21). Factors referred to as TFIIIA, TFIIIB, and TFIIIC are required for proper initiation by RNA polymerase III (4, 16, 18). An internal control region (ICR) extending from +47 to +91 contains the minimal DNA sequences necessary for transcription initiation (1, 8, 15). There is some evidence that somatic or oocyte (S/O) sequence differences near the 5′ boundary of this ICR give rise to the differential expression of these genes (22). However, the findings reported here show that several sequence differences between +47 and +56 are only partially responsible for the differential expression in vitro in whole oocyte S150 extracts. The findings further show that these base differences result in increased affinity for a transcription factor(s), other than TFIIIA, which is stably associated with active transcription complexes.

The 5S RNA genes used in this study are the *X. laevis* somatic 5S gene in pXls11 (11) and the major oocyte 5S gene in pXlo176 (5, 22). Oligonucleotide-directed mutagenesis (24) was used to change the oocyte 5S sequence at positions +47, +53, +55, and +56 to a somatic-type sequence in the hybrid pXlo/s47-56. pXlo/s40 contains a hybrid somatic 5S gene with oocyte-type (pXlo176) sequences preceding the gene and at +30 and +37 (13). Each of these 5S DNAs was inserted into pUC 18.

Transcription assays were carried out in whole oocyte S150 extracts prepared as described by Glikin et al. (6). These assays were performed in a 50-μl reaction mixture containing 40 μl of oocyte S150 extract in 70 mM KCl–20 mM Tris hydrochloride (pH 7.9)–7 mM $MgCl_2$–0.8 mM dithiothreitol–1.6 mM each ATP, CTP, and UTP–20 μM GTP–10 μCi of [α-$^{32}$P]GTP. Reaction mixtures were incubated at room temperature for 2 h.

**Effect of S/O sequence differences within the ICR on transcriptional efficiency in oocyte S150 extracts.** The ICR extends from approximately +47 to +91 and contains those sequences which are essential for 5S transcription (1, 8, 15). Wormington et al. (22) previously reported that a fourfold lower competition strength of the oocyte 5S gene in oocyte nuclear extracts was solely attributable to base differences near the 5′ boundary of the ICR. The transcriptional properties of oocyte nuclear extracts differ from those of whole oocyte extracts; in nuclear extracts, the transcriptional advantage of the somatic 5S gene is only 4- to 10-fold, whereas in whole oocyte extracts, the somatic 5S gene is 50- to 100-fold more active than the oocyte 5S gene (9, 10), thus approaching the 1,000-fold advantage of the endogeneous somatic 5S genes in embryonic and adult cells. To determine whether this transcriptional advantage is attributable to the several conserved S/O sequence differences near the 5′ boundary of the ICR (Fig. 1B), I constructed a hybrid 5S gene. Oligonucleotide-directed mutagenesis was used to change the major oocyte 5S sequence at positions +47, +53, +55, and +56 to a somatic-type sequence. In transcription assays in the S150 extract, this pXlo/s47-56 hybrid gene had intermediate activity, 10-fold higher than that of the oocyte 5S gene but 10-fold lower than that of the somatic 5S gene (Fig. 1A, lanes 1 to 3). Clearly, these base differences are only partially responsible for the 100-fold somatic transcriptional advantage in the S150 extract. This finding is consistent with an earlier report that S/O sequence differences preceding the ICR are important for this differential expression (13); a somatic 5S hybrid gene (pXlo/s40) having oocyte-type sequences at positions +30 and +37 and preceding the initiation site was 5- to 10-fold less active than the somatic 5S gene (Fig. 1A, lane 4).

