Tissue-Specific Synthesis of Yolk Proteins in Caenorhabditis elegans

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The primary site of yolk protein synthesis in the nematode, Caenorhabditis elegans, has been determined. In animals containing no gonadal cells (obtained by laser ablation of the gonadal precursor cells early in development), yolk proteins are present in abundance. This demonstrates that yolk proteins are made outside the gonad. An examination of proteins present in tissues isolated by dissection, and a comparison of proteins synthesized by isolated tissues incubated in vitro have identified the intestine as the major site of yolk protein synthesis. We propose that yolk proteins are synthesized in the intestine, secreted from the intestine into the body cavity, and taken up from the body cavity by the gonad to reach oocytes. The site of yolk protein synthesis has also been examined in four mutants that have largely male somatic tissues, but a hermaphroditic germ line. Here again, yolk proteins are produced by intestines in a hermaphroditic-specific manner. This suggests that sex determination is coordinately regulated in intestinal and germ line tissues.

INTRODUCTION

The yolk proteins of the nematode, Caenorhabditis elegans, have been identified (Klass et al., 1979; Sharrock, 1982). They comprise a group of proteins with approximate molecular weights of 170K, 115K, and 88K which are called yp170, yp115, and yp88, respectively. Peptide mapping of these proteins (Sharrock, 1982) has demonstrated that yp170, yp115, and yp88 represent three distinct species. The 170K yolk protein may be resolved into a doublet on polyacrylamide-sodium dodecyl sulfate (SDS)-gels; however, the two bands of the doublet have been shown to be closely related by peptide analysis (Sharrock, 1982).

In all egg-laying animals studied to date, synthesis of yolk proteins, or their precursors, occurs in a tissue outside the gonad. Once synthesized, the proteins are secreted into the circulation and transported to the ovary (reviewed by Tata and Smith, 1979). In this paper, we report the site of synthesis of yolk proteins in C. elegans. This work provides necessary background information for further genetic and biochemical studies of yolk protein regulation. In addition, we have extended a technique for microdissection used to isolate pharynges (Waterston et al., 1974) to separate most of the rest of the animal's tissues, and we have demonstrated synthesis of proteins by isolated tissues in vitro for the first time in C. elegans.

MATERIALS AND METHODS

Strains

The nomenclature used in this paper conforms to published guidelines (Horvitz et al., 1979). The wild-type strain used is Caenorhabditis elegans var. Bristol, strain N2; the mutant strains are tra-1(e1489)III; tra-2(b202)II; isx-1(hc17)IV; him-8(e1489)IV; and isx-2(b245)III; him-1(e879)I.

Maintenance

Strains were maintained as described by Brenner (1974). Temperature sensitive mutants were kept at the permissive temperature (16°C) except for shifts to restrictive temperature (25°C) as described in Results. N2 and other mutants were kept at 20°C.

Laser Ablation

The laser microbeam system and the procedure for killing individual cells in C. elegans have been described elsewhere (Sulston and White, 1980).

Dissection

To isolate individual tissue (Fig. 2) animals were cut with a scalpel blade behind the rear bulb of the pharynx in about 2 ml of an isotonic medium (Chan and Gehring, 1971). An eyelash was used to press the contents of the animal into the dissecting medium, and tungsten needles were used to separate the intestine and gonads from each other and from the body wall. The intestines and body walls were removed from the medium by eyelash; gonads and embryos were taken up in a few microliters of the solution to which they were being transferred.

To observe secretion, a cut was made through the pharynx, and the animal was pulled out of the medium repeatedly on an eyelash. The break in surface tension is sufficient to bring the intestine about halfway out of the body (Fig. 5).
Labeling in Vivo

Whole animals were labeled as groups of 3–10 individuals as follows: *Escherichia coli* NA22 was grown for 24 hr at 37°C in 10 ml of low-sulfate medium containing 100 μCi/ml 35S-35SO4. The bacterial suspension was centrifuged, and the pellet resuspended in about 0.1 ml M9 salts (Brenner, 1974). The bacterial suspension was distributed to four to six plates in as small drops as possible, and allowed to dry down. Worms were placed on this bacterial lawn 6 hr at 25°C, 8–10 hr at 20°C, or 12 hr at 15°C (these are nearly equivalent periods of time with respect to development of *C elegans* at the various temperatures). This period of labeling was chosen as a reasonable approximation of steady-state labeling. After labeling for 8–10 hr, animals had incorporated 10,000–15,000 cpm into TCA-precipitable material, corresponding to a specific activity of about 7.5 × 104 cpm/μg protein. After 12 hr (20°C), incorporation decreased, presumably due to dilution of label as a result of bacterial growth. The animals were transferred to unlabeled bacteria for 15–30 min before freezing in aliquots of M9 salts on dry ice.

