such as P/CAF and SRC1 (reviewed in Coqueret, 2002) depending on the promoter context, so it will be critical to determine the acetylation status of both C/EBPβ and histones on cyclin D1 target gene promoters. A third important question is why should LIP activate these target genes? Does it merely displace or antagonize the transcriptional activity of the bound LAP isoforms? LIP has been reported to exhibit an increased DNA binding affinity relative to the LAP isoforms of C/EBPβ. Does this then imply that there is a reciprocal relationship between cyclin D1 and LIP expression in cancer? In breast cancers, LIP was reported to be predominantly overexpressed in ER-negative tumors (Milde-Langosch et al., 2003; Zahnow et al., 1997), while cyclin D1 is usually elevated in ER-positive tumors. Curiously, overexpression of the translation initiation factor eIF4e has been shown to increase cyclin D1 expression, while decreased expression of eIF4e and eIF2α has been correlated with increased LIP expression (Calkhoven et al., 2000). However, other mechanisms involving both gene amplification and transcriptional activation are involved in the overexpression of cyclin D1 in cancer. A more thorough examination of the mechanisms regulating the expression of different C/EBPβ isoforms and their activities on different target gene promoters is, however, clearly warranted. Finally, what are the functions of the specific cyclin D1 target genes identified in this study in the etiology of cancer? Thus, while numerous interesting questions remain to be answered, the future for combining molecular genetic and data mining approaches to discover novel interactions and pathways in human cancer appears bright.

**Jeffrey M. Rosen**

**Department of Molecular and Cellular Biology**
**Baylor College of Medicine**
**1 Baylor Plaza**
**Houston, Texas 77030**

**Selected Reading**

Calkhoven, C.F., Muller, C., and Leutz, A. (2000). Genes Dev. *14*, 1920–1932.

Coqueret, O. (2002). Gene *299*, 35–55.

Hui, R., Cornish, A.L., McClelland, R.A., Robertson, J.F., Blamey, R.W., Musgrove, E.A., Nicholson, R.I., and Sutherland, R.L. (1996). Clin. Cancer Res. *2*, 923–928.

Kowenz-Leutz, E., and Leutz, A. (1999). Mol. Cell *4*, 735–743.

Lamb, J., Ramaswamy, S., Ford, H.L., Contreras, B., Martinez, R.V., Kittrell, F.S., Zahnow, C.A., Patterson, N., Golub, T.R., and Ewen, M.E. (2003). Cell *114*, this issue, 323–334.

Milde-Langosch, K., Loning, T., and Bamberger, A.M. (2003). Breast Cancer Res. Treat. *79*, 175–185.

Xu, M., Nie, L., Kim, S.H., and Sun, X.H. (2003). EMBO J. *22*, 893–904.

Zahnow, C.A. (2002). Breast Cancer Res. *4*, 113–121.

Zahnow, C.A., Younes, P., Laucirica, R., and Rosen, J.M. (1997). J. Natl. Cancer Inst. *89*, 1887–1891.

Zhu, S., Yoon, K., Sterneck, E., Johnson, P.F., and Smart, R.C. (2002). Proc. Natl. Acad. Sci. USA *99*, 207–212.

# TFIIS and GreB: Two Like-Minded Transcription Elongation Factors with Sticky Fingers

**How the structurally distinct transcription factor TFIIS from eukaryotes and its bacterial counterpart GreB act to convert their cognate RNA polymerases into ribonucleases has been a longstanding question. Now, two new structures of these factors bound to their respective RNA polymerases (Opalka et al. and Kettenberger et al. [this issue of *Cell*]) suggest how they accomplish this feat.**

Eukaryotic TFIIS (also known as SII) and its bacterial counterpart GreB are unique among all transcription factors: they are the only known transcription factors capable of restarting arrested RNA polymerases (Wind and Reines, 2000; Uptain et al., 1997). TFIIS is expressed ubiquitously in eukaryotes, where it acts specifically to reactivate arrested RNA polymerase II to ensure efficient synthesis of mRNA. GreB performs a similar task in bacteria.

The tendency to arrest is an inherent property of RNA polymerases. Upon arrest, an RNA polymerase stops transcribing, refuses to budge even in the presence of sufficient concentrations of ribonucleoside triphosphates to support further transcript elongation, and clings tenaciously to its DNA template and nascent transcript, presenting a potential impediment to other RNA polymerases.

