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A test of 'polymerase handover' as a mechanism for stimulating initiation by RNA polymerase I

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ABSTRACT

On the tandemly linked ribosomal genes of Xenopus laevis, the RNA polymerase transcribes past the 3' end of the 40S coding region and terminates at T3 just upstream of the gene promoter. The close proximity of T3 to the gene promoter, and the functional interdependence of these two elements, has led to the suggestion that polymerase terminating at T3 might be passed directly to the gene promoter. Such a mechanism might be necessary to maintain the characteristic high rate of transcription initiation seen on the ribosomal genes. We have performed a direct test of this model by introducing chain-terminating psoralen adducts into a circular plasmid containing a single gene promoter with its attendant T3 region upstream. We find that the psoralen adducts can completely prevent polymerase from traveling around the template circle (and thus prevent polymerase from approaching the promoter from upstream) without affecting the rate of transcription initiation at the gene promoter. This result suggests that recycling of polymerase from T3 to the promoter is not a significant mechanism in maintaining high initiation rates.

INTRODUCTION

RNA polymerase I can approach the gene promoter (initiation site for transcription of the 40S rRNA precursor) of the Xenopus laevis ribosomal genes via at least three different routes. The first route is by capture from the free pool of unbound polymerase. A second route is via polymerase read-through from the preceding tandemly linked gene. We (1) and others (2) have shown that little, if any, release of polymerase occurs when an elongating complex reaches the 3' end of the 40S rRNA coding region. Instead, the polymerase continues on across the intergenic spacer where it terminates at the T3 terminator just upstream of the gene promoter (see Figure 1). A third route is via initiation at one of the spacer promoters. The Xenopus ribosomal genes contain multiple polymerase I promoters in the intergenic spacer region and these promoters are highly active in some cell types (3,4,5). Polymerase initiating at these spacer promoters also terminates at the T3 terminator just upstream of the gene promoter.

There has been some speculation concerning a possible transcriptional

role for polymerase traversing the intergenic spacer and for polymerase initiating at the spacer promoters. An attractive model (reviewed in reference 6) is one in which polymerase from either source might help to maintain the dense packing of polymerase that is normally observed on the gene region. Thus, it was proposed that polymerase terminates at T3 but following termination the polymerase might be handed over to the gene promoter without being lost to the free pool. It is this latter possibility, the conservative handing over of polymerase from terminator to promoter, which we have attempted to test in this paper.

We have devised the following test. We have constructed a circular plasmid (diagramed in Figure 1) which contains a single gene promoter attached to a ribosomal minigene. In this construct the T3 terminator is present in its normal location upstream of the promoter. If handover occurs, then this promoter should be able to feed itself. Polymerase which initiates from the free pool would travel around the circle, terminate at T3, be handed over to the promoter and continue around to repeat the process. Once a polymerase initiated at this promoter it would never be lost. Thus, a promoter at which handover can occur should have a large advantage over a promoter where handover is prevented. We reasoned that we could prevent handover by preventing polymerase from chain elongating around the plasmid. The method we chose to induce premature termination was to introduce random psoralen adducts into the circle, a procedure which we (in this paper) and others (7,8) have shown blocks polymerase elongation.

We show that introducing psoralen adducts into the circle has little effect on initiation rates and in some cases actually stimulates initiation. We conclude that this experiment provides no support for the handover model. A similar conclusion has also been reached in experiments where ultraviolet light was used to block chain elongation on the endogenous ribosomal genes in tissue culture cells (Labhart and Reeder, submitted).

