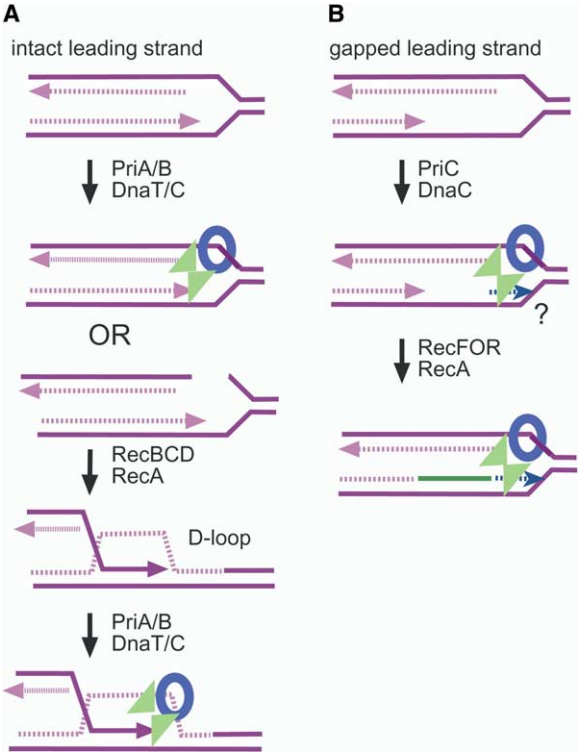

Figure 1. Alternate Replication Restart Pathways

(A) In the presence of an intact leading strand, PriA/B/DnaT/C can reassemble DnaB helicase (blue ovals) and DNA polymerase III holoenzyme (green triangles) on the fork. Alternatively, after fork breakage, recombination via RecABCD can form a D loop intermediate, a substrate for PriAB/DnaT/C restart, with an intact 3′ leading strand.

(B) In a gapped fork, PriC/DnaC can reload DnaB (blue ovals) and DNA polymerase II holoenzyme (green triangles) and possibly reprime the leading strand. RecAFOR recombination is required to fill replication gaps.

the biochemistry. Contrary to expectations from genetic analysis (Sandler, 2000), Heller and Marians (2005) did not observe any requirement for the Rep helicase protein in the PriC-dependent in vitro restart pathway. Perhaps in vivo, Rep is necessary to unwind the lagging strand or dislodge proteins to reveal a DnaB loading site, not an issue in the in vitro reactions. Furthermore, there appear to be more than these two systems for DnaB loading, including PriA/PriC, PriA/PriB/PriC, and even DnaA/PriC-dependent pathways (Hinds and Sandler, 2004; Sandler, 2005; Sandler et al., 1999). This suggests that all four proteins, DnaA, PriA, PriB, and PriC, can be used to provide specificity in a combinatorial fashion, dependent on the initiation substrate.

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Transcriptional Activation: Mediator Can Act after Preinitiation Complex Formation

The Mediator complex connects transcriptional activators bound at enhancers with RNA polymerase (Pol) II. Wang et al. (2005) demonstrate that Mediator also has an important role in transcriptional activation after recruitment of the Pol II machinery to promoters.

Transcriptional activator proteins bound (directly or indirectly) at their target sites stimulate the synthesis of specific mRNAs by three fundamental, and not mutually exclusive, mechanisms. First, activator proteins stimulate the "recruitment" of the basic transcription machinery (i.e., RNA polymerase and associated factors) with the core promoter by directly interacting with a component(s) of this machinery. Second, in a "postrecruitment" mechanism, activators stimulate the activity of the basic transcription machinery at a step after its association with the promoter in a preinitiation complex. Third, activators can modify the DNA template by interacting with chromatin-modifying activities and targeting them to the cognate promoters. The resulting local alterations in chromatin structure can stimulate preinitiation complex formation or a postrecruitment step. For purposes here, the terms recruitment and

postrecruitment refer to the basic transcription machinery and are not to be confused with recruitment of chromatin-modifying activities.

Experimentally, the distinction between recruitment and postrecruitment mechanisms has been addressed in living cells by determining the relationship between transcriptional activity and the amount of RNA Pol II and general transcription factors at the promoter as assayed by chromatin Immunoprecipitation (ChIP). In yeast cells, transcriptional activity is very strongly correlated with the association of Pol II and initiation factors, indicating that transcription of the vast majority of genes is regulated at the recruitment step (Kuras and Struhl, 1999; Li et al., 1999). In flies and mammals, there are specific examples of activators stimulating transcription at either the level of recruitment or postrecruitment (Rougvie and Lis, 1988; Agalioti et al., 2000), although it is unknown which mechanism predominates.

