# A gp330/megalin-related protein is required in the major epidermis of

# *Caenorhabditis elegans* for completion of molting

**John Yochem[1,2,3,*], Simon Tuck[2], Iva Greenwald[3,‡] and Min Han[1,4]**

[1]Department of Molecular, Cellular and Developmental Biology, [4]Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309 USA
[2]Umeå Center for Molecular Pathology, Umeå University, S-901 87 Umeå, Sweden
[3]Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA
[‡]Present address: Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
[*]Author for correspondence (e-mail: john.yochem@colorado.edu)

## SUMMARY

A genetic analysis of a gp330/megalin-related protein, LRP-1, has been undertaken in *Caenorhabditis elegans*. Consistent with megalin's being essential for development of mice, likely null mutations reveal that this large member of the low density lipoprotein receptor family is also essential for growth and development of this nematode. The mutations confer a striking defect, an inability to shed and degrade all of the old cuticle at each of the larval molts. The mutations also cause an arrest of growth usually at the molt from the third to the fourth larval stage. Genetic mosaic analysis suggests that the *lrp-1* gene functions in the major epidermal syncytium hyp7, a polarized epithelium that secretes cuticle from its apical surface. Staining of whole mounts with specific monoclonal antibodies reveals that the protein is expressed on the apical surface of hyp7. Sterol starvation can phenocopy the *lrp-1* mutations, suggesting that LRP-1 is a receptor for sterols that must be endocytosed by hyp7. These observations indicate that LRP-1 is related to megalin not only structurally but also functionally.

Key words: gp330, Megalin, LRP-2, Sterols, *Caenorhabditis elegans*, Molting

## INTRODUCTION

In mammals, megalin (also called gp330 or LRP-2, for the second low density lipoprotein (LDL) receptor-related protein) is one of two > 500 kDa members of the LDL receptor family of integral membrane proteins (Saito et al., 1994). Megalin is expressed on the apical surface of certain epithelia and is thought to be a clearance receptor that maintains lipid homeostasis and regulates the activity of extracellular proteases (for review, see Krieger and Herz, 1994; Strickland et al., 1995). It can bind, in vitro, a variety of particles and molecules, including proteases, complexes of proteases and their inhibitors, and lipoprotein particles. If endocytosed from extracellular fluids, the ligands are often degraded following dissociation from the receptor, which is then returned to the cell surface. Although LRP, the other large member of the LDL receptor family, is not expressed on the apical surface of epithelia, it is also an endocytotic receptor and can bind many of the same ligands as megalin (Krieger and Herz, 1994).

Megalin has been shown to endocytose LDL particles themselves, suggesting that it may be a high-capacity receptor that is used by certain tissues that require large amounts of cholesterol (Stefansson et al., 1995). This proposal is consistent with the phenotype of mice that are homozygous for

a null mutation of the gene. They die soon after birth with malformations of the skull (a holoproencephalic phenotype) that resemble, but are weaker than, those that result when the biosynthesis of cholesterol is perturbed either by genetic disease or by the application of certain teratogens (Willnow et al., 1996). Although these observations suggest a major role for megalin in the uptake of cholesterol, the genetic analysis has also suggested a role in regulation of proteases: megalin-deficient lungs have malformed alveolar sacs that resemble those associated with emphysema or with an experimental perturbation of proteolysis (Willnow et al., 1996). The precise role, if any, of megalin in the regulation of extracellular proteases remains unknown, however.

Like mammals, the free-living nematode *Caenorhabditis elegans* has at least two genes that can encode > 500 kDa members of the LDL receptor family. One of these has recently been revealed by the genome sequencing consortium (Waterston and Sulston, 1995). Although sharing an overall structure with megalin and to a slightly greater extent LRP, its predicted product is unusual (unpublished observations). The second gene has been called *lrp-1* (Yochem and Greenwald, 1993), but its predicted product was later discovered to be more closely related to rat gp330/megalin than to LRP (Saito et al., 1994), suggesting that worm LRP-1 and mammalian megalin

might be counterparts. The apparently early appearance of these large proteins in metazoan evolution suggests that they have a fundamental function that might be revealed by genetic analysis in a simple animal. In order to investigate this possibility, likely null mutations have been isolated in the *lrp-1* gene, revealing that the megalin-related protein is essential for growth and development of *C. elegans*. The phenotype of the mutations is discussed with respect to the pattern of expression of the gene.

## MATERIALS AND METHODS

### Genetic markers and media

N2 variety Bristol was the wild-type strain, and the following genetic deficiencies or recessive mutations were used: Linkage Group (LG) I: *unc-13(e1091)* (Waterston and Brenner, 1978), *gld-1(q266)* (Francis et al., 1995), *ozDf5* (Francis et al., 1995), *nDf24* (Ferguson and Horvitz, 1985); LGIV: *him-8(e1489)* (Hodgkin et al., 1979). Chromosomal rearrangements included *szT1*, a reciprocal translocation between LGI and LGX (Fodor and Deàk, 1985; Edgley et al., 1995); and *nDp4*, a duplication of a large segment of LGI that is attached to LGV (McKim et al., 1992). *nDp4* should contain the wild-type *lrp-1* locus and is largely maintained in the heterozygous state. If not otherwise indicated, strains were propagated as described by Brenner (1974) on nematode growth medium (NGM) that contained a supplement of 5 µg/ml cholesterol, either DifcoBacto or Sigma type A7002 agar, and lawns of the food source, *Escherichia coli* strain OP50. Sterol-deficient medium was similar to NGM but had the following alterations: bactopeptone was eliminated, agarose (SeaKem GTG) replaced agar, and cholesterol was not added. Control medium was identical except for the presence of 50 µg/ml of cholesterol (Sigma; C-8667; >99% pure) added from a stock solution (5 mg/ml in 95% ethanol; an equivalent volume of 95% ethanol lacking cholesterol was added to the sterol-deficient medium). Before being applied to either medium, overnight cultures of the OP50 bacteria were washed twice with M9 buffer (Brenner, 1974) and then concentrated 20-fold in M9 buffer.

### Isolation of the *ku156* and *ku157* alleles of *lrp-1*

BW1707 (provided by J. A. Powell-Coffman and W. Wood, University of Colorado), which has the genotype *unc-13(e1091)/nDf24 (I)*; *him-8(e1489) (IV)*, was the parental strain of the *ku156* and *ku157* alleles of *lrp-1*. Its use was based on the physical proximity of *lrp-1* to *unc-13* (A. Coulson, personal communication; Yochem and Greenwald, 1993) and the assumption that *lrp-1* would be essential for viability or fertility because its product is conserved with mammalian megalin. Based on previously described methods (Rosenbluth et al., 1985), BW1707 was treated with gamma rays (1900 or 3800 rads), and fully coordinated first-generation larvae were placed one to a plate so that the progeny of each could be examined for the presence of sterile Unc-13 segregants or for the absence of Unc-13 segregants, indicating a lethal mutation closely linked to *unc-13(e1091)* (Fig. 1).

Heterozygous males were crossed to BS585 (provided by T. Schedl, Washington University, St. Louis), a strain having the genotype *unc-13(e51) ozDf5 I*; *nDp4(I,V)/+*, in order to test complementation between candidate mutations and *ozDf5*, a small deficiency that deletes genes to the right of *unc-13* including *lrp-1* DNA (Francis et al., 1995). Mutations that failed to complement and that could therefore be within the *lrp-1* gene were next tested for their ability to be rescued by an integrated array (*kuIs18*) that is composed of *rol-6(su1006)* DNA and two overlapping cosmid clones (F29D11 and C27G12) that contain the *lrp-1* gene (A. Coulson, personal communication).