MATERIALS AND METHODS

Plasmid construction

The structure of pG40-T2 is shown in Figure 1. This plasmid has the same ribosomal minigene insert as does the previously described ψ40-T2 (9) but the insert has been removed from pBR322 and inserted between the Sal I and Eco RI sites of the polylinker of pGEM4 (Promega Biotec). This arrangement allows us to transcribe the minigene either from the ribosomal gene promoter or from the phage T7 promoter that is now located upstream of the minigene. A companion plasmid, pG403-T2, is identical to pG40-T2 except that sequences from -245 to

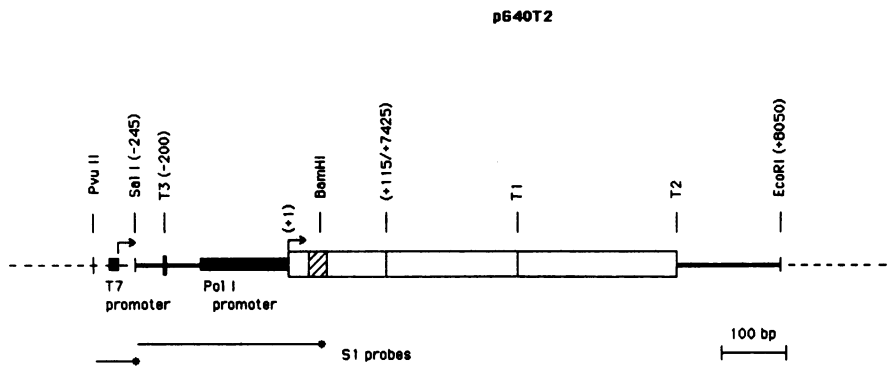

Figure 1. Structure of the ribosomal minigene plasmid pG40-T2. The minigene resides between the Sal I and Eco RI sites of pGEM4 and contains the T3 termination site (-200), the gene promoter (-142 to +6), the 5′ end of the 40 S precursor coding region (up to +115) and the 3′ end of the coding region (+7425 to +8050). Within the 3′ fragment are sites T1 (3′ end of the 28S coding region) and T2 (3′ end of the 40 S precursor). At position +31 a 40 bp linker has been inserted to allow design of an S1 probe specific for transcripts from this minigene. The T7 promoter is located in the pGEM4 vector sequences upstream of the minigene and the arrow indicates the direction of transcription. In a related plasmid, pG403-T2, ribosomal DNA sequences from -245 to -160 have been deleted in order to remove T3. In pG40 and pG403 the ribosomal gene sequences from the Bam HI to the Eco RI site have been deleted.

The control plasmid that was co-injected with the experimental plasmids was ψ52-T2, a plasmid identical to ψ40-T2 except that it contains a 52 bp linker inserted at position +31 (9). The end-labeled S1 probes used to detect transcription and readthrough at the T7 and polymerase I promoters are shown under the diagram. Numbers in brackets are coordinates relative to the transcription initiation site at +1.

-160 upstream of the ribosomal gene promoter have been deleted. This deletion removes the entire T3 terminator region but does not damage the 5′ boundary of the gene promoter (located at about -140).

pG40 and pG403 are identical to pG40-T2 and pG403-T2 except that in each case the Bam HI to Eco RI fragment has been removed (this removes sites T1 and T2; see Figure 1).

The control plasmid, co-injected with every experimental plasmid, was ψ52-T2 (described in the legend to Figure 1).

Psoralen photoreaction

2 µg of supercoiled DNA in 30 µl of Tris-HCl, 0.5 mM EDTA, pH 7.6, were supplemented with 2 µl of a 10 µg/ml stock solution (in ethanol) of 4,5′,8-trimethylpsoralen (Sigma) and were irradiated on ice in 1.5 ml Eppendorf

tubes with ultraviolet light at 366 nm (model UVL-21, UVP, Inc, San Gabriel CA). The light source was mounted at a distance of 20 cm above the DNA solutions and the irradiation times were as indicated in the Figures. The irradiated samples were then made up to 150 mM NaCl, extracted 3 times with chloroform and precipitated with ethanol.

In some experiments, after the psoralen-treated DNA was purified away from free psoralen as described above, the samples were re-irradiated with the same light source for 30 min at a distance of 7 cm.

The extent of crosslinking was analyzed by linearizing the plasmids with Eco RI, end labeling the DNA with the Klenow fragment of DNA polymerase, and separating crosslinked from noncrosslinked molecules by electrophoresis on an alkaline agarose gel (10). For analysis of photoreacted plasmids recovered from micro-injected oocytes the alkaline agarose gel was soaked twice for 15 min in 0.25 M HCl and then was blotted and hybridized with ^{32}P nick translated pBR322.

