THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 15, Issue of April 9, pp. 15339–15347, 2004
Printed in U.S.A.

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

Received for publication, September 29, 2003, and in revised form, January 13, 2004
Published, JBC Papers in Press, January 15, 2004, DOI 10.1074/jbc.M310731200

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The general transcription factor TFIID sets the mRNA start site and consists of TATA-binding protein and associated factors (TAF_{II}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_{II}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_{II}s contribute to metazoan transcription *in vivo*, especially at developmental and tissue-specific genes. Previously, we investigated functions of four shared TFIID/SAGA TAF_{II}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 metazoan-specific promoters. Here we show evidence 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)[1] 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 metazoans, 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_{II}s). TAF_{II}s interact with core promoter elements and contact a diverse array of upstream transactivators (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_{II}, 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_{II}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_{II}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_{II} occupancy only at TFIID-dependent genes (12, 13). These studies suggest that in yeast a major proportion of transcription involves a TAF_{II}-independent form of TFIID and that the TFIID-specific TAF_{II}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_{II} functions in yeast, it is a distinct question how TAF_{II}s contribute to transcription *in vivo* in metazoans, 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 metazoans involves a more complex interplay between promoters and long range elements, as well as additional PIC components and TAF_{II} isoforms that are not present in yeast (2). Loss of TAF_{II} function in metazoans has been difficult to study because TAF_{II}s are expressed both maternally and zygotically, thus

* This work was supported by grants from the National Institutes of Health (to T. K. B. and Y. S.) and the March of Dimes (to T. K. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PIC, preinitiation complex; CBP, cAMP-responsive element-binding protein-binding protein; CTD, C-terminal domain; dsRNA, double strand RNA; GFP, green fluorescent protein; Inr, initiator; pol II, RNA polymerase II; RNAi, RNA interference; RT, reverse transcription; SAGA, SPT-ADA-GCN5; TAF_{II}, TATA-binding protein-associated factor; TBP, TATA-binding protein; TF, transcription factor; WT, wild type.

complicating 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), a 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_{II}s. If transcription is prevented in the early *C. elegans* embryo, maternally 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 TAF_{II}s 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 isoform TLF(TRF2), which does not associate with TAF_{II}s (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, or -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-2 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 TAF_{II}s 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. TAF_{II}s are named according to Tora (27), a nomenclature different from that described previously for *C. elegans* TAF_{II}s (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× phosphate-buffered saline, 1% Triton X-100, 1% bovine serum albumin) prior to staining. TAF-1 antibody staining was competed by the cognate but not heterologous peptides (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) (Covance), and CBP-1 (29) was performed as 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 (yk6h7) 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 (*glp-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 M 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 α-pol II was ARNA3 (Research Diagnostics). Secondary antibodies used were goat anti-rabbit IgM (Kirkegaard and Perry Laboratories) for anti-phospho-Ser-5 and anti-phospho-Ser-2, goat anti-mouse (Jackson Immunologicals) for α-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. 1*A*). A specific antiserum 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. 3*D*). In *taf-1*(*RNAi*) embryos, nuclear TAF-1 antibody staining was eliminated (Fig. 2), and *taf-1* mRNA levels were reduced dramatically (Fig. 3*D*), indicating that TAF-1 expression was decreased significantly. In contrast, levels of multiple other TAF_{II}s, 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 TAF_{II}s or PIC components such as *ama-1* and *ttb-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). *taf-1*(*RNAi*) embryos arrested development at a similar stage, apparently without differentiation (Fig. 3*A*). To evaluate maternal RNA storage we monitored early cell divisions and performed parallel experiments in a transgenic strain that expressed a fusion of the maternally derived germ line protein PIE-1 to GFP. Appropriate localization of PIE-1::GFP depends on at least 20 maternal genes (32). In *taf-1*(*RNAi*) embryos PIE-1::GFP expression and localization patterns were normal at every stage (Fig. 3*B*; not shown), suggesting that storage of maternal gene products was likely to be intact. Early cell division timing and cleavage planes were also normal in these RNAi embryos, except that the cell cycle

