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Post-Transcriptional Regulation of RNA Polymerase II Levels in *Caenorhabditis elegans*

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ABSTRACT

To investigate the regulation of RNA polymerase II levels in *Caenorhabditis elegans*, we have constructed nematode strains having one, two, or three copies of *ama-1*, the gene for the largest subunit of RNA polymerase II. Steady-state levels of RNA polymerase II polypeptides and solubilized enzyme activity are invariant with gene dosage, indicating regulatory compensation. However, steady-state levels of *ama-1* mRNA are directly proportional to gene dosage. These results imply that RNA polymerase II levels in *C. elegans* are regulated post-transcriptionally.

RNA polymerase II (Rpo II) is the core of the transcription apparatus responsible for mRNA synthesis in eukaryotes. Although capable of template-dependent RNA synthesis, the enzyme requires both general and gene-specific transcription factors to direct regulated transcription initiation (GREENBLATT 1991). Recent focus on the interaction between such factors and Rpo II has generated renewed interest in features of the enzyme (YOUNG 1991). In all eukaryotes, Rpo II consists of two large (~200 kD and ~140 kD) polypeptides and 6–10 smaller polypeptides (<50 kD). Several of the smaller subunits are shared with RNA polymerases I and III (reviewed by SAWADOGO and SENTENAC 1990). In *Saccharomyces cerevisiae*, molecular genetic analyses have shown that most Rpo II subunits are required for transcription *in vivo* (YOUNG 1991).

Although new information about Rpo II structure and function is emerging rapidly, little is known about the regulation of Rpo II biosynthesis and assembly. The central role of this enzyme in gene expression suggests that its intracellular concentration should generally be maintained at steady levels, which might be modulated by cellular growth rates and developmental transitions. The existence of multiple subunits, some of which are common to all three classes of RNA polymerase, raises the question of how subunit levels are coordinated.

Experiments with mammalian cell lines containing α-amanitin-resistant Rpo II have provided evidence that biosynthesis of mammalian Rpo II is directly or indirectly autoregulated by the concentration of enzyme (SOMERS, PEARSON and INGLES 1975; GUIALIS *et al.* 1977). Growth in the presence of α-amanitin, a specific inhibitor of Rpo II, results in degradation of wild-type, α-amanitin-sensitive enzyme. Hybrid cell lines containing both α-amanitin-sensitive and α-amanitin-resistant forms of Rpo II respond to growth in α-amanitin by accelerating synthesis of enzyme subunits. The result is a twofold increase in steady-state levels of the drug-resistant enzyme, compensating for loss of the wild-type enzyme (GUIALIS, MORRISON and INGLES 1979). The level at which this regulation occurs (whether transcriptional or post-transcriptional) has not been determined.

Progress in the molecular genetics of eukaryotic RNA polymerases has made it possible to reinvestigate the regulation of their biosynthesis. Genes for Rpo II subunits have been isolated from several organisms, notably from yeast, where genes for all 11 subunits have been identified and sequenced (YOUNG 1991). The nematode *Caenorhabditis elegans* has recently been used as a model system for the molecular genetics of RNA polymerases. Purified Rpo II from *C. elegans* resembles that from other organisms, having two large subunits (205 kD and 135 kD) and several smaller (<40 kD) subunits (SANFORD, PRENGER and GOLOMB 1985). The gene (*ama-1 IV*) encoding the largest subunit of the enzyme has been isolated and sequenced (BIRD and RIDDLE 1989). Various α-amanitin-resistant, lethal and sublethal alleles of *ama-1* have been described (SANFORD, GOLOMB and RIDDLE 1983; ROGALSKI, BULLERJAHN and RIDDLE 1988; ROGALSKI, GOLOMB and RIDDLE 1990) and fine-structure mapped (BULLERJAHN and RIDDLE 1988), and chromosomal duplications and deficiencies that include the *ama-1* locus are available (ROGALSKI and RIDDLE 1988).

In preliminary experiments, one of us observed that crude extracts from a strain hemizygous for *ama-1*

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contained wild-type levels of solubilized Rpo II activity and polypeptides, as though regulatory compensation were occurring (TULLIS 1986). To investigate the regulation of this enzyme, we constructed stable strains of *C. elegans* containing one, two, or three copies of *ama-1*, and compared them with respect to enzyme activity, subunit levels, and *ama-1* mRNA content. In crude extracts from synchronized cultures of third-stage (L3) larvae, levels of Rpo II activity and *ama-1* polypeptide were invariant with gene dosage, suggesting that the steady-state concentration of the *ama-1* gene product is regulated. In contrast, steady-state levels of *ama-1* mRNA varied directly with gene dosage, indicating that regulation of levels of the largest subunit is post-transcriptional.

MATERIALS AND METHODS

Genetics: Two chromosomal rearrangements, *mDf10 IV* and *mDp1(IV;f)* were used to construct nematode strains carrying one, two, or three copies of the *ama-1 IV* gene (see Figure 1). The chromosomal deficiency, *mDf10*, entirely deletes *ama-1* and nearby genes, including *dpy-13* (ROGALSKI and RIDDLE 1988; Figure 1). Mutations in the *dpy-13* cuticle collagen gene (VON MENDE *et al.* 1988) result in a dumpy (short, fat) body shape, and this provides a convenient visible marker closely linked to *ama-1*. Wild-type alleles of *ama-1* and *dpy-13* are present on the free chromosomal duplication *mDp1*, which duplicates the left arm of chromosome *IV* (ROGALSKI and RIDDLE 1988).