Transcription by T7 RNA polymerase

100 ng of plasmid DNA was transcribed with 20 units of T7 RNA polymerase (BRL) for 45 min in 20 µl of BRL transcription buffer supplemented with 5 mM DTT and 400 µM each of ATP, GTP, CTP, and UTP.

Transcription by a X. laevis S-100 extract

S-100 extracts were made from a line of cultured kidney cells and transcription was carried out as described previously (11) with the following modifications. 20 µl aliquots of the extract were preincubated with 200 ng of pBR322 for 10 min on ice followed by a further 10 min incubation on ice with 50 ng of minigene template before adding the nucleotides and starting the reaction. Reactions were incubated for 3 hrs at 24°C.

Oocyte injection and S1 nuclease protection

The oocyte injection solution contained equimolar amounts of a control and a test plasmid (total DNA concentration 25 µg/ml), 500 µg/ml α-amanitin, 50 mM NaCl, 10mM Tris-HCl, pH 7.6, and 0.2 mM EDTA. Approximately 40 nl was injected per nucleus (1 ng of DNA per nucleus) and the oocytes were then incubated at room temperature for a further 8 to 14 hrs before extracting the RNA and analyzing it by S1 nuclease protection as previously described (1). The end-labeled single-stranded probes used for S1 nuclease protection are shown in Figure 1. The probe to detect transcription from the T7 promoter was a 64 nucleotide Pvu II-Sal I fragment kinase labeled at the Sal I site. The probe used to detect initiation at the ribosomal gene promoter was a 298 nucleotide Sal I-Bam HI fragment kinase labeled at the Bam HI site.

RESULTS

Crosslinking the DNA of ribosomal minigene plasmids with psoralen.

Figure 1 shows the structure of pG40-T2, the ribosomal minigene plasmid that was used in most of the experiments reported in this paper. To introduce chain-terminating psoralen adducts into this plasmid it was mixed with psoralen and UV irradiated for various lengths of time as described in the Methods section. Figure 2A shows analysis of the treated DNA by electrophoresis on an alkaline agarose gel after linearization at a unique Eco RI site. Untreated DNA (lane 1) runs as a single band as expected. At increasing UV doses, however, a slower moving band is seen which represents molecules where the two DNA strands have become joined together by at least one crosslink. At the highest UV dose (lane 6) all of the DNA has at least one crosslink and runs at the slow moving position. Increasing UV doses actually cause the slower band to speed up a bit, presumably because multiple crosslinks make the DNA mass more compact.

UV irradiation of DNA in the presence of psoralen leads initially to the formation of covalent monoadducts. Further irradiation causes a fraction of the monoadducts to crosslink (12). Thus, in Figure 2A, lanes 2 and 3, where no crosslinks are detectable, the DNA does have psoralen monoadducts attached to it. To demonstrate that this is so, DNA from the experiment in Figure 2A was purified away from non-covalently linked psoralen and re-irradiated for a longer time. As shown in Figure 2B, this re-irradiation converts many of the monoadducts into additional crosslinks and crosslinked molecules are now seen

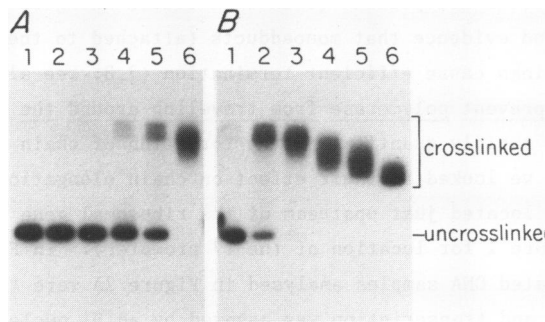

Figure 2. Detection of psoralen monoadducts and crosslinks. A) pG40-T2 was UV irradiated for 0, 0.5, 1, 2, 4, and 10 min (lanes 1-6, repectively) in the presence of psoralen as described in Materials and Methods. The plasmids were then purified, digested with Eco RI, end-labeled, and electrophoresed on a 1% alkaline agarose gel. B) After UV irradiation and purification, an aliquot of each sample shown in panel A was further irradiated before being restricted, end labeled, and electrophoresed.

