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A Broad but Restricted Requirement for TAF-5 (Human $TAF_{II}100$) for Embryonic Transcription in *Caenorhabditis elegans**

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As conserved components of the transcription factor (TF) IID- and TFTC/SAGA-related complexes, TATA-binding protein-associated factors (TAF_{II}s) are important for eukaryotic mRNA transcription. In yeast, genetic analyses suggest that, although some individual TAF_{II}s are required for transcription of most genes, others have highly specialized functions. Much less is known about the functions of TAF_{II}s in metazoans, which have more complex genomes that include many tissue-specific genes. TAF-5 (human (h) $TAF_{II}100$) is of particular interest because it is predicted to have an important structural role. Here we describe the first genetics-based analysis of TAF-5 in a metazoan. By performing RNA interference in *Caenorhabditis elegans* embryos, which can survive for several cell generations without transcription, we found that *taf-5* is important for a significant fraction of transcription. However, TAF-5 is apparently not essential for the expression of multiple developmental and other metazoan-specific genes. This phenotype remarkably resembles the previously described effects of similarly depleting two *C. elegans* histone fold TAF_{II}s, TAF-9 ($hTAF_{II}31/32$) and TAF-10 ($hTAF_{II}30$), but is distinct from the widespread transcription block caused by TAF-4 ($hTAF_{II}130$) depletion. Our findings suggest that TAF-5, TAF-9, and TAF-10 are part of a functional module of TFIID- and TFTC/SAGA-related complexes that can be bypassed in many metazoan-specific genes.

Eukaryotic mRNA transcription requires the coordinate activity of gene-specific activators, coactivator proteins, general transcription factors (TFIIA,[1] TFIIB, TFIID, TFIIE, TFIIF, and TFIIH), Mediator complexes, and RNA polymerase II (pol II) (1–3). This complexity allows the transcription machinery to communicate with gene-specific regulators through an extraordinary diversity of combinatorial interactions. Genetic studies performed in yeast indicate that, although many transcription machinery components are essential, others seem to perform more specialized roles in regulating subgroups of genes (4–6). In general, genes involved in maintenance of cell viability are shared by all eukaryotes, suggesting that aspects of their regulation are likely to be conserved between yeast and metazoans. However, most metazoan genes, including those controlling development and differentiation, are not conserved in single cell eukaryotes and may require alternative regulatory strategies (7, 8).

The general transcription factor TFIID is composed of the TATA-binding protein along with 12–14 additional polypeptides, the TATA-binding protein-associated factors (TAF_{II}s)[2] (5, 9, 10). The TAF_{II}s are generally conserved in eukaryotes (11). TFIID has various functions during initiation; it appears to possess enzymatic activities, and TAF_{II}s have been implicated in essential interactions with gene-specific activators and with core promoter sequences located near the transcription start site (5, 9, 12, 13). Many TAF_{II}s contain a domain that is related to the histone fold, through which they form dimers within a conserved TFIID structure (14–17). Some TAF_{II}s are also constituents of complexes that lack TATA-binding protein but share some functions with TFIID, including SAGA and the related metazoan complexes TFTC and PCAF (18–21). TFTC can substitute for TFIID during transcription initiation (20), and *in vivo* studies suggest that, in yeast, the TFIID and SAGA complexes function redundantly in many genes (6).

In yeast, individual TAF_{II}s are required for cell viability, but studies involving conditional mutations or shutoff systems indicate that TAF_{II}s differ significantly in the extent to which they are required for transcription. A consensus has emerged from these studies that a significant fraction of yeast genes can be transcribed independently of TAF_{II}s, that TAF_{II} dependence maps to core promoters, and that TAF_{II}s that are present in both the TFIID and SAGA complexes are more broadly required than those present in TFIID alone (5, 6, 9, 12, 13, 22, 23). These models remain a subject of investigation and debate, however (24, 25).

The striking conservation of TFIID structure predicts that TAF_{II} functions are likely to be conserved between yeast and humans. It is an open question, however, how TAF_{II}s contribute to regulation of developmental and other metazoan-specific genes. Investigation of metazoan TAF_{II} functions *in vivo* is difficult not only because of cell lethality, but also because TAF_{II}s are generally expressed maternally in the embryo, making their mutant phenotypes complex (26, 27). To circumvent these issues, we have used RNA interference (RNAi) (28) to inhibit both maternal and zygotic expression of TAF_{II}s in the *Caenorhabditis elegans* embryo. In the early embryo, maternally produced mRNAs maintain viability until the 100-cell stage in the absence of transcription, making it feasible to manipulate expression of even essential transcription factors (29, 30). We have previously studied three histone fold TAF_{II}s:

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[1] The abbreviations used are: TF, transcription factor; pol II, RNA polymerase II; TAF, TATA-binding protein-associated factor; RNAi, RNA interference; GFP, green fluorescent protein; CTD, C-terminal domain; MAPK, mitogen-activated protein kinase.