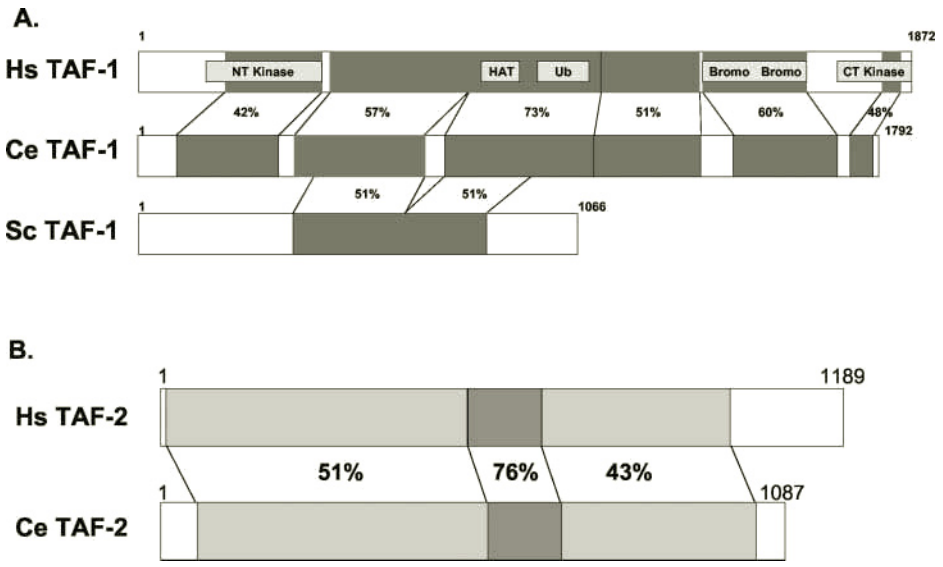

FIG. 1. **Predicted *C. elegans* TAF-1 and TAF-2 proteins.** *A*, *C. elegans* (*Ce*) TAF-1 (amino acids *numbered* above) compared with its human (*Hs*) and yeast (*Sc*) counterparts. CeTAF-1 includes predicted N-terminal and C-terminal kinase domains (*NT Kinase*, *CT kinase*, respectively), histone acetyltransferase (*HAT*) and ubiquitin conjugation (*Ub*) domains, and a bromodomain pair. The bromodomains and CT-kinase have been found only in metazoan TAF-1 orthologs (57). *Percentages* indicate similarity to human and yeast TAF-1, respectively. *B*, CeTAF-2 compared with HsTAF-2 as in *A*. CeTAF-2 is similarly related to *S. cerevisiae* Taf-2 throughout its length (not shown).

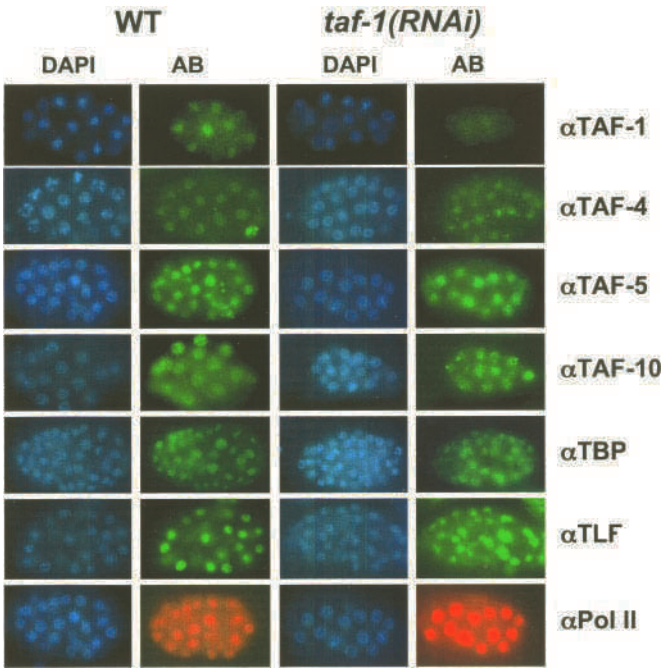

FIG. 2. **Expression of TAF$_{II}$s, TBP, and TLF in *taf-1(RNAi)* embryos.** Representative WT or *taf-1*(*RNAi*) embryos (designated above the *columns*) were stained with antibodies as indicated, along with DAPI to visualize DNA. Embryos measure ~50 μm.

