Gene loops have been described in different organisms from yeast to human and form through interaction between components of the transcription pre-initiation complex and Ssu72, a member of the 3’ end cleavage and polyadenylation complex. A recent study by Tan-Wong et al. reports a new role for gene loops in promoting ORF transcription directionality from otherwise bidirectional promoters.

Many yeast promoters are bidirectional and produce, in addition to the protein coding mRNA, non-coding RNAs (ncRNAs) from the opposite strand in a divergent orientation.1,2 This feature, shared by about 50% of S. cerevisiae promoters, contributes to the genome pervasive transcription. While a small subset of these ncRNAs has been attributed a regulatory role, genetic evidence for functionality is lacking for the vast majority of these transcripts. It is therefore reasonable to think that the cell has evolved mechanisms to keep this potential transcriptional noise under control.

Different strategies to maintain transcriptome steady-state levels have been described, which mainly involve RNA decay pathways. Indeed many non-coding RNAs are unstable and detected only in strains defective in the machineries responsible for their rapid elimination. Thus, a large fraction of ncRNAs called cryptic unstable transcripts (CUTs) are degraded by the 3’ to 5’ exonuclease activity of the nuclear exosome component Rrp6,1-3 while others are degraded by the cytoplasmic exonuclease Xrn1 (XUTs).4 Another proposed option to reduce the amount of divergent transcripts is to force the transcription orientation of a bidirectional promoter toward the coding sequence. Evidence supports that regulation of chromatin modifications and nucleosome remodelling may influence transcription directionality.5,6

The recent study by Tan-Wong et al. reports a new mechanism able to restrain divergent ncRNA synthesis from bidirectional promoters.7 The study provides evidence that gene loops resulting from the transcription-induced interaction of the promoter with the 3’ end of protein coding units enhance transcriptional directionality. Based on chromatin conformation capture (3C) experiments and transcript quantifications, the authors show that disruption of the gene loop in the ssu72–2 mutant leads to increased divergent transcription from the FMP27 promoter region. Ssu72 is a phosphatase part of the 3’ end cleavage and polyadenylation factor (CPF) that was implicated in the maintenance of the gene loop structure through its ability to also interact with promoter elements.9

Tan-Wong et al. extend the observation on FMP27 to a more global analysis using tiling arrays in ssu72–2 and Δrrp6 single and double mutants. They identify a series of new transcripts defined as SRTs (Ssu72-restricted transcripts) in addition to the CUTs revealed by loss of the nuclear exosome component Rrp6. The authors then restrict the analysis to pairs of spaced tandem genes to demonstrate that the promoter associated SRTs (pSRTs) originate from the bidirectional promoter of the downstream ORF and are distinct from antisense transcripts potentially initiating within the transcription termination region of the upstream ORF (Fig. 1). Furthermore RNA PolII occupancy experiments indicate that the...
pSRTs appearing in ssu72-2 result from de novo transcription initiation.

Interestingly, inspection of published genome-wide histone acetylation levels reveals that pSRT-associated promoters show significantly reduced histone H4 acetylation. Moreover, the authors detect an increase in promoter acetylation when abrogating gene loop formation in a ssu72-2 mutant background. These observations suggest that gene loops may favor the recruitment of a histone deacetylase (HDAC) in order to maintain the promoter in a deacetylated state limiting firing of divergent pSRTs.

The identification of the HDAC responsible for this deacetylation is not addressed in this paper, but the authors exclude the involvement of the Rpd3 small (Rpd3s) H4 deacetylation complex. Rpd3s is recruited via its Eaf3 or Rco1 subunits on histone H3 methylated on lysine 36 (H3K36me) by Set2 in the body of transcribed genes. Using a nascent transcript sequencing (NET-Seq) approach in Δrco1, Rpd3s was recently proposed by Churchman et al. to contribute to promoter directionality by repressing divergent transcription following its recruitment at the 3’ region of the upstream transcribed gene methylated on H3K36. However, comparing the entire set of pSRTs with the Rco1-restricted transcripts (RRTs) identified by Churchman et al. or by performing additional tiling arrays in Δrco1 and Δrco1Δrrp6 mutants, Tan-Wong et al. conclude that these transcripts have different features. While the pSRTs are associated with the transcription start site (TSS) of the downstream ORF, the RRTs are linked and antisense to the transcription termination site (TTS) of the upstream ORF in tandem pairs (Fig. 1).