[2] The TAF_{II} nomenclature follows that of Tora (11) and differs from the *C. elegans* TAF_{II} names we used in our previous work (31).

FIG. 1. **Domain conservation in *C. elegans* TAF-5.** *C. elegans* (*Ce*) TAF-5 (amino acids numbered above) includes six WD-40 motifs (*red*) and three other conserved domains (*CD1–CD3*; *blue*), all of which are present in yeast and other metazoan TAF-5 proteins (34). Percent similarity to human (*h*) TAF-5 is shown in each *box*, whereas percentages indicated below indicate similarity to human PAF-65β.

TAF-4, TAF-9, and TAF-10 (31). Surprisingly, TAF-4 appears to be required for essentially all early embryonic transcription. In contrast, TAF-9 and TAF-10 are required for a significant fraction of transcription, but not for expression of many metazoan-specific genes, implying the existence of a functional TAF_{II} subgroup that is not essential at many metazoan promoters.

Given the difference between these two classes of *C. elegans* TAF_{II} phenotypes, it is of interest to study TAF-5 (human $TAF_{II}100$), which may mediate critical interactions within the TFIID and SAGA complexes. TAF-5 contains WD-40 repeats, which generally form a protein-protein interaction module, and it binds to TAF-9 and multiple other TAF_{II}s (32–35). Expression shutoff experiments and analysis of certain conditional *taf5* mutations suggest that, in yeast, Taf5 is necessary for a small fraction of pol II transcription, but that other *taf5* mutations have more severe effects (6, 36, 37). A particular *taf5* mutation that broadly inhibits transcription destabilizes most TFIID components and impairs SAGA function, consistent with Taf5 having important functions in these complexes (37). Metazoan TAF-5 orthologs have not been studied *in vivo*, but a *Drosophila* TAF-5-related protein (Cannonball) is expressed specifically in primary spermatocytes and is required for expression of spermatid differentiation genes (38). The importance of Cannonball for this particular developmental transcription program raises the question of whether TAF-5 similarly may be critical for developmental gene regulation.

In this study, we have investigated requirements for *taf-5* in the early *C. elegans* embryo. We found that TAF-5 is broadly required for transcription, but is remarkably similar functionally to TAF-9 and TAF-10. In contrast to TAF-4, TAF-5 does not appear to be required for the expression of multiple metazoan-specific promoters or for a significant fraction of early transcription. In addition, RNAi co-inhibition experiments suggest that simultaneous lack of *taf-5* and *taf-10* does not cause a broader transcription defect. We conclude that TAF-5, TAF-9, and TAF-10 form part of a functional module of TFIID- and TFTC/SAGA-related complexes that is not required at many developmental and other metazoan-specific genes.

EXPERIMENTAL PROCEDURES

C. elegans and Bioinformatics—*C. elegans* strains were maintained as described (31). The wild-type strain was N2. Green fluorescent protein (GFP) reporter strains were provided to us as cited previously (31). *C. elegans taf-5* was identified by searching WORMpep and genomic data bases (Sanger Center) with human and *Saccharomyces cerevisiae* protein sequences. Alignments were produced by Megalign (DNASTAR, Inc.). The *taf-5* open reading frame is F30F8.8.

Immunostaining and Fluorescence Analysis—Rabbit antisera that were raised against the N-terminal TAF-5 peptide THNNSAMEDNLLSRPMNNES with an N-terminal Cys added were affinity-purified (31). For TAF-5 staining, embryos were subjected to 2% paraformaldehyde fixation and freeze-cracked before treating with methanol. Washes and antibody incubations were performed in 1× phosphate-buffered saline, 1% Triton X-100, and 1% bovine serum albumin prior to staining. Anti-TAF-5 antibody staining was competed by the cognate (but not heterologous) peptides (data not shown). Staining with other antibodies, including anti-TAF-9, anti-TAF-10, anti-pol II (POL 3/3) (39), anti-phospho-Ser-5 (P-CTD) (40), and anti-phospho-Ser-2 (H5) (Babco), was performed as described (31). For GFP analysis, embryos were transferred to 2% agarose pads. Images were captured with 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 Version 5.0.