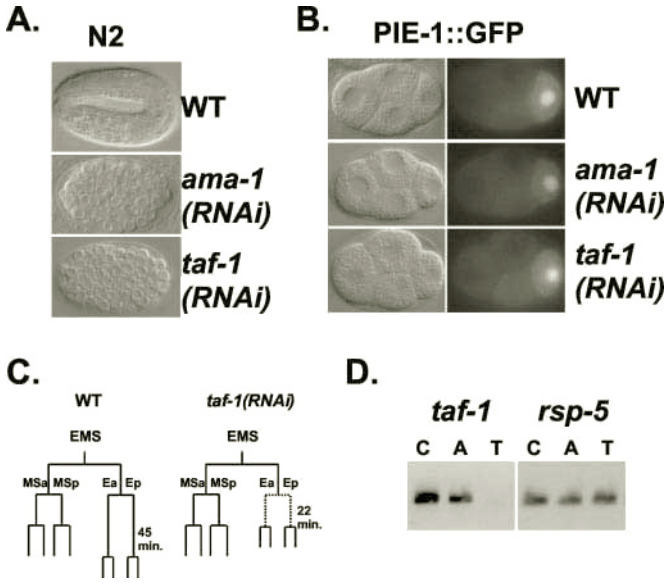

FIG. 3. **Terminal and early cell division phenotypes of *taf-1(RNAi)* embryos.** *A*, *taf-1*(*RNAi*) phenotype. Representative terminally arrested RNAi embryos were examined by differential interference microscopy and compared with a WT embryo that was about to hatch. *ama-1*(*RNAi*) and *taf-1*(*RNAi*) embryos each ceased development with 90–100 cells ($n = 4$). *ama-1* encodes the pol II large subunit. *B*, PIE-1::GFP expression in WT and RNAi embryos, examined by fluorescence microscopy. In *taf-1*(*RNAi*) embryos, each aspect of PIE-1::GFP germ line and subcellular localization was indistinguishable from WT, including the presence of PIE-1 in germ line RNA-protein P granules (32). *C*, shortened E2 cell cycle in *taf-1*(*RNAi*) embryos. Lineage analysis of *taf-1*(*RNAi*) embryos revealed that their early cell division planes were normal except that the E2 cells (*Ea* and *Ep*) divided prematurely. Only the EMS cell lineage is shown. *D*, depletion of *taf-1* mRNA in *taf-1*(*RNAi*) embryos. RT-PCR was performed with RNA from control (*C*), *ama-1*(*RNAi*) (*A*), or *taf-1*(*RNAi*) (*T*) embryos. The moderate decrease in *taf-1* mRNA in *ama-1*(*RNAi*) embryos is consistent with inhibition of zygotic but not maternal *taf-1* expression, but in *taf-1*(*RNAi*) embryos both maternal and zygotic expression of *taf-1* was largely prevented. In contrast, expression of the strictly maternally expressed gene *rsp-5* (39, 40) was unaffected by knock-down of either *taf-1* or *ama-1*.

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).

