Distinct requirements for *C. elegans* TAF\textsubscript{II}s in early embryonic transcription

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TAF\textsubscript{II}s are conserved components of the TFIID, TFTC and SAGA-related mRNA transcription complexes. In yeast (\textit{y}), \textit{y}TAF\textsubscript{II}17 is required broadly for transcription, but various other TAF\textsubscript{II}s appear to have more specialized functions. It is important to determine how TAF\textsubscript{II}s contribute to transcription in metazoans, which have larger and more diverse genomes. We have examined TAF\textsubscript{II} functions in early *Caenorhabditis elegans* embryos, which can survive without transcription for several cell generations. We show that \textit{taf-10} (\textit{y}TAF\textsubscript{II}17) and \textit{taf-11} (\textit{y}TAF\textsubscript{II}25) are required for a significant fraction of transcription, but apparently are not needed for expression of multiple developmental and other metazoan-specific genes. In contrast, \textit{taf-5} (\textit{h}TAF\textsubscript{II}130) seems to be required for essentially all early embryonic mRNA transcription. We conclude that TAF-10 and TAF-11 have modular functions in metazoans, and can be bypassed at many metazoan-specific genes. The broad involvement of TAF-5 in mRNA transcription \textit{in vivo} suggests a requirement for either TFIID or a TFTC-like complex.

\textbf{Keywords:} *C. elegans*/gene regulation/TAF\textsubscript{II}s/TFIID/transcription

Introduction

Eukaryotic mRNA transcription requires assembly of a multiprotein pre-initiation complex (PIC) at promoters. This machinery includes RNA polymerase (Pol II), general transcription factors (GTFs) required for Pol II activity (TFIIA, B, D, E, F and H) and a mediator-related complex (Hampsey, 1998; Lemon and Tjian, 2000; Malik and Roeder, 2000). Some PIC components are essential for transcription, but in yeast others may act as modular interfaces through which gene groups can be regulated coordinately (Holstege \textit{et al.}, 1998; Green, 2000; Lee \textit{et al.}, 2000). In metazoans, additional PIC components and transcription cofactors have evolved that are not present in yeast (Lemon and Tjian, 2000). Most metazoan genes do not appear to correspond directly to yeast genes, even though many encode conserved domains (Chervitz \textit{et al.}, 1998; Rubin \textit{et al.}, 2000; Rubin, 2001). Given these differences, it is important to determine how conserved PIC components contribute to transcription in metazoans.

The GTF TFIID, which recognizes the transcription start site, is remarkably conserved from yeast to humans (Burley and Roeder, 1996; Albright and Tjian, 2000; Green, 2000). TFIID consists of the TATA-binding protein (TBP), along with ~12 polypeptides known as the TAF\textsubscript{II}s (TBP-associated factors). Some TAF\textsubscript{II}s interact with core promoter sequences, and various individual TAF\textsubscript{II}s can bind a diverse array of upstream transactivators. In addition, human (h) TAF\textsubscript{II}250 and its orthologs have enzymatic activities that include a conserved histone acetyl transferase (HAT) (Albright and Tjian, 2000; Green, 2000; Matangkasombut \textit{et al.}, 2000; Pham and Sauer, 2000). The TAF\textsubscript{II}s may thus provide a functional link between proximal and distal promoter regions, and activities that promote transcription. Consistent with this idea, a TFIID structure reveals surfaces that could mediate extensive core promoter and protein–protein contacts (Andel \textit{et al.}, 1999; Brand \textit{et al.}, 1999a). Some TAF\textsubscript{II}s are also present in the human TBP-free TAF\textsubscript{II}-containing complex (TFTC), and in the related complexes PCAF and STAGA (human) and SPT–ADA–GCN5 (SAGA) (yeast) (Martinez \textit{et al.}, 1998; Ogryzko \textit{et al.}, 1998; Wieczorek \textit{et al.}, 1998; Brand \textit{et al.}, 1999b; Sterner and Berger, 2000). We refer to these as TFTC-related complexes. They lack TBP, and contain either the GCN5 or PCAF HAT instead of an hTAF\textsubscript{II}250 ortholog. In addition to TAF\textsubscript{II}s, TFTC-related complexes contain subunits that are related to TFIID-specific TAF\textsubscript{II}s, suggesting possible functional overlap. Supporting this idea, TFTC is structurally similar to TFIID, and can mediate transcription initiation \textit{in vitro} (Wieczorek \textit{et al.}, 1998; Brand \textit{et al.}, 1999a).

Analysis of conditional yeast mutants indicates that expression of most genes depends upon either the TFIID or SAGA HAT, and that many yeast genes may be regulated through the action of either complex (Lee \textit{et al.}, 2000). Individual yeast TAF\textsubscript{II}s are each necessary for cell viability, but the extent to which they are required for Pol II transcription \textit{in vivo} remains controversial (Albright and Tjian, 2000; Green, 2000; Kuras \textit{et al.}, 2000; Li \textit{et al.}, 2000). Some yeast TAF\textsubscript{II}s are broadly required, but others appear to have more specific functions that derive from interactions with core promoters, and possibly with other proteins.