From a set of 10,000 F$_1$ progeny, six essential mutations were isolated that could not be complemented by *ozDf5*. Of these, three could be complemented by *kuIs18*. DNA was isolated from balanced strains containing these three mutations and from the parental strain, and Southern blots were probed for alterations within *lrp-1*. The two mutations in *lrp-1* that were isolated have been designated *ku156* and *ku157*. Each was backcrossed seven times to the N2 strain before further analyses. The *ku156* mutation was separated from the *unc-13* mutation by identifying a spontaneous recombinant. The rarity of the recombination (1/1296) is consistent with the measured linkage of *unc-13* and *gld-1*, the nearest marker to the right of *lrp-1* (Francis et al., 1995).

### Transformation rescue of *lrp-1* mutations

Extrachromosomal arrays were formed in vivo following microinjection of cloned DNA into the syncytial germ line (Mello and Fire, 1995). The plasmid pRF4 (*rol-6(su1006dm)*) (Mello and Fire, 1995) was used at a concentration of 100 µg/ml as a dominant marker for transformation. For construction of MH#jy209b, a plasmid specific for the 5′ part of the *lrp-1* gene, a 16.5 kb *Sma*I/*Hpa*I DNA fragment (Fig. 2) was isolated from the cosmid F29D11. For MH#jy207b, a plasmid specific for the 3′ part, a 14 kb *Spe*I/*Sal*I DNA fragment (Fig. 2) was isolated from the same cosmid. The fragments were inserted into BlueScript vectors, and the resulting plasmids were each injected at 2.5 µg/ml. Because the plasmids share 2 kb of DNA from *lrp-1*, homologous recombination within this region can create a gene of full length when the plasmids are injected together (Mello and Fire, 1995). Rescue of *ku156* or *ku157* was assessed by injecting MH204 (genotype *unc-13(e1091) lrp-1(ku156)*; *nDp4/+*) or MH972 (genotype *unc-13(e1091) lrp-1(ku157)*; *nDp4/+*) with a mixture of the DNA clones and then determining whether transgenic lines, based on rolling behavior conferred by pRF4, could produce healthy Unc-13 progeny that could themselves establish stable lines. Rescue of *szT1* by *lrp-1* DNA was determined by genetic crosses employing extrachromosomal arrays formed from the injections of MH204 or MH972. Control injections lacking *lrp-1* DNA but containing pRF4 confirmed specificity of the rescue.

### Genetic mosaic analysis

Based on the approach of Lackner et al. (1994), spontaneous mosaics were generated in the progeny of a strain, MH1066, having the genotype *lrp-1(ku156)*; *him-8(e1489)*; *kuEx76[SUR-5GFP(NLS); lrp-1(+)]*. The extrachromosomal array *kuEx76* was formed in vivo following microinjection (Mello and Fire, 1995) of three DNA clones into the syncytial germline of a heterozygote balanced for *ku156*. F29D11 and C27G12, the two overlapping cosmid clones that contain genomic DNA from the *lrp-1* region, were injected at 2.5 µg/ml each for rescue of chromosomal copies of *lrp-1(ku156)*. (Subsequent work demonstrated that the C27G12 clone is superfluous for rescue of *lrp-1* mutations.) The establishment of trangenic lines and the detection of mosaicism were based on injecting pTG96 at 100 µg/ml for expression of SUR-5(GFP)NLS (Yochem et al., 1998), a marker that expresses a form of the green fluorescent protein (GFP) in many nuclei. Normal growth plates containing MH1066 were first examined at magnifications of 40-100× with a dissecting microscope equipped with a mercury lamp for vigorous L3 and L4 larvae or young adults that had mosaic patterns of green fluorescence. Using 1000× magnification, mosaicism was assessed by examining certain nuclei for the presence or absence of fluorescence as previously described (Yochem et al., 1998). The mosaics were then examined with Nomarski optics for the presence or absence of all aspects of the Lrp-1 mutant phenotype.

### Preparation of monoclonal antibodies against LRP-1

Because the LRP-1/megalin protein is largely composed of many copies of motifs that are also present in a number of other proteins, the carboxyl-most region was used for the production of monoclonal

antibodies in an effort to minimize the chances for cross-reactivity. A 700 bp *Eco*RI fragment from a cDNA clone encoding the final amino acids, the stop codon, and 3′ untranslated DNA was cloned into the vector pGEX-1 (Smith and Johnson, 1988) such that the final 117 amino acids of LRP-1 are fused to the carboxyl terminus of glutathione S-transferase (GST). The fusion protein was produced in bacteria and isolated by its binding to glutathione-conjugated beads. Injections of mice and production of hybridomas were performed by M. Marlow at the Monoclonal Antibody Facility at Princeton University. In order to avoid antibodies against GST, supernatants were assayed by western blotting for reactivity to a fusion protein containing the same moiety of LRP-1 and β-galactosidase in place of GST. Two independent monoclonal antibodies, 1H6 and 4H5, were used for staining whole mounts.

### Indirect immunofluorescence

Mixed-stage populations of worms were fixed and made permeable to antibodies as previously described (Bettinger et al., 1996). A 1:1000 dilution of an ascites preparation of the mouse monoclonal antibody MH27 was used for detection of adherens junctions between epithelia (Waterston, 1988; Podbilewicz and White, 1994). Non-ascites preparations of 1H6 (1:200) and 4H5 (1:150) were used together for enhancement of the LRP-1 staining. Binding of the antibodies was detected with a 1:1600 dilution of a Cy3-conjugated antibody against mouse IgG (Jackson ImmunoResearch). Incubations (16 hours at 4°C) and washes (20°C over the course of several hours) were as previously described (Finney and Ruvkun, 1990). Whole mounts were examined at 1000×, and micrographs were generated with a CCD camera and Adobe Photoshop.
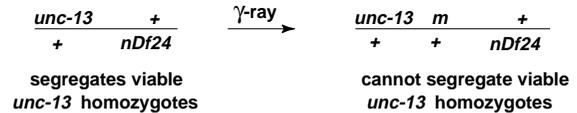
## RESULTS

### The *lrp-1* gene is essential for growth

Two recessive mutations, *ku156* and *ku157*, have been isolated in the *lrp-1* gene following gamma-irradiation of a strain, BW1707, that is balanced for mutations linked to *unc-13* (Fig. 1). The mutations fail to complement each other and confer the same phenotype. Homozygotes segregating from heterozygous mothers hatch and initiate growth and development. Most, however, lack vigor, and an arrest of growth and development usually ensues at the molt from the third (L3) to the fourth (L4) larval stage if not earlier. The homozygotes are moderately Dumpy (short and fat), have darkened intestines, are small for their developmental state, and are often encased in old cuticle or have pieces of old cuticle that remain attached to parts of the epidermis. Nomarski observations reveal an undefined but unhealthy appearance, and there is an accumulation of refractile material in the rectum. Lastly, strains with simple genotypes such as MH210 (*lrp-1(ku156)/gld-1(q266)*) fail to segregate dead embryos, demonstrating that *lrp-1* homozygotes are able to complete embryonic development (and usually early larval development) when they segregate from heterozygous mothers.
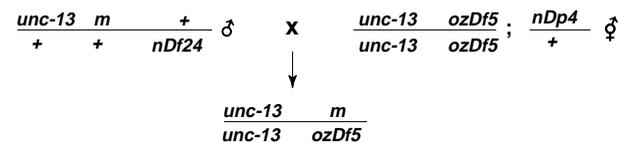
Genetic, molecular, and immunocytochemical data prove that these are mutations in *lrp-1* and suggest that this description is likely to be the null phenotype. One indication that these are mutations in *lrp-1* is the ability to rescue the phenotype of either mutation completely following co-injection of two plasmids, MH#jy207b and MH#jy209b, that contain overlapping subclones of genomic DNA that should derive only from the *lrp-1* locus (Fig. 2). More importantly, the *ku157* mutation is associated with an alteration of the *lrp-1* coding region when restricted DNA is analyzed by Southern
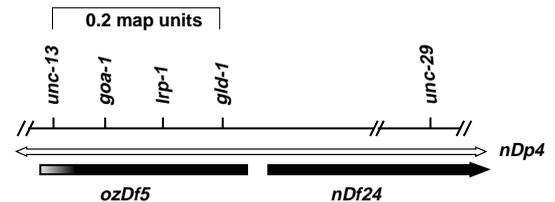