**Effect of the S/O sequence differences between +47 and +56 on the binding of factors other than TFIIIA.** Factors TFIIIA, TFIIIB, and TFIIIC together form a stable complex with 5S DNA which is resistant to challenge by a second template and remains associated throughout successive rounds of transcription (2). This complex forms the basis for second-template exclusion assays. Oocyte and hybrid pXlo/s47-56 5S DNAs were preincubated in the S150 extract for 1 h to allow the formation of stable factor-DNA complexes (Fig. 2). A second template, somatic 5S DNA, was then introduced. A lower concentration of pXlo/s47-56 DNA was required to deplete a limiting factor, thereby inhibiting subsequent somatic 5S transcription. This result was most apparent in lanes 2 and 7; preincubation with 100 ng of pXlo/s47-56 was sufficient to substantially reduce the somatic 5S signal, whereas this same concentration of oocyte 5S DNA had no significant effect on somatic 5S transcription. These results indicate that the somatic-type base changes between +47 and +56 facilitate the stable binding of factors into transcription complexes. Note that at higher DNA concentrations, the oocyte 5S signal equaled that of pXlo/s47-56.

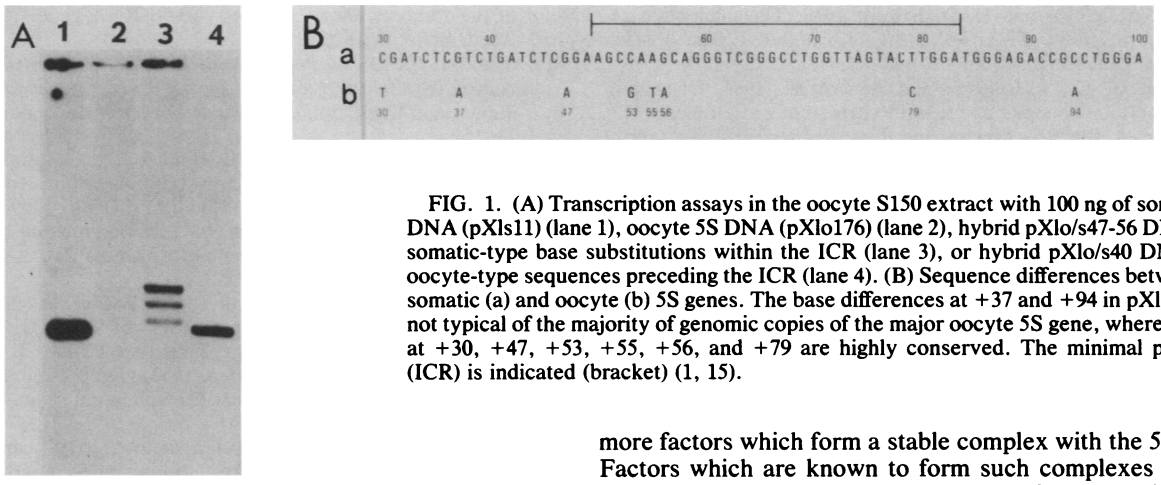


FIG. 1. (A) Transcription assays in the oocyte S150 extract with 100 ng of somatic 5S DNA (pXls11) (lane 1), oocyte 5S DNA (pXlo176) (lane 2), hybrid pXlo/s47-56 DNA with somatic-type base substitutions within the ICR (lane 3), or hybrid pXlo/s40 DNA with oocyte-type sequences preceding the ICR (lane 4). (B) Sequence differences between the somatic (a) and oocyte (b) 5S genes. The base differences at +37 and +94 in pXlo176 are not typical of the majority of genomic copies of the major oocyte 5S gene, whereas those at +30, +47, +53, +55, +56, and +79 are highly conserved. The minimal promoter (ICR) is indicated (bracket) (1, 15).

This result suggests that higher DNA concentrations compensate for reduced factor affinity, such that the same maximal number of active complexes are formed with either gene.

