

RESEARCH HIGHLIGHT

It's Sno'ing on Pol III at nuclear pores

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Abstract

Integral nuclear pore proteins associate with subsets of snoRNA and tRNA genes transcribed by RNA polymerase III and promote 3' transcript processing in nematodes.

The spatial organization of RNA polymerase III (Pol III)-transcribed genes in metazoans is poorly understood. A recent report by Ikegami and Lieb [1] indicates that, intriguingly, a large number of genes encoding small nucleolar RNAs (snoRNAs) and transfer RNA (tRNAs) are associated with integral nuclear pore proteins, suggesting that these genes reside within nuclear pores.

Pol III-transcribed genes in three dimensions

Pol III transcribes tRNA genes as well as the genes encoding U6 snRNA, 5S rRNA and a limited number of other short noncoding RNAs. tRNA and some other Pol III-transcribed genes contain sequence-specific internal promoter elements known as A and B boxes that have been highly conserved through eukaryotic evolution. The A and B boxes serve as principal binding sites for the multisubunit transcription factor TFIIC (TFIIIC), which recruits TATA binding protein (TBP) and other TFIIB subunits to a region just upstream of the Pol III transcription start site (TSS) [2]. For tRNAs, the A and B box sequence elements are incorporated into the transcript and are recognized by processing (and other) factors. For example, RNase Z - the endonuclease that removes the 3' trailer from precursor-tRNA - recognizes the pseudouridine stem loop (Ψ loop) that is derived from the B box (Figure 1a, b).

Recent studies in yeast have shown that tRNA genes also play roles in chromatin and higher order genome organization [3]. TFIIC binds to condensin and leads to clustering of tRNA genes in the nucleolus [4]. We

understand a bit about gene clustering, but apart from that our general understanding of genome organization is poor - for all metazoans, mammals included, and for all Pol III-transcribed genes, tRNA included [5].

