Transcriptional Activation: Getting a Grip on Condensed Chromatin

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Chromosomal transcription involves the concerted action of enormous, multisubunit chromatin remodeling complexes. A recent study, however, suggests a different and surprising viewpoint in which these protein assemblies are quite dynamic and individual subunits play key roles in chromatin remodeling.

Biochemical studies over the past ten years have continued to reinforce the view that transcriptional activators control the initiation of transcription by orchestrating the recruitment of large multisubunit protein complexes (reviewed in [1]). For example, ATP-dependent chromatin remodeling enzymes such as SWI/SNF, histone acetyltransferase complexes such as SAGA, and the RNA polymerase II holoenzyme are believed to function in vivo as pre-assembled protein complexes that have native molecular weights in the millions of Daltons. In a recent issue of Current Biology, Memedula and Belmont [2] describe a powerful in vivo system that directly visualizes the activator-dependent recruitment of multisubunit complexes to a target promoter. Surprisingly, they provide compelling evidence that chromatin remodeling complexes may not function as pre-assembled units in vivo; rather, their data suggest that individual catalytic subunits perform obligatory roles during transcriptional activation that cannot be carried out by the 1–2 Mda complexes.

In order to visualize how a transcriptional activator functions in the context of a condensed chromatin fiber in vivo, Belmont and colleagues [3] created a novel Chinese hamster ovary (CHO) cell line (A03.1) with an amplified chromosomal region of approximately 90 Mbp containing arrays of lacI repressor binding sites, the gene for dihydrofolate reductase (DHFR) and co-amplified genomic DNA (Figure 1). When imaged in live cells by decoration with a fusion protein consisting of the lacI repressor (lacI) linked to the green fluorescent protein (lacI–GFP), this 90 Mbp tract appears as a condensed foci approximately 1 µm in diameter. When these cells are transiently transfected with an expression vector that produces a fusion of LacI–GFP to the potent VP16 transcriptional activator domain, the fluorescent foci undergo dramatic decondensation (Figure 1). This activator-directed decondensation is accompanied by increased acetylation of all four core histones, recruitment of PCAF, Gcn5 and CBP/p300 histone acetyltransferases, and activation of transcription [3]. Inhibition of transcription with α-amanitin does not block the dramatic decondensation of the lacI array, indicating that these structural changes do not require transcriptional activity.

Memedula and Belmont [2] have now followed up these initial studies by monitoring the recruitment kinetics of several different chromatin remodeling enzymes. Rather than use transient transfection to introduce the lacI–GFP–VP16 fusion, cells were loaded with glass beads pre-coated with the purified fusion protein. Using this methodology, binding of the lacI–GFP–VP16 fusion throughout the condensed lacI array was detectable by GFP fluorescence immediately following initiation of the bead loading procedure. Likewise, VP16-dependent recruitment of the TRRAP protein, a subunit of multiple histone H3 and H4 acetyltransferase complexes [4,5], was detected within 5 minutes at the lacI array. Surprisingly, recruitment of several other histone acetyltransferase complex subunits, including the Gcn5, PCAF, and CBP/p300 catalytic subunits, was not detectable until 20–30 minutes after binding of the activator. The recruitment of SWI/SNF subunits was also temporally distinct — the catalytic ATPase subunits, Brg1 and bbrm, arrived within 5–10 minutes after activator binding, whereas the BAF155 and BAF170 subunits were not detectable until at least 20–30 minutes.

Thus, although TRRAP, Brg1 and bbrm are generally believed to be obligatory subunits of large complexes, their early arrival at the lacI array — with respect to other histone acetyltransferase or hSWI/SNF subunits — suggests that the VP16 activation domain promotes their recruitment as either isolated polypeptides or as components of smaller subcomplexes.

Why should an activator direct recruitment of individual components of multisubunit complexes? One possibility is that the intact, 1–2 Mda complexes may simply be too large to gain access to the interior volume of the condensed lacI locus. If this were the case, however, one might expect to observe recruitment of histone acetyltransferase and SWI/SNF complexes to the periphery of the amplified lacI array. This type of localization was clearly not observed [2]. Alternatively, the chromatin structure of the amplified lacI array may block the binding of intact complexes even to nucleosomes positioned on the exterior of the condensed mass.