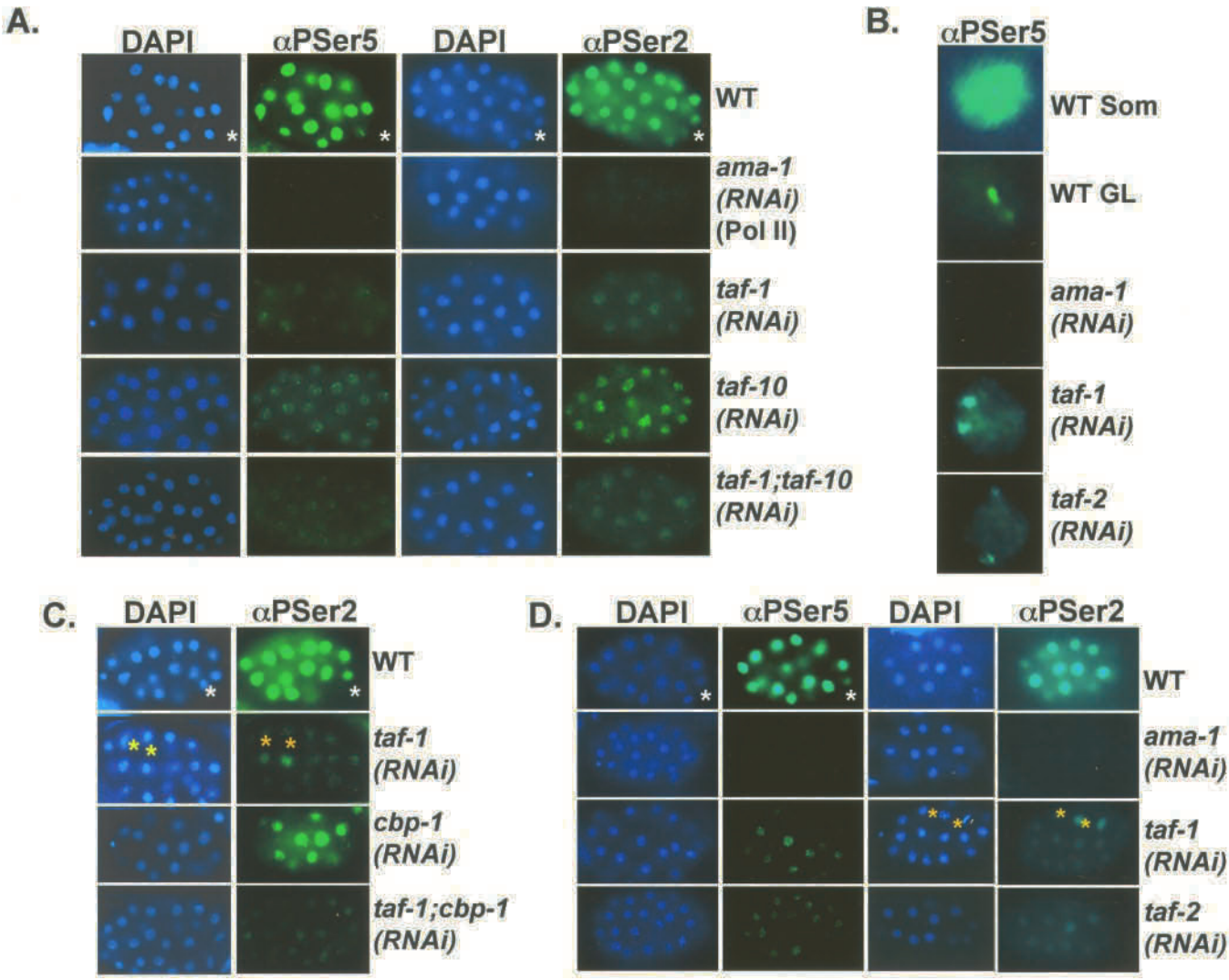

FIG. 4. **Reduced pol II CTD phosphorylation in individual *taf-1(RNAi)* embryos.** *A*, decreased CTD Ser-2 and Ser-5 phosphorylation in *taf-1(RNAi)* embryos. Prior to developmental arrest, WT or RNAi embryos (in *rows*) were stained with anti-phospho-Ser-2 or anti-phospho-Ser-5 antibodies and DAPI 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 TAF_{II} 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 an *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, *αPSer5* refers to staining with the P-CTD (*A* and *B*) and H14 (*D*) antibodies, which stain with highly similar patterns (not shown).

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 nucleus (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. 4*A*). 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. 4*A*) (22, 23), suggesting that most pol II transcription had been prevented. Accordingly, in *taf-1(RNAi)* embryos nucleoplasmic anti-phospho-Ser-5 staining was decreased proportionally to anti-phospho-Ser-2 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. 4*A*), indicating that the residual CTD phosphorylation in *taf-1(RNAi)* embryos was not sensitive to depletion of an additional TAF_{II} 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

