moter". Here we present considerations that tend to invalidate Moss' interpretation of his data as a general model.

The major flaw in Moss' model is that it is based on a characterization of spacer transcription that disregards quantitative analysis of *in vivo* transcripts. Specifically, spacer transcripts are too rare to be a component of normal ribosomal DNA (rDNA) expression. This has been apparent in electron microscopic analyses of spacer activity in *Xenopus* oocytes, where short ribonucleoprotein matrices ('prelude transcripts') are infrequently observed in the rDNA spacer[2]. Following identification of the site of initiation of *Xenopus* rRNA precursor synthesis[3], and the observation of homology between repeated spacer elements (the so-called *Bam* islands) and the initiation region[4], Trendelenburg[5] reexamined spacer transcripts in detail. One of his findings was that, while prelude transcripts could be found in 50–70% of the spacers of a few frogs (3/25), in most frogs, only 2–5% of rDNA spacers had transcription matrices, and yet other frogs apparently had none. Thus, in the most favourable of examples from individual frogs, only two-thirds of the spacers showed prelude transcription, and in a randomly selected frog, the frequency was $\leq 1/20$ this fraction. Such frequencies indicate that spacer transcription is generaly rare in *Xenopus*, and, therefore, unlikely to be "the driving force by which polymerase is delivered to the ribosomal gene promoter".

The scarcity of spacer transcripts is also shown by biochemical estimates of their abundance in both *Xenopus* and *Drosophila*. Sollner-Webb and Schultz (cited in ref. 6) have found that in RNA of different frogs, the amount of 5′ end of spacer transcripts relative to the amount of 5′ end of 40S RNA varies from ~1% to <0.05%. This is in good agreement with the electron microscopic observations. In *Drosophila*, a variable number of copies of initiation sequences are tandemly repeated immediately upstream of the rRNA transcription unit[7]. In both *Drosophila melanogaster* and *Drosophila virilis*, we have found short nuclear RNAs that result from transcription of these NTS repeats. The transcripts are rare, their steady-state level being ~1% that of the steady-state level of the external transcribed spacer portion of the ribosomal RNA precursor. These quantitative points argue against a major role of spacer transcription in the expression of rRNA genes.

Although transcriptional activity of the NTS repeats is rare, the possibility remains that multiple copies of promoter sequences in some way facilitate rRNA precursor initiation. However, the duplication of promoter sequences seen in the spacers of *Xenopus*, *Drosophila* and some other organisms is not a ubiquitous feature of rRNA genes, as such duplications are absent from the rDNA of yeast[8] and humans[9]. Therefore, the existence of multiple sites for potential transcription falls short as a general model. An alternative explanation is that the spacer repeats of *Xenopus* and *Drosophila* have been generated through unequal crossing-over at points of adventitious sequence homology during the course of evolution, but serve no function in a regulatory capacity. As the repeats contain signals for transcription initiation, accidental transcripts may be generated. Present evidence neither supports nor eliminates either of these models, and assignment of any function to the spacer transcripts is premature.

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MOSS REPLIES—The criticisms of Murtif and Rae stem mainly from a misunderstanding of the details of the *Xenopus laevis* ribosomal spacer model[1]. The model proposed for the function of this spacer was developed from biochemical observations of spacer transcription *in vivo*[1] and from earlier studies of the primary structure of this spacer[2]. However, spacer promotion alone is insufficient to explain the strong competition observed between spacer-containing and spacer-less genes[1]. It was therefore postulated[1] that the highly repetitive spacer sequences were also active in this competition. As these sequences would be unable to initiate spacer transcription but show some promoter homologies, it was suggested that they may act as RNA polymerase binding sites. Hence in the model a certain level of transcription, initiated at the spacer promoters, acts to drive bound, but uninitiated, polymerase to the major (40S) gene promoter. Each spacer need direct only one transcript at a time for its optimal functioning by this mechanism. Therefore, the number of spacer transcripts produced at maximal gene activity could be as low as 5% of the 40S RNA transcription (the ratio of spacer promoters to repetitive polymerase binding sites) or even 2.5% if close-packing of uninitiated polymerase were possible. Sollner-Webb and Schultz (cited in ref. 3) estimate the number of 5′ termini of spacer transcripts in *X. laevis* tissue as between 0.05 and 1% of that of 40S RNA 5′ termini at steady state. I have observed very similar levels in *X. laevis* tissue culture (ref. 1 and my unpublished observations). However, I have also observed in the same RNA preparations that the number of 3′ termini of these spacer transcripts is ~10% of 40S 5′ termini. Therefore the 5′ and 3′ termini of the spacer transcripts have very different half lives within the cell. Thus, Sollner-Webb and Schultz probably greatly underestimate the steady-state level of these transcripts.

It is, of course, not the steady-state RNA levels, but the relative rates of 40S RNA and spacer RNA initiation which are relevant to tests of the model of spacer function. My preliminary results indicate that compared with the 40S RNA, spacer transcripts are more highly represented in pulse-labelled RNA than in steady-state RNA. They are therefore more rapidly degraded than the 40S RNA and any measurements made on steady-state RNA levels will underestimate the true rate of spacer transcription.

Data on ribosomal spacer transcription obtained from electron microscope analyses are by necessity rather selective. Additionally, electron microscope analyses are subject to the same problems as are the steady-state biochemical analyses if, as is likely, the half life of the spacer transcripts is about the same as, or less than, the sample preparation time. The work of Rungger *et. al.*[4] demonstrates that agents assumed to slow RNA processing or degradation also increase the spacer transcription detected in electron microscope spreads. Thus, spacer transcripts may be degraded during normal spreading. Nevertheless, Scheer *et al.*[5] studying *Triturus*, not *Xenopus*, say of their observations "Frequently ... lateral fibrils (transcripts) are seen attached to the spacer segments ...".

It was not suggested in ref. 1 that the proposed model is generally applicable, nor should one necessarily expect it to be so. If the model is appropriate to other *Xenopus* species and *Drosophila melanogaster*, this will be deduced from functional assays. The usefulness of the model in *X. laevis* is not reduced by its apparent inapplicability to yeast and human ribosomal spacers, neither of which have been completely sequenced.

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