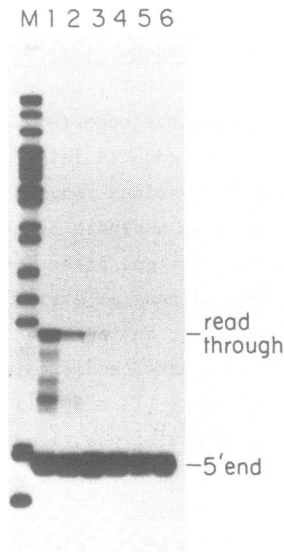

Figure 3. Detection of elongation blocks on psoralen-reacted templates. The same psoralen-reacted pG40-T2 plasmids analyzed in Figure 2A were transcribed in vitro by T7 RNA polymerase and the RNA was analyzed by S1 mapping using the 5' end-labeled Pvu II-SalI probe shown in Figure 1. Full length probe protection indicates transcription read-through due to polymerase elongating around the entire circle. Correct initiation is indicated by the presence of a protected 33 nucleotide 5' end fragment. Lane M: Hpa II digest of pBR322.

in all of the treated samples (lanes 2 through 6). It is important to realize that these monoadducts are present (even when crosslinking is not observed) since there is good evidence that monoadducts (attached to the coding strand) as well as crosslinks cause efficient termination (7,8; see also Figure 3). Psoralen adducts prevent polymerase from traveling around the plasmid.

As a means of rapidly quantitating the formation of chain-terminating psoralen adducts, we looked at their effect on chain elongation from the phage T7 promoter located just upstream of the ribosomal gene promoter in pG40-T2 (see Figure 1 for location of the T7 promoter). In Figure 3 the same psoralen-treated DNA samples analysed in Figure 2A were transcribed by T7 RNA polymerase and transcription was assayed by an S1 nuclease protection probe. Initiation of transcription is indicated by a specifically protected band (labeled "5' end" in Figure 3) while transcription elongation clear around the DNA circle is indicated by full length protection of the probe (labeled "read through"). It is clear that even the lowest level of psoralen photoreaction, a level which yielded no detectable crosslinks in Figure 2A,

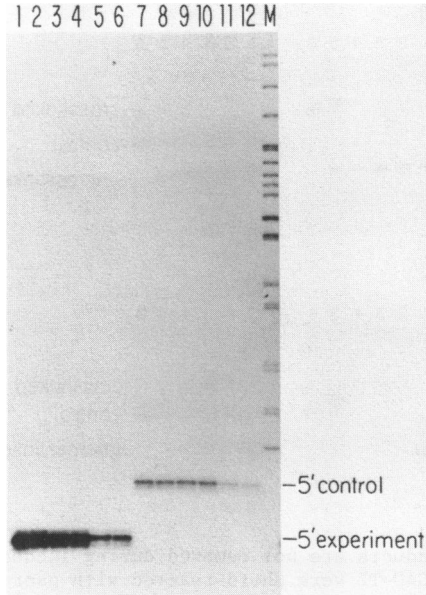

Figure 4. Transcription of psoralen-reacted plasmids after injection into oocyte nuclei. The same set of psoralen-reacted plasmids analyzed in Figures 2A and 3 were also injected into oocyte nuclei with an equimolar amount of a control plasmid, ψ52-T2 (see legend to Figure 1 for structure of ψ52-T2). Transcription was assayed by S1 protection using the 5′ end-labeled SalI-Bam HI probe from pG40-T2 (lanes 1-6) and the analogous probe from ψ52-T2 (lanes 7-12). Lane M: Hpa II digest of pBR322. Note that in lanes 3 and 4 transcription initiation on both the psoralen-reacted and control plasmids remains at control levels even though RNA chain elongation is completely prevented from proceeding around the DNA circle on these templates (as indicated by the absence of read-through in lanes 3 and 4, Figure 3). Higher levels of psoralen-reaction reduce initiation on both control and experimental plasmids for unknown reasons (lanes 5 and 6, 11 and 12).

lane 2, was sufficient to severely inhibit chain elongation by T7 RNA polymerase. Direct evidence that amphibian RNA polymerase I is similarly blocked by psoralen will be presented below in Figures 6 and 7.