It appears likely that individual metazoan TAF\textsubscript{II}s function analogously to yeast TAF\textsubscript{II}s in regulating genes that are conserved in all eukaryotes. It is an open question, however, to what extent they are important at genes that do not have yeast counterparts, which we refer to as metazoan-specific genes. Analysis of metazoan TAF\textsubscript{II}s function \textit{in vivo} has been hampered by cell lethality, and
by the complexity of terminal developmental phenotypes (Zhou et al., 1998; Pham et al., 1999; Wassarman et al., 2000). To circumvent the problem of cell lethality, we are studying metazoan TAF\textsubscript{I\textalpha}s in the Caenorhabditis elegans embryo. *Caenorhabditis elegans* embryonic mRNA transcription appears to begin at the 4-cell stage, but in its absence maternally produced mRNAs maintain viability until around the 100-cell stage (Powell-Coffman et al., 1996; Seydoux and Dunn, 1997). In this context, we can investigate the functions of otherwise essential transcription factors in living cells.

We have used RNA-mediated interference (RNAi) to investigate the functions of three *C. elegans* TAF\textsubscript{I\textalpha}s: TAF-5, TAF-10 and TAF-11 (Figure 1; Table I). TAF-10 is of considerable interest because it is orthologous to *Saccharomyces cerevisiae* TAFII130 (Saluja et al., 1998; Michel et al., 1998; Moqtaderi et al., 1998; Lee et al., 2000) (Figure 1B). TAF-11 corresponds to yTAFII25, which has been proposed to be either universally (Sanders et al., 1999) or narrowly (Lee et al., 2000) required (Figure 1C). TAF-5 corresponds to yTAFII48, the requirements for which are unknown (Sanders and Weil, 2000). TAF-5 is particularly interesting because it corresponds to hTAF\textsubscript{II}130, which contains metazoan-specific motifs that are targeted directly by numerous activators (Figure 1A) (Saluja et al., 1998; Rojo-Niersbach et al., 1999). In addition to being present in TFIIID, TAF-10 and TAF-11 orthologs are found in all TFTC-related complexes, and a TAF-5 ortholog is present in TFTC (see Brand et al., 1999b). We show that *C. elegans* taf-10 and taf-11 are required for a significant proportion of embryonic transcription but, strikingly, are not limiting for activation of multiple developmental and other metazoan-specific genes. In contrast, taf-5 appears to be essential for virtually all early transcription. Our findings suggest that TAF-10 and TAF-11 form part of a functional module that is not required for activation of many metazoan-specific genes, and that TAF-5 may have a more fundamental mechanistic role.

### Results

*Caenorhabditis elegans* TAF\textsubscript{I\textalpha}s

By searching *C. elegans* databases, we have identified at least one well-conserved homolog for each TAF\textsubscript{I\textalpha} in human TFIIID (Table I). We have named these *C. elegans* TAF\textsubscript{I\textalpha}s in order of their predicted molecular weights. Each contains conserved sequence motifs found in their metazoan and yeast orthologs (not shown), including the

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<tr>
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*Caenorhabditis elegans* homologs of TAF\textsubscript{I\textalpha}s were identified by searching WORMpep or genomic databases (Sanger Centre) with human, *Drosophila* or *S. cerevisiae* sequences. The open reading frame, predicted molecular weight and gene name are listed for the *C. elegans* homologs. *Caenorhabditis elegans* TAF\textsubscript{I\textalpha}s have been identified and described independently by Aoyagi and Wassarman (2000).
histone fold domains through which multiple TAFs form heterodimers (Burley and Roeder, 1996; Gangloff et al., 2000, 2001a,b). TAF-5, TAF-10 and TAF-11 each contain a characteristic histone fold (Figure 1), through which they are each predicted to pair with a different TAFII (Burley and Roeder, 1996; Gangloff et al., 2000, 2001b). These similarities predict that TFIID structure and function have been conserved in *C. elegans*. We have also searched for *C. elegans* orthologs of other TFTC-related complex components. *Caenorhabditis elegans* encodes a GCN5/PCAF-related HAT (Y.Shi, unpublished) and a TRA1/TRRAP homolog (not shown) but, by our search criteria (Materials and methods), we did not identify orthologs of ADA/SPT proteins (ADA1, ADA2, ADA3, SPT3, SPT7, SPT8 and SPT20), which are not essential for yeast viability (Sterner and Berger, 2000). In *C. elegans*, ADA/SPT functions may be fulfilled by more distantly related proteins, or a streamlined version of TFTC may be formed by TAFIIs, the GCN5/PCAF-related HAT and TRA1/TRRAP.