The one-copy strain, DR961, carries two *mDf10* chromosomes and the *mDp1* duplication. The only copy of *ama-1* in this strain is on the duplication. Several steps were required to construct DR961. First, *dpy-13(e184)/+* males were crossed with heterozygous hermaphrodites of genotype *mDf10/nT1 IV*; *+/nT1 V*, and dumpy cross-progeny (*dpy-13/mDf10*) were selected for mating with *unc-17(e113) dpy-13(e184)/+ +*; *mDp1* males. These latter males were generated by crossing wild-type (N2) males with *unc-17(e113) dpy-13(e184)*; *mDp1* hermaphrodites. The *dpy-13(e184)* mutation is semi-dominant, so that *dpy-13(e184)/+* heterozygotes are semi-dumpy, but *dpy-13(e184)/+/+* animals are normal or nearly normal in body length. Hence, males of genotype *unc-17 dpy-13/+ +*; *mDp1* were specifically identified on the basis of normal body length. When crossed with the *dpy-13(e184)/mDf10* dumpy hermaphrodites, they produced semi-dumpy cross-progeny of four different genotypes, including *unc-17 dpy-13/+ mDf10*; *mDp1*. Nematodes with this genotype were identified, and a wild-type segregant (*mDf10/mDf10/mDp1*) was used to establish the DR961 strain.

The two copy strain used here was DR680, of genotype *dpy-13(e184) ama-1(m118)*. This is a dumpy derivative of DR432, the reference α-amanitin-resistant strain on which previous biochemical studies have been reported (SANFORD, GOLOMB and RIDDLE 1983). The three-copy strain, DR962, carried the wild-type *ama-1* gene on the *mDp1* duplication as well as two chromosomal *ama-1(m118)* genes. A lethal mutation, *let-276(m240)*, closely linked to *ama-1* (ROGALSKI and RIDDLE 1988) prevented loss of the duplication in the strain, because the *let-276(+)* allele on the duplication was required for viability. The DR962 strain, *dpy-13(e184) ama-1(m118) let-276(m240) IV*; *mDp1(IV;f)*, is semi-dumpy because of the two chromosomal *dpy-13(e184)* mutations. It was constructed by crossing *unc-17(e113) dpy-13(e184)/+ +*; *mDp1* males with *unc-17(e113) + + + unc-5(e53)/+ dpy-13(e184) ama-1(m118) let-276(m240) +* hermaphrodites. Semi-dumpy F_1 hermaphrodite progeny with normal movement were selected, and their self-progeny screened for the presence of dumpy, uncoordinated (*dpy-13 unc-17*) animals to identify a population segregating the desired genotype. A semi-dumpy sibling of genotype *dpy-13(e184) ama-1(m118) let-276(m240)*; *mDp1* was used to establish the DR962 strain.

Growth of nematodes: The wild-type Bristol strain (N2) and derivatives were cultured at 20° on NG plates with *E. coli* strain OP50 (BRENNER 1974) and were subsequently transferred to liquid cultures of S medium (SULSTON and BRENNER 1974) containing *E. coli* strain X1666 (SANFORD, GOLOMB and RIDDLE 1983). During the growth of DR962 on plates, rare recombination events between the chromosomal duplication *mDp1* and chromosome *IV* were scored by loss of the semi-dumpy phenotype and discarded. Strain DR961 was tested for the presence of *mDf10* by complementation testing with *dpy-13/+* males. Following the transfer of animals to liquid medium, such screening was no longer possible. Therefore, to avoid contamination with genetic recombinants, growth in liquid medium was carried out for only three to four generations. To establish synchronously developing cultures of larvae, embryos were obtained from gravid adults by hypochlorite treatment (EMMONS, KLASS and HIRSH 1979) and were hatched in the absence of food, which arrests the worms as L1 larvae. Hatched L1 larvae were allowed to feed in liquid culture for 24 hr (L3 stage) and were then collected, washed in distilled water, and settled by gravity. This procedure removes bacteria as well as homozygous *mDf10* segregants which are in DR961 and DR962 cultures and arrest as L1 larvae.

Genomic Southern blot analysis: DNA was extracted from each culture of *C. elegans* according to the method of EMMONS, KLASS and HIRSH (1979) except that the worms were not ground by mortar and pestle prior to digestion with Proteinase K. DNA (5 μg) was digested with *Eco*RI, electrophoresed on a 1% agarose gel and transferred to nitrocellulose. Southern blots were hybridized with the following nick-translated probes in 50% formamide, 5 × SSC at 42°: DR#27, a 4.9-kb *Eco*R1 fragment of the *ama-1* gene in pUC18 (BIRD and RIDDLE 1989) and pRW28 (pCeMyoH5.5B/322), a 5.5-kb *Bam*HI fragment of the *unc-54* (myosin heavy chain) gene in pBR322 (MACLEOD, KARN and BRENNER 1981). Blots were washed at 65° with 0.2 × SSC, 0.1% SDS and exposed to Kodak XAR film in the presence of a Cronex Lightning Plus intensifying screen at −80°. The autoradiogram was scanned with a Pharmacia LKB Ultroscan XL enhanced laser densitometer.

