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REVIEW ARTICLE

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How is polyadenylation restricted to 3′-untranslated regions?

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Abstract

Polyadenylation occurs at numerous sites within 3′-untranslated regions (3′-UTRs) but rarely within coding regions. How does Pol II travel through long coding regions without generating poly(A) sites, yet then permits promiscuous polyadenylation once it reaches the 3′-UTR? The cleavage/polyadenylation (CpA) machinery preferentially associates with 3′-UTRs, but it is unknown how its recruitment is restricted to 3′-UTRs during Pol II elongation. Unlike coding regions, 3′-UTRs have long AT-rich stretches of DNA that may be important for restricting polyadenylation to 3′-UTRs. Recognition of the 3′-UTR could occur at the DNA (AT-rich), RNA (AU-rich), or RNA:DNA hybrid (rU:dA- and/or rA:dT-rich) level. Based on the nucleic acid critical for 3′-UTR recognition, there are three classes of models, not mutually exclusive, for how the CpA machinery is selectively recruited to 3′-UTRs, thereby restricting where polyadenylation occurs: (1) RNA-based models suggest that the CpA complex directly (or indirectly through one or more intermediary proteins) binds long AU-rich stretches that are exposed after Pol II passes through these regions. (2) DNA-based models suggest that the AT-rich sequence affects nucleosome depletion or the elongating Pol II machinery, resulting in dissociation of some elongation factors and subsequent recruitment of the CpA machinery. (3) RNA:DNA hybrid models suggest that preferential destabilization of the Pol II elongation complex at rU:dA- and/or rA:dT-rich duplexes bridging the nucleotide addition and RNA exit sites permits preferential association of the CpA machinery with 3′-UTRs. Experiments to provide evidence for one or more of these models are suggested.

KEYWORDS
3' end formation, 3' UTR, cleavage/polyadenylation, Pol II elongation, polyadenylation, transcription

1 | INTRODUCTION

The 3′-ends of eukaryotic messenger RNAs (mRNAs) are generated during the process of transcriptional elongation by RNA polymerase II (Pol II). During elongation, the nascent transcript is cleaved followed by addition of a poly(A) tail (Bentley, 2014; Kumar et al., 2019; Proudfoot et al., 2002; Tian & Manley, 2013, 2017). Cleavage and polyadenylation occur at numerous sites within the 3′-untranslated region (3′-UTR), thereby generating ~50 3′ mRNA isoforms for a typical yeast gene (Moqtaderi et al., 2013; Ozsolak et al., 2010; Pelechano et al., 2013). As Pol II traverses the gene, there is a kinetic competition between the Pol II elongation and cleavage/polyadenylation (CpA) machineries. The level of CpA also depends on the RNA sequence.

In accord with kinetic competition, analysis of Pol II mutants indicates that the rate of Pol II elongation regulates the profile of poly(A) sites. Slow Pol II elongation causes a preference for usage of proximal poly(A) sites in yeast, fly, and human cells (Geisberg et al., 2020, 2022; Liu et al., 2017; Pinto et al., 2011; Yague-Sanz et al., 2020). Yeast cells undergoing the diauxic response have a slow

Pol II elongation rate, resulting in an upstream-shifted poly(A) profile that is strikingly similar to that caused by slow Pol II mutants (Geisberg et al., 2020). Conversely, fast Pol II elongation in yeast shifts polyadenylation towards distal sites (Geisberg et al., 2020, 2022). Although the Pol II speed mutants shift the relative abundance of 3′ isoforms, they do not generate new poly(A) sites. The upstream and downstream shifts in poly(A) profiles occur continuously at the single nucleotide level (Geisberg et al., 2022). This indicates that the CpA and Pol II elongation complexes are spatially, and perhaps physically, coupled in vivo during the process of CpA, in accord with functional interactions between the transcription and CpA machineries (Bentley, 2014; He et al., 2003; Licatalosi et al., 2002; Nordick et al., 2008). This coupling strongly suggests that polyadenylation occurs rapidly upon emergence of the nascent RNA from the Pol II elongation complex (Geisberg et al., 2022).

