TAF4 takes flight

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The eukaryotic mRNA transcription machinery is exceedingly complex, perhaps reflecting the requirement for response to diverse environmental and developmental signals. Some of the machinery is common to all mRNA genes and includes RNA polymerase II (Pol II) and a set of general transcription factors (GTFs) (1). Signal-specific gene expression patterns are defined by sequence-specific DNA-binding proteins (activators) that bind cognate sites in the promoter and enhancers of target genes. In most cases activators are not sufficient for stimulation of transcription. Instead, these DNA-binding proteins initiate the ordered assembly of large multiprotein complexes (the coactivators), in addition to the general factors, at the promoters of target genes (2). A cursory examination of the complexes involved in signal-responsive activated transcription in a chromatin environment indicates that >100 different polypeptides are involved in the process. Many of these proteins occur in stable multiprotein complexes. One of the central players in this process is TFIID. TFIID consists of the TATA-binding protein (TBP) and 12–13 TBP-associated factors (TAFs) (3). TFIID forms the heart of the preinitiation complex and DNA-binding proteins directly contact TFIID to stimulate the rate of transcription of target genes. An important remaining question in this field is to uncover how each individual polypeptide contributes to gene activation within the context of the entire complex. Experiments designed to test this are complicated because loss of one critical subunit of a complex often leads to disintegration of the entire complex (4–6). In a recent issue of PNAS, Wright and Tjian circumvent this problem and manage to dissect the role of a single TFIID subunit, TAF4 in the context of an otherwise intact holo-TFIID complex (7). They identify a single metazoan-specific domain of TAF4 that is important for activation of wingless (Wg/Wnt) target genes in *Drosophila*. The work not only defines an in vivo role for this domain but also sets the groundwork for the utilization of novel methods to dissect the function of individual subunits of large protein assemblies.

TAF4 is considered a core subunit because it is required for the integrity of the holo-TFIID complex. It is conserved from yeast to man, but the region of conservation is limited to a histone H2A homology region at the carboxyl terminus of the protein (Fig. 1*A*). The metazoan homologues of TAF4 contain an extended amino terminus with two additional conserved regions: a glutamine-rich region and a more recently defined ETO-TAFH domain.

The glutamine-rich region was one of the first coactivator domains of TFIID to be defined (8). It interacts with a number of activators including Sp1 and CREB (8, 9). Additionally, disruption of such essential activator–coactivator interactions in the glutamine rich region of TAF4 plays a role in the progression of Huntington's disease (10).

This work further cements TFIID as an important target of transcriptional activators.

The ETO-TAFH domain was originally defined based on sequence similarity with the 8;21 (ETO) family of proteins (11). Its structure has been solved and it forms a compact 5-helix "wedge" with a binding pocket for hydrophobic peptides (12). Although this domain can interact with a number of transcription factor in vitro, no clear in vivo role for the ETO domain has been defined.

To investigate the role of TAF4 in a physiological response, Wright and Tjian used the Wingless/Wnt (Wg/Wnt) signaling pathway (7). Wg/Wnt signaling plays important roles in cell fate determination during animal development and has also been implicated in control of adult stem cell proliferation. Importantly, there are a number of well-defined wingless targets in *Drosophila*. The Tjian group had previously shown that the carboxy-terminal histone fold is required for holo-TFIID complex formation, consistent with work on human TAF4 (6, 13). Building on this knowledge, they engineered modified versions of TAF4 that lacked regions of the amino terminus. Importantly, all of these modified TAF4 proteins can still incorporate into the TFIID complex. This strategy allowed them to show that the TAF4 ETO-TAFH domain was required for activation of the wingless target gene naked cuticle (*nkd*) both in cultured cells and in larval imaginal discs. They further showed that this domain directly interacts with the amino terminus of the *pygopus*, a dedicated transcription factor for wingless signaling. Interestingly, the amino terminus of *pygopus* has previously been defined as absolutely necessary for Wg/Wnt signaling (14). These findings place TAF4 at the end of a chain of protein–protein interactions that are required for activation of Wg/Wnt signaling target genes (Fig. 1*B*).

This work further cements TFIID as an important target of transcriptional activators. Moreover, these studies show that TAF4 itself contains at least 3 different functional domains. The amino terminus may allow multiple contacts with TFIID through the combinatorial action of multiple activators: each interacts with either the glutamine-rich region or the ETO domain. In contrast, the histone fold domain plays an essential structural role in TFIID stability.