**taf**(_RNAi_) embryos arrest development early, without differentiation

*Caenorhabditis elegans* embryonic development is orchestrated initially by maternally derived proteins and mRNAs, which establish early cell asymmetries and patterns of new embryonic transcription (Newman-Smith and Rothman, 1998). To determine whether TAF-5, TAF-10 and TAF-11 proteins are present in the early embryo, we examined their expression by staining with peptide-derived antibodies. Under staining conditions that were optimized for early embryos (Figure 2 and data not shown), TAF-5 was apparent in nuclei from the 2-cell stage through early morphogenesis, and TAF-10 and TAF-11 were readily detectable in nuclei from the 4-cell stage through late gastrulation. TAF-5, TAF-10 and TAF-11 were similarly detectable in adult germline and oocyte nuclei (not shown), suggesting that they are maternally expressed.

To investigate *taf-5, taf-10* and *taf-11* functions in the early embryo, we inhibited their expression by RNAi (Fire et al., 1998). As a benchmark for phenotypes caused by pleiotropic transcription defects, we compared *taf**(_RNAi_) embryos with *ama-1(RNAi)* and *ttb-1(RNAi)* embryos. *ama-1* encodes the Pol II large subunit (Powell-Coffman et al., 1996) and *ttb-1* encodes TFIIB, a Pol II GTF required for transcription initiation (Lemon and Tjian, 2000). We determined whether maternal gene expression was generally intact in these RNAi embryos by monitoring early cell division patterns and timing, and by performing parallel RNAi experiments in a transgenic strain that expresses a fusion of the maternally derived germline protein PIE-1 to green fluorescent protein (GFP). This PIE-1::GFP protein recapitulates the complex patterns of PIE-1 expression and localization, which depend upon >20 other maternal genes (Tenenhaus et al., 1998; Reese et al., 2000b).

All *ama-1, ttb-1, taf-5, taf-10* and *taf-11(RNAi)* embryos arrested development at 90–100 cells and lacked signs of differentiation (Figure 3A), as reported previously for *ama-1* (Powell-Coffman et al., 1996). At every stage prior to terminal arrest, maternal PIE-1::GFP expression and localization patterns appeared normal in these RNAi embryos (Figure 3A and data not shown). Their early cell division timing and cleavage planes were also generally normal, except for the cell cycle period of the two E cell daughters (E2 cells), which form the endoderm (Figure 3B and data not shown). For gastrulation to occur, the E2 cells must divide after 45 min instead of the 22 min characteristic of their cousins, the two MS2 cells. This cell cycle lengthening requires new mRNA transcription and endodermal specification (Powell-Coffman et al., 1996; Zhu et al., 1998). In *ama-1(RNAi), ttb-1(RNAi)* and each set of *taf**(_RNAi_) embryos, the E2 cells divided immediately...
after the MS2 cells (Figure 3B). Our findings suggest that in taf$_{5}(RNAi)$ embryos, maternal mRNA stores appear generally to be intact, but new mRNA transcription may be severely impaired.

Nuclear antibody staining for TAF-5, TAF-10 or TAF-11 was eliminated in each respective set of RNAi embryos (Figure 2), indicating a penetrant loss of function. In yeast, loss of some TAF$_{18}$ destabilizes other TFIIID components (Apone et al., 1998; Michel et al., 1998; Moqtaderi et al., 1998; Chen and Manley, 2000). To investigate whether this might have occurred, we stained taf$_{5}(RNAi)$ embryos with antibodies against each TAF$_{11}$ that we analyzed (Figure 2). TAF-10 and TAF-11 were both present at normal levels in taf$_{5}(RNAi)$ embryos. Interference with either taf-10 or taf-11 did not affect TAF-5 expression, but caused loss of both TAF-10 and TAF-11. Because we have not detected evidence of maternal gene expression defects in these taf$_{5}(RNAi)$ embryos, we conclude that TAF-10 and TAF-11 proteins may each depend upon each other for stability.

**Inhibition of Pol II CTD phosphorylation in taf$_{5}(RNAi)$ embryos**

To investigate overall transcription levels in taf$_{5}(RNAi)$ embryos, we analyzed phosphorylation of the Pol II large subunit C-terminal domain (CTD). The CTD consensus repeat (YSPTSPS; 42 copies in C.elegans) is phosphorylated on actively transcribing Pol II (Hirose and Manley, 2000). CTD phosphorylation is important for promoter clearance, elongation and integration of transcription with mRNA processing. At the promoter, yeast Pol II is phosphorylated on Ser5 of the CTD repeat by the TFIIB kinase (Komarnitsky et al., 2000; Schroeder et al., 2000). As Pol II moves away from the start site, the distribution of CTD phosphorylation shifts to Ser2, but the kinase responsible has not been identified (Komarnitsky et al., 2000). In C.elegans embryos, CTD phosphorylation patterns are tightly correlated with transcriptional activity (Seydoux and Dunn, 1997; Tenenhaus et al., 1998). In somatic nuclei, antibody staining first detects Ser5 phosphorylation as a bright punctate pattern at the 4-cell stage, when transcription begins (Seydoux and Dunn, 1997) (Figure 4, columns 2 and 3). In the transcriptionally silent early germline precursor nucleus, this staining is confined to two distinct foci. Ser2 phosphorylation is first detectable at the 4-cell stage, and is absent in the early embryonic germline (Seydoux and Dunn, 1997) (Figure 4, column 5).

