Nuclear receptor NHR-25 is required for cell-shape dynamics during epidermal differentiation in *Caenorhabditis elegans*

Marie Šilhánková[1,2], Marek Jindra[1,3] and Masako Asahina[2,*]

[1]Department of Molecular Biology, Faculty of Biological Sciences, University of South Bohemia, CZ – 370 05, Czech Republic
[2]Institute of Parasitology and [3]Institute of Entomology ASCR, České Budějovice, 37005 Czech Republic
*Author for correspondence (e-mail: masako@paru.cas.cz)

Accepted 26 October 2004
Journal of Cell Science 118, 223-232 Published by The Company of Biologists 2005
doi:10.1242/jcs.01609

Summary

Epithelial cell shape changes underlie important events in animal development. During the postembryonic life of the nematode *Caenorhabditis elegans*, stem epidermal seam cells lose and actively renew mutual adherens junction contacts after each asymmetric division that separates them. The seam cell contacts are important for epidermal differentiation, but what regulates the cell-shape changes that restore them is unknown. Here, we show that NHR-25, a transcription factor of the nuclear receptor family, is expressed in the seam cells and is necessary for these cells to elongate and reach their neighbors after the asymmetric divisions. A failure to do so, caused by *nhr-25* RNA interference, compromises the subsequent fate of seam-cell anterior daughters. Unexpectedly, the lack of cell-cell contacts does not prevent a unique seam cell to produce a neuroblast, even though a homeotic gene (*mab-5*) that normally prevents the neuroblast commitment is ectopically expressed in the absence of *nhr-25* function. Seam cells lacking mutual contacts display reduced expression of a Fat-like cadherin marker *cdh-3::gfp*. Although some seam cells retain the ability to fuse at the final larval stage, the resulting syncytium shows gaps and bifurcations, translating into anomalies in cuticular ridges (alae) produced by the syncytium. *nhr-25* RNAi markedly enhances branching of the alae caused by a mutant cuticular collagen gene *rol-6*. Silencing of *nhr-25* also disturbs epidermal ultrastructure, which is probably the cause of compromised cuticle secretion and molting. Cell shape dynamics and molting thus represent distinct roles for NHR-25 in epidermal development.

Key words: *Caenorhabditis elegans*, Epidermis, Cell motility, Cell fate, Nuclear receptor, NHR-25/FTZ-F1

Introduction

Developmental processes involve shape changes of epithelial cells. These cellular movements depend upon cytoskeletal actin and cell-adhesion molecules, and are regulated by conserved pathways. In the nematode *Caenorhabditis elegans*, the best-studied process of epithelial cell motility is the embryonic ventral closure (Chin-Sang and Chisholm, 2000; Simske and Hardin, 2001). Considerably less is known about the regulation of cellular movements that shape *C. elegans* epidermis during the postembryonic development.

The postembryonic epidermis of *C. elegans* offers an excellent model to study dynamic modulation of cell morphology, cell contacts and cell fusion. The worm epidermis consists of the hyp7 syncytium, hyp cells of the head and tail, the ventral P cells and the lateral seam cells. Ten seam cells initially present on each side of a freshly hatched worm serve as stem cells, dividing during the four larval stages (L1-L4) to generate epidermal cells that join the hyp7 syncytium, neuroblasts and additional seam cells (Sulston and Horvitz, 1977) (Fig. 1). During the L1 stage, seam cells V1-V6 are connected via adherens junctions to the next seam cell in the row and to six pairs of the P cells. The initial V-cell division produces anterior daughters that first separate the pairs of P cells and then fuse with the hyp7 syncytium, thus creating gaps between seam cells. The seam cells elongate in the anteroposterior direction to re-establish adherens junctions with their neighbors (Austin and Kenyon, 1994; Podbilewicz and White, 1994). The cycle of loss and reconstitution of contacts between adjacent seam cells reiterates once per larval stage, whenever newborn cells fuse with the hyp7 syncytium.

Regaining contacts between seam cells is important for at least four reasons. First, reaching the contact is thought to be a signal for the seam cells to stop elongating (Austin and Kenyon, 1994). Second, cell-ablation experiments have shown that a unique seam cell, V5.p, requires its neighbors to produce a neuroblast (Sulston and White, 1980; Austin and Kenyon, 1994). Third, the integrity of the seam is crucial for the proper anchoring of vulval and uterine cells, and for the attachment of vulval muscles (i.e. conditions for the normal functioning of the egg-laying system) (for a review, see Michaux et al., 2001). Finally, after the connected seam cells cease dividing by the L4 stage, they fuse into a syncytium, which makes adult-specific cuticular ridges called alae. Understandably, gaps in the syncytium cause gaps in the alae (Singh and Sulston, 1978).

Regulation of the active process of seam-cell reconnection is poorly understood. We find that a transcription factor, the

nuclear receptor NHR-25, is responsible for the renewal of seam-cell contacts following the asymmetric cell division. RNA interference (RNAi) silencing of *nhr-25* blocks the elongation of seam cells that consequently remain isolated within the hyp7 syncytium. The lack of cell contacts caused by the loss of *nhr-25* function has profound consequences for the ensuing differentiation of the larval and adult epidermis. Although some of these consequences resemble the effects of seam-cell ablation (Sulston and White, 1980), the V5.p cell devoid of contacts with its neighbors surprisingly still gives rise to the anterior neuroblast.

Of the enormous family of 284 *C. elegans* nuclear receptors, NHR-25 is one of the few that have been ascribed a biological role, and is also one that is evolutionarily conserved (Asahina et al., 2000; Gissendanner and Sluder, 2000; Maglich et al., 2001). The mammalian NHR-25 counterpart SF-1/FTZ-F1 regulates steroid-hormone synthesis (Parker and Schimmer, 1997) and is essential for adrenal and male gonad development in mice (Luo et al., 1994; Sadovsky et al., 1995). In *Drosophila*, two FTZ-F1 protein isoforms play distinct developmental roles. Interaction of maternal αFTZ-F1 with a Hox protein [Fushi tarazu (FTZ)] is necessary for embryonic segmentation (Guichet et al., 1997; Yu et al., 1997; Suzuki et al., 2001; Yussa et al., 2001). The other isoform, βFTZ-F1, is a key regulator of the steroid-dependent replacement of the larval cuticle (molting) and of metamorphosis that transforms the larva into the pupa and the adult (Broadus et al., 1999; Yamada et al., 2000; Thummel, 2001).

NHR-25 has previously been implicated in several aspects of epidermal development in *C. elegans*, including embryogenesis, molting, dauer formation and the differentiation of the vulva (Asahina et al., 2000; Gissendanner and Sluder, 2000; Chen et al., 2004; Gissendanner et al., 2004; Hwang and Sternberg, 2004). Here, we show that NHR-25 plays an essential role in postembryonic epidermal-cell elongation and junction that ensure proper differentiation of seam cells and their epidermal descendants. Loss of *nhr-25* function also affects epidermal-cell morphology to the point that secretion of a new cuticle and consequently molting are compromised.

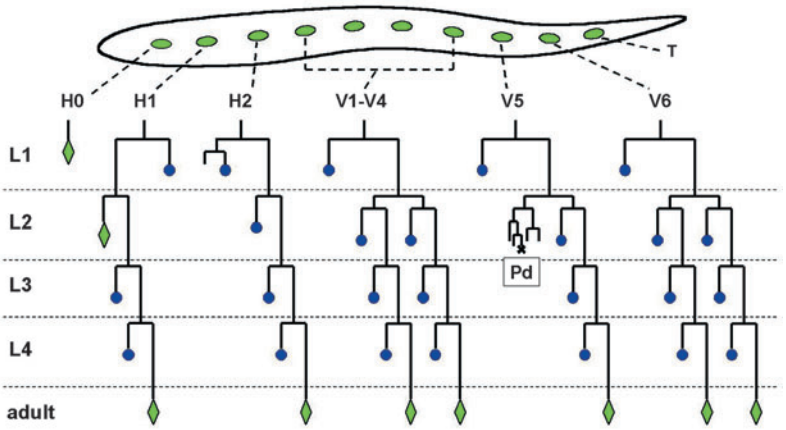

Fig. 1. A schematic drawing of seam-cell lineages. Ten seam cells in a freshly hatched worm (top) will produce 16 seam cells of the adult. The lineage of the tail seam cell T is not shown. Blue circles represent anterior daughters that fuse with the hyp7 syncytium and green diamonds denote stem seam cells. Pd indicates descendants of the V5.pa cell forming the postdeirid. Anterior is to the left. Based on Sulston and Horvitz (Sulston and Horvitz, 1977).