2 | HOW DOES ELONGATING POL II RECOGNIZE 3′-UTRS? THE PARADOX

The large number of poly(A) sites within 3′-UTRs indicates that the CpA machinery is quite promiscuous with respect to sequence preferences. In this regard, polyadenylation of random-sequence RNAs (generated from a random-sequence chromosome) occurs at many sites, with the pattern of poly(A) sites being similar (though not identical) to that of *bona fide* yeast mRNAs (Gvozdenov et al., 2023). Despite the promiscuity of the CpA machinery, there is very little polyadenylation within coding regions (~1% of the level within 3′-UTRs) even though coding regions are much longer than 3′-UTRs. This very low level of polyadenylation in coding regions is not due to rapid degradation, because half-lives of poly(A)-containing RNAs within coding regions are similar to those of mRNAs on an overall basis (Geisberg et al., 2014).

These observations lead to the following paradox. How does Pol II travel through long coding regions without generating poly(A) sites, yet then permits promiscuous polyadenylation once it reaches the 3′-UTR? At one level, the paradox is explained by chromatin immunoprecipitation experiments showing strong and preferential association of the CpA machinery with 3′-UTRs (Kim et al., 2010; Nedea et al., 2003) that depends on phosphorylation of the Pol II C-terminal tail by Ctk1 kinase (Ahn et al., 2004). However, the key question of how CpA complex recruitment is largely restricted to 3′-UTRs during the process of Pol II elongation remains unanswered. The CpA complex associates with coding regions at modest levels (Kim et al., 2010; Nedea et al., 2003), but it is unknown why this association leads to much lower levels of polyadenylation than expected.

3′-UTRs and coding regions must have different sequence properties so that the Pol II elongation machinery can distinguish between them. Presumably, these differences allow the CpA machinery to be recruited to and/or function efficiently within 3′-UTRs but not within coding regions. At present, very little is known about the molecular mechanism(s) involved in this discrimination.

3 | SEQUENCES THAT DISTINGUISH CODING REGIONS FROM 3′-UTRS

3′-UTRs are modular entities that are generally sufficient to determine polyadenylation profiles of the vast majority of yeast genes. Specifically, in 26 out of 27 cases tested, poly(A) profiles are essentially identical when either (1) the entire coding region is deleted, (2) a large gene is inserted in frame between the coding region and 3′-UTR, or (3) coding regions of genes from different chromosomes are swapped (Lui et al., 2022). Thus, in addition to coding regions and 3′-UTRs being functionally distinct with respect to translation via sense and nonsense codons, they are also independent and functionally distinct modules with respect to polyadenylation (and subsequent transcriptional termination that follows the cleavage step).

At the DNA level, polyadenylation in yeast 3′-UTRs is associated with a long degenerate motif characterized by a long AT-rich stretch followed by shorter A- and T-rich sequences; this motif is absent in coding regions (Moqtaderi et al., 2013). Mutations within these regions reduce the efficiency of 3′-end formation (Hyman et al., 1991). In addition, the choice of specific poly(A) sites is influenced by a local sequence that relies on A residues after the cleavage point (Moqtaderi et al., 2013). The restriction of poly(A) sites to 3′-UTRs is also true for metazoans even though poly(A) site selection is influenced by an AAUAAA motif 10–30 nt upstream of the poly(A) site (Colgan & Manley, 1997; Proudfoot & Brownlee, 1976; Zhao et al., 1999). However, numerous AAUAAA motifs in coding regions and introns are not used for polyadenylation, and there is extensive 3′-end heterogeneity even when AAUAAA motifs are present (Geisberg et al., 2022).

Although deletion of the coding region does not generally affect poly(A) site selection, the poly(A) profile of an exceptional mutant strongly suggests that the long AT-rich stretch plays a critical role in distinguishing 3′-UTRs from coding regions (Lui et al., 2022). Specifically, deletion of the *BYE1* coding region juxtaposes an abnormally AT-rich region within the 5′-UTR to the 3′-UTR, and this derivative causes a dramatic upstream shift in the poly(A) profile. In this exceptional situation, the AT-rich stretch is shifted further upstream, thereby establishing a functional connection between this stretch and poly(A) site utilization.