Two recent structural studies of yeast SWI/SNF and RSC remodeling complexes are consistent with this possibility [6,7]. In these studies, single particle reconstructions yielded three-dimensional structures which indicate that mononucleosomes bind within a large cavity of SWI/SNF or RSC that is composed of multiple protein densities. As nucleosomes within condensed chromatin fibers may have only a limited interaction surface, the ability of SWI/SNF-like complexes to ‘envelope’ a nucleosome might only be possible in the context of decondensed, nucleosomal arrays. In contrast, a smaller, isolated Brg1 or bbrm ATPase subunit — which may lie at the base of the
SWI/SNF cavity — might be capable of a wider range of nucleosomal interactions that are not hindered by chromatin folding. The intrinsic chromatin remodeling activity of the isolated ATPase subunit may then be sufficient to disrupt condensation of the lacI foci, promoting subsequent assembly or recruitment of remodeling complexes [8].

The recruitment of individual chromatin remodeling subunits also suggests that the rates of assembly or disassembly of multisubunit complexes may play a key role in transcriptional control. For instance, the available pool of ‘free’ Brg1/hbrm or TRRAP subunits may influence the rate or extent of gene induction. This pool is expected to be quite small, as virtually all of the Brg1 detected in whole cell extracts appears to be complexed with BAF subunits (A.N. Imbalzano, personal communication). Furthermore, studies in yeast have shown that individual SWI/SNF subunits are unstable and rapidly degraded [9]. Alternatively, stable pools of individual subunits may not exist, but novel disassembly activities may control the localized production of key subunits or subcomplexes. Likewise, the completion of complex assembly or the subsequent recruitment of a pre-assembled complex may also involve novel forms of regulation.

The sequential recruitment of TRRAP, Brg1/hbrm and histone acetyltransferase catalytic subunits may reflect functional interdependencies among these isolated subunits (Figure 2). For instance, several previous studies have reported that the VP16 activation domain is unable to contact or recruit human SWI/SNF complexes in purified in vitro systems [10–12]. The rapid VP16-dependent recruitment of TRRAP in vivo, however, may generate a novel surface that is uniquely competent for subsequent recruitment of Brg1 and hbrm. Similarly, the recruitment of Brg1/hbrm before the histone acetyltransferase catalytic subunits may reflect an obligatory requirement for SWI/SNF-dependent remodeling in histone acetyltransferase recruitment. This novel order of events may be determined by the heterochromatin-like state of the lacI array, as in other cases histone acetyltransferase subunits are clearly recruited to a mammalian target before Brg1 [13].

Interestingly, the VP16-dependent series of factor recruitments are reminiscent of those detected during activation of a large group of yeast genes during late mitosis [14]. In this case, prior SWI/SNF remodeling was proposed to promote subsequent histone acetyltransferase binding by driving the release of histone amino-terminal tail domains from nucleosome–nucleosome interactions involved in mitotic condensation. Consistent with this type of model, Memedula and Belmont [2] also observe a partial decondensation of the lacI array following Brg1/hbrm recruitment. Thus, chromatin unfolding may be the event that triggers histone acetyltransferase recruitment, as well as the subsequent assembly or recruitment of complete remodeling complexes.

How general is this apparent stepwise assembly phenomenon? Unfortunately, even the very best studies that follow in vivo recruitment typically monitor only one subunit of a putative complex (for example see [13]). Recently, Martens and Winston [15] have described what may be a case of stepwise recruitment involving the yeast SWI/SNF complex. These authors found that transcriptional repression of SER3 required Swi2p/Snf2p, the ATPase subunit of yeast SWI/SNF, but repression was nearly independent of other SWI/SNF subunits (including the homolog of BAF155/170, Swi3p). Furthermore, although an intact SWI/SNF complex appeared to be present at the promoter, the authors demonstrated that Swi2p could be recruited to the SER3 promoter region even in the absence of other SWI/SNF subunits. Thus, yeast Swi2p has the capacity to be recruited, and to function, as an individual subunit — or at least as a smaller subcomplex — much like Brg1/hbrm at the amplified lacI array in the work of Medulla and Belmont [2].

An even more unexpected example of the dynamic nature of multisubunit complexes comes from a recent study from Misteli and colleagues [16]. These investigators used an in vivo microscopy method, fluorescence recovery after photobleaching (FRAP), to...
follow the kinetics for recruitment of RNA polymerase I subunits at endogenous ribosomal genes. Surprisingly, their data indicate that RNA polymerase II may not function as a pre-assembled complex in vivo, as previously believed, but that individual subunits or smaller subcomplexes assemble in a sequential manner at a ribosomal target gene. If true for RNA polymerase I, why not RNA Polymerase II? Indeed, if the subunit organization of RNA polymerases is dynamic, then all reports of ‘stable’ multisubunit complexes may need to be reinterpreted with a touch of caution. Although the transcription field may never revert back to a strict ‘step-wise assembly’ model for gene regulation [17], it seems that simplified, holoenzyme recruitment models are also unlikely to accurately describe this process.

References