Materials and Methods

Nematode strains

C. elegans strains were reared at 20°C according to standard protocols (Brenner, 1974). The N2 variety Bristol was used as the wild type. The following transgenic strains were used.

GS2806 – *dpy-20(e1362)*; *arIs51[dpy-20(+)*; *cdh-3::gfp]* (Pettitt et al., 1996) (gift from I. Greenwald, Columbia University, NY).

RG242 – *veIs13[col-19::gfp*; *rol-6(su1006)]* (Abrahante et al., 1998) (gift from A. E. Rougvie, University of Minnesota, Minneapolis, MN).

JR667 – *unc-119(e2498::Tc1)*; *wIs51[unc-119(+)*; *scm::gfp]* (Terns et al., 1997) (from the *Caenorhabditis* Genetics Center).

SU93 – *jcIs1[ajm-1::gfp*; *unc-29(+)*; *rol-6(su1006)]* (Mohler et al., 1998) (from the *Caenorhabditis* Genetics Center).

CF237 – *muIs[mab-5::lacZ, unc-31(+)] unc-31(e169)* (Salser and Kenyon, 1992) (from the *Caenorhabditis* Genetics Center).

NL2099 – *rrf-3(pk1426)* (Simmer et al., 2002) (from the *Caenorhabditis* Genetics Center).

DNA transformation

An *nhr-25::gfp* construct encoding the first six amino acid residues of NHR-25 fused to green fluorescent protein (GFP) was prepared by fusing a region from a *Pst*I site 10,635 bp upstream of the *nhr-25* start codon (Asahina et al., 2000) with *gfp* in the pPD95.79 vector without the nuclear localization signal. Transformation was performed as described previously (Mello and Fire, 1995).

RNAi

A 900-bp *Xho*I-*Eco*RI fragment of an *nhr-25* cDNA clone yk342d8 (from Y. Kohara, National Institute of Genetics, Mishima, Japan) that excluded a sequence encoding the conserved DNA-binding domain was cloned into pBluescript KS$^-$ for double-stranded RNA (dsRNA) synthesis in vitro or the pPD129.36 vector (a gift from A. Fire, Carnegie Institution of Washington, Baltimore, MD) for dsRNA expression in *Escherichia coli* HT115 (a gift from D. Court, National Cancer Institute, Frederick, MD). For *nhr-23* RNAi, dsRNA was synthesized from a linearized vector 4666 (Kostrouchova et al., 2001). RNAi silencing by dsRNA injection into the adult gonad was done as described previously (Asahina et al., 2000); RNAi by feeding was performed essentially as described (Timmons et al., 2001). Expression of dsRNA in *E. coli* HT115, grown to an optical density at 600 nm (OD_{600}) of 0.4 in LB medium (Sambrook et al., 1989) containing 50 μg ml^{-1} ampicillin or carbenicillin and 12.5 μg ml^{-1} tetracycline, was induced for 4 hours with 0.4 mM isopropyl-β-D-thiogalactoside (IPTG). Bacteria were then concentrated by centrifugation and seeded on nematode growth medium (NGM; Brenner, 1974)/agarose plates containing both antibiotics and 0.4 mM IPTG. Worms were placed on the seeded plates and kept at 20°C. Bacteria carrying pPD129.36 without an insert were used for control.

Immunostaining

Eggs laid by control or RNAi-treated adults 14 hours after dsRNA injection were collected at 12-hour intervals. Larvae 0-24 hours after hatching were then subjected to immunostaining as described by Bossinger et al. (Bossinger et al., 2001). Worms were washed in distilled water, transferred onto slides coated with 0.1% poly-L-lysine, 0.2% gelatin, 0.4 mM potassium chromium (III) sulfate, squashed with a cover slip and frozen in liquid nitrogen. Samples were then fixed and rehydrated by the following steps: 10 minutes in 100%

methanol, 20 minutes in 100% acetone, 10 minutes in 90% ethanol, 10 minutes in 60% ethanol (all incubations at –20°C) and, finally, 10 minutes in 30% ethanol at room temperature. Slides were washed three times for 10 minutes each in TBT (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween-20). Incubations were done in TBT containing 1% bovine serum albumin and 1% skimmed milk at 4°C overnight for primary antibodies and 2-3 hours at room temperature for Alexa-488- or Cy3-conjugated secondary antibodies. A rabbit antiserum against a portion of the ligand-binding domain [amino acids 294-475 (Asahina et al., 2000)] of bacterially expressed NHR-25 protein was used at a dilution of 1:1000. The anti-DLG-1 antibody (kindly provided by O. Bossinger, Heinrich-Heine Universität, Düsseldorf, Germany) was diluted 1:400 and the MH27 antibody (Francis and Waterston, 1985) recognizing AJM-1 was diluted 1:500. Stained worms were observed using either the Zeiss LSM 410 laser-scanning microscope or with Zeiss Axioplan 2 equipped for epifluorescence.

β-Galactosidase detection

Worms were washed with distilled water, frozen in liquid nitrogen and vacuum dried for 45 minutes. Following incubation in acetone for 3 minutes at –20°C, worms were vacuum dried for 10 minutes. β-Galactosidase activity was detected by incubation at room temperature in 200 mM sodium phosphate (pH 7.5), 1 mM $MgCl_2$, 0.004% sodium dodecyl sulfate (SDS), 10 mM potassium ferricyanide/ferrocyanide, 75 μg ml^{-1} kanamycin, 0.04% X-gal. The reaction was terminated by washing with S-basal (100 mM NaCl, 50 mM potassium phosphate, 5 mg/l cholesterol) medium and samples were mounted in 90% glycerol. This procedure has been adopted from http://cobweb.dartmouth.edu/~ambros/worms/17.html.

Electron microscopy

Control and *nhr-25(RNAi)* adults were washed in S-basal medium and anesthetized in 8% ethanol. Worms were fixed in 0.2 M cacodylate buffer (pH 7.2) with 2.5% glutaraldehyde at 4°C for 4 hours followed by post-fixation in 2% OsO_4 at 4°C for 2 hours. For transmission electron microscopy (TEM), samples were dehydrated by an acetone series and embedded in Epon resin. Ultrathin sections were double stained with uranyl acetate and lead citrate, and viewed with a JEOL 1010 transmission electron microscope. For scanning electron microscopy (SEM), samples were subjected to critical-point drying, coated with gold and viewed with a JEOL 6300 scanning electron microscope.

Results

nhr-25 is required for the active renewal of contacts by seam cells

The lateral seam cells of the *C. elegans* epidermis are arranged in a row, with each seam cell being connected via adherens junctions to its anterior and posterior neighbor, and to the surrounding hyp7 syncytium. During each larval stage, the seam-cell contacts are interrupted by an asymmetric division of the V1-V6 seam cells, whose newborn anterior daughters fuse with the hyp7 syncytium, leaving temporary gaps (Austin and Kenyon, 1994) (Fig. 1). Staining of adherens junctions with antibodies against DLG-1 or AJM-1 (data not shown) shows that the V1.p-V6.p posterior daughters actively bridge the gaps to restore their contacts in normally developing L1 animals (Fig. 2A-D).

Because *nhr-25* is essential in the epidermis (Asahina et al., 2000), we tested its role in the dynamic changes of seam-cell shape. Worms were subjected to RNAi by injection of adult hermaphrodites with *nhr-25* dsRNA. L1 progeny stained shortly after hatching showed wild-type pattern of adherens junctions (Fig. 2E). Seam cells then divided and their daughters fused normally with the hyp7 syncytium, leaving the seam cells separated by gaps (Fig. 2F,G). However, the seam cells then failed to extend processes and remained isolated in nearly all of the *nhr-25(RNAi)* worms (Fig. 2H). Some animals displayed gaps between all seam cells whose parents produced a hyp daughter during the L1 division, others only between some of these cells, probably reflecting an uneven RNAi penetrance. Gaps never occurred between the head seam cells H0 and H1.a (Fig. 3C), because H1 produces its hyp daughter posteriorly (Fig. 1). Consequently, the H2.p seam cell was isolated, whereas H0 and H1.a stayed in contact throughout the L1 and L2 stages.