RNAi Analysis—A *taf-5* cDNA (yk348c7) that covers >90% of the predicted coding region was obtained from Yuji Kohara (National Institute of Genetics, Mishima, Japan). *In vitro* synthesized double-stranded RNA (Ribomax, Promega) was injected at 0.6–1.0 μg/μl into young adults (two to eight 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, 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-5* and *taf-10* RNAi was performed with a 1:1 mixture of double-stranded RNAs. In parallel, a 1:1 dilution of each individual double-stranded RNA with either TE or an unrelated double-stranded RNA (*glp-1*) resulted in appropriate terminal arrest, END-1::GFP expression, and CTD epitope staining levels (data not shown). For heat shock, *hsp-16.2*::*gfp* embryos were incubated at 37 °C for 1 min in 10 μl of M9 medium. Fluorescence was examined 1 h later.

RESULTS

taf-5 Is Essential during Early Embryonic Development—Data base searches of the *C. elegans* genome revealed a single, well conserved *taf-5* ortholog (Fig. 1), which re-identified the corresponding human (*$TAF_{II}100$*) and yeast (*$TAF_{II}90$*) genes as its closest relatives in the GenBank™/EBI Data Bank. The predicted TAF-5 protein contains six WD-40 motifs as well as three additional domains that are conserved in yeast, human, and *C. elegans* (Fig. 1). Although yeast Taf5 is present in both TFIID and SAGA (41), human PCAF complexes contain a related protein, PAF-65β (19). Using the above search criteria, we did not detect a PAF-65β ortholog, suggesting that, in *C. elegans*, TAF-5 is utilized in both TFIID- and TFTC/SAGA-related complexes. We also did not identify a *C. elegans* ortholog of *Drosophila* Cannonball.

To evaluate the distribution of TAF-5 in early *C. elegans* embryos, we examined its expression by antibody staining. TAF-5 was present in all embryonic nuclei (Fig. 2). We also noted that TAF-5 was present in oocytes and the adult germ line (data not shown), suggesting that it is maternally expressed. Inhibition of *taf-5* expression by RNAi eliminated embryonic staining with the anti-TAF-5 antibody (Fig. 2), suggesting that a significant depletion of the TAF-5 protein occurred. In contrast, levels of TAF-4 and TAF-10 did not appear to be affected in *taf-5(RNAi)* embryos (Fig. 2). Similarly, antibody staining indicated that TAF-5 levels were approximately normal in *taf-4(RNAi)*, *taf-9(RNAi)*, and *taf-10(RNAi)* embryos (data not shown).

Maternally deposited RNAs control early developmental patterns and sustain the *C. elegans* embryo during early embryogenesis (42). When maternal expression and zygotic expression

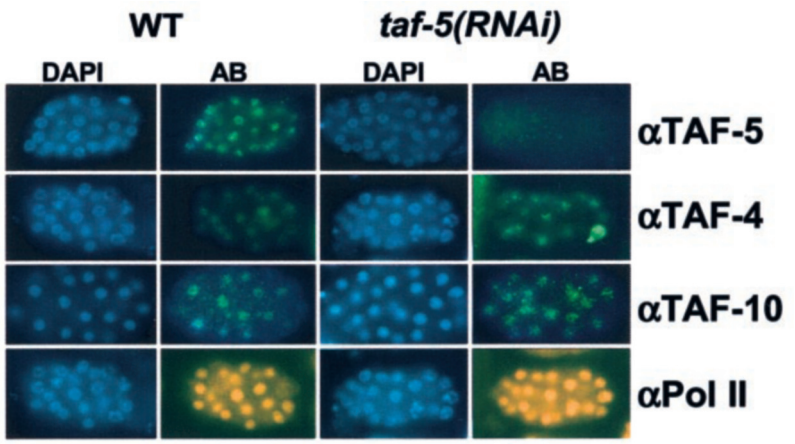

FIG. 2. **Expression of TAF-5, TAF-4, and TAF-10 in wild-type and *taf-5(RNAi)* embryos.** Representative wild-type (*WT*) or *taf-5(RNAi)* embryos were stained with antibody (*AB*) to TAF-5, TAF-4, TAF-10, or the pol II large subunit AMA-1 (antibody POL 3/3), along with 4,6-diamidino-2-phenylindole (*DAPI*) to visualize DNA. Embryos measure ~50 μm.

of essential general transcription factors, including *ama-1* (pol II large subunit), *ttb-1* (TFIIB), *taf-4*, *taf-9*, *taf-10*, *cdk-9*, and *rgr-1*, are inhibited by RNAi, embryonic development arrests at ~100 cells without differentiation (29, 31, 43, 44). The development of *taf-5(RNAi)* embryos arrested at a similar stage without signs of differentiation (Fig. 3*A*), suggesting a broad defect in zygotic transcription.

