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Transcription Elongation: TLKing to Dispatch Chromatin?

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The tousled-like kinases have been implicated in chromatin deposition, but surprising new findings in *Caenorhabditis elegans* indicate they have a role in transcription elongation. Are these apparently distinct functions of tousled-like kinases related?

Tousled-like kinases (TLKs) have important functions during the cell cycle in eukaryotes. The TLK family was named after its founding member, the product of the *TOUSLED* gene which is essential for normal development of the plant *Arabidopsis thaliana* [1]. More recently, these kinases have been implicated in deposition of chromatin following DNA replication. During S phase, nucleosomes must be assembled onto newly synthesized DNA in a manner that faithfully maintains the epigenetic active or silenced states of the chromatin. This process is facilitated by chaperones that include the anti-silencing function 1 protein (Asf1) [2,3]. TLKs phosphorylate Asf1 during S phase, when their activity peaks [4], and recent analyses in *Drosophila* strongly support a link between TLK and Asf1 *in vivo* [5]. The *tlk* and *asf1* genes in *Drosophila* interact functionally, and their overexpression in the salivary gland leads to chromatin abnormalities and decreased DNA endoreduplication; loss of *tlk* causes cell-cycle arrest and apoptosis in embryos, or mitosis abnormalities. While TLKs thus appear to be involved in regulating Asf1 and chromatin assembly in S phase; they can also phosphorylate the histone H3 tail, suggesting they may have other cell-cycle functions [6].

As reported recently in *Current Biology*, Han et al. [7] have now used RNA interference (RNAi) to study the single TLK gene, *tlk-1*, of the nematode *Caenorhabditis elegans* during embryogenesis. A significant fraction of *tlk-1(RNAi)* embryos showed signs of aberrant chromosome morphology, consistent with a chromatin-related function for the kinase. Neither RNAi nor *in vitro* experiments, however, gave any indication that *C. elegans* TLK-1 contributes to embryonic histone H3 phosphorylation. Remarkably, the *tlk-1(RNAi)* embryos were consistently characterized by a broad inhibition of mRNA transcription. They arrested development at 100 cells without differentiation, a classic sign of a widespread transcription defect, and they showed markedly reduced expression of individual genes. Interestingly, the *tlk-1(RNAi)* embryos were also characterized by dramatically decreased levels of two protein modifications that are specifically associated with transcription elongation.

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Transcription of mRNA begins with assembly of a pre-initiation complex which includes RNA polymerase II and a set of general transcription factors that establish the start site (Figure 1) [8]. The transition from initiation to elongation is coordinated through phosphorylation of the RNA polymerase II carboxy-terminal domain (CTD), a unique repeat based upon the consensus YSPTSPS. Through the CTD, transcription is physically coupled to the capping, processing and nuclear export events that yield a mature mRNA [9]. During initiation, the CTD is phosphorylated on serine 5, after which RNA polymerase II appears to be subjected to a checkpoint which establishes that the machinery for productive elongation is in place (Figure 1). The CTD is then phosphorylated on serine 2, a modification that predominates on elongating RNA polymerase II [11]. Chromatin modifications that may provide a transcription 'memory' also occur during this process (Figure 1). For example, the H3 tail is methylated on lysine 36 specifically during elongation [13,14]. During both initiation and elongation phases of transcription, chromatin modification or remodeling complexes may be required to overcome chromatin barriers to transcription [8].

Downregulation of *tlk-1* by RNAi apparently did not broadly prevent the initiation of transcription, but rather seemed to affect a later step. In the *tlk-1(RNAi)* embryos, CTD serine 5 phosphorylation levels were grossly normal [7], a significant observation because this phosphorylation appears to require the presence of

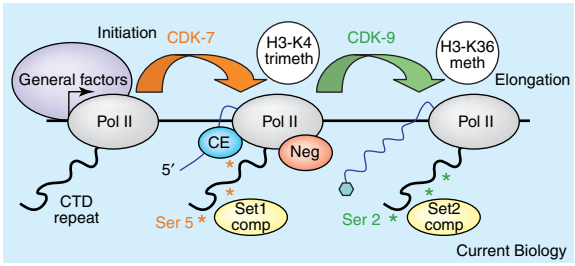

Figure 1. A partial view of the RNA polymerase II transcription cycle.