To see whether the observed cellular defects were specific to NHR-25, adherens junctions were stained in L1 worms

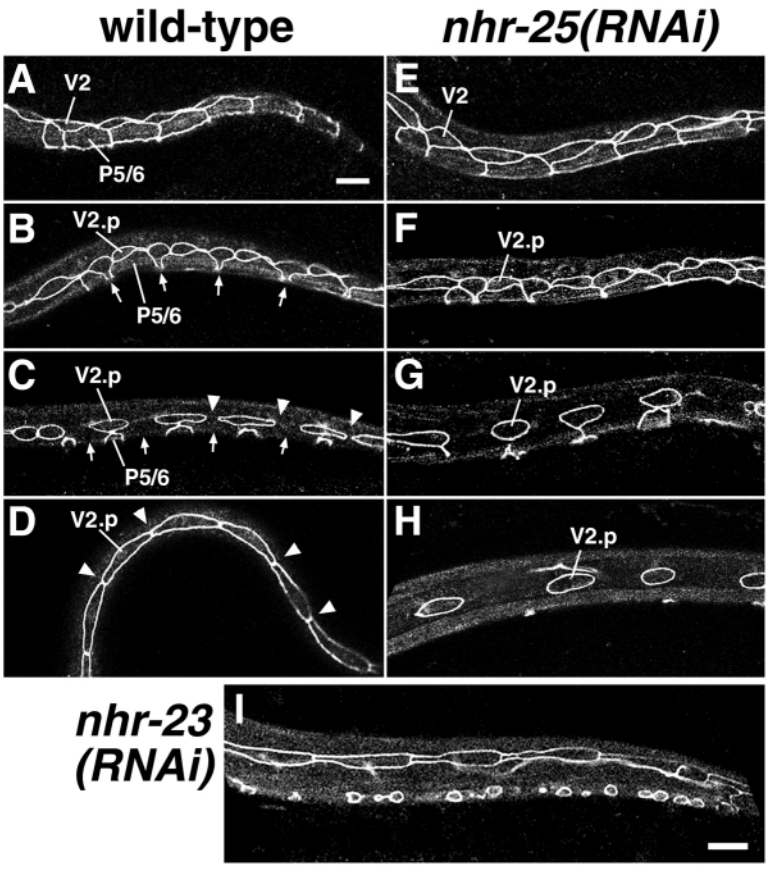

Fig. 2. Seam cells fail to restore contacts in *nhr-25(RNAi)* L1 worms. Confocal sections are shown with adherens junctions visualized by using the anti-DLG-1 antibody (Bossinger et al., 2001). The images were taken approximately 3 hours (A), 5 hours (B), 7 hours (C) and 9 hours (D) after hatching. Examples of V and P cells are shown. Anterior seam-cell daughters fusing with the hyp7 syncytium are indicated with arrows. Arrowheads mark seam-cell elongation and the restoration of their mutual contacts. Anterior is to the left, dorsal up. Bars, 10 μm; bar in A applies to all panels except I.

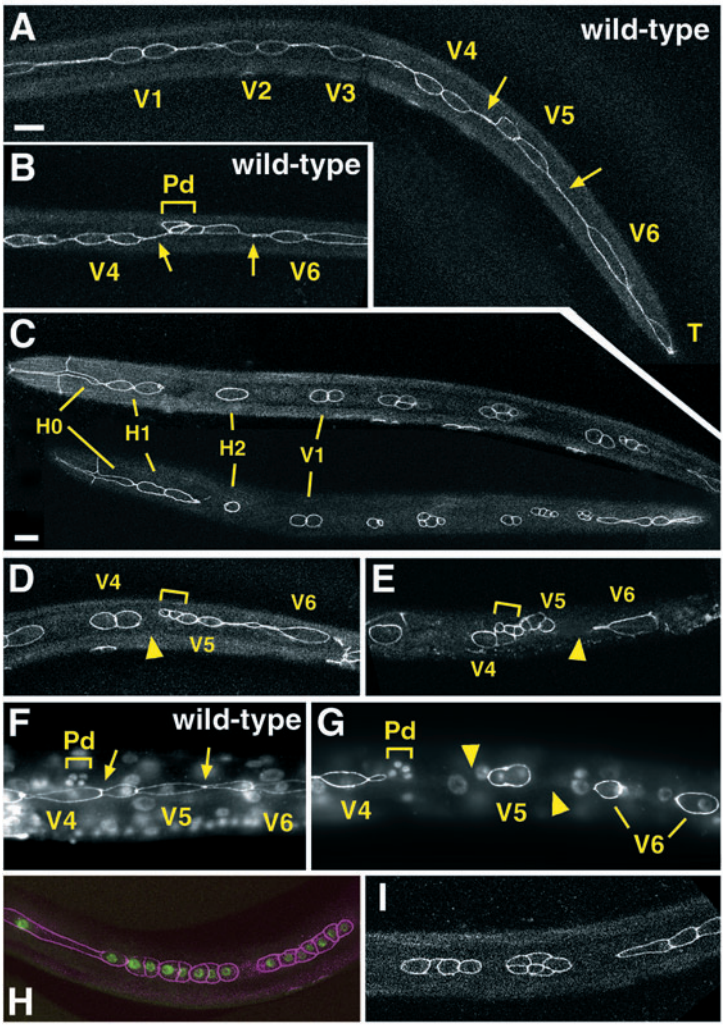

Fig. 3. The lack of cell-cell contacts in *nhr-25(RNAi)* L2 worms affects the fate of the seam-cell anterior daughters but not the ability of V5.p to produce a postdeirid neuron. Adherens junctions were stained with the anti-DLG-1 antibody. For simplicity, V cells are labeled with the names of their L1-stage ancestors. (A,B) In wild-type L2 worms, all seam cells remain in contact before and throughout their doubling division. V5.p produces an anterior neuroblast that is in the process of cell divisions, forming the future postdeirid (Pd). Contacts with descendants of V4.p and V6.p are shown by arrows. (C) In *nhr-25(RNAi)* animals, dividing seam cells remain round and isolated except for H1, whose contact with H0 is not interrupted because H1 had produced its hyp7 daughter towards the posterior. (D,E) The V5.pa daughters (brackets) divide and migrate dorsally in a postdeirid-like fashion regardless of the lack of contacts (arrowheads) with either V4.p (D) or V6.p (E) descendants in L2 *nhr-25(RNAi)* larvae. (F,G) Simultaneous propidium iodide and DLG-1 staining at the early L3 stage reveals the nuclei of the four postdeirid cells (Pd) and the normal adherens junctions (arrows) between V cells (F). The postdeirid structure differentiates normally in the absence of both anterior and posterior cell contacts of V5.p descendants (G, arrowheads) in *rrf-3(pk1426)*; *nhr-25(RNAi)* worms. (H,I) Superfluous seam cells in *nhr-25(RNAi)* L2 worms express the *scm::gfp* marker (nuclear signal in H) and might form clusters (I). In all images, anterior is to the left and dorsal up. Bars, 10 μm; bar in A applies to all panels except C.

following RNAi silencing of a functionally related nuclear receptor, NHR-23 (Kostrouchova et al., 1998; Kostrouchova et al., 2001). Although the expected *nhr-23(RNAi)* anomalies such as molting defects and dumpy (Dpy) resulted (not shown), silencing of *nhr-23* did not perturb the ability of seam cells to re-establish contacts after the asymmetric division and hyp cell fusion (Fig. 2I). Therefore, NHR-25 has a unique role in epidermal development.

Next, we asked whether the ability of seam cells to restore their contacts depends on a postembryonic effect of NHR-25 or whether *nhr-25* RNAi perturbs some earlier embryonic function. Seam cells normally regain their contacts within 1 hour after their anterior daughters have fused with hyp7 (Austin and Kenyon, 1994) (i.e. by 8 hours after hatching). RNAi applied by feeding freshly hatched larvae of the RNAi-sensitive *rrf-3(pk1426)* strain on *E. coli* expressing *nhr-25* dsRNA produced seam-cell contact phenotypes identical to those obtained by injection (e.g. Fig. 3G), indicating that NHR-25 is required after hatching.

