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Cell-Specific Gene Expression in the Nematode

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I. Introduction

The problem of cell-specific gene expression has long been a major concern to developmental biologists. Why and how specific genes are expressed only in certain differentiated cells and not in others are of vital importance. Many well-documented examples of differentiated cell types expressing quantitative and/or qualitative changes in gene expression now exist. For example, Galau *et al.* (1976) demonstrated that different sets of genes are expressed during development and in different adult tissues of the sea urchin. More recently, Angerer and Davidson (1984) have used *in situ* hybridization of specific DNA probes to demonstrate the expression of lineage-specific genes long before morphological differentiation. Other examples include the ovalbumin gene, known to be expressed only in hormone-stimulated oviducts (Harris *et al.*, 1973; O'Malley and Means, 1974), and the globin genes expressed at various developmental stages in differentiating erythrocytes (Bastos and Aviv, 1977). Many other examples of cell-specific gene expression are known, including the silk moth chorion proteins (Paul and Kafatos, 1975; Reiger *et al.*, 1978), the glue proteins in *Drosophila* (Beckendorph and Kafatos, 1976), and α-amylase in mammals (Young *et al.*, 1981; Flavell, 1981).

Detailed molecular analysis of genes has provided important information on the mechanisms of gene expression. For example, numerous studies have examined the role of chromatin structure (Weintraub and Groudine, 1976; Garel and

Axel, 1976; Weisbrod and Weintraub, 1981) as well as the significance of specific sequences in the transcription and translation of eukaryotic genes (e.g., Ziff and Evans, 1978; Grosschedl and Birnstiel, 1980). Furthermore, studies of the globin, actin, immunoglobulin, histone, and silk moth chorion genes have demonstrated the existence of gene families with suggested importance for the evolution of new functions for old genes (Brown, 1981). In addition, the detailed study of multigene families has provided vital information on the mechanisms of cell-specific gene expression as seen, for example, in the temporal and spatial regulation of different members of the actin gene family (Buckingham and Minty, 1983; Fryberg *et al.*, 1983).

Although these findings help to explain a great deal that was previously unknown about eukaryotic gene expression, genetic analysis may play a necessary role in identifying genetic elements not detectable by molecular techniques alone. If, for example, there exist genetic elements controlling the expression of other genes or of entire pathways, these might be missed by even the most detailed molecular study. Genetic analyses in the past have played an important role in identifying important genetic events. For instance, McClintock (1967) first demonstrated the existence of movable genetic elements and their effects on gene expression by a genetic analysis of maize. In addition, Dryer and Bennett (1965), using a combination of amino acid sequencing data and genetic analysis, proposed a model whereby one immunoglobulin protein molecule could be encoded by the rearrangement of two previously separated genes. Similarly, genetic analysis (mutational analysis) could serve to identify specific elements necessary for complex developmental pathways that could be studied at the molecular level. Gene regulation mechansisms should therefore be investigated in systems like *Drosophila* and *Caenorhabditis*, which are amenable to both genetic and molecular analysis.

The free-living soil nematode *Caenorhabditis elegans* is an excellent organism for the multidisciplinary study of gene regulation during development. *C. elegans* is a genetically manipulable, multicellular eukaryote that differentiates specific tissue types. Hypodermal, muscle, nerve, intestinal, and gonadal tissues are all present in the worm. Therefore, by implication, it undergoes extensive and specific gene regulation. A detailed description of the organism can be found in Brenner (1974), Edgar and Wood (1977), Riddle (1978), and Zuckerman (1980).

The study of the genetics of *C. elegans* has continued to expand from Brenner's first detailed description in 1974 to a system that now offers amber tRNA suppressors (Waterston and Brenner, 1978; Waterston, 1981; Wills *et al.*, 1983), crossover suppressors (Herman, 1978), duplications (Herman *et al.*, 1979), transposable elements (Emmons *et al.*, 1983; Liao *et al.*, 1983; Rosenzweig *et al.*, 1983; Eide and Anderson, 1985b), and 437 mapped loci, as well as hundreds of mutants in embryogenesis, spermatogenesis, cell lineages, muscle development, behavior, sexual development, meiosis, and virtually all aspects of the life

cycle. *C. elegans* can be easily cultured in the laboratory either axenically or monoxenically in large quantities for biochemical studies (Brenner, 1974).

