Acid connections

With which components of the transcription machinery do the acidic regions of eukaryotic transcriptional activator proteins make contact? Several candidates have come into focus.

Expression of eukaryotic genes is regulated in response to developmental and environmental cues by a wide variety of transcriptional activator proteins. Such proteins contain both a DNA-binding domain, which recognizes specific promoter DNA sequences, and a physically separate transcriptional activation domain, which stimulates the initiation of mRNA synthesis by RNA polymerase II. It is generally believed, though unproven, that the DNA-binding domain serves primarily to bring the protein to the promoter, so that the activation domain can directly contact some component(s) of the basic transcription machinery. In principle, the target of an activation domain could be any of the proteins that constitute the complex transcription machinery, such as the ten subunits of RNA polymerase holoenzyme TFIID — which binds to the promoter's TATA element — or the auxilliary, general transcription factors TFIIA, TFIIB, TFIIE, TFIIF and TFIIG (recently reviewed in [1]). Alternatively, activation domains might indirectly affect the function of the basic machinery by interacting with a distinct 'adaptor' protein. Finally, the target could be a component of the chromatin template, such as histones or associated non-histone proteins. Here, I will discuss the apparently conflicting recent evidence in support of TFIID, TFIIB, adaptor proteins or chromatin as the immediate target for the transcriptional activation domains (Fig. 1).

The transcription machinery

The first step in assembling an active transcription complex is the binding of TFIID to the TATA promoter element, after which assembly of the complex appears to proceed in an ordered manner [2]. TFIIA associates first and, in combination with TFIID, alters the interaction with the TATA element. Then, TFIIB joins the complex, in which it appears to bridge the region between the TATA element and initiation site. Finally, RNA polymerase II and TFIIE/F come on board to form a preinitiation complex capable of synthesizing mRNA. Although this TATA-dependent basic machinery accurately initiates transcription *in vitro*, it is essentially inactive *in vivo*, where it must be stimulated by the activation domain of a promoter-bound transcriptional activator protein to allow efficient gene transcription.

The best characterized transcriptional activation domains are defined by short acidic regions that show little primary sequence similarity (reviewed in [3,4]). Acidic activation regions are universal in that they stimulate transcription in all eukaryotic organisms tested. However, two structurally distinct activation regions, defined either as glutamine-rich or proline-rich, have also been identified [5,6]. As transcriptional activation domains are defined operationally, the important issue of whether the two distinct types represent fundamentally different transcriptional mechanisms remains to be solved. This article will focus on the most likely targets of acidic activation regions.

TFIID

In this article, TFIID will be defined as a highly conserved polypeptide (27–31 kD depending on the species) that binds the TATA element and supports basal transcription in combination with the auxiliary factors. Note, however, that as isolated from mammalian cells, TFIID behaves as a large, chromatographically complex entity that has never been purified despite considerable effort.

A functional interaction between acidic activation regions and TFIID was initially proposed from the observation that transcriptional induction by yeast GCN4 and GAL4 proteins is restricted to specific TATA elements [4,7]. GCN4 binds about 50 base pairs upstream of the multiple TATA elements of both the divergently transcribed *his3* and *pet56* genes, yet it only induces a subset of normal *his3* transcripts. The basis for this discordant regulation is that GCN4 (and GAL4) activation is observed only in combination with a conventional TATA element that interacts with TFIID; the other TATA-like elements in this region are only able to support basal transcription [8]. In a related set of experiments, the GCN4 activation region was shown to be required specifically for *his4* transcription that depends on a conventional TATA element, whereas GCN4 derivatives with parts of their acidic region deleted, stimulated low levels of TATA-independent transcription [9]. Thus, acidic activation domains appear to function efficiently only in combination with conventional TATA elements that are TFIID interaction sites. Finally, there are several other examples of functionally distinct TATA elements that respond differentially to specific upstream activators.

