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Internal lectin binding patterns in the nematodes *Caenorhabditis elegans, Panagrolaimus superbus* and *Acrobeloides maximus*

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Summary – Using ten different lectins, the binding patterns were studied in three free-living rhabditid nematodes : *Caenorhabditis elegans, Panagrolaimus superbus,* and *Acrobeloides maximus*. Although all the nematode tissues showed binding by one or more of the lectins used, considerable differences were noticed between the three nematode species. Yolk, four of the coelomocytes, and the oocytes bind most often with the lectins used. Although the intestinal brush border interacts with a lot of foreign materials, it stained only with few lectins. The lectin binding pattern of the yolk indicated that, at the time of incorporation of the yolk in embryos or shortly thereafter, a processing of the yolk occurs resulting in changes in lectin-binding characteristics of the yolk protein.

Résumé – *Modalités de liaison des lectines chez les nématodes* Caenorhabditis elegans, Panagrolaimus superbus *et* Acrobeloides maximus – Les modalités de liaison des lectines sont étudiées chez trois nématodes Rhabditides libres – *Caenorhabditis elegans, Panagrolaimus superbus* et *Acrobeloides maximus* – en utilisant dix lectines différentes. Bien que tous les tissus des nématodes se lient à une ou plus des lectines utilisées, des différences considérables sont observées entre les trois espèces de nématodes. Le vitellus, quatre des coelomocytes et les oocytes se lient le plus souvent avec les lectines testées. Quoique réagissant à de nombreuses substances étrangères, la bordure en brosse de l'intestin n'est colorée que par quelques lectines. Les modalités de liaison du vitellus indiquent qu'au moment de l'incorporation de ce dernier dans l'embryon – ou quelque peu après celle-ci – il se produit dans le vitellus un processus qui modifie les caractéristiques de liaison des lectines avec son contenu protéinique.

Key-words : *Acrobeloides maximus, Caenorhabditis elegans,* immunohistochemistry, lectin histochemistry, nematodes, *Panagrolaimus superbus.*

Lectins are proteins or glycoproteins of non-immune origin extracted mostly from plants and from some animals, which reversibly bind with high specificity to carbohydrates. This property has led to the extensive use of lectins to study cell and tissue glycoconjugates (Roth, 1986).

In nematology, lectins have mainly been used for the study of carbohydrates on the exterior surfaces or exudates of mainly plant-parasitic nematodes (Spiegel *et al.*, 1982; Bowman *et al.*, 1988; Aumann & Wyss, 1989). Although the use of lectins to characterize *Caenorhabditis elegans* proteins has been reported (Sharrock, 1983; Francis & Waterston, 1991), to date no systematic analysis has been performed of the internal lectin-binding sites in nematodes.

Considering the huge body of information already present on *C. elegans,* its use may facilitate future more detailed studies of lectin-glycoconjugate interactions. Furthermore, the application of information obtained from this nematode to other economically important nematodes has already been indicated (Ward, 1988). In the present study, the binding patterns of ten fluorescent-labeled lectins were analysed in three rhabditid nematodes, *i.e., C. elegans* (Rhabditidae), *Panagrolaimus superbus* (Panagrolaimidae), and *Acrobeloides maximus* (Cephalobidae).

Material and methods

NEMATODES

C. elegans var. *Bristol* was obtained from T. Bogaert (MRC) and *him-8* (*e1489* IV CB1489) from J. Hodgkin through J. Vanfleteren (Belgium), *P. superbus* was provided by B. Sohlenius (Sweden) and *A. maximus* by P. Baujard (Sénégal).

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Monoxenic and axenic culture

All nematodes were sterilised for monoxenic or axenic culture using alkaline hypochlorite solution according to Sulston and Hodgkin (1988). Nematodes were cultured on *Escherichia coli* and generally handled according to Brenner (1974). Stock cultures were kept at 20 °C. *C. elegans* was cultured axenically according to Vanfleteren *et al.* (1990). *A. maximus* cannot be sustained indefinitely on this axenic medium and requires an additional 250 µl hemoglobin solution/100 ml axenic medium. Alternatively, haeme could be excluded if 30 % of the culture fluid consisted of heated liver extract (HLE) prepared based on Sayre *et al.* (1961). Commercially available liver was cut into small pieces, autolysed for 24 h at 4 °C, and homogenized 1 : 1 with water at 4 °C. The homogenate was heated for 4 min at 56 °C and centrifuged at 8832 *g* for 30 min at 4 °C in a Sorvall RC-5B Refrigerated Superspeed Centrifuge. After centrifugation the HLE supernatant was filter sterilized (0.2 µm), and frozen at – 20 °C until use. No suitable axenic medium was identified which allowed indefinite culture of *P. superbus*.

Embryo preparation

Following Goh and Bogaert (1991) embryos of different developmental stages were collected by washing nematodes (gravid adults and eggs) from 5 cm plates with 0.8 ml of M9 buffer (22 mM KH_2PO_4, 33 mM Na_2HPO_4, 86 mM NaCl, 1mM $MgSO_4$). The suspension was mixed with 0.4 ml of alkaline hypochlorite (4 vol. 2 M NaOH : 3 vol. 14 % (v/v) NaOCl) in an Eppendorf tube. After 8-10 min with occasional mixing, the tube was spun in an Eppendorf centrifuge (1 min at 3180 *g*). The pellet was taken up in 0.4 ml of M9 buffer and mixed with the same volume of ice-cold 2 M sucrose/100 mM NaCl. After 0.2 ml of distilled water was placed on top of the sucrose cushion and the embryos floated to the interface after 5 min centrifugation at 3180 *g*. The embryos were removed from the sucrose cushion with a Pasteur pipette and washed once in PBS (50 mM Na_2HPO_4, 140 mM NaCl, pH 7.2). Embryos were collected by centrifugation and resuspended in distilled water.

Embryo centrifugation

Embryo centrifugation was carried out according to Schlicht and Schierenberg (1991). A drop of distilled water containing eggs of all developmental stages was put on a poly-L-lysine (MW > 300 000, Sigma, St. Louis, MI, USA) coated slide. The slide was put in a plastic holder with two incisures to tightly maintain the slide. The plastic holder with the slide neatly fitted in the metal centrifuge beaker of a Sorvall RC 5B refrigerated superspeed centrifuge. The beaker was filled with distilled water. The slide was centrifuged at 14 926 *g* for 15 min at 10 °C. After centrifugation the eggs were put under a coverslip, freeze-cracked and incubated with lectins as described further in the text.

Whole nematode/embryo staining

Lectin binding : a detailed analysis of the different fixation and treatment of nematode tissue for optimal binding has been described previously. All results reported here were carried out after freeze cracking followed by acetone only fixation (Borgonie *et al.*, 1994). Nematodes were incubated overnight at 4 °C with 100 µg/ml fluorescent-labeled lectins, dissolved in PBS containing 0.5 % Triton X-100. For visualization of nuclei, 4, 6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MI, USA) was added to the lectin at a final concentration of 1 µg/ml. The slides were washed five times in PBS pH 8.0 and mounted in 50 % glycerol-PBS (pH 8.0). The coverslip was sealed with clear nail polish. Controls were carried out by preincubating the lectins with 0.3 M of complementary sugar for 1 h at room temperature prior to incubation. Asialofetuin was used at a final concentration of 1 mg/ml. A second control was done by preincubating freeze-cracked nematodes/embryos overnight in the dark in 50 mM $NaIO_4$ in 100 mM potassium acetate buffer (pH 4.5) and subsequent washing three times in cold PBS (4 °C) prior to lectin incubation.