An additional advantage of the nematode as an experimental system is its simple and extensively characterized anatomy. All of the somatic cell lineages, both embryonic and postembryonic, have been recorded (Sulston and Horvitz, 1977; Deppe *et al.*, 1978; Kimble and Hirsh, 1979; Sulston *et al.*, 1983). The descendants, parental lineage, and migration patterns of all of the somatic cells in the nematode are known. This information is not available for any other multicellular eukaryote and constitutes a significant advantage for any study involving the cellular aspects of differentiation and development. For example, this information has made possible the identification of heterochronic mutants in *C. elegans*, in which specific cell lineages are temporally altered, causing the delay or advance of specific cell divisions (Ambros and Horvitz, 1984). The identification of such mutants has obvious implications for the mechanism of phylogenetic change.

Finally, a number of laboratories are currently studying the molecular structure and organization of a number of different nematode genes, e.g., the actin and collagen genes (Files *et al.*, 1983; Kramer *et al.*, 1982, 1985; Cox *et al.*, 1984, 1985), myosin genes (MacLeod *et al.*, 1977, 1981; Waterston *et al.*, 1982a,b; Karn *et al.*, 1983), yolk protein genes (Sharrock, 1983, 1984; Kimble and Sharrock, 1983; Blumenthal *et al.*, 1984), heat shock genes (Snutch and Baillie, 1984), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes (R. Hecht, personal communication), in addition to the major sperm protein (MSP) genes of *C. elegans* (Klass *et al.*, 1982, 1984; Burke and Ward, 1983). Clone banks of genomic and cDNA sequences, as well as expression vectors, are available for various DNA analyses. In addition, it has recently been demonstrated that transformation is possible in the nematode by injection of specially designed plasmid vectors into the gonad. Furthermore, transposition of the transposable element Tc1 has been demonstrated in both the *unc-54* and *unc-22* genes of *C. elegans* var. Bergerac (BO) (Eide and Anderson, 1985b; D. Moerman and R. Waterston, 1984, personal communication).

Because of these advantages, many laboratories have chosen the nematode as an experimental system for the genetic and molecular analysis of the cell-specific expression of genes. A brief description of the culture methods, development, and genetics of the organism is provided below, followed by a detailed description of a number of genes in *C. elegans* whose tissue-specific regulation is currently being investigated.

A. Laboratory Culture Methods

C. elegans can be easily cultured in the laboratory and is routinely grown on agar plates and fed *Escherichia coli* (Brenner, 1974). Optimum growth temperature is 20°C. Permissive and restrictive temperatures used for temperature-

sensitive mutants are 16 and 25.5°C, respectively. The worm can also be grown either in a rich chemical medium (Dougherty *et al.*, 1959; Rothstein, 1974; Gandhi *et al.*, 1980) or in a liquid medium supplemented with *E. coli*, and large quantities can be obtained for biochemical studies (6 g wet weight/liter). All wild-type and mutant strains can be stored indefinitely in liquid nitrogen or in a ultralow (−80°C) freezer to maintain a 10–50% viability.

B. Anatomy and Development

One of the major advantages of the nematode as an experimental system is its transparent cuticle and simple anatomy. This advantage has facilitated the complete recording of the entire cell lineage from fertilization to adulthood (Sulston and Horvitz, 1977; Deppe *et al.*, 1978; Kimble and Hirsh, 1979; Sulston *et al.*, 1983). The eggs of *C. elegans* are prolate spheroids approximately 40 × 60 μm surrounded by a transparent shell. After embryogenesis, a first-stage larva (L1) hatches from the egg. An L1 contains 558 somatic cells and a four-cell gonadal primordium (Sulston *et al.*, 1983). Somatic cells consist of nervous, gonadal, intestinal, and hypodermal tissues. The first-stage larva (240 μm long) begins to feed and develop, undergoing four larval molts as it grows to sexually mature adulthood. Growth and development to maturation take place in approximately 72 hours at 20°C. If, during its early development, the larva is subjected to starvation conditions, it will enter a developmentally arrested state, called the dauer larva stage, during the L2 to L3 molt. Dauer larvae live for extended periods of time, an average of 45 days at 20°C compared to 14 days for nondauer worms. Upon refeeding, dauer larvae enter the normal developmental pathway and grow to be sexually mature adults (Cassada and Russell, 1975) and display normal adult life spans (Klass and Hirsh, 1976).