Loss of *nhr-25* allows neuroblast differentiation in the absence of seam cell contacts

At the L2 stage, seam cells V1.p-V4.p and V6.p duplicate, whereas V5.p generates an anterior neuroblast (V5.pa) that in turn gives rise to a sensory organ composed of four cells, which will hereafter be referred to as the postdeirid (Fig. 1). Based on V-cell laser ablation experiments, postdeirid formation requires a physical contact of V5.p with both V6.p and V4.p or another anterior seam cell that can extend far enough to reach V5.p (Sulston and White, 1980; Austin and Kenyon, 1994). The killing of V6 leads to ectopic expression of the homeotic gene *mab-5* in V5.p and its descendants, which prevents postdeirid differentiation (Hunter et al., 1999). The absence of V5.p neighbors does not preclude postdeirid formation in *mab-5* mutant background (Austin and Kenyon, 1994).

Staining of the adherens junctions confirmed that during and immediately after the L2 doubling division all the seam and future postdeirid cells maintained contacts in wild-type worms (Fig. 3A,B). By contrast, in *nhr-25(RNAi)* animals, the Vn.p cells remained isolated during and after their duplication (Fig. 3C). Interestingly, the V5.pa cells in these animals divided in a postdeirid neuroblast manner regardless of the absence of V5.p contacts with V4.p or V6.p (Fig. 3D,E). By the time that the V cells underwent their next asymmetric division in early L3 control animals, the four postdeirid cells have lost adherens junctions (Fig. 3F). The same pattern of postdeirid formation was observed in *nhr-25(RNAi)* worms even when V5 descendants lacked contacts with both their anterior and their posterior neighbors (Fig. 3G). Thus, *nhr-25* silencing had a similar effect to *mab-5* mutation, in that it allowed the postdeirid to form in the absence of the cell-cell contacts. However, this effects was not due to the loss of *mab-5* expression, because a *mab-5::lacZ* construct was ectopically active in seam cells positioned anterior to V6.p in *nhr-25(RNAi)* worms (Fig. 4).

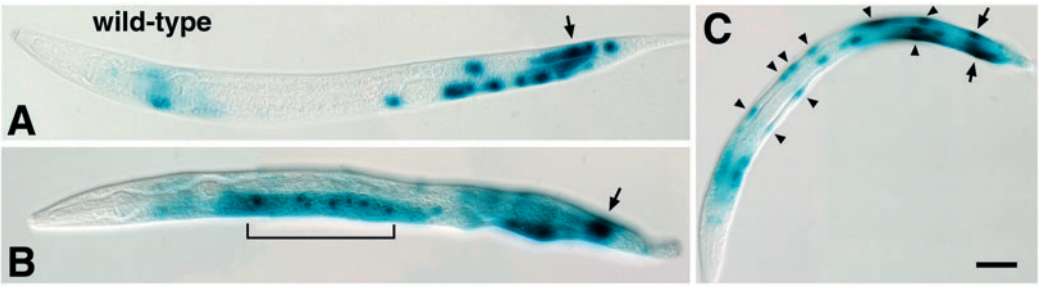

Fig. 4. A *mab-5::lacZ* reporter gene is ectopically expressed in seam cells anterior to V6.p in *nhr-25(RNAi)* L2 larvae. Bracket in (B) shows ectopic β-galactosidase activity in seam cells and their daughters. V6.p cells are indicated by arrows, arrowheads in (C) mark other seam cells on lateral sides. Additional staining comes from ventral P cells and neuroblasts. Anterior is to the left and dorsal up in (A,B); the animal in (C) is viewed from the ventral side. Tails of the *nhr-25(RNAi)* larvae show typical malformations. Bar, 20 μm.

Loss of *nhr-25* perturbs the fate of seam-cell daughters

Following the round of proliferative division at the L2 stage, the isolated couples of seam cells in *nhr-25(RNAi)* animals divided further to produce cells that should normally assume the hyp7 fate (Fig. 1). However, instead of fusing with the syncytium, the newborn cells remained attached to the Vn.p stem cells via abnormal anteroposterior and dorsoventral adherens junctions (Fig. 3C,H,I). The presumed hyp7 cells within these clusters continued to express a seam-cell-specific nuclear marker *scm::gfp* in the JR667strain (Fig. 3H) and could not be distinguished from the original seam cells. Some of the clusters contained more than the four cells normally generated by a single Vn.p during the L2 stage (Fig. 1), implying additional cell divisions of the incorrectly specified daughters (Fig. 3H,I).

Seam cells produce hyp7-fusing anterior daughters once per larval stage and thus need to elongate and reconnect periodically (Fig. 1). It was of interest to know whether the effect of *nhr-25* RNAi on cell-contact renewal was limited to L1 or whether it also affected later stages. By transferring 14-hour-old larvae from control bacteria to bacteria expressing *nhr-25* dsRNA, we applied RNAi about 6 hours after seam cells have regained their contacts (Austin and Kenyon, 1994). Loss of NHR-25 at this time thus could not affect seam-cell elongation following the first L1 division, yet it resulted in gaps between seam cells visible at the L4 stage (Fig. 5C). When worms were subjected to RNAi at 20 hours after hatching, gaps were observed in the adult seam (data not shown). These results suggested that *nhr-25* is required throughout development for the periodic restoration of mutual seam-cell contacts.

Effects of *nhr-25* RNAi on seam development

By the late L4 stage, seam cells had fused into a continuous syncytium in control animals (Fig. 5A). By contrast, gaps between seam cells prevented the formation of such an uninterrupted syncytium in *nhr-25(RNAi)* worms (Fig. 5B,C,E). Interestingly, the expression of a reporter derived from the *C. elegans cdh-3* cadherin gene (*cdh-3::gfp*) was reduced or undetectable in the isolated seam cells, in contrast to those of control animals (Fig. 5D,E). When some seam cells in *nhr-25(RNAi)* worms eventually fused, their incorrect alignment along the anteroposterior axis caused bifurcations of the syncytium (Fig. 5F). In addition, *nhr-25(RNAi)* adults contained on each lateral side up to 26 (20±3 on average, n=50) nuclei positive for the seam-cell-specific marker (Fig. 5H) as

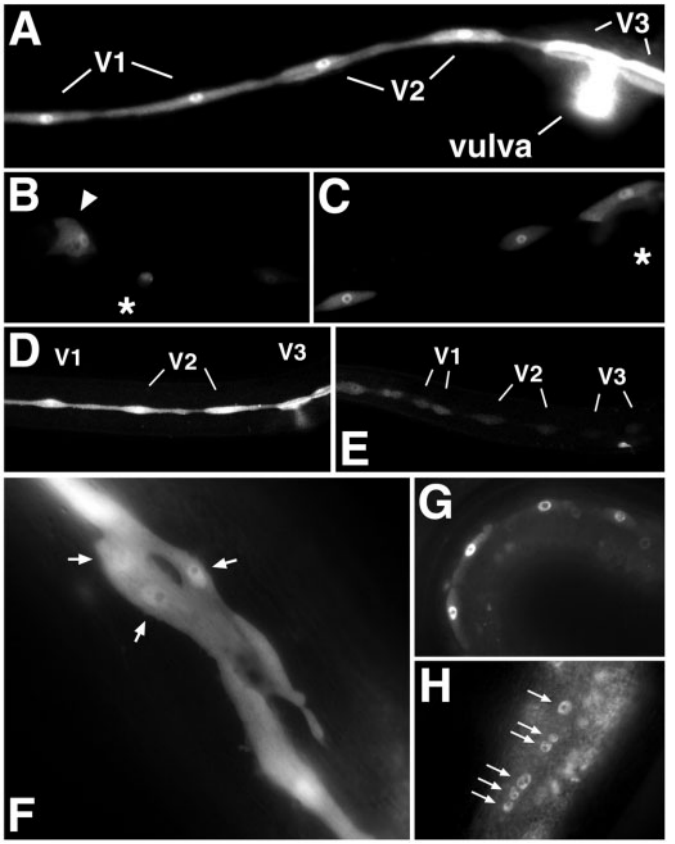

Fig. 5. *nhr-25* RNAi affects development of L4-adult seam epidermis and expression of a cadherin *cdh-3::gfp* reporter. (A) Direct GFP fluorescence of *cdh-3::gfp* reveals continuity of the seam and proper formation of the vulva in control L4 larvae. (B,C) Large gaps separate seam cells in worms subjected to *nhr-25* RNAi by feeding since hatching (B) or starting at 14 hours after hatching (C). Arrowhead points to a seam cell that failed to elongate in the anteroposterior direction, asterisks mark the positions where the vulva would normally develop. (D,E) Confocal images acquired under identical settings show that *cdh-3::gfp* expression is reduced or nearly undetectable in *nhr-25* dsRNA-fed L4 animals (E) compared with controls (D). (F) Fusion of incorrectly aligned seam cells in late L4 *nhr-25(RNAi)* larvae (obtained by dsRNA injection of their parents) causes bifurcations and loops in the syncytium, as revealed by *cdh-3::gfp*. Arrows point to three seam nuclei. (G,H) L4 progeny of adults injected with *nhr-25* dsRNA contain superfluous, irregularly spaced seam-cell nuclei marked by *scm::gfp* (H, arrows) compared with controls (G).

