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Fidelity of Transcription of *Xenopus laevis* Globin Genes Injected into *Xenopus laevis* Oocytes and Unfertilized Eggs

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The *Xenopus laevis* α1- and β1-globin genes were injected into oocytes and unfertilized eggs of *X. laevis*. In oocytes, the injected globin genes were actively transcribed, but the majority of the transcripts were incorrectly initiated. In unfertilized eggs, the injected genes were transcribed at a low level but only from the correct start sites. In oocytes, the injected circular plasmid DNA containing the cloned globin genes persisted but did not replicate. In contrast, DNA injected into unfertilized eggs replicated up to 15-fold within a 22-h period. We suggest that the ability of the egg to selectively transcribe the injected *X. laevis* globin genes from the correct promoter sites may be related to differences in chromatin structure between the oocyte and the unfertilized egg.

In *Xenopus laevis*, oocytes and eggs are related yet very different cell types. An oocyte is a growing egg cell that can be surgically removed from the ovary of an adult female, whereas the term "egg" refers to a mature gamete laid by the female frog after hormonal induction. Oocytes are very active in RNA synthesis, but after maturation into eggs, very little RNA is synthesized. Active transcription does not resume until fertilized eggs reach midblastula transition. Another major difference between *Xenopus* oocytes and eggs lies in DNA replication. The oocytes does not replicate its chromosomes, but when the egg is "activated," either by fertilization or by pricking with a micropipette, rapid chromosomal replication begins. Although very different in transcriptional activity and capacity to replicate their chromosomes, oocytes and eggs have similar compositions. Both contain large amounts of the components, such as ribosomes, histones, and polymerases, which will be needed for rapid development of the early embryo (reviewed in references 12 and 22).

Given these fundamental differences in metabolism between oocytes and eggs, each has proven to be experimentally useful in different ways. When purified DNA is injected into unfertilized eggs, it replicates semiconservatively (8, 11, 21), thus providing a model system for studying eucaryotic DNA replication (13). For transcriptional studies, genes are usually injected into oocytes rather than unfertilized eggs, because there is generally 10 to 20 times more transcription of the injected genes in oocytes (26). Only some genes, however, are transcribed with sufficient fidelity to make oocyte injection a suitable functional assay. Oocytes do not accurately transcribe the rabbit β-globin gene (32) or the ovalbumin gene (38). The inability of the oocyte to faithfully transcribe these genes may be related to the fact that they encode specialized proteins that are normally only expressed in specific cell types (12).

We show that this phenomenon extends to the *Xenopus* genes. Neither the *Xenopus* α1- nor β1-globin gene is accurately transcribed in oocytes. Rather surprisingly, past experiments involving injection of the same *Xenopus* β-globin gene into fertilized eggs showed that the injected gene was transcribed in developing embryos at low levels but from the correct promoter (2, 3). To further investigate the difference between transcriptional expression of the injected *Xenopus* globin genes in oocytes and embryos, the *Xenopus* adult major α- and β-globin genes were injected into oocytes and unfertilized eggs. Significant differences were found between the transcripts produced in the two cell types.

MATERIALS AND METHODS

DNA injected. *Xenopus* oocytes or unfertilized eggs were injected with pXGαβ1 plasmid DNA (Fig. 1). The plasmid contains the *X. laevis* major adult α- and β-globin genes (α1 and β1), which were originally cloned by inserting a 14.3-kilobase-pair (kbp) *Eco*RI fragment of *X. laevis* genomic DNA into a bacteriophage λ vector (30). From λXGαβ1, the 14.3-kbp *Eco*RI fragment containing the globin genes was recloned into the *Eco*RI site of the plasmid vector pAT153 (36).

Injection and sample preparation. Ovarian tissue was surgically removed from anesthetized mature female *X. laevis* (Xenopus Ltd., South Nutfield, Surrey, United Kingdom), incubated in Ca^{2+}-depleted OR-2 medium (37) containing 0.2% collagenase (Sigma Chemical Co., St. Louis, Mo.) for 2 h at 20 to 22°C, and then gently washed with modified Barth medium (10) supplemented with 10 μg of penicillin per ml and 5 μg of streptomycin per ml. Individual stage VI oocytes (6) were selected for injection. To obtain unfertilized eggs, mature females were injected with 500 IU of gonadotrophin, and 15 to 22 h later, eggs were squeezed out through the cloaca (4). The eggs were decapsulated in 2.0% cysteine hydrochloride (pH 7.8), washed gently with MMR medium (5), a modified Ringer's solution supplemented with penicillin and streptomycin as above, and injected immediately.

The DNA to be injected was purified by two bandings in CsCl gradients and dissolved at the desired concentration in injection buffer (0.1 M KCl, 0.01 M Tris [pH 7.5]). A volume of 15 nl per oocyte or egg was injected as previously described (20). Oocytes were centrifuged before injection to bring the nucleus to the surface where it could be easily visualized and injected. In unfertilized eggs, the DNA was injected into the cytoplasm because during maturation of oocytes into eggs, the nuclear membrane disappears. Injected oocytes or unfertilized eggs were incubated in modified Barth or MMR medium, respectively, at 18°C. Samples of 10 to 20 oocytes or eggs were taken after the indicated incuba-

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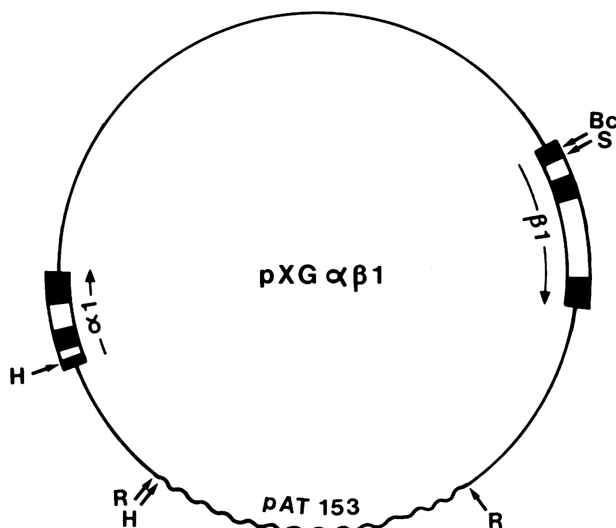

FIG. 1. Diagram of recombinant plasmid pXGαβ1. The plasmid contains the major adult α- and β-globin genes of *X. laevis* on a 14.3-kbp *Eco*RI fragment of genomic DNA. Wavy lines represent bacterial plasmid DNA sequences, and straight lines represent *Xenopus* genomic DNA sequences. The α1- and β1-globin genes are shown as boxes, with the filled-in portions corresponding to exons. Arrows indicate the direction of transcription. A few relevant restriction sites are indicated as follows: Bc, *Bcl*I; H, *Hin*dIII; R, *Eco*RI; and S, *Sau*96.

tion time and stored at −70°C. Samples were later homogenized in SET (1% sodium dodecyl sulfate, 5 mM EDTA, 10 mM Tris [pH 7.5]) and treated with proteinase K (100 μg/ml, 37°C, 1 h). Nucleic acids were then isolated by phenol extraction, chloroform extraction, and ethanol precipitation.

Analysis of transcription. Transcripts of the *X. laevis* β1-globin gene were detected by primer extension as previously described (2). RNA from three injected oocytes or eggs was hybridized to a molar excess of a single-stranded primer, a ^{32}P-labeled 52-nucleotide *Bcl*I-*Sau*96 DNA fragment derived from the first exon of the β1-globin gene (see Fig. 1). With reverse transcriptase, the hybridized primers were extended to the 5′-ends of the β1-globin gene transcripts. The extension products were analyzed on 10% acrylamide-urea gels. Correctly initiated *X. laevis* β1-globin gene transcripts give major primer extension products 127 and 129 nucleotides in length (2, 3, 31).

Transcripts of the *X. laevis* α1-globin gene were detected by an S1 nuclease hybridization assay (2). RNA from three injected oocytes or eggs was hybridized to an excess of a ^{32}P 5′-end-labeled *Hin*dIII fragment that extends from a site within the first exon of the α1-globin gene to well upstream of the 5′-end of the gene (Fig. 1). Samples were digested with S1 nuclease and analyzed on 10% acrylamide-urea gels. Correctly initiated *X. laevis* α1-globin gene transcripts protect a fragment 128 nucleotides in length (2).