The lectins used were : the lectins from *Canavalia ensiformis* (Con A), *Phaseolus vulgaris* erythroagglutinin (PE) and *P. vulgaris* leucoagglutinin (PHA-L), *Lens culinaris* (LCA), *Ricinus communis* (RCA-II), *Ulex europaeus* (UEA-I), *Solanum tuberosum* (STA), *Arachis hypogaea* (AHA), *Glycine max* (SBA), and *Limulus polyphemus* (LPA) were obtained from Sigma (St. Louis, MI, USA). All lectins were FITC labeled except UEA-I which was TRITC labelled. All the lectin stainings, including the controls, were done with lectin coming from one and the same preparation.

Sugars and glycoproteins : used for controls were : (Con A) : α-methyl-D-mannopyranoside, (SBA) : N-Acetyl-D-galactosamine, (AHA and RCA-II) : lactose, (STA) : Triacetyl chitotriose and (PHA-E, PHA-L, LPA, UEA-I and LCA) : asialofetuin were from Sigma (St. Louis, MI, USA). Table 1 summarizes the sugar specificity, inhibition and control experiments performed for each of the lectins.

Immunohistochemistry

After freeze-cracking, the slide was dipped in precooled methanol (– 20 °C) for 5 min and then in acetone (– 20 °C) for 5 min and air dried. The slides were washed four times for 15 min in buffer (PBS, 0.01 % (v/v) Tween-20, 5 % (v/v) dried milk). Monoclonal anti-yolk antibodies (generously provided by Susan Strome, Indiana University, Bloomington, IN, USA) were diluted in the same buffer 1:20 and incubated overnight at 4 °C. The slides were washed three times for 15 min in buffer. The worms were then incubated in

Table 1. *Sugars and glycoproteins used in control experiments to block lectin staining.*

Lectin	Affinity	Inhibiting sugar(s) used	Sugar giving complete inhibition	50 mM $NaIO_4$
CON A	α-D-man, α-D-glc	α-meman	α-meman	+
RCA-II	β-D-galNAc, α-D-gal	lac	lac	+
PHA-E	oligosaccharides	asialofetuin	asialofetuin	+
PHA-L	oligosaccharides	asialofetuin	asialofetuin	+
LCA	α-D-man	α-D-man, asialofetuin	– – – (*)	–
STA	triacetyl chitotriose	triacetyl chitotriose	triacetyl chitotriose	+
UEA-I	α-L-fuc	α-L-fuc & asialofetuin	asialofetuin	+
AHA	β-D-gal (1-3)-D-galNAC	lac	lac	+
SBA	D-galNAc	D-galNAc	D-galNAc	+
LPA	fetuin > NeuNAc	asialofetuin	asialofetuin	+

Sugar affinity as reported by the manufacturer (SIGMA) (Man : mannose; glc : Glucose; α-meman : α-methyl-D-mannopyranoside; galNac : N-Acetyl-D-galactosamine; lac : lactose; glcNAc : N-Acetyl-D-glucosamine; fuc : fucose; NeuNAc : N-Acetyl neuraminic acid). (+) : Complete inhibition of staining; (–) : no inhibition of staining. Data presented are from acetone-only fixed nematodes, using overnight incubation at 4 °C. (*) asialofetuin inhibited all staining, except anterior brush border staining in *C. elegans*.

FITC-labeled goat anti-mouse IgG (Sigma) at a 1:50 dilution for 4 h at room temperature. Slides were washed three times 15 min each in buffer and one time in PBS for 15 min before being mounted as described above. Three anti-yolk monoclonal antibodies were used; PIIa A3 C9 (recognizes yolk protein (yp) 170B), OI A5 D9 (recognizes yp170) and OI C1 D3 (recognizes yp170A, yp115 and yp88) (Sharrock *et al.*, 1990).

To identify neurons, a goat anti-horseradish peroxidase (Type VI, Sigma, St. Louis, MI, USA) antibody was used since this antibody has been reported to identify cells of the nervous system (among others) in *C. elegans* and *Drosophila melanogaster* (Siddiqui & Culotti, 1991; Wang *et al.*, 1994). Previously it was reported that this antibody recognizes 27 of the 302 neurons, besides several non-neuronal cells in *C. elegans* (Siddiqui & Culotti, 1991). Preparation of the nematode tissue was as described above except that the washing and incubation buffer contained 0.1 % (v/v) Triton X-100 instead of Tween-20. The primary antibody was diluted 1:25. The secondary antibody was a rabbit anti-goat IgG FITC conjugate (Sigma, St. Louis, MI, USA) diluted 1:50.

Collagenase digestion

Collagenase digestion of freeze cracked nematodes was performed by incubating the nematodes with 2 mg/ml collagenase (Sigma type IV, 460 units/mg, Sigma, St. Louis, MI, USA) in 100 mM Tris HCl pH 7.5, 1 mM CaCl2 at 37 °C for 1 h. After washing five times in PBS, nematodes were incubated with lectin overnight as described above.

For lectins and immunostainings, mixed cultures (containing eggs, all four juvenile stages, young and old adults) of all three nematode species were stained. Since males in *A. maximus* are rare, none were used for the study.

Observations were made using a Leitz Diaplan (Wetzlar, Germany) microscope equipped for fluorescence microscopy. Photographs were taken on Kodak ektachrome 160T film and processed commercially.

Results

A summary of the binding patterns obtained is given in Table 2. Some additional or in depth analyses are described in the next paragraphs.

Con A

Identification of the vesicles (Fig. 1A, B) was aided by centrifugation of single cell embryos of the three nematodes and subsequent staining of the embryo with Con A. Centrifugation results in three cytoplasmic bands (yolk, granule-free and lipids) in the embryo as described by Schlicht and Schierenberg (1991). Using TEM, they observed that yolk accumulates in the centrifugal pole. This approach led to concentration of the fluorescent stain in the centrifugal pole in *C. elegans* and *P. superbus* but led to staining of the two polar bands in *A. maximus* with the strongest staining in the centrifugal pole (Fig. 1C).

Using the anti-yolk monoclonal antibodies, staining of the yolk in the nematode *C. elegans* gave a slightly different binding pattern. Staining with a mixture of the antibodies resulted in strong staining in the developing oocytes and embryos, far less in the intestinal cells and pseudocoelomatic cavity (Fig. 1D). None of the antibodies, raised against *C. elegans* yolk proteins, bind to yolk proteins either from *P. superbus* or *A. maximus*.