A sexually mature adult hermaphrodite is approximately 1200 μm in length and 100 μm in diameter (Fig. 1A). The adult hermaphrodite contains 959 somatic cells and a gonad containing approximately 2600 nuclei (Sulston *et al.*, 1983; Hirsh *et al.*, 1976). The worm is surrounded by a collagenous cuticle covered by a proteinaceous cortical layer (Josse and Harrington, 1964; Cox *et al.*, 1981a). The nervous system (Ward *et al.*, 1975; Ware *et al.*, 1975; White *et al.*, 1976; Lewis and Hodgkin, 1977; Albertson and Thomson, 1976) and the body musculature (Epstein *et al.*, 1977; Garcea *et al.*, 1978; MacLeod *et al.*, 1977, 1981; Harris and Epstein, 1977; Harris *et al.*, 1977) have been studied in great detail.

As a sexually mature adult, *C. elegans* produces approximately 300 progeny by self-fertilization over a 3.5-day period. The mean life span of hermaphrodites is 23 days at 16°C, 14.5 days at 20°C, and 8.9 days at 25.5°C, with some variation encountered between experiments (Klass, 1977). The hermaphrodite reproductive system is composed of a bilateral, symmetrical structure that produces sperm during the fourth larval stage and then oocytes, which continue to be

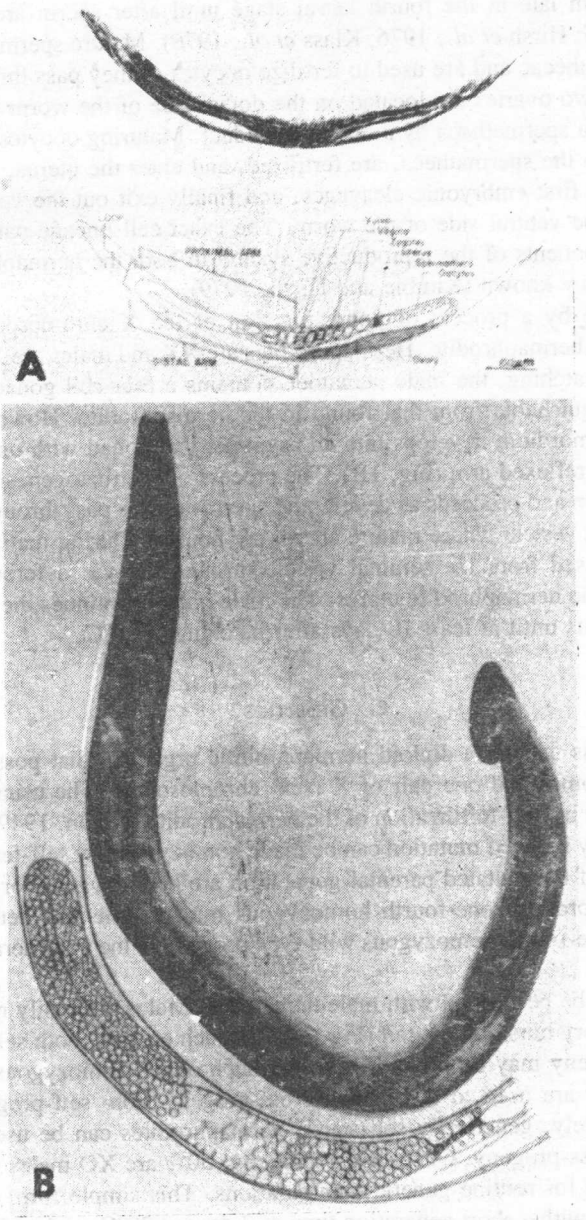

FIG. 1. (A) A photomicrograph of an adult hemaphrodite with an accompanying diagram of the reproductive system. Reprinted by permission from Hirsh *et al.* (1976). (B) A photomicrograph of an adult male with an accompanying diagram of the reproductive system. Reprinted by permission from Wolf *et al.* (1978).