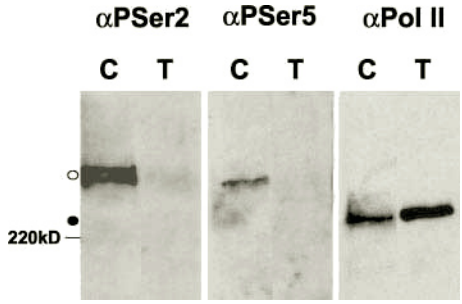

FIG. 5. **Reduced pol II CTD phosphorylation in *taf-1*(*RNAi*) embryo populations.** Immunoblots of protein extracts prepared from control (*C*) or *taf-1*(*RNAi*) (*T*) embryos were probed with anti-phospho-Ser-2, anti-phospho-Ser-5 (H14), and α-pol II antibodies (see "Experimental Procedures"). Hyper- and hypophosphorylated forms of pol II are designated with an *open* and *closed circle*, respectively. In this *taf-1*(*RNAi*) sample the dramatically decreased CTD phosphorylation levels that were present are representative, but levels of a loading control (CBP-1) were higher than WT (not shown), suggesting that the seemingly elevated level of total pol II (*right panel*) is unlikely to be significant.

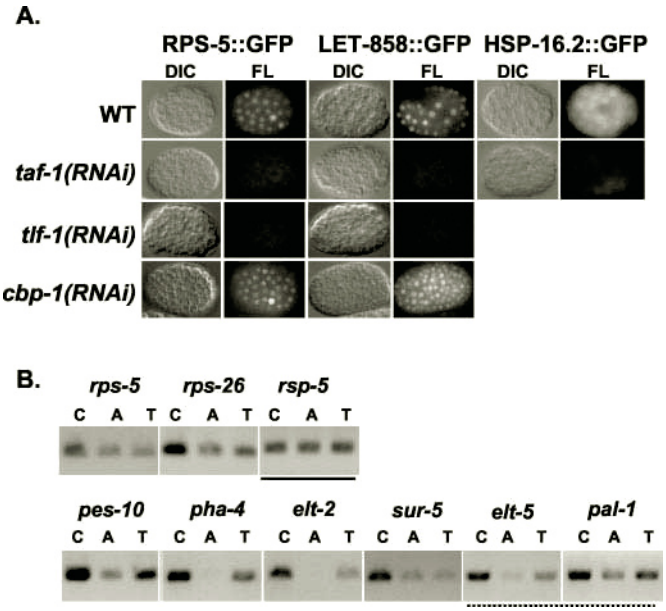

FIG. 6. ***taf-1*- and *tlf-1*-dependent expression of individual genes.** *A*, reduced expression of generally conserved eukaryotic genes in *taf-1* and *tlf-1*(*RNAi*) embryos. Differential interference (*DIC*) and fluorescent (*FL*) images are shown of WT or RNAi embryos (in *rows*) from the indicated reporter strains. In a representative experiment the nonintegrated RPS-5::GFP reporter was expressed in 18 of 40 WT embryos and 23 of 41 *cbp-1*(*RNAi*) embryos, but in none of >50 of each set of *taf-1*(*RNAi*) or *tlf-1*(*RNAi*) embryos. Embryos shown are otherwise representative of the entire population (>40 embryos) analyzed in each of multiple independent experiments. *B*, reduced expression of endogenous genes in *ama-1* and *taf-1*(*RNAi*) embryos. RT-PCR was performed with total RNA from control (*C*), *ama-1*(*RNAi*) (*A*), or *taf-1*(*RNAi*) (*T*) embryos. Genes shown on the *top row* are conserved all among eukaryotes, and those on the *bottom row* are metazoan-specific. In *C. elegans* the presence of embryonic *rsp-5* (*solid underline*) mRNA does not depend upon embryonic AMA-1 or TAF-1 because this gene is transcribed specifically in the maternal germ line (39, 40). *elt-5* and *pal-1* (*dotted underline*) may be expressed both maternally and zygotically (39, 40, 63, 64). Each primer set was tested against at least two independent RNA preparations in multiple experiments. To assure linearity of the reactions, primer sets were used with multiple cDNA dilutions and at different amplification cycle numbers. *rsp-5* was tested in each independent experiment to control for the amount of RNA present.

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 TAF_{II}s (7, 11). Expression of the corresponding GFP reporters was abolished in *taf-1*(*RNAi*) embryos (Fig. 6*A*). Accordingly, levels of endogenous *rps-5* and *rps-26* mRNA were also lower in *ama-1* and *taf-1*(*RNAi*) embryos (Fig. 6*B*). 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. 6*B*).