To investigate whether the *taf-5(RNAi)* phenotype involves a general defect in maternal mRNA stores, we evaluated early cell division patterns and performed parallel experiments in a transgenic strain that expresses a fusion of the maternally derived germ line protein PIE-1 and GFP. PIE-1::GFP recapitulates the endogenous PIE-1 localization pattern, which depends upon at least 20 maternal genes (45). As in *ama-1(RNAi)* embryos, in *taf-5(RNAi)* embryos, PIE-1::GFP expression and localization patterns were normal at every stage (Fig. 3*B* and data not shown). Early cell division timings and cleavage planes were also generally normal in *taf-5(RNAi)* embryos, except for the cell cycle period of the two E daughters (E2 cells), which give rise to the endoderm. When early mRNA transcription is broadly inhibited, as in *ama-1(RNAi)* embryos, the E2 cell cycle length is shortened from 45 to ~22 min (29, 31). The E2 cells similarly divided after 22 min in *taf-5(RNAi)* embryos. Together, our findings suggest that depletion of embryonic TAF-5 does not detectably influence maternal mRNA stores, but may significantly impair new mRNA transcription.

Reduced pol II CTD Phosphorylation in taf-5(RNAi) Embryos—To investigate how mRNA transcription is affected in *taf-5(RNAi)* embryos, we analyzed phosphorylation of the pol II large subunit CTD. The CTD consists of multiple repeats that are based on the consensus YSPTSPS (46). Pol II is recruited to promoters with the CTD in an unphosphorylated form; then during transcription, the CTD is first phosphorylated at Ser-5 of the repeat by the TFIIH kinase (40, 47). During elongation, the distribution of CTD phosphorylation shifts to Ser-2 (47, 48), which, in metazoans, is phosphorylated by the positive-transcription elongation factor b (P-TEFb) kinase (43, 49). CTD Ser-5 and Ser-2 phosphorylation can be specifically detected in *C. elegans* embryonic nuclei by staining with antibodies P-CTD and H5, respectively (30, 31, 40), which we refer to as anti-phospho-Ser-5 and anti-phospho-Ser-2 for clarity (Fig. 4).

In the *C. elegans* embryo, the presence of nuclear anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining is dependent upon transcription. Staining with these antibodies is not detectable until the 3–4-cell stage, when new mRNA transcription begins; then at later stages, the patterns and intensity of this staining appear to parallel transcription activity (30, 31). CTD Ser-5 phosphorylation was detected as a punctate pattern

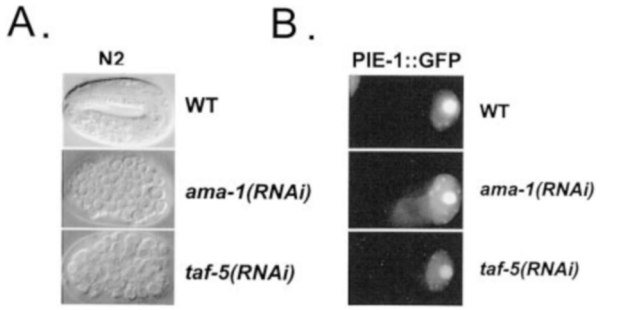

FIG. 3. **Terminal and early cell division phenotypes of *taf-5(RNAi)* embryos.** *A*, *taf-5(RNAi)* phenotype. Representative terminally arrested RNAi embryos were examined by differential interference microscopy and compared with a wild-type (*WT*) embryo that was about to hatch. *ama-1(RNAi)* and *taf-5(RNAi)* embryos each ceased development with 90–100 cells ($n = 5$). *ama-1* encodes the pol II large subunit. *B*, PIE-1::GFP expression in wild-type and RNAi embryos, examined by fluorescence microscopy. In *taf-5(RNAi)* embryos, each aspect of the PIE-1::GFP germ line and subcellular localization was indistinguishable from that in wild-type embryos, including the presence of PIE-1 in germ line RNA-protein P granules (45).

in the transcriptionally active somatic nuclei, but was limited to two bright foci in the transcriptionally silent germ line nucleus (Fig. 4, *A* and *B*). These germ line anti-phospho-Ser-5 foci depend upon the essential initiation factor TFIIB (TTB-1) and the Mediator component RGR-1, suggesting that they may correspond to aborted or stalled transcription events (31, 44). Phosphorylation of CTD Ser-2 was detected only in somatic cells (Fig. 4*A*). Both anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining levels are reduced to background levels when transcription initiation is inhibited in *ttb-1(RNAi)* or *rgr-1(RNAi)* embryos (31, 44).

