SCF^{Met30} substrate, and now, E3 substrate-recognition factor—into one protein effectively coordinates the activity of two multisubunit complexes, the SCF^{Met30} ligase complex and Met4 transcriptional complex, in response to rapid changes in extracellular conditions. In the current model, Met4 function is completely shut down during normal cell growth by a double kill; polyubiquitylation of Met4 blocks it from accessing the target promoters and degradation of its cofactors prevents any residual transactivation. In response to sulfur deprivation or heavy-metal stress, a simple and quick release of Met4 from the SCF^{Met30} ligase results from the dissociation of Met30 and Skp1 and allows cells to rapidly activate Met4 target genes by simultaneously stabilizing its cofactors and allowing Met4 to access its target genes.

A remarkable feature of cullin-family proteins is the ability of individual cullins to associate with different motifs that are evolutionarily conserved and present in many proteins, such as binding of BC box by CUL2 and CUL5, BTB-domain proteins by CUL3, and WD40-repeat proteins by CUL4. The presence of these motifs in numerous proteins—mammalian cells express as many as or more than 70 F box-, 50 BC box-, 200 BTB-, and 90 DDB1-interacting WD40 proteins—already suggested the assembly of as many as 400 to 500 distinct CRLs. The current finding that a SCF/CRL1 E3 ligase can use its own substrate to recruit another substrate could further widen the scope of CRLs assembly. In fact, as the authors noted, there have been several examples where recognition of a SCF substrate involves an additional factor besides the F box protein (Figure 1): Cks1 and cyclin T1 in targeting p27 and Cdk9 for ubiquitylation by SCF^{Skp2}, respectively (Hao et al., 2005; Kiernan et al., 2001). TAZ, also a transcription factor that plays a key role in Hippo pathway, has previously been reported to facilitate polycystin 2 ubiquitylation by $SCF^{\beta\text{-}Trcp}$ (Tian et al., 2007) and was recently identified itself to be the substrate of the same $SCF^{\beta\text{-}Trcp}$ (Liu et al., 2010). These intriguing observations suggest the possibility that other CRLs may be equally versatile and further expand the potential pool of substrates targeted by CRLs.

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The Three Rs of Transcription: Recruit, Retain, and Recycle

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The dynamic protein interactions required for transcription are functionally important yet poorly understood; in this issue of *Molecular Cell*, Zobeck et al. (2010) resolve the sequential recruitment and selective recycling of transcription factors at an actively transcribing locus in *Drosophila*.

Transcription of protein-coding genes by RNA polymerase II (Pol II) is a highly dynamic process considered to have several distinct steps in which Pol II function is regulated by transient protein interactions. These steps include preinitiation complex assembly, transcription initiation, promoter escape, processive transcription elongation, and transcription termination (Selth et al., 2010). Much of our knowledge about the assembly and movement of the Pol II transcription machinery comes from relatively static biochemical and molecular studies, in which transcription reactions have been initiated, allowed to proceed for a certain amount of time, and then stopped for off-line analysis (Voss and Hager, 2008). While these studies have collectively provided many mechanistic insights into transcription, they are fundamentally static snapshots of a highly dynamic process. However, recent technological advances in microscopy now allow for

live-cell imaging of Pol II during transcription, with greatly enhanced temporal and spatial resolution (Yao et al., 2007). In this issue of *Molecular Cell*, Lis and colleagues use this technology for real-time kinetic analyses of transcription factor (TF) recruitment to an actively transcribing locus.

The structural dynamics that accompany the transcription process are readily visualized in polytene chromosomes from *Drosophila* salivary glands. When these large multistrand chromosomes form, multiple copies of the same gene remain closely associated, thus spatially amplifying sites of active transcription (Lis, 2007). One of the best-characterized models to study transcription in *Drosophila* are the *Hsp70* loci, which form chromosomal puffs upon heat shock (HS) that are indicative of rapid and robust activation of transcription (Lis, 2007). While many regulators of *Hsp70* expression have been identified (Fuda et al., 2009), a complete understanding of regulation itself requires an understanding of how these factors are recruited and assembled onto the activated genes.

