Strange bedfellows: polyadenylation factors at the promoter

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One of the more unexpected discoveries in the field of gene expression during the last few years has been the degree to which transcription of mRNA precursors is coupled to their subsequent processing into mature mRNAs. Much of this linkage involves the transcribing enzyme itself, RNA polymerase II (RNAP II), and specifically the repetitive C-terminal domain of its largest subunit (known simply as the CTD). A large body of evidence now indicates that the CTD has a significant role in enhancing the efficiency of all three of the major processing reactions—capping, splicing, and polyadenylation (for review, see Hirose and Manley 2000; Bentley 2002; Maniatis and Reed 2002; Proudfoot et al. 2002). This was initially surprising—all three reactions were known to occur just fine in vitro in the absence of transcription, or for that matter RNAP II and the CTD—but in retrospect it makes sense as a way to ensure accurate, efficient, and rapid processing of nascent pre-mRNAs. Precisely how the CTD functions is not entirely understood, especially in the more complex splicing and polyadenylation reactions, but a number of interactions with specific processing factors have been documented and these likely serve to help recruit the processing machinery to the pre-mRNA and then to stabilize or enhance the activity of these complexes.

Interactions between the transcription and processing machineries extend beyond those by which RNAP II and the CTD stimulate processing (for review, see Manley 2002). For example, yeast capping enzymes seem able to repress transcription reinitiation, perhaps as a checkpoint to ensure that capping has been completed (Myers et al. 2002), whereas mammalian splicing factors can function in vitro to stimulate transcription initiation (Kwek et al. 2002) or elongation (Fong and Zhou 2001). In this brief review, we discuss evidence supporting the existence of a complex, evolutionarily conserved set of interactions that link even more tightly events that occur at the promoter to define the transcription start site and mRNA 5′ end with those that occur at the other end of the gene to produce the mRNA 3′ end and ultimately the transcription stop site.

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TFIID, RNA polymerase II, and the polyadenylation machinery

The story began back in 1997 with the discovery that the general transcription factor (GTF) TFIID, which has a central role in promoter recognition during transcription initiation, could be copurified with CPSF, a multisubunit factor known to function in an a similar way in selection of the site of polyadenylation in the mRNA precursor (Dantonel et al. 1997). In reconstituted transcription reactions, it was shown that the interaction with TFIID recruits CPSF to the promoter and brings about its transfer to RNAP II concomitant with transcription initiation (Fig. 1). These interactions were envisioned as part of a mechanism to ensure efficient association of the polyadenylation machinery with the transcriptional apparatus, ultimately facilitating recognition of the polyadenylation signal on the nascent mRNA. These ideas were consistent with a number of related findings, including the demonstration that transcripts produced in transiently transfected cells by RNAP II lacking a CTD are not processed efficiently and that the CTD binds CPSF and another processing factor, CstF, in vitro (McCracken et al. 1997), and that the CTD can in fact be required for the 3′ cleavage reaction in vitro even in the absence of transcription (Hirose and Manley 1998; see Fig. 2 for a depiction of the complex polyadenylation machinery). Furthermore, RNAP IIO (the phosphorylated, transcribing form of the enzyme) and CPSF and/or CstF frequently colocalize on lampbrush chromosomes in frog oocytes (Gall et al. 1999) and polytene chromosomes in flies (Osheim et al. 2002).

The above studies were all conducted with metazoan organisms. It initially seemed possible that the linkages between transcription and 3′ processing might not be necessary in yeast, perhaps because of its simpler genomic organization and reflected for example in the simpler and shorter CTD (e.g., 26 relatively homogeneous heptapeptide repeats in yeast vs. 52 more divergent ones in mammals). The yeast CTD, however, has been shown to interact physically with subunits of the yeast equivalents of CstF and CPSF (Barilla et al. 2001; Dichtl et al. 2002a). Additionally, another study described a genetic