As a positive control in both primer extension assays and S1 nuclease hybridization assays, known amounts of total erythrocyte RNA prepared from anemic adult *X. laevis* were analyzed in parallel with experimental samples. To estimate the number of transcripts in experimental samples, we compared the relative intensities of the autoradiographic signals from experimental samples with those from control samples. We assumed that 2% of the total RNA is polyadenylated and that 50% of the polyadenylated RNA in adult erythrocytes is α1-globin gene transcripts and the other 50% is β1-globin gene transcripts.

Transcripts of the herpes simplex virus (HSV) thymidine kinase (*tk*) gene were detected by an S1 nuclease hybridization assay (24). RNA from injected oocytes or eggs was hybridized to an excess of ^{32}P-labeled, 131-nucleotide *Bgl*II-*Eco*RI, single-stranded probe DNA. The probe DNA extends across the 5′-end of the HSV *tk* gene. RNA-DNA hybrids were digested with S1 nuclease (10 U/μl, 37°, 30 min) and analyzed on 10% acrylamide-urea gels. Authentic HSV *tk* gene transcripts protect 54 to 56 nucleotides of the probe DNA (24).

Analysis of replication. After injection, oocytes or eggs were immediately transferred to medium (Barth or MMR). In those experiments involving the inhibition of DNA replication, aphidicolin was added to the medium at a concentration of 2, 10, or 20 μg/ml. (Aphidicolin was supplied by A. H. Todd, Imperial Chemical Industries, Ltd.) The oocytes or eggs were incubated, and samples were collected and processed as described above. Total DNA from the equivalent of 0.5 oocyte or egg was electrophoresed through a 0.8% agarose horizontal slab gel in 0.5× Tris-borate buffer (0.045 M Tris-borate, 0.045 M boric acid, 0.001 M EDTA) at 5.5 V/cm for 4 h. After electrophoresis, DNAs in the gel were depurinated, denatured, transferred onto nitrocellulose membranes, and hybridized to pAT153 DNA, which was ^{32}P-labeled by nick translation (17). To obtain quantitative results, areas of the filter hybridizing to the pAT153 DNA were cut out, and radioactivity was measured by liquid scintillation counting.

RESULTS

Transcription of *Xenopus* β1-globin genes injected into *Xenopus* oocytes and unfertilized eggs. The *Xenopus* major adult α- and β-globin genes were injected into oocytes or unfertilized eggs on an 18-kbp circular recombinant plasmid DNA (Fig. 1). In most cases, 4 ng per oocyte or egg was injected. This amount of DNA is well below the saturating levels for transcription in oocytes (26) and for chromatin assembly in either oocytes (39) or eggs (23). After a 20- to 22-h incubation period, total RNA was extracted from the injected oocytes and eggs and analyzed by primer extension for transcripts of the *Xenopus* β1-globin gene. The 52-nucleotide DNA primer hybridizes within the first exon of β1-globin gene transcripts. When hybridized to RNA from *Xenopus* adult erythroblasts, the primer was extended to give two major fragments 127 and 129 nucleotides in length (Fig. 2, lanes 5 and 6; Fig. 3, lanes 4 and 5). These fragments are the sizes expected for correctly initiated transcripts of the *X. laevis* major adult β-globin gene which displays heterogeneity in the start point of transcription (31). When this primer extension assay was used to analyze RNA from injected oocytes, there was a large array of extended fragments ranging in size from just slightly larger than the 52-nucleotide primer to more than 500 nucleotides in length (Fig. 2, lanes 8 and 10). Included within this array were extended fragments of the size predicted for transcripts initiating from the correct promoter, but these constituted only a minority of the total extension products. The extended fragments that are shorter than the normal 127- and 129-nucleotide extended fragments may represent either transcripts initiated downstream of the true promoter or artifacts caused by premature termination of the reverse transcriptase reaction. It is difficult to distinguish between these two possibilities. However, the extension products that are long-

er than 127 nucleotides can only represent transcripts of the injected β1-globin gene that have been initiated at sites upstream of the normal start sites. In contrast to the diversity of extended fragments seen with RNA from oocytes injected with the β1-globin gene, RNA from unfertilized eggs injected with the same DNA gave only two major extended fragments which are identical to those observed with adult erythroblast RNA (Fig. 2, lanes 1 and 3). It appears that in unfertilized eggs, the injected *Xenopus* β1-globin gene is accurately transcribed from the correct promoter; in oocytes, however, the injected β1-globin gene is actively but inaccurately transcribed with the majority of the transcripts initiating at incorrect start sites.

In oocytes, the ratio of correct versus incorrect transcription of the injected β1-globin gene varied from experiment to experiment. To distinguish between experimental variability and variability in the oocytes themselves, oocytes from five different females were injected in parallel with plasmid

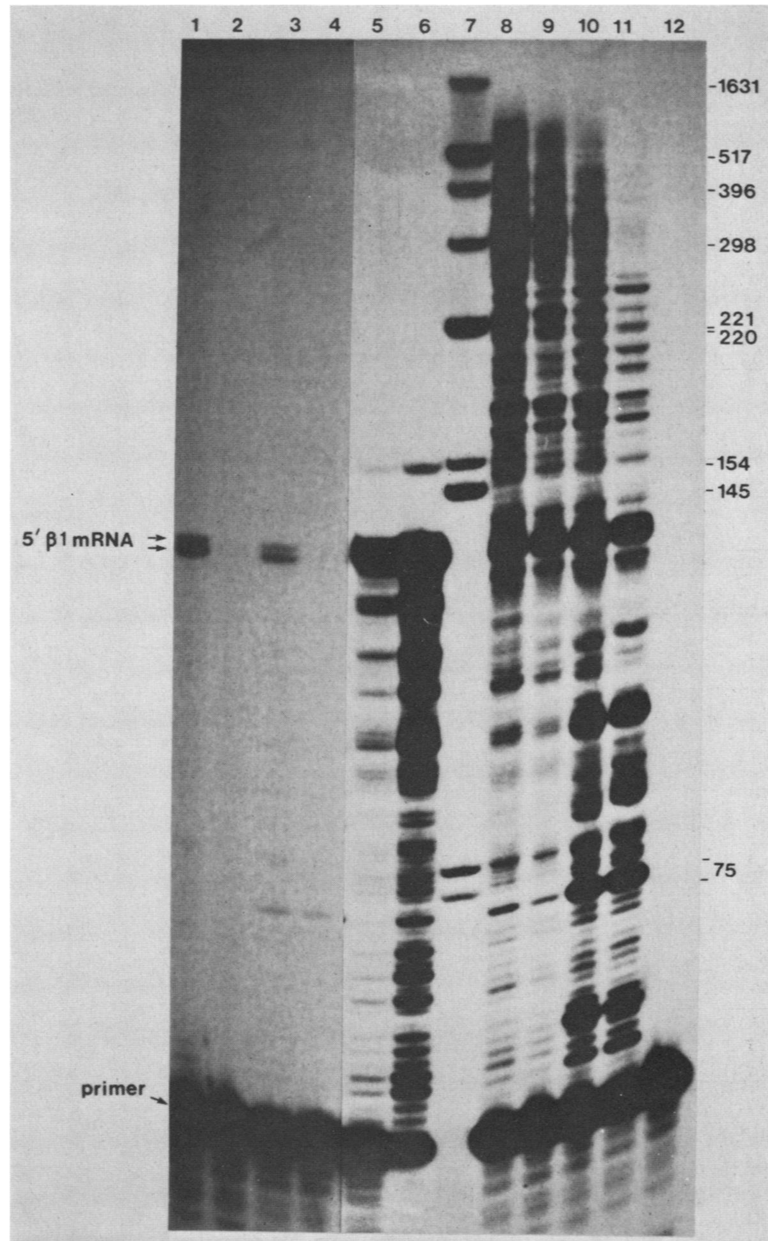

FIG. 2. Detection of β1-globin gene transcripts in unfertilized eggs and oocytes injected with pXGαβ1 DNA. Transcripts were detected by primer extension analysis (see the text). The positions of the primer and of the extension products from correctly initiated β1-globin gene transcripts are indicated on the left. The size of the pAT153/*Hin*fI fragments present in lane 7 are listed on the right. Lanes 1 to 4, Unfertilized eggs injected with 4 ng (lanes 1 and 2) or 24 ng (lanes 3 and 4) of pXGαβ1 DNA; lanes 2 and 4, eggs incubated for 22 h in the presence of 10 μg of aphidicolin per ml; lanes 1 and 3, eggs incubated for 22 h in the absence of aphidicolin. Lanes 8 to 12, Oocytes injected with 4 ng (lanes 8 and 9) or 24 ng (lanes 10 and 11) of pXGαβ1 DNA; lane 12, control with uninjected oocytes; lanes 9 and 11, oocytes incubated for 22 h in the presence of 10 μg of aphidicolin per ml; lanes 8 and 10, oocytes incubated for 22 h in the absence of aphidicolin. Controls with 5.0 and 50 ng of total RNA from adult erythrocytes are shown in lanes 5 and 6, respectively.

