LETTERS TO THE EDITOR

Is there a *Xenopus* transcription factor that can substitute for TFI_{II}A?

Re: Two TFI_{II}A activities regulate expression of the *Xenopus* 5S RNA gene families

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To the Editor:

Blanco et al. (1989) describe findings that lead them to consider a mechanism for the differential expression of 5S RNA genes in *Xenopus* that is radically different from one we have favored (see Wolffe and Brown 1988). They partly purified an abundant 42-kD protein from oocytes which they refer to as a “42-kD TFI_{II}A.” They report that the 42-kD protein replaces TFI_{II}A as a transcription factor for somatic 5S RNA genes and suggest that it represses the transcription of oocyte 5S RNA genes. The assignment of these activities rests in part on the purity of the 42-kD protein, especially their contention that their 42-kD protein preparation lacks TFI_{II}A contamination. The 42-kD protein is abundant in somatic cells and oocytes and, therefore, cannot be the somatic form of TFI_{II}A that we know is absent in oocytes (Kim et al. 1990). Joel Gottesfeld, one of the co-authors of this disputed research, sent me samples of the anti-TFI_{II}A sera that were used in their paper to show that the 42-kD preparation is free of bona fide TFI_{II}A. He also sent me two different partly purified extracts which he estimated contain between 10 and 20% 42-kD protein.

Their anti-TFI_{II}A antisera (including that used in the experiments reported in the Blanco et al. paper) are two orders of magnitude weaker than our own, providing a possible explanation for why they could not detect low levels of TFI_{II}A contamination. I compared the activity of the two 42-kD protein extracts with in vitro-synthesized TFI_{II}A (Vrana et al. 1989; Kim et al. 1990) for their ability to complement a *Xenopus* oocyte nuclear extract depleted of endogenous TFI_{II}A (with our anti-TFI_{II}A antibody) and thus to support transcription of oocyte and somatic 5S RNA genes. There is a concentration range of added TFI_{II}A that gives a linear response in its support of 5S RNA synthesis (Kim et al. 1990). The first of Gottesfeld’s 42-kD preparations had no detectable activity whatsoever. This 42-kD preparation neither stimulated somatic 5S RNA gene transcription nor repressed oocyte 5S RNA gene transcription (when mixed with TFI_{II}A), although at high concentrations it inhibited transcription generally. I could have detected 1% of the activity of TFI_{II}A on a mole/mole basis.

The second 42-kD preparation complemented a low level of 5S RNA transcription activity. Estimated on a mole/mole basis, the 42-kD protein was about 1% as active as TFI_{II}A, and the ratio of somatic to oocyte 5S RNA gene transcription in this low level of activity was

the same as that seen with TFI_{II}A complementation. This level of activity is so low that any number of the components in the impure extract could have been responsible.

In summary, I have been unable to reproduce the two results claimed in the Blanco et al. (1989) paper—neither the ability of a 42-kD protein at any reasonable stoichiometrical amount to replace TFI_{II}A in the support of somatic 5S RNA gene transcription nor its purported activity as a specific repressor of oocyte 5S RNA gene transcription. The experiments reported by Blanco et al. give no reason to believe that the 42-kD protein has any relationship to TFI_{II}A other than the fact that both proteins are found in oocytes.

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References

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To the Editor:

A controversy has arisen concerning a paper by Blanco et al. (1989) in which we described the identification of a 42-kD protein found in mature *Xenopus* oocytes, embryos, and somatic cells. We reported that this protein binds the internal control region (ICR) of the 5S rRNA genes and supports transcription of the somatic class of 5S genes in a manner analogous to the well-characterized 39-kD protein transcription factor IIIA (TFI_{II}A). The 42-kD protein also binds to the oocyte-type 5S genes but fails to support transcription of these genes in our in vitro systems. From these data we hypothesized that the 42-kD protein may play a central role in the differential transcription of the two classes of 5S genes observed during *Xenopus* embryogenesis.

We have sent Dr. D. Brown two recently prepared samples of the 42-kD protein, both of which were active in our laboratory in 5S DNA binding and transcription of

the somatic-type 5S RNA gene in a reconstituted system (as previously described by Blanco et al. 1989). After sending the first sample, we found that it contained substantial levels of 39-kD TFIIIA (as 7S RNPs). The second sample contained less than 1% TFIIIA compared to the 42-kD protein, as determined by Western blots probed with an anti-TFIIIA antibody sent to us by Dr. Brown. Dr. Brown has used these 42-kD protein samples in transcription experiments with a TFIIIA-depleted oocyte nuclear extract and found them to be inactive relative to in vitro-synthesized TFIIIA. We noted previously that both TFIIIA and the 42-kD protein sediment at the 7S region of glycerol gradients and that RNase treatment of the 42-kD protein is required for DNA binding, suggesting that the 42-kD protein may also be in an RNP particle (Blanco et al. 1989). We believe that Dr. Brown's results are due to the comparison of the 42-kD protein without RNase treatment to 5S RNA-free TFIIIA.

Dr. Brown has suggested that our results are due to contamination of the 42-kD protein fractions with authentic 39-kD TFIIIA and that we have failed to detect such contamination due to the weakness of our anti-TFIIIA antisera. We agree with Dr. Brown that his anti-TFIIIA antibody is indeed 50- to 100-fold stronger than either serum used in our original study ($\alpha 469$ and $\alpha 470$). Furthermore Brown's antibody detects contamination of the 42-kD protein with 39-kD TFIIIA, which is not detected with our anti-TFIIIA sera ($\alpha 469$ and $\alpha 470$, at least in their current state). It is thus conceivable that the degree of TFIIIA contamination of the 42-kD protein samples used by Blanco et al. was underestimated and that some of the results presented by Blanco et al. could have been due to this contamination. These points, however, cannot be addressed directly as the preparations of the 42-kD protein used by Blanco et al. are no longer available. An independent demonstration of the transcriptional activity of the 42-kD protein, which cannot be attributed to TFIIIA cross-contamination, will be available when the cloning and expression of a cDNA for this protein has been achieved. Such studies are currently under way in our laboratories.

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