RNA isolation and Northern blot analysis: Worms (1 g) were suspended in 3 volumes of a solution containing 4 M guanidine thiocyanate, 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 100 mM 2-mercaptoethanol and 0.5% Sarkosyl, frozen in liquid nitrogen, transferred to a small (37 ml) blender cup (precooled to −80°), and blended to a fine powder. RNA was purified according to MEYER and CASSON (1986). The concentration of mRNA within each total RNA preparation was estimated by hybridization of oligo([^{32}P] dT) to RNA samples immobilized on nitrocellulose slot blots (HARLEY 1987). Total RNA preparations containing equal amounts of mRNA were electrophoresed through a 1% agarose-formaldehyde denaturing gel (DAVIES, DIBNER and BATTEY 1986), transferred to nitrocellulose and hybridized in 50% formamide, 5 × SSC at 42° with the following probes labeled by nick-translation: DR#27 (described above) and pCeA103, a 210-bp gene-specific *Hinc*II-*Hinf*I fragment derived from the 3′ end of the *act-1* (actin) gene in pUC9 (FILES, CARR and HIRSH 1983). Blots were washed at 65°

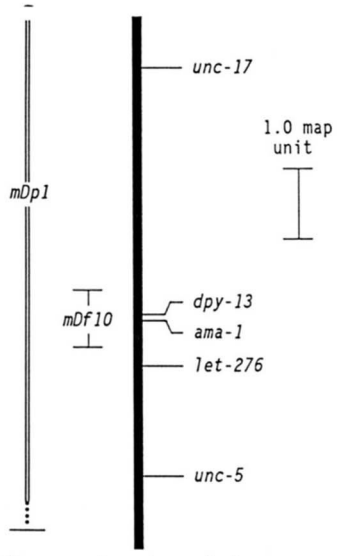

FIGURE 1.—The *ama-1* region of chromosome *IV*. A partial genetic map of the region around *ama-1 IV* showing the positions of the genes, the deficiency (*mDf*) and the duplication (*mDp*) used in the construction of nematode strains described in the text. In this vertical map, the left arm of chromosome *IV* is up and the right arm is down.

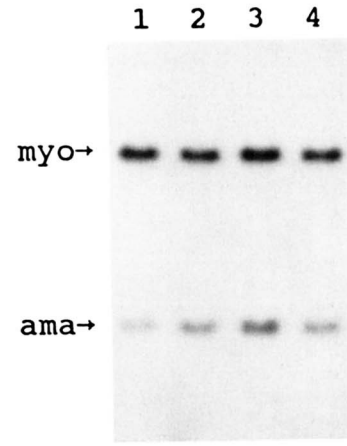

FIGURE 2.—DNA from strains differing in *ama-1* gene dosage. Approximately 5 μg of genomic DNA from DR961 (one *ama-1* gene copy; lane 1), DR680 (two copies; lane 2), DR962 (three copies; lane 3) and N2 (wild-type; lane 4) was digested with *Eco*RI, fractionated on a 1% agarose gel and transferred to nitrocellulose. The filter was hybridized with nick-translated plasmid probes containing *ama-1* and *unc-54 I* ("myo") sequences. Laser densitometry results are given in Table 1.

in 0.2 × SSC, 0.1% SDS and exposed to Kodak XAR film in the presence of a Cronex Lightning Plus intensifying screen.

RNA polymerase II enzyme activity assay: RNA polymerase II was extracted from worms (1 g) by ultrasonic disruption. Cellular debris was removed by centrifugation and passage of the supernatant over Miracloth (SANFORD, GOLOMB and RIDDLE 1983). Protein concentrations were determined following trichloroacetic acid precipitation (LOWRY *et al.* 1951). Crude extracts were allowed to incorporate [^{32}P]CTP into RNA for 20 min at 25° under standard conditions in the absence and presence of 7.5 μg/ml α-amanitin using calf thymus DNA as a template (SANFORD, GOLOMB and RIDDLE 1983). Incorporated radioactivity was determined in a DE81 filter assay (ROEDER 1974).

Quantitative Western blot analysis: Equal amounts of protein (20 μg) from crude extracts of each strain were subjected to SDS-polyacrylamide gel electrophoresis in a 5–15% gradient gel (LAEMMLI 1970). Protein bands were blotted onto nitrocellulose as described by TOWBIN, STAEHELIN and GORDON (1979), and the largest subunit (*ama-1* gene product) and second largest subunit of Rpo II were visualized following sequential addition of goat IgG directed against *Drosophila melanogaster* Rpo II (WEEKS, COULTER and GREENLEAF 1982), rabbit-anti-goat IgG and ^{125}I-Protein A. As a control, α-tubulin was identified following the sequential addition of α-tubulin monoclonal antibody (Amersham), rabbit-anti-mouse IgG and ^{125}I-Protein A. Blots were exposed to Kodak XAR film in the presence of a Cronex Lightning Plus intensifying screen and the relative levels of polypeptides representing the largest and second largest subunits of RNA polymerase II were determined by scanning the autoradiogram with a laser densitometer.