Arrest occurs when the 3′-end of a nascent transcript loses critical base pair contacts with the DNA template and is displaced from, or in some cases, completely extruded from the polymerase active site through a pore or channel that is situated directly beneath the primary catalytic magnesium ion and through which incoming ribonucleoside triphosphates are believed to enter the active site (Komissarova and Kashlev, 1997; Nudler et al., 1997; Cramer et al., 2000, 2001; Zhang et al., 1999). Elegant biochemical studies have shown that TFIIS and GreB restart arrested RNA polymerases by a remarkable mechanism that proceeds with TFIIS- or GreB-promoted ribonucleolytic cleavage of the displaced 3′-end of the nascent transcript, producing a new 3′-end that is properly base paired with the DNA template in the active site and, thus, can be extended by polymerase (Wind and Reines, 2000; Uptain et al., 1997). TFIIS and GreB are capable of promoting endonucleolytic removal of as many as 17 nucleotides from the 3′-ends of nascent transcripts in arrested RNA polymerase elongation complexes while still allowing efficient reextension of those transcripts by polymerase. In part because pyrophosphoryolysis—chemically the reverse of the polymerization reaction—can also result in removal of large oligonucleotides from the 3′-ends of transcripts in arrested elongation complexes, it was proposed that transcription elongation factor-promoted removal of the 3′-ends of nascent transcripts is carried out by the polymerase's own active site (Rudd et al., 1994).

Exactly how TFIIS and GreB can produce in their cog-

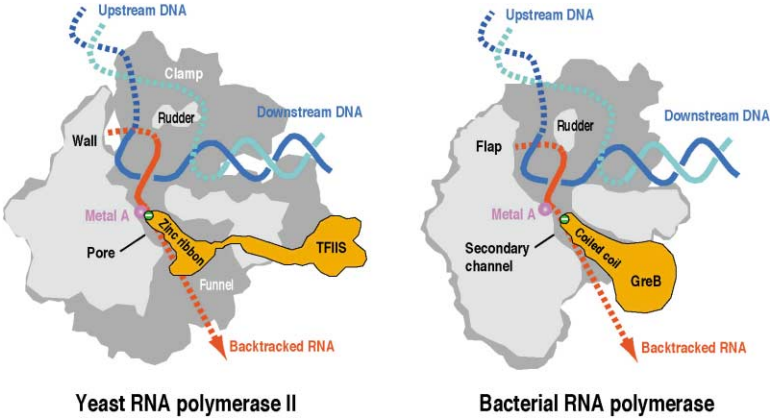


Figure 1. Cutaway View of Complexes of Arrested RNA Polymerase II and TFIIS (Left) and Arrested Bacterial RNA Polymerase and GreB (Right)

Shown are major features of the complexes, including the RNA polymerase II pore and bacterial RNA polymerase channel, TFIIS, and GreB (orange), and the primary catalytic magnesium ion (Metal A, pink). The positions of the conserved acidic residues in TFIIS and GreB are indicated by the green circles. The nascent transcript is shown in red, and the DNA template is blue. The presumed locations of backtracked RNAs are indicated by the dashed arrows.

nate RNA polymerases a change so profound that it converts their active sites from "polymerizing" to "ribonucleolytic," however, has been a mystery and the subject of intense speculation. Complicating matters is that while TFIIS and GreB apparently evolved to perform similar critical tasks in mRNA synthesis, they are quite unrelated proteins, in both primary amino acid sequence and three-dimensional structure. TFIIS is composed of a conserved N-terminal domain, which is dispensable for transcript cleavage activity, and a central three-helix bundle joined by an extended flexible linker to a C-terminal zinc ribbon domain composed of a three-stranded $\beta$ sheet stabilized by a tetrad of four cysteine residues that chelate a single zinc ion. GreB is composed of two domains: an N-terminal domain composed of an extended antiparallel $\alpha$-helical coiled-coil dimer and a C-terminal globular domain composed of a $\beta$ sheet.

Given the dramatic structural differences between TFIIS and GreB, it seemed that nothing short of detailed information on their structures in association with their cognate RNA polymerases would ultimately yield the kind of insights necessary to determine once and for all how these remarkable transcription factors accomplish their missions. Two articles in this issue of Cell provide just such analyses.

