**Three Stories:** **Evolution of Promoter Directionality; The Role of Mediator; and Dramatically Different Structure of mRNA 3’ Isoforms in yeast**

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**Promoter directionality**: The Pol II machinery inherently initiates transcription in one direction, yet eukaryotic promoter regions are frequently transcribed bidirectionally. Are divergent RNA transcripts simply a mechanistic byproduct of Pol II transcription or do they serve an evolved biological function? To address this, we used a functional evolutionary approach that involves S. cerevisiae strains containing large segments of foreign, and hence evolutionarily irrelevant, yeast DNA. Promoter regions in foreign species environments lose the directionality they have in their native species. Strikingly, fortuitous promoter regions that occur frequently in foreign DNA produce equal transcription in both directions. Thus, the existence of divergent RNA transcripts is an expected mechanistic feature of promoter regions, and it does not imply a functional role for these transcripts. These and other results indicate that promoter regions are intrinsically bidirectional and are shaped by evolution to bias transcription of coding transcripts while suppressing non-coding antisense transcription.

**Mediator function**: Mediator is a transcriptional co-activator recruited to enhancers by DNA-binding activators, and it also interacts with Pol II as part of the PIC. A single Mediator complex physically bridges the enhancer and core promoter. However, the Mediator kinase module associates with the enhancer, but not with the core promoter, and it dissociates from the enhancer upon depletion of TFIIH kinase. Thus, Mediator undergoes a compositional change in which the kinase module dissociates from Mediator to permit association with Pol II and the PIC. Thus, Mediator acts as a dynamic bridge.

Mediator is often viewed as a general transcription factor, but depletion of essential head or middle subunits causes only a modest transcriptional decrease.  In contrast, simultaneous depletion of all Mediator modules drastically decreases transcription.  Depletion of head or middle subunits, but not tail subunits, causes a downstream shift in the Pol II profile, suggesting that Mediator inhibits promoter escape.  Interestingly, PIC formation and Pol II transcription can occur when Mediator is not detected at core promoters.  These results indicate that Mediator is essential for Pol II transcription and stimulates PIC formation, but it is not a required component of the PIC in vivo.

**3’ isoform structure**: Alternative polyadenylation generates numerous 3’ mRNA isoforms that can vary in biological properties such as stability and localization. We develop methods to obtain transcriptome-scale structural information and protein binding on individual 3’ mRNA isoforms in vivo. Strikingly, near-identical mRNA isoforms can possess dramatically different structures throughout the 3’UTR. Analyses of identical mRNAs in different species or refolded in vitro indicate that structural differences in vivo are often due to trans-acting factors. The level of Pab1 binding to poly(A) containing isoforms is surprisingly variable, and differences in Pab1 binding correlate with the extent of structural variation for closely-spaced isoforms. A pattern encompassing single-strandedness near the 3’ terminus, double-strandedness of the poly(A) tail, and low Pab1 binding is associated with mRNA stability. Thus, individual 3’ mRNA isoforms can be remarkably different physical entities in vivo. Sequences responsible for isoform-specific structures, differential Pab1 binding, and mRNA stability are evolutionarily conserved, indicating biological function.