RESULTS

Verification of *ama-1* gene dosage in nematode strains: Three derivatives of wild-type (N2) *C. elegans* with different *ama-1* gene dosages were constructed. The one-copy strain (DR961), has a single, wild-type *ama-1* allele on the free duplication *mDp1 (IV;f)* and is homozygous for the short deficiency *mDf10 IV* including *ama-1* and nearby genes (Figure 1). The two copy strain (DR680) is homozygous for *ama-1(m118)*, an allele that lowers sensitivity to α-amanitin 150-fold (SANFORD, GOLOMB and RIDDLE 1983), and for *dpy-13(e184)*, a semidominant mutation that results in a dumpy (short, fat) body shape. The three copy strain (DR962) is a semi-dumpy derivative of DR680 containing two chromosomal *ama-1(m118)* alleles and a wild-type *ama-1* allele on the free duplication *mDp1 (IV;f)*. The *m118* mutation does not significantly affect solubilized enzyme activity or stability (SANFORD, GOLOMB and RIDDLE 1983), nor does it affect brood size or development (ROGALSKI, GOLOMB and RIDDLE 1990). The *mDp1* duplication recombines rarely with the normal homologue of chromosome *IV* (ROGALSKI and RIDDLE 1988).

Because of the possibility of gain or loss of *ama-1* alleles by recombination during propagation of strains carrying *mDp1*, the gene dosage in each population used for RNA and protein analysis was confirmed by quantitative Southern blot analysis. Filters were probed simultaneously with nick-translated recombinant plasmids containing sequences for the *ama-1* and *unc-54* (myosin heavy chain) genes (Figure 2). The *unc-54* gene on chromosome I served to normalize the amount of DNA in each lane. The autoradiogram in Figure 2 was scanned with a laser densitometer, and the *ama-1* gene dose was determined by comparing the signal intensity of the *ama-1* band to that of *unc-54* ("myo"). The three strains had the *ama-1* gene dosage expected for their genotypes (Table 1).

RNA polymerase II activity is invariant with *ama-1* gene dosage: Solubilized Rpo II activity in a non-

TABLE 1

Confirmation of *ama-1* gene dosage in nematode strains

Strain	*ama-1* gene dosage	
	Expected	Observed
N2	2	2
DR961	1	1.0
DR680	2	2.1
DR962	3	3.2

The autoradiogram from Figure 2 was scanned with a laser densitometer. For each strain, the gene dosage was determined by normalizing the signal intensity of the *ama-1* band to that of the *unc-54* (myosin) band and expressing the result relative to that obtained for N2 (assigned the diploid value of two). Repeat experiments with each culture used in these studies gave measurements to within 10% of the expected values.

specific transcription assay provides an estimate of intracellular enzyme concentration (ROEDER 1976). The intracellular concentration of Rpo II in nematodes was determined by measuring enzyme activity in crude extracts, thereby avoiding purification steps which could result in variable enzyme recovery. We had previously shown that the enzyme activity relative to the amount of the largest subunit of Rpo II in crude extracts of *C. elegans* is equal to that of the purified enzyme (SANFORD, PRENGER and GOLOMB 1985). Thus, crude extracts do not contain inhibitors of RNA chain elongation.

In order to compare Rpo II levels from different strains, it was important to prepare extracts from identically staged cultures. Relative to total protein, solubilized Rpo II activity and polypeptide levels remain fairly constant throughout larval development; however, elevated levels have been noted in embryos (DALLEY and GOLOMB 1992). When independently grown cultures of a given strain are staged identically, the variation in Rpo II activity and polypeptide levels among cultures is small (not shown). Mid-larval (L3) cultures were chosen as a source of extracts for all experiments described here.

Crude extracts were prepared from three independently grown cultures of each strain, and Rpo II activity was measured in a nonspecific transcription assay using calf thymus DNA as a template. The various classes of RNA polymerase in *C. elegans* can be distinguished by their differential sensitivity to α-amanitin (wild-type Rpo II is 50% inhibited at 7 ng/ml, *ama-1(m118)*-derived Rpo II at 1 μg/ml, Rpo III at ~25 μg/ml and Rpo I is insensitive (SANFORD, GOLOMB and RIDDLE 1983; ROGALSKI, GOLOMB and RIDDLE 1990)). Extracts were allowed to incorporate [^{32}P]CTP into RNA in the absence and presence of 7.5 μg/ml α-amanitin, a concentration that completely inhibits wild-type Rpo II and inhibits 88% of *m118* Rpo II activity. At this concentration, the contribution of Rpo III to α-amanitin sensitive activity was negligible. Under these conditions, Rpo II in extracts of N2 and DR961 should be completely inhibited, whereas DR962 and DR680 Rpo II should be inhibited 92% and 88%, respectively. Table 2 shows that the Rpo II activity detected in each of the nematode strains is close to that expected if *C. elegans* compensates for differences in *ama-1* gene copy number.

TABLE 2

Comparison of RNA polymerase II activity levels among nematode strains carrying one, two, or three copies of the *ama-1* gene

Strain	*ama-1* gene dose	*ama-1* alleles	Observed activity ratio		Expected activity ratio	
			Mean	SEM	If dosage independent	If dosage dependent
N2	2	2 S	1.0	0.14	1	1
DR961	1	1 S	0.99	0.15	1.00	0.50
DR680	2	2 R	0.82	0.08	0.88	0.88
DR962	3	2 R:1 S	0.92	0.18	0.92	1.38