Because TAF4 is a well-known target of activators, it is not surprising to uncover a coactivator function. The thought-provoking finding is that the region defined for Wg/Wnt responsiveness, a metazoan-specific pathway, maps to a metazoan-specific region of TAF4. The fact that the other coactivator region of TAF4 (the glutamine-rich region, involved in response to metazoan-specific activators like Sp1) also maps to the amino terminus suggests that metazoans have expanded the TAF components of TFIID to help interpret diverse metazoan-specific signals. In their current article, together with their previous work, Wright and Tjian have been able to separate the structural requirements of TAF4 from the coactivator function of TAF4 and they find that the more ancient region of TAF4 (the histone fold) is required for structural integrity and the newer region of TAF4 has acquired coactivator function (6, 7).

A second important observation is the finding that TFIID and Pol II are pre-

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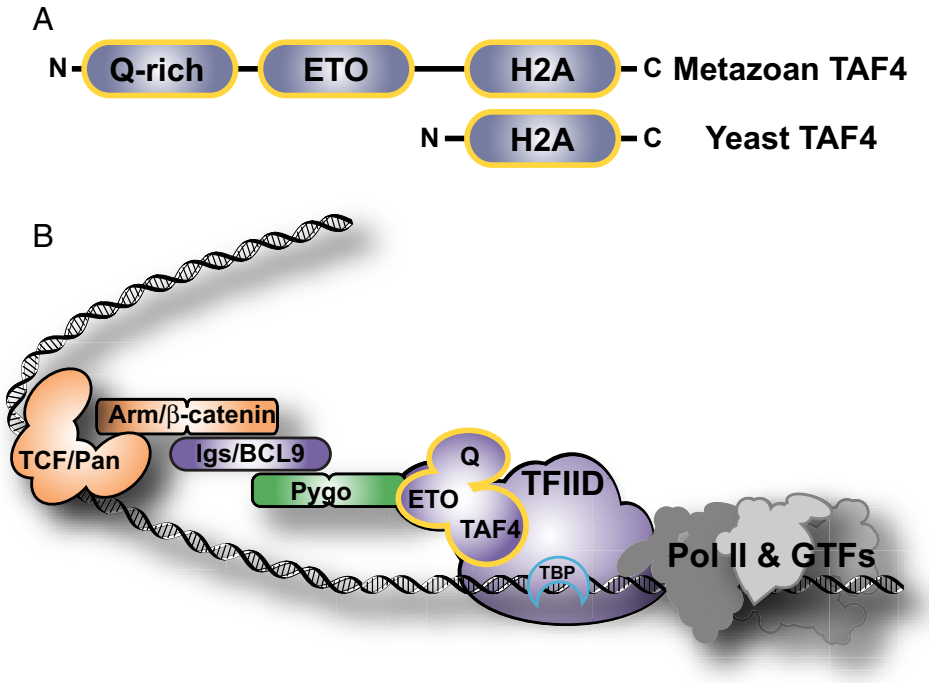

Fig. 1. Role of TAF4 in activation of wingless target genes. (*A*) Domain structure of TAF4. All TAF4 homologues contain a sequence similarity to histone H2A (H2A) at their carboxyl terminus. Only the metazoan TAF4 homologues contain an extended amino terminus. The amino terminus contains 2 regions that are targeted by transcription regulators. A glutamine-rich region close to the amino terminus (Q-rich) and an ETO-TAFH (ETO) domain in the middle of the protein. (*B*) Wingless/Wnt target genes are under the control of the TCF/*pangolin* (TCF/Pan) family of DNA-binding proteins. When the pathway is active, *Armadillo*/β-Catenin (Arm/β-Catenin) is stabilized and moves to the nucleus where it finds target genes by interacting with TCF/Pan. Legless/BCL9 (lgs/BCL9) and pygopus (Pygo) bridge the DNA-binding protein TCF to the basal machinery through interaction with TAF4 ETO domain.

loaded and paused on the inactive *nkd* gene. Stimulation of the pathway leads to only a slight increase in TFIID and Pol II promoter occupancy but a great increase in mRNA synthesis. This is important in the light of new genomic analyses that show Pol II may be preloaded at a significant portion of the genome (15–17). Thus, the TAF4-pygopus contact might play a role in releasing paused polymerases from Wg/Wnt target genes, a novel function for TFIID.

Going forward, this work lays out an experimental plan to look at individual subunits in larger complexes. By first mapping the regions required for integrity of the complex, and then investigating the functional role of other domains, it is possible to identify small domains required for function in megadalton-size complexes. This work also raises several new questions about TFIID function in transcription activation. Is the TAF4 *pygopus* interaction required for setting up the preloaded polymerase or in allowing polymerase escape? If RNA polymerase is present with TFIID at the promoter, what is the mechanism of RNA polymerase release? It is also clear that TAF1 plays a role in wingless signaling, but what role? Further experiments will be needed to address these issues.

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