How can the combinatorial specificity between enhancer and TATA elements be explained? It could involve allosteric interactions between TFIID and transcriptional activator proteins. TFIID might undergo sequence-specific conformational changes on DNA-binding and some conformations may prevent interaction with particular activators. Conversely, activator proteins might differentially affect the DNA-binding specificity or transcrip-

tional function of TFIID. In this regard, functional TATA elements that support equivalent levels of basal TFIID-dependent transcription *in vitro* respond extremely differently to GAL4-mediated activation *in vivo* [10]. Finally, there could be multiple TATA-binding proteins that differ with respect to their DNA recognition sequences and responsiveness to activator proteins. All of these models imply that there is functional interaction between acidic activation regions and TFIID, but do not address whether this interaction is direct or mediated through another protein.

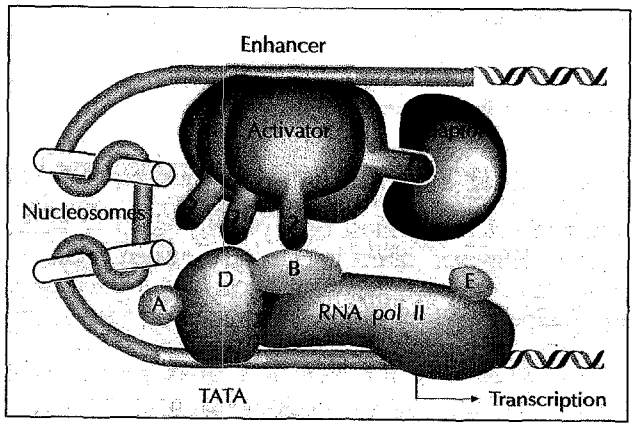

Fig. 1. A typical eukaryotic promoter, showing the likely targets of acidic activation regions (?).

The hypothesis that TFIID is the direct target of activation regions is supported by a variety of biochemical experiments [11,12]. Dissociation rate measurements and DNase I footprinting experiments are indicative of cooperative interactions between TFIID and activator proteins that seem to depend on the acidic activation region. Moreover, prior binding of both the activator and TFIID facilitates the subsequent binding of RNA polymerase II, TFIIB, and TFIIE to the promoter. Although these observations suggest a direct contact between activators and TFIID, the impurity of the protein preparations leaves open the possibility of an indirect effect, especially given the structural and functional differences between partially purified TFIID fractions and the cloned protein.

Additional evidence for direct contact is the specific retention of TFIID on an affinity chromatography column containing the acidic activation domain of the herpesvirus VP16 protein [13]. The value of this assay is perhaps lessened by the fact that TFIIB seems to bind more avidly to the same column (see below). Moreover, the possibility of non-specific ionic interactions is very real because the VP16 domain is highly acidic and TFIID is highly basic. Taken together with the other genetic and biochemical observations, these experiments provide a good, but not compelling, case that TFIID is the target of acidic activation domains.

TFIIB

Although most of the effort concerning the target of acidic activation regions has focused on TFIID, some recent biochemical experiments have implicated TFIIB as a target [14]. Partially assembled pre-initiation complexes were formed on biotinylated DNA templates, purified by affinity chromatography, and characterized for their transcriptional properties. In the absence of an acidic activator, TFIID is the only general transcription factor that stably assembles on the template. This suggests that the next step, assembly of TFIIB into the complex, is rate limiting. However, in the presence of both the acidic activator and TFIID, TFIIB stably associates with DNA, implying that the acidic region recruits TFIIB to the template. These results indicate a functional interaction between acidic activation regions and TFIIB, but do not conclusively prove a direct contact. For example, TFIID could respond directly to activation region but then undergo a conformational change that allows the recruitment of TFIIB. In addition, the ordered nature of the *in vitro* assembly process makes it impossible to determine the effect of activation regions on factors that function at later steps.

