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# Distinct requirements for *C.elegans* $TAF_{II}$s in early embryonic transcription

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**$TAF_{II}$s are conserved components of the TFIID, TFTC and SAGA-related mRNA transcription complexes. In yeast (y), y$TAF_{II}$17 is required broadly for transcription, but various other $TAF_{II}$s appear to have more specialized functions. It is important to determine how $TAF_{II}$s contribute to transcription in metazoans, which have larger and more diverse genomes. We have examined $TAF_{II}$ functions in early *Caenorhabditis elegans* embryos, which can survive without transcription for several cell generations. We show that *taf-10* (*y$TAF_{II}$17*) and *taf-11* (*y$TAF_{II}$25*) are required for a significant fraction of transcription, but apparently are not needed for expression of multiple developmental and other metazoan-specific genes. In contrast, *taf-5* (*y$TAF_{II}$48*; human *$TAF_{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 *in vivo* suggests a requirement for either TFIID or a TFTC-like complex.**

*Keywords*: *C.elegans*/gene regulation/$TAF_{II}$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 *et al*., 1998; Green, 2000; Lee *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 *et al*., 1998; Rubin *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 $TAF_{II}$s (TBP-associated factors). Some $TAF_{II}$s interact with core promoter sequences, and various individual $TAF_{II}$s can bind a diverse array of upstream transactivators. In addition, human (h) $TAF_{II}$250 and its orthologs have enzymatic activities that include a conserved histone acetyl transferase (HAT) (Albright and Tjian, 2000; Green, 2000; Matangkasombut *et al*., 2000; Pham and Sauer, 2000). The $TAF_{II}$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 *et al*., 1999; Brand *et al*., 1999a). Some $TAF_{II}$s are also present in the human TBP-free $TAF_{II}$-containing complex (TFTC), and in the related complexes PCAF and STAGA (human) and SPT–ADA–GCN5 (SAGA) (yeast) (Martinez *et al*., 1998; Ogryzko *et al*., 1998; Wieczorek *et al*., 1998; Brand *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 h$TAF_{II}$250 ortholog. In addition to $TAF_{II}$s, TFTC-related complexes contain subunits that are related to TFIID-specific $TAF_{II}$s, suggesting possible functional overlap. Supporting this idea, TFTC is structurally similar to TFIID, and can mediate transcription initiation *in vitro* (Wieczorek *et al*., 1998; Brand *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 *et al*., 2000). Individual yeast $TAF_{II}$s are each necessary for cell viability, but the extent to which they are required for Pol II transcription *in vivo* remains controversial (Albright and Tjian, 2000; Green, 2000; Kuras *et al*., 2000; Li *et al*., 2000). Some yeast $TAF_{II}$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 $TAF_{II}$s function analogously to yeast $TAF_{II}$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 $TAF_{II}$ function *in vivo* has been hampered by cell lethality, and

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**Table I.** *Caenorhabditis elegans* $TAF_{II}$s

| Human | *Drosophila* | *Saccharomyces cerevisiae* | *C.elegans* clone | Predicted mol. wt | *C.elegans* name |
|---|---|---|---|---|---|
| 250 | 250/230 | 130/145 | W04A8.7 | 204.0 | *taf-1* |
| 150 | 150 | 150 | Y37E11B.4 | 137.0 | *taf-2* |
| 80/70 | 60/62 | 60 | W09B6.2 | 80.9 | *taf-3.1* |
| 80/70 | 60/62 | 60 | Y37E11AL.8 | 91.8 | *taf-3.2* |
| 100 | 80/58 | 90 | F30F8.8 | 72.1 | *taf-4* |
| 130 | 110 | 48 | R119.6 | 60.4 | *taf-5* |
| 18 | | 19 | C14A4.10 | 47.0 | *taf-6* |
| 28 | 30β | 40 | F48D6.1 | 37.8 | *taf-7.1* |
| 28 | 30β | 40 | K10D3.3 | 37.6 | *taf-7.2* |
| 28 | 30β | 40 | F43D9.5 | 24.5 | *taf-7.3* |
| 55 | | 67 | F54F7.1 | 29.2 | *taf-8.1* |
| 55 | | 67 | Y111B2A.16 | 26.4 | *taf-8.2* |
| 20 | 30α | 68/61 | Y56A4.3 | 28.0 | *taf-9* |
| 31/32 | 40/42 | 17 | T12D8.7 | 20.6 | *taf-10* |
| 30 | 24 | 25 | K03B4.3 | 19.6 | *taf-11* |

*Caenorhabditis elegans* homologs of $TAF_{II}$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* $TAF_{II}$ genes have been identified and described independently by Aoyagi and Wassarman (2000).