RNA polymerase II in crude extracts of three independently grown cultures of the indicated nematode strains was allowed to incorporate [^{32}P]CTP for 20 min at 25°, using calf thymus DNA as a template. Incorporated radioactivity was determined from triplicate DEAE-cellulose filter assays, and RNA polymerase II activity was calculated as the difference in cpm incorporated in the absence and presence of 7.5 μg/ml α-amanitin. Typical data, for a single N2 culture, are as follows (mean of triplicate determinations ± standard error): activity in absence of α-amanitin (total activity), 17,670 ± 589 cpm; activity in presence of α-amanitin, 11,987 ± 570 cpm; calculated RNA polymerase II activity, 5683 cpm; protein concentration, 2.395 ± 0.081 mg/ml. In other extracts, which contained various concentrations of protein, total activity ranged from 13,842 to 21,534 cpm, and RNA polymerase II activity from 4766 to 8249 cpm. For each extract, RNA polymerase II activity was expressed relative to protein concentration (specific activity). The "observed activity ratio" refers to the mean specific activity for all three cultures of a strain, normalized to that for N2 (assigned a value of one). Expected activity ratios for DR680 and DR962 were calculated assuming a K_i of 1 μg/ml for *ama-1(m118)*-derived enzyme (SANFORD, GOLOMB and RIDDLE 1983). Abbreviations: S, wild-type *ama-1* allele; R, *ama-1(m118)* allele; SEM, standard error of the mean for all determinations for a strain.

***ama-1* polypeptide levels do not vary with gene dosage:** Quantitative Western blotting was used to compare the levels of Rpo II largest subunit between strains. Equal amounts of protein extracted from three independently grown cultures of each strain were subjected to gel electrophoresis, and anti-Rpo II IgG was used to detect both the largest subunit (205 kD) and the second largest subunit (135 kD) of Rpo II. Levels of *ama-1* polypeptide equivalent to wild-type were found in extracts of the one copy strain DR961, the two-copy strain DR680, and the three copy strain DR962 (Figure 3, A and B). Levels of the second largest subunit (135 kD), expected to be independent of *ama-1* gene dosage, were also found to be invariant in all strains (Figure 3C). Similar results were obtained with extracts from staged cultures of fourth-stage (L4) larvae (not shown).

The anti-*Drosophila* Rpo II antibody used here (WEEKS, COULTER and GREENLEAF 1982) cross-reacts with the highly conserved Rpo II subunits, but with