Activator proteins can interact with many components of the basic Pol II machinery in vitro, but it has been difficult to establish which interactions are physiologically relevant. However, there is considerable evidence that the Mediator complex is a key, and perhaps the major, target of activator proteins within the basic Pol II machinery (Malik and Roeder, 2000; Myers and Kornberg, 2000). The Mediator complex consists of 25–30 proteins, and it is conserved throughout eukaryotes (Sato et al., 2004). Although Mediator is not required for basal Pol II transcription from a core promoter in vitro, and hence is not usually classified as a general initiation factor, it should be considered as a component of the basic Pol II transcription machinery. In particular, several subunits of Mediator are essential for general Pol II transcription in yeast cells, Mediator can stimulate basal Pol II transcription in vitro, and Mediator associates with Pol II to generate a stable complex sometimes called the Pol II holoenzyme (Malik and Roeder, 2000; Myers and Kornberg, 2000). Many activator proteins can directly interact with Mediator in vitro, and most importantly, cells containing mutations of specific Mediator subunits show severely diminished responses to selected activators without a general effect on Pol II transcription.

It has been generally believed that Mediator functions at the recruitment step, possibly by stabilizing an activator-dependent reinitiation intermediate such that multiple rounds of transcription occur from a initial preinitiation complex (Struhl, 1996; Yudkovsky et al., 2000). In yeast, Mediator is required for formation/stability of preinitiation complexes that are dependent on a functional activator protein (Kuras and Struhl, 1999; Li et al., 1999). In addition, activator-dependent association of Mediator with promoters in vivo can occur independently of and prior to Pol II association (Cosma et al., 2001; Park et al., 2001). Thus, Mediator serves as a bridge that connects the activator protein bound to specific genomic sites with the function of the basic Pol II machinery at the promoter.

In a recent issue of *Molecular Cell*, Arnold J. Berk's laboratory showed that Mediator also functions at a postrecruitment step (Wang et al., 2005). This laboratory previously identified the Med23 subunit of Mediator as a direct and physiologically relevant target for the ELK1 and E1A activators (Stevens et al., 2002). Of particular importance, ES cells lacking Med23 contain a normal Mediator complex (except for Med23) and support transcription of most genes, but they are severely defective in activation by ELK1 and E1A. In vitro, the interaction of these activators with Med23 stimulates preinitiation complex formation, indicating that Mediator functions at the recruitment step under these conditions. To determine which steps are controlled by this activator-mediator interaction in vivo, Wang et al. (2005) first used whole-genome transcriptional profiling to identify *Egr1* as a gene that depends on Med23 for activation by ELK1 in response to serum stimulation. By using ChIP, the association of many proteins involved in transcription, chromatin-modifying complexes, and histone modifications at the endogenous *Egr1* locus were compared in wild-type and Med23-deficient cells.

The key observation is that Med23 dramatically stimulates recruitment of Mediator and transcriptional activity, but it has only a modest effect on recruitment of Pol II and other general factors at the promoter. In part, the modest effect is due to ELK1-independent association of the basal Pol II machinery at the promoter. Importantly, however, the presence of Med23 confers a 5-fold increase in the rate of initiation by Pol II molecules bound at the promoter. As assayed by salt sensitivity in vivo, there appears to be a pause between Pol II association at the promoter and the early phase of elongation; the mechanistic basis of this pause is unknown but distinct from the paused Pol II that occurs at the *Drosophila Hsp70* gene (Rougvie and Lis, 1988).

The mechanism by which the activator-Mediator interaction stimulates a postrecruitment step is unknown, but several possibilities appear unlikely. Med23 is not important for the ELK1-dependent recruitment of the Swi/Snf nucleosome-remodeling complex, increased histone acetylation and histone methylation at H3-K4, or loss of nucleosome density; hence, the Med23-dependent postrecruitment step does not appear to involve changes in chromatin structure. In addition, Med23 does not affect phosphorylation of serines 2 or 5 of the C-terminal domain of Pol II, nor does it affect the relative amount of several elongation factors (NELF, DSIF, and P-TEFb) with Pol II. It is tempting to speculate that the postrecruitment step involves isomerization of the preinitiation complex, promoter melting, or promoter clearance. Perhaps this postrecruitment step involves conformational effects on Mediator upon interaction with Pol II, particularly because the Mediator-Pol II interaction involves the Pol II C-terminal domain that has multiple roles in the transcriptional process.

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