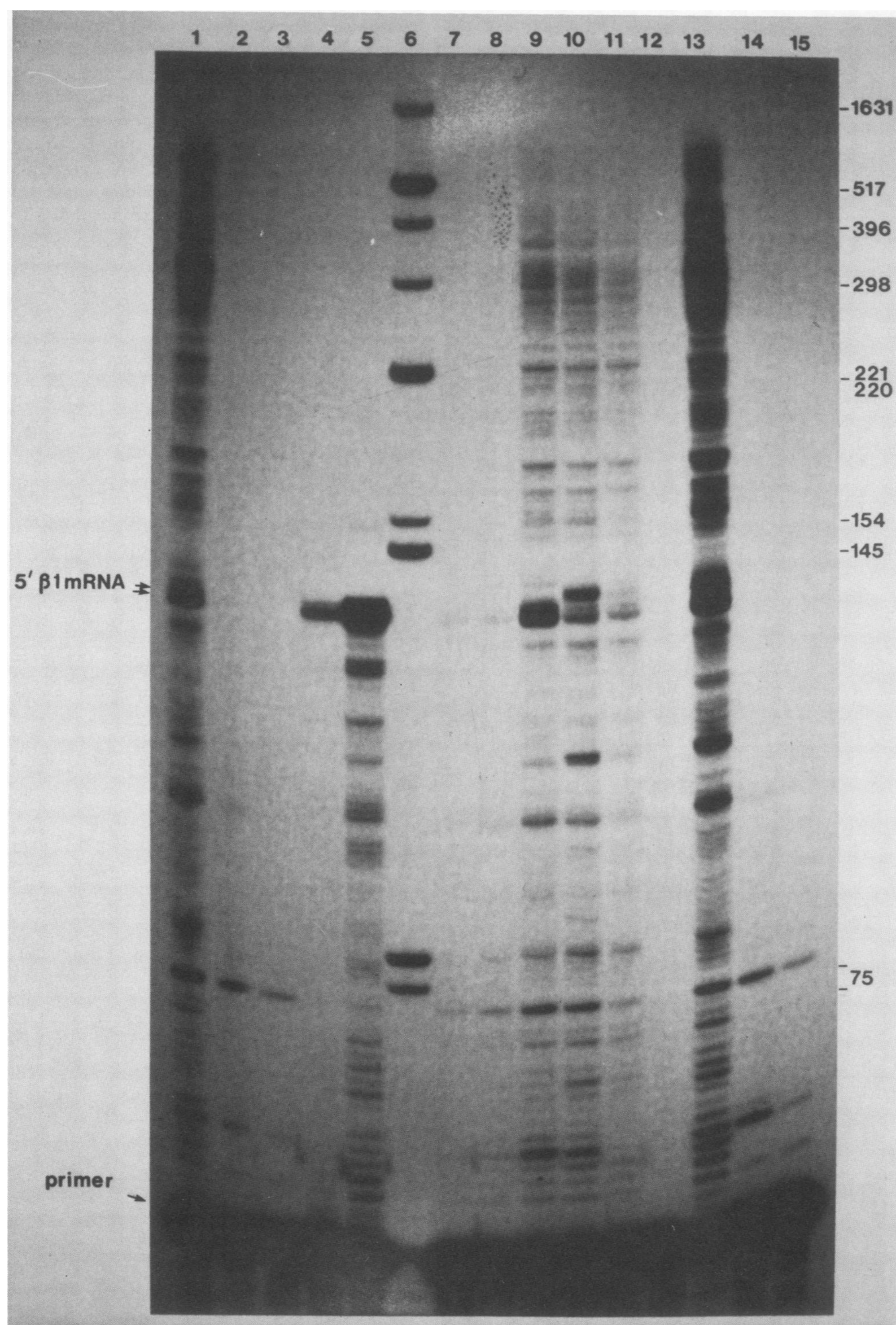

FIG. 3. Variability in the transcription of injected β1-globin genes in oocytes from five different females and the effect of α-amanitin on the transcription of β1-globin genes injected into oocytes. As in Fig. 2, the positions of the primer and of the extension products from correctly initiated β1-globin gene transcripts are indicated on the left; on the right, the size of the pAT153/*Hin*fI fragments present in lane 6 are listed. Lanes 7 to 11, primer extension analysis of RNA from oocytes of five different females injected in parallel with 4 ng of pXGαβ1 DNA per oocyte; lane 12, control with RNA from uninjected oocytes. Positive controls with 0.5 and 5.0 ng of total RNA from adult erythrocytes are shown in lanes 4 and 5, respectively. Lanes 1 to 3 and 13 to 15, RNA analyzed from oocytes coinjected with 4 ng of pXGαβ12 and 0 (lanes 1 and 13), 0.2 (lanes 2 and 14), or 20 ng (lanes 3 and 15) of α-amanitin.

pXGαβ1 DNA. RNA from uninjected oocytes was also prepared and analyzed in parallel. The results of this primer extension assay show considerable variability among the transcripts produced in the five sets of oocytes (Fig. 3, lanes 7 to 11). There was variability from female to female in the total amount of correct transcripts per oocyte as well as variability in the ratio of correct to incorrect transcripts. In oocytes from one female (Fig. 3, lane 11), the bands corresponding to transcripts with the correct 5′-ends were not any more intense than the numerous other bands corresponding to incorrectly initiated transcripts. In oocytes from another female (Fig. 3, lane 9), the most intense bands were those which correspond to normal transcripts; however, a wide assortment of incorrect transcripts was also present. This variability in transcription of the injected genes was not due to irreproducibility in the amount of DNA injected or to differences in the persistence of the injected DNA. The same samples that were analyzed in the primer extension assay were analyzed by nitrocellulose blotting for the amount and state of the injected plasmid DNA. No significant differences were found among the five samples (data not shown). It seems that oocytes from different females vary in their relative ability to recognize the correct promoter of the injected β1-globin gene. In contrast, eggs from different females showed little variability in that in all cases, the injected β-globin genes were transcribed at low levels and exclusively from the normal site of initiation.

Estimates of the amount of correct transcription of the injected β1-globin gene in oocytes and eggs were made by comparing the intensity of the bands corresponding to extended fragments from correctly initiated transcripts with the intensity of the bands observed with known amounts of control RNA. By comparison of the experimental lanes (Fig. 3, lanes 7 to 11) with the control lanes (Fig. 3, lanes 4 and 5), there were from 1 to 20 pg of correctly initiated transcripts per three oocytes injected. Considering that 4 ng of DNA (2 $\times 10^8$ gene copies) was injected per oocyte, this represents 0.005 to 0.1 correct transcripts per gene during the 20-h incubation period. Compared with the transcription of some other polymerase II-transcribed genes injected into oocytes, this is a very low level of correct transcription. McKnight et al. (24) estimated that for the HSV *tk* gene, there was a minimum of 3.0 specific transcripts per gene copy per day, and Etkin and Maxson (7) estimated that injected sea urchin histone genes were transcribed at ca. 2.4 transcripts per gene copy per day. The rate of correct transcription of the β1-globin gene injected into unfertilized eggs has also been estimated. In the experiment shown in Fig. 2, three eggs, each injected with 4 ng of pXGαβ1 DNA, produced ca. 2 pg of correctly initiated transcripts in 22 h. This represents a low level of transcription (0.01 transcripts per gene in 22 h) which is within the range of correct transcription observed for the β1-globin gene injected into oocytes. Thus, there is a comparable low level of correctly initiated transcription of the injected β1-globin gene in both eggs and oocytes. The principal difference between transcription of the β1-globin gene in eggs and oocytes is that oocytes produced, in addition to a low level of correctly initiated transcripts, a large number of β1-globin gene transcripts initiated from incorrect start sites.