Injection of psoralen-treated plasmids into oocytes.

Aliquots of the psoralen-treated DNA samples analyzed in Figure 2A were mixed with a non-treated control plasmid and injected into the nuclei of X. laevis oocytes. Transcription initiation was measured by S1 nuclease protection with the results shown in Figure 4. Figure 4, lanes 1 through 6, shows the result of increasing psoralen treatment on initiation at the ribosomal gene promoter of pG40-T2, lanes 7 through 12 shows the initiation signal from the co-injected control plasmid. Two results emerge from this

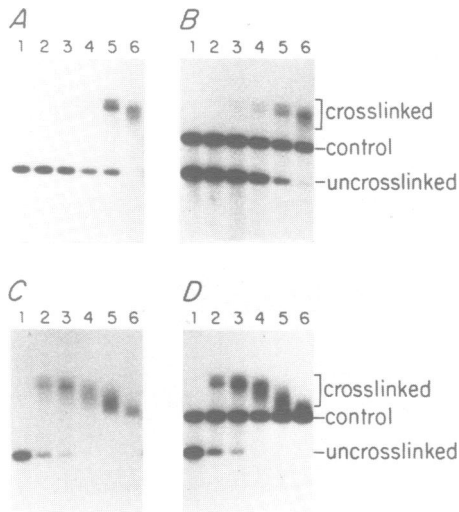

Figure 5. Psoralen adducts are not removed during incubation in oocytes. In panel A aliquots of pG40-T2 were photo-reacted with psoralen for 0, 0.5, 1, 2, 5, or 10 minutes (lanes 1-6 respectively) and then analyzed on an alkaline agarose as described in Figure 2. In panel B the same psoralen-reacted series was injected into oocytes, reisolated, and analyzed on an alkaline gel. Note that incubation in the oocytes has not removed any crosslinks. (The control band is from the untreated ψ52-T2 that was co-injected as a control.) In panel C the same series of samples analyzed in panel A were purified away from unreacted psoralen and then further irradiated with UV to demonstrate the presence of uncrosslinked monoadducts. In panel D a similar series of samples were recovered from injected oocytes and subjected to further UV irradiation before gel analysis. These experiments demonstrate that the oocytes remove neither crosslinks nor monoadducts.

experiment. First, at higher levels of psoralen treatment (Figure 4, lanes 5 and 6) initiation at the ribosomal gene promoter is impaired. However, to our surprise we also note that the initiation signal from the untreated, co-injected control is also depressed in these samples (Figure 4, lanes 11 and 12). We have seen this trans inhibition effect in many independent experiments and at present have no explanation for the effect. Injection of a highly crosslinked DNA (even when it is a non-specific DNA such as pBR322) depresses transcription from a co-injected control plasmid even though we can detect no covalent modification of the control plasmid (data not shown). The presence of this trans inhibition leads us to discount the decrease in initiation we see in lanes 5 and 6 of Figure 4 as being due to artifact.

The second result, however, that emerges from the experiment in Figure 4 is that transcription initiation is not inhibited by lower levels of psoralen

treatment which are still sufficient to block all chain elongation around the plasmid. In Figure 4, lanes 3 and 4, transcription initiation is still at untreated control levels even though no polymerase is able to travel entirely around these circles (as is shown in Figure 3, lanes 3 and 4). Our overall conclusion, despite the trans inhibition seen at high psoralen levels, is that it is not necessary for polymerase to travel clear around the DNA circle to maintain high initiation rates on these plasmids.

Oocytes do not remove psoralen adducts.