The best evidence for direct contact is that TFIIB is specifically retained on a column containing the strong VP16 activation domain, whereas it does not bind to columns containing a transcriptionally inactive derivative of the VP16 region that contains a single amino acid substitution. This correlation between association *in vitro* and function *in vivo* strongly supports the model that TFIIB is the target. However, the association of TFIID with a VP16 column (see above) is also sensitive to the same substitution mutation. The VP16 acidic region seems to interact more strongly with TFIIB than TFIID, but this parameter may be irrelevant for transcriptional activity. The observed interactions with both TFIID and TFIIB could be specific, but the possibility remains that chromatography reflects artifactual ion exchange rather than a protein–protein interaction. Although the inactive VP16 mutant has the same overall charge, its proline substitution is likely to disrupt the overall structure resulting in altered chromatographic properties. Establishing the functional relevance of an *in vitro* interaction is a very difficult problem.

Adaptors

Three independent lines of evidence suggest the involvement of a distinct 'adaptor' protein that functionally connects the acidic region to the basic machinery. First, TFIID prepared by overexpression of the cloned gene, unlike partially purified endogenous TFIID activity, does not respond to acidic activation domains. Although differences in protein modification or inherent specific activity have not been excluded, this functional distinction has been ascribed to an adaptor protein present in the impure TFIID fraction. Second, the potent VP16 activation region 'squelches' transcription that depends on acidic regions but does not affect the basal TATA-dependent reaction [15,16]. This result has been interpreted as a removal of the adaptor from the DNA-bound transcription complex by a protein–protein interaction with the excess VP16 domain in solution. Third, it has recently been shown that human TFIID cannot substitute for the

essential function of yeast TFIID *in vivo* [17,18]. As yeast and human TFIID are indistinguishable in a simple TATA-dependent assay, the species specificity is more likely to be related to the activation process, that is, species-specific adaptors.

To date, it has been impossible to separate TFIID from the putative adaptor in extracts of mammalian or *Drosophila* cells. Indirect assays of the adaptor have involved competition experiments between endogenous and recombinant TFIIDs [19] as well as heat inactivation, which is hypothesized to inactivate TFIID but not the adaptor [20]. In both cases, the results suggest that the functional interaction between TFIID and the adaptor is species-specific, with the non-conserved amino-terminal region being the critical determinant. However, these suggestions do not easily explain why the amino-terminal region of yeast TFIID is dispensable for functional activity *in vivo* [17,18]. Given the large apparent molecular weight of endogenous TFIID activity, the TATA-binding protein encoded by the cloned gene may be a subunit of a stable multi-protein complex that includes the adaptor.

Recent experiments in yeast provide the most compelling evidence for an adaptor protein that can be separated away from the components of the basic machinery [21]. The adaptor has been purified almost 1000-fold from the crude extract; the resulting preparation is nearly devoid of the basic factors. Most importantly, this adaptor is required for activation by GCN4 and GAL4, whose acidic regions differ completely in primary sequence, but is unnecessary for basal TATA-dependent transcription. Although the ease of separating an adaptor from TFIID is much greater in yeast than in higher eukaryotic cells, this probably represents a minor distinction in the strength of a protein–protein interaction rather than a fundamental mechanistic difference.

Chromatin

As chromatin has a strong and repressive effect on transcriptional initiation, acidic activation domains might function by increasing the ability of pre-initiation complexes to compete with nucleosomes for occupancy of the DNA template. It has recently been reported [22] that transcriptional enhancement by various GAL4 activator proteins *in vitro* is much higher under conditions of nucleosome assembly than in the absence of histones. In other words, these GAL4 activator proteins specifically alleviate nucleosome-dependent repression. Pre-binding of the GAL4 derivatives to the promoter permits preinitiation complex formation even after nucleosome assembly; importantly, this effect requires the acidic activation region. Unlike in standard *in vitro* transcription experiments, bacterially produced TFIID can respond to acidic activation regions when reactions are performed on nucleosomal templates [22]. However, impure TFIID preparations that contain the putative adaptor proteins are still much more responsive to acidic activators than TFIID itself.