**Fig. 1.** A screen for lethal mutations in the *ozDf5* region of Linkage Group I. Approximate locations of the genetic deficiencies *ozDf5* and *nDf24* are indicated with black bars (Francis et al., 1995). Although *ozDf5* was isolated in *cis* to *unc-13(e51)*, the speckled region indicates that the left end of the deletion is not precisely known.

hybridizations: a *Hin*dIII fragment migrates more slowly than the corresponding 5.1 kb fragment of the N2 or BW1707 strains (Fig. 3). Because the flanking *Hin*dIII fragments are not altered, *ku157* is an insertion of 0.8 kb of DNA into an internal region of the *lrp-1* gene (Figs 2, 3). Although Southern blot analysis of *Eco*RI- or *Hin*dIII-digested DNA has failed to reveal an alteration associated with *ku156*, immunocytochemical data presented below prove that it is also a severe mutation in *lrp-1*. Lastly, genetic tests suggest that *ku156* and *ku157* are likely null mutations: animals with the genotype *unc-13(e1091) lrp-1(ku156* or *ku157)* are similar to those having the genotype *unc-13(e1091) lrp-1(ku156* or *ku157)/unc-13(e51) ozDf5*.

A pre-existing mutation in *lrp-1* was revealed by serendipity. In an attempt to balance an unrelated mutation on LGI with *szT1*, a reciprocal translocation between LGI and LGX whose physical breakpoint on LGI maps just to the right of *unc-13* (McKim et al., 1988), Dumpy segregants were noticed that had all of the features of the phenotype described above for *ku156* and *ku157*. Although *szT1* homozygotes had been described as dead embryos (Fodor and Deàk, 1985; Edgley et al., 1995), similar larvae were seen in three distinct strains containing *szT1*, suggesting that these larvae are the segregants that fail to inherit the untranslated LGI that normally complements an essential gene on LGI that is disrupted by the translocation. As

**Fig. 2.** A schematic representation of the *lrp-1* gene and its predicted product. The coding region (Yochem and Greenwald, 1993) is indicated in relation to 30 kb of DNA from the cosmid clone F29D11, the complete sequence of which has been detemined by the *C. elegans* sequencing consortium and is available from GenBank or ACeDB (A *C. elegans* Data Base) (Waterston and Sulston, 1995). The true 5′-end of the genomic DNA in the cosmid clone corresponds to the zero position. The 3′-ends of the two genes predicted to flank *lrp-1* are indicated with arrows. The 16.5 kb *Sma*I/*Hpa*I fragment and the 14 kb *Spe*I/*Sal*I fragment used for constructing the plasmids MH#jy207b and MH#jy209b are indicated with brackets below the restriction-enzyme map. The region used as a hybridization probe for the Southern blot shown in Fig. 3B is indicated with a line, and the *Hin*dIII restriction fragments affected by the *ku157* or *szT1* mutations are represented as bars. The parts of the predicted protein encoded by exons 23 to 27 are shown because of their relevance to the mutations. The motifs present in LRP-1 and rat gp330/megalin are indicated as follows: boxes, Class A (ligand binding) repeats; white ovals, class B.2 epidermal growth factor (EGF) repeats, black ovals, class B.1 EGF repeats; cross-hatched lines, YWTD spacers (Saito et al., 1994).
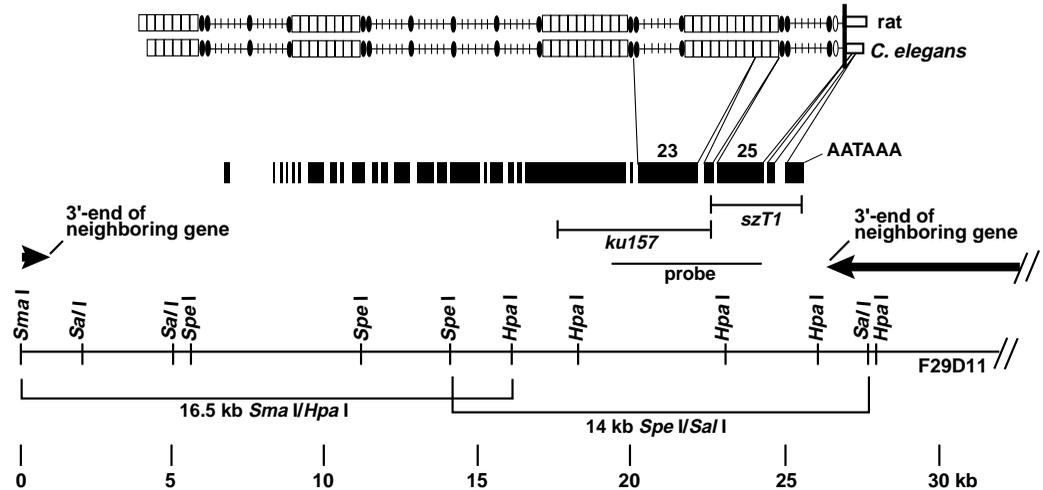
expected from the phenotype, genetic tests demonstrate that the breakpoint is within the *lrp-1* gene (unpublished observations). Briefly, *szT1* cannot complement *ku156* or *ku157*, but extrachromosomal arrays containing the *lrp-1*-specific plasmids can rescue the translocated chromosomes. Also, Southern blot analysis reveals an alteration in a 2.8 kb *Hin*dIII fragment that contains four exons from the *lrp-1* gene (unpublished observations; summarized in Fig. 2). Thus, three mutations, each likely to be severe, have been isolated in the *lrp-1* gene, revealing that it is essential for growth and development.

## *lrp-1* is required for completion of molting

A striking effect seen with *ku156*, *ku157*, or *szT1* homozygotes is a failure of many to shed all of the old cuticle when molting from one larval stage to the next. This failure can result in worms being completely encased in old cuticle that has become displaced from the anterior end (and sometimes from the extreme posterior end) but not from the rest of the body (Fig. 4A). These worms can have one of two fates. Many of them remain stuck in this cuticle but continue to live for several days. Others are able to break through the old cuticle, but it or parts of it remains attached to the body; these worms therefore have rings of old cuticle that encircle the body or have a trail of old cuticle that remains attached to the posterior part of the body just anterior to the anus (Fig. 4B,C). In contrast to the shed cuticle of wild-type *C. elegans*, the displaced cuticle of an *lrp-1* homozygote does not degrade within the lifetime of the worm.

Observations with a dissecting microscope sometimes reveal Dumpy, nonvigorous segregants that appear not to have old cuticle associated with them. When examined at high magnification with Nomarski optics, many of these can be seen to have small pieces of old cuticle that remained attached to their bodies. Others, however, do not have old cuticle associated with them. If allowed to resume growth, they arrest by the time of the L3 to L4 molt and have become encased in old cuticle or have trails of old cuticle.