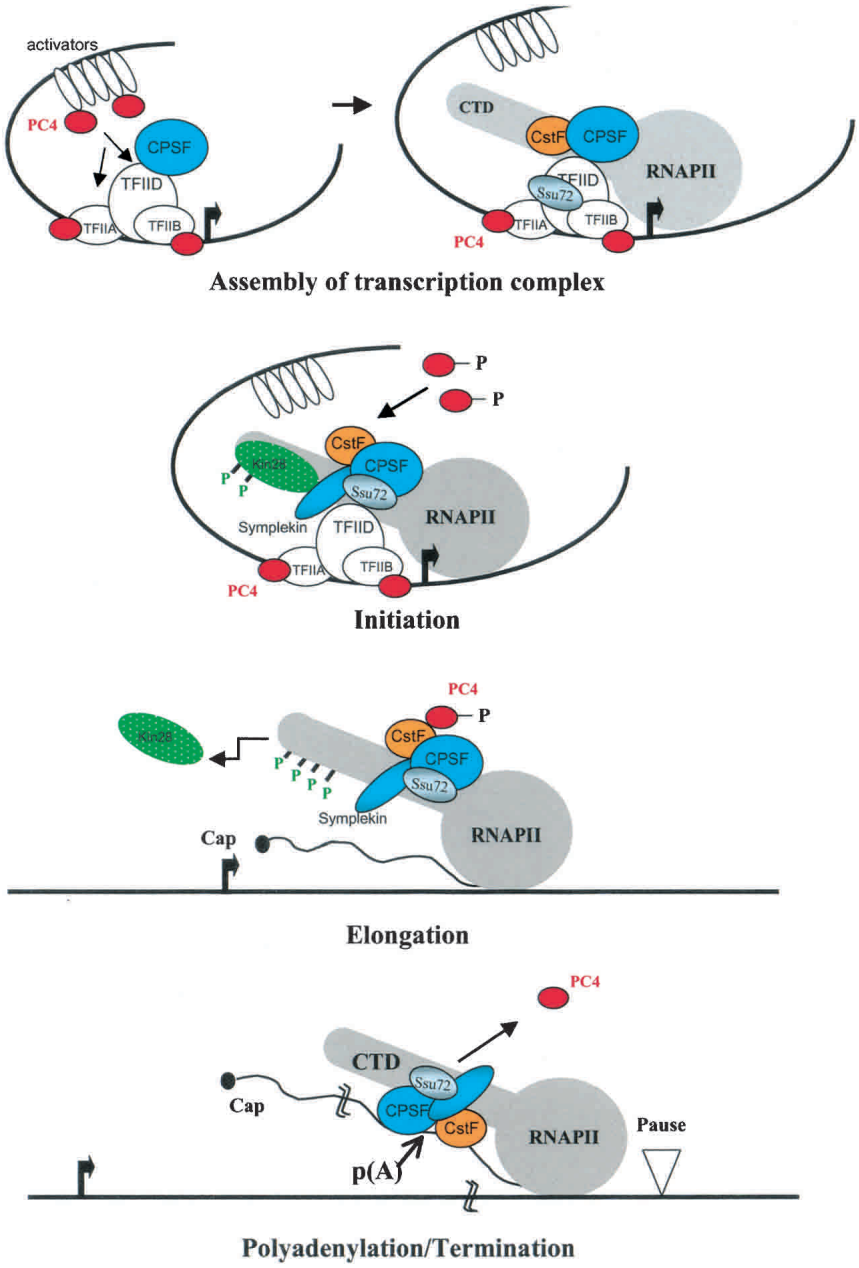

Figure 1. Transcription and polyadenylation factors: links from the beginning to the end. The transcription cycle starts with the recruitment and assembly of the preinitiation complex at the promoter. The transcriptional coactivator PC4 (red) spheres facilitate the recruitment of GTFs (TFIID/TFIIB/TFIIA are shown), possibly through interaction with TFIIB. TFIID brings CPSF to the preinitiation complex and CstF may also be recruited simultaneously by PC4 (Sub1), symplekin (Pta1), and/or other CPSF subunits. At this point Ssu72 joins the complex via interactions with TFIIB and symplekin. This either displaces PC4 or occurs at promoters that don't require PC4, as the interactions with symplekin are mutually exclusive (see text). Recruitment of RNAP II and other GTFs, including TFIIH (only the Kin28/cdk7 subunit is shown here) follows. The RNAP II CTD, and likely other factors, are phosphorylated as transcription initiates. PC4, perhaps now phosphorylated, interacts with CstF-64 and functions to help prevent premature termination. TFIIH/Kin28 dissociates, although other CTD kinases and at least one phosphatase (Fcp1) regulate RNAP II phosphorylation levels during elongation. When elongating RNAP II reaches the polyadenylation signals, which are recognized by CPSF and CstF, PC4 dissociates; the polyadenylation complex, including Ssu72 and symplekin, is activated; and 3′ cleavage and poly(A) synthesis occurs. The RNAP II elongation complex becomes termination-competent and eventually terminates, by poorly understood mechanisms, likely involving downstream pause sites. The transcription start site (arrow), polyadenylation site [p(A)], and mRNA cap are indicated.

interaction supporting the CTD–CstF interaction, chromatin immunoprecipitation assays suggesting that polyadenylation factors can be associated along the length of a transcribed gene and data indicating that the efficiency of 3′ end formation of some transcripts is reduced in strains containing a truncated CTD (Licatalosi et al. 2002). Therefore it seems that coupling between the transcription and polyadenylation machinery is conserved throughout eukaryotes, although it may well be less pervasive in yeast (e.g., see Dower and Rosbash 2002).

