An Extensive Requirement for Transcription Factor IID-specific TAF-1 in Caenorhabditis elegans Embryonic Transcription*

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The general transcription factor TFIID sets the mRNA start site and consists of TATA-binding protein and associated factors (TAF<sub>18</sub>s), some of which are also present in SPT-ADA-GCN5 (SAGA)-related complexes. In yeast, results of multiple studies indicate that TFIID-specific TAF<sub>18</sub>s are not required for the transcription of most genes, implying that intact TFIID may have a surprisingly specialized role in transcription. Relatively little is known about how TAF<sub>18</sub>s contribute to metazoan transcription in vivo, especially at developmental and tissue-specific genes. Previously, we investigated functions of four shared TFIID/SAGA TAF<sub>18</sub>s in Caenorhabditis elegans. Whereas TAF-4 was required for essentially all embryonic transcription, TAF-5, TAF-9, and TAF-10 were dispensable at multiple developmental and other mRNAs. In the promoters we studied, we show that in C. elegans embryos transcription of most genes requires TFIID-specific TAF-1. TAF-1 is not as universally required as TAF-4, but it is essential for a greater proportion of transcription than TAF-5, -9, or -10 and is important for transcription of many developmental and other metazoan-specific genes. TAF-2, which binds core promoters with TAF-1, appears to be required for a similarly substantial proportion of transcription. C. elegans TAF-1 overlaps functionally with the coactivator p300/CBP (CBP-1), and at some genes it is required along with the TBP-like protein TLF(TRF2). We conclude that during C. elegans embryogenesis TAF-1 and TFIID have broad roles in transcription and development and that TFIID and TLF may act together at certain promoters. Our findings imply that in metazoans TFIID may be of widespread importance for transcription and for expression of tissue-specific genes.

Eukaryotic mRNA transcription involves formation of a preinitiation complex (PIC) at the core promoter, which directs initiation. The PIC includes a set of general transcription factors (TFIIA, B, D, E, F, and H) and a mediator complex, along with RNA polymerase II (pol II) (1, 2). In Saccharomyces cerevisiae many PIC components have surprisingly specific roles at particular gene subsets (3, 4). Much less is known about how individual PIC components contribute to transcription regulation in metazoa, which have evolved a greater complexity of stage- and tissue-specific gene control and additional genes that are not present in yeast.

The general transcription factor TFIID is of particular interest because it establishes the start site and provides enzymatic activities that may regulate transcription (5, 6). TFIID is comprised of the TATA-binding protein (TBP) along with ~14 TBP-associated factors (TAF<sub>18</sub>s). TAF<sub>18</sub>s interact with core promoter elements and contact a diverse array of upstream trans-activators (5–8). TAF-1 and TAF-2 together bind directly to the initiator (Inr) element, which encompasses the start site (9). TAF-1, the largest TAF<sub>18</sub>, is also necessary for TFIID stability and possesses histone acetyltransferase, kinase, and ubiquitin conjugating activities (10). TAF-1 is unique to TFIID, but many other TAF<sub>18</sub>s are also found in the SPT-ADA-GCN5 (SAGA)-related complexes (5, 6), which are similar in structure to TFIID but lack TBP and contain a GCN5-related histone acetyltransferase instead of TAF-1.

In S. cerevisiae conditional mutation or shut-off analyses suggest that many individual TAF<sub>18</sub>s have surprisingly specific functions (5, 6). For example, whole genome analyses indicate that TFIID-specific taf-1 and taf-2 are essential for expression of only 14 and 3% of genes, respectively (3, 4, 11), and chromatin immunoprecipitation has detected significant TAF<sub>18</sub> occupancy only at TFIID-dependent genes (12, 13). These studies suggest that in yeast a major proportion of transcription involves a TAF<sub>18</sub>-independent form of TFIID and that the TFIID-specific TAF<sub>18</sub>s are each required to transcribe only a modest fraction of the genome, although this model remains a subject of investigation and debate (14). In contrast, expression of the majority of yeast genes is prevented by conditional loss of either TAF-9, which is shared between TFIID and SAGA, or of Taf-1 and the SAGA histone acetyltransferase GCN5 simultaneously, suggesting that TFIID and SAGA are redundant at many genes (4–6).

Although considerable information has been obtained about TAF<sub>18</sub> functions in yeast, it is a distinct question how TAF<sub>18</sub>s contribute to transcription in vivo in metazoa, particularly in the context of the complex processes of tissue development or differentiation. The three-dimensional structure of TFIID is conserved among eukaryotes (15–17), predicting a similar conservation of function. However, transcription in metazoa involves a more complex interplay between promoters and long range elements, as well as additional PIC components and TAF<sub>18</sub> isomers that are not present in yeast (2). Loss of TAF<sub>18</sub> function in metazoa has been difficult to study because TAF<sub>18</sub> are expressed both maternally and zygotically, thus...
completing interpretation of mutant phenotypes. For example, in Drosophila taf-1 mutants have pleiotropic defects, but the consequences of eliminating both maternally and zygotically expressed TAF-1 have not been determined (18). In hamster cells a conditional taf-1 mutation decreased expression of \(-18\%\) of genes and caused apoptosis (19), finding that is consistent with yeast data but does not appear to involve complete ablation of TAF-1 function.