We also analyzed expression of GFP reporters for tissue-specific genes involved in development of the mesentoderm (*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. 7*A*) (41), then *pha-4* and *elt-5* are expressed slightly later (42, 43). *end-1* regulation appears to be complex (Fig. 7*A*), 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 (Fig. 7, *B* and *C*, and Table I). This residual MED-1::GFP expression was also seen in *taf-1, taf-10*(*RNAi*) embryos (Fig. 7*B*), 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. 7*C* and, Table I), a difference that may derive from the multiple inputs that act at this promoter (Fig. 7*A*). The robustness of this *end-1* expression suggests that the reductions in transcription of other genes seen in *taf-1*(*RNAi*) embryos reflects a

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.

	PES-10::GFP (early zygotic)	MED-2::GFP (endoderm)	PHA-4::GFP (digestive tract)	CKI-2::GFP (cell cycle)	SUR-5::GFP (MAP[a] kinase pathway)
WT	+++	+++	+++	+++	+++
ama-1(RNAi)	−	−	−	−	−
taf-9(RNAi)	++	+++	+++	+++	+++
taf-1(RNAi)	+	+	+	+	+

[a] MAP, mitogen-activated protein.

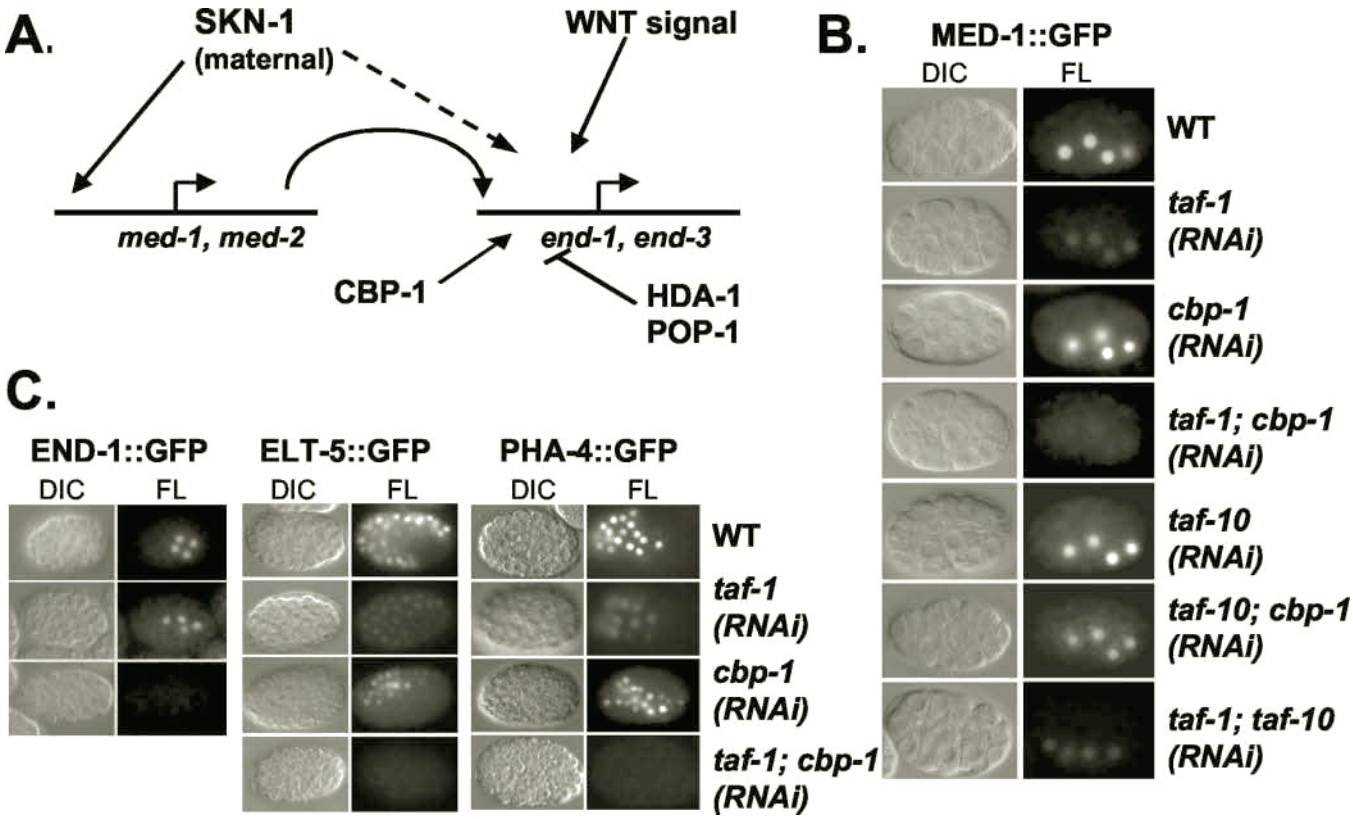

FIG. 7. **Defects in expression of developmental genes in *taf-1(RNAi)* embryos.** *A*, pathways controlling early mesendodermal gene expression in *C. elegans*. Regulation of *end-1* depends on multiple maternal and zygotic inputs (see "Results"). *B*, expression of MED-1::GFP in RNAi embryos, analyzed as in Fig. 5. *C*, developmental gene expression in *taf-1* and *cbp-1*(*RNAi*) embryos. END-1::GFP is expressed specifically in the E cell (endodermal) lineage. In a representative experiment, END-1::GFP was expressed at the E4 and E8 stages at normal levels in (70%; $n = 68$) of *taf-1*(*RNAi*) embryos. *end-1* is unique among genes that we have analyzed in that END-1::GFP expression is not necessarily uniform within sets of TAF_{II} RNAi embryos (22, 23). In each RNAi embryo set, E2 descendants were mislocalized to the posterior edge of the embryo because of defective gastrulation (Fig. 3). In all mixed dsRNA experiments a uniform total RNA concentration was maintained using unrelated dsRNA.

