A Broad but Restricted Requirement for TAF-5 (Human TAFII100) 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 (TAFIIs) are important for eukaryotic mRNA transcription. In yeast, genetic analyses suggest that, although some individual TAFIIs are required for transcription of most genes, others have highly specialized functions. Much less is known about the functions of TAFIIs in metazoans, which have more complex genomes that include many tissue-specific genes. TAF-5 (human (h) TAFII100) 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 TAFIIs, TAF-9 (hTAFII31/32) and TAF-10 (hTAFII30), but is distinct from the widespread transcription block caused by TAF-4 (hTAFII130) depletion. Our findings suggest that TAF-5, TAF-9, and TAF-10 are part of a functional module of TFIIID- 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 (TFIIF), TFIIF, TFIID, TFIE, TFIIIE, and TFIIHI), 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 TFIIID is composed of the TATA-binding protein along with 12–14 additional polypeptides, the TATA-binding protein-associated factors (TAFIIs)5 (5, 9, 10). The TAFIIIs are generally conserved in eukaryotes (11). TFIIID has various functions during initiation; it appears to possess enzymatic activities, and TAFIIs 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 TAFIIIs contain a domain that is related to the histone fold, through which they form dimers within a conserved TFIIID structure (14–17). Some TAFIIIs are also constituents of complexes that lack TATA-binding protein but share some functions with TFIIID, including SAGA and the related metazoan complexes TFTC and PCAF (18–21). TFTC can substitute for TFIIID during transcription initiation (20), and in vivo studies suggest that, in yeast, the TFIIID and SAGA complexes function redundantly in many genes (6).

In yeast, individual TAFIIIs are required for cell viability, but studies involving conditional mutations or shutoff systems indicate that TAFIIIs 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 TAFIIIs, that TAFII dependence maps to core promoters, and that TAFIIIs that are present in both the TFIIID and SAGA complexes are more broadly required than those present in TFIIID alone (5, 6, 9, 12, 13, 22, 23). These models remain a subject of investigation and debate, however (24, 25).

The striking conservation of TFIIID structure predicts that TAFII functions are likely to be conserved between yeast and humans. It is an open question, however, how TAFIIIs contribute to regulation of developmental and other metazoan-specific genes. Investigation of metazoan TAFII functions in vivo is difficult not only because of cell lethality, but also because TAFIIIs 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 TAFIIIs 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 TAFIIIs:

5 The TAFII nomenclature follows that of Tora (11) and differs from the C. elegans TAFII names we used in our previous work (31).
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**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 (TAF5/100) and yeast (TAF5/90) 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 Ta5 is present in both TFIIID 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 TFIIID- and TFC/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. 1). 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-5(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. 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.

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**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 WormPept and genomic data bases (Sanger Center) with human and *Sacrocyces cerevisiae* protein sequences. Alignments were produced by Megalign (DNASTAR, Inc.). The taf-5 open reading frame is F39F8.8.

**Immunostaining and Fluorescence Analysis—**Rabbit antisera that were raised against the N-terminal TAF-5 peptide THNNSMED-NLLSRPMNNE 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 1X 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 (gpl-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.
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. 3A), 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. 3B 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 in the CTD with 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). Ser-2 antibody staining levels are reduced to background levels when transcription initiation is inhibited in taf-5(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. 4A) (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).
anti-phospho-Ser-5 and anti-phospho-Ser-2 antibody staining levels similarly did not decrease further when \textit{taf-5} and \textit{taf-10} were inhibited simultaneously by RNAi (\textit{taf-5},\textit{taf-10}(RNAi)) embryos (Fig. 4), suggesting that \textit{taf-5}, \textit{taf-9}, and \textit{taf-10} may be required for transcription of highly overlapping sets of genes.

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

We first investigated the expression of two groups of genes that are widely expressed in the \textit{C. elegans} embryo, \textit{rps-5}, \textit{let-858}, and the heat shock gene \textit{hsp-16.2} each have orthologs in unicellular eukaryotes as well as in metazoans. In yeast, expression of \textit{rps-5} and many other ribosomal protein genes is highly dependent on TAFIIs (12, 13). Expression of GFP reporters that correspond to these three conserved genes was abolished in \textit{taf-5}(RNAi) embryos (Fig. 5A 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. \textit{cki-2} (cyclin-dependent kinase inhibitor) and \textit{sur-5} (MAPK pathway) are conserved in metazoans and are expressed early in the \textit{C. elegans} embryo. The corresponding GFP reporters were expressed at wild-type levels in \textit{taf-5}(RNAi) embryos (Table I). \textit{pes-10}, which has been identified only in \textit{C. elegans}, is activated at the onset of embryonic transcription. \textit{Pes-10}:GFP expression was reduced significantly (but not eliminated) in \textit{taf-5}(RNAi) embryos (Table I). Significantly, each of these various genes was expressed at levels comparable to those that are characteristic of \textit{taf-9}(RNAi) or \textit{taf-10}(RNAi) embryos (31).
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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. 5B 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 (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 (31). PHA-4::GFP was expressed comparably in taf-5(RNAi) and taf-5, taf-10(RNAi) embryos (Fig. 5B). 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 TAF5) 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 penetration, 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. 5A). Finally, the taf-5(RNAi) phenotype did not appear to be enhanced by simultaneous RNAi inhibition of taf-10 (Figs. 4A and 5B), 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 TFII D and SAGA (36, 37). A particular yeast taf5 mutation that causes a very broad transcription defect is also associated with destabilization of most other TFII D subunits (37). We cannot currently address whether the TFII D 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 TFII D or TFC/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. 4A and B and 5B) further supports the model that TAF-5 and TAF-10 are functionally linked. Our evidence that many 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 TAFPs (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. 5B and Table I) (31). We conclude that these three TAFPs 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 TAFPs (Fig. 5A), 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 TFII D or TFC/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 TAFPs contribute to transcription.

The apparent parallels between TAF-5, TAF-9, and TAF-10
functions suggest that these TAFIIs 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 TAFIIs (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 TAFIIs, coupled with a more detailed understanding of TFIID and TFTC complex structure, should reveal mechanisms through which individual TAFIIs and different domains of TFIID and TFTC complexes are utilized or bypassed during transcription of metazoan genes.

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