In the Caenorhabditis elegans embryo, it is possible to use RNA interference (RNAi) (20) to inhibit both maternal and zygotic expression of C. elegans TAF-\(_{\text{p gs}}\). If transcription is prevented in the early C. elegans embryo, maternal supplied mRNAs maintain viability until the 100-cell stage, making it feasible to block expression of even essential transcription factors (21). Using this strategy, we determined previously that TAF-4 is required for essentially all early embryonic transcription (22). In contrast, TAF-5, TAF-9, and TAF-10 were required for significant and comparable fractions of early transcription but appeared to be dispensable at most metazoan-specific promoters (22, 23). Each of the TAFpgs we have analyzed is shared between TFIID and SAGA-like complexes, leaving open the question of how broadly TFIID is required in the embryo. This issue is of particular interest because a major fraction of C. elegans embryonic transcription requires the TBP isomorph TLF (TRF2), which does not associate with TAF-1 (24–26).

In this study we have determined that TFIID-specific TAF-1 is essential for most transcription in the developing C. elegans embryo. In contrast to the shared TFIID/SAGA TAF-5, -9, and -10, TAF-1 is needed for many metazoan-specific genes to be expressed at appropriate levels. TAF-1 does not appear to be universally essential for early embryonic transcription, however, unlike TAF-4. TAF-4 appears to be required for a similarly extensive fraction of embryonic transcription as TAF-1. We have also obtained evidence for functional overlap between TAF-1 and the C. elegans CBP/p300 ortholog cbp-1. We conclude that in the early C. elegans embryo TFIID and promoter recognition by TAFpgs are important for transcription of most genes, including many that require TLF.

EXPERIMENTAL PROCEDURES

C. elegans and Bioinformatics—C. elegans strains were provided to us and maintained as described previously (22). The wild-type (WT) strain was N2. TAFpgs are named according to Tora (27), a nomenclature different from that described previously for C. elegans TAFpgs (22). C. elegans taf-1 and taf-2 each reidentified their corresponding human and Drosophila counterparts in GenBank data bases. Alignments were produced by Megalign (DNastar).

Immunostaining and Fluorescence Analysis—Rabbit antisera were raised against the TAF-1 peptide VSQKPHKDENATPVPVKKLVT with an N-terminal Cys added and affinity purified (22). For TAF-1 staining, embryos were fixed with 1% paraformaldehyde and 0.1% glutaraldehyde before treating with methanol. Washes and antibody incubations were performed in PBT (1/10 phosphate-buffered saline, 1% Triton X-100, 0.1% bovine serum albumin) prior to staining. TAF-1 antibody staining was competed by the cognate but not heterologous peptide (not shown). Staining with α-TAF-9, α-TAF-10, α-pol II (pol 3/3) (22), P-CTD (anti-phospho-Ser-5) (28), H5 (anti-phospho-Ser-2) (Convance), and CBP-1 (29) was performed as indicated in Ref. 22. α-TBP-1 and α-TLF-1 immunostaining was performed as in Ref. 25. Staining with the H14 antibody was performed as for H5 and provided results identical to those with the P-CTD antibody. Green fluorescent protein (GFP) analysis, image capture, and manipulation were performed as described by Walker et al. (22).

RNAi Analysis—For injection of dsRNA, cDNA fragments for taf-1 (nucleotides 3066–3942 and 4329–4936) and taf-2 (nucleotides 385–1347) were generated by PCR from a C. elegans cDNA library (gift of Marc Vidal). Identical results were obtained from both taf-1 cDNAs as well as from a taf-1 clone (yk617) obtained from Yuji Kohara (NIG, Japan). dsRNA synthesis was synthesized in vitro with Megascript (Ambion). Injection and analysis of embryos were performed as described by Walker et al. (22). Simultaneous double RNAi was performed with a 1:1 mixture of dsRNAs. In parallel, a 1:1 dilution of each individual dsRNA with an unrelated dsRNA (gfp-1) resulted in appropriate terminal arrest, reporter gene expression, and CTD phosphorylation levels (not shown). For feeding of dsRNA, cDNA fragments for taf-1 (nucleotides 2791–3408) and ama-1 (nucleotides 1254–2259) were inserted into the feeding vector pPD129.36 (gift of Andy Fire). Synchronized L4 larvae were placed on bacteria expressing dsRNA to gfp (pPD128.110, gift of Andy Fire), ama-1, or taf-1 for 36 h. taf-1 and ama-1(RNAi) embryos produced from feeding dsRNA at 36 h had anti-phospho-Ser-2 staining patterns and LET-858:GFP expression similar to injected dsRNA at 24 h (not shown).