These results suggest that chromatin is not simply a non-specific repressor of transcription, but is more directly involved in the activation process. It is consistent with this view that small amino-terminal deletions of yeast histone H4 specifically interfere with the silencer function [23], and complete removal of histone H4 increases transcription from promoters lacking enhancer elements [24]. This suggests that nucleosome loss can compensate, at least in part, for the absence of an activator protein and perhaps implies that activator proteins function by facilitating nucleosome displacement. It is unlikely, however, that acidic activator proteins function solely by increasing accessibility of the basic machinery to the chromatin template because GAL4 cannot activate transcription by T7 polymerase [25].

Reconciling the disparate views

The fact that acidic activator proteins can stimulate transcription *in vitro* in the absence of any nucleosome formation strongly suggests that a component of the transcription machinery must be a primary target of acidic regions. However, direct interaction between acidic regions and chromatin might still occur, and it is tempting to imagine negatively charged activation domains counteracting the repressive effects of positively charged histones. Whether or not there is a direct interaction, it seems very likely that the nucleosomal template magnifies the inherent activation mechanism. A simple model for this is cooperative binding of the activator and TFIID to the promoter resulting from disruption of the chromatin. As an activator protein and a nucleosome cannot simultaneously bind the same DNA sequence, the binding of the activator should (at least transiently) generate a nucleosome-free region, thus increasing the chance for TFIID to interact with the TATA element. Such cooperative binding differs from the classical mechanism involving specific protein–protein interactions.

In considering the remaining candidates, a critical question is whether acidic activator proteins can directly stimulate the basic machinery. Although an adaptor protein appears essential for activation in standard reactions *in vitro*, it seems non-essential during conditions of nucleosome assembly. At face value, this result indicates that there must be a direct interaction of acidic regions that does not involve the adaptor. If we ignore the trivial possibility of an adaptor contamination in one of the non-TFIID containing fractions that can only be detected under the magnifying conditions of nucleosome assembly, the conclusion would be that TFIID and/or TFIIB is a direct target. This certainly does not preclude a direct interaction between acidic regions and the adaptor. Moreover, the adaptor is clearly important for full transcriptional activation to be achieved even during nucleosome assembly. However, the adaptor might function indirectly by augmenting the interaction between the acidic domain and the 'true' target.

What about the relative merits of TFIID and TFIIB? Although recruitment of TFIIB to the promoter seems to be rate-limiting for activation *in vitro*, it is difficult to assess whether this is a direct or indirect effect of the acidic region. The arguments favoring TFIIB over TFIID

are that conformational changes of TFIID or the adaptor do not have to be invoked and that its interaction with the acidic region is stronger. The arguments favoring TFIID are less obvious, but perhaps more compelling. First, acidic activation regions function in essentially all eukaryotic species, indicating that the target must be highly conserved, and the structural and functional similarities among eukaryotic TFIIDs are extensive, whereas yeast and mammalian TFIIB activities do not cross-complement. It is possible, however, that TFIIBs share a common surface for contacting the acidic region but differ in their interactions with other basic transcription factors and/or adaptor. Second, the TFIID hypothesis also more easily explains the combinatorial specificity between specific enhancer and TATA sequences.

In summary, none of the arguments in support of a favored target is very persuasive. Moreover, although the above discussion makes the simplifying assumption of a single direct target, there are many plausible models in which acidic regions directly interact with more than one component. The rather non-specific sequence requirements and repeating structure of an activation region could be viewed as a mechanism to allow distinct proteins to interact simultaneously with adjacent subregions. In this way, semi-specific interactions with the acidic surface would increase the local concentrations of the basic components (and possibly the adaptor), thus increasing the rate of complex assembly and subsequent transcriptional initiation. Given the intensity of research on these questions, it is likely that answers will be forthcoming in the relatively near future.