To determine whether the incorrectly initiated transcripts in oocytes were the products of polymerase II or III, oocytes were coinjected with pXGαβ1 DNA and two different amounts of α-amanitin. The lower concentration (0.2 ng per oocyte) inhibits polymerase II but not polymerase III, and the higher concentration (20 ng per oocyte) inhibits both polymerase II and III (reviewed in reference 12). The results of injecting oocytes with 4 ng of pXGαβ1 DNA plus 0, 0.2, or 20 ng of α-amanitin are shown in Fig. 3, lanes 1 to 3 and 13 to 15. Both levels of α-amanitin abolish nearly all transcription of the β1-globin gene, indicating that polymerase II is responsible for the incorrect, as well as the correct, transcription of the injected β1-globin gene in oocytes.

Transcription of *Xenopus* α1-globin genes injected into *Xenopus* oocytes and unfertilized eggs. The samples analyzed in Fig. 2 for β1-globin gene expression have also been analyzed for α1-globin gene expression. Transcripts of the α1-globin gene were detected by an S1 nuclease assay. When hybridized to RNA from *Xenopus* adult erythroblasts and digested with S1 nuclease, the 1,700-nucleotide probe gives a 128-nucleotide-long protected fragment which represents α1-globin gene transcripts with the correct start point of transcription (Fig. 4, lanes 5 and 6). Analyzing RNA from injected oocytes in this way gave a large number of protected fragments, most of which were much longer than the size expected for properly initiated transcripts of the α1-globin gene (Fig. 4, lanes 8 and 9). The 5′-end of the cloned α1-globin gene lies 1,550-base pairs from the junction of the *Xenopus* and plasmid vector DNA (Fig. 1). It is likely, therefore, that much of the erroneous transcription of the injected α1-globin gene in oocytes is initiated within plasmid DNA. Polymerase II was responsible for this erroneous transcription of the α1-globin gene in oocytes, since low levels of α-amanitin (0.2 ng per oocyte) effectively blocked almost all transcription of the α1-globin gene (data not shown).

Analyzing RNA from injected eggs gave a very different result. In unfertilized eggs, the injected α1-globin gene was transcribed at low levels but from the correct start site (Fig. 4, lanes 1 and 3). These results are similar to those observed for the α1-globin gene injected into fertilized eggs of *X. laevis* (2). Thus, the injected *Xenopus* α1-globin genes, like the β1-globin genes, are transcribed correctly but at low levels in eggs, whereas in oocytes, the level of transcription is much higher, but most of the transcripts are incorrectly initiated.

Replication and topology of injected DNA. Oocytes and unfertilized eggs were injected with pXGαβ1 DNA and then incubated in media containing 0 or 10 μg of aphidicolin per ml. Aphidicolin is a specific, direct inhibitor of animal DNA polymerase α (16), and DNA replication in cell-free systems derived from *Xenopus* eggs is 95% inhibited by as little as 10 μg of aphidicolin per ml (25; M. Mechali, personal communication). Total nucleic acids were extracted from samples taken immediately after injection and after incubation for 20 to 22 h at 18°C. The amount of pXGαβ1 DNA present in the various samples was determined by hybridization of ^{32}P-labeled pAT153 DNA to nitrocellulose blots of the samples separated on agarose gels (Fig. 5). The relative amounts of pXGαβ1 DNA present in the various samples was determined by liquid scintillation counting of the appropriate areas of the radioactively labeled filters (Table 1).

When injected into oocytes, the circular pXGαβ1 DNA did not replicate during the 22-h incubation period, but 35 to 50% of the DNA injected persisted 22 h after injection. The presence of aphidicolin did not affect the state or persistence of the DNA injected into oocytes (Fig. 5; Table 1). In unfertilized eggs, there was extensive replication of the DNA. When 4 ng was injected, there was within 22 h, a 6.3-fold increase in the amount of pXGαβ1 DNA, and when 24 ng was injected, there was a 1.5-fold increase (Fig. 5b; Table 1). This experiment and others involving injection of differ-

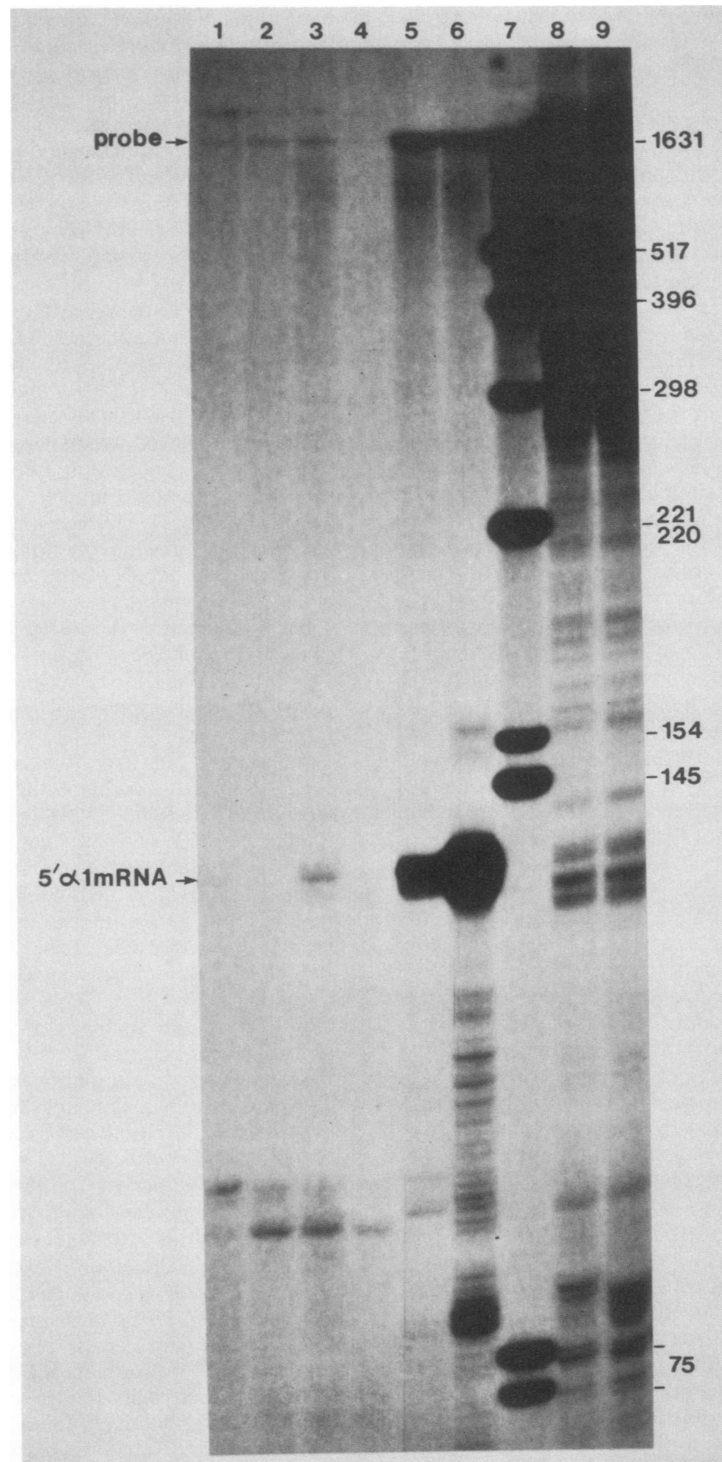

FIG. 4. Detection of α1-globin gene transcripts in unfertilized eggs and oocytes injected with pXGαβ1 DNA. Transcripts were detected by an S1 nuclease hybridization assay (see the text). The positions of the probe and of the fragment protected by correctly initiated α-globin gene transcripts are indicated on the left. The sizes of the pAT153/*Hin*fI fragments shown in lane 7 are listed on the right. Lanes 1 to 4, Unfertilized eggs injected with 4 ng (lanes 1 and 2) or 24 ng (lanes 3 and 4) of pXGαβ1 DNA; lanes 2 and 4, eggs incubated for 22 h in the presence of 10 μg of aphidicolin per ml; lanes 1 and 3, eggs incubated in the absence of aphidicolin. Lanes 8 and 9, Oocytes injected with 4 ng of pXGαβ1 DNA; lane 9, oocytes incubated for 22 h in the presence of aphidicolin; lane 8, oocytes incubated in the absence of aphidicolin. Controls with 0.5 and 5.0 ng of total RNA from adult erythrocytes are shown in lanes 5 and 6, respectively.