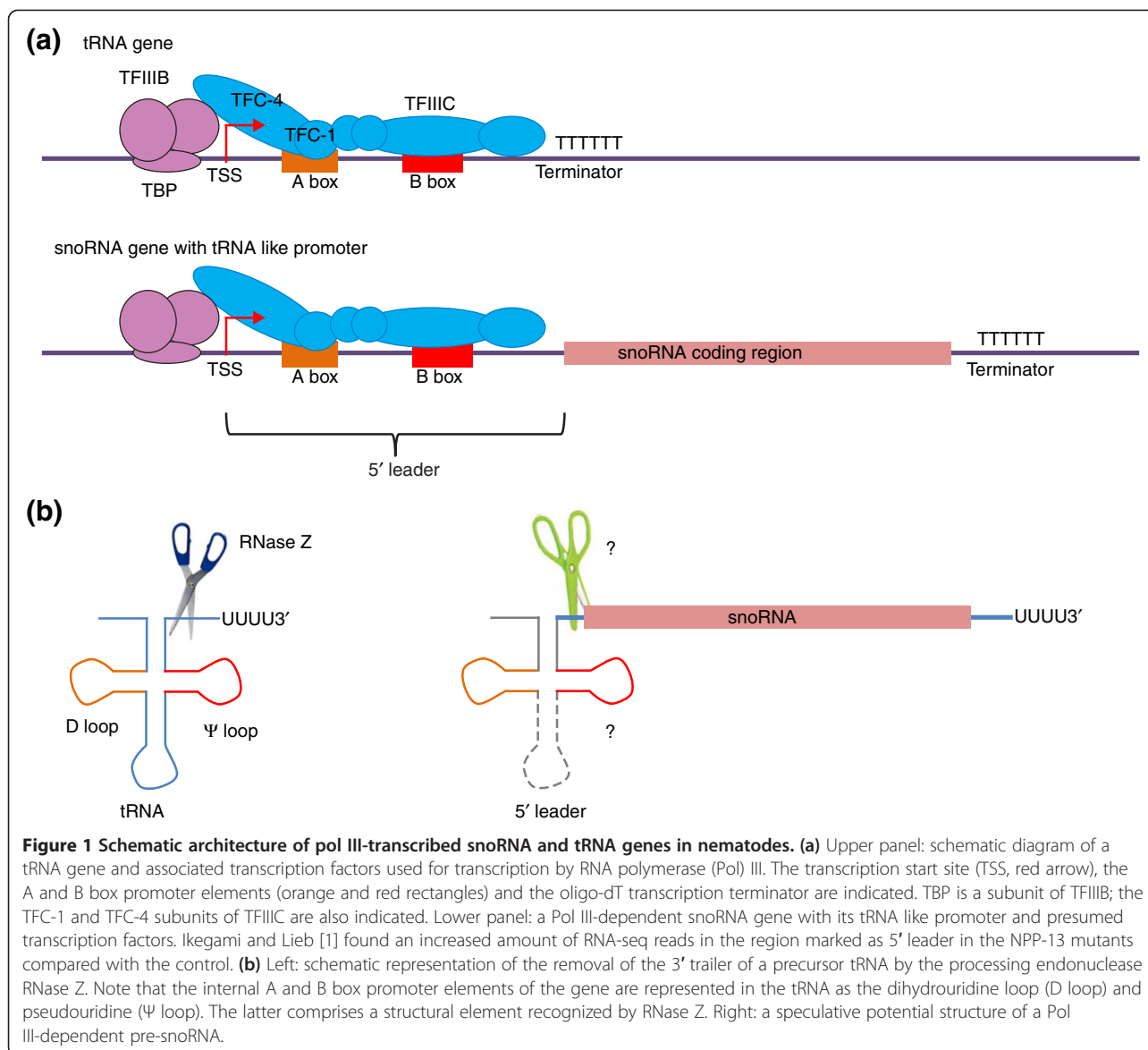
In some species, variable fractions of the numerous genes that encode snoRNAs are under the control of Pol III, utilizing A and B box promoters [6]. The snoRNAs can be classified into two major classes: ones that direct pseudouridylation and others that direct 2'-O-ribose methylation of rRNAs. In yeast, only one of the 75 snoRNA genes is transcribed by Pol III while the rest rely on Pol II, and all of the approximately 450 human snoRNAs are transcribed by Pol II, either independently or as parts of introns of larger transcripts [6]. It is important to note that *Caenorhabditis elegans* is exceptional in this regard since almost half of its approximately 140 annotated snoRNA genes contain typical Pol III A and B box promoters downstream of the TSS, followed by the snoRNA sequence. Accordingly, the A and B box sequences are transcribed as part of a 5' leader RNA that must be removed to liberate the snoRNA [6]. In the case of plant snoRNAs that are transcribed by Pol III, this pathway is reminiscent of the 3' processing pathway for precursor-tRNAs that contain long 3' trailers [7].

Upon activation in yeast, several inducible genes have been shown to be relocated to the nuclear pore and this is required for their activation [8]. This relocation also helps with transcriptional memory, a property by which the kinetics of a second round of activation occurs more quickly than the first. In mammalian cells, silent chromatin associates with the nuclear periphery, although whether nuclear pores play a role in transcriptional regulation is unknown.

Nuclear pore components are required for efficient processing of Pol III snoRNAs

In their recent article, Ikegami and Lieb [1] report that knocking down NPP-13 (homolog of vertebrate nucleoporin 93 (Nup93)), an integral component of the *C. elegans* nuclear pore, as well as other pore components, leads to a defect in the maturation of 51 of the 59

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snoRNAs transcribed by Pol III. RNA-seq and other genomics approaches indicate that these snoRNAs retain the 5' leader sequence, suggesting the processing activity that removes the 5' leader is compromised. A knee-jerk reaction to these findings would be to query whether this effect is indirect, perhaps due to disrupted transport of a key Pol III transcription or RNA processing factor. However, attempts to address this by examining mRNA levels of known RNA processing factors did not support this hypothesis.

Examination of global RNA-seq data also revealed that two tRNA genes with weak Pol III terminators retained their 3' trailers. Although the extent of these 3' trailer retentions was not as robust as for the snoRNAs, this

alerted the authors to examine tRNA genes more closely.

What determines which Pol III gene subsets associate with nuclear pore proteins?