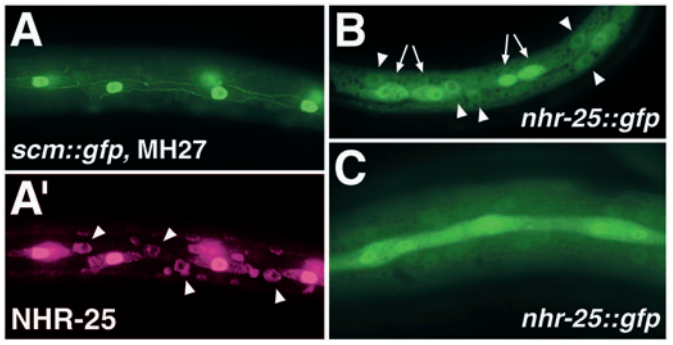

Fig. 6. NHR-25 is expressed in the seam cells throughout development. (A,A′) An antibody against NHR-25 stains nuclei of seam cells, marked with *scm::gfp* and outlined by MH27 antibody staining of adherens junctions in the same L1 larva. (B,C) Expression of an *nhr-25::gfp* construct in seam cells of L2 (B, arrows) and L4 animals. In all panels, anterior is to the left. Arrowheads point to hyp7 daughters of seam cells.

opposed to the 16±0.3 (n=50) nuclei in controls (Fig. 1). The superfluous nuclei were smaller than normal and were close to each other (Fig. 5H), suggesting that they arose by extra cell divisions of the incorrectly specified seam-cell daughters (Fig. 3H).

NHR-25 is expressed in the seam cells

The effects of *nhr-25* could be autonomous to seam cells if *nhr-25* is expressed there. To test this possibility, we used an antibody against the NHR-25 protein. This antibody stained the same nuclei lit by the *scm::gfp* seam-cell-specific marker at L1 (Fig. 6A) and later stages (data not shown). A weaker signal was observed in the nuclei of hyp7 cells produced by the first asymmetric division of the V cells. A transgenic *nhr-25::gfp* construct confirmed that *nhr-25* was expressed in the seam cells from L1 (not shown) until adulthood (Fig. 6B,C); consistent with the antibody staining, this construct was less active in the hyp7 daughters.

Adult cuticle secretion, epidermal ultrastructure and molting

The seam syncytium makes a cuticular structure called the alae, which consists of three continuous ridges on each side of the worm (Fig. 7A,C). Compared with controls, alae of *nhr-25(RNAi)* adults were shallow (Fig. 7B) and the middle ridge was interrupted (Fig. 7D). Gaps (Fig. 7E) and minor branching (data not shown) in *nhr-25(RNAi)* alae corresponded with gaps and bifurcations in the underlying seam syncytium, respectively (Fig. 5). Branching of the alae has been known to occur in mutants for certain genes, such as *rol-6*, which encodes a cuticular collagen (Cox et al., 1980; Thein et al., 2003). The *rol-6(su1006)* mutation is routinely used as a dominant visible marker for *C. elegans* transformation (Mello and Fire, 1995) and by itself caused moderate alae branching in about 47% of cases in two independent strains (Fig. 7F, Table 1). *nhr-25* RNAi in the *rol-6(su1006)* background dramatically enhanced the severity of branching (Fig. 7G) and increased its incidence to over 83% (Table 1).

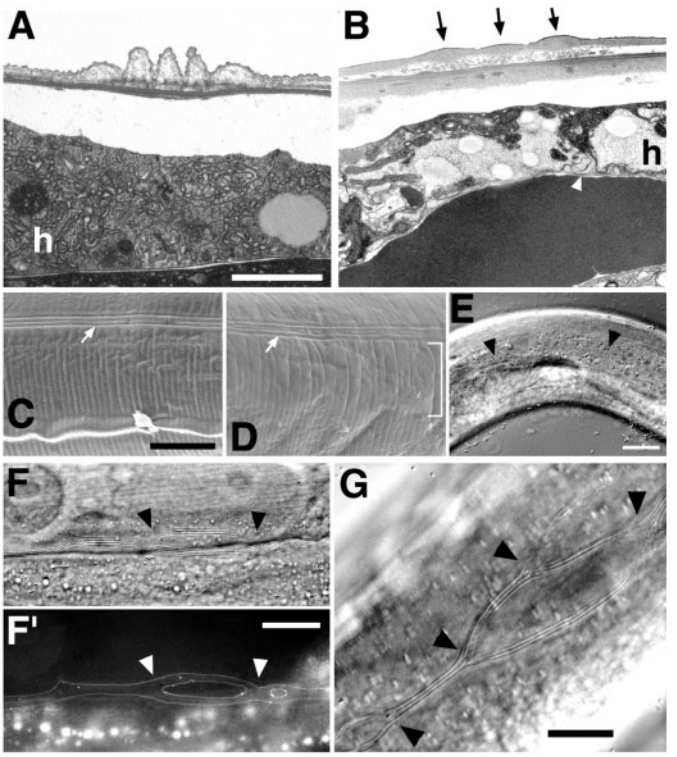

Fig. 7. *nhr-25* RNAi disrupts formation of the adult alae and the lateral epidermis. (A,B) Transmission electron microscopy sections reveal aberrant cuticle with shallow alae (arrows) in *nhr-25* RNAi adults (B). The lateral hyp7 syncytium (h) has an abnormal structure, with much of the dense membrane system missing. The dark material (B) is yolk not taken up by the gonad. (C,D) Scanning electron micrographs of wild-type (C) and *nhr-25(RNAi)* (D) adults viewed from the lateral side. Alae are shown with arrows, bracket (D) indicates a lateral region of sparse annuli. (E) A gap in the alae (delimited by arrowheads) caused by *nhr-25* RNAi. (F,G) Branching of the adult alae caused by a dominant mutation of the cuticle gene *rol-6* in the SU93 strain (F) is enhanced by *nhr-25* RNAi (G); a severe case is shown, corresponding to '>1 small loops' in Table 1. The underlying seam adherens junctions, marked by expression of the *ajm-1::gfp* reporter (F′) mirrors the shape of the alae. Arrowheads indicate the branching points. Bars, 2 μm (A,B), 10 μm (C,D), 20 μm (E-G).