We stained embryos with the P-CTD antiserum to detect phospho-Ser5 (Schroeder et al., 2000), the H5 antibody to detect phospho-Ser2 (Bregman et al., 1995; Patturajan et al., 1998) and the 8WG16 antibody to detect the unphosphorylated CTD (Patturajan et al., 1998). To avoid confusion, we refer to these antibodies as α-PSer5, α-PSer2 and α-UnP CTD, respectively. In contrast to ama-1(RNAi) embryos, in which staining with α-UnP CTD was abolished, in ttb-1(RNAi) and taf$_{5}(RNAi)$ embryos Pol II levels were not significantly affected (Figure 4, column 7). α-PSer5 staining of wild-type embryos (Figure 4, columns 2 and 3) recapitulated the pattern obtained previously with the phospho-Ser5 antibody H14 (see above) (Seydoux and Dunn, 1997). In contrast, nuclear α-PSer5 staining was reduced to background levels in ama-1(RNAi) and ttb-1(RNAi) embryos. In taf$_{5}(RNAi)$ embryos, diffuse nucleoplasmic α-PSer5 staining was reduced similarly, but each somatic nucleus contained two distinct foci like those in the transcriptionally silent germline cell (Figure 4, columns 2 and 3). In parallel to staining with α-PSer5, α-PSer2 reactivity was comparably severely reduced in ama-1(RNAi), ttb-1(RNAi) and taf$_{5}(RNAi)$ embryos (Figure 4, column 5). These staining reductions were representative of these RNAi embryos from the 4-cell stage until arrest (not shown), suggesting that in ama-1(RNAi), ttb-1(RNAi) and taf$_{5}(RNAi)$ embryos overall transcription levels are extremely low at each embryonic stage.

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**Fig. 3.** Terminal and early cell division phenotypes of ama-1 (RNA pol II), ttb-1 (TFIIB), taf-5, taf-10 and taf-11 RNAi embryos. (A) TAF$_{18}$ RNAi embryo phenotypes. RNAi embryos produced by N2 (wild-type) or pie-1::gfp mothers were examined by differential interference (DIC) or fluorescence (FL) microscopy. Typical examples of wild-type (WT) or RNAi embryos are shown, as indicated to the right of each row. The left column compares terminally arrested RNAi embryos with a wild-type embryo that is about to hatch. ama-1(RNAi), ttb-1(RNAi), taf-5(RNAi), taf-10(RNAi) and taf-11(RNAi) embryos each arrested with 90±100 cells (n = 5). The right two columns show 4-cell pie-1::gfp WT and RNAi embryos. In these RNAi embryos, each aspect of PIE-1::GFP germline and subcellular localization was indistinguishable from wild type, including the presence of PIE-1 in germline RNA–protein P granules (Reese et al., 2000b). Embryos measure ~50 μm. (B) Shortened E2 cell cycle in taf$_{5}(RNAi)$ embryos. Lineage analysis of each set of taf$_{5}(RNAi)$ embryos (n >5) revealed that their early cell division planes and times were normal, except that their E2 cells (Ea and Ep) divided prematurely. Only the EMS cell lineage is shown.
In contrast, CTD phosphorylation was less severely affected in taf-10(RNAi), taf-11(RNAi) and taf-10(RNAi); taf-11(RNAi) embryos. In the somatic nuclei, at all embryonic stages, two α-PSer5 foci were accompanied by nucleoplasmic staining that was decreased, but more prominent than in taf-5(RNAi) embryos (Figure 4, columns 2 and 3; not shown). In taf-10(RNAi), taf-11(RNAi) and taf-10(RNAi); taf-11(RNAi) embryos, α-PSer2 staining levels were similarly not eliminated (Figure 4, column 5). The comparable reduction in CTD phosphorylation accompanying simultaneous interference with taf-10 and taf-11 is consistent with the interdependence of TAF-10 and TAF-11 protein levels (Figure 2). These findings suggest that some transcription can occur independently of taf-10 and taf-11.