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 $TAF_{II}$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* $TAF_{II}$s: TAF-5, TAF-10 and TAF-11 (Figure 1; Table I). TAF-10 is of considerable interest because it is orthologous to yeast (y)$TAF_{II}17$, which is very broadly essential for *Saccharomyces cerevisiae* transcription (Apone *et al*., 1998; Michel *et al*., 1998; Moqtaderi *et al*., 1998; Lee *et al*., 2000) (Figure 1B). TAF-11 corresponds to $yTAF_{II}25$, 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 $yTAF_{II}48$, the requirements for which are unknown (Sanders and Weil, 2000). TAF-5 is particularly interesting because it corresponds to $hTAF_{II}130$, 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 TFIID, 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.

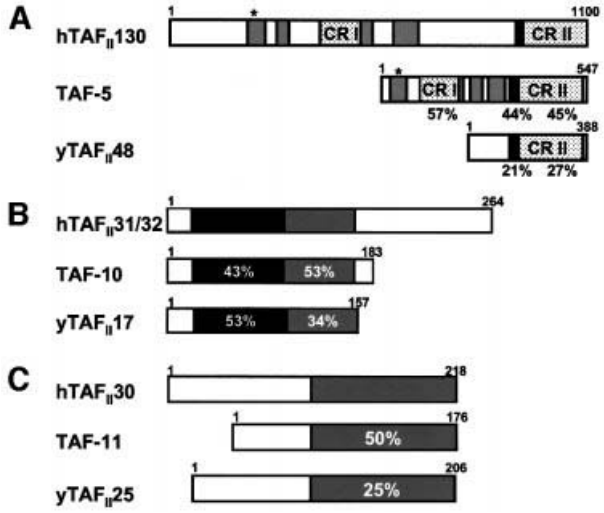


**Fig. 1.** Similarities between *C elegans* $TAF_{II}$s and their human and yeast counterparts. (**A**) *C.elegans* TAF-5 is compared with $hTAF_{II}130$ and $yTAF_{II}48$. TAF-5 includes conserved regions (CR) I and II (speckled boxes), the predicted histone fold (black box) (Gangloff *et al*., 2000) and glutamine-rich regions (gray boxes) present in $hTAF_{II}130$. The metazoan-specific conserved elements are important for activator binding by $hTAF_{II}130$ (Saluja *et al*., 1998; Rojo-Niersbach *et al*., 1999), particularly a small motif indicated by an asterisk. In (A), (B) and (C), the percentage similarity to the corresponding human $TAF_{II}$ is indicated below or within the relevant motifs. (**B**) TAF-10 is related to $hTAF_{II}31/32$ and $yTAF_{II}17$ within the histone fold (dark gray) (Burley and Roeder, 1996) and an adjacent conserved region (light gray). (**C**) TAF-11, $hTAF_{II}30$ and $yTAF_{II}25$ are most similar within the histone fold region (in gray) (Gangloff *et al*., 2001b).

