Published in final edited form as:

Science. 2014 August 1; 345(6196): 524. doi:10.1126/science.1254246.

Response to Comment on “Mechanism of eukaryotic RNA polymerase III transcription termination”

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Abstract

Arimbasseri *et al.*, in their Comment, suggest that to terminate transcription in vivo, RNA polymerase III uses a mechanism other than hairpin-dependent termination and that properties of purified polymerase may depend on preparation procedure. Evidence suggests that our preparation is indeed different from that of other methods. Our new data suggest that, apart from hairpin-dependent termination, one or more “fail-safe” termination mechanisms may exist in the cell.

RNA polymerase III (Pol III) synthesizes short RNAs, such as tRNAs and 5S RNA. In Nielsen *et al.*, we proposed that termination of synthesis by Pol III is facilitated by folding of the transcript (1). Arimbasseri *et al.* provide evidence that other RNA Pol III preparations do not require this structure (2). We suggest that different mechanisms exist for different preparations of the enzyme, such that in vivo secondary structure-facilitated termination may be redundant.

We believe that the point raised by Arimbasseri *et al.* is valid. It is clear from their Comment that the activity during termination may depend on the preparation of Pol III, with different protocols of purification leading to different forms of the enzyme. The description of Pol III preparation in our study was unclear and consisted of more steps than preparations in the Comment. To this end, we would like to clarify this purification procedure. The cleared lysate of *Saccharomyces cerevisiae* NZ16 (6x His-tagged Pol III) cells, grown to OD₆₀₀ 5 to 6, was fractionated with ammonium sulfate (38 g/100 mL), and the formed pellet was dissolved in Hepes pH 7.8 buffer containing 1 M KCl and 5 mM MgCl₂ and applied to 100-mL Ni-nitrilotriacetic acid column. The column was washed with the same buffer containing 10 mM imidazole. The fraction that eluted with 250 mM imidazole was diluted and applied to the Heparin column, and fractions eluting between the 400 mM and 600 mM of ammonium sulfate gradient in Hepes buffer were collected. These fractions were dialyzed to a Hepes buffer containing 100 mM KCl and 20% glycerol and loaded to a 1-mL Mono-Q column. After washing, the fractions that eluted in the 100- to 1000-mM gradient of KCl were collected and tested for RNA polymerase (RNAP) activity in assays of elongation complexes. Glycerol was added (to 50%) to the active fractions, and they were stored at –80°C. Preparations were further tested in the following way: In our elongation complex assay (3), RNAP activity was inhibited by Tagetitoxin (4, 5); the RNAP preparation

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possessed phosphodiester bond hydrolytic activity, several orders of magnitude higher than Pol II (6, 7). The preparation also almost lacked iterative (repetitive slippage) synthesis, which is observed with Pol I on some sequences (8) (Fig. 1A).

We suggest that there is likely more than one “fail-safe” mechanism that exists to ensure efficient and robust termination by Pol III, which is critical for the functioning of this transcription machine. This idea is supported by our new result with nuclear lysate. In our work on lysates, figure 2B of Nielsen *et al.* (1) and Fig. 1B (left panel), the immobilized 17-nucleotide (nt) oligomer elongation complex, formed by transcription on Pol III promoter-containing template, was washed to remove nucleoside triphosphates (NTPs), nonbound (such as abortive and cleavage) products, and Pol III not engaged in elongation before the further extension, which is a conventional procedure when investigating, for example, bacterial termination. This washing allows full synchronization of RNAPs and prevention of reinitiation, which may obscure the results. The washing must have saved all DNA binding factors but might have led to loss of some soluble or weakly bound ones. In such a setup, we observed that the absence of the secondary structure of the transcript strongly slowed down the release. Given the possibility of the presence of some additional termination factors, discussed above, we attempted to perform a similar experiment of extension of formed 17-nt oligomer elongation complexes without washing. Indeed, as seen in Fig. 1B (middle panel), the release of termination complexes was much more efficient even in the absence of the secondary structure in the transcript. However, even in this new setup, the release on the transcript with the unstructured region was still slightly diminished. This result supports the above idea that there exist one or possibly more “fail-safe” mechanisms for Pol III termination that may act in parallel to ensure efficient termination by Pol III.

ACKNOWLEDGMENTS

This work was supported by UK Biotechnology and Biological Sciences Research Council.

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Fig. 1.

(A) Pol III preparation lacks iterative synthesis characteristic to Pol I. Assembled elongation complexes were extended in the presence of uridine triphosphate on a T₁₀ template. Note differences in cleavage and pausing patterns. (B) Different setups of the experiments with nuclear lysates. In the left panel, the experiment presented in figure 2B in (1). The 17-nt oligomer stable elongation complexes (EC17), obtained by transcription from the Pol III promoter in nuclear lysate on the linear template coding for tRNA^{Tyr} with or without unstructured region (UN), were either washed or not washed before the extension in the presence of all NTPs. The sizes of the termination products were controlled by RNA markers obtained with T7 RNAP on the same transcribed sequence (as, for example, shown on the far right panel).