Binding is also visible at the position of the developing pharynx lumen and proceeds from the invaginating anterior end towards the middle (Fig. 2F, G). In *A. maximus* two additional thin processes were stained originating dorsally and ventrally and running parallel with the

Table 2. *Summary of interior lectin binding.*

Lectin	Structure	*C. elegans*			*P. superbus*			*A. maximus* (1)	
		E	F	M	E	F	M	E	F
CON A	oocyte surface	na	−−	na	na	−−	na	na	+++
	yolk	++	++ (2)	na	++	++ (2)	na	+++	+++
UEA-II	yolk	+	++	na	+	++	na	++	+++
	epidermis	−−	−−	−−	−−	+	+	−−	−−
PHA-E	yolk	+	+	−−	++	+++ (3)	−−	+	++
PHA-L	yolk	+	+	−−	++	+++	−−	+	+
	sperm	na	−−	−−	na	na	+	na	na
RCA-II	oocyte surface	na	+++ (4)	na	na	+++ (4)	na	na	+++ (4)
	oocyte cytoplasm	na	++ (5)	na	na	−−	na	na	++ (5)
	intest. lumen embryo	+	na	na	+++	na	na	−−	na
	intest. brush border	+	+++ (6)	+++ (6)	+++	+++	+++	−−	−−
	developing pharynx	+++	na	na	+++	na	na	+++	na
	developing rectum	++ (7)	na	na	++ (7)	na	na	++ (7)	na
	secr./excr. duct	+++ (7)	na	na	+++ (7)	na	na	+++ (7)	na
	adult pharynx	na	+++	+++	na	+	+	na	+++
	nervous system	na	−−	−−	++	+++	+++	+++	+++
	outer layer nerve ring (8)	+	+++	+++	+	+++	+++	−−	−−
	outer cuticle lining pharynx (8)	−−	++	++	−−	++	++	−−	++
	coelomocytes (9)	+	+	+	+	++	+	+	++
LCA	yolk	++	−−	na	++	−−	na	−−	−−
	oocyte yolk	na	+++	na	na	+++	na	na	+
	outer nerve ring (8)	++	++	++	++	++	++	++	++
	pharynx (8)	++	++	++	++	++	++	++	++
	rectum	+++	+	+	+++	+	+	+++	+
	coelomocytes (9)	+	+++	+++	+	+++	+++	+	+++
STA	pharynx precursors?	+	na	na	+	na	na	+	na
	outer intestine layer	−−	na	na	−−	na	na	+	na
	secr./excr. system	−−	+++	+++	−−	−−	−−	−−	−−
	coelomocytes	+	+++	+++	+	+++	+++	+	+++
	intest. brush border	−−	−−	−−	−−	−−	−−	+	+++
	intest. cytoplasm	−−	−−	−−	−−	−−	−−	−−	+++
	coelomocytes (9)	−	++	++	−−	++	++	−−	++
AHA	yolk	−−	−−	−−	−−	−−	−−	+	−−
	surface epid. cells	−−	−−	−−	−−	−−	−−	+	−−
	head neurons	−−	−−	−−	−−	−−	−−	+	−−
	coelomocytes (9)	−−	+	+	−−	+	+	++	+++
	uterus lining	−−	+++	na	na	−−	na	na	−−
	epidermal nerves	−−	+++	+	−−	−−	−−	−−	−−
	microtubule cells AVM, PVM	−−	+++	−−	−−	−−	−−	−−	−−
SBA	yolk	−−	+++	−−	−−	+	−−	−−	+++
	oocyte surface	na	+++	na	na	+	na	na	+++
	gonad precursors	na	−−	na	na	−−	na	na	++
	rectum	++	−−	−−	+	−−	−−	++	−−
	sperm	na	++	++	na	−−	+	na	−−
	vulva	na	+++	na	na	+	na	na	−−
LPA		no specific staining							

Data presented are from acetone-only fixed nematodes, using overnight incubation at 4 °C. E : embryo; F : female; M : male; + : weak binding; ++ : moderate binding; +++ : strong binding; − − : no binding; NA : not applicable. (1) *A. maximus* males are rare, none were used for this analysis. (2) No yolk staining in cells immediately posterior to the pharynx. (3) Strong staining in cells immediately posterior to the pharynx. Gradual decrease in staining from first to fourth juvenile stage. (4) Staining gradually disappears as the oocyte enters the uterus and is completely absent when the eggshell is formed. (5) Staining disappears when the eggshell is formed. (6) Except the anterior ring of four cells. (7) Variable staining obtained, not all specimens exibited staining. (8) Binding abolished after collagenase treatment. (9) Only four coelomocytes stain in any of the three nematode species.