A comparsion with wild-type molting indicates that the displaced cuticle of the *lrp-1* homozygotes represents old cuticle that should have been shed and degraded during molts
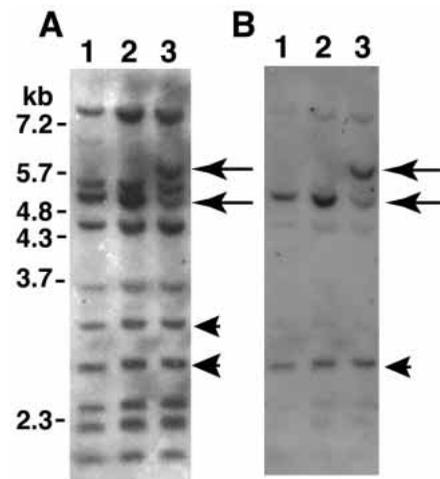


**Fig. 3.** Southern blot analysis of *ku157* DNA. (A) Hybridization of radiolabeled F29D11 to *Hin*dIII-digested DNA. Lane 1, the wild-type pattern present in DNA from N2 or the parental strain BW1707. Lane 2, DNA from a strain with the genotype *unc-13(e1091); lrp-1(ku156); nDp4/+*. Lane 3, DNA from a strain with the genotype *unc-13(e1091) lrp-1(ku157); nDp4/+*. The wild-type 5.1 kb fragment and the 5.9 kb fragment associated with *ku157* are indicated with arrows, and the 3.2 and 2.8 kb fragments that flank the 5.1 kb fragment are indicated with arrowheads. (B) Proof that the altered 5.9 kb band is specific for the *lrp-1* coding region was obtained by removing most of the F29D11 signal and then re-hybridizing the filter with a probe covering the region of *lrp-1* indicated in Fig. 2. Note that the 5.1 kb fragment hybridizes with half the intensity of the 5.9 kb fragment; most of the progeny should have inherited two copies of the *ku157* allele but only one copy of the wild-type *lrp-1* gene because they are heterozygous for the balancer *nDp4* (McKim et al., 1992).

rather than an aberrant blistering of the cuticle of animals not undergoing molts as is seen with mutations in the six *bli* genes (Kramer, 1997). During normal molts (Singh and Sulston, 1978), new cuticle is first synthesized under the old cuticle. Old cuticle from the anterior half of the pharynx is then expelled, and old body cuticle with the pharyngeal cuticle attached separates from the anterior end of the body. That the *lrp-1* homozygotes have displaced these cuticles is very evident (Fig. 4A). Moreover, the cuticle of the rectum, the excretory duct, and regions of the body from which cuticle has not detached are clearly thicker than normal, suggesting that new cuticle has been synthesized as for a normal molt.

During normal molts (Singh and Sulston, 1978), the entire animal rotates within the old cuticle, presumably to loosen it from the midbody. This is following by a rupturing of the anterior cuticle, which is associated with contractions of the head, and emergence of the animal from the old cuticle which disappears. Like the wild-type worms, *lrp-1* homozygotes can undergo contractions of the head after displacement of the phayngeal and anterior cuticles. These contractions, however, appear weak relative to those of wild-type worms, and the rotation of the entire body is not seen. Taken together, these observations indicate that the homozygotes initiate the process of molting but are unable to complete it. In particular, they are unable to degrade the old cuticle.

## Other aspects of the mutant phenotype

Lrp-1 segregants from *szT1* strains or from strains containing *ku156* no longer in *cis* to *unc-13(e1091)* are usually stationary although some are capable of moving a few worm lengths and can respond appropriately to harsh touch with a metal wire or to gentle touch with a cilium. Occasional worms, including some that are completely incased in old cuticle, can locomote for several millimeters, but they eventually become stationary and usually arrest growth at the L3 to L4 molt.

Examination with a dissecting microscope reveals that the *lrp-1* mutations confer a darkening of the intestine. Because specialized L3 larvae termed dauers also have darkened intestines (Riddle and Albert, 1997), the possibility exists that the mutant homozygotes have entered this state of diapause which is induced by starvation and high population densities.

Examination with Nomarski optics indicates that this is not the case. Some of the vesicles in the mutant intestine are highly refractile and have a necrotic appearance; these vesicles exactly correspond to dark particles seen with bright-field illumination. In contrast, the intestines of dauers were seen to be packed with dense vesicles that lack high refractility. Furthermore, alae and a constricted pharynx, both morphological features of dauers (Riddle and Albert, 1997), are absent in the *lrp-1* homozygotes. Although not resembling the intestines of dauers, the mutant intestines do resemble those of wild-type worms that have been starved for bacteria but have not entered the dauer state. Thus, the darkened intestine may be a consequence of not feeding well, especially for homozygotes whose mouths are obstructed by undegraded cuticle.

Nomarski observations of *szT1*, *ku156*, or *ku157* homozygotes reveal an accumulation of refractile material in the rectum in those animals that have not shed the old cuticle from this region (Figs 4, 5). The rectal hypodermal cells K.a and K′, which produce some of the rectal cuticle (Sulston et al., 1983), are enlarged and have a refractile sphere – perhaps a Golgi apparatus – near their nuclei (Fig. 5). There is often a posterior displacement of the nuclei of the rectal cells B and F and of other cells in the tail that perhaps results from the enlargement of K.a and K′.
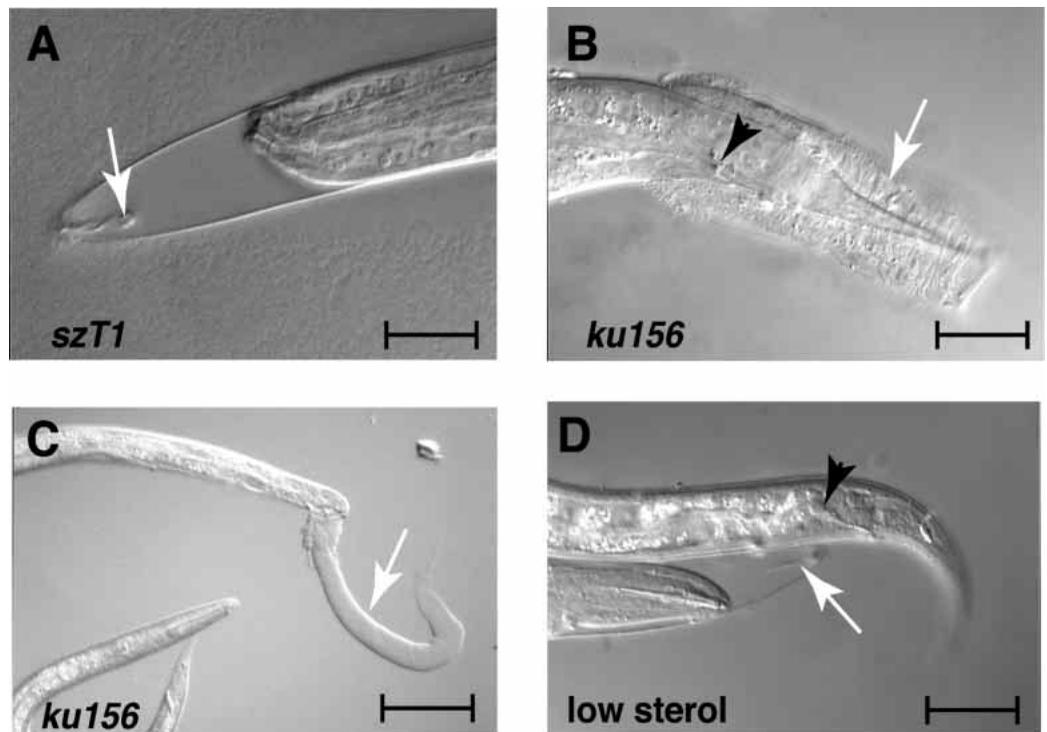