Only a subset of snoRNA and tRNA genes show evidence of association with the nuclear pore complex (124 of the 609 tRNA genes and 51 of the 59 Pol III snoRNA genes). ChIP-chip and ChIP-seq data showed that three nuclear pore proteins, NPP-13, NPP-3 and importin-β protein (IMB-1), produced nearly identical patterns of Pol III gene loci - strong evidence that the subsets selected were not random. It is known that NPP-13 and NPP-3 interact directly with each other, while IMB-1

connects via other channel proteins, arguing that the Pol III loci interact with the nuclear pore rather than the free proteins. Knockdown of the gene encoding IMB-1 did not affect snoRNA maturation, suggesting an indirect connection to the Pol III genes, with NPP-13 involved in more functional interactions.

An outstanding question is how only a subset of Pol III genes differentially associates with the pore proteins. While the sequences of the A and B boxes of the pore-targeted genes were analyzed, these were not compared with the non-targeted genes and so we do not know if A and/or B box sequence composition may be a factor. In this regard, it is interesting that TFIIC occupancy was significantly less correlated with the nuclear pore proteins compared with stronger occupancy by TFIIB and Pol III on the Pol III snoRNA genes [1]. While upstream TATA boxes are found in a variable number of tRNA genes in different species, this was not addressed for the tRNA and snoRNA genes here. Not all mammalian Pol III genes are active at a given time, suggesting gene subset-specific activation pathways, yet the mechanisms involved are largely unknown.

About 30 tRNA genes in *C. elegans* contain introns that must be removed by dedicated tRNA splicing enzymes, which in other metazoa are believed to localize to the nuclear membrane. In addition, tRNA modification enzymes have been localized to the nuclear inner membrane [9]. Presumably, tRNA intron retention was not a feature of the NPP-13 knockdown.

Another question that arises is whether the snoRNA genes transcribed by Pol III are predominantly of the pseudouridylation or ribose methylation class. In either case, it would also be interesting to know if the snoRNA-associated proteins, comprising the H/ACA small nucleolar ribonucleoproteins (snoRNPs; pseudouridylation) and the C/D snoRNPs (methylation), are required for efficient processing in this system, or if the nascent pre-assembled snoRNA is the substrate for processing.

How would it work: kinetic coupling of initiation and/or elongation and processing?

One of the most interesting ChIP-seq findings reported by Ikegami and Lieb [1] is that Pol III was found at two peaks on the snoRNA genes in normal embryos: one near the TSS and the other further downstream, toward the snoRNA processing site. However, in NPP-13-depleted embryos, the TSS peak was significantly reduced, while the downstream peak increased. Although these observations might suggest decreased Pol III initiation in the mutants, steady state RNA levels were increased upon NPP-13 depletion. Nonetheless, as the difference in Pol III position is the only difference observed in the transcriptional machinery itself, it may be

argued that it is mechanistically related to the RNA processing defect. One can envision two scenarios, both involving kinetic coupling. The authors suggest that the increased downstream Pol III peak may reflect a pause due to lack of processing. An explanation for the markedly reduced peak at the TSS in the NPP-13 mutants may reflect faster promoter escape by Pol III. Thus, NPP-13 may somehow increase the dwell time of Pol III in pre-initiation mode, providing more of a kinetic window to recruit RNA processing factors. For vertebrate U1 and U2 snRNAs, it is known that proper RNA 3' processing depends on the promoter element [10]. In any case, it will be interesting to know the mechanisms by which NPP-13 affects Pol III transcription and/or RNA processing.

The big question: Sno job or is Pol III working at nuclear pores?

Nuclear pores have a core that contains the NPP-13 protein, for which both physical and functional effects on snoRNA biogenesis were shown. Their cumulative observations led the authors to suggest the likely interpretation that the affected Pol III genes may be actively transcribed within nuclear pores. However, cross linking followed by immunoprecipitation does not necessarily reflect direct interaction or sub cellular localization. It is an exciting possibility that the transcription and/or maturation of these RNAs occurs within the nuclear pores themselves. As with other thought-provoking studies, further investigations are required to advance these results. Direct visualization and other approaches will be necessary to determine whether Pol III transcription and/or nascent transcript processing occurs in the nuclear pores.

Abbreviations

Pol III: RNA polymerase III; snoRNA: Small nucleolar RNA; snoRNP: Small nucleolar ribonucleoprotein; snRNA: Small nuclear RNA; TBP: TATA binding protein; TFIIC: Transcription factor IIIC; tRNA: Transfer RNA; TSS: Transcription start site.

Competing interests

The authors declare that they have no competing interests.

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