During the initiation of transcription, phosphorylation of the polymerase II (Pol II) CTD on serine 5 by the CDK-7 kinase is required for Pol II to clear the promoter, and for recruitment and activation of the mRNA capping enzyme machinery (CE) [8]. Serine 5 phosphorylation also recruits the Set1 methylase, which trimethylates the histone H3 tail on lysine 4 (H3-K4 trimeth) within 5′ coding regions [13,14]. During the transition to elongation, inhibition by negative factors (red) enforces a checkpoint which may insure that appropriate capping, elongation and processing factors are in place [8,10,12,15]. This inhibition is relieved when CTD serine 2 is phosphorylated by CDK-9, the kinase within the essential elongation factor P-TEFb [12,16,17]. Among the factors recruited to serine 2-phosphorylated RNA polymerase II is the Set2 complex, which methylates H3 on lysine 36 (H3-K36 meth) [13,14]. The CTD is dephosphorylated by phosphatases (not shown) that appear to act along the gene, so that unphosphorylated RNA polymerase II is available to be recruited for further transcription [11].

the transcription initiation apparatus [10]. In contrast, CTD serine 2 phosphorylation was dramatically reduced in *tlk-1(RNAi)* embryos [7]. Previous studies indicated that serine 2, but not serine 5, phosphorylation is lost when the serine 2 kinase is lacking [11,12], and that serine 2 phosphorylation is partially reduced by RNAi downregulation of the mRNA capping enzyme, suggesting that mRNA capping may be important for the elongation checkpoint (Figure 1) [10]. The *tlk-1(RNAi)* phenotype similarly might derive from a failure to clear that checkpoint, or from a later elongation defect such that normal levels of serine-phosphorylated RNA polymerase are not maintained. Also consistent with an elongation defect, the *tlk-1(RNAi)* embryos showed decreased levels of H3-lysine 36 methylation (Figure 1), but not of the non-specific euchromatin marker dimethyl H3-lysine 4 [7].

These fascinating *in vivo* studies in *C. elegans* and *Drosophila* [5,7] raise the questions of whether the chromatin deposition and transcription elongation functions of TLKs are conserved and perhaps important in many biological contexts, and whether the two functions might be related. Earlier results have indicated that disruption of chromatin structure may be important for the efficiency of elongation [8]. A simple model to explain the current findings is that the elongation defect of *tlk-1(RNAi)* embryos might derive from abnormal S-phase chromatin deposition, so that the new chromatin structure is refractory to elongation. Alternatively, TLKs might have a role in modulating chromatin structure directly at active genes during elongation. Finally, TLKs might have a regulatory role during the elongation checkpoint or a later transcription step, in which they modulate activity, localization or stability of a key elongation or mRNA processing regulator (Figure 1).

A number of experiments are now feasible that will help discriminate among these models. It will be important to characterize further the chromosome abnormalities reported in the new *C. elegans* study [7], and to investigate whether the *Drosophila tlk-1* mutant embryonic phenotype involves transcription defects. Are TLKs and possibly Asf1 localized to active genes during elongation? How does absence of TLKs influence recruitment of elongation or processing factors, and accumulation of other chromatin markers such as trimethyl H3-lysine 4, which appears before the elongation checkpoint (Figure 1). Of course, it will also be crucial to determine whether loss of Asf1 activity in *C. elegans* mimics or influences the *tlk-1(RNAi)* elongation defect, and whether *C. elegans* TLK-1 regulates Asf1 activity *in vivo*. Finally, it may be informative to investigate how *tlk-1* interacts functionally with other genes that are important for transcription elongation. Whatever new questions and ideas are raised by these future experiments, further analysis of TLKs is bound to yield important new information about both transcription elongation and chromatin assembly.

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