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 and greatly reduced in *taf-1*(*RNAi*) embryos (Fig. 6*B*). 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. 6*B*). 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. 6*B* and 7*C* and Table I), suggesting that in general the decreases in reporter expression which we observed in living embryos reflected comparably reduced endogenous e transcription.

This broad requirement for a TFIID-specific TAF_{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 TAF_{II}s (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 spermiogenesis 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. 6*B* and 7*C*). 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. 6*B* 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-

TABLE II
Requirements for yeast and C. elegans TAF$_{II}$s for transcription in vivo
In vivo analyses of TAF$_{11}$ function in *C. elegans* and *S. cerevisiae* are summarized.

	C. elegans embryo	*S. cerevisiae*
TAF-1	Most transcription; more extensive than TAF-5, -9, or -10[a]	14% of genes[d,e]
TAF-2	Comparable to TAF-1[a]	3% of genes[d,e]
TAF-4	Essentially complete[b]	Broad, but apparently not complete[f]; 11% of genes[e]
TAF-5	Significant fraction; not including most metazoan-specific genes[c]	Small or broad fraction[g]; 8% of genes[e]
TAF-9	Significant fraction; not including most metazoan-specific genes[b]	Broad or essentially complete[h]; 59% of genes[d,e]
TAF-10	Significant fraction; not including most metazoan-specific genes[b]	Essentially complete,[h] 16–19% of genes[d,e]

[a–c] Approximate proportion of transcription requiring the indicated TAF$_{II}$ as indicated by the following studies in *C. elegans:* [a]this study, [b]Ref. 22, [c]Ref. 23.
[d–g] Percentages of *S. cerevisiae* genes requiring a particular TAF$_{II}$, as indicated by whole genome [d]Ref. 4,[e] Ref. 11, or individual gene [f]Ref. 65, [g]Refs. 66 and 67 analysis.
[h] Approximate proportion of genes or bulk transcription requiring TAF-9 (yTAF$_{II}$17) (68–70) or TAF-10 (yTAF$_{II}$25) (71).

ent and conserved in yeast, which lack TLF. We conclude that in the embryo transcription of various individual genes involves both TLF and TFIID.