It is evident that certain polyadenylation factors can interact with the CTD, that this enhances polyadenylation efficiency, and that the interaction can begin back at the promoter. But this raises a number of questions, such as: How extensive are these interactions at the promoter? Can GTFs, or other promoter-associating factors, affect 3′ end formation? And can polyadenylation factors influence transcription initiation? The answers to these questions are beginning to emerge, and the picture that they paint is more complex, and interesting, than anyone would have predicted.

Transcription initiation and termination: the role of PC4/Sub1

Before continuing on with interactions at the promoter, we need to consider briefly what happens downstream of the polyadenylation signal; that is, what makes RNAP II terminate transcription. This remains a difficult and

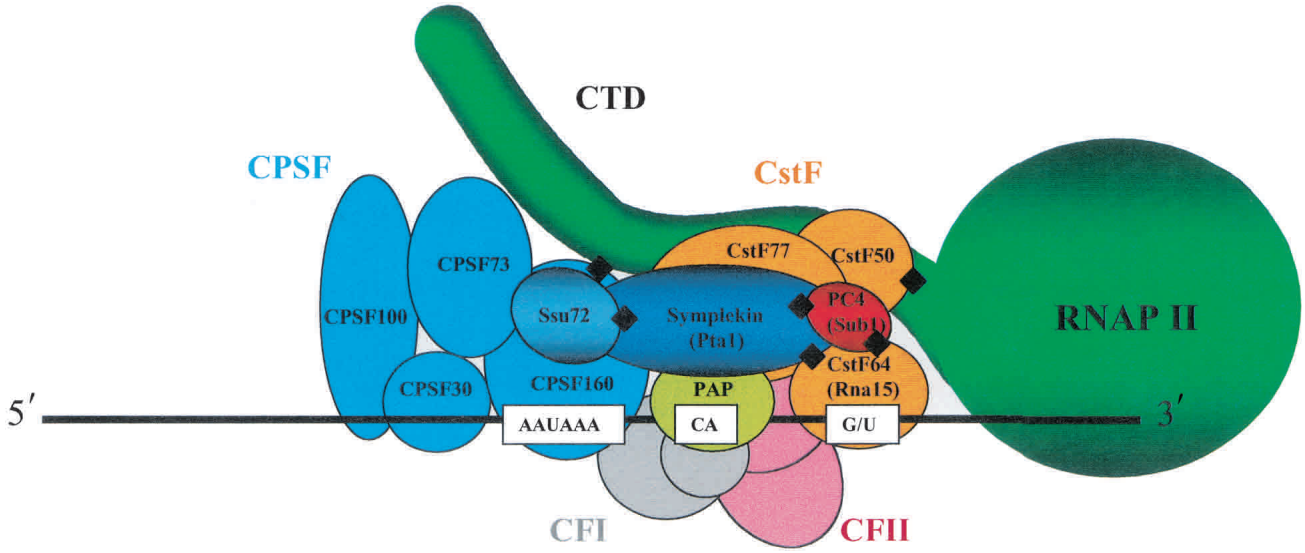

Figure 2. Schematic representation of the polyadenylation machinery. The majority of the components of the mammalian and yeast polyadenylation complexes are conserved, including all currently known factors that function in the transcription connection. For simplicity, only the mammalian nomenclature is depicted; the yeast names of factors that have important roles in the events described here are also indicated. (Note that although an apparent human homolog of Ssu72 exists, it has not yet been characterized functionally). ◆, documented protein–protein interactions that help link transcription and 3′ processing (see text). Polyadenylation signal sequences (upstream AAUAAA, CA cleavage site consensus, and downstream G/U-rich region) are boxed. CPSF, cleavage-polyadenylation specificity factor; CstF, cleavage stimulation factor; CFI and CFII, cleavage factors I and II, respectively; PAP, poly(A) polymerase.