At present, the hypothesis that the long AT-rich stretch is critical for restricting polyadenylation to 3′-UTRs is based on a single genetic circumstance. In addition, it is unclear what features of the AT-rich region (e.g., length, nucleotide composition, short sequence motifs) are functionally important. It is noteworthy that the AT-rich stretch in natural yeast genes has a bias to T residues on the coding strand, and the abnormally AT-rich region in the exceptional *BYE1* derivative also has a very strong bias to T residues in the coding strand. Such T stretches on the coding strand are associated with transcriptional termination by bacterial (Ray-Soni et al., 2016) and archaeal (Maier & Marchfelder, 2019) RNA polymerases and by eukaryotic RNA polymerase III (Arimbasseri & Maraia, 2015). Perhaps, long AT-rich stretches with a bias to T residues on the coding strand play an

analogous, but intermediate, role in Pol II termination by affecting CpA, which triggers termination.

4 | DOES THE DISTINCTION BETWEEN 3′-UTRS AND CODING REGIONS OCCUR AT THE DNA OR RNA LEVEL OR BOTH?

In principle, the 3′-UTR could be defined at the DNA (AT-rich), RNA (AU-rich), or RNA:DNA hybrid level (Figure 1). While cleavage and polyadenylation occur on RNA, this doesn't mean that the 3′-UTR is distinguished from coding regions via RNA, because CpA is directly linked to Pol II elongation, which occurs on DNA (Geisberg et al., 2020). The nucleic acid critical for recognition of the 3′-UTR is linked to molecular models for why polyadenylation is restricted to 3′-UTRs. As the CpA machinery preferentially associates with 3′-UTRs, DNA-, RNA-, or RNA-DNA hybrid-based models are distinguished by the mechanism of preferential recruitment/stabilization of the CpA machinery to 3′-UTRs. All the models discussed below assume that the long AT/U-rich stretch plays a critical role (Figure 1).

5 | RNA-BASED MODELS

Cleavage and polyadenylation ultimately occur on RNA, so RNA-based models seem the most straightforward and represent the prevailing view. The simplest model is that the CpA complex directly (or indirectly through one or more intermediary proteins) binds long AU-rich stretches that are exposed after Pol II passes through these regions. In yeast, the AU-rich sequence is very degenerate, so recognition is unlikely to involve a defined sequence motif. Metazoans have an AAUAAA motif that is important for recruitment of the CpA complex (Chan et al., 2014; Schonemann et al., 2014; Sun et al., 2018), but many AAUAAA sequences in introns, 3′-UTRs, and coding regions do not support polyadenylation (Geisberg et al., 2022).

For RNA-based models, the long, degenerative motif linked to polyadenylation (Moqtaderi et al., 2013) suggests that a relatively long AU-rich sequence must be exposed after passage of Pol II so that polyadenylation occurs at downstream sites. This poses questions about the nature of CpA complex recruitment as well as how CpA occurs at multiple sites downstream of the AU-rich stretch. Regarding recruitment of the CpA complex, the relatively long length of the AU-rich stretch might reflect a requirement for multivalent and weak RNA-protein interactions over the entire AU-rich stretch (Gross & Moore, 2001). Alternatively, RNA:RNA interactions within the AU-rich stretch could generate structures recognized by the CpA machinery.

The mechanism by which the recruited CpA complex functions at multiple downstream sites must be linked to the nucleotide-level linkage and presumed stereochemical relationship between the Pol II elongation and CpA machineries (Geisberg et al., 2022). One possibility is that CpA recruitment to a particular site leads to a stable interaction with the Pol II elongation machinery that permits threading of the DNA template and newly synthesized RNA. In such a model, the CpA machinery occupies a single location within the AU-rich stretch, and threading allows the same physical entity to perform CpA at multiple sites. Alternatively, the CpA machinery might translocate to multiple positions within the AU-rich stretch as elongating Pol II traverses the gene. In this regard, AU-rich stretches persist 20-30 nt downstream from poly(A) sites (Moqtaderi et al., 2013).

6 | DNA-BASED MODELS

3′-UTRs might be recognized at the DNA level by virtue of their nucleosome-depleted status (Fan et al., 2010). High AT-rich sequences within 3′-UTRs are both intrinsically inhibitory to nucleosome formation (Peckham et al., 2007; Sekinger et al., 2005) and are a substrate for the RSC complex, which mediates nucleosome depletion (Lorch et al., 2014). Pol II and its associated factors travel