In *taf-5(RNAi)* embryos, nucleoplasmic anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining levels were significantly reduced in parallel, and two anti-phospho-Ser-5 foci like those present in the germ line were also prominent in somatic nuclei (Fig. 4, *A* and *B*). This pattern suggests a partial but significant reduction in overall embryonic CTD phosphorylation and mRNA transcription levels, and it is strikingly similar to the pattern seen in *taf-9(RNAi)* or *taf-10(RNAi)* embryos (Fig. 4*A*) (31). These decreases in staining are distinct, however, from the more dramatic effects observed in somatic cells in *taf-4(RNAi)* embryos, in which anti-phospho-Ser-5 antibody staining was reduced to only the two foci, and anti-phospho-Ser-2 staining was undetectable (31). Previously, we observed that the effects of inhibiting expression of *taf-9* and *taf-10* simultaneously by RNAi were not distinguishable from the effects of inhibiting either gene individually (31). Significantly,

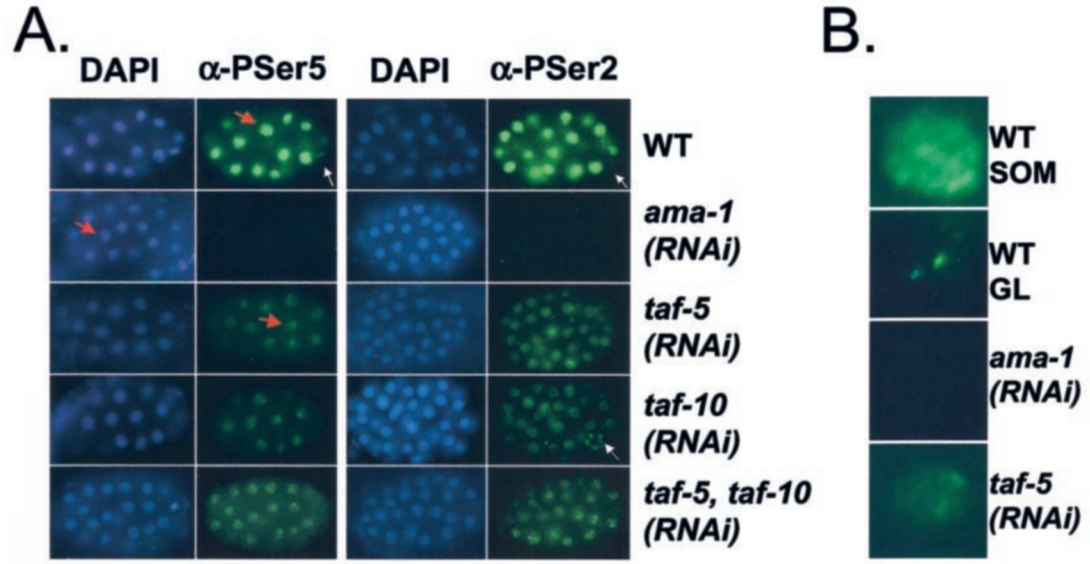

FIG. 4. **Reduced pol II CTD phosphorylation in *taf-5(RNAi)* embryos.** *A*, comparably decreased CTD Ser-5 and Ser-2 phosphorylation in *taf-5(RNAi)* and *taf-10(RNAi)* embryos. Prior to terminal developmental arrest, wild-type (*WT*) or RNAi embryos (in *rows*) were stained with anti-phospho-Ser-5 (*α-PSer5*) or anti-phospho-Ser-2 (*α-PSer2*) antibody and 4,6-diamidino-2-phenylindole (*DAPI*) to visualize DNA. Representative embryos of comparable stages are presented. In parallel experiments, staining with an antibody against a different pol II region revealed that pol II levels were equivalent in wild-type and TAF_{11}(RNAi) embryos (Fig. 2). The relative differences in anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining intensities between wild-type and RNAi embryos were comparable between the activation of transcription at the 4-cell stage and terminal arrest, and when embryos were photographed at multiple different exposure times. Germ line nuclei that are in the focal plane shown are marked with *white arrows*, and somatic nuclei depicted in *B* are indicated by a *red arrow*. Anti-phospho-Ser-2 antibody variably cross-reacted with germ line RNA-protein P granules, as in the *taf-10(RNAi)* embryo. *B*, detailed view of anti-phospho-Ser-5 antibody-stained wild-type somatic (*SOM*) and germ line (*GL*) nuclei, along with *ama-1(RNAi)* and *taf-5(RNAi)* somatic nuclei. In the wild-type germ line nucleus (*A*, *white arrows*), note the presence of two discrete foci and the lack of nucleoplasmic staining that is evident in somatic nuclei. In *taf-5(RNAi)* somatic nuclei, these foci are present along with significantly reduced nucleoplasmic staining.

anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining levels similarly did not decrease further when *taf-5* and *taf-10* were inhibited simultaneously by RNAi (*taf-5,taf-10(RNAi)* embryos) (Fig. 4), suggesting that *taf-5*, *taf-9*, and *taf-10* may be required for transcription of highly overlapping sets of genes.

Expression of Many Metazoan-specific Genes in taf-5(RNAi) Embryos—To evaluate the importance of TAF-5 for expression of individual genes, we performed RNAi experiments in a set of *C. elegans* strains that carry transgenic reporters. These reporters include intact regulatory regions fused to GFP coding regions and are expressed in the embryo in parallel to the corresponding endogenous genes. Expression of each of these reporters is undetectable or reduced to similar trace levels in *ama-1(RNAi)* and *taf-4(RNAi)* embryos (31).

We first investigated the expression of two groups of genes that are widely expressed in the *C. elegans* embryo. *rps-5*, *let-858*, and the heat shock gene *hsp-16.2* each have orthologs in unicellular eukaryotes as well as in metazoans. In yeast, expression of *rps-5* and many other ribosomal protein genes is highly dependent on TAF_{II}s (12, 13). Expression of GFP reporters that correspond to these three conserved genes was abolished in *taf-5(RNAi)* embryos (Fig. 5*A* and Table I), consistent with a significant reduction in overall transcription levels (Fig. 4). In contrast, TAF-5 did not appear to be essential in some widely expressed metazoan-specific genes. *cki-2* (cyclin-dependent kinase inhibitor) and *sur-5* (MAPK pathway) are conserved in metazoans and are expressed early in the *C. elegans* embryo. The corresponding GFP reporters were expressed at wild-type levels in *taf-5(RNAi)* embryos (Table I). *pes-10*, which has been identified only in *C. elegans*, is activated at the onset of embryonic transcription. PES-10::GFP expression was reduced significantly (but not eliminated) in *taf-5(RNAi)* embryos (Table I). Significantly, each of these various genes was expressed at levels comparable to those that are characteristic of *taf-9(RNAi)* or *taf-10(RNAi)* embryos (31).