To study the dynamic recruitment of TFs to the *Hsp70* loci in real time, Zobeck et al. (2010) utilized transgenic fly lines that each express an eGFP- or mRFP-tagged TF involved in HS gene activation and monitored TF recruitment to HS-induced chromosomal puffs using various microscopy methods. Three proteins were studied: P-TEFb, a kinase that targets Pol II; Spt6, a nucleosome chaperone; and Topo I, which unwinds supercoiled DNA. Each of these transgenic lines coexpresses a subunit of Pol II (Rpb3), tagged to be complementary to the TF (either eGFP or mRFP). Initial laser scanning confocal microscopy (LSCM) experiments confirmed that all three TFs are recruited together with Pol II to the *Hsp70* loci in living *Drosophila* salivary gland cells upon HS treatment (hence studying them "on the fly, in the fly"). The authors then used spinning disk confocal microscopy (SDCM) to acquire images at frame rates 3-fold higher than LSCM, improving the temporal resolution up to six frames/min to refine the precise timing of recruitment for each TF. Data were collected from several independent salivary gland nuclei over a period of 20 min after HS. Remarkably, there was little cell-to-cell variation in the calculated times and rates of recruitments for each factor. By fitting the buildup of fluorescence intensities to obtain rates of TF recruitment, the authors found that the process initiated with the arrival of the heat shock factor (HSF) (Figure 1A) seconds after HS starts (17 ± 1 s). P-TEFb and Pol II were recruited at similar times after HS induction (95 ± 3 s and 103 ± 2 s, respectively). Spt6 and Topo I followed afterwards, at 115 ± 2 s post-HS for Spt6 and 132 ± 4 s for Topo I. Together, these data demonstrate that TFs follow a sequential order of recruitment to the activated *Hsp70* loci and that this process occurs synchronously within a population of cells.

Once initiated, TF recruitment continues for 8–10 min postactivation, as revealed by total fluorescence intensity measurements of P-TEFb, Spt6, and Topo I. Notably, this is longer than needed to fully saturate the binding of these same factors to their chromatin binding sites—chromatin immunoprecipitation (ChIP) data for P-TEF-b and Spt6 show saturation by ~3 min. Similar results were also obtained for Pol II, confirming the initial report of this recruitment phenomenon (Yao et al., 2007), thus supporting a model where both general and gene-specific factors are recruited in stoichiometric excess and retained at sites of active transcription.

To investigate a possible kinetic basis for this retention effect, Zobeck et al. measured the local exchange dynamics of the three TFs at the *Hsp70* loci using fluorescence recovery after photobleaching (FRAP) experiments. Their data show that these dynamics vary considerably as a function of the TF and time post-HS. For example, at a point early in the HS treatment, both P-TEFb and Topo I rapidly recover after photobleaching (~80% recovery within 2 min), while Spt6 recovery is slower (~50% recovery). However, at later times (40–60 min into HS), recruitment of all of these proteins is dramatically slowed (~40% recovery of P-TEFb and Topo I; no recovery for Spt6). Thus, as transcription progresses, a portion of the TF population remains associated with the *Hsp70* loci, suggesting they are actively retained and that diffusion into the area is limited. Similar results obtained for Pol II itself led to the proposal of a so-called "transcription compartment" that could generate high local concentrations of TFs readily available for subsequent rounds of transcription (Yao et al., 2007).

How might such a "compartment" be dynamically formed near actively transcribing genes? One candidate protein that could regulate this process is the enzyme poly(ADP-ribose) polymerase (PARP), which attaches ADP-ribose molecules to target proteins and creates polymers of ADP-ribose (Tulin and Spradling, 2003). Given that PARP is required after HS to induce expression of *Hsp70* genes in *Drosophila* and for the formation of chromosomal puffs at this locus (Tulin and Spradling, 2003), Zobeck et al. directly examined the role of PARP activity in the local retention of TFs. Strikingly, treatment with the PARP inhibitor PJ34 significantly reduced Pol II retention near the *Hsp*70 locus, effectively eliminating the reduced FRAP observed in late HS without PJ34 treatment. These results suggest that PARP's generation of ADP-ribose polymers is essential for retaining Pol II at the transcription site. Analogous noncovalent matrices that restrain protein diffusion have been proposed for other biological processes (Buchan and Parker, 2009), although differing in composition and mechanism of assembly.

Collectively, these data favor a model where individual TFs are recruited to activated genes in a series of sequential steps, implying that controlling the recruitment efficiency of any of these TFs could significantly impact transcriptional yield. In contrast, studies done in other experimental systems suggest that activated genes are recruited to foci enriched in preassembled complexes containing phospho-Pol II and various TFs (Chakalova et al., 2005). It is not yet known under what circumstances each of these model mechanisms is favored over the other, but one could imagine that it might be gene or cell-type specific. Interestingly, just as polytene chromosomes have multiple copies of genes that allow amplified transcription of regulated genes, it has been proposed that colocalization to these "transcription factories" in diploid cells boosts the expression of coregulated genes (Chakalova et al., 2005).