produced from late in the fourth larval stage until after sperm are exhausted (Honda, 1925; Hirsh *et al.*, 1976; Klass *et al.*, 1976). Mature sperm are housed in the spermathecae and are used to fertilize oocytes as they pass through to the uterus. The two ovaries are located on the dorsal side of the worm and each is connected to a spermatheca by a reflexed oviduct. Maturing oocytes pass down the oviduct to the spermatheca, are fertilized, and enter the uterus, where they undergo their first embryonic cleavages, and finally exit out the vulva located midway on the ventral side of the worm. The exact cell lineage pattern for all somatic components of the reproductive system in both the hermaphrodite and the male is now known (Kimble and Hirsh, 1979).

Males arise by a process of nondisjunction of the X chromosomes during meiosis in the hermaphrodite. Hermaphrodites are XX and males are XO. Immediately after hatching, the male nematode contains a four-cell gonadal primordium indistinguishable from that found in the hermaphrodite. However, in the male, this primordium develops into an asymmetrical gonad with only a single testis and one reflexed arm (Fig. 1B). The process of spermatogenesis begins in the testis region and proceeds as developing spermatocytes pass through the loop to the seminal vesicle where mature sperm are housed. During mating, mature sperm are passed from the seminal vesicle through the vas deferens and are injected into the hermaphrodite uterus. The male gonad continues the process of spermatogenesis until at least 10 days after hatching at 20°C.

C. Genetics

C. elegans is usually a diploid hermaphroditic organism that possesses five pairs of autosomes and one pair of X (sex) chromosomes. The usual mode of reproduction is by self-fertilization of the hermaphrodite (Nigon, 1949; Brenner, 1974). A newly induced mutation can be made homozygous by self-fertilization. The progeny of the mutated parental generation are heterozygous for the mutation and will produce one-fourth homozygous mutant, one-half heterozygous mutant, and one-fourth homozygous wild-type progeny in the F_2 generation (Fig. 2A).

Crosses can be performed with male nematodes. Males are easily recognized by the copulatory bursa on the tail (Fig. 1B). In such crosses, both self-progeny and cross-progeny may be produced, so hermaphrodites homozygous for a recessive marker are used to distinguish cross-progeny from self-progeny (Fig. 2B). Alternatively, genetically self-sterile hermaphrodites can be used to produce 100% cross-progeny. Of the cross-progeny, 50% are XO males which are used in crosses for routine genetic manipulations. This simple form of inheritance, together with a short generation time of 3 days at 20°C, makes *C. elegans* ideally suited for genetic studies.