References

1. SAWADOGO M, SENTENAC A: **RNA Polymerase B (II) and general transcription factors.** *Ann Rev Biochem* 1990, 59:711–754.
2. BURATOWSKI S, HAHN S, GUARENTE L, SHARP PA: **Five intermediate complexes in transcription initiation by RNA polymerase II.** *Cell* 1989, 56:549–561.
3. PTASHNE M: **How eukaryotic transcriptional activators work.** *Nature* 1988, 335:683–689.
4. STRUHL K: **Molecular mechanisms of transcriptional regulation in yeast.** *Ann Rev Biochem* 1989, 58:1051–1077.
5. COUREY AJ, TJIAN R: **Analysis of Sp1 *in vivo* reveals multiple transcriptional domains, including a novel glutamine-rich activation motif.** *Cell* 1988, 55:887–898.
6. MERMOD N, O'NEILL EA, KELLY TJ, TJIAN R: **The proline-rich transcriptional activator of CTF/NF-1 is distinct from the replication and DNA binding domain.** *Cell* 1989, 58:741–753.
7. STRUHL K: **Constitutive and inducible *Saccharomyces cerevisiae* promoters: evidence for two distinct molecular mechanisms.** *Mol Cell Biol* 6:3847–3853.
8. PONTICELLI AS, STRUHL K: **Analysis of yeast *his3* transcription *in vitro*: Biochemical support for multiple mechanisms of transcription.** *Mol Cell Biol* 1990, 10:2832–2839.
9. PELLMAN D, MCLAUGHLIN ME, FINK GR: **Function of the TATA element at *his4*.** *Nature* 1990, 348:82–86.
10. WOBBE CR, STRUHL K: **Yeast and human TATA-binding proteins have nearly identical DNA sequence requirements for transcription *in vitro*.** *Mol Cell Biol* 1990, 10:3859–3867.
11. SAWADOGO M, ROEDER RG: **Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region.** *Cell* 1985, 43:165–175.
12. HORIKOSHI M, HAI T, LIN Y-S, GREEN M, RODER RG: **Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex.** *Cell* 1988, 54:1033–1042.
13. STRINGER KF, INGLES CJ, GREENBLATT J: **Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID.** *Nature* 1990, 345:783–786.
14. LIN Y-S, GREEN MR: **Mechanism of action of an acidic transcriptional activator *in vitro*.** *Cell* 1991, 64:971–981.
15. BERGER SL, CRESS WD, CRESS A, TRIEZENBERG SJ, GUARENTE L: **Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors.** *Cell* 1990, 61:1199–1208.
16. KELLEHER RJI, FLANAGAN PM, KORNBERG RD: **A novel mediator between activator proteins and the RNA polymerase II transcription apparatus.** *Cell* 1990, 61:1209–1215.
17. CORMACK BP, STRUBIN M, PONTICELLI AS, STRUHL K: **Functional differences between yeast and human TFIID are localized to the highly conserved region.** *Cell* 1991, 65:341–348.
18. GILL G, TJIAN R: **A highly conserved domain of TFIID displays species specificity *in vivo*.** *Cell* 1991, 65:333–340.
19. PUGH BF, TJIAN R: **Mechanism of transcriptional activation by Sp1: evidence for coactivators.** *Cell* 1990, 61:1187–1197.
20. PETERSON MG, TANESE N, PUGH F, TJIAN R: **Functional domains and upstream activation properties of cloned human TATA binding proteins.** *Science* 1990, 248:1625–1630.
21. FLANAGAN PM, KELLEHER RJI, SAYRE MH, TSCHOCHNER H, KORNBERG RD: **A mediator required for activation of RNA polymerase II transcription *in vitro*.** *Nature* 1991, 350:436–438.
22. WORKMAN JL, TAHLOR ICA, KINGSTON RE: **Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes.** *Cell* 1991, 64:533–544.
23. KAYNE PS, KIM U-J, HAN M, MULLEN JR, YOSHIZAKI F, GRUNSTEIN M: **Extremely conserved histone H4 N-terminus is dispensible for growth but essential for repressing the silent mating loci in yeast.** *Cell* 1988, 55:27–39.
24. HAN M, GRUNSTEIN M: **Nucleosome loss activates yeast downstream promoters *in vivo*.** *Cell* 1988, 55:1137–1145.
25. CHEN W, TABOR S, STRUHL K: **Distinguishing between mechanisms of eukaryotic transcriptional activation with bacteriophage T7 RNA polymerase.** *Cell* 1987, 50:1047–1055.

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