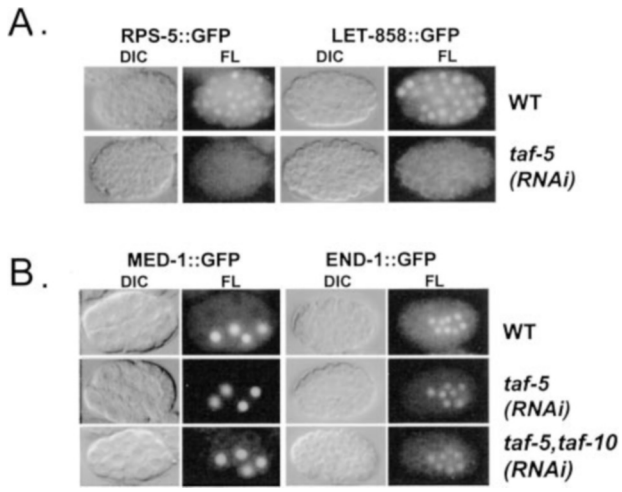

FIG. 5. **Expression of individual conserved and metazoan-specific genes in *taf-5(RNAi)* embryos.** *A*, *taf-5*-dependent conserved genes. Differential interference (*DIC*) and fluorescent (*FL*) images are shown of wild-type (*WT*) and RNAi embryos (in *rows*) from the reporter strains indicated. Each of these reporters was expressed in most embryonic cells. In a representative experiment, the RPS-5::GFP reporter, which is nonintegrated, was expressed in 21 of 45 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. *B*, expression of developmental genes in *taf-5(RNAi)* embryos. Expression of MED-1::GFP and END-1::GFP was examined in wild-type and RNAi embryos. In a representative experiment, END-1::GFP was expressed at normal levels (30%; $n = 71$) in *taf-5(RNAi)* embryos. *end-1* is unique of the genes that we have analyzed in that END-1::GFP expression is not necessarily uniform within sets of TAF_{11}(RNAi) embryos (31); this may reflect its highly complex regulation, which involves multiple signal inputs (50, 51). In each RNAi embryo set, E2 descendants were mislocalized to the posterior edge of the embryo due to defective gastrulation caused by the reduced E2 cell cycle time (see "Results").

TABLE I
Requirements for taf-5 for early embryonic gene expression

	PES-10::GFP (early zygotic)	MED-2::GFP (endoderm)	ELT-5::GFP (ectoderm)	PHA-4::GFP (pharynx)	CKI-2::GFP (cell cycle)	SUR-5::GFP (MAPK pathway)	HSP-16.2::GFP[a] (conserved eukaryotic)
Wild-type	+[b]	+	+	+	+	+	+
ama-1(RNAi)[c]	−	−	−	−	−	−	−
taf-10(RNAi)[c]	−/+	+	+	+[d]	+	+	−
taf-5(RNAi)	−/+	+	+	+[d]	+	+	−

[a] *hsp-16.2* is referred to as "conserved eukaryotic" because it is common to yeast and metazoans. All other genes listed are metazoan-specific.
[b] Reporter strains were scored as + when GFP was expressed at wild-type levels in all embryos and as − when GFP was undetectable or present at comparable trace levels in taf_{II}*(RNAi)* and *ama-1(RNAi)* embryos. −/+ indicates detection of low level GFP expression above background levels in *ama-1(RNAi)* embryos. For each data set, >40 embryos were analyzed in multiple independent experiments.
[c] Results from *ama-1(RNAi)* and *taf-10(RNAi)* experiments are from Ref. 31.
[d] In *taf-5(RNAi)* and *taf-10(RNAi)* embryos, PHA-4::GFP was expressed at normal levels, but in fewer cells than in wild-type embryos.

We also analyzed expression of a group of developmental genes in *taf-5(RNAi)* embryos. These genes specify or promote differentiation of the mesendoderm (*med-1* and *med-2*), endoderm (*end-1*), pharynx (*pha-4*), and epidermis (*elt-5*). GFP reporters that correspond to *med-1*, *med-2*, and *elt-5* were expressed at wild-type levels in all *taf-5(RNAi)* embryos, and END-1::GFP was similarly expressed in ~30% of these RNAi embryos (Fig. 5*B* and Table I). PHA-4::GFP was also robustly expressed in *taf-5(RNAi)* embryos, but in fewer cells than in wild-type embryos (Table I). Significantly, in each case, these expression patterns closely paralleled those observed previously in *taf-9(RNAi)* and *taf-10(RNAi)* embryos (31), with the exception that END-1::GFP was expressed in a higher proportion (80–90%) in *taf-9(RNAi)* and *taf-10(RNAi)* embryos. In addition, both MED-1::GFP and END-1::GFP were expressed comparably in *taf-5(RNAi)* and *taf-5,taf-10(RNAi)* embryos (Fig. 5*B*). Together, our findings indicate that TAF-5 is essential for a significant proportion of early embryonic transcription, but not for expression of many metazoan-specific genes, a phenotype that is remarkably similar to the previously described requirements for TAF-9 and TAF-10.

DISCUSSION

Much remains to be learned about how general transcription machinery components participate in regulating different types of genes in metazoans. In this study, we have obtained evidence that, in the early *C. elegans* embryo, TAF-5 (human $TAF_{II}100$) is required for a significant fraction of pol II transcription, but does not appear to be essential for expression of many metazoan-specific genes. Overall levels of pol II CTD Ser-5 and Ser-2 phosphorylation were substantially reduced (but not eliminated) throughout development of *taf-5(RNAi)* embryos (Fig. 4, *A* and *B*), implying a significant but incomplete defect in pol II transcription. Accordingly, in these RNAi embryos, a set of conserved genes was not expressed detectably, but various developmental and other metazoan-specific genes were transcribed at significant levels (Fig. 5 and Table I).