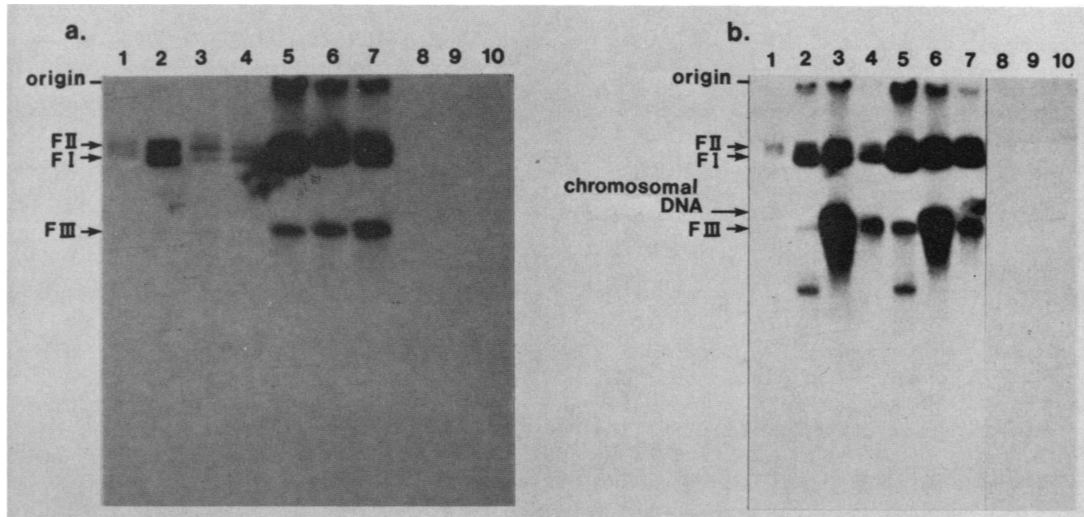

FIG. 5. Analysis of the persistence and replication of pXGαβ1 DNA injected into oocytes or unfertilized eggs. Replication of the injected DNA was detected by hybridizing nitrocellulose blots of agarose gels with ^{32}P-labeled pAT153 DNA (see the text). Each lane contains DNA extracted from the equivalent of 0.5 oocytes (a) or eggs (b). Lane 1, control sample of 0.2 ng of pXGαβ1 DNA (both panels); lanes 2 to 4, samples from oocytes (a) or eggs (b) injected with 4 ng of pXGαβ1 DNA; lanes 2, samples taken immediately after injection; lanes 3 and 4, samples taken after 22 h of incubation in the presence (lanes 4) or absence (lanes 3) of 10 μg of aphidicolin per ml; lanes 5 to 7, samples from oocytes (a) or eggs (b) injected with 24 ng of pXGαβ1 DNA; lanes 5, samples taken immediately after injection; lanes 6 and 7, samples taken after 22 h of incubation in the presence and absence of aphidicolin, respectively; lanes 8 to 10, samples from uninjected oocytes (a) or buffer-injected eggs (b); lanes 8, samples taken at the time of injection; lanes 9 and 10, samples taken after 22 h of incubation in the presence and absence of aphidicolin, respectively. To the left of the panels, the positions of chromosomal DNA and pXGαβ1 plasmid DNA are indicated. FI, supercoiled circular plasmid DNA; FII, relaxed circular plasmid DNA; and FIII, linearized plasmid DNA.

ent amounts of DNA into unfertilized eggs indicate that replication of the injected DNA probably ceases when a certain, perhaps toxic, concentration of DNA (25 to 35 ng per egg) is reached. The majority of the newly replicated DNA in unfertilized eggs comigrated with high-molecular-weight cell DNA (Fig. 5b, lanes 3 and 6). These results are similar to those found in fertilized *Xenopus* eggs in which the injected DNA replicates and eventually comigrates with chromosomal DNA (1–3, 33). When the unfertilized eggs were exposed to 10 μg of aphidicolin per ml, replication of the injected DNA was effectively blocked (Fig. 5b; Table 1).

It has been suggested that globin gene transcription may be related in some way to DNA replication (35). Given that DNA injected into eggs replicates and DNA injected into oocytes does not, the same samples analyzed for the persistence and replication of the injected pXGαβ1 DNA (Fig. 5) were also analyzed for the transcription of the injected *Xenopus* β1-globin gene in oocytes. In injected oocytes, in the presence or absence of aphidicolin, there was a certain amount of correctly initiated transcription admidst a heavy background of incorrect transcription (Fig. 2, lanes 8 to 11). In unfertilized eggs injected with pXGαβ1 DNA, only transcripts of the β1-globin gene with the correct start sites were observed, and these transcripts were synthesized only in the absence of aphidicolin (Fig. 2, lanes 1 to 4). The effect of aphidicolin on α1-globin gene expression was also examined. Incubation of the injected oocytes in aphidicolin did not affect transcription of the α1-globin gene (Fig. 4, lane 9). However, when the injected eggs were incubated in aphidicolin, no α1-globin gene transcripts were detected (Fig. 4, lanes 2 and 4).

It is difficult, however, to assess the significance of this inhibition because the drug may have side effects in unfertilized eggs. Although aphidicolin is reported to block DNA replication without interfering with RNA or protein synthesis (16), our control experiments indicate that, at least in this system, aphidicolin can interfere with transcription. The HSV *tk* gene is actively and accurately transcribed when injected into oocytes (24) in which no replication of injected DNAs occurs. When the HSV *tk* gene was injected into unfertilized eggs, *tk* transcripts were detected only when the eggs were incubated without aphidicolin (data not shown). Since DNA replication is known not to be necessary for *tk* transcription, it appears that aphidicolin is interfering with HSV *tk* transcription in eggs in some other way. Given the results of these control experiments, we cannot say whether the failure to detect α1- and β1-globin gene transcripts in unfertilized eggs incubated in aphidicolin is the result of aphidicolin inhibiting DNA replication or of its interfering in some other way with transcription.

The activity of polymerase II-transcribed genes injected into *Xenopus* oocytes is much higher when the genes are carried on a circular DNA molecule than on a linear molecule. The higher transcriptional activity from circular templates is not attributable merely to rapid degradation of the

TABLE 1. Effect of aphidicolin on persistence and replication of pXGαβ1 DNA injected into *Xenopus* oocytes and eggs[a]

Cultures	pXGαβ1 DNA (cpm) at:		
	0 h	22 h (0 μg/ml)	22 h (10 μg/ml)
Oocytes			
4 ng of DNA injected	25,900	8,990	10,000
24 ng of DNA injected	130,000	65,500	66,300
Eggs			
4 ng of DNA injected	35,000	220,000	28,700
24 ng of DNA injected	122,000	187,000	77,700

[a] Cultures were exposed to concentrations of aphidicolin of 0 μg/ml at 0 h and 0 and 10 μg/ml at 22 h.