Research teams led by Patrick Cramer (University of Munich) and Seth Darst (Rockefeller University) report structures of complexes of TFIIS and GreB with their respective RNA polymerases (Kettenberger et al., 2003; Opalka et al., 2003) and provide, for the first time, a glimpse of the inner workings of TFIIS and GreB transcriptional activities (Figure 1). Surprisingly, their results provide support for the idea that, despite the lack of conservation between their primary and tertiary structures, TFIIS and GreB use very similar strategies to convert the active sites of their RNA polymerases into ribonucleases.

After diffusing TFIIS into preformed crystals of 12 subunit yeast RNA polymerase II, Cramer and colleagues were able to derive a 3.8 Å model of a TFIIS-pol II complex from X-ray diffraction data. In the TFIIS-pol II complex, the zinc ribbon domain of TFIIS is inserted deeply into the RNA polymerase II pore, positoning two highly conserved acidic residues at the tip of a thin $\beta$ hairpin turn in the enzyme's active site. In their paper, Darst and colleagues describe a model of GreB bound to *E. coli* RNA polymerase built by fitting high-resolution RNA polymerase and GreB structures to a 15 Å structure of the GreB-RNA polymerase complex determined by cryo-electron microscopy and image processing of helical crystals. In their model, the GreB N-terminal coiled-coil reaches deep into a channel corresponding to the RNA polymerase II "pore." At the tip of the GreB coiled-coil are two conserved acidic residues, which again are placed into the polymerase active site. In both models, the conserved acidic residues are adjacent to the primary catalytic magnesium ion, where they are perfectly placed to position (or reposition) a second, more loosely bound catalytic magnesium ion critical for ribonuclease activity. Thus, these findings provide support for the intriguing model that TFIIS and GreB function not simply as effectors that interact with their RNA polymerases and act at a distance to induce conformational conversion of their active sites from a polymerase to a ribonuclease, but as direct participants that become themselves integral components of the ribonuclease active sites.

These new and elegant structural studies of RNA polymerases in association with transcription factors that regulate their activities join recently reported high-resolution structures of bacteriophage, bacterial, and eukaryotic RNA polymerase at the dawn of a new era in transcription research, when we begin to view the interplay of RNA polymerases and the transcription factors that control them at atomic resolution.

**Ronald C. Conaway,[1,2] Stephanie E. Kong,[1] and Joan Weliky Conaway[1,2,3]**
[1]Stowers Institute for Medical Research
Kansas City, Missouri 64110
[2]Department of Biochemistry and Molecular Biology
Kansas University Medical Center
Kansas City, Kansas 66160
[3]Department of Biochemistry and Molecular Biology
University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma 73190

**Selected Reading**

Cramer, P., Bushnell, D.A., Fu, J., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R., and Kornberg, R.D. (2000). Science *288*, 640–649.

Cramer, P., Bushnell, D.A., and Kornberg, R.D. (2001). Science *292*, 1863–1875.

Kettenberger, H., Armache, K.-J., and Cramer, P. (2003). Cell *114*, this issue, 347–357.

Komissarova, N., and Kashlev, M. (1997). Proc. Natl. Acad. Sci. USA *94*, 1755–1760.

Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, A. (1997). Cell *89*, 33–41.

Opalka, N., Chlenov, M., Chacon, P., Rice, W.J., Wriggers, W., and Darst, S.A. (2003). Cell *114*, this issue, 335–345.

Rudd, M.D., Izban, M.G., and Luse, D.S. (1994). Proc. Natl. Acad. Sci. USA *91*, 8057–8061.

Uptain, S.M., Kane, C.M., and Chamberlin, M.J. (1997). Annu. Rev. Biochem. *66*, 117–172.

Wind, M., and Reines, D. (2000). Bioessays *22*, 327–336.

Zhang, G., Campbell, E.A., Minakhin, L., Richter, C., Severinov, K., and Darst, S.A. (1999). Cell *98*, 811–824.