## Results

### *Caenorhabditis elegans* $TAF_{II}$s

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

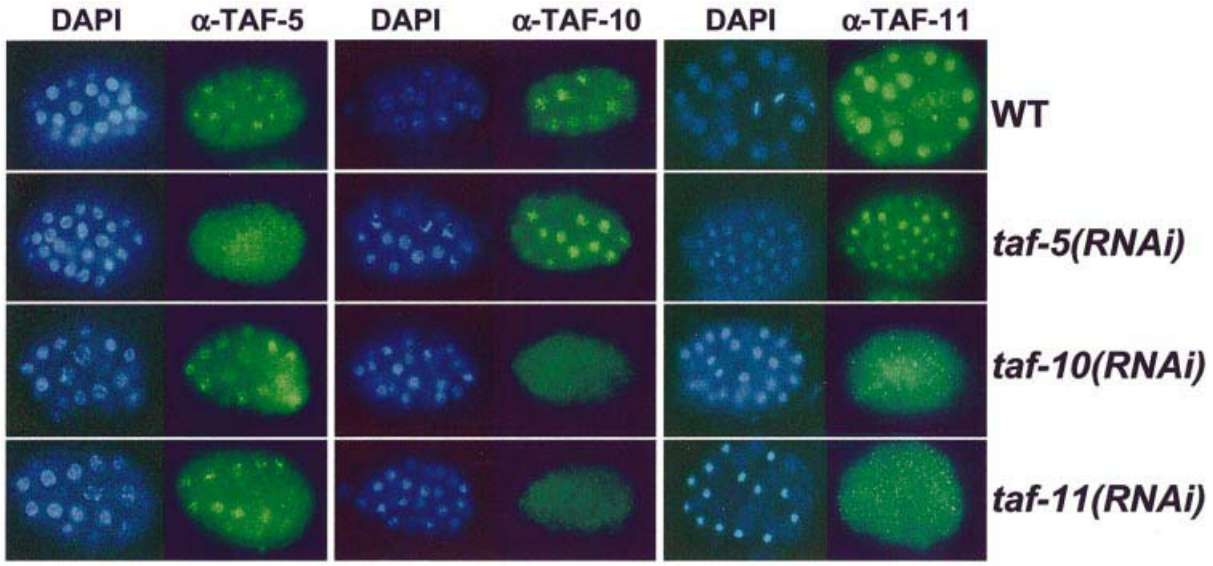


**Fig. 2.** Expression of TAF-5, TAF-10 and TAF-11 in wild-type and RNAi embryos. Representative wild-type or $TAF_{II}$ RNAi embryos (indicated in rows) were stained with antibodies to TAF-5, TAF-10 or TAF-11, or with DAPI to visualize DNA, as indicated above the columns. RNAi embryos were collected 24 h after injection of hermaphrodite mothers, when a uniformly affected population was being produced (see Materials and methods).

histone fold domains through which multiple $TAF_{II}$s 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 $TAF_{II}$ (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 $TAF_{II}$s, the GCN5/PCAF-related HAT and TRA1/TRRAP.

### *$taf_{II}$(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 *$taf_{II}$(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 *$taf_{II}$(RNAi)* embryos, the E2 cells divided immediately

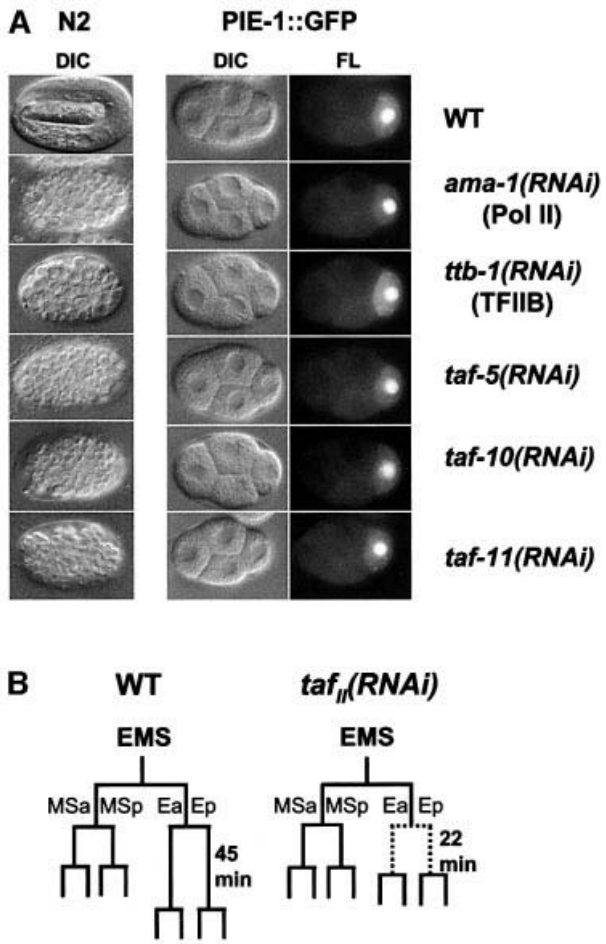


**Fig. 3.** Terminal and early cell division phenotypes of *ama-1* (RNA pol II), *ttb-1* (TFIIB), *taf-5*, *taf-10* and *taf-11* RNAi embryos. (**A**) $TAF_{II}$ RNAi embryo phenotypes. RNAi embryos produced by N2 (wild-type) or *pie-1::gfp* mothers were examined by differential interference (DIC) or 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 $taf_{II}$*(RNAi)* embryos. Lineage analysis of each set of $taf_{II}$*(RNAi)* embryos ($n \geqslant 5$) 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.