Although the distinction between the two classes can only be established when the tandem genes are more than 400 bp apart, the results indicate that pSRTs, but not RRTs, derive from bidirectional promoters identifying Ssu72 rather than Rco1 as a major contributor to promoter directionality. In support of this view, the ssu72-2 mutation also induces a weak downregulation of the downstream ORF in a pair when a pSRT is present in between. Although the reported decrease in mRNA levels is modest, this observation suggests that promoters have an intrinsic ability to fire in both directions but the loop structure imposes transcription directionality limiting the assembly of two adjacent divergent PICs as recently proposed. Thus a single PIC may form in nucleosome free regions associated with promoters engaged in loops. In sufficiently spaced tandem ORFs, a second independent PIC may form that promotes synthesis in the opposite direction to generate antisense RRTs associated with the 3’ end of genes in antisense orientation.

**Figure 1.** Ssu72-dependent gene loops form upon ORF transcription, which results in reduced (Set3 dependent?) histone H4 acetylation at the promoter, restricting divergent pSRT transcription. pSRTs arise in ssu72-2 and are distinct from RRTs generated in the Rpd3s mutant Δrco1 at the 3’ end of genes in antisense orientation.
of transcribed genes by di-methylated H3K4 demethylated by the multienzyme complex Smr3-Set1,20,21 as a potential HDAC to promote histone deacetylation at bidirectional promoters. Although no experiments were performed to confirm this hypothesis, Set3 appears as a plausible candidate due to its localization and already demonstrated ability to repress cryptic promoters in 5’-transcribed regions.21 This possibility raises the question of the signal responsible for recruiting this HDAC in the context of gene loops. Overall, the findings from both Churchman et al. and Tan-Wong et al. highlight the importance and the generality of deacetylation activities to control mRNA production.

Bidirectional promoters are the major source of cryptic transcription. Indeed, Snu172 is a phosphatase involved in the dephosphorylation of Ser5P in the RNA Pol II C-terminal domain (CTD). This Snu172 activity enhances productive transcription elongation by negatively regulating the association of the Ndt1-Nab3-Sm3 (NNS) early termination complex with RNA Pol II CTD.22,23 Furthermore, recent PAR-CLIP data reveal the presence of NNS at the 5’ end of many ORFs.24 Thus, the observed repression effect of the snu172 mutant on ORFs could be due, at least in part, to the effect of this mutation on transcription elongation and NNS dependent termination. snu172-2 is not the only mutant resulting in gene loop disruption and increased pSRT transcription. Indeed, Tan-Wong et al. tested other polyadenylation complex components with similar results, underlining the importance of a functional active 3’ end processing machinery for the maintenance of the gene loop structure and hence absence of divergent transcription. Moreover, the authors show that exchanging the poly A signals with an endonucleolytic Rnt1 cleavage site similarly disrupts the loop configuration and increases pSRT production. The effect of this cis-mutation was observed both on plasmid and in a yeast genomic context as well as on an integrated β-globin gene construct in human HEK 293 cells, indicating a similar and conserved gene loop dependent transcriptional directionality in higher eukaryotes. The observation that abrogation of normal 3’ end formation results in gene loop disruption and concomitant 5’-fold upregulation of divergent pSRTs, also points to the existence of a coordinated recycling of factors from the terminator back to the promoter that favors ORF transcription, in agreement with previously published data.25

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