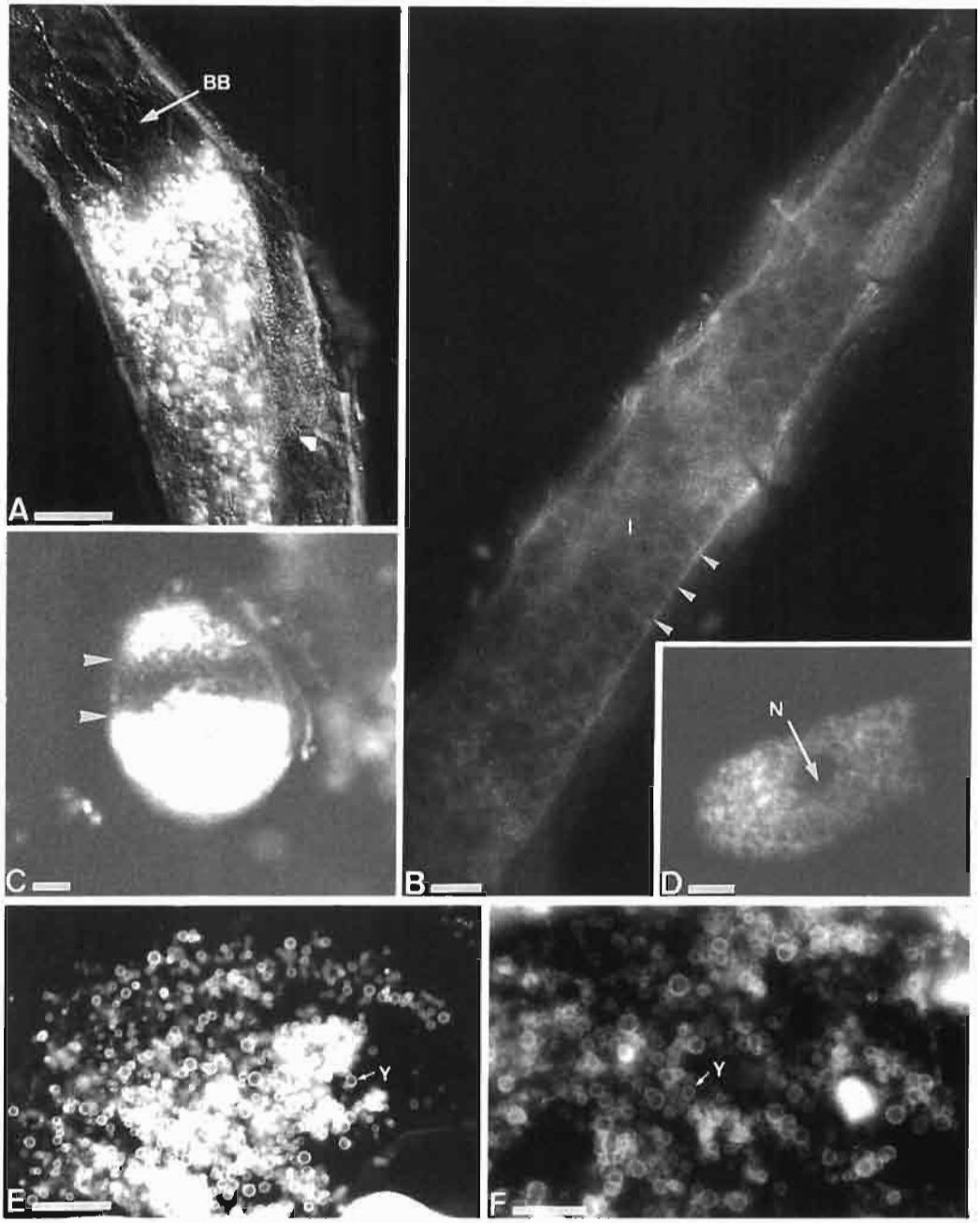

Fig. 1. *A : Strong staining of intestinal yolk vesicles in the nematode* Panagrolaimus superbus *using PHA-E; to demonstrate the strength of the staining, this epifluoresence photograph was taken with the microscope light on, at its lowest setting; anterior top; B : Staining of an* Acrobeloides maximus *specimen by Con A showing a concentration of staining near the basal sides of the intestine in the pseudocoelom; anterior top; C : Centrifuged* A. maximus *single cell embryo, showing two distinct bands (arrowheads) after staining with Con A; D :* Caenorhabditis elegans *embryo still inside the uterus after staining with monoclonal anti-yolk antibodies; E : Peripheral staining of yolk spheres with LCA revealed in* A. maximus *after squashing embryos; F : Same as E, but at higher magnification (BB : Basal bulb, I : intestine, NU : nucleus, Y : yolk vesicle). (A-B : bar = 20 µm; C-D : bar = 10 µm; E-F : bar = 5 µm).*

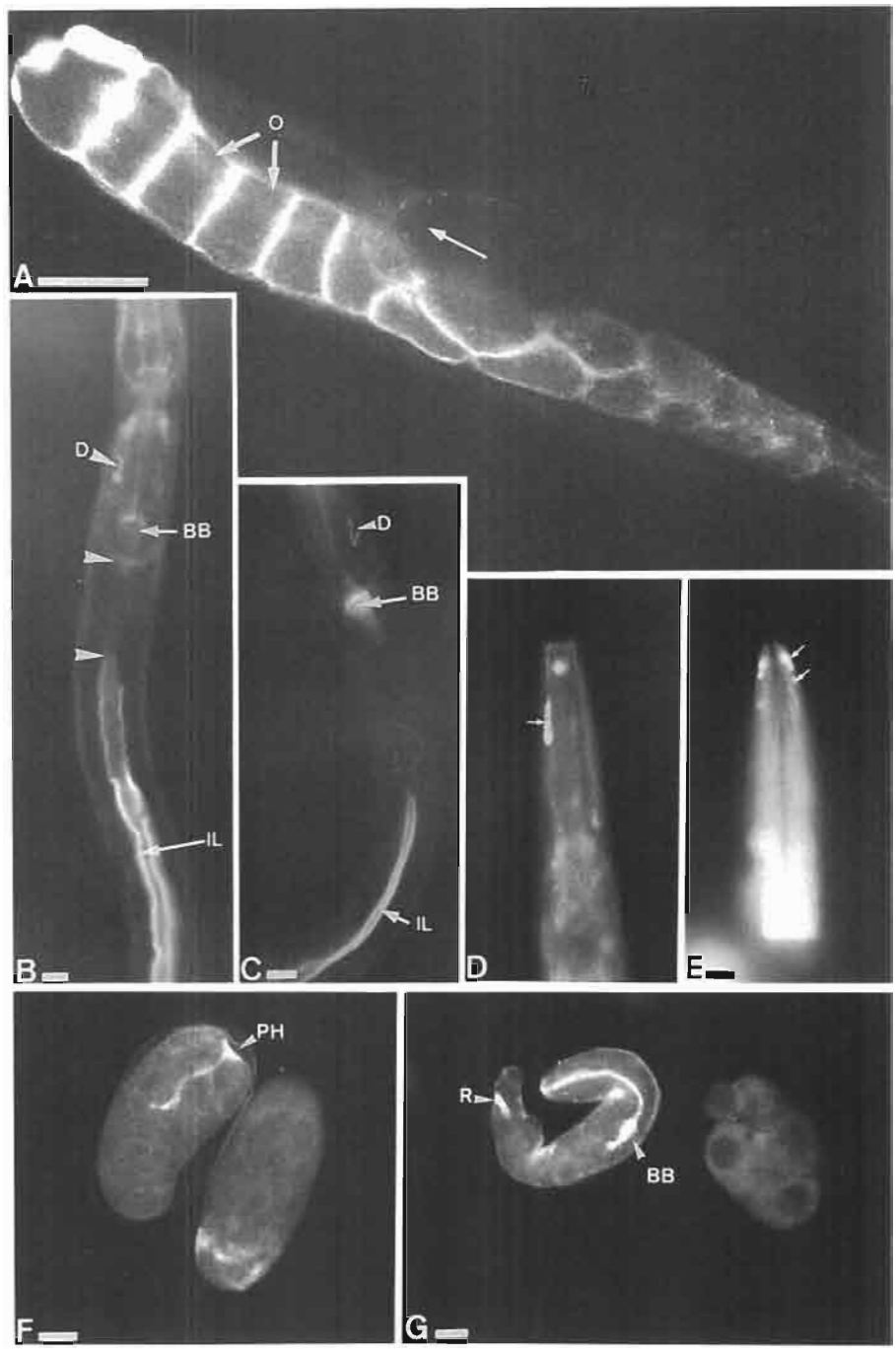

Fig. 2. *A : Staining of dissected ovary from* Acrobeloides maximus *by RCA-II. Surface staining of oocytes increases as development proceeds (arrow); B : Staining by RCA-II of the layer surrounding pharynx, the excretory duct and strong staining of the intestinal brush border in* Caenorhabditis elegans; *note the absence of staining in the cells immediately posterior to the basal bulb (between arrowheads); the adjacent cells stain weak; anterior top; C : Staining of* C. elegans *after collagenase digestion; note absence of peri-pharyngeal staining; anterior top; D-E : Difference of structures stained in* A. maximus *by LCA; (D arrow) and a polyclonal anti-horseradish peroxidase antibody; (E arrows); anterior top; F-G : Progressive staining of the developing pharynx in* C. elegans *embryos; the staining of the rectum was not always present (O : oocytes; D : excretory duct; BB : basal bulb; I : intestine; PH : pharynx; R : rectum). (A-E : bar = 20 μm; F-G : bar = 10 μm).*