Brenner (1974) initiated genetic analysis of *C. elegans* by characterizing ~200

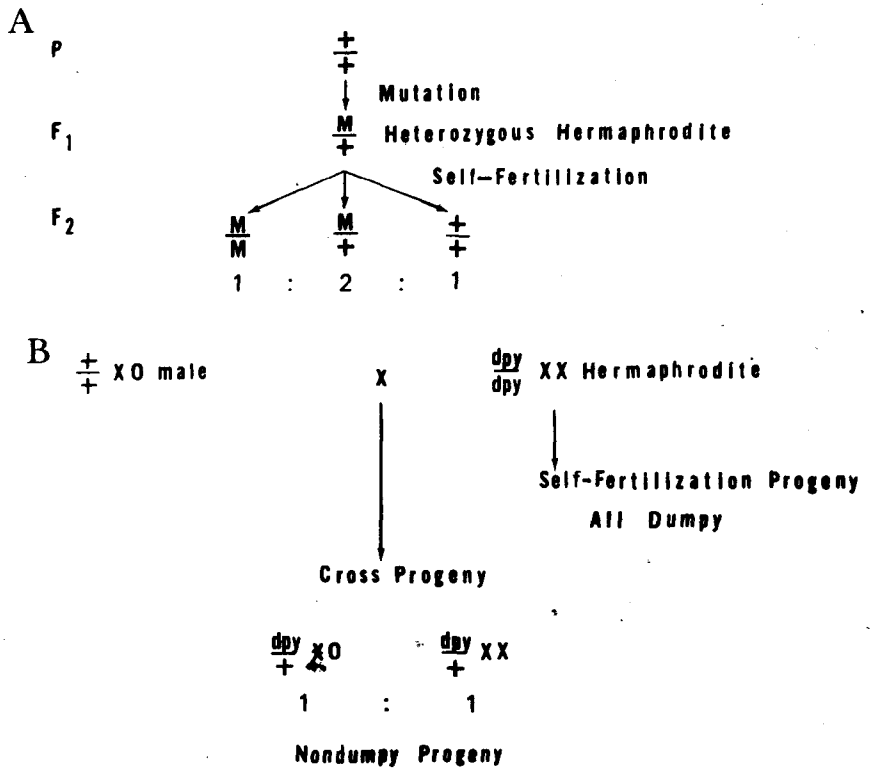

FIG. 2. (A) Diagram of the hemaphrodite mode of inheritance in *C. elegans*. M, Hypothetical recessive mutation; P, parental generation; F_1, first generation; F_2, second generation. (B) Diagram of mating between wild-type male and homozygous recessive *dpy*/*dpy* hermaphrodite; *dpy*, recessive mutation causing "dumpy" phenotype. Reprinted by permission from Klass and Johnson (1985).

mutations which were induced by the mutagen ethyl methanesulfonate (EMS) and defined ~100 genes. Brenner (1974) calculated an average forward mutation rate of 5×10^{-4} per gene by dividing the frequency of appearance of mutations by the total number of complementation groups. The ability for detailed genetic analysis is now being extended by the isolation of balancer stocks, deletions, duplications, and lethal mutations (Herman, 1978; Herman *et al.*, 1976), as well as by the recent identification of the only mutation identified in a metazoan which causes the synthesis of an amber suppressor tRNA (Waterston and Brenner, 1978; Wills *et al.*, 1983). The genetics of *C. elegans* has been reviewed by Herman and Horvitz (1980).

All of the mutant strains that have been characterized and mapped are available from the *Caenorhabditis elegans* Stock Center at the University of Missouri

at Columbia. To date, 437 loci have been mapped and hundreds of mutants have been isolated and characterized that affect various aspects of gonadogenesis, spermatogenesis, development, chemotaxis, dauer larva formation, sexual dimorphism, cell lineage, cell cycle, and the nervous system. Approximately 300 temperature-sensitive (ts) mutants that interrupt the life cycle of *C. elegans* have been isolated and used to analyze development (Hirsh and Vanderslice, 1976; Vanderslice and Hirsh, 1976). Use of these and other mutants to study cellular differentiation in *C. elegans* is described in the next section.

II. Analysis of Tissue-Specific Gene Expression in the Nematode

A. General Approach

Many different approaches have been used for the study of the cell-specific expression of genes. Generally, a gene product is identified either by its electrophoretic mobility or enzymatic activity. Most analyses then use a specific antibody to demonstrate the tissue-specific location and temporal regulation of the protein. After the temporal and spatial regulations have been determined, a number of different experimental avenues can be taken. In the majority of cases, attempts are made to clone the gene or genes in question. This is simplified if the gene codes for an abundant mRNA which can be at least partially purified to make a cDNA probe to isolate the gene from a genomic clone bank. Alternatively, genes exhibiting a high degree of sequence conservation from other species can be used as probes under less stringent conditions to isolate the same genes in the nematode. Another more general approach that has been used is to construct a cDNA clone bank followed by differential screening to identify genes expressed at specific developmental stages or in certain tissues. Such a procedure has been used to identify genes expressed during spermatogenesis in the nematode.

Once the gene has been isolated, it can be used as a probe to determine when and where the RNA transcripts first appear. This type of data provides valuable information on the transcriptional regulation of the gene. Having a probe also allows determination of the number of copies of the gene in the genome, presence of introns, and DNA as well as amino acid sequence. In addition, analysis of various regulatory mechanisms (e.g., methylation, rearrangement, and amplification) can be performed.

One of the ultimate goals of the study of the cell-specific expression of genes is to determine what sequences specify the temporal and/or spatial expression of the gene in question. One approach is to develop an *in situ* promoter assay to allow modification of various regions of the promoter and their subsequent effect on expression. Such methods are currently being developed for *C. elegans*.