after the MS2 cells (Figure 3B). Our findings suggest that in $taf_{II}$*(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 $TAF_{II}$s destabilizes other TFIID 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 $taf_{II}$*(RNAi)* embryos with antibodies against each $TAF_{II}$ 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 $taf_{II}$*(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 $taf_{II}$(RNAi) embryos*

To investigate overall transcription levels in $taf_{II}$*(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 TFIIH 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 $taf_{II}$*(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.

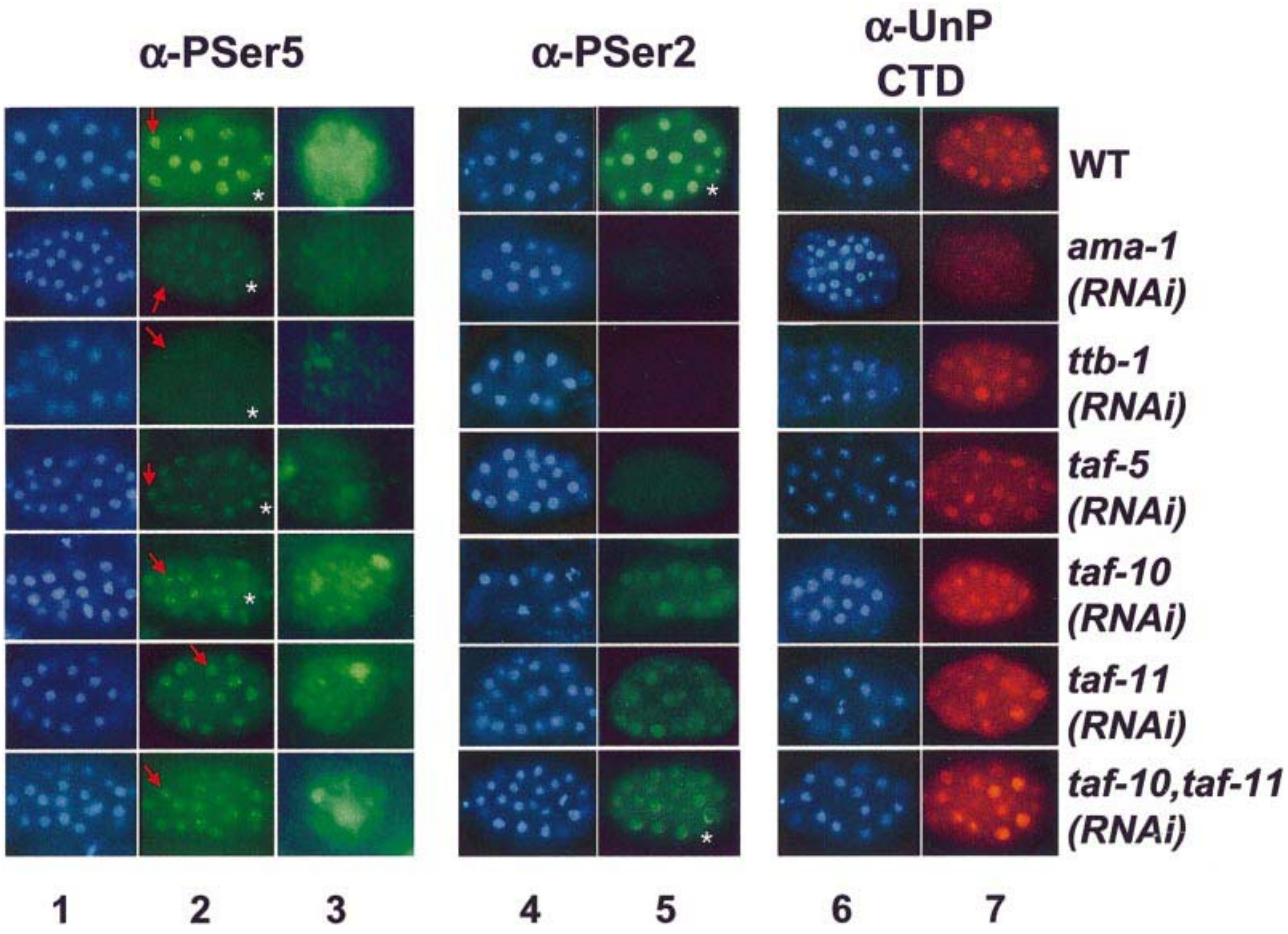


**Fig. 4.** Broader requirement for *taf-5* than *taf-10* or *taf-11* for Pol II CTD phosphorylation. Wild-type or RNAi embryos were stained with Pol II CTD antibodies (see text) prior to terminal developmental arrest. Representative embryos of comparable stages are presented in rows, as indicated. Columns 1, 4 and 6 show nuclei stained by DAPI. α-P-Ser5 staining is shown in column 2, and an expansion of the nucleus marked by the red arrow is shown in column 3. Column 5 shows embryos stained with the α-P-Ser2 antibody. Column 7 shows staining with α-UnP CTD; identical results were obtained with an antibody against a different Pol II region (POL 3/3; not shown). α-PSer5 and α-PSer2 recognize Pol II isoforms associated