**taf-10 and taf-11 are not rate limiting for many metazoan-specific promoters**

To investigate how these TAFII genes are involved in expression of individual genes, we performed RNAi experiments in *C. elegans* that carry transgenic reporters that are transcribed in the early embryo. These reporters include intact regulatory regions along with GFP-fused coding regions, and are expressed in patterns that parallel the corresponding endogenous genes. We examined expression of three genes that are common to yeast and metazoans: *let-858*, *rps-5*, and *hsp16.2*, a heat shock gene. In yeast, *rps-5* transcription is highly dependent upon TAFII genes (Li et al., 2000). Interference with taf-5, taf-10 or taf-11 abolished LET-858::GFP and RPS-5::GFP expression, and significantly decreased expression of HSP-16.2::GFP in response to heat shock (Figure 5). Interference with each of these *tafi* genes comparably impaired expression of these reporters (Figure 5), suggesting that the less severe decrease in CTD phosphorylation associated with interference with taf-10 or taf-11 (Figure 4) reflects a difference in function, not RNAi penetrance.

We next tested how interference with these TAFII genes affects expression of genes that are not present in yeast, but are widely expressed. pes-10 has been identified only
in *C. elegans*, and is expressed at the onset of embryonic transcription (Seydoux and Fire, 1994). PES-10::GFP expression was severely affected by interference with *ama-1* or *taf-5* expression, and was decreased in *taf-10*(RNAi) or *taf-11*(RNAi) embryos (Figure 5). cki-2 (CDK inhibitor) and *sur-5* (MAP kinase pathway component) are broadly conserved among metazoans (Gu et al., 1998; Hong et al., 1998). cki-2 and *sur-5* reporters required *ama-1* and *taf-5*, but were unaffected by interference with either *taf-10* or *taf-11* expression (Table II).

![Fig. 5](image-url)

Fig. 5. Comparable requirements for *taf-5*, *taf-10* and *taf-11* at conserved genes. GFP fluorescence was examined in wild-type or *taf_{10,RNAi}* embryos (in rows) that were produced by the reporter strains indicated above the columns. Each of these reporters was expressed in most embryonic cells. In a representative experiment, the RPS-5::GFP reporter, which is non-integrated, was expressed in 23/47 wild-type embryos but in none of >50 of each set of RNAi embryos. Embryos shown are otherwise representative of the entire population analyzed in each of multiple independent experiments, in which >40 embryos were scored per reporter strain. HSP16.2::GFP expression varied slightly within each set of embryos, but those depicted correspond to average levels of expression and to representative differences between WT and RNAi embryos. Genes that are conserved between yeast and metazoans are indicated at the bottom.

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<th>Table II. Requirements for TAF_{10,11} for metazoan-specific gene expression</th>
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Reporter strains were scored as + when GFP was expressed at wild-type levels in all embryos. They were scored as − when GFP was undetectable, or was present at comparable trace levels in *taf_{10,11}* and *ama-1* RNAi embryos. In each case, >40 embryos were analyzed in multiple independent experiments. In three independent reporter strains, PHA-4::GFP was expressed at normal levels, but in fewer cells than wild type.

We also investigated the importance of these TAF_{10,11}s for activation of cell type-specific genes. The redundant GATA factor-encoding genes *med-1* and *med-2* specify mesendodermal lineages (Maduro et al., 2001), and are required for expression of the related gene *end-1*, which specifies the endoderm (Zhu et al., 1998). *pha-4* is a *forkhead*-family gene that later specifies the pharynx and rectum (Hornet et al., 1998; Kalb et al., 1998), and *elt-5* encodes an epidermally expressed GATA factor (J.Rothman, unpublished). In *taf-5*(RNAi) embryos, GFP reporters corresponding to these genes were not expressed above the trace or undetectable levels characteristic of *ama-1*(RNAi) embryos (Figure 6; Table II). In contrast, *med-1*, *med-2* and *elt-5* reporters were expressed robustly in all *taf-10*(RNAi) and *taf-11*(RNAi) embryos (Table II). All *taf-10*(RNAi) and *taf-11*(RNAi) embryos also expressed PHA-4::GFP in many cells (Table II), a striking finding because *pha-4* transcription requires upstream zygotic gene expression (Hornet et al., 1998; Kalb et al., 1998). END-1::GFP normally appears in the E2 cells, then persists in their E4–E8 descendants (Figure 6 and data not shown). As predicted from their shortened E2 cell cycle (Figure 3B), in most *taf-10*(RNAi), *taf-11*(RNAi) and *taf-10*(RNAi) embryos, END-1::GFP initially appeared at normal levels in E4 cells, then was present in E8 cells (Figure 6). These reporter experiments confirm the general importance of *taf-5*, and suggest that *taf-10* and *taf-11* are required for a significant fraction of embryonic transcription, but not for expression of many metazoan-specific genes.

**Discussion**

We have investigated how three TAF_{10,11}s contribute to gene expression in the developing early *C. elegans* embryo. We have found that TAF-10 and TAF-11 are required for a significant but not complete fraction of Pol II transcription, indicating that they have broad but modular functions. This conclusion is consistent with models suggested by some studies of the TAF-10 ortholog yTAFII17, which appears to be as broadly required for transcription as any TAFII studied in *S. cerevisiae* (Table III) (Apone et al., 1998; Michel et al., 1998; Moqtaderi et al., 1998). Our findings extend these yeast models, however, by indicating that many developmental and other metazoan-specific genes are regulated independently of TAF-10 and TAF-11. In contrast, TAF-5 appears to be generally essential for early embryonic transcription, suggesting that it has a
broader role in transcription than has been demonstrated previously for other TAFII s.