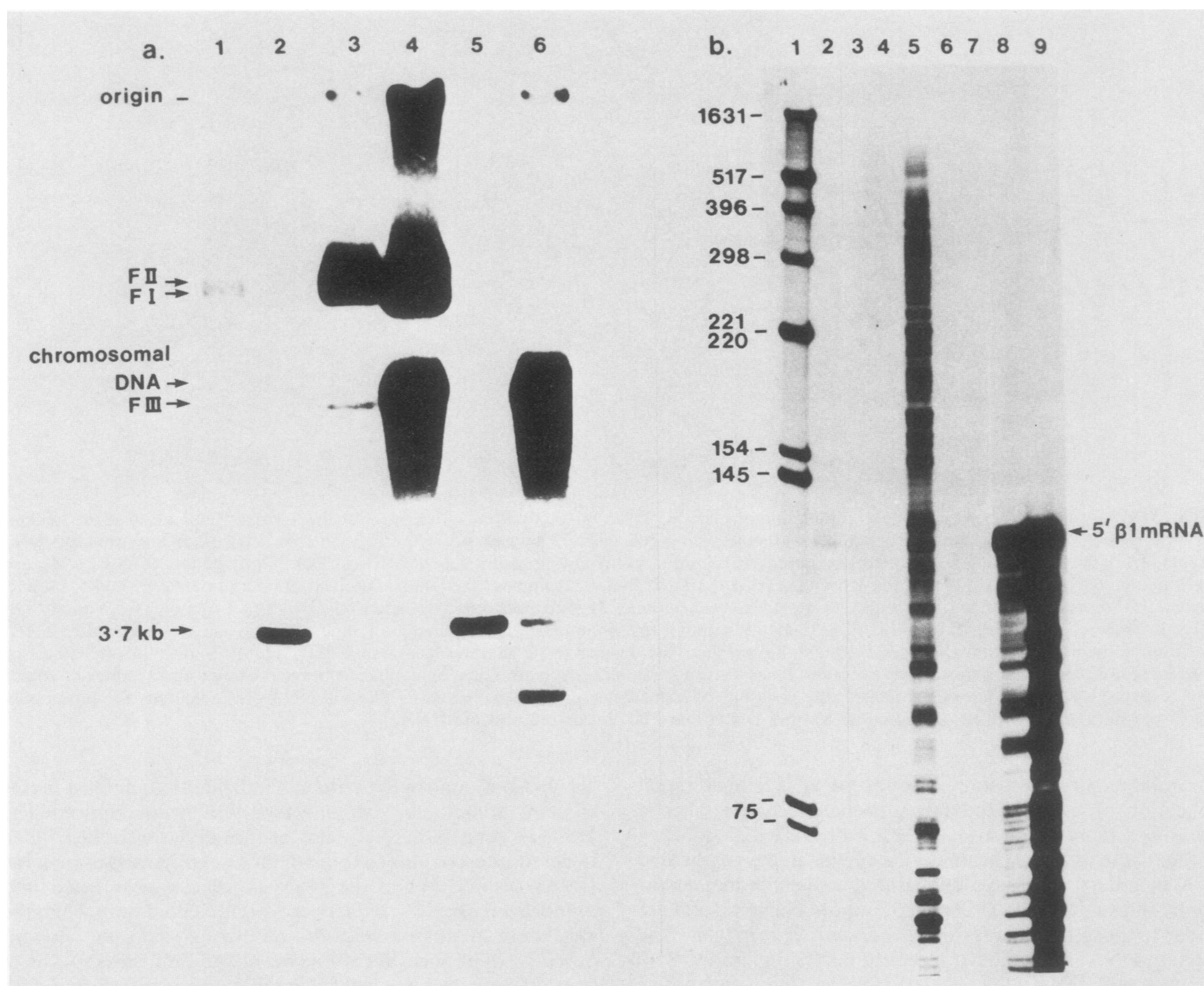

FIG. 6. Analysis of the replication and expression of β1-globin gene when injected as a circular or linear DNA. Unfertilized eggs and oocytes were injected with 4 ng of undigested pXGαβ1 plasmid DNA or *Eco*RI-digested pXGαβ1 DNA. Samples were taken 0 and 22 h after injection and analyzed for replication of the injected DNA (a) and for transcription of the injected β1-globin gene (b). (a) Replication of the injected DNA in eggs (see the text). Lanes 1 and 2, Controls showing 0.2 ng of undigested and *Eco*RI-digested pXGαβ1 DNA; lanes 3 and 4, samples from eggs injected with undigested DNA and incubated 0 and 22 h, respectively; lanes 5 and 6, samples from eggs injected with *Eco*RI-digested DNA and incubated 0 and 20 h, respectively. The positions of chromosomal DNA, FI, FII, and FIII pXGαβ1 DNA and the 3.7-kbp *Eco*RI fragment of pXGαβ1 DNA are indicated. The 14.3-kbp *Eco*RI fragment of pXGαβ1 DNA does not hybridize with the pAT153 probe DNA and is not visible. (b) Transcription analysis of the injected β1-globin gene in eggs and oocytes. Lanes 2, 3, and 4, Eggs injected with undigested and *Eco*RI-digested pXGαβ1 DNA and mock injected, respectively; (The band in lane 3 is too weak to be seen here but on the original film is identical in size to although less intense than the band seen in lane 2.) lanes 5, 6, and 7, oocytes injected with undigested pXGαβ1 DNA, *Eco*RI-digested DNA, and uninjected, respectively. Controls with 5.0 and 50 ng of total RNA from adult erythrocytes are shown in lanes 5 and 6, respectively. The position of extension products from correctly initiated β1-globin gene transcripts are indicated on the right. The size of the pAT153/*Hin*fI fragments present in lane 1 are listed on the left.

linear templates but appears to be related to the topological state of the DNA (14, 27). Because of the difference in topology of the DNA in oocytes and unfertilized eggs, we decided to compare the effect of injecting linear or circular DNA on the transcription of the *Xenopus* β1-globin gene. pXGαβ1 DNA was injected either as circular DNA or after digestion with *Eco*RI (Fig. 1). In oocytes, the circular DNA was transcribed efficiently but incorrectly (Fig. 6b, lane 5). Transcription from linear DNA was not detected (Fig. 6b, lane 6). In unfertilized eggs, however, injecting DNA in either form gave a low number of correctly initiated transcripts, with the level of transcription from linear DNA being less than that from circular DNA (Fig. 6b, lanes 2 and 3). Transcription from linear or circular DNA probably results from the fact that after injection into eggs, both linear and circular DNAs replicate and form a high-molecular-weight concatemers (Fig. 6a, lanes 4 and 6). Linear DNAs injected into oocytes are not efficiently transcribed by polymerase II, most probably because a topologically constrained template is necessary. In eggs, however, injected linear and circular DNAs both seem to achieve the necessary chromatin conformation for correct transcription during their replication and assembly into high-molecular-weight DNA.

Viability of injected unfertilized eggs. Unfertilized eggs

have been reported not to survive for more than 5 or 6 h after being activated by pricking (12). Our experiments involving the collection of samples at 5 and 22 h postinjection showed that the injected unfertilized eggs were very active in both DNA replication and RNA transcription during the 5- to 22-h incubation period. Unfertilized eggs were each injected with 4 ng of circular 13.1-kbp plasmid DNA and then incubated for 0, 5, or 22 h in media containing 0, 2, or 20 μg of aphidicolin per ml. DNA from the various samples was extracted and analyzed by nitrocellulose blotting as previously described. In the absence of aphidicolin, there was after 5 h a 1.4-fold increase in the amount of injected DNA sequences. By 22 h, there was a 5.6-fold increase (Fig. 7, lanes 3 and 6). Thus, there was an appreciable amount of DNA replication taking place between 5 and 22 h postinjection. The presence of aphidicolin at concentrations of either 2 or 20 μg/ml effectively blocked most DNA replication (Fig. 7, lanes 4, 5, 7, and 8).

Samples taken 5 to 6 h or 18 to 22 h postinjection have also been analyzed for the transcription of injected *Xenopus* α-globin gene, *Xenopus* β-globin gene, and HSV *tk* gene. In all cases, no transcription of the injected gene was detected at the early time point, but transcription was always detectable at the later time point. The HSV *tk* gene is transcribed relatively efficiently in both *Xenopus* oocytes (24) and eggs (our results). We detected no transcripts of the injected HSV *tk* gene 5 h postinjection but readily detected transcripts 18 h postinjection (Fig. 8, lanes 4 and 5). From these results, we conclude that the injected unfertilized egg continues to be biologically active even 5 or 6 h after piercing.

