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

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Nielsen et al. (Reports, 28 June 2013, p. 1577) characterized their RNA polymerase III (Pol III) preparation and concluded that it requires an RNA hairpin/duplex structure for terminating transcription. We could not corroborate their findings using bona fide Pol III from two laboratory sources. We show that Pol III efficiently terminates transcription in the absence of a hairpin/duplex in vitro and in vivo.

A biochemical analysis of Saccharomyces cerevisiae–derived RNA polymerase III (Pol III) by Nielsen et al. (1) showed that although a fraction of the enzyme paused at the known termination signal, oligo(T), it failed to release the transcript in the absence of a terminator-proximal RNA hairpin or other RNA duplex structure. This work contrasts with that of others that concluded that no hairpin or dyad structure is required for termination by Pol III (2, 3). Soren Nielsen and Nikolay Zenkin graciously provided us with a sample of their Pol III. Our analyses reveal that the Nielsen Pol III fails to terminate in the absence of a terminator-proximal RNA hairpin or other RNA duplex structure, as they reported, but this polymerase preparation differs substantially from Pol III purified independently by A. Arimbasseri and G. Kassavetis using different methods in different laboratories.

Using the template specified by Nielsen et al. to produce a transcript lacking secondary structure, we found that our S. cerevisiae Pol III efficiently released transcripts at oligo(T). Further analyses indicated that the disparity must lie in the polymerases rather than variables of the assays. This was confirmed by direct comparisons of the two polymerases, the Nielsen Pol III (NP) and ours (AP) (4), using two different templates and two immobilization methods (i) through the

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**Fig. 1. Direct comparisons of NP polymerase and AP Pol III for termination and transcription factor TFIIIB+TFIIIC–dependent initiation.** (A) Elongation complexes (EC) were assembled on templates A and B and immobilized, through the RNA polymerase His6 tag, on Ni-NTA (nitrilotriacetic acid) agarose. NTPs were added, incubated for 10 min, and the released (R) and bound (B) transcripts separated, as indicated above each lane. (B) Comparison of elongation complex formation and transcription by AP and NP on immobilized template B. The quantities of AP and NP used are the same as in (A); reactions were for 1 min. Lanes 1 to 4 and 9 to 12 were done as described (4), whereas lanes 5 to 8 and 13 to 16 were done in the absence of EDTA and 40°C incubation. (C) Comparison of transcript release by three different preparations of Pol III. AP was purified on NiNTA agarose (4) (used in other figures), whereas untagged KP was highly purified by sequential ion-exchange chromatography (6). The quantities of AP and NP used are the same as in (A) and (B). Released, bound, and total (T) transcripts are indicated. (D) Pol III–specific transcription assay. Pre-initiation complexes were formed by incubating a SUP4 tRNA gene plasmid with S. cerevisiae S100 extract deficient in endogenous Pol III activity, followed by the addition of AP or NP, as indicated above the lanes. Reactions were started by the addition of NTPs (with α-[32P] GTP) and MgCl2 and incubated for 20 min.
polymerase His6 tag (4) (Fig. 1A) or (ii) through the 5′-biotin–tagged nontemplate strand DNA (Fig. 1B) (7). For the experiment shown in Fig. 1A, templates A and B contain differently positioned 9T and 10T terminators, respectively, producing 5′-32P-labeled RNA primer-directed transcripts predicted to lack secondary structure; AP efficiently released transcripts at the T tracts of both templates (lanes 1 and 3). In contrast, NP produced more read-through transcripts (RT), and most of the transcripts with 3′ ends at the T tract were in the bound fractions (lanes 6 and 8). For the experiment shown in Fig. 1B, we used immobilized DNA, unlabeled RNA primer and α-[32P]GTP (guanosine triphosphate) incorporation (I), and the same amounts of polymerases as in Fig. 1A. In this assay, NP was far more active than AP and also produced more RT. Transcripts paused at oligo(T) were not released by NP but were efficiently released by AP (Fig. 1B). It is noteworthy that substantial RT was also produced by NP for transcripts with a hairpin and on even longer T tracts (Fig. 2C in (I)), reflecting unexpectedly low termination efficiency relative to what would result with other preparations of Pol III (with comparable nucleoside triphosphate (NTP) concentrations; see (3–5)). We also compared NP and AP with an untagged preparation of Pol III (KP) (6), using immobilized DNA (Fig. 1C). KP was nearly indistinguishable from AP, whereas NP again produced mostly bound transcripts, arrested at oligo(T). It is likely important that NP was substantially more active than AP in the immobilized DNA assay relative to the immobilized polymerase assay. NP also differed in

Fig. 2. Pol III termination is independent of transcript terminator–proximal RNA structure in vivo. (A) Schematic of the different suppressor tRNA constructs used. pSer7T is the parent construct (8). Promoter elements and other features are indicated (arrow, start site; box A; intron; box B; 7T or 3T terminators; T2, read-through terminator). The dashed vertical lines delineate the 5′ and 3′ matured/processed ends of the tRNA. An unstructured spacer (US) or a spacer that forms a stable hairpin (HP) was introduced within the 3′ trailer, immediately upstream of the terminator. On the right side is a schematic of the resultant predicted pre-tRNA products. (B) Patch suppression assay analysis of S. pombe colonies carrying the different suppressor constructs. Red color indicates lack of suppression, and white color indicates efficient suppression. (C) Northern blot analysis of the nascent tRNAs produced by the different suppressor constructs using an intron-specific probe. 7T- and 7T-US/HP designations to the left side indicate the position of the nascent 7T-terminated pre-tRNAs of pSer7T and pSer7T-US/HP constructs, respectively. “Endo” indicates the nascent transcript of the endogenous dimeric tRNAser-tRNAmet gene. 3T-T2 indicates the 3′ extended transcript generated by read-through transcription of the constructs with the 3T terminator. The arrow indicates a partially processed pre-tRNA intermediate before nuclear export. The differences in the profiles in lanes 2 and 3 likely reflect alternative 3′ processing pathways (15). The lower panel shows the same blot probed for the pol II-transcribed US snRNA as a sample recovery control.
for proliferation, growth, and development (12, 13). High productivity and additional features also make Pol III a compelling choice for therapeutic expression of small interfering RNAs for RNA interference (RNAi) and other purposes (14). In this regard, our data indicate that a terminator-proximal hairpin is not essential for termination, either in vivo or in vitro.

REFERENCES AND NOTES

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