# Eukaryotic DNA Replication Origins: Reconciling Disparate Data

**In this issue of *Cell*, Anglana and coworkers provide new insight into the nature of mammalian replication origins that helps to reconcile the divergent views that emerged over the past decade. Taken together with other studies, we can see how replication origins have evolved in response to the demands of animal development.**

About 13 years ago, three papers appeared in *Cell*, each claiming to have mapped initiation sites for DNA replication in Chinese hamster cells within the intergenic region downstream of the DHFR gene. Remarkably, two of these studies concluded that initiation events originated from specific genomic sites (Burhans et al., 1990; Handeli et al., 1989), while the third concluded that initiation events were distributed uniformly throughout the intergenic region (Vaughn et al., 1990). But despite the development and application of novel origin mapping methods and the analysis of a variety of genomic sites in different organisms, a paradox remained (DePamphilis, 1999). Studies employing 2D gel fractionation of total genomic DNA to detect replication bubbles or to map the polarity of replication forks generally concluded that initiation events were distributed uniformly over regions as large as 55 kb ("initiation zones"), whereas methods that mapped either the relative distribution or the relative abundance of nascent DNA strands along the genome invariably concluded that initiation events originated within specific loci of ~1 kb or less. Furthermore, site specificity was not detected in the early embryos of frogs, flies, and fish, and the question of whether or not these genomes, like those in yeast, contain sequences that impart autonomous replication to extrachromosomal DNA (ARS elements) remained controversial. True, mammalian replication origins do contain "replicators" (genetically required sequences that impart origin activity when translocated to other chromosomal sites), but while these replicators can be inactivated by internal deletions, they lack an identifiable, genetically required consensus sequence, such as the ARS consensus sequence in budding yeast replicators (Prioleau et al., 2003). How might such disparate data be reconciled?

A new insight is revealed by Anglana et al. (2003): nucleotide pools can modulate initiation site selection within single cells. The authors begin by confirming that a novel method for mapping initiation sites on single DNA fibers ("molecular combing") maps the same specific 0.3 kb origin downstream of the GNA13 gene in Chinese hamster cells that they had previously identified by other methods. Using molecular combing, they identify five secondary (low frequency) initiation sites within 128 kb of the single, primary (high frequency) oriGNA13. Remarkably, their results reveal that initiation at the primary origin represses initiation at the secondary origins. Moreover, increasing the nucleotide pool by addition of DNA precursors to the culture medium reduces the frequency of initiation at secondary origins, whereas reducing the nucleotide pool by addition of hydroxyurea (a specific inhibitor of ribonucleotide reductase) increases the frequency of initiation at secondary origins. This implies that synchronization of cells at their G1/S boundary by reducing nucleotide pools would favor the appearance of initiation zones, a caveat that may account for some of the data in the literature. It also implies that the frequency of initiation sites in mammals is similar to the frequency in yeast and in frog eggs (1/20 to 1/30 kb).

How might nucleotide pools affect initiation site selection? Four possibilities come to mind. First, the ability of ORC to assemble pre-RCs at specific DNA sites may depend on dNTPs, as it does on ATP (Bell and Dutta, 2002). Second, previous studies have shown that initiation at one origin will prevent initiation at an identical origin many kb away, presumably because replication forks passing through replication origins prevent either assembly or activation of pre-RCS. Reducing the concentration of dNTPs reduces the rate of DNA synthesis, thus allowing more time for initiation at secondary origins. Third, inhibiting DNA synthesis can result in extensive DNA unwinding in the absence of concomitant DNA synthesis. In that event, DNA polymerase $\alpha$:DNA primase, the enzyme that initiates DNA synthesis de novo, may begin on single-stranded DNA some distance away from the site where replication forks were assembled. This would create the appearance of an initiation zone with the primary origin at its center. Fourth, the existence at pre-RCs of a molar excess of Mcm(2-7), the hexameric DNA helicase that unwinds the DNA, allows the possibility that some helicases may translocate from primary initiation sites to secondary initiation sites when dNTP pools are low. Only time will tell which mechanism is correct.

What might distinguish primary from secondary origins? Primary and secondary origins clearly exist in yeast, where some origins are activated once each cell division cycle while others are not, and in bacteriophage such as T7 where deletion of the primary origin simply shifts replication to a secondary origin. The origins identified by Anglana et al. (2003) each contain AT-rich sequences of the type commonly found at matrix attachment regions and in the replication origins of fission yeast, flies, and mammals. Fission yeast origins are similar in size to metazoan origins and exhibit similar genetic