**Fig. 4.** Undegraded cuticle is associated with mutations in *lrp-1*. Longitudinal views with Nomarski optics, dorsal side up and anterior usually to the left (as is also the case for all subsequent micrographs). (A) An *szT1* homozygote at the L2 stage that had been stuck for several hours in old cuticle. Expulsion of the pharyngeal cuticle (arrow) is evident. (B) Old cuticle (arrow) that remained attached to the posterior of an Lrp-1 segregant, from the strain MH210, that was arrested at the molt to the L4 stage. Refractile material in the rectum can also be seen (arrowhead). (C) Cuticle that remained attached to the tail of an L3 larva from MH210 had become filled with excrement, emphasizing the enduring nature of the old cuticle. (D) Growth of the wild-type N2 strain on medium deficient in sterols resulted in a young L3 larva having refractility of the rectum (arrowhead) and a displaced anterior and pharyngeal cuticles (arrow). Scale bars (A,B,D) 20 µm; (C) 100 µm.
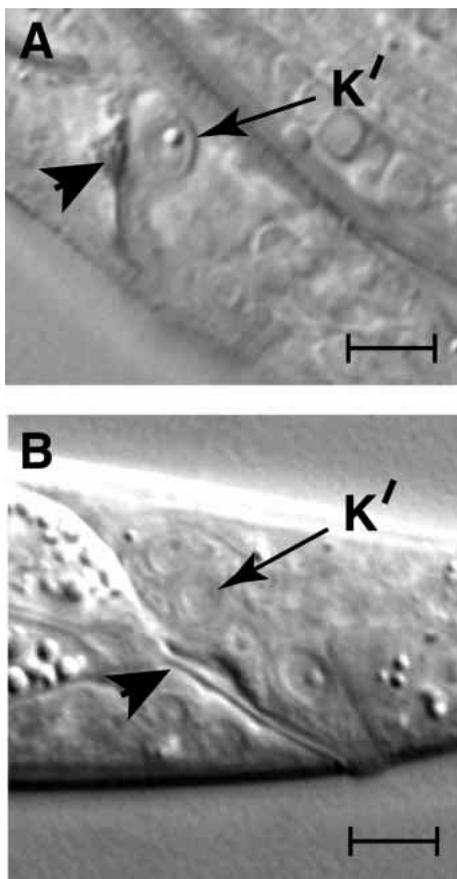
**Fig. 5.** *lrp-1* affects the rectum. (A) Nomarski view of an enlarged K′ cell (arrow) and of refractile material in the rectum (arrowhead) of a mid-L3 larva from MH210. (B) A wild-type rectum is present in a non-mutant L3 larva from MH210. Scale bars, 5 μm.



**Fig. 6.** Parts of the early cell lineage in which inheritance of wild-type *lrp-1* DNA can correct the mutant phenotype. Data for 15 mosaics that lacked features of the Lrp-1 mutant phenotype are summarized schematically with respect to the early cell lineage and the early progenitors of the hyp7 syncytium (Sulston et al., 1983). Each of the 14 squares represents one animal and depicts the only cell that established a genotypically wild-type clone of cells in that animal. An additional animal had two wild-type clones (circles), one of which (ABal) should not have contributed to the hyp7 syncytium.

## Mosaic analysis suggests that the epidermal syncytium hyp7 is the focus of *lrp-1* activity

An analysis of mosaics, individuals having both genotypically wild-type and genotypically mutant cells (Herman, 1995), permitted a genetic assessment of the cells that require the function of *lrp-1*. Spontaneous mosaics in which an extrachromosomal array containing wild-type *lrp-1* DNA failed to disjoin during embryogenesis, were identified among the progeny of a strain, MH1066, having the genotype *lrp-1(ku156); him-8(e1489); kuEx76[SUR-5GFP(NLS); lrp-1(+)]*. Inheritance of the array by AB, $P_1$, ABarp, ABpla, $P_2$, or Ca is sufficient for rescue of all aspects of the mutant phenotype (Fig. 6). In particular, the rectal defects are corrected even if the rectal cells themselves fail to inherit the array. Although other arguments can be put forth (for instance, inheritance of *lrp-1* activity by any cell might be sufficient), inheritance by hyp7 is the only lineal commonality of the mosaics, suggesting that this syncytium, which is formed by the fusion of cells that descend from ABa, ABp, and C (a granddaughter of $P_1$) (Sulston et al., 1983), is the focus of the gene's activity (see also Herman and Hedgecock, 1990).

A maternal contribution of wild-type *lrp-1* activity could explain the ability of most *lrp-1* homozygotes that segregate from heterozygous mothers to develop to the L3 to L4 molt. Furthermore, a member of the LDL receptor family might be
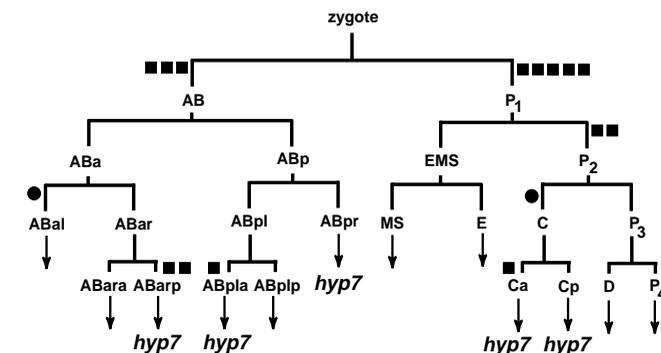
expected to function in the endocytosis of yolk proteins by oocytes. In order to test for a maternal contribution, mosaics in which the germ line did not inherit the *kuEx76* array were examined for the production of progeny. In all four cases, progeny were produced, and they exhibited a phenotype indistinguishable from that of the homozygotes segregating from heterozygotes. Thus, activity of *lrp-1* is not absolutely required for endocytosis of yolk proteins, nor is it absolutely required for embryonic development. An incompletely expressed effect on embryogenesis cannot be excluded, however, because MH1066 contains a *him-8* mutation that results in death of embryos (Hodgkin et al., 1979), and these could have masked embryonic lethality caused by the germ-line mosaicism.

## LRP-1 is present on the apical surface of hyp7

By means of monoclonal antibodies that had been produced against the carboxyl terminus, expression of the *lrp-1* gene product was investigated in mixed-stage populations of N2 worms using conditions (Bettinger et al., 1996) in which 99% of whole mounts stained with both anti-LRP-1 antibodies and MH27. Most of the staining is punctate, has an appearance consistent with circular or spheroidal aggregates of the protein, and is evident only in hyp6 and hyp7 (Fig. 7), two syncytia that compose most of the epidermis (White, 1988). Co-staining with MH27, an antibody that recognizes adherens junctions (Waterston, 1988; Podbilewicz and White, 1994), demonstrates that the staining is confined to the dorsal and ventral ridges and to wider sections that are adjacent to the lateral seam cells, which themselves fail to stain. Therefore, the regions of staining correspond to the thick regions of this epidermis (with respect to a cross-sectional view of worms); staining is absent (Fig. 7) from longitudinal bands that correspond to the thin regions of hyp7 to which body muscles attach (White, 1988). The staining is evident in both sexes at hatching, in each of the four larval stages, and in adults. The staining is often brighter in hyp6 in L1 larvae and in the posterior part of hyp7 in adult males. Wild-type worms that

have been starved of bacteria for 4 days also stain, albeit less intensely than unstarved worms.