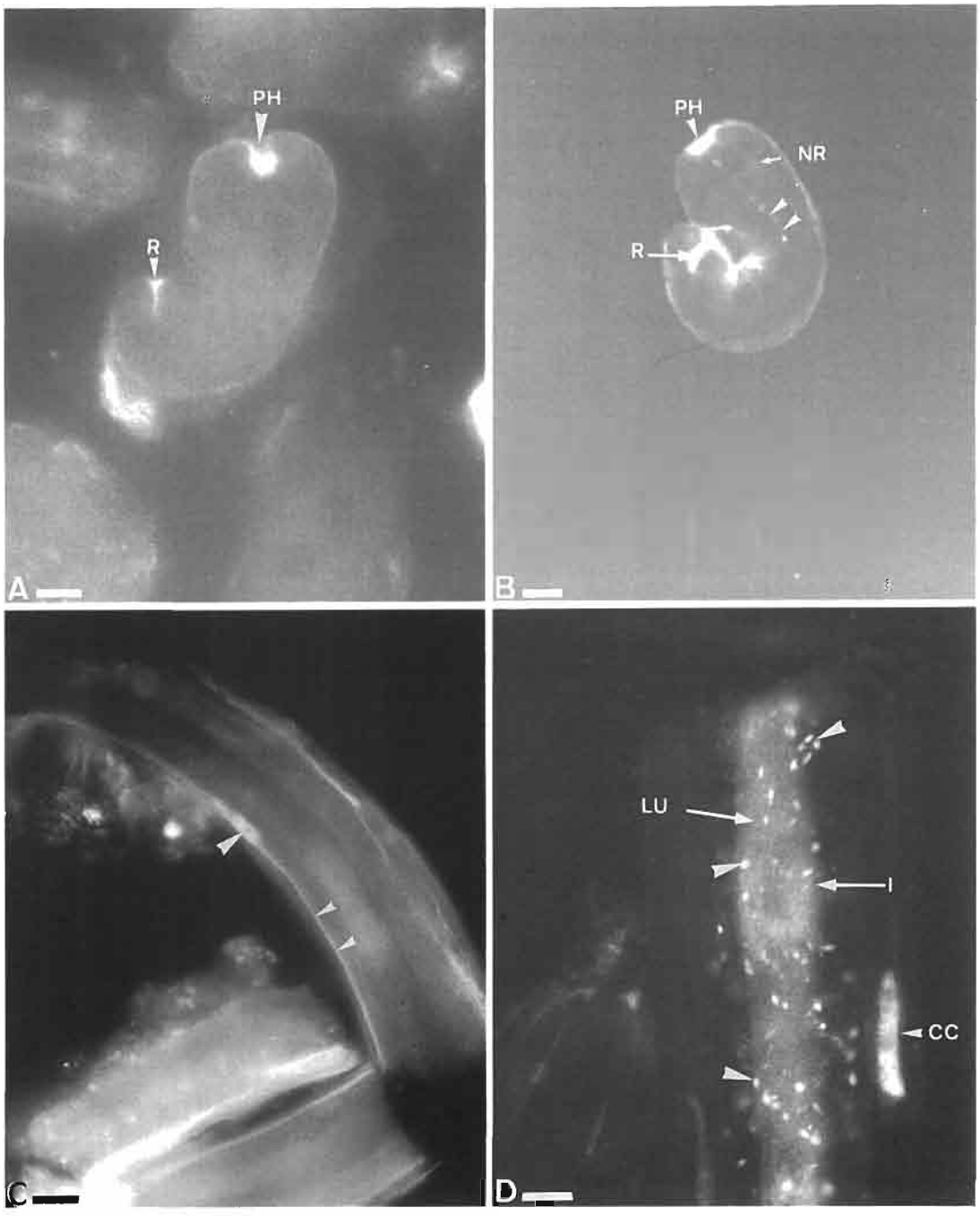

Fig. 3. *A : Staining of the invaginating pharynx and rectum early in development in* Acrobeloides maximus *using LCA; B : LCA faintly stains the developing nerve ring; in comparison to RCA-II, the pharynx stains much weaker in* A. maximus; *C : Staining of epidermis associated nerves in* A. maximus *(small arrowheads) and neurons (large arrowhead) by LCA; D : Staining of the coelomocytes and intestine in* A. maximus *by STA; besides stain being present in the entire lumen, a large number of small cytoplasmic vesicles stained, close to the intestinal brush border (CC : coelomocyte; I : intestine; PH : pharynx; R : rectum; NR : nerve ring; LU : intestinal lumen; anterior top). (A-B : bar = 10 μm; C-D : bar = 20 μm).*