RT-PCR—N2 hermaphrodites were fed dsRNA for taf-1, ama-1, or gfp (control). Adults were washed five times in phosphate-buffered saline, and embryos were collected by bleaching. After lysing embryos in 0.5% SDS, 5% β-mercaptoethanol, 10 mM EDTA, 10 mM Tris-HCl, pH 7.5, and 0.5 mg/ml proteinase K (Invitrogen), RNA was extracted with Tri-Reagent (Sigma). cDNA was produced from 1 μg of control RNA and from equivalent numbers of ama-1 or taf-1(RNAi) embryos (Superscript II, Invitrogen). PCR was performed using HotMix (Eppendorf). Each primer set was tested on cDNA produced from at least two independent RNA preparations. At least three dilutions of cDNA were tested, and multiple cycle numbers were used to assure linearity of reaction. Primers were designed to span at least one intron (sequences available upon request).

Immunoblot Analysis—Control, taf-1(RNAi), or ama-1(RNAi) embryos from feeding were collected as for RT-PCR, then lysed by sonication in 100 mM Tris, pH 7.9, 3 mM MgCl\(_2\), 0.3 mM KCl, 0.1% Nonidet P-40, 1 mM dithiothreitol, and 20% glycerol. Proteins were separated on 6.5% gels, transferred to nitrocellulose, and probed with the antibodies indicated in Fig. 5. In this experiment anti-phospho-Ser-5 was H14 and pol II was ARNAS (Research Diagnostics). Secondary antibodies used were goat anti-rabbit IgG (Kirkegaard and Perry Laboratories) for anti-phospho-Ser-5 and anti-phospho-Ser-2, goat anti-mouse (Jackson Immunologicals) for pol II, and goat anti-rabbit (Jackson Immunologicals) for α-CBP-1. Blots were visualized by enhanced chemiluminescence (Amersham Biosciences).

RESULTS

taf-1 Is Essential during Early Embryonic Development—To investigate TFIID functions in the early C. elegans embryo, we inhibited TAF-1 expression by RNAi. C. elegans TAF-1 is significantly related to hTAF-1 throughout its length, including predicted functional domains (Fig. 1A). A specific antisemur detected TAF-1 in all WT embryonic nuclei, in oocytes, and in the adult germ line, indicating that taf-1 is maternally expressed (Fig. 2 and data not shown). Accordingly, taf-1 mRNA levels were only modestly reduced when zygotic transcription was prevented by RNAi knock-down of ama-1, the pol II large subunit (Fig. 3D). In taf-1(RNAi) embryos, nuclear TAF-1 antibody staining was eliminated (Fig. 2), and taf-1 mRNA levels were reduced dramatically (Fig. 3D), indicating that TAF-1 expression was decreased significantly. In contrast, levels of multiple other TAFpgs, TBP, and AMA-1 were similar to WT in taf-1(RNAi) embryos (Fig. 2 and data not shown).

C. elegans embryonic development is initially sustained by maternally provided gene products (30). Interference with maternal and zygotic expression of other TAFpgs or PIC components such as ama-1 and tfb-1 (TFIIB) arrests embryonic development at about 100 cells without signs of differentiation, a phenotype that is characteristic of a broad zygotic transcription defect (21–23, 31). In taf-1(RNAi) embryos, nuclear TAF-1 antibody staining was eliminated (Fig. 2), and taf-1 mRNA levels were reduced dramatically (Fig. 3D), indicating that TAF-1 expression was decreased significantly. In contrast, levels of multiple other TAFpgs, TBP, and AMA-1 were similar to WT in taf-1(RNAi) embryos (Fig. 2 and data not shown).

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period of the two E daughters (E2 cells), which give rise to the endoderm, was decreased by approximately half (Fig. 3 C). The last phenotype is characteristic of a broad transcription defect (21, 22). Our findings suggest that depletion of embryonic TAF-1 does not detectably alter maternal mRNA stores but may significantly impair embryonic mRNA transcription.