with transcription initiation and elongation, respectively (Komarnitsky *et al*., 2000; Schroeder *et al*., 2000), and the α-UnP CTD antibody provides a Pol II expression control. In columns 2 and 5, germline nuclei that are in the focal plane shown are marked with an asterisk. In α-P-Ser5-stained germline nuclei, note the lack of nucleoplasmic staining and the presence of two discrete dots (Seydoux and Dunn, 1997). Some germline nuclei stained with α-PSer2 have perinuclear background deriving from cross-reactivity of the secondary antibodies with P granules. The relative differences in α-PSer5 and α-PSer2 staining intensities were comparable when embryos were photographed at multiple different exposure times.

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 $TAF_{II}$s 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 $TAF_{II}$s (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 $taf_{II}$s 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 $TAF_{II}$ 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).

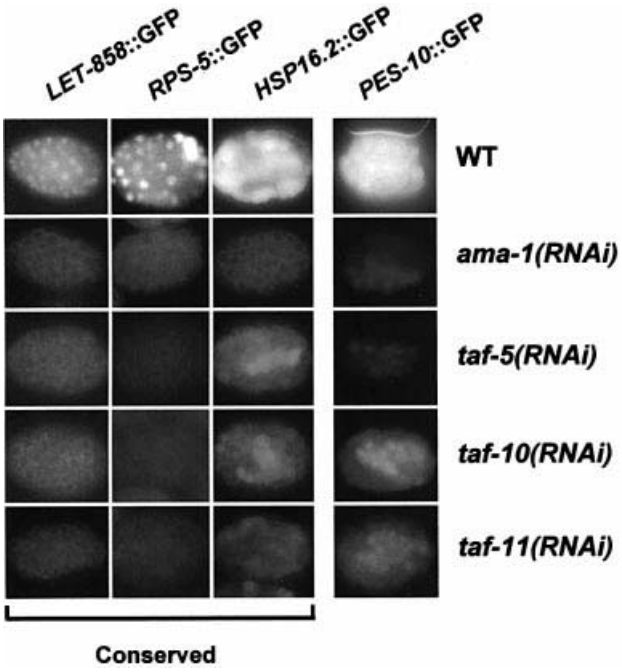


**Fig. 5.** Comparable requirements for *taf-5*, *taf-10* and *taf-11* at conserved genes. GFP fluorescence was examined in wild-type or $taf_{II}$*(RNAi)* embryos (in rows) that were produced by the reporter strains indicated above the columns. Each of these reporters was expressed in most embryonic 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.