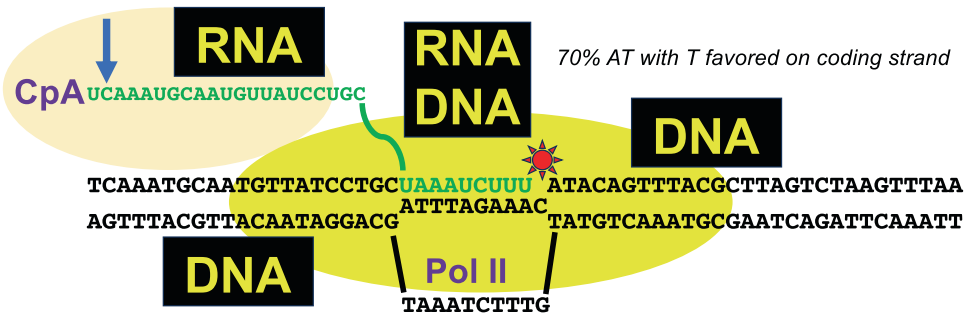

FIGURE 1 Models for how polyadenylation is restricted to 3′-UTRs. Diagram of RNA polymerase II (yellow oval) transcribing through an AT-rich (70% A + T with a preference of T residues on the coding strand) stretch of DNA in the 3′-UTR. DNA (black sequence), RNA (green sequence), RNA-DNA hybrid (black/green duplex), dissociated nontemplate strand (sequence below the duplex), and Pol II active site (red pointed circle) are indicated. The cleavage/polyadenylation (CpA) machinery (orange oval) is stereo-positioned (ovals touching) with respect to Pol II and a CpA site (blue arrow). DNA, RNA, and RNA:DNA hybrid models are shown with respect to their site of action; DNA models could involve sequences upstream and/or downstream of the active site.

efficiently through coding regions, but some factors dissociate from the template at the 3′-UTR (Kim et al., 2004; Mayer et al., 2010), perhaps due to low histone density. Dissociation of these factors from the elongating Pol II machinery might facilitate recruitment of the CpA complex and subsequent formation of mRNA 3′ ends. Conversely, the CpA complex might preferentially associate with the elongating Pol II complex at nucleosome-depleted regions, thereby causing dissociation of some components of the Pol II elongation machinery within the 3′-UTR.

Alternatively, 3′-UTR recognition and CpA complex recruitment might be due to changes in the composition and/or the conformation of the elongating Pol II machinery when it traverses an extended region of high AT content. In this regard, Pol II occupancy (assayed by native elongation transcription sequencing, NET-seq) in human cells decreases around the AT-rich region, presumably reflecting a local increase in Pol II speed or perhaps a change in the nature of the elongating Pol II machinery (Geisberg et al., 2022). In contrast to decreased Pol II occupancy just before CpA, Pol II occupancy increases after CpA occurs, reflecting a slowdown in elongation associated with the termination process (Geisberg et al., 2022).

7 | RNA:DNA HYBRID MODELS

At every moment during Pol II elongation, there is an ~8 nucleotide RNA:DNA hybrid located just upstream of the nucleotide addition site and just downstream of the region where RNA emerges from the elongation complex. This RNA:DNA hybrid is the primary stability determinant of the Pol II elongation complex (Kireeva et al., 2000; Wilson et al., 1999), and shortening of the RNA:DNA hybrid causes dissociation of the complex and leads to termination in vitro (Komissarova et al., 2002; Wilson et al., 1999). RNA:DNA hybrids involving AT/U-rich stretches are much less stable than those involving GC-rich stretches (Casey & Davidson, 1977), so long AT/U stretches are expected to reduce stability of elongation complexes. In addition, rU:dA duplexes are less stable than rA:dT duplexes (Casey & Davidson, 1977), perhaps explaining why Pol II termination occurs preferentially at T-rich stretches on the coding strand (Han et al., 2023), and why AT-rich stretches in 3′-UTRs are biased toward T residues on the coding strand. Thus, preferential destabilization of RNA:DNA duplexes throughout long AT-rich sequences could explain how the Pol II elongation machinery distinguishes 3′-UTRs from coding regions. In this model, local destabilization of the Pol II elongation machinery at AT-rich sequences does not cause termination per se, but rather renders these regions permissive for CpA recruitment and/or activity.