Severely Reduced pol II CTD Phosphorylation Levels in taf-1(RNAi) Embryos—We investigated how mRNA transcription was affected in taf-1(RNAi) embryos first by analyzing phosphorylation of the pol II large subunit CTD. The CTD consists of multiple repeats that are based upon the consensus YSPTSPS (33). Polymerase II is initially recruited in an unphosphorylated form, then at the promoter its CTD repeat is phosphorylated on Ser-5 by the TFIIH kinase (28, 34). During elongation the distribution of CTD phosphorylation shifts to Ser-2 (34, 35), which is phosphorylated by the P-TEFb kinase (31, 36). CTD Ser-5 and Ser-2 phosphorylation can be specifically detected in C. elegans embryonic nuclei by staining with the H14 (or P-CTD) and H5 antibodies, respectively (22, 28, 37), which we refer to as anti-phospho-Ser-5 and anti-phospho-Ser-2 for clarity (Figs. 4 and 5).
In the *C. elegans* embryo, the levels and patterns of anti-phospho-Ser-5 and anti-phospho-Ser-2 staining parallel overall transcription activity (22, 23, 31, 37, 38). Nuclear staining with these antibodies first appears at the three- to four-cell stage, when new mRNA transcription begins. CTD Ser-2 phosphorylation is detected only in the transcriptionally active somatic cells (Fig. 4, A, C, and D) (37). CTD Ser-5 phosphorylation is apparent as a partially punctate nucleoplasmic pattern in interphase somatic nuclei but is limited to two discrete foci in the transcriptionally silent germ line nuclei (Fig. 4, A, B, and D). These germ line foci depend upon the general transcription factor TFIIB and the mediator component RGR-1, suggesting that they require PIC formation (22, 31).

In *taf-1*(RNAi) embryos nucleoplasmic anti-phospho-Ser-5 and anti-phospho-Ser-2 staining was dramatically and consistently reduced in all somatic cells (Fig. 4A). The level of anti-phospho-Ser-2 staining in these RNAi embryos was only slightly higher than the background seen in transcriptionally silent *ama-1*(RNAi) or *taf-4*(RNAi) embryos and was significantly lower than in *taf-5*, *taf-9*, or *taf-10*(RNAi) embryos (Fig. 4A) (22, 23), suggesting that most pol II transcription had been prevented. Accordingly, in *taf-1*(RNAi) embryos nucleoplasmic anti-phospho-Ser-2 staining was decreased proportionally to anti-phospho-Ser-5 staining, except that two anti-phospho-Ser-5 foci like those normally present in the germ line were prominent in somatic cells (Fig. 4, A and B), as had been observed previously in *taf-4*, *taf-5*, *taf-9*, and *taf-10*(RNAi) embryos. Anti-phospho-Ser-2 and anti-phospho-Ser-5 staining levels were affected similarly when *taf-1* and *taf-10* were inhibited simultaneously by RNAi (*taf-1; taf-10*(RNAi); Fig. 4A), indicating that the residual CTD phosphorylation in *taf-1*(RNAi) embryos was not sensitive to depletion of an additional TAFII and was unlikely to derive from a partial RNAi effect. Significantly, in individual *taf-1*(RNAi) embryos CTD phosphorylation levels were proportionally decreased between the onset of transcription at the four-cell stage until terminal arrest (data not shown).

**Fig. 4. Reduced pol II CTD phosphorylation in individual *taf-1*(RNAi) embryos.** A, decreased CTD Ser-2 and Ser-5 phosphorylation in *taf-1*(RNAi) embryos. Prior to developmental arrest, WT or RNAi embryos (in rows) were stained with anti-phospho-Ser-2 or anti-phospho-Ser-5 antibodies and DAPI to visualize DNA. Representative embryos of comparable stages are presented. In parallel experiments, staining with an antibody against a different RNA pol II region revealed that pol II levels were equivalent in WT and TAFII RNAi embryos (Fig. 2). The relative differences in anti-phospho-Ser-5 and anti-phospho-Ser-2 staining intensities between WT and RNAi embryos were comparable between the onset of transcription at the four-cell stage and terminal arrest and when embryos were photographed at multiple exposure times. Germ line nuclei that are in the focal planes shown are marked with a white asterisk. B, expanded anti-phospho-Ser-5-stained somatic nuclei. In *taf-1*(RNAi) somatic nuclei, nucleoplasmic anti-phospho-Ser-5 staining is dramatically reduced, but two bright foci are present as in the WT germ line. C, CTD Ser-2 phosphorylation in *taf-1*(RNAi) and *cbp-1*(RNAi) embryos, analyzed as in A. Yellow asterisks indicate cells in early stages of mitotic chromosome condensation, in which anti-phospho-Ser-2 stains a pol II-independent cross-reactive epitope (37). D, CTD phosphorylation in *taf-2*(RNAi) embryos. In this figure, αP Ser5 refers to staining with the P-CTD (A and B) and H14 (D) antibodies, which stain with highly similar patterns (not shown).
shown), suggesting that this reduction derived from a continuous broad decrease in pol II transcription and not a stage-specific abnormality.