Another cuticular structure affected by *nhr-25* RNAi were the annuli, transverse rings around the whole body that are interrupted only by the lateral alae. Compared with wild-type controls, the annuli were shallower and sparser in all *nhr-25(RNAi)* adults, particularly on the lateral sides, where a thick layer of hyp7 syncytium underlies the cuticle (Fig. 7D). The subcellular morphology of the lateral part of the adult syncytium displayed striking anomalies (Fig. 7B). On the dorsolateral and ventrolateral sides, the hyp7 syncytium was abnormally enlarged instead of forming a thin layer between the cuticle and muscles (Fig. 8A,B). Epidermal attachments (fibrous organelles that normally fasten muscles to the cuticle) could not be found in *nhr-25(RNAi)* adults, and the structure of the epidermal basement membrane and the muscle morphology were altered as well. In addition, no struts that would link the two layers of the adult cuticle were visible. Instead, unusual vesicles that might represent faulty cuticle secretion were

Table 1. Enhancement of alae branching in two strains carrying the *rol-6(su1006)* mutation by *nhr-25* RNAi

	SU93		RG242	
Branching severity	Control *n*=50	*nhr-25* RNAi *n*=55	Control *n*=50	*nhr-25* RNAi *n*=50
Normal	58	20	48	14
Single loop	38	22	46	28
>1 Small loop	4	34	4	34
>1 Large loop	0	20	2	20
Extensive branching	0	4	0	4

Numbers are % incidence of loops or branches.

trapped between the cuticle layers (Fig. 8B). These anomalies of the epidermal ultrastructure are probably the cause of defective L4-to-adult molt, observed in about 30% of wild-type and in 100% of *rrf-3(pk1426)* worms that had been fed on *nhr-25* dsRNA-expressing bacteria from hatching. Unshed L4 cuticle remained attached to their heads or tails and caused body constrictions in these *nhr-25(RNAi)* adults (Fig. 8C,D).

Discussion

Role of the transcription factor NHR-25 in seam-cell contacts

By preventing seam cells from restoring mutual contacts after asymmetric divisions, *nhr-25* RNAi emulated the effect of V-cell ablation. Anterior daughters of the isolated seam cells assumed the wrong fate: they failed to fuse with the hyp7 syncytium, continued expressing a seam-specific transgene and occasionally divided, thus resembling their parent stem cells. These results were consistent with the early observations of Sulston and White (Sulston and White, 1980) that ‘a gap in the seam can cause the remaining cells to proliferate more than usual’, this being ‘due to some of the anterior daughters, which normally fuse with the hypodermal syncytium, persisting as seam cells and subsequently dividing again’. It is not clear yet why the absence of the cell contact conserves the seam fate in the anterior daughters. However, the extra seam cells were not produced by additional heterochronic divisions, such as those occurring in mutants for another nuclear receptor DAF-12 (Antebi et al., 2000).

Consistently with its transcriptional function, we have detected NHR-25 in the nuclei of seam and, at a lower level, in hyp7 epidermal cells. This finding supports the possibility that the role of NHR-25 in the renewal of seam-cell contacts might be autonomous to the seam cells, although we still cannot exclude the possibility that NHR-25 is required in the surrounding hyp7 syncytium. Moderate antibody staining and activity of *nhr-25::gfp* constructs (Gissendanner and Sluder, 2000) (this study) in hyp7 suggest that NHR-25 also plays a role in this part of the epidermis. Such a role is apparent from the effect of *nhr-25* RNAi on the hyp7 subcellular structure.

The failure of seam cells to restore contacts might be caused by their inability to emit a mutually attractive signal, incompetence to sense such a signal, disrupted cytoskeleton or impaired recognition and adhesion to their neighbors. However, we have seen normal expression and localization of DLG-1, which is required for proper cytoskeleton organization (Bossinger et al., 2001; Firestein and Rongo, 2001; McMahon et al., 2001) and of AJM-1, an essential component of adherens junctions (Koppen et al., 2001), in seam cells affected by *nhr-25* RNAi. Also, E-cadherin (HMR-1), α-catenin (HMP-1) and β-catenin (HMP-2), which connect cytoskeleton with the extracellular matrix (Costa et al., 1998), have appeared undisturbed by a loss of *nhr-25* function (Chen et al., 2004). We have found that *cdh-3::gfp*, which encodes a promoter fusion of a Fat-like cadherin, is underexpressed in seam cells that have lost mutual adherens junctions as a result of *nhr-25* RNAi. CDH-3 is required for differentiation of the hyp10 syncytium and has also been suggested to play a role in cell-cell recognition, adhesion and the maintenance of the seam integrity (Pettitt et al., 1996). Reduced *cdh-3* expression could therefore offer a plausible explanation for the loss of cell-cell contacts in *nhr-25(RNAi)* animals. However, although *cdh-3* is expressed in seam cells throughout development, seam-cell morphology appears normal in *cdh-3* mutants, possibly because of gene(s) compensating for CDH-3 function (Pettitt et al., 1996). Thus, *cdh-3* might not be the sole gene downstream of NHR-25 action.

Another candidate NHR-25 target is *acn-1*, which encodes

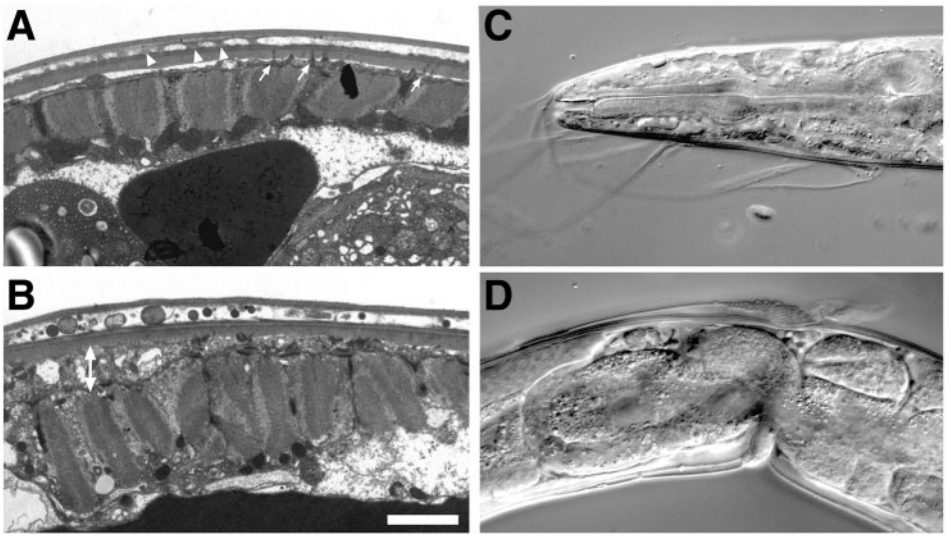

Fig. 8. *nhr-25* RNAi disrupts the ultrastructure of the epidermis and molting. (A,B) Compared with a control (A), the ventrolateral hyp7 epidermis is extremely thick in *nhr-25(RNAi)* adult (B, double arrow), thus separating muscles from the cuticle. The fibrous organelles that normally attach muscles to the cuticle (arrows) are not apparent (B). Likewise the struts between the cuticle layers (arrowheads) are missing; instead, abnormal vesicles of unknown content are present in *nhr-25(RNAi)* animals (B). Bar, 2 μm. (C,D) Wild-type worms fed on *nhr-25* dsRNA-expressing *E. coli* from the time of hatching display molting defects. Examples show unshed L4 cuticle, attached to the head (C) or causing constrictions (D) in affected adults.

a peptidase of the angiotensin-converting enzyme family. Expression of an *acn-1::gfp* reporter was reduced in seam cells upon *nhr-25* RNAi, and some phenotypes caused by silencing of *acn-1*, including abnormal alae and molting defects, resembled those induced by *nhr-25* RNAi (Brooks et al., 2003). However, although we reproduced these *acn-1* RNAi phenotypes, we found that Vn.p cells re-established their contacts normally, therefore excluding ACN-1 as an effector of NHR-25 in this aspect of development. Gaps in the seam were observed in mutants for a transmembrane protein of the plexin family PLX-1 (Fujii et al., 2002). Plexins are receptors for semaphorins, secreted or transmembrane cues for axon guidance (Raper, 2000). Of two *C. elegans* plexin and three semaphorin genes, *plx-1* and *mab-20* [encoding semaphorin 2a (Roy et al., 2000)] are active in the larval seam cells.

The disrupted seam cell contacts are probably unrelated to molting defects seen in *nhr-25(RNAi)* worms, because gaps between seam cells occur even in *nhr-25(RNAi)* animals that had molted normally and molting defects have not been reported for *plx-1* mutants that display the seam-cell phenotype.

Neuroblast differentiation suggests NHR-25 involvement in the Wnt pathway

Laser ablations (Sulston and White, 1980) suggested that the physical contact of V5.p with V6.p and V4.p was a signal for V5.p to produce an anterior neuroblast V5.pa and hence the postdeirid. Austin and Kenyon (Austin and Kenyon, 1994) made a strong case for cell-cell contact, as opposed to a diffusible signal, by showing that a delay in the V5.p commitment was equal to the time needed for a distant seam cell to reach V5.p. Without the contact, no postdeirid was formed. We were therefore surprised to see that, in *nhr-25(RNAi)* L2 worms, the V5.pa cells divided in the neuroblast fashion even when V5.p was disconnected from its neighbors.