Staining of segregants from a strain, MH210 (*lrp-1(ku156)/gld-1(q266)*), demonstrated that the epitope(s) of 1H6 and of 4H5 are not present in fixed *ku156* homozygotes. In contrast to the N2 strain, 26% of 137 segregants completely lacked the LRP-1 pattern but did stain with the MH27 antibody, demonstrating that they were fixed and permeable to antibodies. Thus, the proportion not staining agrees with the predictions of Mendelian genetics. Moreover, 14 segregants could be unambiguously identified as *lrp-1* homozygotes because they had refractile material in their recta. Each of these completely failed to stain with the anti-LRP-1 antibodies but did stain with MH27 (Fig. 7). The 1H6 and 4H5 monoclonal antibodies are therefore specific for the LRP-1 protein, and the absence of staining of the homozygotes suggests that *ku156* is a likely null mutation, in agreement with the genetic tests.

Because MH27 recognizes an epitope associated with zonulae adherens that form at the apical junctions of polarized epithelia such as hyp6, hyp7, and the lateral seam cells (Waterston, 1988; Podbilewicz and White, 1994), a final conclusion is possible: location of >90% of the LRP-1 staining to the same focal plane as the MH27 staining demonstrates that LRP-1 is located in the apical region of hyp6 and hyp7 (Fig. 7). In particular, the basolateral surfaces are never seen to stain. Thus, the resemblance between LRP-1 and mammalian megalin extends to their localization at the apical surface of polarized epithelia.

## Sterol starvation can phenocopy *lrp-1* mutations

The resemblance of LRP-1 to mammalian megalin suggests that the worm protein endocytoses sterols like cholesterol. Because sterols are an essential nutrient for *C. elegans* (Chitwood, 1992), a

failure of *lrp-1* mutants to endocytose sterols could explain the critical nature of the mutant phenotype. Wild-type N2 worms growing on medium containing a suboptimal level of sterols were therefore examined for any resemblance to the mutant phenotype. After transfer to deficient medium, about 5% of
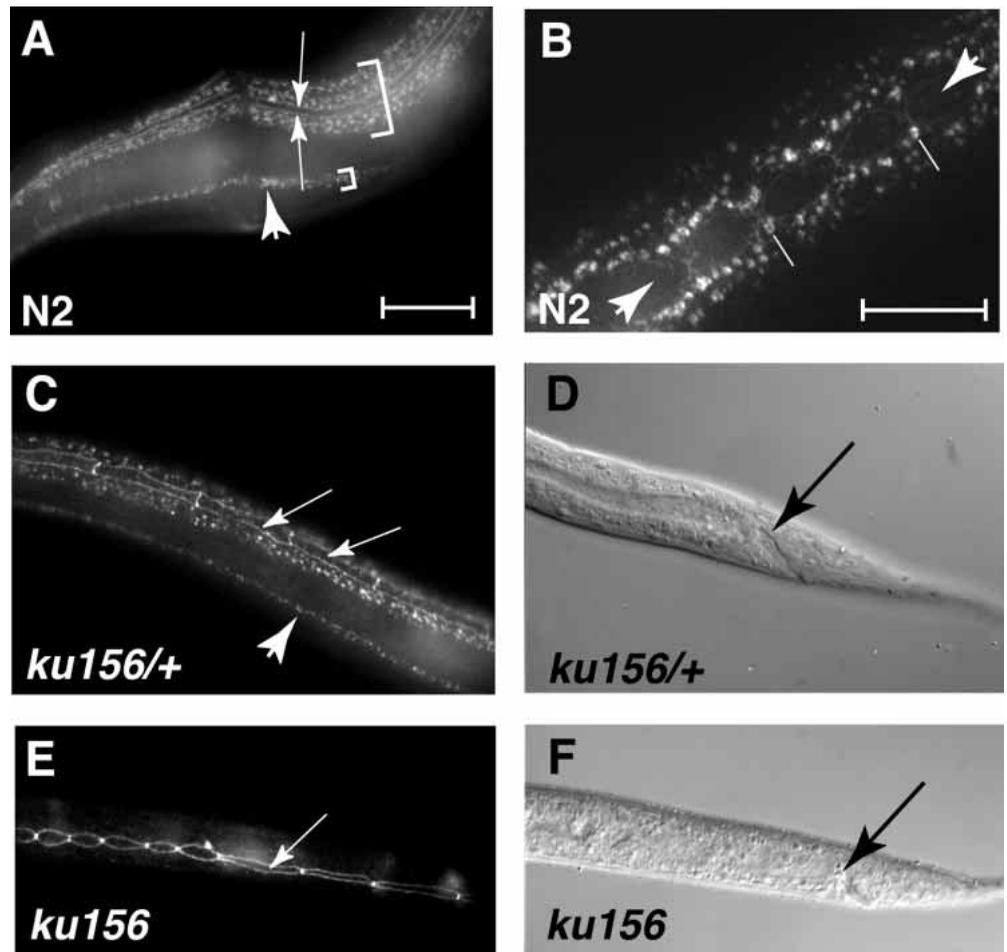


**Fig. 7.** Expression of the *lrp-1* gene product in apical regions of hyp7. Each specimen has been stained for LRP-1 with the 1H6 and 4H5 monoclonal antibodies. Except for B, each has also been stained for adherens junctions with MH27. All fluorescence micrographs represent the extreme left focal plane. (A) Bands of apical, punctate staining of LRP-1 are present along the entire length of hyp7 (only an anterior section is shown) in an adult N2 hemaphrodite. Two wide bands of the LRP-1 staining (large bracket) flank MH27 staining of the boundary between hyp7 and the lateral seam syncytium (arrows) (compare with E for an MH27 pattern in the absence of the LRP-1 pattern). A small bracket depicts the band of LRP-1 staining in the ventral ridge of hyp7; also shown is MH27 staining of the junction between the excretory pore and hyp7 (arrowhead). Note the absence of staining in the zone to which body muscles attach (between the brackets). (B) The LRP-1 pattern in the midbody of an N2 hermaphrodite in the absence of staining with MH27. The circular nature of the punctate staining is particularly evident (thin lines). Based on staining of the dorsal and ventral ridges, in focal planes not shown, the central zone (between arrowheads) that lacks staining corresponds to lateral seam cells. (C) LRP-1 staining in hyp7 on either side of the lateral seam in the midbody of a non-mutant L2 segregant from MH210. Co-staining with MH27 reveals the apical junctions of the lateral seam, two cells of which are indicated (arrows). LRP-1 staining in the ventral ridge of hyp7 is also evident (arrowhead). (D) An interior view with Nomarski optics of a more posterior region of the same animal as in C, demonstrating a lack of refractile material in the rectum. (E) A mutant L2 segregant of MH210 lacks LRP-1 staining. MH27 staining of the apical junctions between hyp7 and the lateral seam cells, one of which is indicated with an arrow, demonstrates that the specimen was permeable to antibodies. (F) An interior view with Nomarski optics of a more posterior region of the same animal as in E, revealing refractile material in the rectum, indicating homozygosity of the *lrp-1* mutation. Scale bars (A,B) 20 μm; (C-F) same scale as A.

larvae of the second generation but none of the first generation were Dumpy, had problems shedding old cuticle during molts, and sometimes had refractile material in their recta (Fig. 4D). In addition, some adults of the second generation exhibited an abnormal loopy movement of their bodies and were unable to move effectively. (Similar behavior is occasionally displayed by adult segregants of transgenic lines in which the *szT1* or *ku156* mutations are rescued with extrachromosomal arrays. This congruence may indicate that the mutations were incompletely rescued by the array in these animals.) In contrast, N2 worms that had been grown on otherwise identical medium that had been supplemented with 50 µg/ml cholesterol lacked all of these defects. Although the starvation was not absolute (worms continued to propagate at half the rate of control populations on cholesterol-supplemented medium) and two generations were required before effects were evident, the ability of the starvation to cause the same defects as the Lrp-1 mutant phenotype leaves open the possibility that LRP-1 endocytoses sterols.