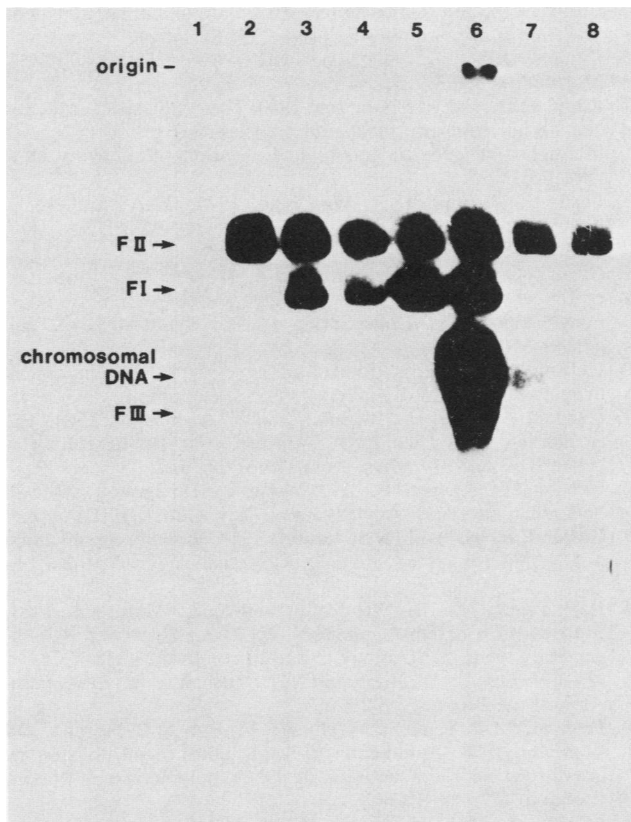

FIG. 7. Analysis of the replication of DNA injected into unfertilized eggs. Eggs were injected with 4 ng of pJKd⁻* plasmid DNA (33) and incubated in 0, 2, or 20 μg of aphidicolin for 0, 5, or 22 h. Samples were analyzed as described in the text. Lane 1, Control showing 0.2 ng of pJKd⁻* DNA; lane 2, sample taken immediately after injection; lanes 3, 4, and 5, samples incubated for 5 h in 0, 2, or 20 μg of aphidicolin per ml, respectively; lanes 6, 7, and 8, samples incubated for 22 h in 0, 2, and 20 μg of aphidicolin per ml, respectively. The positions of chromosomal DNA and FI, FII, and FIII pJKD⁻* DNA are indicated on the left.

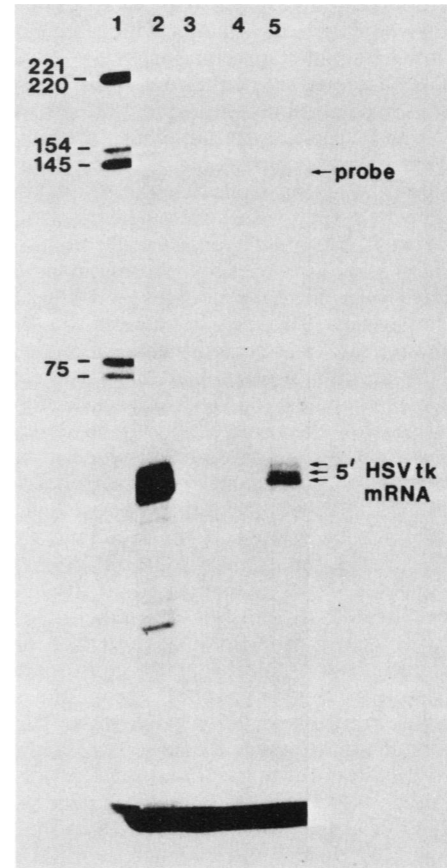

FIG. 8. Detection of HSV *tk* gene transcripts in unfertilized eggs and oocytes. Eggs and oocytes were injected with 2 ng of a plasmid DNA that contains the HSV *tk* gene. Lane 2, Oocytes injected and incubated for 20 h; lane 3, control with mock-injected oocytes; lanes 4 and 5, eggs injected and incubated for 5 and 18 h, respectively. The positions of the probe DNA and of correctly initiated HSV *tk* gene transcripts are indicated on the right. On the left, the sizes of the pAT153/*Hin*fI fragments shown in lane 1 are listed.

DISCUSSION

It is not immediately apparent why injected *Xenopus* globin genes should be faithfully transcribed from the correct promoters in unfertilized and fertilized eggs (2, 3) and promiscuously transcribed in oocytes. Eggs are less transcriptionally active than oocytes, and a variety of genes, when injected into unfertilized eggs, produce 10 to 20 times fewer hybridizable transcripts than when injected into oocytes (26). Thus, an overall lower level of transcription of the injected globin genes in eggs was predicted, but the higher degree of transcriptional fidelity in eggs was unexpected. Another possible, but we believe unlikely, explanation for these results is differential transcript stability, with incorrectly initiated transcripts being selectively degraded in eggs

but not in oocytes. Investigation of this possibility would involve pulse-labeling experiments which are not feasible, given the low levels of transcription.

Naked DNA injected into either oocytes or eggs is assembled into a chromatin-like structure (23, 39). Chromatin organization and structure are thought to play an important role in governing gene expression and regulation (reviewed in reference 15). It is possible that the difference between oocytes and eggs in their transcriptional expression of injected globin genes is due to differences in chromatin assembly. Differences in the properties of chromatin assembled on DNA injected into *Xenopus* oocytes and eggs have been observed previously (9). DNA containing the *Dropsophila* histone gene repeat was injected into *Xenopus* oocytes and eggs, and the chromatin assembled on the injected DNAs was analyzed. In occytes, the DNA was shown by micrococcal nuclease and DNaseI digestion to be assembled into chromatin with a 180-base-pair nucleosome periodicity, but in eggs, the injected DNA became resistant to both micrococcal nuclease and DNaseI digestion. The resistance to digestion is thought to be related to the fact that, in eggs, the injected DNA replicates in the cytoplasm; in oocytes, however, the injected DNA is deposited in the nucleus and remains unreplicated. Recent work from the same laboratory (34) has shown that a proportion of the DNA injected into oocytes adopts an unusual chromatin conformation. Their work suggests that this "dynamic" chromatin is the transcriptionally active form for RNA polymerase III. It may be that this torsionally strained, dynamic chromatin may also permit the initiation of transcription by RNA polymerase II in regions other than the authentic globin gene promoter.

The amount of replication of injected DNA in unfertilized eggs that we observed is much higher than has previously been reported. Harland and Laskey (13) injected 5 ng of simian virus 40 DNA per unfertilized egg and observed an increase of 1.5 ng of simian virus 40 DNA after 5 h of incubation. In our experiments involving the injection of 4 ng of pXGαβ1 DNA, we observed, on the average, a 5- to 10-fold increase in the amount of pXGαβ1 DNA after 20 to 22 h of incubation (an increase of 16 to 36 ng of DNA). Besides the longer incubation time, the increased amount of replication may be attributable to the fact that we removed the jelly coat before injection by chemical decapsulation and never treated the unfertilized eggs with UV irradiation as did Harland and Laskey (13).

During *Xenopus* development, embryos are transcriptionally inactive until the midblastula transition (28). Newport and Kirschner (29) suggested that transcriptional activation is triggered by the amount of nuclear DNA present by midblastula transition (24 ng) and have shown that a yeast tRNA gene injected into unfertilized eggs can be induced to become transcriptionally active when the DNA content of the egg is artificially increased to 24 ng per egg. We found that injecting 24 ng of pXGαβ1 DNA rather than 0.5 or 4 ng did not cause a significant increase in the number of globin gene transcripts. The number of transcripts produced in unfertilized eggs appeared to be most directly related to gene copy number (taking replication into account).

The variability in transcription of the injected *Xenopus* β1-globin gene by oocytes from different females was surprising, especially since the globin genes were from the same species. Oocytes from different females, however, have been reported to differ significantly. Jones et al. (18) injected oocytes with a chimeric gene consisting of the chloramphenicol-3-*O*-acetyltransferase gene structural sequences linked to the simian virus 40 early gene promoter. The level of chloramphenicol-3-*O*-acetyltransferase activity in injected oocytes from different females varied by almost 30-fold, but oocytes from an individual female gave consistent results. Korn et al. (19) have observed that oocytes from some females are capable of reactivating oocyte-type 5S DNA in injected *Xenopus* erythroid cell nuclei, although oocytes from other females are not. Given the variability in transcriptional behavior between oocytes from different females and also the high degree of inaccurate initiation of transcription of globin genes injected into oocytes, injection into unfertilized eggs appears to be a more generally useful system in which to study authentic transcription.