Overlapping Requirements for TAF-1 and CBP-1 at Some Metazoan-specific Genes—In *S. cerevisiae* TAF-1 and GCN5 appear to have redundant functions at many genes (4). Surprisingly, RNAi knock-down of the single *C. elegans* GCN5-related histone acetyltransferase (CeGCN5; ORF Y47G6A.6) depleted its mRNA but did not impair development and did not detectably reduce gene expression in the *taf-1* RNAi background.[2] This finding does not suggest major functional overlap between CeGCN-5 and TAF-1, although contributions from persistent maternal CeGCN-5 protein cannot be ruled out. Because the human SAGA-like complex TFTC can function cooperatively with the metazoan histone acetyltransferase p300/CBP (48), we tested whether TAF-1 and the *C. elegans* p300/CBP ortholog CBP-1 might have overlapping functions.

CBP-1 is required for all non-neuronal differentiation in *C. elegans* (49), but *cbp-1* RNAi did not detectably reduce embryonic CTD phosphorylation levels (Fig. 4*C*), 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. 6*B* and 7, *B* and *C*). CTD phosphorylation levels were not distinguishably different between *taf-1*(*RNAi*) and *taf-1; cbp-1*(*RNAi*) embryos (Fig. 4*C*), 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.

Comparably 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 TFTC complex, which can substitute for TFIID to initiate transcription (50). When expression of *C. elegans* TAF-2 (Fig. 1*B*) 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. 4*D*), indicating that TAF-1 and TAF-2 are required for similarly extensive proportions of *C. elegans* embryonic transcription.

[2] A. Walker, P. Dufourcq, and F. Gay, unpublished observations.

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 nucleoplasmic 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. 6*B* 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-858*, *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. 4*A* and 7*B*) and were highly consistent and accompanied by depletion of TAF-1 protein (Fig. 2) and mRNA (Fig. 3*D*). 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. 6*B* 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/SAGA TAF$_{II}$ group represented by TAF-5, -9, and -10, however, because those TAF$_{II}$s are dispensable widely at metazoan-specific genes (Figs. 6*B* and Fig. 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. 4*A*) (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. 4*B*) (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 TFIIB, 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 TAF_{II}s 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 TAF_{II}s have been reported to be essential for transcription of 14 and 3% of the genome, respectively (Table II). In addition, most studies indicate that in yeast the shared TFIID/SAGA TAF_{II}s 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 TFIID/SAGA TAF_{II}s 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 TAF_{II}s have been studied in conditional mutant strain populations in the context of ongoing mRNA production, but we depleted TAF_{II}s 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 metazoans but not yeast, TAF-1 contains a C-terminal kinase and a double bromodomain that targets TFIID to acetylated nucleosomes (Fig. 1*A*) (56). In yeast, related domains are present in the Bdf1 protein, which interacts substoichiometrically with TFIID and in its genetically redundant relative Bdf2 (57). Bdf1 and Bdf2 have metazoan orthologs that are distinct from TAF-1, however, and apparently have TFIID-independent functions in euchromatin maintenance (58, 59). Although it is possible that the TAF-1 bromodomains might have some TFIID-independent functions, the comparable requirement for TAF-2 which we have observed (Fig. 4*D*) argues against this view.

The simplest model to explain our findings is that a higher percentage of promoters require TFIID in the *C. elegans* embryo than in yeast. In yeast it seems that a TAF_{II}-independent TBP form is sufficient at many genes and that TFIID occupancy is proportional to transcription only where the TATA element is weak or nonexistent (12, 13). TFIID 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 TFIID-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 TFIID, 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 TFIID-independent promoters (26), in the *C. elegans* embryo various genes require both TLF and TAF-1 (see "Results") and possibly TFIID. 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 TFIID, 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. 4*C* 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. 7*A*).

The broad role in *C. elegans* embryonic transcription played by TAF-1 and TAF-2 suggests that these TAF_{II}s and TFIID may generally be of greater importance for transcription in metazoans than predicted from yeast studies. Although TFIID 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.

Acknowledgments—We thank Andrew Fire for constructs; Grace Gill, Steve Buratowski, Johnathan Whetstine, and the Blackwell laboratory members for helpful discussions or critical reading of this manuscript; and Leslie Tu for technical assistance.

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