These results indicate that the somatic-type base differences between +47 and +56 increase the affinity for one or more factors which form a stable complex with the 5S gene. Factors which are known to form such complexes include TFIIIA, TFIIIB, and TFIIIC (7, 17). McConkey and Bogenhagen (8) have demonstrated that TFIIIA affinity is not affected by the sequence differences between the major oocyte and somatic 5S genes. In agreement with that study, the oocyte and hybrid pXlo/s47-56 5S genes were found to have the same affinity for TFIIIA, as measured by gel retardation and footprint competition assays (data not shown). This result indicates that a factor(s) other than TFIIIA, most probably TFIIIB or TFIIIC, is influenced by the base differences at +47 to +56. TFIIIC appears to interact with the region from +47 to +56; Pielar and coworkers (12) showed that a point mutation at +51 was detrimental to binding by TFIIIC. In addition, the sequences between +50 and +64 exhibit homology to the consensus A block of the tDNA promoter which is protected by TFIIIC in DNase I footprinting assays (3).

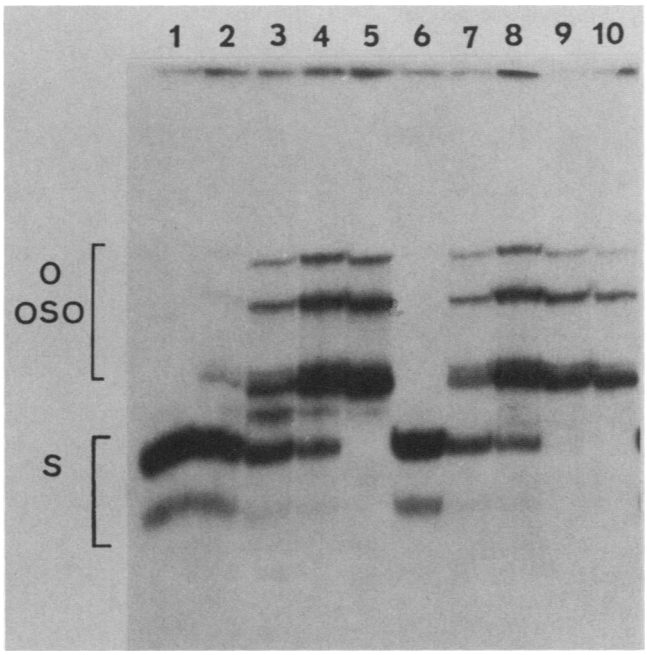


FIG. 2. Effect of S/O sequence differences between +47 and +56 on stable complex formation. Second-template exclusion assays contained 0, 100, 200, 400, or 800 ng of oocyte 5S DNA (O, lanes 1 to 5) or hybrid pXlo/s47-56 5S DNA (OSO, lanes 6 to 10). These DNAs were preincubated for 1 h in the oocyte S150 extract and were then incubated with 400 ng of somatic 5S DNA (S) and labeled nucleotide triphosphates for 2 h. In this partially denaturing gel system (19), the different 5S RNAs are resolved on the basis of secondary structure. The three transcript lengths of oocyte (O) and pXlo/s47-56 (OSO) DNAs are due to the imperfect termination signal of the oocyte 5S gene. The 1-h preincubation period allowed the preformation of transcription complexes on the oocyte and pXlo/s47-56 5S DNAs but not on the somatic 5S DNA. For this reason, the maximal signals of oocyte and pXlo/s47-56 5S DNAs were as high as that of somatic 5S DNA.

It was previously reported that the +50 region (more precisely +52) of the oocyte, but not the somatic, 5S gene was hypersensitive to DNase I when TFIIIA was bound (14). Footprinting assays with the pXlo/s47-56 mutant indicated that the sequence differences at +47 to +56 are responsible for this differential sensitivity; the somatic-type base substitutions resulted in reduced sensitivity at +52 (data not shown). Interestingly, it was previously found that the +50 region of the oocyte, but not the somatic, 5S gene was also hypersensitive to S1 nuclease as naked supercoiled DNA (14). Experiments are in progress to determine the basis by which these several S/O base differences affect active complex formation.

I thank K. Azer for technical assistance, S. Evans, J. Gottesfeld, and J. Blanco for helpful discussions, and L. Elder for help in preparing the manuscript.

This study was supported by Public Health Service grant GM 34888 from the National Institutes of Health.

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