## DISCUSSION

Genetic, molecular, and immunocytochemical data indicate that null mutations have been isolated in the *lrp-1* gene. A striking effect of these mutations is an inability to shed and degrade all of the old cuticle during larval molts. The cuticle is a collagen-rich exoskeleton that functions as a barrier to the environment (Kramer, 1997), and an inability to degrade it is consistent with a perturbation of extracellular proteolysis. Perhaps the extracellular part of LRP-1 is required for activiation of collagenase or other proteases that might be secreted during molts. Alternatively, LRP-1 may be needed for proteolytic processing of procollagens or other components of the cuticle. Insufficient maturation of the precursors might produce a cuticle that is resistent to degradation and lacks pliability, rendering worms unable to move well. Aberrant synthesis of the cuticle could also account for the Dumpy phenotype (Kramer, 1997). Because homozygotes that have managed to shed most of the old cuticle are also Dumpy, it might be an intrinsic property of the newly synthesized cuticle.

The presence of LRP-1 on the apical surface of hyp6 and hyp7 is completely consistent with the failure of the mutants to shed and degrade old cuticle. The hyp7 syncytium composes most of the epidermis (also called the hypodermis) of *C. elegans* (White, 1988). (Because the much smaller hyp6 syncytium fuses with hyp7 during the L2 to L3 molt (Yochem et al., 1998), for simplicity only hyp7 will be emphasized.) The apical plasma membrane of hyp7 is the outermost plasma membrane for most of the length of the body, and from this apical membrane is secreted the components for most of the body cuticle (Kramer, 1997). Before a cycle of endocytosis is initiated, the large extracellular part of LRP-1 must therefore be located just beneath the overlying cuticle or in contact with it. A cycle of endocytosis from the apical surface of hyp7 should also be associated with coated pits and endocytotic vesicles; in the case of mammalian megalin, the latter are particularly large multivesicular bodies that have been termed apical vesicles (Chatelet et al., 1986; Willnow et al., 1996). Thin-section electron microscopy of *C. elegans* has revealed large multivesicular bodies in the syncytial epidermis that are similar in appearance to apical vesicles (White, 1988); detection of these vesicles by the anti-LRP-1 antibodies would be a straight-forward interpretation of the punctate, circular pattern of staining. Thus, although LRP-1 has not been proved to be a receptor, the available data suggest that it functions as such in a location that is consistent with the mutant phenotype and with the proposal that one function of this class of receptor is for the regulation of extracellular proteolysis (Krieger and Herz, 1994; Strickland et al., 1995; Willnow et al., 1996).

Perhaps a more likely explanation for the mutant phenotype, however, is indicated by the effect of sterol starvation: the fact that starvation phenocopies the mutations suggests that LRP-1 endocytoses dietary sterols like cholesterol. The presence of LRP-1 in the apical region of hyp7 may appear at first glance to be inconsistent with such a function, as the sterols would have to pass through the cuticle before encountering the receptor. Experiments with the large parasite *Ascaris suum* have, however, indicated that nematodes primarily absorb sterols through the epidermis rather than through the intestine (Fleming and Fetterer, 1984). The presence of LRP-1 in the apical region of a polarized epithelium may therefore serve the same function as the major one deduced for mammalian megalin: the endocytosis of cholesterol from extensive fluids that are external to these epithelia (Willnow et al., 1996).

Although propagation of *C. elegans* is known to require dietary sterols (Chitwood, 1992), the morphological effects of sterol deprivation have not been previously reported. Sterol starvation of other nematodes has been reported to cause incomplete molts (Bottjer et al., 1984; Coggins et al., 1985), but a comparison with the Lrp-1 mutant phenotype is difficult because the effects on molting were not described in detail. As was the case described here for *C. elegans*, sterol starvation of the free-living nematodes *Panagrellus redivivus* and *C. briggsae*, a close relative of *C. elegans*, required two generations before arrest of growth (Hieb and Rothstein, 1968; Bottjer et al., 1985). This lag is at variance with the manifestation of the Lrp-1 phenotype in homozygotes as they segregate from heterozygous mothers. One explanation is that sterols had not been sufficiently eliminated from the media; it is difficult to starve nematodes of sterols because microbiological media are often contaminated with them, and nematodes can convert plant or yeast sterols to useful forms, including cholesterol (Chitwood, 1992). It is possible that the first generation were unaffected because of a combination of a maternal supply in the eggs from which they arose and an ability to scavenge contaminating sterols from the medium. The level of contaminating sterols may have been too low, however, for an adequate maternal contribution to the next generation.

Because it is not known why sterols are essential for nematodes (Chitwood, 1992), interpretation of the effect of the deprivation on molting remains speculative. Perhaps a lack of cholesterol in cell membranes renders worms too weak to complete the process. Also, degradation of old cuticle may be activated by a developmental signal that requires a cholesterol modification such as occurs for the *Drosophila* protein hedgehog (Porter et al., 1996). Another possiblity is that a steroid hormone such as 20-hydroxyecdysone regulates molting in *C. elegans*. It is interesting to note in this regard that 'RNA interference' (Fire et al., 1998) of CHR3, an orphan nuclear hormone receptor, results in a failure to shed and

degrade all of the old cuticle during molts (Kostrouchova et al., 1998). CHR3 closely resembles DHR3, a *Drosophila* protein that affects metamorphosis and is induced upon treatment with 20-hydroxyecdysone (Lam et al., 1997; White et al., 1997). If expression of CHR3 also requires ecdysone, a failure of *lrp-1* mutants to endocytose sterols could produce the same phenotype. It is controversial, however, whether normal molting of nematodes is regulated by ecdysone, and production of ecdysone from cholesterol has yet to be demonstrated for any nematode (Chitwood, 1992). By what ever means, the genetic mosaic analysis of *lrp-1* is consistent with signaling between cells, because the rectal defects were corrected in mocaics even when the rectal cells themselves failed to inherit the wild-type gene.

The effects of sterol starvation on nematodes suggests that LRP-1 endocytoses dietary sterols that are needed for an unknown function during molting. Nevertheless, the alternative must be considered that the effects of the starvation arise from perturbation of a component that acts in parallel to or in conjuction with LRP-1 during molts. Also, it is not clear why the structures of LRP-1 and mammalian megalin are so closely conserved if they are only required for the uptake of sterols. We have begun a genetic approach to the question of function by isolating mutations in other genes that confer the same phenotype. Also, mutations in the genes *let-454*, *let-462*, and *let-473* have been mentioned as affecting molting (Johnsen and Baillie, 1991). The analysis and cloning of these and further analysis of CHR3 (Kostrouchova et al., 1998) may also help reveal the process in which LRP-1 functions and why this process is essential for growth and completion of molting.

# REFERENCES

**Bettinger, J. C., Lee, K. and Rougvie, A. E.** (1996). Stage-specific accumulation of the terminal differentiation factor LIN-29 during *Caenorhabditis elegans* development. *Development* **122**, 2517-2527.

**Bottjer, K. P., Weinstein, P. P. and Thompson, M. J.** (1984). Effects of azasteroids on growth and development of the free-living stages of *Nippostrongylus brasiliensis* and *Nematospiroides dubius*. *Comp. Biochem. Physiol.* **78B**, 805-811.