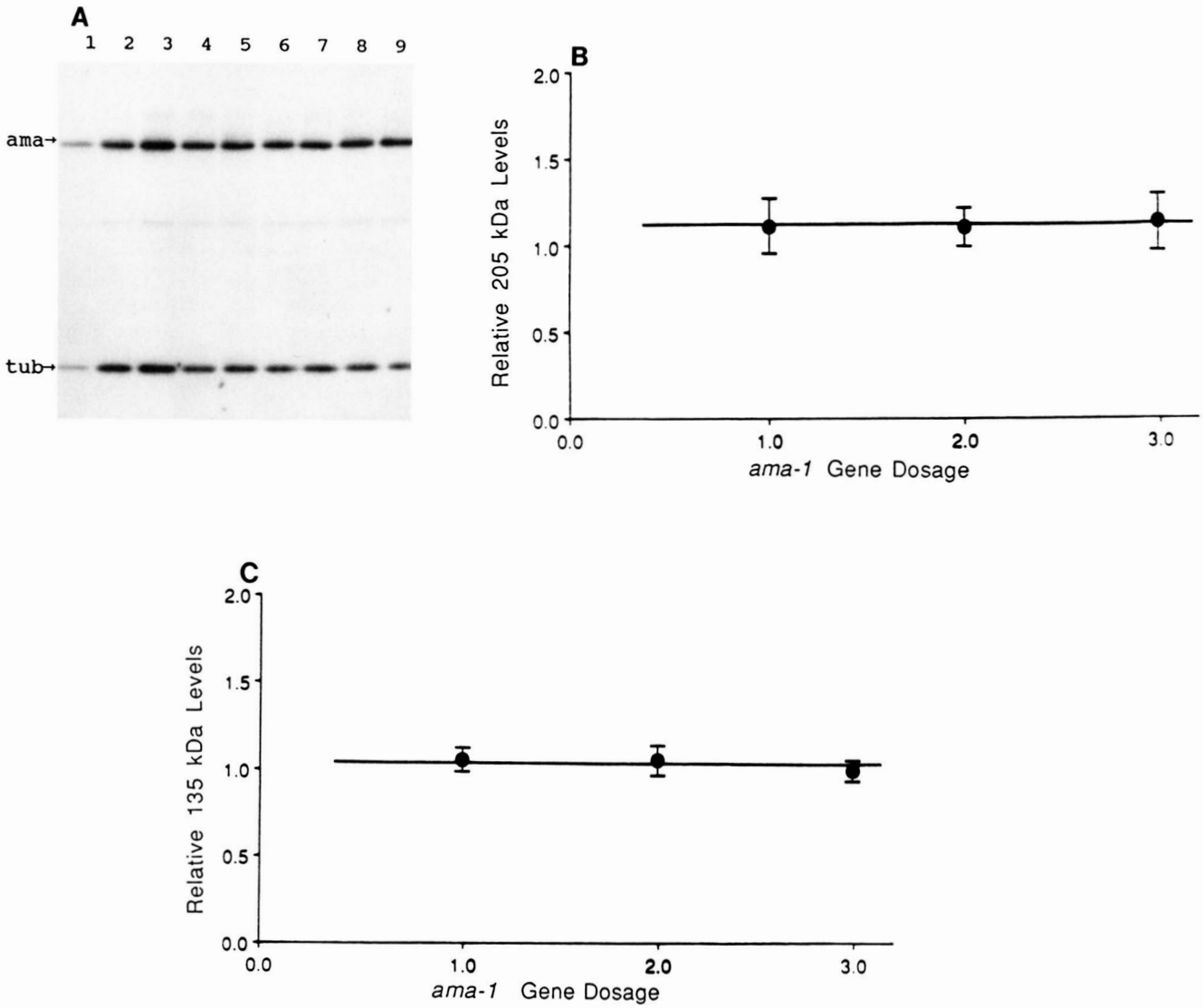

FIGURE 3.—Comparison of RNA polymerase II largest subunit levels among nematode strains differing in *ama-1* gene dosage. A, Equal quantities of total protein (20 μg) from crude extracts made from synchronized cultures of third-stage larvae of DR961 (one *ama-1* gene copy; lanes 4 and 7), DR680 (two copies; lanes 5 and 8) and DR962 (three copies; lanes 6 and 9) were compared with an extract from wild-type (N2) third-stage larvae (lane 1, 10 μg; lane 2, 20 μg; and lane 3, 30 μg) after electrophoresis on a 5–15% gradient SDS-polyacrylamide gel, transfer to nitrocellulose and reaction with anti-RNA polymerase II IgG to detect *ama-1* polypeptide. The blot was subsequently reacted with anti-α-tubulin IgG ("tub"). The second largest subunit of Rpo II (135 kDa) can be seen as a light band below "ama"; longer exposures were used for quantitation of this subunit. B, Total protein from the indicated nematode strains was subjected to Western blot analysis as in (A), followed by scanning densitometry. Three independently grown cultures of each strain were analyzed, representing a total of nine determinations of subunit levels per strain. Each determination was made by scanning the autoradiogram with a laser densitometer and determining the mean signal intensity of the *ama-1* polypeptide (205 kDa subunit) for each strain. These values were normalized relative to the mean value for N2 in the same blot (assigned a value of one). Each data point represents the mean value of all determinations for a strain. C, The relative levels of the 135-kD subunit of Rpo II for each strain were measured on the western blots from (B). These values were calculated in a manner analogous to that for the 205 kD subunit. Bars indicate the SEM for all determinations for a strain.

few other nematode proteins in crude extracts (SANFORD, PRENGER and GOLOMB 1985). The detection of polypeptides in an N2 extract was linear in the range of interest (Figure 3A, lanes 1–3). As a control, the blot was also reacted with a monoclonal antibody directed against α-tubulin ("tub"); this protein is present at similar levels in each lane. The observation that strains N2 and DR680 (two *ama-1* gene copies) had approximately equal levels of largest subunit polypeptide (Figure 3A, compare lane 2 with lanes 5 and 8) shows that the *m118* mutation in DR680 does not affect subunit levels.

RNA polymerase II is post-transcriptionally regulated: To ask whether control of *ama-1* polypeptide synthesis is transcriptional or post-transcriptional, we compared the steady-state levels of *ama-1* mRNA from staged cultures of the strains differing in *ama-1* gene copy number. The results show that *ama-1* transcript levels are directly proportional to the *ama-1* gene dose in each strain (Figure 4).

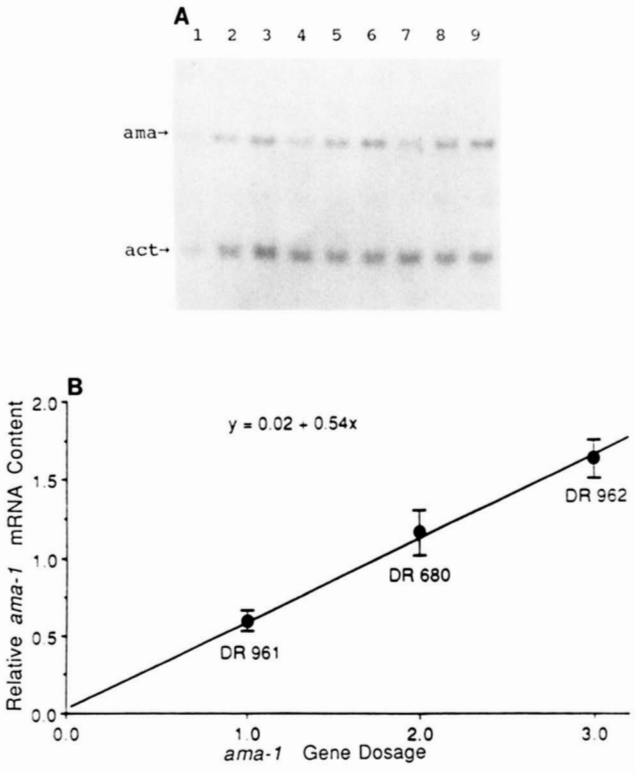

FIGURE 4.—The effect of gene dosage on *ama-1* mRNA levels. Total RNA was extracted from the indicated nematode strains and mRNA quantities assessed by poly(A) content. A, Total RNA containing equal quantities of mRNA from DR961 (one *ama-1* gene copy; lanes 4 and 7), DR680 (two copies; lanes 5 and 8) and DR962 (three copies; lanes 6 and 9) was separated on a 1% agarose-formaldehyde gel. Lanes 1–3 contained different amounts of N2 mRNA. Lane 2 contained an mRNA quantity equivalent to that in lanes 4–9; lane 1 contained 0.5-fold that amount and lane 3 contained 1.5-fold. RNA was transferred to nitrocellulose and hybridized with gene-specific probes containing *ama-1* and *act-1* (actin) sequences. B, RNA from three independently grown cultures of each strain were subjected to Northern blot analysis as in (A), followed by scanning densitometry, representing a total of 13 determinations of relative *ama-1* mRNA content per strain. Each determination was made by normalizing the band intensity of *ama-1* mRNA to that of *act-1* mRNA in the same lane, and expressing the result relative to the mean ratio for N2 in the same blot (assigned a value of one). Each data point represents the mean value of all determinations for a strain. Bars indicate the SEM for all determinations for a strain.