enigmatic problem. RNAP II needs to be able to transcribe genes as long as a mega-base pair or more, so it's perhaps not surprising that it's hard to stop, and it is likely that multiple factors will eventually be found to contribute to termination. It has been known for many years, though, that mutation of the signals that specify polyadenylation can prevent subsequent termination by RNAP II (for review, see Proudfoot 1989; Hirose and Manley 2000), indicating a link between 3′ processing and termination. In addition, a combination of yeast genetics and transcriptional run-on assays provided evidence that at least several of the proteins required for 3′ processing are also necessary for termination (Birse et al. 1998; Dichtl et al. 2002a). This includes subunits of the yeast equivalents of both CstF and CPSF, and therefore, together with data described above, links factors that function (by a still poorly defined mechanism) in termination with events that occur at the promoter.

The story continues with the unexpected discovery that the RNA-binding subunit of human CstF, CstF-64 (which recognizes the G/U-rich sequence element that lies downstream of the 3′ cleavage site; Fig. 2), interacts with the biochemically well-characterized transcriptional coactivator PC4 (Calvo and Manley 2001; Fig. 2). Both proteins have yeast counterparts (RNA15 is equivalent to CstF-64 and Sub1 to PC4) and the interaction is conserved in yeast. The significance of the physical interaction is supported by genetic interactions, as most notably overexpression of Sub1 was found to cause lethality with a specific allele of *RNA15*, *rna15-1*. The mutation in the rna 15-1 protein results in an amino acid change in the evolutionarily conserved terminus, and significantly was found to tighten the interaction between RNA15 and Sub1. Transcriptional run-on experiments indicated that Sub1 functions as an antiterminator (Fig. 1), likely acting to suppress or control a termination activity associated with the RNA15 C-terminal domain (Aranda and Proudfoot 2001).

These new properties of PC4/Sub1—its ability to interact directly with a polyadenylation factor and to influence the ability of RNAP II to elongate/terminate transcription—are especially interesting in light of its well-documented functions at the promoter. As mentioned above, PC4 was discovered as a coactivator; that is, as a protein that allows sequence-specific transcription activators to function in reconstituted transcription reactions, presumably by facilitating interactions between the activator and GTFs (Ge and Roeder 1994; Kretzschmar et al. 1994). The ability of PC4 to interact with GTFs is supported further by observations that the protein facilitates early steps in preinitiation complex assembly, but represses transcription from such incomplete complexes, suggesting that PC4 may have multiple roles leading to formation of activated transcription complexes (Malik et al. 1998; Werten et al. 1999). PC4 also binds both single- and double-stranded DNA, with an especially high affinity for melted regions in duplex DNA (Werten et al. 1998, 1999), although how this contributes to activity remains unknown.

Initial studies in yeast were for the most part consistent with this overall picture of PC4 function in transcription initiation, but also provided evidence for an

additional interaction that, as we shall see, helps to tighten the links with 3′ processing. Using biochemical approaches similar to those that resulted in the discovery of PC4, Tsp1 (a.k.a. Sub1) was purified as a factor capable of enhancing in vitro transcription, with the additional and significant finding that Tsp1 interacts directly with the GTF TFIIB (Knaus et al. 1996). Sub1 was discovered genetically, as a high-copy suppressor of specific TFIIB defects. Deletion of *SUB1* (which itself is nonessential) was synthetically lethal with these same alleles, and Sub1 overexpression increased activated expression from several specific genes (Henry et al. 1996). Therefore, a combination of genetics and biochemistry support what are likely evolutionarily conserved interactions with both general transcription (TFIIB) and 3′ processing (CstF-64) factors.

The TFIIB/Ssu72 connection

The next link in the developing network of promoter-processing interactions also involves TFIIB. TFIIB has key roles in transcription initiation, helping to assemble and stabilize the preinitiation complex and to position the complex to allow accurate initiation by RNAP II (Woychik and Hampsey 2002). Yeast TFIIB was identified initially by mutations that have the effect of altering the start site of transcription. In characterizing this property further, Sun and Hampsey (1996) identified mutations in an essential, evolutionarily conserved gene they called *Ssu72* that enhanced both the start site defects and the growth phenotype of one of the TFIIB mutations. These interactions were similar to those observed with Sub1 and TFIIB, and a subsequent study analyzing interactions between *sub1* and *ssu72* alleles with a panel of mutations affecting TFIIB strengthened the similarity. In each case, interactions were detected with a subset of mutations that affect start site selection, and significantly, the allele specificity was almost identical (Wu et al. 1999). Therefore, Sub1 and Ssu72, which are unrelated in primary structure, share a common function, which is to help the transcriptional machinery identify the start site of transcription through a mechanism that involves a direct interaction with TFIIB.