**Similarly restricted requirements for taf-10 and taf-11 in vivo**

The reduced but significant levels of Pol II CTD Ser2 and Ser5 phosphorylation in taf-10(RNAi) and taf-11(RNAi) embryos (Figure 4 and data not shown) predicted a substantial but incomplete transcriptional defect. Accordingly, these RNAi embryos expressed multiple reporter transgenes at normal levels (Figure 6; Table II). Various lines of evidence indicate that this restricted transcriptional requirement for taf-10 and taf-11 reflects their biological functions, rather than incomplete RNAi penetrance. These RNAi effects were highly reproducible, appeared with consistent timing after injection, were accompanied by lack of TAF-10 and TAF-11 antibody staining (Figure 2) and were not enhanced by simultaneous interference with taf-10 and taf-11 expression (Figures 4 and 6). Finally, expression of the conserved genes let-858, rps-5 and hsp-16.2 was decreased as severely in these RNAi embryos as in taf-5(RNAi) embryos (Figure 5; Table II). Although our experiments do not eliminate the possibility that trace levels of TAF-10 and TAF-11 remain in these RNAi embryos, they suggest that these TAFII s are not required for transcription of a significant proportion of C. elegans embryonic genes.

In yeast, taf-10 and taf-11 orthologs have been inactivated conditionally by mutation or expression shut-off (Table III). Our findings are consistent with evidence that yTAFII17 is broadly required for transcription (Apone et al., 1998; Moqtaderi et al., 1998; Lee et al., 2000), but argue against the model that yTAFII17 and yTAFII25 are generally essential (Michel et al., 1998; Sanders et al., 1999). *Caenorhabditis elegans* TAF-10 and TAF-11 levels are mutually dependent (Figure 2). This finding suggests that the discrepancy between evidence that yTAFII25 is required very broadly (Sanders et al., 1999) or to transcribe 16% of yeast genes (Lee et al., 2000) might involve effects on stability of other TAFII s. While some yeast heat shock genes are yTAFII17 independent (Apone et al., 1998; Moqtaderi et al., 1998), hsp-16.2 was partially dependent upon taf-10 and taf-11 (Figure 5), making it of interest to investigate the TAFII dependence of other *C. elegans* heat shock genes.

A significant aspect of our findings is that taf-10 and taf-11 generally did not appear to be required for expression of the metazoan-specific genes that we tested, including multiple developmental genes (Figures 5 and 6; Table II). The single exception was pes-10 (Figure 5), which was partially dependent upon these TAFII s. In contrast, taf-10 and taf-11 were essential for expression of each gene we analyzed that is conserved between metazoans and fungi (Figure 5). In these TAFII RNAi

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**Table III. Requirements for yeast and C.elegans TAFII s for transcription in vivo**

<table>
<thead>
<tr>
<th>S.cerevisiae</th>
<th>C.elegans embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFII</td>
<td>Transcriptional requirement</td>
</tr>
<tr>
<td>TAFII48</td>
<td>unknown</td>
</tr>
<tr>
<td>TAFII17</td>
<td>59% of genes(^a); broad or essentially complete(^b)</td>
</tr>
<tr>
<td>TAFII25</td>
<td>16% of genes(^a); essentially complete(^b)</td>
</tr>
</tbody>
</table>

*Results of the present study are compared with in vivo analyses of S.cerevisiae TAFII17 and TAFII25 (see text).*

\(^a\)Percentages indicate the proportion of yeast genes that required the indicated TAFII for normal expression, as indicated by microarray analysis (Lee et al., 2000).

\(^b\)In separate studies, the approximate proportion of transcription that appeared to depend upon yTAFII17 (Michel et al., 1998; Moqtaderi et al., 1998) or yTAFII25 (Sanders et al., 1999).
embryos, proliferation ceased at ~100 cells (Figure 2), presumably as maternal proteins that are critical for cell division or function became limiting, suggesting that taf-10 and taf-11 are needed to express genes that are required for these functions. We conclude that taf-10- and taf-11-dependent genes are likely to include many conserved genes involved in cell division or viability, but a much smaller proportion of the specialized genes that have evolved in metazoans. Consistent with our results, murine embryonic carcinoma cells from which TAFII30 (TAF-11) was depleted conditionally could be induced by retinoic acid to leave the cell cycle and differentiate into endodermal cells, but otherwise underwent apoptosis (Metzger et al., 1999). Conditional depletion of a chicken TAF-10 ortholog from a cell line also triggered apoptosis, in conjunction with an apparently modest reduction in mRNA transcription (Chen and Manley, 2000).