Many developmentally regulated genes in the nematode *C. elegans* are currently being investigated to determine the underlying mechanism(s) of regulation. In the following sections we will describe a number of genes that have been isolated and studied in *C. elegans* that demonstrate both temporal and spatial regulation. The purpose of this review is not to provide a detailed description of all of the genes investigated in *C. elegans,* but rather, to describe those cases of cell-specific gene expression that have the potential of providing valuable insight into the possible genetic and molecular mechanisms involved, and secondly, to provide information on the types of analyses that are possible in the nematode.

B. Specific Examples

1. *Vitellogenin Genes*

Oocytes in *C. elegans* were shown to contain four specific proteins whose time of synthesis and abundance suggested that they were yolklike proteins (Klass *et al.*, 1979). This observation has been sustantiated by the purification and characterization of four proteins, a 170,000-Da doublet (yp 170 A and B), a single protein of 115,000 Da (yp 115) and a smaller single protein of 88,000 Da (yp 88). The 170-kDa yolk protein appears as a doublet on SDS electrophoresis gels; however, the two bands are closely related by peptide analysis. The 115-kDa and 88-kDa proteins are cleaved from a 180-kDa precursor *in vivo* (Sharrock, 1983, 1984). In keeping with the previously established nomenclature for insects and other species, the 180-kDa yolk protein precursor is currently called VIT-180. Actual genes are referred to as *vit-1, 2, 3, 4,* and *5* (see below).

The yolk proteins of *C. elegans* show an interesting pattern of temporal and spatial regulation. The synthesis of *C. elegans* yolk proteins, similar to that of other organisms, does not occur in the developing oocytes but takes place in the intestine, beginning at the early stages of oogenesis. The intestine, therefore, plays the role of "nurse cell" to the oocyte. Kimble and Sharrock (1983), using a combination of laser ablation of gonadal precursor cells and a comparison of proteins synthesized by isolated tissues, identified the intestine as the site of yolk protein synthesis. Their data indicate that the yolk proteins are synthesized in the intestine, independent of the presence or absence of the gonad, and are secreted from the intestine to the coelom, where they are taken up by the gonad.

The vitellogenin genes were isolated by cloning cDNA synthesized from a poly(A) RNA fraction enriched for yp 170 mRNA (Blumenthal *et al.*, 1984). Recombinant clones were screened by a differential hybridization technique using labeled cDNA made from poly(A) RNA enriched for yp 170 RNA and a labeled cDNA made from total poly(A) RNA. Three colonies that hybridized more strongly to the yp 170-enriched probe were selected for further analysis. Each of these hybridized to high-molecular-weight RNA from adults but not

larvae. These cDNA clones were then used to select genomic sequences. Positive identification of these sequences as vitellogenin genes was provided by a hybrid arrest translation assay using single-strand DNA from M13 subclones. A family of five genes which encode the 170-kDa yolk proteins has been identified. The genes and mRNAs are about 5×10^3 bp in length. Using labeled cDNA from poly(A) RNA from adult hermaphrodites, adult males, and larvae as probes in a Southern blot, it was demonstrated that RNA homologous to the cloned genes is present in hermaphrodites but absent in males and larvae. This developmental pattern matches that of the yolk proteins. Furthermore, RNA isolated from dissected intestines is highly enriched for sequences which hybridize these genes, indicating that this gene family is expressed only in the intestine of the adult hermaphrodite—a finding recently substantiated by the *in situ* hybridization of vitellogenin gene probes to RNA in the intestine (M. Krause and T. Blumenthal, personal communication).

The vitellogenin genes can be divided into two subfamilies: *vit-1* and *vit-2* (which encode yp 170 B) and *vit-3*, *vit-4*, and *vit-5* (which encode yp 170 A). A final gene, *vit-6*, has recently been cloned, which encodes the VIT-180 precursor to yp 115 and yp 88. It appears to be a single copy gene of 5.5 kb, is developmentally regulated, and codes for an mRNA of 5.2 kb. *Vit-6* is distantly related to *vit-1* through *-5*, suggesting that all of the *C. elegans* vitellogenin genes evolved from a common ancestor.