But what does all this have to do with links between transcription initiation and 3′ end formation? Remarkably, it now seems that Ssu72, like Sub1, also functionally interacts with the 3′ end machinery (Fig. 2). The first indication of this came from a proteomic analysis designed to identify protein complexes in yeast cells. Using a tagged version of a subunit of the yeast CPSF complex (Pta1, which we will discuss in more detail below), a complex containing the known CPSF subunits as well as several additional proteins, including Ssu72, was isolated (Gavin et al. 2002). These findings were confirmed first by Dichtl et al. (2002b), who also provided evidence that Ssu72 functions in some way to enhance 3′ end formation and/or termination. For example, accumulation of 3′ extended transcripts was observed for some genes. But more conclusive evidence that Ssu72 functions in 3′ end formation was provided by He et al. (2003), who showed that Ssu72 is in fact required for the actual 3′ cleavage reaction. They first demonstrated a defect in 3′ processing in vivo in cells containing a conditional *Ssu72* allele, and then more significantly showed that extracts prepared from cells lacking Ssu72 were defective in cleavage, which could be rescued by addition of purified recombinant Ssu72. Therefore, Ssu72 has an important but unknown role in the assembly and/or function of the polyadenylation machinery to facilitate endonucleolytic cleavage of the pre-mRNA.

Sub1 and Ssu72 therefore display remarkable similarities in function. Both are active at the promoter and function at least in part to assure proper assembly of the transcription complex, through very similar if not identical interactions with TFIIB. Both also associate with components of the polyadenylation machinery to influence downstream events, although here there is a bit of divergence: Sub1 functions through a CstF component to modulate its termination activity, whereas Ssu72 works in the context of CPSF to facilitate cleavage. These findings, together with the previously documented role of the RNAP II CTD in both transcription and processing, point to an intricate and complex network of interactions that link events that occur at the 5′ and 3′ ends of the gene. Although there is still a great deal to learn about precisely how all these dynamic interactions are orchestrated, we conclude by describing an additional factor, called Pta1 (or symplekin in mammals), that appears to help coordinate these diverse interactions, and then speculate on how this network works and why it exists.

Pta1/symplekin: integrating all the factors

Pta1 was identified as a necessary component of yeast CPSF, and seems to be a core component (Preker et al. 1997; Zhao et al. 1999) more tightly associated than, for example, Ssu72. In mammals, symplekin is less tightly associated with CPSF, but exists in a larger complex containing both CstF and CPSF (Takagaki and Manley 2000). The way in which symplekin was found to be a component of the polyadenylation complex provides some clues about its function, both in polyadenylation per se and, as we will see, in integrating the polyadenylation and transcription machineries. Specifically, symplekin was found to interact avidly with CstF-64 (recall that this is the CstF subunit that interacts with PC4, although the domains involved are distinct; Fig. 2). This interaction, however, is mutually exclusive with an interaction with CstF-77, which is the subunit required for integrity of the CstF complex. Therefore it was proposed that symplekin functions to facilitate or maintain a properly assembled CstF, and by extension, to help hold together the much larger, complete polyadenylation complex.

For the link to transcription, we need to return to yeast. First, the interaction that holds Ssu72 in the CPSF complex is with Pta1, as indicated by both biochemical and genetic studies (Dichtl et al. 2002b; He et al. 2003). Importantly, the same methods established that Sub1

also interacts with Pta1, but these two interactions are, again, mutually exclusive: Sub1 and Ssu72 both interact with Pta1, but only one at a time (He et al. 2003). Finally, Pta1 also provides an important link to elongating RNAP II and specifically to its phosphorylated CTD. Both *Pta1* and *Rbp1* (which encodes the RNAP II large subunit), interact with *Kin28*, which encodes a kinase that is a component of the GTF TFIIH and that phosphorylates the CTD early during the elongation phase of transcription (Rodriguez et al. 2000). These results suggest that Pta1 facilitates interactions of the transcription and/or polyadenylation machinery with RNAP II, specifically the CTD, at the time of transcription initiation.