A possible objection to the preceding conclusion might be that oocytes are able to rapidly remove psoralen adducts. Figure 5 shows that this objection is not valid; oocytes are not able to remove either crosslinks or monoadducts from the DNA template. Figure 5A shows alkaline agarose gel electrophoresis of a series of increasingly crosslinked DNA samples similar to those shown in Figure 2A. In Figure 5B these same samples have been injected into oocyte nuclei (along with a non-crosslinked control plasmid), recovered from the oocytes, blotted onto a nitrocellulose membrane, and hybridized with radioactive pBR322. There is no conversion or loss of crosslinked material during incubation in the oocytes. Figure 5, C and D shows that, likewise, monoadducts are also not removed by the oocytes. In Figure 5C, the same DNA samples shown in Figure 5A were re-irradiated to demonstrate the presence of additional monoadducts that were not crosslinked in the original UV exposure. Figure 5D shows the same experiment except the DNA was injected into oocytes and recovered before re-irradiation. This experiment demonstrates that the oocytes also cannot remove monoadducts. We conclude from this that our original conclusion was valid; chain elongation around the DNA circle is not required to maintain high levels of transcription initiation.

The effect of the T3 terminator.

The X. laevis ribosomal genes contain a strong terminator of transcription, termed T3, located about 200 bp upstream of the site of transcription initiation at the gene promoter (see Figure 1). In addition to terminating essentially all polymerases that approach from upstream of the promoter, T3 also has a stimulatory effect on the gene promoter itself (11). This dual terminator-promoter stimulation effect of T3 was one of the original reasons why we considered the possibility of polymerase handover from terminator to promoter. We thought it would be interesting to see what effect psoralen adducts would have on initiation at a promoter that is lacking the T3 region. The result is shown in Figure 6. Lane 1 shows the transcription signal from an untreated sample of pG40 after injection into

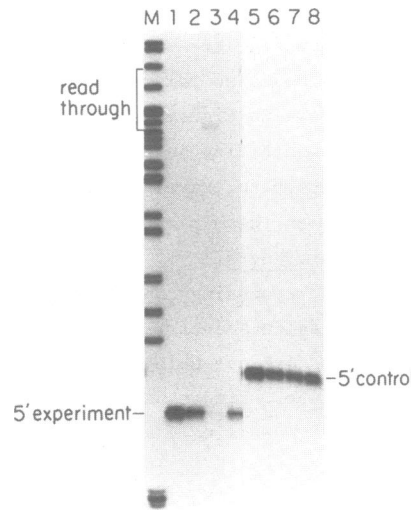

Figure 6. Effect of psoralen reaction on transcription of minigenes with and without a T3 termination site upstream of the promoter. Oocyte injection and transcription assay by S1 protection are the same as in Figure 4.The injected templates are: Lane 1, unreacted pG40 (T3 termination site present); Lane 2, psoralen-reacted pG40; Lane 3, unreacted pG403 (termination site deleted; note decrease in initiation signal and increase in read-through); Lane 4, psoralen-reacted pG403 (note increase in initiation signal and loss of read-through). Lanes 5-8 show the transcription from the control plasmid ψ52-T2 that was co-injected with the experimental plasmids in lanes 1-4, respectively.

oocyte nuclei. Lane 2 shows a similar sample that has been irradiated for 2 mins in the presence of psoralen. Lanes 5 and 6 show the transcription signal from a co-injected control plasmid. These lanes essentially repeat the experiment shown in Figure 4, lanes 1 and 4, 7 and 10, and show that blocking elongation around the circle has no effect on transcription initiation on pG40. (Lane 2 actually shows a lower signal than does lane 1. However, when lanes 1 and 2 are normalized to the signal from the coinjected control plasmid, lanes 5 and 6, there is no decrease in initiation due to psoralen treatment.) In lane 3 the injected plasmid was pG403, a plasmid identical to pG40 except that the entire T3 region has been deleted (sequences from -245 to -160). Deletion of the T3 region has two effects. The first effect is that the 5′ initiation signal decreases. The magnitude of this decrease varies among different oocyte batches. In this particular experiment the initiation signal has decreased at least 10-fold. The second effect is that significant readthrough transcription is now seen, presumably due to loss of the termination function of T3. In lane 3 the read through