The conclusions of these antibody staining experiments were supported by immunoblot analyses of embryo extracts, which demonstrated that in taf-1(RNAi) embryo populations CTD Ser-2 and Ser-5 phosphorylation was only barely detectable (Fig. 5). In contrast, and also consistent with immunofluorescence experiments (Fig. 2), the levels of total pol II present in taf-1(RNAi) embryos were at least equivalent to the levels detected in control extracts (α-pol II, Fig. 5). The dramatic decreases in pol II CTD phosphorylation which accompanied taf-1 RNAi knock-down indicated that taf-1 is required for the majority of pol II transcription in the early embryo.

Decreased Expression of Conserved and Metazoan-specific Genes in taf-1(RNAi) Embryos—To evaluate the importance of TAF-1 for expression of individual genes in vivo, we used two types of assay. First, we used RNAi to inhibit taf-1 expression in C. elegans strains that carry transgenic reporter genes. These transgenes include intact regulatory regions fused to GFP and are expressed in parallel to the corresponding endogenous genes. A unique advantage of this system is that it allows analysis of de novo gene expression in individual living embryos. Each of these reporters is fully dependent upon taf-4, but in taf-5, taf-9, and taf-10(RNAi) embryos the metazoan-specific reporters we have analyzed are expressed at WT levels (22, 23). Second, we used RT-PCR to measure the expression of endogenous genes in control and RNAi embryos.

We first investigated the expression of two groups of genes that are expressed widely within the embryo. rps-5, let-858, and the heat shock gene hsp-16.2 each has orthologs in unicellular eukaryotes as well as in metazoans. In C. elegans, their expression requires taf-5, taf-9, and taf-10 in addition to taf-4 (22, 23), and in yeast expression of rps-5 and other ribosomal protein genes is dependent upon many TAFII8 (7, 11). Expression of the corresponding GFP reporters was abolished in taf-1(RNAi) embryos (Fig. 6A). Accordingly, levels of endogenous rps-5 and rps-26 mRNAs were also lower in ama-1 and taf-1(RNAi) embryos (Fig. 6B). The residual rps-5 and rps-26 mRNA that was detected in ama-1 and taf-1(RNAi) embryos is likely to be derived from the previously described maternal expression of these genes (39, 40), which would not be affected in our assays. In addition, and in contrast to TAF-5, -9, and -10, TAF-1 was also critical for expression of the widely expressed metazoan-specific genes cki-2 (CDK inhibitor) and sur-5 (mitogen-activated protein kinase kinase pathway) (Table I and Fig. 6D).

We also analyzed expression of GFP reporters for tissue-specific genes involved in development of the mesoderm (med-1 and -2), endoderm (end-1), pharynx (pha-4), and epidermis (elt-5) in taf-1(RNAi) embryos. As embryonic transcription begins med-1 and -2 are induced by maternally provided SKN-1 (Fig. 7A) (41), then pha-4 and elt-5 are expressed slightly later (42, 43). end-1 regulation appears to be complex (Fig. 7A), involving MED proteins and the actions of CBP-1 and WNT signaling, which together relieve repression mediated by histone deacetylase (HDA-1) and POP-1 (29, 41, 44). In taf-5, taf-9, and taf-10(RNAi) embryos these genes were expressed at WT levels (Table I) (22, 23). In contrast, in taf-1(RNAi) embryos the med-1, med-2, pha-4, and elt-5 reporters were expressed in normal patterns but at significantly reduced levels (Figs. 7, B and C, and Table I). This residual MED-1::GFP expression was also seen in taf-1, taf-10(RNAi) embryos (Fig. 7B), indicating that it is independent of taf-10. END-1::GFP was expressed at WT levels in ~70% of taf-1(RNAi) embryos, however (Fig. 7C and, Table I), a difference that may derive from the multiple inputs that act at this promoter (Fig. 7A). The robustness of this end-1 expression suggests that the reductions in transcription of other genes seen in taf-1(RNAi) embryos reflects a
requirement for TAF-1 at those genes and not a nonspecific abnormality.

RT-PCR experiments demonstrated that expression of many endogenous metazoan-specific genes was reduced similarly in taf-1 (RNAi) embryos. Expression of the zygotically expressed genes pha-4 and elt-2 was undetectable in ama-1 (RNAi) embryos (Fig. 6B). Expression of genes that appear to be expressed both maternally and zygotically expressed (sur-5, elt-5, and pal-1) was reduced but not eliminated in both ama-1 and taf-1 (RNAi) embryos (Fig. 6B). Significantly, mRNA production from the corresponding endogenous genes paralleled expression of the PHA-4, ELT-5, and SUR-5::GFP reporters in these RNAi embryos (Figs. 6B and 7C and Table I), suggesting that in general the decreases in reporter expression which we observed in living embryos reflected comparably reduced endogenous transcription.