8 | RESOLVING THE MODELS

Several types of experiments would be useful in distinguishing between and evaluating the contributions of these classes of models: (1) determining the sequence preferences of AT-rich stretches for generating poly(A) profiles and linking these profiles to biochemical properties (e.g., RNA:DNA duplex stability, nucleosome occupancy, association with RNA-binding proteins) via analysis of many derivatives of AT-rich stretches; (2) determining poly(A) profiles in cells treated with analogues of thymine or uracil to create conditions that respectively have modified DNA or RNA; (3) analyzing poly(A) profiles in strains defective in a wide variety of mutant strains that affect Pol II elongation, the CpA complex, mRNA processing, mRNA turnover, chromatin as well as Pol II mutants that affect known structure and/or functions (e.g., RNA:DNA hybrids). Importantly, the three classes of models are not mutually exclusive and indeed may all contribute to why polyadenylation is very strongly restricted to 3′-UTRs.

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DATA AVAILABILITY STATEMENT

This paper has no data; it is a perspective.

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REFERENCES

Ahn, S. H., Kim, M., & Buratowski, S. (2004). Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3′-end processing. *Molecular Cell*, *13*, 67–76. https://doi.org/10.1016/s1097-2765(03)00492-1

Arimbasseri, A. G., & Maraia, R. J. (2015). Mechanism of transcription termination by RNA polymerase III utilizes a non-template strand sequence-specific signal element. *Molecular Cell*, *58*, 1124–1132. https://doi.org/10.1016/j.molcel.2015.04.002

Bentley, D. L. (2014). Coupling mRNA processing with transcription in time and space. *Nature Reviews Genetics*, *15*, 163–175. https://doi.org/10.1038/nrg3662

Casey, J., & Davidson, N. (1977). Rates of formation and thermal stabilities of RNA:DNA and DNA:DNA duplexes at high concentrations of formamide. *Nucleic Acids Research*, *4*, 1539–1552. https://doi.org/10.1093/nar/4.5.1539

Chan, S. L., Huppertz, I., Yao, C., Weng, L., Moresco, J. J., Yates, 3rd J. R., Ule, J., Manley, J. L., & Shi, Y. (2014). CPSF30 and Wdr33 directly bind to AAUAAA in mammalian mRNA 3′ processing. *Genes & Development*, *28*, 2370–2380. https://doi.org/10.1101/gad.250993.114

Colgan, D. F., & Manley, J. L. (1997). Mechanism and regulation of mRNA polyadenylation. *Genes & Development*, *11*, 2755–2766.

Fan, X., Moqtaderi, Z., Jin, Y., Zhang, Y., Liu, X. S., & Struhl, K. (2010). Nucleosome depletion at yeast terminators is not intrinsic and can occur by a transcriptional mechanism linked to 3′-end formation. *Proceedings of the National Academy of Sciences*, *107*, 17945–17950.

Geisberg, J. V., Moqtaderi, Z., Fan, X., Ozsolak, F., & Struhl, K. (2014). Global analysis of mRNA isoform half-lives reveals stabilizing and destabilizing elements in yeast. *Cell*, *156*, 812–824.

Geisberg, J. V., Moqtaderi, Z., Fong, N., Erickson, B., Bentley, D. L., & Struhl, K. (2022). Nucleotide-level linkage of transcriptional elongation and polyadenylation. *eLife*, *11*, e83153.

Geisberg, J. V., Moqtaderi, Z., & Struhl, K. (2020). The transcriptional elongation rate regulates alternative polyadenylation in yeast. *eLife*, *9*, e59810.

Gross, S., & Moore, C. L. (2001). Rna15 interaction with the A-rich yeast polyadenylation signal is an essential step in mRNA 3′-end formation. *Molecular and Cellular Biology*, *21*, 8045–8055. https://doi.org/10.1128/MCB.21.23.8045-8055.2001

Gvozdenov, Z., Barcutean, Z., & Struhl, K. (2023). Functional analysis of a random-sequence chromosome reveals a high level and the molecular nature of transcriptional noise in yeast. *Molecular Cell*, *83*, 1786–1797.