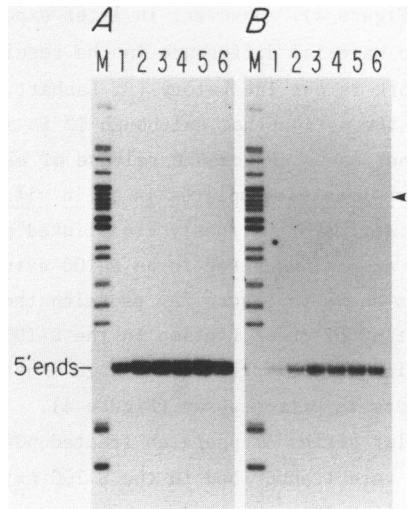

Figure 7. In vitro transcription of psoralen-reacted templates. Panel A, the same series of psoralen-reacted pG40-T2 samples analyzed in Figures 2A, 3, and 4A were also transcribed in an S-100 cell free extract as circular templates and analyzed by S1 protection. Note that psoralen-reaction has a slight stimulatory effect on initiation. Panel B, transcription of an analogous psoralen-reacted series of the T3-deleted template, pG403-T2. Psoralen-reaction eliminates a faint read-through band (arrow head) and causes a considerable stimulation of initiation.

signal is greater than the initiation signal, probably because the readthrough signal is due to multiple rounds of transcription around the plasmid circle. Introduction of psoralen adducts into pG403 also has two effects. The readthrough signal disappears, confirming our expectation that psoralen adducts block chain elongation by eukaryotic polymerase as well as elongation by T7 polymerase. And the initiation signal is stimulated at least 5-fold. We interpret this result to mean that one function of T3 is to prevent occlusion of the gene promoter by polymerase running over it from upstream. On pG403, where there is no protective terminator, occlusion keeps the initiation signal low and prevention of readthrough by psoralen adducts allows an increase in initiation. The initiation signal does not increase to wild-type levels possibly because T3 has other positive effects on initiation other than simple prevention of promoter occlusion (11), and these other positive effects cannot be mimicked by psoralen treatment.

Note that in the experiment shown in Figure 6 we have used minigene derivatives that lack the T2 region from the 3′ end of the gene. When we began this work we included T2 in the constructs because it seemed closer to

the natural situation (Figure 4). However, in later experiments (Figure 6) we omitted T2 and see no material difference in the results. This is consistent with other work in our laboratory (P. Labhart, unpublished) that continues to strengthen the notion that, although T2 is a strong site of 3' end formation, it does not cause detectable release of elongating polymerase.

Transcription of psoralen-treated templates in an in vitro S-100 extract.

Samples of the same series of variously crosslinked pG40-T2 templates shown in Figure 2A were also transcribed in an S-100 extract made from X. laevis kidney cells. As shown in Figure 7A, psoralen treatment actually caused a slight stimulation of transcription in the S-100 system and we did not observe the inhibition of transcription at high crosslinking levels that was observed in the oocyte injection assay (Figure 4).

In Figure 7B a similar series of psoralen treated pG403-T2 templates (lacking the T3 region) were transcribed in the S-100 extract. In Figure 7B, lane 1, we see that deleting T3 severely depresses transcription and there is roughly as much readthough signal as there is initiation signal. Increasing psoralen treatment abolishes the readthrough signal and considerably stimulates the initiation signal (but not to the same level as a plasmid with T3).

DISCUSSION

What happens when chain elongating RNA polymerase encounters a psoralen adduct covalently linked to the template? Previous work has shown that if it is a mono adduct on the coding strand or a crosslink, then the polymerase stops synthesizing RNA at that point (7,8). Our results, although less extensive, are in complete agreement with that conclusion (see Figures 3, 6 and 7). In addition, our data argue that the polymerase releases from the template rather than being simply stalled by the psoralen. We infer this from the fact that stopping chain elongation does not decrease the rate of initiation. Polymerases are tightly packed on active ribosomal gene plasmids injected into oocytes (about one polymerase per 85 bp; see reference 13) and if release did not occur, the polymerases would rapidly crowd up against each other and initiation would cease. Since initiation does not cease, we infer that psoralen adducts cause polymerase release in addition to blocking chain elongation.