Determining what TFs are specifically ADP-ribosylated by PARP and to what

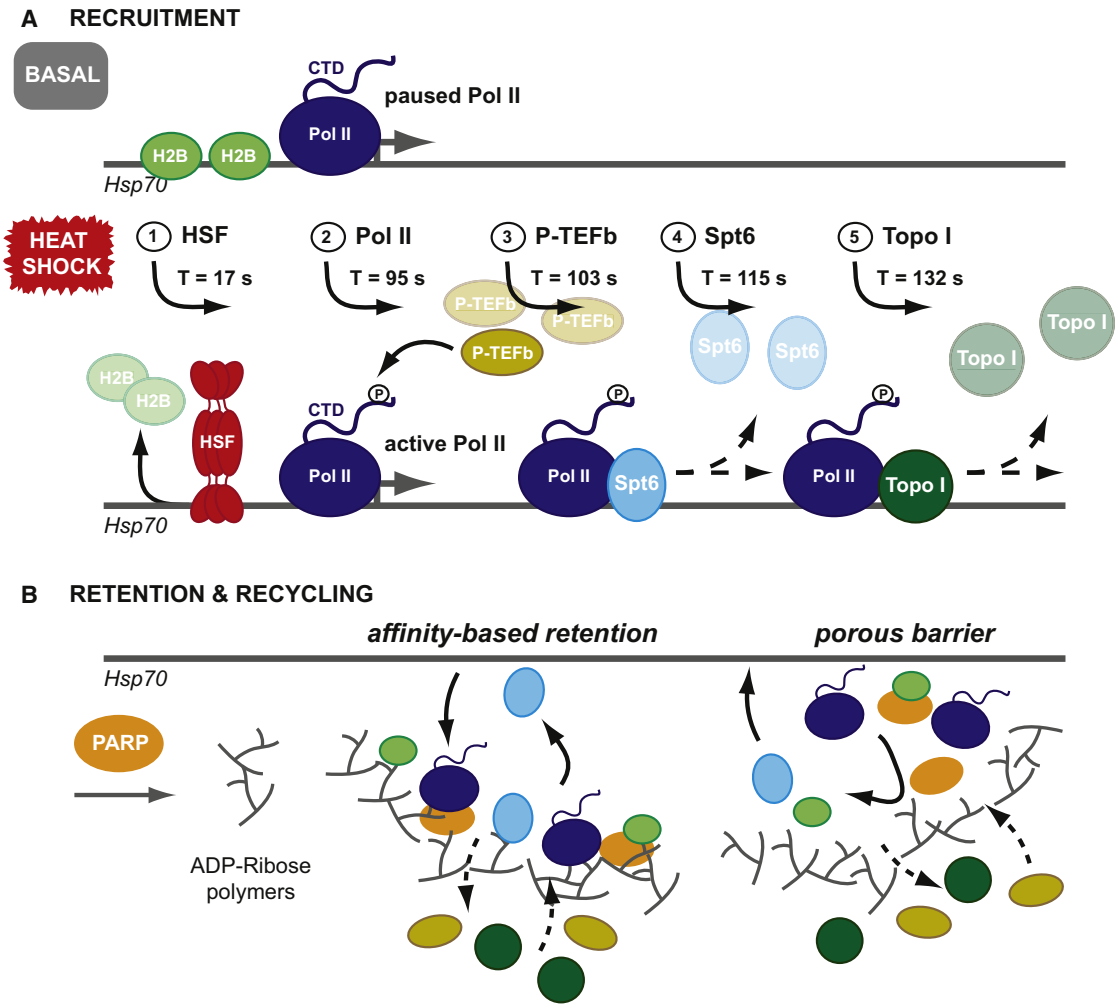

Figure 1. Sequential Recruitment and Selective Retention of Transcription Factors at the *Hsp70* Locus after Heat Shock
(A) Heat shock induces sequential recruitment of TFs to *Hsp70*, beginning with HSF. Additional TFs are recruited in synchronous waves across independent nuclei and to stoichiometric excess over chromatin binding sites.
(B) FRAP data demonstrate that TFs are selectively retained at the *Hsp70* loci throughout HS. PARP activity is required for TF retention in this "transcription compartment," possibly through the formation of a matrix of polymerized ADP-ribose. This matrix could participate in TF retention and recycling by affinity-based retention of TFs for the ADP-ribose polymers, formation of a porous barrier, or some combination of both models.