**Bottjer, K. P., Weinstein, P. P. and Thompson, M. J.** (1985). Effects of an azasteroid on growth, development and reproduction of the free-living nematodes *Caenorhabditis briggsae* and *Panagrellus redivivus*. *Comp. Biochem. Physiol.* **82B**, 99-106.

**Brenner, S.** (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

**Chatelet, F., Brianti, E., Ronco, P., Roland, J. and Verroust, P.** (1986). Ultrastructural localization by monoclonal antibodies of brush border antigens expressed by glomeruli. II. Extrarenal distribution. *Am. J. Pathol.* **122**, 512-519.

**Chitwood, D. J.** (1992). Nematode sterol biochemistry. In *Physiology and Biochemistry of Sterols* (ed. G. W. Patterson and W. D. Nes). pp. 257-293. American Oil Chemists' Society, Champaign, IL.

**Coggins, J. R., Schaefer III, F. W. and Weinstein, P. P.** (1985). Ultrastructural analysis of pathologic lesions in sterol-deficient *Nippostrongylus brasiliensis* larvae. *J. Invertebr. Pathol.* **45**, 288-297.

**Edgley, M. L., Bailie, D. L., Riddle, D. L. and Rose, A. M.** (1995). Genetic

**Ferguson, E. L. and Horvitz, H. R.** (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics* **110**, 17-72.

**Finney, M. and Ruvkun, G.** (1990). The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. *Cell* **63**, 895-905.

**Fire, A., Xu, S. Q., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C.** (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811.

**Fleming, M. W. and Fetterer, R. H.** (1984). *Ascaris suum*: continuous perfusion of the pseudocoelom and nutrient absorption. *Exp. Parasitol.* **57**, 142-148.

**Fodor, A. and Deàk, P.** (1985). The isolation and genetic analysis of a *Caenorhabditis elegans* translocation (*szT1*) strain bearing an *X*-chromosome balancer. *J. Genet.* **64**, 143-157.

**Francis, R., Barton, M. K., Kimble, J. and Schedl, T.** (1995). *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. *Genetics* **139**, 579-606.

**Herman, R. K.** (1995). Mosaic analysis. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism* (ed. H. F. Epstein and D. C. Shakes). pp. 123-146. Academic Press, San Diego.

**Herman, R. K. and Hedgecock, E. M.** (1990). Limitation of the size of the vulval primordium of *Caenorhabditis elegans* by *lin-15* expression in surrounding hypodermis. *Nature* **348**, 169-171.

**Hieb, W. F. and Rothstein, M.** (1968). Sterol requirement for reproduction of a free-living nematode. *Science* **160**, 1778-1780.

**Hodgkin, J. A., Horvitz, H. R. and Brenner, S.** (1979). Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67-94.

**Johnsen, R. C. and Baillie, D. L.** (1991). Genetic analysis of a major segment [*LGV*(left)] of the genome of *Caenorhabditis elegans*. *Genetics* **129**, 735-752.

**Kostrouchova, M. K., Krause, M., Kostrouch, Z. and Rall. J. E.** (1998). CHR3: a *Caenorhabditis elegans* orphan nuclear hormone receptor required for proper epidermal development and molting. *Development* **125**, 1617-1626.

**Kramer, J. M.** (1997). Extracellular matrix. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 471-500. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

**Krieger, M. and Herz, J.** (1994). Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu. Rev. Biochem.* **63**, 601-637.

**Lackner, M. R., Kornfeld, K., Miller, L. M., Horvitz, H. R. and Kim, S. K.** (1994). A MAP kinase homologue, *mpk-1*, is involved in ras mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev.* **8**, 160-173.

**Lam, G. T., Jiang, C. and Thummel, C. S.** (1997). Coordination of larval and prepupal gene expression by the DHR3 orphan receptor during *Drosophila* metamorphosis. *Development* **124**, 1757-1769.

**McKim, K. S., Howell, A. M. and Rose, A. M.** (1988) The effects of translocations on recombination frequency in *Caenorhabditis elegans*. *Genetics* **120**, 987-1001.

**McKim, K. S., Starr, T. and Rose, A. M.** (1992). Genetic and molecular analysis of the *dpy-14* region in *Caenorhabditis elegans*. *Mol. Gen. Genet.* **233**, 241-251.

**Mello, C. and Fire, A.** (1995). DNA transformation. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism* (ed. H. F. Epstein and D. C. Shakes). pp. 451-482. Academic Press, San Diego.

**Podbilewicz, B. and White, J. G.** (1994). Cell fusions in the developing epithelia of *C. elegans*. *Dev. Biol.* **161**, 408-424.

**Porter, J. A., Young, K. E. and Beachy, P. A.** (1996). Cholesterol modification of hedgehog signaling proteins in animal development. *Science* **274**, 255-259.

**Riddle, D. L. and Albert, P. S.** (1997). Genetic and environmental regulation of dauer larva development. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess). pp. 739-768. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

**Rosenbluth, R. E., Cuddeford, C. and Baillie, D. L.** (1985) Mutagenesis in *Caenorhabditis elegans*: II. A spectrum of mutational events induced with 1500 R of gamma-radiation. *Genetics* **109**, 493-511.

**Saito, A., Pietromonaco, S., Loo, A. K.-C. and Farquhar, M. G.** (1994). Complete cloning and sequencing of rat gp330/'megalin,' a distinctive

**balancers.** In *Caenorhabditis elegans: Modern Biological Analysis of an Organism* (ed. H. F. Epstein and D. C. Shakes), pp. 147-184. Academic Press, San Diego.

member of the low density lipoprotein receptor gene family. *Proc. Natl. Acad. Sci. USA* **91**, 9725-9729.

**Singh, R. N. and Sulston, J. E.** (1978). Some observations on moulting in *Caenorhabditis elegans*. *Nematologica* **24**, 63-71.

**Smith, D. B. and Johnson, K. S.** (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione *S*-transferase. *Gene* **67**, 31-40.

**Stefansson, S., Chappell, D. A., Argraves, K. M., Strickland, D. K. and Argraves, W. S.** (1995). Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B100. *J. Biol. Chem.* **270**, 19417-19421.

**Strickland, D. K., Kounnas, M. Z. and Argraves, W. S.** (1995). LDL receptor-related protein: a multiligand receptor for lipoprotein and proteinase catabolism. *FASEB J.* **9**, 890-898.

**Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N.** (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.

**Waterston, R. H.** (1988). Muscle. In *The Nematode Caenorhabditis elegans* (ed. W. B. Wood). pp. 281-335. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

**Waterston, R. H. and Brenner, S.** (1978). A suppressor mutation in the nematode acting on specific alleles of many genes. *Nature* **275**, 715-719.

**Waterston, R. and Sulston, J.** (1995). The genome of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **92**, 10836-10840.

**White, J.** (1988). The anatomy. In *The Nematode Caenorhabditis elegans* (ed. W. B. Wood). pp. 81-122. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

**White, K. P., Hurban, P., Watanabe, T. and Hogness, D. S.** (1997). Coordination of *Drosophila* metamorphosis by two ecdysone-induced nuclear receptors. *Science* **276**, 114-117.

**Willnow, T. E., Hilpert, J., Armstrong, S. A., Rohlmann, A., Hammer, R. E., Burns, D. K. and Herz, J.** (1996). Defective forebrain development in mice lacking gp330/megalin. *Proc. Natl. Acad. Sci. USA* **93**, 8460-8464.

**Yochem, J. and Greenwald, I.** (1993). A gene for a low density lipoprotein receptor-related protein in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U S A* **90**, 4572-4576.

**Yochem, J., Gu, T. and Han, M.** (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics* **149**, 1323-1334.