We also investigated the importance of these $TAF_{II}$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 (Horner *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 (Horner *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)*; *taf-11(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 $TAF_{II}$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 $yTAF_{II}17$, which appears to be as broadly required for transcription as any $TAF_{II}$ 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

**Table II.** Requirements for $TAF_{II}$s for metazoan-specific gene expression

| | SUR-5::GFP (MAP kinase pathway) | CKI-2::GFP (cell cycle) | MED-1::GFP (endoderm/mesoderm) | MED-2::GFP (endoderm/mesoderm) | PHA-4::GFP (digestive tract) | ELT-5::GFP (ectoderm) |
|---|---|---|---|---|---|---|
| Wild type | + | + | + | + | + | + |
| *ama-1(RNAi)* | – | – | – | – | – | – |
| *taf-5(RNAi)* | – | – | – | – | – | – |
| *taf-10(RNAi)* | + | + | + | + | + | + |
| *taf-11(RNAi)* | + | + | + | + | + | + |

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 $taf_{II}$ 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.

broader role in transcription than has been demonstrated previously for other $TAF_{II}$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 $TAF_{II}$s are not required for transcription of a significant proportion of *C.elegans* embryonic genes.

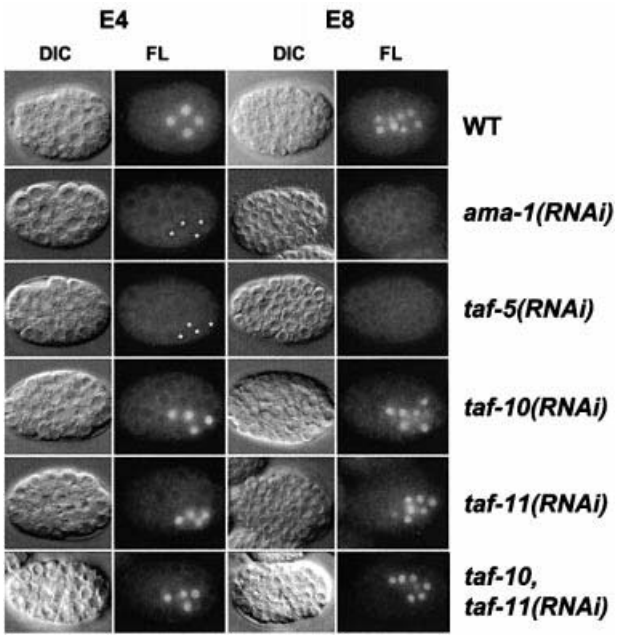


**Fig. 6.** *taf-5*, *taf-10* and *taf-11* are essential for gastrulation, but vary in importance for END-1::GFP expression. END-1::GFP expression was examined in RNAi embryos, as indicated to the right of each row. Differential interference (DIC) and fluorescent (FL) images of an E4 and an E8 stage embryo from each set are shown. In a representative experiment, END-1::GFP was not expressed in any *ama-1(RNAi)* or *taf-5(RNAi)* embryos ($n$ >100), but at the E4 and E8 stages was expressed at normal levels in most *taf-10(RNAi)* (90%; $n$ = 71) and *taf-11(RNAi)* (80%; $n$ = 82) embryos. In parallel experiments, END-1::GFP was expressed in a similar proportion of *taf-10(RNAi)*; *taf-11(RNAi)* embryos. Within these last RNAi embryo sets, only a small proportion (<5%) expressed END-1::GFP in E2 cells (not shown). Lineage analysis ($n$ >5) revealed that all END-1::GFP-positive cells derived from the E cell. Asterisks mark the E4 cells in the *ama-1(RNAi)* and *taf-5(RNAi)* embryos. In each of these RNAi embryo sets, E2 descendants were mislocalized to the posterior edge of the embryo because their abnormally short cell cycle resulted in defective gastrulation.