A network of interactions link the beginning with the end

The studies described above suggest that an extensive network of interactions couples events at the promoter that lead to transcription initiation with subsequent events, including elongation, polyadenylation, and termination. Although mechanistic details remain unknown, and it is likely that additional important factors and interactions will be uncovered, it is possible to begin to put together a model that envisions how it all works (Fig. 1). An important point is that all of the factors involved are conserved from yeast to humans, and, although there will undoubtedly be differences in the details, the underlying mechanisms are likely universal. In the following, for simplicity we use the mammalian terminology.

The earliest events in the assembly of a coupled transcription/termination complex involves the function of PC4 as a coactivator, facilitating recruitment of a TFIID/TFIIB complex, perhaps by direct interaction with TFIIB. TFIID corecruits CPSF, likely via direct interaction between CPSF and TFIID subunits as well as possibly a symplekin-PC4 interaction. We suggest that CstF is recruited essentially simultaneously, via interactions with symplekin, PC4 and/or other subunits of CPSF (e.g., see Murthy and Manley 1995). Ssu72 also seems to function at this point. As mentioned above, its interaction with symplekin is mutually exclusive with that of PC4, and in light of the correspondence in allele specificities, the same is likely true with respect to TFIIB. Therefore Ssu72 could function either as a switch, displacing PC4, or in place of PC4 at PC4-independent promoters. All of these interactions not only ensure proper loading of these components of the polyadenylation machinery onto the transcriptional apparatus, but also facilitate proper assembly of the preinitiation complex, as illustrated by the observations that defects can alter the start site of transcription. Following assembly of the remainder of the preinitiation complex, transcription begins, accompanied by CTD phosphorylation by cdk7 (i.e., Kin28). This is sensed by symplekin, which mediates, together with other components, transfer of the CPSF–CstF complex to the elongating polymerase complex. We suggest that PC4 remains associated with the CstF-64 C terminus, where it functions to prevent premature termination. On reaching the 3′ processing site (and there are undoubtedly numerous additional factors and interactions required along the way), PC4 dissociates, allowing, for activation of the large polyadenylation complex by a process involving Ssu72, the RNAP II CTD and other factors. These changes are likely triggered by recognition of the poly(A) signals in the RNA by the RNA-binding components of CPSF and CstF, and/or by phosphorylation/dephosphorylation of key components. Two very recent studies have presented unexpected evidence that Ssu72 possesses protein phosphatase activity (Ganem et al. 2003; Meinhart et al. 2003), and it may be that this is important for the protein's function at both ends of the gene. Eventually, and here the details are entirely unclear, RNAP II terminates transcription and is recycled.

Lessons from *Escherichia coli*

The above model, although on the one hand vague with respect to mechanistic details and on the other probably requiring significant revision in the future, does account for all the biochemical, genetic, and functional data that places polyadenylation factors at the promoter and initiation factors at the polyadenylation signal. The idea that factors involved in 3′ end formation should have such conspicuous roles at the promoter still seems a bit audacious, in particular because transcription can be reconstituted without these factors and 3′ end formation until recently also seemed to have its own set of factors. But perhaps these sorts of linkages should have been predictable from studies that began over a quarter century ago. Specifically, pioneering work on the mechanisms of gene expression identified the fascinating and mysterious N and Q proteins encoded by bacteriophage λ that function as antitermination factors but which act initially, to modify RNAP, at sites near the promoter (Santangelo and Roberts 2002). A number of proteins with related properties exist in *E. coli*, such as RfaH (Artsimovitch and Landick 2002). RfaH contacts paused RNAP in an interaction that also requires contacts with the nontemplate DNA strand in the transcription bubble. By an unknown mechanism, this modifies RNAP so that it elongates more rapidly and is capable of overcoming downstream pause elements. It is intriguing that these properties resemble some of those of PC4—to act as an antiterminator and to bind melted DNA. Whether or not these similarities are meaningful, these and other studies in *E. coli* provide interesting precedents for the emerging results in eukaryotes. It is likely, however, that the much more complex eukaryotic transcription/processing machinery requires significantly more elaborate and sophisticated mechanisms than those employed by prokaryotes. Given that the exact mechanisms by which the *E. coli* antiterminators function is still mysterious, even after 25 years of study, it seems likely that understanding how all the factors described here work will keep molecular biologists occupied for years to come.

References

Aranda, A. and Proudfoot, N. 2001. Transcriptional termination factors for RNA polymerase II in yeast. *Mol. Cell* **7:** 1003–1011.