The developmental stage at which yolk proteins are first observed was determined by labeling worms on 35S-labeled *Escherichia coli* for a period of 2 hr in consecutively staged groups throughout L4 (fourth larval stage) and into adulthood (until the first embryo was produced). Before molting, worms undergo a 1.5- to 2-hr period of “lethargus” during which they do not eat. Therefore, one group of worms was placed on labeled bacteria for 1 hr before lethargus and then taken off within 15 min after the molt. Worms were staged using standard morphological markers as in Kimble and White (1981).

Protein Synthesis in Isolated Tissues

Dissected tissues were incubated in a 25-μl drop of medium (Chan and Gehring, 1971) containing 1 mCi/ml [35S]methionine and 100 μM amino acid mixture lacking methionine at 20°C on a siliconized depression slide placed in a wet chamber. Isolated tissues were incubated for 1 hr. Incorporation into tissues was efficient for periods up to 2 hr, but became less efficient after 4 hr presumably due to deterioration. After incubation, pieces were transferred into a drop of lysis solution, 5% SDS, 10% glycerol, 0.15 M Tris, pH 7.0, plus protease inhibitors iodoacetate (20 mM) and phenylmethylsulfonyl fluoride (2 mM), placed on a siliconized well slide, and frozen immediately on dry ice.

Electrophoresis

Electrophoresis in SDS-containing gels of polyacrylamide was performed by the method of Laemmli (1970) using a 5–15% gradient of polyacrylamide. Gels were autoradiographed using hypersensitized film after fluorography following the procedure of Laskey and Mills (1975). The percentage of total labeled protein represented by individual bands was estimated using a Joyce-Loebel densitometer.

RESULTS

The Yolk Proteins Are Made Outside the Gonad

To test whether *C. elegans* yolk proteins (yp170, yp115, and yp88) might be made outside the gonad, the four precursor cells that give rise to the entire gonad (two precursor cells of the somatic gonad and two germ line precursor cells) (Kimble and Hirsh, 1979) were killed by laser ablation in L1 hermaphrodites. (Development of nematodes involves four larval stages, L1, L2, L3, and L4, after embryogenesis and before adulthood.) The adult animals obtained after this operation possess no gonadal cells at all, and accumulate large amounts of amorphous refractive material in the body cavity. Separation of the proteins from such animals by electrophoresis shows many differences from the intact animal, as would be expected after elimination of an entire organ. However, the gonadless animals do possess the prominent bands of typical yolk proteins (170K, 115K, and 88K daltons molecular weight) (Fig. 1A). This result suggests that yolk proteins are normally made outside the gonad, and that their synthesis is independent of gonadal cues.

If the gonadal precursor cells are ablated in L1 males instead of hermaphrodites, neither yolk proteins nor a 15K protein typical of males (Klass and Hirsh, 1981) are observed in the resultant adults (Fig. 1B). The absence of the yolk proteins indicates that the presence of the male gonad does not inhibit their production in males. The absence of the 15K male protein from gonadless males is consistent with evidence of Klass and Hirsh (1981) that this protein is made in the male gonad.

The Yolk Proteins Are Synthesized by and Secreted from the Intestine

In simplified view, *C. elegans* is made up of three tubes (Fig. 2): the outer body wall consists of a cuticle-producing hypodermis together with an underlying layer of muscle; the intestine is composed of a single layer of endodermal cells; and the gonad is made up of germ line cells partially ensheathed in a tube of somatic gonadal cells. These tubes can be separated by dissection to obtain isolated tissues (Fig. 2B). The distribution of yolk proteins among the tissues of *C. elegans* was determined by dissecting body walls, intestines, gonads, and embryos from worms radioac-
tively labeled in vivo by growth on a lawn of $^{35}$S-labeled E. coli. The proteins of the dissected parts were then separated by SDS-gel electrophoresis. Each tissue of the animal exhibits a characteristic and reproducible pattern of bands (Fig. 3A). In intestines, yp170 is prominent, yp88 faint, and yp115 apparently absent; in body walls, a minor band is present at 170K, and yp115 and yp88 are not detectable. All three proteins are easily observed in whole gonads and embryos, but are faint in distal arms of the gonad (see Fig. 2A for anatomy). Microdensitometer tracings were used to roughly estimate the percentage of total labeled proteins represented by yolk proteins in each tissue: intestine, 10%; gonad, 10%; body wall, 0.5%; embryos, 37%. The trace amount of yolk protein observed in body walls is probably due to our inability to extrude all material from the body cavity upon isolation.