Our data suggest that TAF-10 and TAF-11 are part of a functional module within TFII D- and TFTC-related complexes that can be bypassed during transcription of many, or possibly most, metazoan-specific genes. We predict that at TAF-10- and TAF-11-independent genes, regulatory mechanisms have evolved that depend upon other PIC components, some of which are unique to metazoans. For example, the metazoan-specific co-activator HAT p300/ CBP is targeted by many signal- and tissue-specific activators (Goodman and Smolik, 2000). In sharp contrast to taf-10 and taf-11, the C.elegans p300/CBP ortholog cbb-1 prevents inappropriate cell proliferation, and is required for multiple differentiation pathways (Shi and Mello, 1998). The mediator complex also contains metazoan-specific components (Malik and Roeder, 2000), one of which has been implicated in C.elegans developmental gene expression (Zhang and Emmons, 2000).

**TAF-5 has properties of an essential transcriptional regulator**

In contrast to taf-10 and taf-11, taf-5 is generally required for early embryonic transcription (Table III). In taf-5(RNAi) embryos, at each stage somatic cells were indistinguishable from the transcriptionally silent germ-line precursor in their Pol II CTD Ser2 and Ser5 phosphorylation patterns (Figure 4 and data not shown). taf-5 was also comparable with ama-1 in its importance for reporter gene expression (Figures 5 and 6; Table II). These genes included med-1, med-2 and pes-10, which are expressed approximately when embryonic transcription starts (Seydoux and Fire, 1994; Maduro et al., 2001). TAF-5 appears to be more generally required for early embryonic transcription than even TBP because, in C.elegans and various other metazoans, a considerable proportion of embryonic transcription involves the TBP-related protein TLF (Dantelon et al., 2000; Kaltenbach et al., 2000; Veenstra et al., 2000; Muller et al., 2001). Previous studies implicated the Drosophila TAF-5 ortholog in transcription mediated by the activators Twist and Dorsal (Zhou et al., 1998; Pham et al., 1999), and indicated that an hTAF1130 isoform found in B cells (hTAF1105) is required for some transcription driven by the Dorsal-related factor NF-kB (Yamit-Hezi et al., 2000). Our results suggest that a TAF-5 ortholog or isoform may be generally essential for metazoan mRNA transcription. Interactions between hTAF1130 and polyglutamines encoded by CAG repeat expansions have been implicated in neurodegenerative diseases and neuronal apoptosis (Shimohata et al., 2000). It has been proposed that sequestration of hTAF1130 inhibits CREB-driven transcription, but the broader effect predicted by our results could cause apoptosis analogously to lack of taf-10 or taf-11 orthologs (see above).

In taf-5(RNAi) embryos, α-PSer5 staining appeared in somatic nuclei when transcription normally would begin, but was confined primarily to two discrete foci, as is characteristic of the transcriptionally silent germine cells in early C.elegans and Drosophila embryos (Figure 4 and data not shown) (Seydoux and Dunn, 1997). These foci of α-PSer5 staining may be analogous to structures that appear in mammalian cells when Pol II activity is inhibited (Bregman et al., 1995), and could represent recycling or storage particles (Komarnitsky et al., 2000). These foci did not appear in tlb-1(RNAi) embryos (Figure 4), suggesting that TFII B may be required at an earlier transcription step than TAF-5. Consistent with this idea, during activation of a mammalian gene in vivo, TFII B and the Pol II holoenzyme (including the TFII H kinase) were recruited to the promoter before TFII D (Agalioti et al., 2000). Levels of α-PSer2 staining correlated with nucleoplasmic α-PSer5 staining and reporter expression defects in the respective taf65(RNAi) embryos (Figures 4, 5 and 6; Table II), supporting the model that CTD Ser2 phosphorylation levels reflect early embryonic transcription activity (Seydoux and Dunn, 1997; Tenenhaus et al., 1998). CTD Ser2 can be phosphorylated in vitro by the CDK9 kinase of the elongation factor pTEFB (Zhou et al., 2000), but it has not been determined which kinase is primarily responsible for CTD Ser2 phosphorylation in vivo. Our findings predict that CTD Ser2 phosphorylation is likely to be broadly important for mRNA transcription.

TAF-5 orthologs have been identified in both TFII D and TFTC, each of which can mediate transcription initiation in vitro (Wieczorek et al., 1998; Brand et al., 1999b). Based upon this biochemical framework, our findings suggest that in the early C.elegans embryo, essentially all Pol II transcription may require at least one of these two complexes. Like hTAF1130, TAF-5 could be essential for the structural integrity or assembly of TFII D and TFTC (Furukawa and Tanese, 2000), even though TAF-10 and TAF-11 remained present in taf-5(RNAi) embryos (Figure 2). Alternatively, TAF-5 orthologs might fulfill a critical function of these complexes. In yeast, transcription of most genes appears to depend on either TFII D or SAGA, or both complexes (Lee et al., 2000). Yeast SAGA lacks the TAF-5 ortholog (yTAF148; Figure 1A), but contains a related histone fold protein (ADA-1) (Sterner and Berger, 2000), for which we have not identified a C.elegans ortholog. A critical structural or mechanistic role for TAF-5 predicts that most yeast transcription may require either yTAF148 or ADA-1, each of which pairs with the same histone fold partner (yTAF61/68) (Gangloff et al., 2000; Reese et al., 2000a). It will be of interest to determine whether interactions with activators are important for TAF-5 functions, because these yeast proteins lack metazoan-specific elements that bind activators (Figure 1A). Elucidating how TAF-5 orthologs function in vivo will provide important insights into how
the TFIDF- and TFTC-related complexes contribute to transcription.