Artsimovitch, I. and Landick, R. 2002. The transcriptional regulator RfaH stimulates RNA chain synthesis after recruitment to elongation complexes by the exposed nontemplate DNA strand. *Cell* **109:** 193–203.

Barilla, D., Lee, B.A., and Proudfoot, N.J. 2001. Cleavage/polyadenylation factor IA associates with the carboxyl-terminal domain of RNA polymerase II in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci.* **98:** 445–450.

Bentley, D. 2002. The mRNA assembly line: Transcription and processing machines in the same factory. *Curr. Opin. Cell. Biol.* **14:** 336–342.

Birse, C.E., Minvielle-Sebastia, L., Lee, B.A., Keller, W., and Proudfoot, N.J. 1998. Coupling termination of transcription to messenger RNA maturation in yeast. *Science* **280:** 298–301.

Calvo, O. and Manley, J.L. 2001. Evolutionarily conserved interaction between CstF-64 and PC4 links transcription, polyadenylation, and termination. *Mol. Cell* **7:** 1013–1023.

Dantonel, J.C., Murthy, K.G., Manley, J.L., and Tora, L. 1997. Transcription factor TFIID recruits factor CPSF for formation of 3′ end of mRNA. *Nature* **389:** 399–402.

Dichtl, B., Blank, D., Sadowski, M., Hubner, W., Weiser, S., and Keller, W. 2002a. Yhh1p/Cft1p directly links poly(A) site recognition and RNA polymerase II transcription termination. *EMBO J.* **21:** 4125–4135.

Dichtl, B., Blank, D., Ohnacker, M., Friedlein, A., Roeder, D., Langen, H., and Keller, W., 2002b. A role for SSU72 in balancing RNA polymerase II transcription elongation and termination. *Mol. Cell* **10:** 1139–1150.

Dower, K. and Rosbash, M. 2002. T7 RNA polymerase-directed transcripts are processed in yeast and link 3′ end formation to mRNA nuclear export. *RNA* **8:** 686–697.

Fong, Y.W. and Zhou, Q. 2001. Stimulatory effect of splicing factors on transcriptional elongation. *Nature* **414:** 929–933.

Gall, J.G., Bellini, M., Wu, Z., and Murphy, C. 1999. Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. *Mol. Biol. Cell* **10:** 4385–4402.

Ganem, C., Devaux, F., Torchet, C., Jacq, C., Quevillon-Cheruel, S., Labesse, G., Facca, C., and Faye, G. 2003. Ssu72 is a phosphatase essential for transcription termination of snoRNAs and specific mRNAs in yeast. *EMBO J.* **22:** 1588–1598.

Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M., et al. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415:** 141–147.

Ge, H. and Roeder, R.G. 1994. Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. *Cell* **78:** 513–523.

He, X., Khan, A.U., Cheng, H., Pappas Jr., D.L., Hampsey, M., and Moore, C.L. 2003. Functional interactions between the transcription and mRNA 3′ end processing machineries mediated by Ssu72 and Sub1. *Genes & Dev.* **17:** 1030–1042.

Henry, N.L., Bushnell, D.A., and Kornberg, R.D. 1996. A yeast transcriptional stimulatory protein similar to human PC4. *J. Biol. Chem.* **271:** 21842–21847.

Hirose, Y. and Manley, J.L. 1998. RNA polymerase II is an essential mRNA polyadenylation factor. *Nature* **395:** 93–96.

———. 2000. RNA polymerase II and the integration of nuclear events. *Genes & Dev.* **14:** 1415–1429.

Knaus, R., Pollock, R., and Guarente, L. 1996. Yeast SUB1 is a suppressor of TFIIB mutations and has homology to the human co-activator PC4. *EMBO J.* **15:** 1933–1940.

Kretzschmar, M., Kaiser, K., Lottspeich, F., and Meisterernst, M. 1994. A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators. *Cell* **78:** 525–534.

Kwek, K.Y., Murphy, S., Furger, A., Thomas, B., O'Gorman, W., Kimura, H., Proudfoot, N.J., and Akoulitchev, A. 2002. U1 snRNA associates with TFIIH and regulates transcriptional initiation. *Nat. Struct. Biol.* **9:** 800–805.

Licatalosi, D.D., Geiger, G., Minet, M., Schroeder, S., Cilli, K., McNeil, J.B., and Bentley, D.L. 2002. Functional interaction of yeast pre-mRNA 3′ end processing factors with RNA polymerase II. *Mol. Cell* **9:** 1101–1111.