Han, Z., Moore, G. A., Mitter, R., Lopez Martinez, D., Wan, L., Dirac Svejstrup, A. B., Rueda, D. S., & Svejstrup, J. Q. (2023). DNA-directed termination of RNA polymerase II transcription. *Molecular Cell*, *83*, 3253–3267. https://doi.org/10.1016/j.molcel.2023.08.007

He, X., Khan, A. U., Cheng, H., Pappas Jr, D. L., Hampsey, M., & Moore, C. L. (2003). Functional interactions between the transcription and mRNA 3′ end processing machineries mediated by Ssu72 and Sub1. *Genes & Development*, *17*, 1030–1042. https://doi.org/10.1101/gad.1075203

Hyman, L. E., Seiler, S. H., Whoriskey, J., & Moore, C. L. (1991). Point mutations upstream of the yeast ADH2 poly(A) site significantly reduce the efficiency of 3′-end formation. *Molecular and Cellular Biology*, *11*, 2004–2012. https://doi.org/10.1128/mcb.11.4.2004-2012.1991

Kim, H., Erickson, B., Luo, W., Seward, D., Graber, J. H., Pollock, D. D., Megee, P. C., & Bentley, D. L. (2010). Gene-specific RNA polymerase II phosphorylation and the CTD code. *Nature Structural & Molecular Biology*, *17*, 1279–1286.

Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F., & Buratowski, S. (2004). Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. *The EMBO Journal*, *23*, 354–364.

Kireeva, M. L., Komissarova, N., Waugh, D. S., & Kashlev, M. (2000). The 8-nucleotide-long RNA:DNA hybrid is a primary stability determinant of the RNA polymerase II elongation complex. *Journal of Biological Chemistry*, *275*, 6530–6536.

Komissarova, N., Becker, J., Solter, S., Kireeva, M., & Kashlev, M. (2002). Shortening of RNA:DNA hybrid in the elongation complex of RNA polymerase is a prerequisite for transcription termination. *Molecular Cell*, *10*, 1151–1162. https://doi.org/10.1016/s1097-2765(02)00738-4

Kumar, A., Clerici, M., Muckenfuss, L. M., Passmore, L. A., & Jinek, M. (2019). Mechanistic insights into mRNA 3′-end processing. *Current Opinion in Structural Biology*, *59*, 143–150. https://doi.org/10.1016/j.sbi.2019.08.001

Licatalosi, D. D., Geiger, G., Minet, M., Schroeder, S., Cilli, K., McNeil, J. B., & Bentley, D. L. (2002). Functional interaction of yeast pre-mRNA 3′ end processing factors with RNA polymerase II. *Molecular Cell*, *9*, 1101–1111. https://doi.org/10.1016/s1097-2765(02)00518-x

Liu, X., Freitas, J., Zheng, D., Oliveira, M. S., Hoque, M., Martins, T., Henriques, T., Tian, B., & Moreira, A. (2017). Transcription elongation rate has a tissue-specific impact on alternative cleavage and polyadenylation in *Drosophila melanogaster*. *RNA*, *23*, 1807–1816. https://doi.org/10.1261/rna.062661.117

Lorch, Y., Maier-Davis, B., & Kornberg, R. D. (2014). Role of DNA sequence in chromatin remodeling and the formation of nucleosome-free regions. *Genes & Development*, *28*, 2492–2497. https://doi.org/10.1101/gad.250704.114

Lui, K. H., Geisberg, J. V., Moqtaderi, Z., & Struhl, K. (2022). 3′ untranslated regions are modular entities that determine polyadenylation profiles. *Molecular and Cellular Biology*, *42*, e0024422.

Maier, L. K., & Marchfelder, A. (2019). It's all about the T: Transcription termination in archaea. *Biochemical Society Transactions*, *47*, 461–468. https://doi.org/10.1042/BST20180557

Mayer, A., Lidschreiber, M., Siebert, M., Leike, K., Söding, J., & Cramer, P. (2010). Uniform transitions of the general RNA polymerase II transcription complex. *Nature Structural & Molecular Biology*, *17*, 1272–1278. https://doi.org/10.1038/nsmb.1903

Moqtaderi, Z., Geisberg, J. V., Jin, Y., Fan, X., & Struhl, K. (2013). Species-specific factors mediate extensive heterogeneity of mRNA 3′ ends in yeasts. *Proceedings of the National Academy of Sciences*, *110*, 11073–11078.