Total RNA from strains DR961, DR680, and DR962, containing equal amounts of poly(A)$^+$ RNA, was fractionated on a gel and transferred to nitrocellulose. As a control for linearity of the assay, N2 RNA varying over a three-fold concentration range was applied to adjacent lanes of the gel (Figure 4A, lanes 1–3). The Northern blot was simultaneously probed with nick-translated recombinant plasmids containing sequences for the *ama-1* gene and the gene-specific 3′ untranslated region of the *C. elegans act-1* (actin) gene (Figure 4A). The *act-1* gene is located on chromosome V and is not affected by either of the chromosomal rearrangements used in this study. Hybridization with this probe was used to normalize for the amount of mRNA in each lane. Figure 4B summarizes the data from a number of experiments such as that in Figure 4A.

DISCUSSION

On the basis of experiments involving growth of mammalian tissue culture cells in α-amanitin, SOMERS, PEARSON and INGLES (1975) proposed that mammalian Rpo II levels are autoregulated. Using a different experimental approach, one which avoids the complications of α-amanitin treatment, we have obtained evidence for gene dosage independence of the largest subunit of *C. elegans* Rpo II and, by inference, for feedback regulation of the concentration of the enzyme. Our experiments implicate the largest subunit of *C. elegans* Rpo II directly or indirectly in its own regulation. Because of the evolutionary conservation of Rpo II structure (YOUNG 1991) and the central role this enzyme plays in gene expression, it seems likely that all eukaryotes regulate Rpo II concentration by similar mechanisms.

Enzyme activities were equal in strains having one, two, or three copies of the *ama-1* gene. This result might be expected for a comparison of DR962 (three-copy strain) with DR680 (two-copy strain) because polymerase activity requires assembled enzyme. Additional amounts of largest subunit relative to wild-type would not increase enzyme activity unless the quantity of the largest subunit were limiting in the wild-type relative to all other subunits. However, if *ama-1* polypeptide levels are not regulated, DR961 (the one-copy strain) would have lower Rpo II activity than wild-type. Since the activity from DR961 was the same as wild-type, Rpo II accumulation in this strain must be up-regulated relative to wild-type, or wild-type *C. elegans* must contain a twofold excess of largest subunit relative to other subunits and assembled enzyme.

Measurements of Rpo II subunits in crude extracts differ from solubilized enzyme assays in that they are capable of detecting stably expressed enzyme polypeptides whether free or assembled into Rpo II. In extracts of strains differing in *ama-1* gene copy number, levels of the largest subunit were also independent of gene dosage. If the accumulation of *ama-1* polypeptide were not regulated, DR962 would be expected to contain 1.5-fold the wild-type amount of *ama-1* polypeptide, and DR961, 0.5-fold the wild-type amount. Invariant levels suggest regulation at some level.

In principle, the gene dosage independence described here could result from transcriptional control, or from various post-transcriptional mechanisms. We have found that steady-state levels of *ama-1* transcripts are directly proportional to gene dosage, ruling out mechanisms involving transcriptional regulation, mRNA processing or differential mRNA decay.

These observations could be explained by two alternative mechanisms. The first is translational control, perhaps inhibition of *ama-1* mRNA translation by one or more components of Rpo II, including the largest subunit. The second, less likely, explanation is post-translational degradation of *ama-1* polypeptide. It is possible that the amount of another Rpo II subunit is limiting in assembly, and that excess production of *ama-1* polypeptide simply results in degradation of surplus subunit. Quantitative Western blots on crude extracts, although capable of detecting unassociated *ama-1* polypeptide, are a steady-state measurement that would miss rapidly degraded protein. This explanation would require that *ama-1* polypeptide is produced in at least a two-fold excess in wild-type worms, because DR961 (hemizygous for *ama-1*) contained as much of the largest subunit as wild-type.

Although steady-state measurements cannot rule out post-transcriptional degradation, the results of GUIALIS, MORRISON and INGLES (1979), taken together with ours, favor translational regulation. Degradation of α-amanitin-sensitive Rpo II is followed by an increased rate of synthesis of the largest subunit and several smaller subunits (GUIALIS, MORRISON and INGLES 1979), a result that is more compatible with translational control than with degradation of excess subunit. Although the 5′ end of *C. elegans ama-1* mRNA has not been defined, the corresponding transcripts from yeast (ALLISON *et al.* 1985), *Drosophila* (BIGGS, SEARLES and GREENLEAF 1985), and the mouse (AHEARN *et al.* 1987) have long (>400 base) 5′ untranslated leaders. It will be interesting to examine if the 5′ leader sequences contain regions instrumental in translational control.

Rpo II levels relative to total protein remain fairly constant throughout *C. elegans* larval development (DALLEY and GOLOMB 1992). However, it is likely that critical developmental periods, such as oogenesis or embryogenesis, mandate higher levels of Rpo II. Indeed, *C. elegans* embryos are enriched for solubilized Rpo II activity and polypeptides relative to other stages (DALLEY and GOLOMB 1992). This is reminiscent of *Xenopus laevis* oogenesis, during which large amounts of all three RNA polymerases are synthesized and stored for embryogenesis (ROEDER 1976). *Drosophila* embryos are similarly enriched for Rpo II largest subunit mRNA relative to other stages (BIGGS, SEARLES and GREENLEAF 1985).