The prominence of yp170 in intestines indicates that this may be the extra gonadal site of yolk protein synthesis in C. elegans. Yet, the paucity of the two smaller yolk proteins in tissues outside the gonad presents a paradox since gonadless animals make proteins of all three molecular weights. Intestines and body walls were therefore isolated from gonadless animals (produced by laser ablation as described above). In this experiment, all three bands were easily detected in intestines (Fig. 3B), and again only a minor band at 170K was observed in body walls. This result suggests that the smaller proteins accumulate in the intestine when the animal does not sequester them into embryos. Consistent with this idea, intestines isolated from older intact animals, which no longer are producing embryos, also possess all three proteins (Fig. 3C).

The results presented so far suggest that the yolk proteins may be synthesized in the intestine, but do not exclude two other possibilities. They might be made in the gonad in addition to the intestine, or the intestine might be used as a way station for their modification and/or storage on their way to the gonad from some other site in the body.

The actual site of synthesis of the yolk proteins was determined by analyzing the incorporation of $[^35]$S]methionine into proteins synthesized by isolated tissues in vitro. After a 1-hr incubation, no incorporation was evident by the adult body wall or embryos. However, after the same period of time, both intestines and gonads exhibited significant incorporation (Figs. 4A, B). The proteins synthesized by gonads show a reproducible pattern of bands that clearly lack 170K and 88K proteins (Fig. 4A). (It should be noted that the major bands synthesized by the gonad compare quite well to the bands missing in the gels of gonadless hermaphrodites in Fig. 1.) A protein of slightly slower mobility than 115K is synthesized by gonads, but not one of 115K; a similar protein is observed in larvae, males, and in dissected gonads (including the distal arm). Thus, gonads do not synthesize any of the yolk proteins in detectable quantity.

In six independent experiments, isolated intestines reproducibly showed intense incorporation into a band at 170K, but incorporation into proteins of 115K and 88K was variable (Fig. 4B). In one experiment, clear bands at 115K and 88K were present; in another experiment, a band at 88K but not at 115K was observed; and in others, no detectable incorporation at the 115K and 88K positions was seen. When the lower-molecular-weight proteins were faint or absent, several higher-molecular-weight bands (155K and 165K) appeared. Further analysis is obviously necessary to understand the differences among these results. It is clear, however, that the intestine synthesizes a large amount of protein.

![Fig. 1. Comparison of proteins synthesized by intact (+) and gonadless (–) adult hermaphrodites (A) and males (B). yp170, yp115, and yp88 (170K, 115K, and 88K molecular weights) are present in hermaphrodites both with and without a gonad; they are not present in males either with or without a gonad. Intact hermaphrodites lay eggs containing a large quantity of yolk proteins, whereas gonadless hermaphrodites accumulate yolk proteins. The 15K sperm-specific protein of Klass and Hirsh (1981) is not detectable in gonadless males.](image-url)
with a molecular weight of 170K—roughly 25% of the total labeled protein.

A different kind of dissection was required to show secretion. When animals are cut through the pharynx, intact intestine and gonad are extruded into the incubation medium (Fig. 5). Worms cut in this way were incubated with [³⁵S]methionine for increasing periods of time. Then, the proteins present in the incubated tissues were compared with those in the medium. In such an experiment, proteins of 170K, 115K, and 88K are selectively observed in the medium (Figs. 6A, B) indicating that the yolk proteins are secreted. As in isolated intestines (Fig. 4), the smaller yolk proteins yp115 and yp88 are not detectable in the tissues of this experiment.

The Yolk Proteins Are First Expressed during L4 Lethargus

The developmental stage at which yolk proteins can first be detected was determined next. Whole worms labeled for 2 hr just prior to entry into lethargus do not possess either yp170 or yp88, but worms labeled from 1 hr before lethargus and through it clearly exhibit proteins at both 170K and 88K (Fig. 7). A doublet of approximately 155K molecular weight exhibits the same developmental profile (Fig. 7). In subsequent stages of adulthood, these bands become prominent. yp115 is obscured by a band of slightly slower mobility; however, a faint band at 115K is first seen in the 2-hr time period following the molt.