Nedea, E., He, X., Kim, M., Pootoolal, J., Zhong, G., Canadien, V., Hughes, T., Buratowski, S., Moore, C. L., & Greenblatt, J. (2003). Organization and function of APT, a subcomplex of the yeast cleavage and polyadenylation factor involved in the formation of mRNA and small nucleolar RNA 3′-ends. *Journal of Biological Chemistry*, *278*, 33000–33010. https://doi.org/10.1074/jbc.M304454200

Nordick, K., Hoffman, M. G., Betz, J. L., & Jaehning, J. A. (2008). Direct interactions between the Paf1 complex and a cleavage and polyadenylation factor are revealed by dissociation of Paf1 from RNA polymerase II. *Eukaryotic Cell*, *7*, 1158–1167. https://doi.org/10.1128/EC.00434-07

Ozsolak, F., Kapranov, P., Foissac, S., Kim, S. W., Fishilevich, E., Monaghan, A. P., John, B., & Milos, P. M. (2010). Comprehensive polyadenylation site maps in yeast and human reveal pervasive alternative polyadenylation. *Cell*, *143*, 1018–1029. https://doi.org/10.1016/j.cell.2010.11.020

Peckham, H. E., Thurman, R. E., Fu, Y., Stamatoyannopoulos, J. A., Noble, W. S., Struhl, K., & Weng, Z. (2007). Nucleosome positioning signals in genomic DNA. *Genome Research*, *17*, 1170–1177. https://doi.org/10.1101/gr.6101007

Pelechano, V., Wei, W., & Steinmetz, L. M. (2013). Extensive transcriptional heterogeneity revealed by isoform profiling. *Nature*, *497*, 127–131. https://doi.org/10.1038/nature12121

Pinto, P. A. B., Henriques, T., Freitas, M. O., Martins, T., Domingues, R. G., Wyrzykowska, P. S., Coelho, P. A., Carmo, A. M., Sunkel, C. E., Proudfoot, N. J., & Moreira, A. (2011). RNA polymerase II kinetics in polo polyadenylation signal selection. *The EMBO Journal*, *30*, 2431–2444. https://doi.org/10.1038/emboj.2011.156

Proudfoot, N. J., & Brownlee, G. G. (1976). 3′ Non-coding region sequences in eukaryotic messenger RNA. *Nature*, *263*, 211–214.

Proudfoot, N. J., Furger, A., & Dye, M. J. (2002). Integrating mRNA processing with transcription. *Cell*, *108*, 501–512.

Ray-Soni, A., Bellecourt, M. J., & Landick, R. (2016). Mechanisms of bacterial transcription termination: All good things must end. *Annual Review of Biochemistry*, *85*, 319–347. https://doi.org/10.1146/annurev-biochem-060815-014844

Schönemann, L., Kühn, U., Martin, G., Schäfer, P., Gruber, A. R., Keller, W., Zavolan, M., & Wahle, E. (2014). Reconstitution of CPSF active in polyadenylation: Recognition of the polyadenylation signal by WDR33. *Genes & Development*, *28*, 2381–2393. https://doi.org/10.1101/gad.250985.114

Sekinger, E. A., Moqtaderi, Z., & Struhl, K. (2005). Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. *Molecular Cell*, *18*, 735–748.

Sun, Y., Zhang, Y., Hamilton, K., Manley, J. L., Shi, Y., Walz, T., & Tong, L. (2018). Molecular basis for the recognition of the human AAUAAA polyadenylation signal. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 1419. https://doi.org/10.1073/pnas.1718723115

Tian, B., & Manley, J. L. (2013). Alternative cleavage and polyadenylation: The long and short of it. *Trends in Biochemical Sciences*, *38*, 312–320. https://doi.org/10.1016/j.tibs.2013.03.005

Tian, B., & Manley, J. L. (2017). Alternative polyadenylation of mRNA precursors. *Nature Reviews Molecular Cell Biology*, *18*, 18–30. https://doi.org/10.1038/nrm.2016.116

Wilson, K. S., Conant, C. R., & von Hippel, P. H. (1999). Determinants of the stability of transcription elongation complexes: Interactions of the nascent RNA with the DNA template and the RNA polymerase. *Journal of Molecular Biology*, *289*, 1179–1194. https://doi.org/10.1006/jmbi.1999.2814

Yague-Sanz, C., Vanrobaeys, Y., Fernandez, R., Duval, M., Larochelle, M., Beaudoin, J., Berro, J., Labbé, S., Jacques, P. É., & Bachand, F. (2020). Nutrient-dependent control of RNA polymerase II elongation rate regulates specific gene expression programs by alternative polyadenylation. *Genes & Development*, *34*, 883–897. https://doi.org/10.1101/gad.337212.120

Zhao, J., Hyman, L., & Moore, C. (1999). Formation of mRNA 3′ ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *Microbiology and Molecular Biology Reviews*, *63*, 405–445.

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