extent will be an important step to evaluate what factors make up the "transcription compartment" and to establish how ADP-ribose polymers cause retention of factors (Figure 1B). To this end, additional FRAP experiments combined with PARP inhibitor treatments could assess whether the mobilization of the other TFs is more or less affected than Pol II by PARP inactivation. Because Spt6 was almost completely retained at the *Hsp70* loci after prolonged HS, one would expect this protein to be the most affected by PARP inactivation. If the ADP-ribose polymer/transcription compartment model is correct, then the efficiency of *Hsp70* gene transcription should be dependent on the formation of this compartment; therefore, an inhibitor-mediated decrease in PARP activity should also reduce *Hsp70* transcript levels. Moreover, the fact that PARP remains associated at the *Hsp70* loci at the same level before and after HS indicates that the rate-limiting step in ADP-ribose polymerization (and by extension, formation of the compartment) is not the recruitment of PARP itself, but rather the activation of PARP molecules already localized to the *Hsp70* loci. Insight into what triggers PARP activity during HS and, conversely, what signals repress PARP activity awaits further studies.

The work presented by Zobeck et al. as well as work by others (Voss and Hager, 2008) successfully combines biochemical and biophysical methods to study the mechanistic details of dynamic biological processes. While several features of complexes at the heart of such processes challenge existing methods in their size, dynamic composition, and interactions with surrounding matrices, improved structural and kinetic characterization cannot be far behind.

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Seeking Resolution: Budding Yeast Enzymes Finally Make the Cut

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Genetic studies reported in *Molecular Cell* (Ho et al., 2010) identify Mus81-Mms4 and Yen1 as the structure-specific endonucleases that cleave most Holliday junctions. A failure in this key step has profound effects on mitotic genome stability.

Homologous recombination (HR) is an important DNA repair mechanism that promotes mitotic genome stability and correct chromosome distribution during meiosis. Early HR models were based on meiotic studies in fungi, with the budding yeast *Saccharomyces cerevisiae* emerging as the premier model organism in which to focus on molecular details. Why, then, has it been so difficult to define roles in yeast for the structure-specific endonucleases implicated in completing HR in other model organisms? In this issue of *Molecular Cell*, elegant genetic studies of Ho et al. make headway in resolving this dilemma and provide novel insight into how perturbations at this specific step can compromise genome integrity.

Most mitotic HR is assumed to initiate with a double-strand break (DSB). Repair requires homology-driven invasion of an intact duplex and its subsequent use as a template to synthesize new DNA that spans the break (reviewed by Heyer et al., 2010). As illustrated in Figure 1, interaction of both ends with an intact duplex culminates in formation of two Holliday junctions (HJs) that join the participating chromosomes. A given HJ can be resolved by cleaving either the originally exchanged strands or the non-exchanged strands. The latter mode alters the linkages of genetic markers that flank the HJ, thereby producing reciprocal crossover (CO) products. As an alternative to cleavage, double HJs can be "dissolved" by the combined action of a helicase and topoisomerase, producing only noncrossover (NCO) products. In addition to canonical DSB repair, there exists a second repair mechanism that does not require HJ resolution and yet nevertheless produces a CO product: break-induced replication (BIR). In BIR, one end of the broken chromosome engages the repair template, setting up a replication fork that moves to the end of the intact chromosome; the other broken end is lost. Three properties distinguish BIR from canonical DSB repair: (1) the repaired chromosome *always* has the CO configuration for markers flanking the DSB, (2) only a *single* CO product is generated, and (3) BIR uniquely requires the Pol32 subunit of replicative DNA polymerase δ (Lydeard et al., 2007).

Since the proposal of the HJ intermediate more than 40 years ago (Holliday, 1964), the hunt has been on for "the" eukaryotic enzyme that catalyzes its cleavage. At least four enzymes/complexes with potentially relevant biochemical activity have been characterized: Mus81-Mms4, Slx1-Slx4, Rad1-Rad10, and Yen1 (see Klein and Symington, 2009). While other model organisms seem to rely primarily on one of these proteins/complexes for HJ cleavage in vivo, an ongoing problem for the field has been that the mitotic assays used to study HR in budding yeast have thus far failed to definitively link any of these candidates to CO production.

How do the genetic approaches of Ho et al. begin to resolve this conundrum? The initial insight came from experiments that used a meganuclease to introduce a single DSB in a diploid genome. Because a targeted DSB increases HR many orders of magnitude, it was possible to nonselectively "capture" all the chromosomes present at the time of DSB induction and to follow their segregation into daughter cells. Loss of heterozygosity (LOH), which is diagnostic of a CO event, could then be monitored in both of the daughter cells that individually give rise to half of the resulting colony.