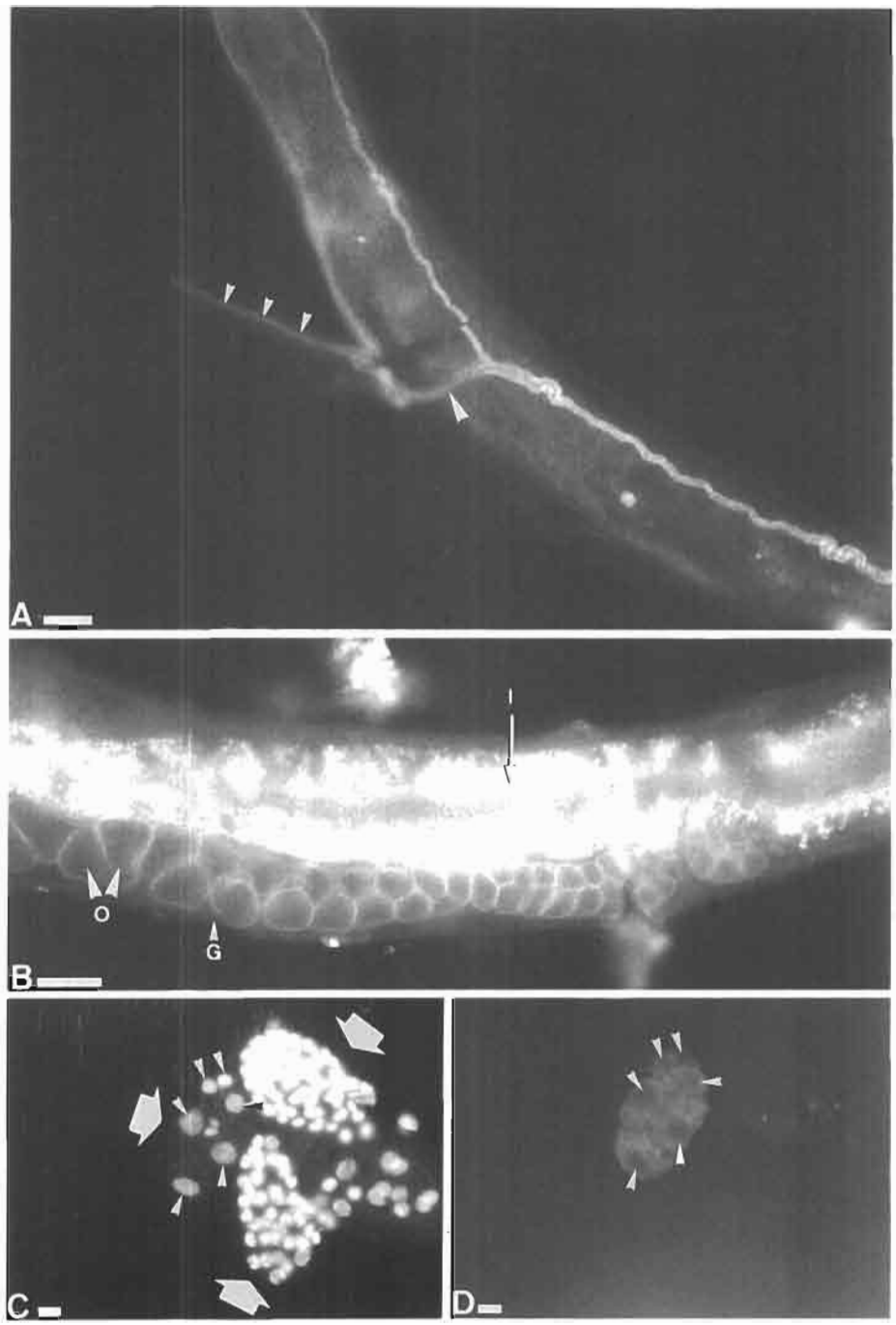

Fig. 4. *A : Staining of the secretory/excretory system in a squashed* Caenorhabditis elegans *by STA; the large arrowhead indicates the transverse duct, the small arrowheads show part of the duct that had partly dislocated as a result of the staining protocol; anterior top; B : Staining of* Acrobeloides maximus *by SBA, massive staining of intestinal yolk and surface staining of the oocytes; C-D : Disappearance of yolk staining during embryonic development in three* A. maximus *embryos (large arrows); the position of the embryo can be compared by nuclear staining (compare small arrowheads) using DAPI in C* versus *the weak yolk staining in D using AHA (G : Gonad; O : oocytes). (A-B : bar = 20 µm; C-D : bar = 10 µm).*

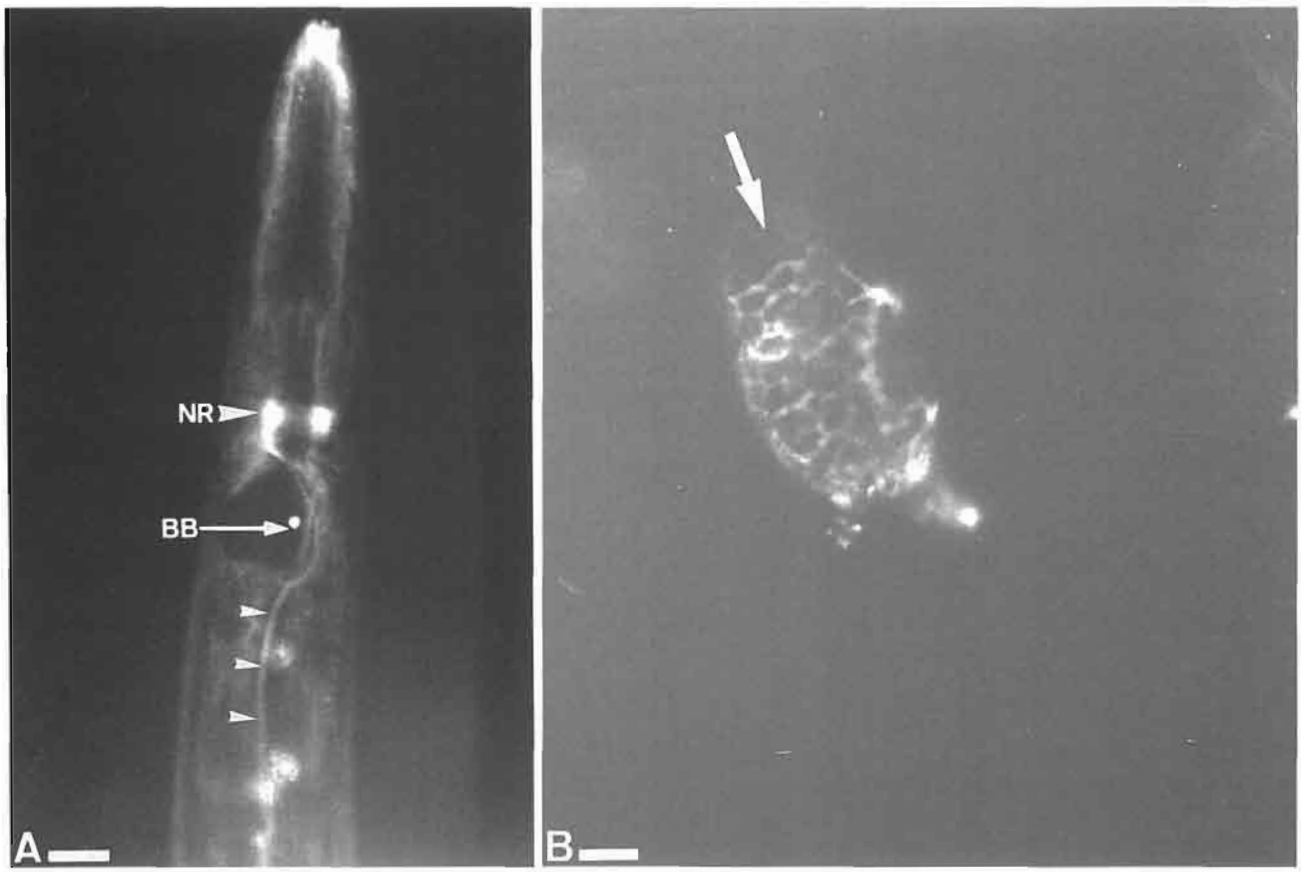

Fig. 5. *A : Staining of the nerve ring and associated nerves (small arrowheads) in* Acrobeloides maximus *by AHA; B : Surface staining of the epidermal cells in* A. maximus *during development by AHA; this staining was only present for a short time; no staining is evident in the head area (arrowhead) (BB : basal bulb; NR : nerve ring). (A : bar = 20 µm; B : bar = 10 µm).*

stained pharynx. The posterior end of that process grew considerably as development proceeded and was present in adult worms as a large, sometimes bilobed triangular structure. Furthermore, the anti-horseradish antibody stained this structure as well.

LCA

In all three nematode species, binding patterns in embryos vary considerably during development. In *C. elegans* and *P. superbus,* yolk is stained in the developing oocytes and early embryonic development. In squashed embryos in which the yolk spheres were released on the slide, it was observed that the lectin-binding of these yolk spheres was strong at the periphery (Fig. 1E, F) while no staining was present in the center. Using the monoclonal anti-yolk antibodies, no such difference in binding patterns could be observed in *C. elegans.* Yolk staining rapidly disappears and the lumen of the developing pharynx, the layer surrounding the rectum, and the nerve ring are stained subsequently. In *A. maximus,* yolk is weakly stained initially, stronger binding gradually appears in the intestinal area in the comma stage, but in later stages of development, yolk staining disappears and the same structures are stained as in the other two species (Fig. 3A, B). In adult *C. elegans,* strong binding to the basal layer of the pharynx and the layer surrounding the nerve ring, also susceptible to collagenase digestion, is evident. Binding to the nerves in *C. elegans* is limited to the cephalic sensilla and amphid-associated structures and some staining could be observed along the nerves coming from these structures. The intestinal yolk, sperm, and the lining of the reproductive system showed weaker binding. The intestinal brush border stains intensively along the entire tract but this staining of the ring of four cells immediately posterior to the pharynx cannot be inhibited by $NaIO_4$ pre-incubation and is therefore considered to be non-specific.