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 $yTAF_{II}17$ is broadly required for transcription (Apone *et al*., 1998; Moqtaderi *et al*., 1998; Lee *et al*., 2000), but argue against the model that $yTAF_{II}17$ and $yTAF_{II}25$ 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 $yTAF_{II}25$ 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 $TAF_{II}$s. While some yeast heat shock genes are *$yTAF_{II}17$* 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 $TAF_{II}$ 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 $TAF_{II}$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 $TAF_{II}$ RNAi

**Table III.** Requirements for yeast and *C.elegans* $TAF_{II}$s for transcription *in vivo*

| *S.cerevisiae* | | *C.elegans* embryo | |
|---|---|---|---|
| $TAF_{II}$ | Transcriptional requirement | $TAF_{II}$ | Transcriptional requirement |
| $TAF_{II}48$ | unknown | TAF-5 | essentially complete |
| $TAF_{II}17$ | 59% of genes[a]; broad or essentially complete[b] | TAF-10, TAF-11 | significant fraction, not including many metazoan-specific genes |
| $TAF_{II}25$ | 16% of genes[a]; essentially complete[b] | | |

Results of the present study are compared with *in vivo* analyses of *S.cerevisiae* $TAF_{II}17$ and $TAF_{II}25$ (see text).
[a]Percentages indicate the proportion of yeast genes that required the indicated $TAF_{II}$ 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 $yTAF_{II}17$ (Michel *et al*., 1998; Moqtaderi *et al*., 1998) or $yTAF_{II}25$ (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 $TAF_{II}30$ (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 TFIID- 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 *cbp-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 germline 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 (Dantonel *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 $hTAF_{II}130$ isoform found in B cells ($hTAF_{II}105$) is required for some transcription driven by the Dorsal-related factor NF-κB (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 $hTAF_{II}130$ 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 sequestering of $hTAF_{II}130$ 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 germline 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 *ttb-1(RNAi)* embryos (Figure 4), suggesting that TFIIB may be required at an earlier transcription step than TAF-5. Consistent with this idea, during activation of a mammalian gene *in vivo*, TFIIB and the Pol II holoenzyme (including the TFIIH kinase) were recruited to the promoter before TFIID (Agalioti *et al*., 2000). Levels of α-PSer2 staining correlated with nucleoplasmic α-PSer5 staining and reporter expression defects in the respective *taf$_{II}$(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 TFIID 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 $hTAF_{II}130$, TAF-5 could be essential for the structural integrity or assembly of TFIID 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 TFIID or SAGA, or both complexes (Lee *et al*., 2000). Yeast SAGA lacks the TAF-5 ortholog ($yTAF_{II}48$; 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 $yTAF_{II}48$ or ADA-1, each of which pairs with the same histone fold partner ($yTAF_{II}61/68$) (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 TFIID- 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* (Gu *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 $TAF_{II}$s or other transcriptional regulators were identified by searching WORMpep or genomic databases (Sanger Centre) with human, *Drosophila* 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, *Drosophila* 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 R119.6, T12D8.7 and K03B4.3, respectively.

### Immunostaining and fluorescence analysis

Rabbit antisera were raised against N-terminal peptides of TAF-5 (CKIAGERSTPGVSTPEPAPPQ), TAF-10 (CDTGEKDTETTASDTD-GHSKE) and TAF-11 (CMNDPEQYEPSSSTESVL) (Cocalico), then affinity purified (Pierce). Staining by each antibody was competed by autologous but not heterologous peptides (not shown). Other antibodies used included P-CTD (α-PSer5) (Schroeder *et al*., 2000), H5 (α-PSer2) (Babco), 8WG16 (α-UnP CTD) (Babco) and POL 3/3 (Bellier *et al*., 1997). For staining, hermaphrodites were cut on polylysine-treated slides. α-PSer5, α-PSer-2 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, *hsp16.2::gfp* embryos were heated to 37°C for 1 min in 10 µl of PBS. 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.

### RNAi analysis

cDNAs corresponding to *taf-5* (yk326f12), *taf-10* (yk163f12), *taf-11* (yk331g8), *ama-1* (yk84f7) and *ttb-1* (yk117e3) were obtained from Yuji Kohara (NIG, Japan). Each covered >90% of the predicted coding region. *In vitro* synthesized double-stranded (ds) RNA (Ribomax; Promega) was injected at 0.6–1.0 µg/µl 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 were scored. Because most analyses were performed before terminal arrest, RNAi effectiveness was confirmed by monitoring sibling embryos that were allowed to develop. Simultaneous *taf-10* and *taf-11* RNAi was performed with a 1:1 mixture of dsRNAs. In parallel, a 1:1 dilution of each individual dsRNA with either TE or an unrelated dsRNA (*glp-1)* resulted in appropriate terminal arrest, END-1::GFP expression and CTD epitope staining levels (not shown).

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