Multiple lines of evidence argue against the notion that this limited requirement for TAF-5 might have derived from incomplete RNAi penetrance, although we cannot eliminate the possibility that trace levels of the TAF-5 protein may remain in these RNAi embryos. These RNAi effects were accompanied by loss of anti-TAF-5 antibody staining and were highly reproducible, and they appeared with consistent timing after injection (Fig. 2 and data not shown). In addition, expression of the conserved genes *let-858*, *rps-5*, and *hsp-16.2* was not detected in *taf-5(RNAi)* embryos (Fig. 5*A*). Finally, the *taf-5(RNAi)* phenotype did not appear to be enhanced by simultaneous RNAi inhibition of *taf-10* (Figs. 4*A* and 5*B*), suggesting that these respective RNAi phenotypes involve overlapping processes and are unlikely to be partial effects.

In *S. cerevisiae*, expression shutoff analyses and various conditional alleles suggest that Taf5 has a limited role in transcription, but other *taf5* alleles are associated with more severe defects that correlate with functional or structural impairment of TFIID and SAGA (36, 37). A particular yeast *taf5* mutation that causes a very broad transcription defect is also associated with destabilization of most other TFIID subunits (37). We cannot currently address whether the TFIID complex is intact in our experiments. In *taf-5(RNAi)* embryos, approximately normal levels of TAF-10 and the broadly essential TAF-4 appeared to be present, however (Fig. 2), suggesting that the *taf-5(RNAi)* phenotype does not involve a general loss of TFIID or TFTC/SAGA subunits.

A particularly interesting aspect of our findings is the remarkable similarity between the *taf-5(RNAi)* phenotype and the previously described effects of inhibiting expression of *taf-9* or *taf-10*, either alone or simultaneously (31). The evidence that *taf-5(RNAi)* and *taf-5,taf-10(RNAi)* embryos are phenotypically similar (Figs. 4 (*A* and *B*) and 5*B*) further supports the model that TAF-5 and TAF-10 are functionally linked. Our evidence that many early embryonic genes are expressed independently of TAF-5, TAF-9, and TAF-10 appears to be consistent with genetic and promoter occupancy studies suggesting that a significant proportion of *S. cerevisiae* genes are transcribed independently of all TAF_{II}s (13, 22, 23). We have previously observed, however, that essentially all early embryonic transcription appears to require *taf-4* (31), indicating that this yeast model may not be fully applicable to *C. elegans*.

It is striking that TAF-5, TAF-9, and TAF-10 do not seem to be required to express nearly all of the developmental and other metazoan-specific genes that we have analyzed (Fig. 5*B* and Table I) (31). We conclude that these three TAF_{II}s are part of a functional subgroup that can be bypassed during transcription of many metazoan-specific genes. Although many of the genes involved in basic cellular functions have been highly conserved between yeast and metazoans, genes that control processes that are specific to multicellular animals, such as development and differentiation, are much more distantly related (7, 8). Our findings suggest that, although many conserved genes such as *rps-5* may have retained regulatory strategies that require these TAF_{II}s (Fig. 5*A*), many metazoan-specific genes have evolved alternative activation mechanisms, perhaps involving different core promoter contexts and activator or coactivator interactions. Because TAF-5 does not appear to be generally required in embryonic developmental gene expression programs, the importance of *Drosophila* Cannonball for spermatid differentiation gene transcription is particularly intriguing (38). Perhaps a specialized form of TFIID or TFTC/SAGA that contains Cannonball has evolved to perform a highly specialized developmental regulatory function. Future elucidation of the differences and parallels between TAF-5 and Cannonball functions may therefore reveal novel mechanistic insights into how TAF_{II}s contribute to transcription.

The apparent parallels between TAF-5, TAF-9, and TAF-10

functions suggest that these TAF_{II}s may be located along a shared surface for protein-protein or protein-DNA interactions within TFIID and TFTC/SAGA complexes. It is consistent with this model that TAF-5 interacts with TAF-9 and with multiple other TAF_{II}s (32, 34, 35). The differences between the apparently general requirement for TAF-4 and the more limited functions of TAF-5, TAF-9, and TAF-10 raise the interesting question of what accounts for these differences at the molecular level. *In vivo* analyses of additional TAF_{II}s, coupled with a more detailed understanding of TFIID and TFTC complex structure, should reveal mechanisms through which individual TAF_{II}s and different domains of TFIID and TFTC complexes are utilized or bypassed during transcription of metazoan genes.

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