There is circumstantial evidence suggesting higher quantitative requirements for Rpo II during *C. elegans* oogenesis or embryogenesis. First, the null phenotype of *ama-1* is developmental arrest during the first larval stage, demonstrating that maternal Rpo II is sufficient to complete embryonic development (ROGALSKI and RIDDLE 1988). Second, the most common phenotype associated with hypomorphic alleles of *ama-1* is adult sterility, indicating that an intermediate level of enzyme activity is sufficient for larval development but not for fertility (ROGALSKI, BULLERJAHN and RIDDLE 1988).

As predicted by regulation of Rpo II levels, the phenotype of strains hemizygous for wild-type *ama-1* or the *m118* allele does not differ from wild-type (ROGALSKI and RIDDLE 1988; ROGALSKI, BULLERJAHN and RIDDLE 1988). However, we have previously described an *ama-1* allele (*m118m526*), which when placed over a deficiency results in a sterile adult, a phenotype more severe that the homozygous *m118 m526* mutant phenotype (ROGALSKI, GOLOMB and RIDDLE 1990). In this case, the hemizygous strain apparently has less Rpo II than the wild-type, at least at some stage of development. However, this apparent exception can be reconciled with our hypothesis by taking into account the properties of the mutant enzyme. The *m118 m526* allele results in a form of Rpo II that has a 20,000-fold reduced sensitivity to α-amanitin. Homozygous *m118m526* animals grow slowly, have a reduced brood size, and are temperature-sensitive sterile. Rpo II isolated from *m118 m526* homozygotes is highly unstable during purification relative to either wild-type or *m118* enzyme. If the mutant enzyme is unstable *in vivo*, severely reduced quantities may place it out of the compensatory range of regulatory mechanisms, especially those required for oogenesis or embryogenesis.

Although the general phenomenon of translational control, including translational repression, has been well documented in eukaryotes (HERSHEY 1991), it would be somewhat surprising to find a nuclear protein such as Rpo II involved in regulation of translation. It is possible that regulation is indirect, or involves free subunit or polymerase subassemblies. Subcomplexes, probably representing assembly intermediates, accumulate in yeast Rpo II assembly mutants and resemble their counterparts in bacterial RNA polymerase assembly (YOUNG 1991).

If the hypothesis of translational control is correct, it would establish strong parallels between regulation of *C. elegans* Rpo II biosynthesis and that of RNA polymerase in *E. coli*. *E. coli* RNA polymerase holoenzyme is composed of four subunits, α, β, β′ and σ (CHAMBERLIN 1976). β′ is homologous to the largest subunit of Rpo II and β to the second largest subunit (YOUNG 1991). Bacterial cells contain a molar excess of α (ENGBAEK, GROSS and BURGESS 1976). Thus assembly of $\alpha_2\beta\beta'$ core enzyme is limited by production of β and β′. The corresponding genes (*rpoB* for β and *rpoC* for β′) map contiguously within the L10 ribosomal protein operon and are autogenously regulated (reviewed by YURA and ISHIHAMA 1979). Overexpression of β and β′ from inducible promoters lowers the efficiency of translation of *rpoBC* mRNA,

but not that of transcription, indicating that these genes are translationally regulated *in vivo* (MEEK and HAYWARD 1986; PASSADOR and LINN 1989). Similarly, underproduction of β subunit (resulting from weak amber mutations in *rpoB*) is offset by increased ribosome loading on the β transcript (DENNIS, NENE and GLASS 1985). An intergenic region 5′ to the *rpoB* gene is implicated in efficient translation of β subunit and in translation repression (DENNIS 1984). *In vitro*, holoenzyme and the $\alpha_2\beta$ subassembly repress translation initiation of β subunit (FUKUDA, TAKETO and ISHIHAMA 1978; KAJITANI, FUKUDA and ISHIHAMA 1980; PEACOCK *et al.* 1982). Additional controls, some of which may be transcriptional, operate to coordinate RNA polymerase subunit synthesis with cellular growth conditions (YURA and ISHIHAMA 1979).

Further studies of Rpo II regulation should reveal how closely it parallels bacterial RNA polymerase regulation. Additional complexity might be expected, because Rpo II contains more subunits than bacterial RNA polymerase, and certain subunits are shared with Rpo I and Rpo III, the levels of which are differentially regulated (ROEDER 1976). The division of labor between the three classes of eukaryotic RNA polymerase also explains the need for controls different from those in bacteria: in *E. coli*, where a major task of RNA polymerase is rRNA synthesis, β and β' are organized into a transcription unit with ribosomal protein genes and coordinately respond to changes in growth conditions that affect the number of ribosomes per cell (YURA and ISHIHAMA 1979). In eukaryotes, physiological transitions are marked by changes in transcriptional activity effected by transcription factors and/or polymerase modification (PAULE *et al.* 1984). However, changes in solubilized enzyme levels accompany certain growth transitions (ROEDER 1976). The characterization and *in vitro* mutagenesis of genes for Rpo II subunits should facilitate studies of regulation. Further definition of the level of regulation for Rpo II will require measurements of the rate of synthesis and degradation of subunits, information on hypothetical regulatory sequences in the mRNA for the largest subunit, and *in vitro* manipulation of translational components.

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