Malik, S., Guermah, M., and Roeder, R.G. 1998. A dynamic model for PC4 coactivator function in RNA polymerase II transcription. *Proc. Natl. Acad. Sci.* **95:** 2192–2197.

Maniatis, T. and Reed, R. 2002. An extensive network of coupling among gene expression machines. *Nature* **416:** 499–506.

Manley, J.L. 2002. Nuclear coupling: RNA processing reaches back to transcription. *Nat. Struct. Biol.* **9:** 790–791.

McCracken, S., Fong, N., Yankulov, K., Ballantyne, S., Pan, G., Greenblatt, J., Patterson, S.D., Wickens, M., and Bentley, D.L. 1997. The carboxy-terminal domain of RNA polymerase II couples mRNA processing to transcription. *Nature* **385:** 357–361.

Meinhart, A., Silberzahn, T., and Cramer, P. 2003. The mRNA transcription/processing factor Ssu72 is a potential tyrosine phosphatase. *J. Biol. Chem.* (in press).

Murthy, K.G. and Manley, J.L. 1995. The 160-kD subunit of human cleavage-polyadenylation specificity factor coordinates pre-mRNA 3′-end formation. *Genes & Dev.* **9:** 2672–2683.

Myers, L.C., Lacomis, L., Erdjument-Bromage, H., and Tempst, P. 2002. The yeast capping enzyme represses RNA polymerase II transcription. *Mol. Cell* **10:** 883–894.

Osheim, Y.N., Sikes, M.L., and Beyer, A.L. 2002. EM visualization of Pol II genes in Drosophila: Most genes terminate without prior 3′ end cleavage of nascent transcripts. *Chromosoma* **111:** 1–12.

Preker, P.J., Ohnacker, M., Minvielle-Sebstia, L., and Keller, W. 1997. A multisubunit 3′ end processing factor from yeast containing poly(A) polymerase and homologues of the subunits of mammalian cleavage and polyadenylation specificity factor. *EMBO J.* **16:** 4727–4737.

Proudfoot, N.J. 1989. How RNA polymerase II terminates transcription in higher eukaryotes. *Trends Biochem. Sci.* **14:** 105–110.

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

Rodriguez, C.R., Cho, E.J., Keogh, M.C., Moore, C.L., Greenleaf, A.L., and Buratowski, S. 2000. Kin28, the TFIIH-associated carboxy-terminal domain kinase, facilitates the recruitment of mRNA processing machinery to RNA polymerase II. *Mol. Cell. Biol.* **20:** 104–112.

Santangelo, T.J. and Roberts, J.W. 2002. RfaH, a bacterial transcription antiterminator. *Mol. Cell* **9:** 698–700.

Sun, Z.W. and Hampsey, M. 1996. Synthetic enhancement of a TFIIB defect by a mutation in SSU72, an essential yeast gene encoding a novel protein that affects transcription start site selection in vivo. *Mol. Cell. Biol.* **16:** 1557–1566.

Takagaki, Y. and Manley, J.L. 2000. Complex protein interac-

tions within the human polyadenylation machinery identify a novel component. *Mol. Cell. Biol.* **20:** 1515–1525.
Werten, S., Stelzer, G., Goppelt, A., Langen, F.M., Gros, P., Timmers, H.T., Van der Vliet, P.C., and Meisterernst, M. 1998. Interaction of PC4 with melted DNA inhibits transcription. *EMBO J.* **17:** 5103–5111.
Werten, S., Wechselberger, R., Boelens, R., van der Vliet, P.C., and Kaptein, R. 1999. Identification of the single-stranded DNA binding surface of the transcriptional coactivator PC4 by NMR. *J. Biol. Chem.* **274:** 3693–3699.
Woychik, N.A. and Hampsey, M. 2002. The RNA polymerase II machinery: Structure illuminates function. *Cell* **108:** 453–463.
Wu, W.H., Pinto, I., Chen, B.S., and Hampsey, M. 1999. Mutational analysis of yeast TFIIB. A functional relationship between Ssu72 and Sub1/Tsp1 defined by allele-specific interactions with TFIIB. *Genetics* **153:** 643–652.
Zhao, J., Kessler, M., Helmling, S., O'Connor, J.P., and Moore, C. 1999. Pta1, a component of yeast CF II, is required for both cleavage and poly(A) addition of mRNA precursor. *Mol. Cell. Biol.* **19:** 7733–7740.