LITERATURE CITED

1. **Bendig, M. M.** 1981. Persistence and expression of histone genes injected into *Xenopus* eggs in early development. Nature (London) **292**:65–67.
2. **Bendig, M. M., and J. G. Williams.** 1983. Replication and expression of *Xenopus laevis* globin genes injected into fertilized *Xenopus* eggs. Proc. Natl. Acad. Sci. U.S.A. **80**:6197–6201.
3. **Bendig, M. M., and J. G. Williams.** 1984. Differential expression of the *Xenopus laevis* tadpole and adult β-globin genes when injected into fertilized *Xenopus laevis* eggs. Mol. Cell. Biol. **4**:567–570.
4. **Billet, F. S., and A. E. Wild.** 1975. Practical studies of animal development. Champan & Hall, London.
5. **Busby, S. J., and R. H. Reeder.** 1982. Fate of amplified nucleoli in *Xenopus laevis* embryos. Dev. Biol. **91**:458–467.
6. **Dumont, J. N.** 1972. Oogenesis in *Xenopus laevis* (Daudin). J. Morphol. **136**:153–180.
7. **Etkin, L. D., and R. E. Maxson.** 1980. The synthesis of authentic sea urchin transcriptional and translational products by sea urchin histone genes injected into *Xenopus laevis* oocytes. Dev. Biol. **75**:13–25.
8. **Ford, C. C., and H. R. Woodland.** 1975. DNA synthesis in oocytes and eggs of *Xenopus laevis* injected with DNA. Dev. Biol. **43**:189–199.
9. **Gargiulo, G., W. Wasserman, and A. Worcel.** 1983. Properties of the chromatin assembled on DNA injected into *Xenopus* oocytes and eggs. Cold Spring Harbor Symp. Quant. Biol. **47**:549–556.
10. **Gurdon, J. B.** 1974. Control of gene expression in animal development. Oxford University Press, London.
11. **Gurdon, J. B., M. L. Birnstiel, and V. A. Speight.** 1969. The replication of purified DNA introduced into living cell cytoplasm. Biochim. Biophys. Acta **174**:614–628.
12. **Gurdon, J. B., and D. A. Melton.** 1981. Gene transfer in amphibian eggs and oocytes. Annu. Rev. Genet. **15**:189–218.
13. **Harland, R. M., and R. A. Laskey.** 1980. Regulated replication of DNA microinjected into eggs of Xenopus laevis. Cell **21**:761–771.
14. **Harland, R. M., H. Weintraub, and S. L. McKnight.** 1983. Transcription of DNA infected into *Xenopus* oocyte is influenced by template topology. Nature (London) **302**:38–43.
15. **Igo-Kemenes, T., W. Horz, and H. G. Zachau.** 1982. Chromatin. Annu. Rev. Biochem. **51**:89–121.
16. **Ikegami, S., T. Taguchi, M. Ohashi, M. Oguro, H. Nagano, and Y. Mano.** 1978. Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-α. Nature (London) **275**:458–460.
17. **Jeffreys, A. J., V. Wilson, D. Wood, J. P. Simons, R. M. Kay, and J. G. Williams.** 1980. Linkage of adult α- and β-globin genes in X. laevis and gene duplication by tetraploidization. Cell **21**:555–564.
18. **Jones, N. C., J. D. Richter, D. L. Weeks, and L. D. Smith.** 1983. Regulation of adenovirus transcription by an E1a gene in microinjected *Xenopus laevis* oocytes. Mol. Cell. Biol. **3**:2131–2142.
19. **Korn, L. J., J. B. Gurdon, and J. Price.** 1982. Oocyte extracts reactivate developmentally inert *Xenopus* 5S genes in somatic nuclei. Nature (London) **300**:354–355.

20. **Kressmann, A., and M. L. Birnstiel.** 1980. Surrogate genetics in the frog oocyte, p. 383–407. *In* J. E. Celis, A. Grassmann, and A. Loyter (ed.), Transfer of cell constituents into eukaryotic cells. Plenum Publishing Corp., New York.
21. **Laskey, R. A., and J. B. Gurdon.** 1973. Induction of polyoma DNA synthesis by injection into frog cytoplasm. Eur. J. Biochem. **37:**467–471.
22. **Laskey, R. A., R. M. Harland, and M. Mechali.** 1983. Induction of chromosome replication during maturation of amphibian oocytes, p. 25–43. *In* R. Porter and J. Whelan (ed.), Ciba Foundation Symposium 98, Molecular biology of egg maturation. Pitman Books, London.
23. **Laskey, R. A., B. M. Honda, A. D. Mills, N. R. Morris, A. H. Wyllie, J. E. Mertz, E. M. DeRobertis, and J. B. Gurdon.** 1978. Chromatin assembly and transcription in eggs and oocytes of *Xenopus laevis*. Cold Spring Harbor Symp. Quant. Biol. **42:**171–178.
24. **McKnight, S. L., E. R. Gavis, and R. Kingsbury.** 1981. Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. Cell **25:**385–398.
25. **Mechali, M., and R. M. Harland.** 1982. DNA synthesis in a cell-free system from Xenopus eggs: priming and elongation on single-stranded DNA in vitro. Cell **30:**93–101.
26. **Mertz, J. E., and J. B. Gurdon.** 1977. Purified DNAs are transcribed after microinjection into *Xenopus* oocytes. Proc. Natl. Acad. Sci. U.S.A. **74:**1502–1506.
27. **Miller, T. J., and J. E. Mertz.** 1982. Template structural requirements for transcription in vivo by RNA polymerase II. Mol. Cell. Biol. **2:**1595–1607.
28. **Newport, J., and M. Kirschner.** 1982. A major developmental transition in early Xenopus embryos. I. Characterization and timing of cellular changes at the midblastula stage. Cell **30:**675–686.
29. **Newport, J., and M. Kirschner.** 1982. A major developmental transition in early Xenopus embryos. II. Control of the onset of transcription. Cell **30:**687–696.
30. **Patient, R. K., J. A. Elkington, R. M. Kay, and J. G. Williams.** 1980. Internal organization of the major adult α- and β-globin genes of X. laevis. Cell **21:**565–573.
31. **Patient, R. K., R. Harris, M. E. Walmsley, and J. G. Williams.** 1983. The complete nucleotide sequence of the major adult β globin gene of X. laevis. J. Biol. Chem. **258:**8521–8523.
32. **Rungger, D., P. D. Matthias, and J. P. Huber.** 1981. Transcription of complex structural genes in the *Xenopus* oocyte system, p. 28–32. *In* H. G. Schweiger (ed.), International cell biology 1980–1981. Springer-Verlag, Berlin.
33. **Rusconi, S., and W. Schaffner.** 1981. Transformation of frog embryos with a rabbit β-globin gene. Proc. Natl. Acad. Sci. U.S.A. **78:**5051–5055.
34. **Ryoji, M., and A. Worcel.** 1984. Chromatin assembly in Xenopus oocytes: in vivo studies. Cell **37:**21–32.
35. **Smithies, O., A. E. Blechl, S. Shen, J. L. Slightom, and E. F. Vanin.** 1981. Co-evaluation and control of globin genes, p. 101–116. *In* G. Stamatoyannopoulos and A. W. Ninehuis (ed.), Organization and expression of globin genes. Alan R. Liss, Inc., New York.
36. **Twigg, A. J., and D. Sherratt.** 1980. Trans-complementable copy-number mutants of plasmid ColE1. Nature (London) **283:**216–218.
37. **Wallace, R. A., D. W. Jared, J. N. Dumont, and M. W. Sega.** 1973. Protein incorporation by isolated amphibian oocytes. III. Optimum incubation conditions. J. Exp. Zool. **184:**321–334.
38. **Wickens, M. P., S. Woo, B. W. O'Malley, and J. B. Gurdon.** 1980. Expression of a chicken chromosomal ovalbumin gene injected into frog oocyte nuclei. Nature (London) **285:**628–634.
39. **Wyllie, A. H., R. A. Laskey, J. Finch, and J. B. Gurdon.** 1978. Selective DNA conservation and chromatin assembly after injection of SV40 DNA into *Xenopus* oocytes. Dev. Biol. **64:**178–188.