Nucleotide sequencing data of the 5′ flanking regions of these genes have provided interesting information on the vitellogenin promoter regions (T. Blumenthal, personal communication). Each gene has a TATA box at position −30 from the transcription initiation site. The 5′ untranslated regions are very short (9–11 bases) but are highly conserved, in contrast to the 3′ flanking sequences, which are highly diverged. However, a number of short, conserved sequences do appear in the first 250 bp of 5′ flanking DNA. These conserved sequences may indicate important controlling elements necessary for the temporal and spatial regulation of the vitellogenin genes.

2. *MSP Genes*

Spermatogenesis in the nematode presents an interesting system for the study of a specific cell differentiation pathway at both the genetic and molecular levels. Spermatogenesis-defective mutants have been isolated and the intracellular changes associated with normal spermatogenesis have been recorded (Wolf *et al.*, 1978; Argon and Ward, 1980; Ward *et al.*, 1981). Furthermore, large quantities of males can be isolated through the use of *him* mutants that cause increases in the rate of nondisjunction of the X chromosomes and a consequent increase in the frequency of XO males (Klass and Hirsh, 1981). Biochemical quantities of sperm can be isolated by a simple procedure involving squashing the males and filtering the sperm (Klass and Hirsh, 1981). Finally, spermatogen-

esis-specific genes have been isolated and are being characterized (Klass *et al.*, 1982, 1984; Burke and Ward, 1983).

Nematode sperm have the interesting characteristic of being amoeboid cells that lack flagella and move by means of pseudopodial extension. Coincident with the absence of flagella is the near-absence of actin and myosin (Nelson *et al.*, 1982). In addition to the major intracellular components of nematode sperm are large, membrane-bound, fibrous bodies (Wolf *et al.*, 1978). These fibrous bodies fill the cytoplasm of the immature spermatid. During spermiogenesis, prior to pseudopodial formation, the fibrous bodies release their fibers into the cytoplasm, where they depolymerize. Similarly, the major protein component of sperm is a 15,000-MW protein with a p*I* of 8.6. Its native state is a dimer with a molecular weight of 30,000 (Klass and Hirsh, 1981). The major sperm protein (MSP) constitutes ~15% of the total sperm protein. Using a gold-labeled anti-MSP antibody for electron microscopy Roberts *et al.* (1985) have shown that the MSP is localized to the fibrous bodies of the immature sperm (Fig. 3). Similarly using fluorescently labeled anti-MSP antibody, Ward and Klass (1982) have demonstrated that MSP becomes localized to the pseudopodia in the mature sperm. These observations have led to the suggestion that MSP is involved in the pseudopodial locomotion of nematode sperm.

One-dimensional SDS gel electrophoresis, as well as analysis by nonequi-

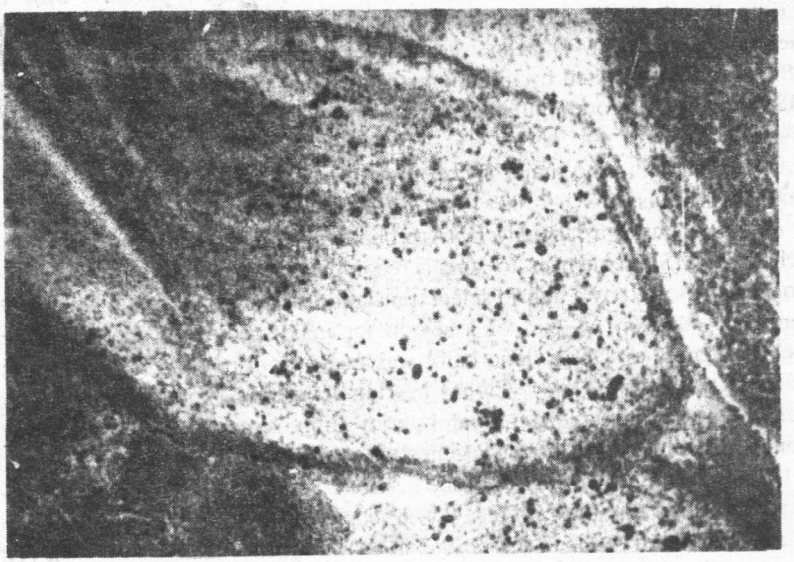

FIG. 3. Thin section through a fibrous body labeled with a colloidal gold conjugate of a monoclonal antibody to MSP. Magnification ×71,700. Reprinted by permission from Roberts *et al.*, 1985.