LPA

Some weak staining was observed along the intestinal brush border of *C. elegans* and *P. superbus,* and along the buccal cavity of *A. maximus.* However, this binding pat-

tern was very variable and inconsistent between different replicas and therefore considered to be non-specific. When in the axenic medium of *C. elegans* and *A. maximus* the haeme was replaced by heated liver extract, no specific binding could be observed.

The two different culture techniques employed gave different results. When using STA on nematodes fed on *E. coli* the bacteria were strongly stained as well and appeared as numerous spots in the intestinal lumen. It was observed that, upon using RCA-II, the intestinal brush border staining was stronger in animals grown on axenic medium than those grown on bacteria.

Discussion

Pharynx

The outer layer which lines the cuticle of the pharynx binds with several lectins at a very early time in development. Initially the entire length is stained but, as development proceeds, the anterior half is stained most consistently. The most posterior half is stained irregularly. Whether this irregularity is the result of changes in carbohydrate composition during development is unknown. Several lectins stained the layer around the pharynx intensely. Only one lectin (STA) was binding to the surface of the embryonic cells that form the pharynx in *A. maximus*. More lectins stained the layer surrounding the pharynx in adults. Support for the identification of this layer as the basal lamina comes from the observation that, when lectin incubation is done after collagenase (Type IV) treatment, the staining of the layer surrounding the pharynx disappears. Since the *C. elegans* basal lamina most likely contains collagen IV (Kingston, 1991), its disappearance after digestion is as would be expected.

Epidermis

No strong binding was observed with any of the lectins used, with the exception of epidermis associated nerves (see nervous system).

Intestine

Of the ten lectins used, RCA-II, STA and LCA bind to the glycocalyx and/or the microvilli of some of the species. The binding pattern of RCA-II in *C. elegans* and STA binding patterns in *P. superbus* and *A. maximus* are most likely linked to feeding. As long as the embryo is encased in an eggshell, hardly any stain is visible in the intestine; however, all hatched nematodes exhibit strong binding. The carbohydrate recognized is most likely secreted, maybe resulting from feeding. In *P. superbus,* RCA-II binding is also strong in embryos inside the eggshell. The binding of LCA to the intestinal brush border of *C. elegans* is considered non-specific since part of the binding at the brush border cannot be inhibited.

Gonad

Staining of the gonad tissue itself is limited. AHA did stain the interior lining of the uterus in *C. elegans* and *A. maximus,* LCA did bind embryonic to precursor cells of the gonad. Most of the binding pattern in the gonad is associated with the outer surface of early oocytes. This uniform superficial membrane staining disappears as the oocyte progresses in the gonad. Repeatedly, no membrane binding was observed once the eggshell was formed. The presence of binding, predominantly in early oocytes, may not be surprising considering the many interactions oocytes undergo at that stage. In males, sperm is recognized by several lectins. Unlike the asymmetrical binding of the sperm surface by Wheat Germ Agglutinin (Roberts & Ward, 1982*a, b*), we only observed uniform surface binding of the sperm surface.

Nervous system

The lectins AHA, LCA and RCA-II bind to several neurons and/or neuronal processes. In the case of AHA this had already been reported (E. Hedgecock and M. Chalfie, pers. comm.). Although the binding to nerves in the head area in *C. elegans* is extensive, far more binding can be observed in *A. maximus*. As a result of the paucity of data available on the nervous structure of that species, comparison with the better known *C. elegans* nervous system is presently not possible. Besides the nerves, amphid associated structures (nerves?) are stained as well and most prominently in *A. maximus*. Despite its large size and consistent staining, we have been unable to identify the structure that is recognized in Fig. 2D. In *A. maximus,* the structure stained with RCA-II is also stained with the anti-horseradish peroxidase antibody, unlike the structure stained with AHA, or LCA. This might indicate that the RCA-II is staining large closely spaced neurons and that the stained structure is not a part of the amphid *sensu stricto* despite its position. Both other lectins bind effectively to the amphidial sac or associated non-nervous structures.

Secretory/excretory system

The secretory/excretory system was only stained in *C. elegans* using STA. In the embryos however RCA-II and SBA did bind occasionally to the excretory duct (duct = the short canal running from the excretory pore to the sinus connecting both lateral canals of the secretory/excretory system) in all three species. In the adults this binding was highly variable. With the RCA-II after collagenase treatment, the excretory duct remained stained (Fig. 2B), indicating that the lectin did not bind to the membrane surrounding the duct and that the lectin bound either to the lining of the duct or to the fluid inside the duct itself. We assume that the lectin bound to fluid present in the duct rather than the duct itself, which would explain the transient nature of the staining pattern. For that same reason we doubt that STA binding to the entire secretory/excretory system (except the

duct) in *C. elegans* is due to the fluid inside the tubular network but represents effective binding of the lectin to a component of the secretory/excretory system. Such a binding would explain the consistent binding pattern observed in the several replicas *versus* the aberrant binding to the duct.

Coelomocytes

In the embryo of the three species studied, strong binding to four coelomocytes was observed. In the adults of all three nematodes, only four coelomocytes are stained. This is surprising since there are more coelomocytes in adults than in embryos. In *C. elegans* two more coelomocytes are present in the adult hermaphrodite (one more in the male) (Sulston & Horvitz, 1977; Sulston *et al.,* 1980). In *A. maximus* there are seven coelomocytes in the female (De Ley & Siddiqi, 1991) and we found the same number in adults of *P. superbus*. The absence of staining in post-embryonically dorsally formed coelomocytes (Mdlpa and Mdrla), indicates that in all three families there are at least two different types of coelomocytes, based on their glycoconjugate composition. No lectin was found to bind to all coelomocytes in any of the three nematode species. Furthermore, not one of the lectins used stained the post-embryonically formed coelomocytes. It is impossible to explain this difference nor to advance any sensible explanation for the differences in staining observed, since the function and structure of coelomocytes in free-living nematodes is still unknown. Several hypotheses have been proposed varying from their implication in cellular defense (phagocytosis) to cells collecting undigestable or poisonous material or stains (for review see Wood, 1988; Bird & Bird, 1991). Other possible functions proposed include coelomocytes as important centers for intermediate metabolism (Turpeenniemi, 1993) or involvement in the correct positioning of the gonad during development (De Ley & Siddiqi, 1991).

Yolk

Several lectins bind strongly to the yolk or yolk precursors in the intestine, yolk proteins secreted in the pseudocoelomic cavity and in embryos. The staining of yolk by several lectins is not exceptional since the binding of Con A to yolk from *C. elegans* and *Dolichorhabditis* sp. had previously been demonstrated by Sharrock (1983) and Winter (1992). However, the strong binding with several lectins confirms the heavily glycosylated nature of the nematode yolk proteins.