Presence of Yolk Proteins in Mutants Affecting Sexual Phenotype

The production of yolk proteins has also been studied in four mutants (Table 1). These mutants were chosen because they exhibit a male-like body, but also make oocytes. We were therefore interested to know if the intestine was hermaphrodite (i.e., if it makes yolk proteins) or male.

In all four of the mutants, both intact and gonadless animals possess all three yolk proteins (Fig. 8). Intestines dissected from [³⁵S]-labeled animals of each mutant are the same as wild-type intestines in that they contain 170K protein, but little or no protein at the 115K and 88K positions (data not shown). These results indicate

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Fig. 2. (A) Schematic of nematode anatomy. Three tubes make up most of the worm’s body: a body wall, an intestine, and a gonad. The distal arm of the gonad consists of immature germ cells; the proximal arm of maturing oocytes. (B) Nomarski micrographs of tissues after their isolation from each other by dissection.
that the yolk proteins are made in the intestine in all
mutants examined.

DISCUSSION

Extragonadal Synthesis of Yolk Proteins in C. elegans

Figure 9 summarizes the most likely route taken by
yolk proteins in C. elegans to reach the oocytes in the
gonad. The evidence for this route is convincing for
yp170, and reasonably good for yp115 and yp88. All three
proteins are clearly synthesized outside the gonad, be-
cause animals with no gonad accumulate them. The iso-
lated intestine synthesizes an abundant quantity of pro-
tein with a mobility of 170K and proteins of 115K and
88K in some experiments. (The variability observed for
yp115 and yp88 is discussed in the next section.) Finally,
all three proteins can be detected in intestines isolated
from labeled animals, and all three are secreted in vitro.

In Drosophila, yolk proteins are synthesized by the
ovary as well as the fat body (Postlethwait et al., 1980;
Gutzeit, 1980). In C. elegans, the gonad clearly does not
synthesize yolk proteins in quantity, but a low level of
synthesis (i.e., less than 5% of the total synthesis of
the proteins) has not been excluded. It seemed possible
that the formation of oocytes in mutants with primarily
male soma might have reflected synthesis endogenous
to the gonad. However, even here, the intestine appears
to be the primary site of yolk protein synthesis.

Production of yp115 and yp88

The extreme abundance of yp115 and yp88 in gonad-
less animals demonstrates that they are made outside
the gonad. Yet, when tissues outside the gonad are ex-
amined, these two proteins are difficult to detect. One
plausible explanation of this paradox is that production
of yp115 and yp88 depends on transport and/or pro-
cessing. The reproducible finding of all three proteins
in the secretion medium of dissected tissues supports
the idea that production of yp115 and yp88 might be
coupled to transport. Processing of yolk proteins from
a larger precursor protein has been observed in many
organisms including insects (Chen et al., 1978), amphi-
bia, and chickens (reviewed by Tata and Smith, 1979).
Processing may also occur in C. elegans. In some ex-
periments, high-molecular-weight proteins (155K and
165K) but not 115K and 88K proteins are synthesized
by isolated intestines; in other experiments, bands at

\begin{tabular}{ccc}
A & 30 & 60 & 120 \\
170K & - & - & - \\
115K & - & - & - \\
88K & - & - & - \\
\end{tabular}

\begin{tabular}{ccc}
B & 30 & 60 & 120 \\
170K & - & - & - \\
115K & - & - & - \\
88K & - & - & - \\
\end{tabular}

FIG. 6. Proteins present in tissues (A) and medium (B) after in-
cubation of cut worms (see Fig. 5) for 30, 60, and 120 min. The yolk
proteins are found selectively in the medium.
115K and 88K are observed, but not the bands at 155K and 165K (Fig. 4). Furthermore, a 155K protein is expressed at the same developmental stage as ypl70 and yp88 (Fig. 7), and a 155K protein accumulates in some sexually transformed mutant strains (Fig. 8). However, further studies, using specific probes for detecting the molecules involved, are necessary to delineate the precise steps in this pathway.