Several suggestions have been made as to how transcription of the intergenic spacer might have an influence on initiation at the ribosomal gene promoter. One suggestion has been that transcription of the spacer might serve to focus polymerase on the gene promoter. Whether the polymerase came

from read through from the preceding repeat unit (1,2) or from initiation at the spacer promoters (4), the polymerase would be delivered to the gene promoter, handed over, and high initiation rates would thereby be maintained. A second suggestion has been that in the act of chain elongation the polymerase sweeps transcription factors, initially bound to the enhancers, down to the promoter (4). And a third suggestion is that transcription through the enhancers might keep them in an active state by "opening up" the chromatin (14).

Experiments reported in this paper directly address only the first of these possibilities, the suggestion that the gene promoter can be fed via hand over of polymerase coming from upstream. We have purposely removed both the enhancers and the spacer promoters from our constructs in order to look for evidence of hand over with as little potential complication as possible from other competing mechanisms. We find that we can completely prevent polymerase from traversing around a minigene circle (and thus from feeding itself) with no adverse effect on the initiation rate nor on its ability to compete with a normal, co-injected minigene. In fact, the converse is true. These results argue against polymerase hand over as a significant mechanism for maintaining high rates of initiation.

In some cases psoralen treatment actually stimulates initiation, presumably by prevention of promoter occlusion. In favor of this interpretation, we note that psoralen only stimulates initiation in cases where we might expect that the normal termination mechanism is damaged or not working at full efficiency. In Figure 6 termination was eliminated by deleting the T3 terminator. In Figure 7 transcription was analysed *in vitro* where the efficiency of termination is probably dependent upon having a saturating amount of a specific termination protein. Grummt and co-workers (16) have shown that S-100 extracts made from mouse cells are impoverished in a termination factor and supplementation of this factor is needed to achieve full termination efficiency. It is likely that a similar situation exists in our own S-100 extracts. Direct evidence that elongating RNA polymerase I can dislodge transcription factors from a promoter has been published recently by Bateman and Paule (17).

Psoralen is a DNA intercalator. The question arises, therefore, whether the promoter stimulation seen in Figures 6 and 7 might be due to topological effects rather than due to prevention of promoter occlusion. We have previously shown (18) that the gene promoter is quite insensitive to intercalators such as chloroquine and ethidium bromide until one reaches doses that simply kill it. Thus, it is unlikely that psoralen would be found

to stimulate the gene promoter by intercalation. In addition, we have performed some experiments (not shown) in which all of the psoralen adducts were introduced into a short fragment which was then ligated to an untreated larger fragment to form a circle. Under these conditions one would expect topological stress due to intercalation would be removed. However, introduction of psoralen under such conditions still stimulates initiation on a plasmid that is lacking T3. These considerations strengthen our conclusion that the stimulation of transcription seen in Figures 6 and 7 is due to prevention of occlusion and is not due to psoralen induced changes in topology.

Our results are in apparent conflict with a previous report by Mitchelson and Moss (15). In their experiments they utilized a construct containing an entire ribosomal gene repeat unit. They observed that when an extra copy of the T3 terminator was placed within the 18S coding sequence on a circular template, initiation at the gene promoter was reduced 80 to 90%. It was suggested that this reduction might be due to the terminator causing premature termination and preventing the recycling of polymerase. An alternative possibility, which they also mention, is that transcription through the spacer has some positive effect. The reason for our differing results might be because we have removed most of the spacer from our constructs.

In related experiments (Labhart and Reeder, submitted) we have used ultraviolet irradiation to introduce transcription terminating lesions into the endogenous ribosomal genes in tissue culture cells where the spacer promoters are highly active. These experiments have led to a conclusion similar to the conclusion reached in this paper: we can prevent all chain elongating polymerase from reaching the gene promoter with no adverse effect on initiation rate. From all of these experiments, we conclude that polymerase hand over is unlikely to be a significant factor in ribosomal gene transcription.

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