For V5.pa to assume the neuroblast fate, a homeotic gene (*mab-5*) acting downstream in the Wnt signaling must be turned off in V5.p. The downregulation of *mab-5* requires V6.p (Hunter et al., 1999) and a negative regulator of the Wnt pathway PRY-1 (Maloof et al., 1999; Korswagen et al., 2002). Ectopic *mab-5* expression that occurs in the absence of V6.p or in a *pry-1(–)* background causes V5.p to divide symmetrically as other Vn.p cells do at the L2 stage. Misexpression of *mab-5* and the resulting inhibition of the postdeirid program requires the normal function of *bar-1*, encoding a β-catenin, and some other components of the Wnt pathway (Hunter et al., 1999; Maloof et al., 1999; Korswagen et al., 2002).

How can a V5.p cell, devoid of contacts with V6.p in *nhr-25(RNAi)* larvae, give rise to a postdeirid-like structure? We hypothesize that blocking of the postdeirid program requires NHR-25 in a similar way to that in which it requires *mab-5* or *bar-1* function. Because *mab-5::lacZ* is ectopically expressed in *nhr-25(RNAi)* background, NHR-25 might act in the Wnt pathway, at the same level as MAB-5 or downstream of it. We propose that NHR-25 might exert its effect by directly interacting with the MAB-5 protein. This possibility is supported by a cooperation between a *Drosophila* NHR-25 ortholog αFTZ-F1 and a MAB-5-related homeodomain protein FTZ during embryonic segmentation (Guichet et al., 1997; Yu et al., 1997; Schwartz et al., 2001; Suzuki et al., 2001; Yussa et al., 2001). Moreover, NHR-25 has been recently shown to interact with the *C. elegans* homeodomain proteins LIN-39 and NOB-1 during vulval and embryonic development, respectively (Chen et al., 2004). Our preliminary data suggest that MAB-5 modulates NHR-25-dependent transcriptional activation in a yeast-based assay (M.S., M. van Gilst, K. Yamamoto, M.J. and M.A., unpublished).

Pleiotropic phenotypes imply a dual role of NHR-25 in the epidermis

Although loss of *nhr-25* causes disruption of seam-cell contacts and compromises the proper differentiation of the seam and the cuticular alae, these effects are unlikely to be directly connected with overall molting defects seen in *nhr-25(RNAi)* worms. Molting is a complex process that can fail at any of several steps, such as expression of a cuticle component, secretion of cuticle by the epidermis, separation of a new cuticle from the old one and behavior leading to the old cuticle removal. Although the effect of *nhr-25* RNAi on shedding cuticle has been well established (Asahina et al., 2000; Gissendanner and Sluder, 2000; Chen et al., 2004; Gissendanner et al., 2004), the studies have not discriminated between the possible causes. Based on the disturbed epidermal ultrastructure and the presence of abnormal vesicles between cuticle layers, we propose that NHR-25 acts generally upon cuticle secretion. Missing cuticular struts and aberrant alae and annuli are examples of cuticular structures that are not properly formed in *nhr-25(RNAi)* adults. Interestingly, silencing of *nhr-25* enhances the effect of a semidominant mutation in the collagen gene *rol-6* on alae branching. Although we could speculate that *rol-6* or other cuticle gene(s) might be regulated by NHR-25, the observed interaction with *rol-6(su1006)* probably results from the effect of *nhr-25* RNAi on increasing the incidence of aberrant seam-cell contacts.

Molting defects, manifested as unshed cuticle, also result from RNAi silencing of the nuclear receptor NHR-23 (Kostrouchova et al., 1998). Reduced mRNA levels from the collagen gene *dpy-7* are observed in *nhr-23(RNAi)* animals and suggest a role of NHR-23 in cuticle synthesis (Kostrouchova et al., 2001). We have previously raised the point that molting relies on the same genetic regulation in insects and nematodes, with NHR-23 and NHR-25 being its evolutionarily conserved core (Asahina et al., 2000). This idea considered the role of the NHR-23 and NHR-25 orthologs DHR3 and βFTZ-F1, respectively, in *Drosophila* molting and metamorphosis, triggered by the steroid hormone ecdysone (Thummel, 2001). In the ecdysone pathway, expression of βFTZ-F1 requires DHR3 (Kageyama et al., 1997; Lam et al., 1997; White et al., 1997). Although a similar epistasis has not been shown for NHR-23 and NHR-25 (Kostrouchova et al., 2001), both factors were recently suggested to activate a common target gene, *acn-1*, whose silencing itself disrupts molting (Brooks et al., 2003). We find that *nhr-23* RNAi does not interfere with the ability of seam cells to restore adherens-junction contacts upon asymmetric divisions. Thus, although NHR-25 and NHR-23 might execute the molting process through a partially common genetic pathway, NHR-25 has a unique role in the epidermis independent of NHR-23. The dual role of NHR-25 (epidermal-cell differentiation and molting) is intriguingly parallel to the

two distinct functions of the *Drosophila* FTZ-F1 isoforms, αFTZ-F1 in segmentation and βFTZ-F1 in steroid-dependent metamorphosis and molting.

We thank the Electron Microscopy Lab at the Institute of Parasitology for technical help, O. Bossinger for providing the anti-DLG-1 antibody and for useful advice, S. Hirose for supporting the preparation of the NHR-25 antibody and M. Labouesse for comments on the manuscript. We appreciate receiving plasmid vectors from A. Fire and *C. elegans* strains from I. Greenwald, A. Rougvie and the *Caenorhabditis* Genetics Center, supported by the National Institutes of Health. The *nhr-25* clone yk342d8 was provided by Y. Kohara and the *nhr-23* plasmid 4666 by M. Kostrouchova. The MH27 antibody was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. This work was supported by grant KJB5022303 and project Z60220518 from the Academy of Sciences of the Czech Republic. M.S. was supported by a graduate studies program 524/03/H133 from the Grant Agency of the Czech Republic.

References

Abrahante, J. E., Miller, E. A. and Rougvie, A. E. (1998). Identification of heterochronic mutants in *Caenorhabditis elegans*. Temporal misexpression of a collagen::green fluorescent protein fusion gene. *Genetics* **149**, 1335-1351.

Antebi, A., Yeh, W. H., Tait, D., Hedgecock, E. M. and Riddle, D. L. (2000). *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. *Genes Dev.* **14**, 1512-1527.

Asahina, M., Ishihara, T., Jindra, M., Kohara, Y., Katsura, I. and Hirose, S. (2000). The conserved nuclear receptor Ftz-F1 is required for embryogenesis, moulting and reproduction in *Caenorhabditis elegans*. *Genes Cells* **5**, 711-723.

Austin, J. and Kenyon, C. (1994). Cell contact regulates neuroblast formation in the *Caenorhabditis elegans* lateral epidermis. *Development* **120**, 313-323.

Bossinger, O., Klebes, A., Segbert, C., Theres, C. and Knust, E. (2001). Zonula adherens formation in *Caenorhabditis elegans* requires *dlg-1*, the homologue of the *Drosophila* gene *discs large*. *Dev. Biol*. **230**, 29-42.

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

Broadus, J., McCabe, J. R., Endrizzi, B., Thummel, C. S. and Woodard, C. T. (1999). The *Drosophila* beta FTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. *Mol. Cell* **3**, 143-149.

Brooks, D. R., Appleford, P. J., Murray, L. and Isaac, R. E. (2003). An essential role in molting and morphogenesis of *Caenorhabditis elegans* for ACN-1, a novel member of the angiotensin-converting enzyme family that lacks a metallopeptidase active site. *J. Biol. Chem.* **278**, 52340-52346.

Chen, Z., Eastburn, D. J. and Han, M. (2004). The *Caenorhabditis elegans* nuclear receptor gene *nhr-25* regulates epidermal cell development. *Mol. Cell. Biol*. **24**, 7345-7358.