This broad requirement for a TFIID-specific TAR II in the early embryo is surprising because in *C. elegans* most embryonic transcription involves the TBP isoform TLF (TRF2) (24, 25), which does not associate with TAFs (26). In *Xenopus* and *zebrafish* embryos TLF and TBP are required at partially overlapping sets of genes (45, 46). In *Drosophila* cells TLF and TFIID can direct initiation from different promoter types, however (26), and in mice TLF is required specifically at spermio genesis genes (47), suggesting that TLF is a promoter specificity factor that may act at different genes from TFIID. However, *C. elegans* TLF is needed for appropriate expression of pha-4 (24, 25), which also requires TAF-1 (Figs. 6B and 7C). To investigate this question further, we examined expression of pes-10, which is activated when embryonic transcription begins and is TLF-dependent and bound by TLF in vivo (25). In taf-1 (RNAi) embryos TLF was present (Fig. 2), but our analysis showed that pes-10 expression was decreased dramatically (Fig. 6B and Table I). Significantly, TLF was also required at rps-5 and let-858 (Fig. 6, A and B), genes that are taf-1-depend-

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**Table I**

**Requirements for taf-1 in metazoan-specific gene expression**

Expression of the indicated reporters is designated as + for no fluorescence or the background levels seen in ama-1 (RNAi) (pol II) embryos, ++ for wild type levels, +++ for intermediate levels of expression, and +++ for very low levels. Results from ama-1 (RNAi) and taf-9 (RNAi) experiments are from Ref. 22. For each data set, more than 40 embryos were analyzed in multiple independent experiments.

<table>
<thead>
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<th>Reporter</th>
<th>AMA-1 (RNAi)</th>
<th>TAF-1 (RNAi)</th>
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<tr>
<td>PES-10::GFP (early zygotic)</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>MED-2::GFP (endoderm)</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>PHA-4::GFP (digestive tract)</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>CKI-2::GFP (cell cycle)</td>
<td>--</td>
<td>+++</td>
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<tr>
<td>SUR-5::GFP (MAP kinase pathway)</td>
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* MAP, mitogen-activated protein.
that during embryonic development CBP-1 has functions that

\[\text{RNAi}\] (Fig. 7, indicating that TAF-1 and TAF-2 are required for similarly

dominates of C. elegans (49), but cbp-1 RNAi did not detectably reduce embrionic CTD phosphorylation levels (Fig. 4C), suggesting a minimal effect on total transcription. Accordingly, in cbp-1(RNAi) embryos many genes were expressed at near normal levels, including some that are upstream of CBP-1-dependent differentiation (med-1, pha-4, and elt-5) (Figs. 6B and 7, B and C). CTD phosphorylation levels were not distinguishably different between taf-1(RNAi) and taf-1; cbp-1(RNAi) embryos (Fig. 4C), suggesting that most TAF-1 and CBP-1 functions are nonredundant, but simultaneous interference with cbp-1 eliminated taf-1-independent med-1, pha-4, and elt-5 expression (taf-1; cbp-1(RNAi)); Fig. 7, B and C). In contrast, in taf-10; cbp-1(RNAi) embryos med-1 and elt-5 were expressed at near WT levels (Fig. 7, B and C, and data not shown). We conclude that during embryonic development CBP-1 has functions that overlap with those of TAF-1, but not necessarily other TAF II s.

Compared Reduced CTD Phosphorylation in taf-1(RNAi) and taf-2(RNAi) Embryos—The broad requirement for TAF-1 we have observed predicts a similarly broad role for TAF-2, which cooperates with TAF-1 to bind to the Inr (9). In yeast taf-2 is required to transcribe only 3% of the genome, however, the smallest fraction of any TAF II (4, 11). TAF-2 is TFIID-specific in yeast, but in humans it is also present within the TPTC complex, which can substitute for TFID to initiate transcription (50). When expression of C. elegans TAF-2 (Fig. 1B) was inhibited by RNAi, embryonic development was arrested similarly to ama-1(RNAi) and taf-1(RNAi) embryos (data not shown). Significantly, at each stage taf-2(RNAi) embryos were indistinguishable from taf-1(RNAi) embryos in their anti-phospho-Ser-2 and anti-phospho-Ser-5 staining levels (Fig. 4D), indicating that TAF-1 and TAF-2 are required for similarly extensive proportions of C. elegans embryonic transcription.

<table>
<thead>
<tr>
<th>TAF-1</th>
<th>C. elegans embryo</th>
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<tr>
<td></td>
<td>Most transcription; more extensive than TAF-5, -9, or -10a</td>
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<tr>
<td></td>
<td>14% of genesb,c</td>
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<tr>
<td>TAF-2</td>
<td>Comparable to TAF-1a</td>
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<tr>
<td></td>
<td>3% of genesd,e</td>
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<tr>
<td>TAF-4</td>
<td>Essentially completea</td>
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<td>Broad, but apparently not completed, 11% of genesf,g</td>
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<tr>
<td>TAF-5</td>
<td>Significant fraction; not including most metazoan-specific genesh</td>
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<tr>
<td></td>
<td>Small or broad fraction; 8% of genesi</td>
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<tr>
<td>TAF-9</td>
<td>Significant fraction; not including most metazoan-specific genesh</td>
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<tr>
<td></td>
<td>Broad or essentially completej; 59% of genesk,l</td>
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<tr>
<td>TAF-10</td>
<td>Significant fraction; not including most metazoan-specific genesh</td>
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<td>Essentially complete, k 16–19% of genesk,l</td>
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a, b Approximate proportion of transcription requiring the indicated TAF II as indicated by the following studies in C. elegans: aRef. 22, et al. 23; bRef. 4, et al. 11, or individual gene Ref. 65, 66 and 67 analysis. c Approximate proportion of genes or bulk transcription requiring TAF-9 (yTAF II17) (68–70) or TAF-10 (yTAF,25) (71).

**DISCUSSION**

We have obtained evidence that taf-1 and taf-2 are each required for most mRNA transcription in the C. elegans embryo. In taf-1(RNAi) and taf-2(RNAi) embryos, at every stage nucleoplasmatic anti-phospho-Ser-2 and anti-phospho-Ser-5 antibody staining was decreased to levels only slightly higher than background (Fig. 4 and data not shown), indicating that continuous broad reductions in pol II transcription had occurred. Immunoblots of embryonic extracts also showed a striking decrease in pol II CTD phosphorylation (Fig. 5). TFIID-specific TAF-1 was also necessary for normal expression of each metazoan-specific gene that we analyzed, with the exception of end-1 (Figs. 6B and 7 and Table I). This requirement for TAF-1 is much more extensive than revealed by previous analyses performed in metazoans, in which TAF-1 function was not completely ablated (18, 19).

It appears unlikely that the limited transcription that occurred in taf-1(RNAi) embryos derived from incomplete RNAi. Expression of the conserved genes let-588, rps-5, and hsp-16.2 was decreased as severely in taf-1(RNAi) embryos as in ama-1(RNAi) embryos (Fig. 6 and data not shown). taf-1(RNAi) phenotypes were not enhanced by simultaneous inhibition of taf-10 (Figs. 4A and 7B) and were highly consistent and accompanied by depletion of TAF-1 protein (Fig. 2) and mRNA (Fig. 3D). We conclude that the residual transcription levels in taf-1(RNAi) embryos derive from a small group of largely taf-1-independent genes, including end-1, and from low level expression of metazoan-specific genes such as med-1, pha-4, and elt-5 (Figs. 6B and 7, B and C).

TAF-1 represents a third functional class of C. elegans TAF II, defined by our experiments (Table II). Unlike TAF-4, TAF-1 does not appear to be generally essential for transcription. TAF-1 is distinct from the TFIID/SGA TAFII group represented by TAF-5, -9, and -10, however, because those TAF II s are dispensable widely at metazoan-specific genes (Figs. 6B and 7 and Table I). Accordingly, in taf-1(RNAi) embryos nucleoplasmic pol II CTD phosphorylation levels were intermediate between those found in taf-4(RNAi) and taf-5, -9, or -10(RNAi) embryos (Fig. 4A) (22, 23). The comparable reductions in CTD phosphorylation found in taf-2(RNAi) embryos suggest that TAF-2 belongs to the same functional class as TAF-1 (Table II).

It is intriguing that in each TAF II RNAi embryo set we have analyzed, somatic nuclei contain two discrete anti-phospho-Ser-5 staining foci (Fig. 4B) (22, 23). Similar foci are normally present in the embryonic germ line, where transcription is blocked by PIE-1 (51), a global repressor that appears to act at a postinitiation step (52, 53). These anti-phospho-Ser-5 foci depend upon the presence of the general transcription factor TFIIIB, the mediator component RGR-1, and the CTD Ser-5 kinase CDK-7 (22, 38, 54), but not upon the mRNA capping enzyme or the elongation kinase P-TEFb, which are required...
specifically for Ser-2 phosphorylation (31, 55). The dependence of these anti-phospho-Ser-5 foci on initiation factors suggests that they might derive from aborted or incomplete transcription events and that the lack of TAFIIα blocks some transcription after PIC formation and CTD Ser-5 phosphorylation have occurred.

The extensive requirements for TAF-1 and TAF-2 for C. elegans transcription are surprising because in S. cerevisiae these TAFIIα have been reported to be essential for transcription of 14 and 9% of the genome, respectively (Table II). In addition, most studies indicate that in yeast the shared TFIIID/SAGA TAFIIα, Taf-5, Taf-9, and Taf-10 are more broadly required than Taf-1 or Taf-2. In striking contrast, in C. elegans these three shared TFIIID/SAGA TAFIIα are needed for a significantly smaller proportion of embryonic transcription than TAF-1 and are dispensable at various metazoan-specific genes that require taf-1 for normal expression levels (Table II). It is possible that some of these differences derive from technical factors. Yeast TAFIIα have been studied in conditional mutant strain populations in the context of ongoing mRNA production, but we depleted TAFIIα before transcription began in individual C. elegans embryos, where maternal mRNAs sustain viability. We believe, however, that these differences may derive from taf-1 and taf-2 having a broader role in C. elegans transcription.

The importance of C. elegans TAF-1 for transcription could derive from its having mechanistic functions that yeast TAF-1 does not. In metazoas but not yeast, TAF-1 contains a C-terminal kinase and a double bromodomain that targets TFIIID to acetylated nucleosomes (Fig. 1A) (56). In yeast, related domains are present in the Bdfl protein, which interacts stoichiometrically with TFIIID and in its genetically redundant relative Bdfl2 (57). Bdfl and Bdfl2 have metazoan orthologs that are distinct from TAF-1, however, and apparently have TFIIID-independent functions in euchromatin maintenance (58, 59). Although it is possible that the TAF-1 bromodomains might have some TFIIID-independent functions, the comparable requirement for TAF-2 which we have observed (Fig. 4D) argues against this view.

The simplest model to explain our findings is that a higher percentage of promoters require TFIIID in the C. elegans embryo than in yeast. In yeast it seems that a TAFIIα-independent TBP form is sufficient at many genes and that TFIIID occupancy is proportional to transcription only where the TATA element is weak or nonexistent (12, 13). TFIIID is recruited to most yeast promoters at low levels, however (7), consistent with it possibly being required more broadly for TBP recruitment in other organisms. Although relatively little is known about C. elegans promoters, fewer than half of surveyed human and Drosophila core promoters contain a TATA element (60), suggesting that TFIIID-dependent promoters may be abundant in metazoans. This requirement may not extend to all core promoter elements, however; TAF-6 and TAF-9 bind the downstream promoter element (DPE), a core promoter motif identified in humans and Drosophila, predicting that recognition of a DPE-like element might not be necessary at the many C. elegans genes that do not require TAF-9 for expression (Table II).

In addition to the possibility that promoter recognition by TAF-1 and TAF-2 may be more important in metazoans than in yeast, the relationship among TFIIID, activators, and other transcription regulatory factors may be more complex in metazoans. For example, in Drosophila the versatility of combinatorial gene regulation is enhanced by pairing of compatible enhancers and core promoters (60). An interesting aspect of the C. elegans embryo is the importance of the TBP-like protein TLF for transcription (24, 25). Although TLF can direct transcription to TFIIID-independent promoters (26), in the C. elegans embryo various genes require both TLF and TAF-1 (see “Results”) and possibly TFIIID. Some of these genes are conserved in unicellular eukaryotes (Fig. 6), which lack TLF. Our findings suggest that in certain contexts TLF has specialized regulatory functions and acts in concert with TFIIID, a model that is consistent with evidence that TLF may influence chromatin organization (26, 61).

It is intriguing that the metazoan-specific histone acetyltransferase CBP-1 apparently has functions that overlap with and complement those of TAF-1 in vivo. Thus, although cbp-1 inhibition eliminated some taf-1-independent gene expression, end-1 is largely taf-1-independent but requires cbp-1 to overcome repression (Fig. 7, A and C) (44). The functional overlap between TAF-1 and CBP-1 could involve their respective histone acetyltransferase functions but could also derive from these proteins contributing to PIC stabilization. It is striking that CBP-1 was required for only a very limited proportion of transcription (Figs. 4C and 7, B and C), given its importance for many differentiation pathways (49). Certain genes, like end-1, may require CBP-1 because their regulation involves particular signals or repressor activities (Fig. 7A).

The broad role in C. elegans embryonic transcription played by TAF-1 and TAF-2 suggests that these TAFIIα and TFIIID may generally be of greater importance for transcription in metazoans than predicted from yeast studies. Although TFIIID and other PIC components are recruited to promoters in vivo with precise timing, the order of these events varies among different genes, presumably so that their regulation can be tailored to fit particular situations (62). Our findings support the idea that the functional relationships among PIC components and coactivators also vary among species and biological contexts. Elucidating these differences is likely to be important for understanding regulation of metazoan transcription, particularly for unraveling the complexities of tissue- and stage-specific gene regulation.

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TAF-1 Required Broadly in C. elegans Transcription

An Extensive Requirement for Transcription Factor IID-specific TAF-1 in *Caenorhabditis elegans* Embryonic Transcription
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