**Yolk Protein Synthesis as an Intestine Specific Marker of Sexual Differentiation**

Anatomical differences are observed between the two sexes of *C. elegans* in all tissues except the intestine. In the gonad, both somatic and germ line tissues differ with sex (Hirsh et al., 1976; Kluss et al., 1976). The most obvious nongonadal sexual specialization are the vulva in hermaphrodites and the copulatory apparatus in males in which particular hypodermal, nerve, and muscle precursor cells are recruited to follow a sex-specific lineage thereby generating a final complex structure (Sulston and Horvitz, 1977).

In contrast, the development and final form of the intestine is identical in hermaphrodites and males (Sulston and Horvitz, 1977; Sulston and Schierenberg, personal communication). However, yolk proteins are produced by hermaphrodite but not male intestines. Hermaphrodite and male intestines can therefore be distinguished at the level of protein synthesis and this feature can now be used to assay sexual differentiation of the intestine.

![Fig. 7. Initial appearance of yolk proteins during development. a, standard provided by a homogenate of hermaphrodites grown in liquid culture. b-f, consecutive developmental stages. Worms were staged according to standard development events (e.g., stages in vulva morphogenesis and the mating cycle) as described in Kimble and White (1982). Hours refer to 20°C hours after hatching as in Sulston and Horvitz (1977). b, 39-41 hr; c, 41-48 hr; d, 42-45 hr (includes L4 lethargus); e, 45-47 hr; f, 47-49 hr; g, 49-51 hr. Bands at 170K and at 88K first appear during L4 lethargus. The appearance of a band at 115K is obscured by a larval protein of slightly slower mobility. A doublet at 155K exhibits the same developmental profile as the yolk proteins (arrowhead).](image)

![Fig. 8. Comparison of proteins produced by intact (+) and gonadless (−) mutants (Table 1). yp170, yp115, and yp88 are synthesized by all mutants examined both with and without gonads. Two mutants (b243 and e1488) accumulate a doublet at 155K in gonadless animals (arrowhead).](image)

### Table 1: Yolk Proteins in Four Mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Gene</th>
<th>XX/XO</th>
<th>Gonadal morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>e1488</td>
<td>tre-1(II)</td>
<td>XX</td>
<td>Hermaphrodite</td>
</tr>
<tr>
<td>b202</td>
<td>tre-2(II)</td>
<td>XX</td>
<td>Intersexual</td>
</tr>
<tr>
<td>hc17</td>
<td>isx-1(IV)</td>
<td>X0</td>
<td>Intersexual</td>
</tr>
<tr>
<td>b243</td>
<td>isx-2(III)</td>
<td>X0</td>
<td>Male</td>
</tr>
</tbody>
</table>

*Note. All mutants have male-like bodies but produce oocytes. The chromosomal complement (XX or XO) and gonadal morphology vary as indicated above. b202 and hc17 animals were shifted to restrictive temperature within a few hours of hatching; b243 animals were shifted to restrictive temperature as young adults. XO males were obtained by using double mutants: hc17 or b243 with a *him* (high incidence of males) mutant as noted under Materials and Methods.*

1 *Hodgkin (personal communication).*
2 *Kluss et al. (1976).*
3 *Nelson et al. (1976).*
4 *Edgar and Hirsh (personal communication).*
Control of Sexual Differentiation in Endoderm and Germ Line Tissues

Normally, XX animals are hermaphrodites and XO animals are males; hermaphrodites make first sperm and then oocytes while males produce only sperm. However, mutations in certain genes alter this pattern. Mutations in transformer genes (tra) change XX animals into phenotypic males (Hodgkin and Brenner, 1977), and mutations in intersex genes (isx) cause an intersexual morphology in the gonads of XO animals (Nelson et al., 1978). Certain alleles of these genes result in a phenotype in which animals exhibit a male-like body but make oocytes (Table 1). The most extreme example involves a mutation in isx-2. In this case, XO adult males raised at permissive temperature begin to produce oocytes when shifted to restrictive temperature (Edgar and Hirsch, personal communication).

Here, we show that yolk proteins are made by the intestine in the four mutants described in Table 1. Thus, intestinal and germ line tissues are hermaphrodite—make yolk proteins and oocytes, respectively—in animals that are otherwise primarily male. This suggests that sexual differentiation is normally regulated coordinate in endodermal and germ line cells. The mechanism by which differentiation of the two tissues is coupled remains unclear. Both tissues may respond independently to the signal to differentiate along the hermaphrodite pathway, or differentiation of the intestine as hermaphrodite may result in an induction of the germ line.

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REFERENCES