Chin-Sang, I. D. and Chisholm, A. D. (2000). Form of the worm: genetics of epidermal morphogenesis in *C. elegans*. *Trends Genet.* **16**, 544-551.

Costa, M., Reich, W., Agbunag, C., Leung, B., Hardin, J. and Priess, J. R. (1998). A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell Biol.* **141**, 297-308.

Cox, G. N., Laufer, J. S., Kusch, M. and Edgar, R. S. (1980). Genetic and phenotypic characterization of roller mutants of *Caenorhabditis elegans*. *Genetics* **95**, 317-339.

Firestein, B. L. and Rongo, C. (2001). DLG-1 is a MAGUK similar to SAP97 and is required for adherens junction formation. *Mol. Biol. Cell* **12**, 3465-3475.

Francis, G. R. and Waterston, R. H. (1985). Muscle organization in *C. elegans*: localization of proteins implicated in thin filaments attachment and I-band organization. *J. Cell Biol.* **101**, 1532-1549.

Fujii, T., Nakao, F., Shibata, Y., Shioi, G., Kodama, E., Fujisawa, H. and Takagi, S. (2002). *Caenorhabditis elegans* PlexinA, PLX-1, interacts with transmembrane semaphorins and regulates epidermal morphogenesis. *Development* **129**, 2053-2063.

Gissendanner, C. R. and Sluder, A. E. (2000). *nhr-25*, the *Caenorhabditis elegans* ortholog of *ftz-f1*, is required for epidermal and somatic gonad development. *Dev. Biol.* **221**, 259-272.

Gissendanner, C. R., Crossgrove, K., Kraus, K. A., Maina, C. V. and Sluder, A. E. (2004). Expression and function of conserved nuclear receptor genes in *Caenorhabditis elegans*. *Dev. Biol*. **266**, 399-416.

Guichet, A., Copeland, J. W., Erdelyi, M., Hlousek, D., Zavorszky, P., Ho, J., Brown, S., Percival-Smith, A., Krause, H. M. and Ephrussi, A. (1997). The nuclear receptor homologue Ftz-F1 and the homeodomain protein Ftz are mutually dependent cofactors. *Nature* **385**, 548-552.

Hunter, C. P., Harris, J. M., Maloof, J. N. and Kenyon, C. (1999). Hox gene expression in a single *Caenorhabditis elegans* cell is regulated by a caudal homolog and intercellular signals that inhibit Wnt signaling. *Development* **126**, 805-814.

Hwang, B. J. and Sternberg, P. W. (2004). A cell-specific enhancer that specifies *lin-3* expression in the *C. elegans* anchor cell for vulval development. *Development* **131**, 143-151.

Kageyama, Y., Masuda, S., Hirose, S. and Ueda, H. (1997). Temporal regulation of the mid-prepupal gene *FTZ-F1*: DHR3 early late gene product is one of the plural positive regulators. *Genes Cells* **2**, 559-569.

Koppen, M., Simske, J. S., Sims, P. A., Firestein, B. L., Hall, D. H., Radice, A. D., Rongo, C. and Hardin, J. D. (2001). Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. *Nat. Cell Biol.* **3**, 983-991.

Korswagen, H. C., Coudreuse, D. Y. M., Betist, M. C., van de Water, S., Zivkovic, D. and Clevers, H. C. (2002). The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt pathway in *C. elegans*. *Genes Dev.* **16**, 1291-1302.

Kostrouchova, M., 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.

Kostrouchova, M., Krause, M., Kostrouch, Z. and Rall, J. E. (2001). Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **98**, 7360-7365.

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.

Luo, X., Ikeda, Y. and Parker, K. L. (1994). A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* **77**, 481-490.

Maglich, J. M., Sluder, A., Guan, X., Shi, Y., McKee, D. D., Carrick, K., Kamdar, K., Willson, T. M. and Moore, J. T. (2001). Comparison of complete nuclear receptor sets from the human, *Caenorhabditis elegans* and *Drosophila* genomes. *Genome Biol.* **2**, 29.1-29.7.

Maloof, J. N., Whangbo, J., Harris, J. M., Jongeward, G. D. and Kenyon, C. (1999). A Wnt signaling pathway controls Hox gene expression and neuroblast migration in *C. elegans*. *Development* **126**, 37-49.

McMahon, L., Legouis, R., Vonesch, J. L. and Labouesse, M. (2001). Assembly of *C. elegans* apical junctions involves positioning and compaction by LET-413 and protein aggregation by the MAGUK protein DLG-1. *J. Cell Sci*. **114**, 2265-2277.

Mello, C. and Fire, A. (1995). DNA transformation. *Methods Cell Biol.* **48**, 451-482.

Michaux, G., Legouis, R. and Labouesse, M. (2001). Epithelial biology: lessons from *Caenorhabditis elegans*. *Gene* **277**, 83-100.

Mohler, W. A., Simske, J. S., Williams-Masson, E. M., Hardin, J. D. and White, J. G. (1998). Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. *Curr. Biol.* **8**, 1087-1090.

Parker, K. L. and Schimmer, B. P. (1997). Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr. Rev.* **18**, 361-377.

Pettitt, J., Wood, W. B. and Plasterk, R. H. (1996). *cdh-3*, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*. *Development* **122**, 4149-4157.

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

Raper, J. A. (2000). Semaphorins and their receptors in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **10**, 88-94.

Roy, P. J., Zheng, H., Warren, C. E. and Culotti, J. G. (2000). *mab-20* encodes Semaphorin-2a and is required to prevent ectopic cell contacts during epidermal morphogenesis in *Caenorhabditis elegans*. *Development* **127**, 755-767.

Sadovsky, Y., Crawford, P. A., Woodson, K. G., Polish, J. A., Clements, M. A., Tourtellotte, L. M., Simburger, K. and Milbrandt, J. (1995). Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands

and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. *Proc. Natl. Acad. Sci. USA* **92**, 10939-10943.

Salser, S. J. and Kenyon, C. (1992). Activation of a *C. elegans* Antennapedia homologue in migrating cells controls their direction of migration. *Nature* **355**, 255-258.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning, A Laboratory Manual* (2nd edn). Cold Spring Harbor Laboratory Press.

Schwartz, C. J. E., Sampson, H. M., Hlousek, D., Percival-Smith, A., Copeland, J. W. R., Simmonds, A. J. and Krause, H. M. (2001). FTZ-Factor1 and Fushi tarazu interact via conserved nuclear receptor and coactivator motifs. *EMBO J.* **20**, 510-519.

Simmer, F., Tijsterman, M., Parrish, S., Koushika, S. P., Nonet, M. L., Fire, A., Ahringer, J. and Plasterk, R. H. A. (2002). Loss of the putative RNA-directed RNA polymerase RRF-3 makes *C. elegans* hypersensitive to RNAi. *Curr. Biol.* **12**, 1317-1319.

Simske, J. S. and Hardin, J. (2001). Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. *BioEssays* **23**, 12-23.

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

Sulston, J. E. and Horvitz, H. (1977). Post-embryonic cell lineages of the nematode, *C. elegans*. *Dev. Biol.* **56**, 110-156.

Sulston, J. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Dev. Biol.* **78**, 577-597.

Suzuki, T., Kawasaki, H., Yu, R. T., Ueda, H. and Umesono, K. (2001). Segmentation gene product Fushi tarazu is an LXXLL motif-dependent coactivator for orphan receptor FTZ-F1. *Proc. Natl. Acad. Sci. USA* **98**, 12403-12408.

Terns, R. M., Kroll-Conner, P., Zhu, J., Chung, S. and Rothman, J. H. (1997). A deficiency screen for zygotic loci required for establishment and patterning of the epidermis in *Caenorhabditis elegans*. *Genetics* **146**, 185-206.

Thein, M. C., McCormack, G., Winter, A. D., Johnstone, I. L., Shoemaker, C. B. and Page, A. P. (2003). *Caenorhabditis elegans* exoskeleton collagen COL-19: an adult-specific marker for collagen modification and assembly, and the analysis of organismal morphology. *Dev. Dyn.* **226**, 523-539.

Thummel, C. S. (2001). Molecular mechanisms of developmental timing in *C. elegans* and *Drosophila*. *Dev. Cell* **1**, 453-465.

Timmons, L., Court, D. L. and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**, 103-112.

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.

Yamada, M., Murata, T., