PERSPECTIVES: MOLECULAR BIOLOGY

Just the Facts of Chromatin Transcription

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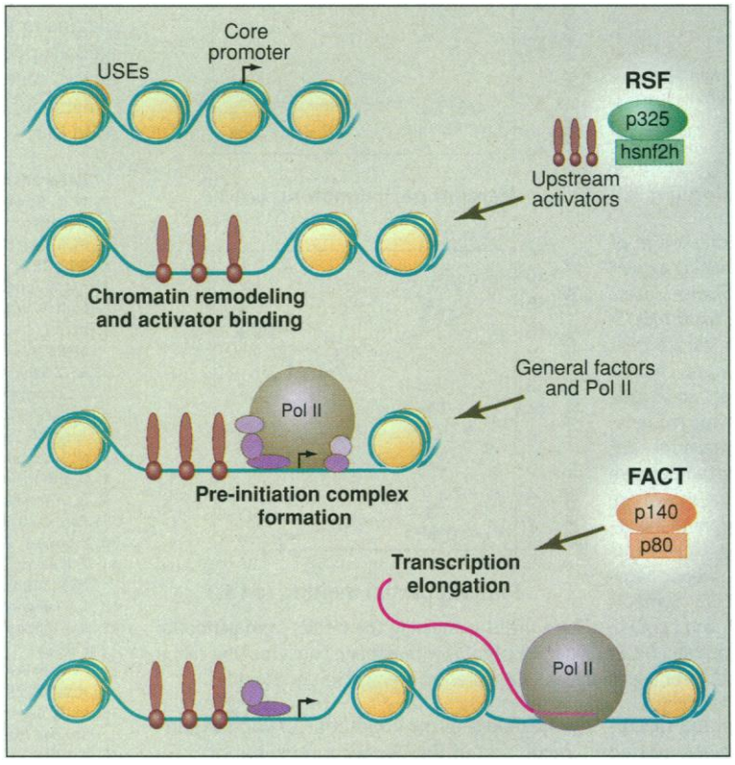
Transcription, the making of RNA polymers inside cells, is a multistep process. It begins with the binding of a DNA-dependent RNA polymerase to the DNA and terminates with the generation of an RNA transcript. In between are two phases: initiation and elongation. For the transcription of most messenger RNAs [mediated by RNA polymerase II (RNAPII)], this process commences as the protein TFIID is loaded onto a promoter, followed by the recruitment of a group of proteins called GTFs or general transcription factors (*1*). The promoter-bound GTFs then recruit RNAPII to form a preinitiation complex. The first phosphodiester bond is synthesized, after which RNAPII elongates along the DNA template, synthesizing RNA.

This rather formidable task of transcribing a gene is compounded further in cells where DNA is organized into chromatin. DNA is wrapped around histone proteins to form nucleosomes, the repeating subunits of chromatin. These structures inhibit transcription at both the initiation and elongation steps (*2*). So, for transcription to proceed the cell must recruit proteins that can alleviate nucleosome-mediated repression. Candidates include new classes of transcriptional regulators, such as SWI/SNF, NURF, CHRAC, ACF, and RSC, that appear to function through modifying chromatin structure (*3*). In an article on page 1900 of this issue (*4*), LeRoy *et al.* add to this list of activities that facilitate activator-driven transcription on chromatin templates. They identify a protein complex, from human cells, termed RSF (remodeling and spacing factor) that facilitates transcription initiation on chromatin templates in vitro. RSF, in conjunction with the previously identified complex FACT (facilitates chromatin transcription) (*5*), which promotes elongation through nucleosomes, overcomes two nucleosome-inhibited steps in transcription (see the figure).



Getting by. A model for the combined action of RSF and FACT in overcoming transcription repression by chromatin. RSF-mediated chromatin remodeling facilitates the binding of activator proteins to upstream elements (USEs). The remodeled template becomes more accessible to components of the general transcription machinery and RNAPII. Polymerase elongation through nucleosomes is facilitated by complexes like FACT. Specialized regulation in vivo will likely be mediated by different combinations of remodeling and elongation complexes.

RSF falls into a growing group of chromatin-remodeling complexes that can alter the structure of nucleosomes in an adenosine triphosphate (ATP)–dependent manner. These complexes include the 2MD SWI/SNF complex, 1MD RSC complex (identified in yeast and humans), as well as a group of smaller (400 to 500 kD) *Drosophila* complexes (NURF, CHRAC, and ACF) (*3*). NURF, CHRAC, and ACF all share the same DNA-dependent adenosine triphosphatase subunit, called ISWI (*6–8*). RSF is also 400 to 500 kD in size and contains only two proteins: an uncharacterized 325-kD protein and a 135-kD protein that was identified as hSNF2h, a human homolog of *Drosophila* ISWI. Thus, RSF is a human complex of the ISWI family, and its biochemical activities resemble those of the *Drosophila* complexes.

RSF can disrupt an ordered array of nucleosomes, resulting in the loss of the periodic spacing between nucleosomes. This remodeling event requires the combined action of RSF and the binding of an activator protein such as Gal4-VP16. Moreover, the disrupted region is confined to an area proximal to the activator binding site. It is unknown whether the activator directly targets RSF to defined regions in chromatin. However, the localized remodeling activity of RSF may be a consequence of its ability to alter nucleosome positioning (for example, spacing). The function of ATP-dependent complexes in mobilizing nucleosomes increases the global accessibility of DNA binding proteins (*9*). Thus, RSF likely facilitates activator binding or rearranges nucleosomes around the bound activator. This is in turn manifested as a persistent localized change in chromatin structure at the promoter, as observed with the *Drosophila* ISWI complexes and with SWI/SNF (*6–8, 10*). LeRoy *et al.* present the important demonstration that such an activity is sufficient to allow GAL4-VP16–driven transcription initiation in a highly purified system containing GTFs, RNAPII, the coactivators PC4 and TFIIA, and a 95% pure nucleosome template. Thus, the remodeled activator-bound template appears to promote the access of GTFs and RNAPII and results in the formation of a complex in chromatin that is competent to initiate transcription.

Chromatin remodeling at promoter regions overcomes just one hurdle that nu-

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cleosomes impose on transcription (see the figure). The large RNAPII complex also has to extend through DNA that is wrapped around the surface of histone octamers. Consequently, nucleosomes tend to impede the passage of polymerases and can result in a stalled polymerase. Earlier work by Orphanides and colleagues (*5*) suggests that transcription elongation through nucleosomes is facilitated by a distinct accessory complex, designated FACT. FACT has only been biochemically defined and, like RSF, has a simple polypeptide composition of two subunits (p140 and p80). FACT does not stimulate transcription initiation and does not require a promoter-bound transcription activator. The only prerequisite for FACT function is a promoter-remodeled template, suggesting that activities like those of RSF have to precede that of FACT. FACT does not fit the pattern of a conventional remodeling activity because ATP hydrolysis is not necessary for FACT-mediated elongation. This complex is also unlikely to function as a conventional elongation factor like TFIIF or TFIIS because neither activity is able to substitute for FACT (*5, 11*).

The report by LeRoy *et al.* illustrates one potential combination of activities (RSF and FACT) that can overcome multistep nucleosome-mediated inhibition of transcription (see the figure). In these experiments RSF remodels the promoter in the presence of GAL4-VP16 and allows the formation of transcription complexes and the initiation of transcription while FACT facilitates productive elongation through downstream nucleosomes. Although LeRoy *et al.* have used a highly purified system, it is formally possible that other activities might have contributed to the transcription activity. In particular, the histones assembled on the template likely represent a combination of endogenous *Drosophila* embryo histones (from the S-190 assembly extract used) and exogenously supplemented human histones. The acetylation state of these histones might have contributed to the active fraction of the templates (*12*).

It will be interesting to see what other chromatin-remodeling complexes will functionally substitute for RSF or FACT in chromatin transcription. LeRoy *et al.* suggest that the homologous *Drosophila* ISWI complexes are likely to have this property. Moreover, the larger SWI/SNF or RSC complexes will likely perform a similar function, perhaps in more specialized circumstances; for example, SWI/SNF participates in chromatin remodeling at the SUC2 promoter in yeast (*13, 14*) and in tissue-specific transcription of the human β-globin gene (*15*). SWI/SNF has also been implicated in contributing to the process of transcriptional elongation (*16, 17*). The situation in vivo is further complicated by the possibility that quite different chromatin-modifying complexes (such as SWI/SNF and the SAGA histone acetyltransferase complex) may be functionally redundant (*18*). Thus, there may be multiple combinations of activities that might be called on by distinct promoters to solve the nucleosome problem during transcription.

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PERSPECTIVES: BIOMEDICINE

The Enigmas of Kaposi's Sarcoma

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No example illustrates the difficulties in understanding tumor biology better than Kaposi's sarcoma (KS). The etiology of this tumor is not easy to define; a metastatic malignancy cannot easily be distinguished from a nonmalignant growth that occurs in multiple sites; and many distinct factors contribute to the pathogenesis of KS.

There are four distinct epidemiological forms of KS. Are these actually the same disease, or are they similar only because the same kinds of cells are involved, as in leukemias, lymphomas, and lung cancers? The first ("classical") form of KS occurs in older males of mainly Mediterranean or Eastern European Jewish backgrounds and has no known contributing environmental factor. A second form, found in parts of equatorial Africa, occurs in all age groups and also has no known precipitating environmental factor. Neither is typically associated with immune deficiency. In contrast, the remaining two types of KS—those associated with organ transplants and with human immunodeficiency virus–type 1 (HIV-1)—are accompanied by immune impairment. Males are predominately afflicted in all forms.

A second problem is the elusive nature of the tumor cell. Many cells in the lesion are clearly normal cells that have infiltrated the tumor, such as leukocytes. The predominant cell in the tumor is a spindle-shaped cell (SC), which is accompanied by abnormal blood vessel development and leakage of blood (see figure). It is reasonable to call the SC the "tumor cell," but there is no direct evidence that this cell is an autonomously growing neoplastic cell rather than a hyperproliferating but otherwise normal cell (hyperplasia) (*1*). Moreover, although most SCs are of endothelial cell origin, there is evidence that some of them arise from other lineages such as macrophages and fibroblasts (*2*). This makes it very likely that only some (if any) SCs are neoplastic because neoplastic cells are usually of one lineage. Some inflammatory cytokines [for example, interferon γ (IFN-γ), which is known to be increased after HIV-1 infection (*3*)] can induce a spindle-like alteration in the shape of endothelial cells and macrophages (*4*). It is therefore an oversimplification to infer that SCs are neoplastic or descended from a single transformed cell clone.

The corollary of this problem raises the third issue: Are any of the cells in the KS lesion neoplastic, or are they all the result of a chronic inflammatory response (*1, 5*)? That is, is KS a malignancy or is it a proliferative inflammatory response, or both? Several lines of evidence indicate that most or all KS proliferative cells are not in fact neoplastic: the three histological features of KS (angiogenesis, inflammation, and proliferation); the absence of a histologically discernible neoplastic cell; the sometimes spontaneous regression of KS; the usual lack of chromosomal abnormalities; the appearance of KS lesions in mul-

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