Materials and methods

Worm strains and maintenance

Caenorhabditis elegans were maintained at 20°C according to standard protocols (Brenner, 1974). N2 was the wild-type strain. We used the following GFP reporter strains: end-1::gfp and elt-5::gfp (J.Rothman, unpublished), med-1::gfp and med-2::gfp (Maduro et al., 2001), pha-4::gfp (Horner et al., 1998), sur-5::gfp (Ga et al., 1998), cki-2::gfp (J.Rothman, unpublished), pie-1::gfp (Reese et al., 2000b), pes-10::gfp (G.Seydoux, unpublished), hsp-16.2::gfp (C.Link, unpublished), let-858::gfp ([Kelly et al., 1997] and rps-5::gfp (A.Fire, unpublished). These reporters differed somewhat in expression intensities, but these differences did not correlate with whether they corresponded to metazoan-specific or conserved genes.

Bioinformatics

The C.elegans homologs of TAF6 or other transcriptional regulators were identified by searching WORMpep or genomic databases (Sanger Centre) with human, Dro sophila or yeast sequences. Searches were performed using full-length and partial sequences, including predicted conserved domains. A C.elegans gene was considered orthologous to a human, Dro sophila or yeast gene only if it re-identified that gene as its closest relative in a search of GenBank redundant databases. Alignments were produced by Megalign (DNASTar). taf-5, taf-10 and taf-11 open reading frames are R1196, T12D8.7 and K03B4.3, respectively.

Immunostaining and fluorescence analysis

Rabbit antisera were generated against N-terminal peptides of TAF5 (CkiaEtrsgpvgttypqppq), TAF10 (Cdtgkrdettasldt-ghske) and TAF11 (Cmndpqepqyppssvcls) (Cocalico), then affinity purified (Pierce). Staining by each antibody was competed by autologous but not heterologous peptides (not shown). Other antibodies used included polyclonal anti-P-CTD (α-Pser5) (Schoeder et al., 2000), H5 (α-Pser2) (Babco), SWG16 (α-Unp CTD) (Babco) and POL 3/3 (Bellier et al., 1997). For staining, hermaphrodites were cut on polystyrene-treated slides. α-Pser5, α-Pser2 and anti-TAF-11 staining was performed as in Seydoux and Dunn (1997). Anti-TAF-5 or 3/3 staining was performed by fixation in paraformaldehyde then methanol, with incubations and washes in PBT [1% phosphate-buffered saline (PBS), 1% Triton X-100, 1% bovine serum albumin (BSA)]. Anti-TAF-10 was incubated in 100 mM Tris pH 7.5, 150 mM NaCl, 5% BSA after fixation in paraformaldehyde, then dimethylformamide. Secondary antibodies used were fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit and anti-mouse IgM, and Cy3-conjugated goat anti-mouse IgG (Jackson). To analyze GFP expression, embryos were transferred to 2% agarose pads. For heat shock, embryos were heated to 37°C using a Zeiss Axioskop2 microscope and AxioCam digital camera, and fluorescence was examined after a 20 min recovery. Embryos without GFP expression were re-evaluated 1±2 h later. Images were captured using a Zeiss AxioSKOP2 microscope and AxioCam digital camera, and GFP or antibody staining intensities were compared over a range of exposure times. Pixel intensities were standardized using Adobe Photoshop 5.0.

RNAS analysis

cDNAs corresponding to taf-5 (yk32612), taf-10 (yk16312), taf-11 (yk331g18), aum-1 (yk8447) and tbl-1 (yk117c3) were obtained from Yuji Kohara (NIH, Japan). Each cDNA covered >90% of the predicted coding region. In vitro synthesized double-stranded (ds) RNA (Ribomax; Promega) was injected at 0.6–1.0 μg/ml into young adults (2–8 fertilized embryos). Uniform populations of terminally arrested embryos appeared 18–22 h later, and evidence of maternal gene expression defects (rounded embryos, equal cell division planes) did not appear until 48 h. For GFP analysis or immunostaining, embryos were collected from dissected hermaphrodites 24 h after injection. Embryos were generally obtained from worm pools, but for END-1::GFP, progeny of individual worms resulted in appropriate terminal arrest, END-1::GFP expression and CTD epitope staining levels (not shown).

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