Processing of the yolk proteins is clearly evident from the changing lectin-binding capabilities during development. Furthermore, if yolk stains in one given species it does not necessarily stain in any of the other species tested, which indicates differences in yolk glycosylation between species of the same family. Several binding patterns were observed; although the various patterns were present in all three nematode species, the most distinct patterns, with the lectins used, were in *A. maximus : i)* with the lectins Con A, UEA-II, PHA-A and PHA-L, the binding is stable throughout development and is present in all developmental stages; *ii)* a second pattern is evident using SBA; strong binding in adult nematodes, weak binding in embryos and none in the J1; *iii)* by contrast, LCA and AHA lectins barely bind to yolk in the adult, but bind to yolk strongly in the developing oocyte; binding has disappeared, however, in the J1 stage. Although both binding patterns (*ii, iii*) are different, they indicate some processing must occur at the time or shortly after incorporation of yolk or its precursors into the maturing oocyte, causing either loss of binding (SBA) or the appearance of new binding sites (LCA, AHA).

Several authors describe yolk spheres as modified " lysosome-like " organelles containing unusual and considerable enzymatic activity (Wall & Meleka, 1985; Fagotto, 1990*a, b,* 1991) necessary in yolk formation by processing carbohydrates of the yolk precursor, vitellogenin (Wall & Meleka, 1985). Removal of the carbohydrates released was considered essential for this processing reaction to proceed in membrane-bound organelles. It has been proposed that the endogenous yolk lectins regulate this reaction (Yoshizaki, 1992). This would lead to a high concentration of sugar residues at the surface of the yolk vacuole where they bind the endogenous lectin. The appearance of the lectin binding at the periphery of the yolk spheres in squashed embryos and the changing binding patterns is reminiscent of results obtained by Yoshizaki (1990, 1992). The peripheral lectin binding and the changing binding patterns could be explained by a similar processing mechanism during incorporation of yolk in the oocyte. The processing of carbohydrates in nematode yolk spheres and their subsequent removal could either lead to existing binding sites disappearing or new binding sites appearing. Ultrastructural analysis of yolk vesicles in the intestine and of developing oocytes of *A. maximus* provide support for this interpretation (Borgonie *et al.,* 1995). In intestinal cells, only yolk vesicles were found which appear uniformily black. However, identical vesicles are found, in the developing oocytes, but also vesicles where the enclosed yolk material exhibits randomly striated oriented tubular cavities, considered by Yoshizaki (1990) in *Xenopus,* to be indicative of yolk cystallization. With one exception, these striated vesicles were exclusively found in the oocytes (in 147 randomly taken micrographs of *A. maximus* sections, only one case of striated vesicles appears in the intestine; see Borgonie *et al.,* 1995).

We believe it is very likely that the " yolk " present in the intestine is the yolk precursor vitellogenin and that the final crystallization to yolk occurs in the oocyte. One can wonder what would be the usefullness of first adding carbohydrate, only to remove it at a later stage. One possibility might be that the added carbohydrates on the

vitellogenin serve as binding sites to ensure correct routing to, and incorporation in the gonad, after which they would become obsolete.

Using lectin binding we observed like Sharrock (1983) and Bossinger and Schierenberg (1992) that considerable amounts of yolk are still present in well developed embryos and newly hatched first stage juvenile. Both reports proposed that this yolk reserve would support post-embryonic development. Bossinger and Schierenberg (1992) also indicated that the yolk reserve in the intestine could be part of a backup energy source allowing survival in periods of starvation. Some of our data support both views but stress the role of yolk in starvation survival. Axenically cultured *C. elegans* contain less yolk since they stain considerably less with the monoclonal anti-yolk antibodies or lectins, and they survive for a shorter time when starved than nematodes cultured on *E. coli* (unpubl. data). These observations support the view of Vanfleteren (pers. comm.) that axenically cultured *C. elegans* are in a continued state of nutritional restriction. By containing less yolk than *C. elegans* cultured on *E. coli,* they have less backup when starved and die sooner. Axenically cultured *C. elegans* are probably unable to produce similar amounts of yolk as when cultured monoxenically because the axenic medium is suboptimal as a food source or as an environment. It has been observed that axenically cultured *C. elegans* produce less offspring when compared to those cultured on *E. coli* (Sulston & Hodgkin, 1988). However, we consistently observed weaker yolk binding in male juvenile specimen as development proceeded. This might further support the view that the surplus yolk is indeed a necessity for completing post embryonic development.

Comparison of the yolk binding pattern between lectins and the anti-yolk monoclonal antibodies gives some information about the target recognized by the antibodies. Relevant is the consistent complete yolk vacuole binding using antibodies in *C. elegans* irrespective of the developmental stage, *versus* the shifting, circumferential, age-dependent shift in staining obtained with some of the lectins. This indicates that the epitopes recognized by the antibodies are either polypeptides or carbohydrates of the yolk protein and its precursor and that these are not involved in the processing occurring in the embryo. These epitopes are not shared by the two other nematode species, as evidenced by the lack of binding with these antibodies.

Specific binding of LPA was not observed when haemoglobin was replaced with liver extract in the axenic culture medium. The absence of binding with LPA is not surprising, given that the results of Bačic *et al.* (1990) had already indicated the absence of sialic acid in *C. elegans* and *Panagrellus redivivus.* However, these authors did obtain evidence for sialic acid in both species using ^{125}I-*Limax flavus* agglutinin in competitive displacement experiments, but only if liver extract was used in the culture medium. We were unable to confirm the observations of difference in binding patterns as result of diet of Bačic *et al.* (1990), possibly because of the reduced sensitivity of direct labeling.

The importance of the two differences in binding patterns observed between animals grown on bacteria and on axenic medium is intriguing but unclear to date. The stronger binding observed with RCA-II could be the result of the presence of bacteria. Nematodes cultured on bacteria have shorter intestinal microvilli than those cultured axenically (unpubl. data). As such there would be fewer binding sites available in the former case.

In comparison with results reported earlier (Link *et al.,* 1988) about external binding patterns in *C. elegans,* there are much more different binding patterns with the lectins used than reported for external lectin binding.

In conclusion, considerable differences exist in lectin-binding patterns between the three free-living nematode species with the same lectin. Furthermore, all tissues in the three species are stained with one or more of the lectins used. In some instances lectins can be used as rapid (direct staining) and cheap alternatives to antibodies (indirect staining) as markers during embryonic development (pharynx, secretory/excretory system, reproductive system in *A. maximus,* yolk). Besides the yolk, four of the coelomocytes and the oocytes are structures which stain most often with the lectins used. In retrospect, this may not be surprising since considering their function, both receive a lot of external cues or interact repetitively with other tissues or substances. Although the intestinal brush border interacts with considerable amounts of foreign material, it is somewhat surprising that the intestinal brush border binds with only a few lectins. However, since acetone was the only fixative with which optimal binding and minimal background staining could be obtained, loss of glycolipids is to be expected under these conditions, it is likely that more binding sites are present and that some data might have been lost during the processing of the tissue. Therefore poorer staining of any given tissue does not necessarily reflect paucity of binding sites.

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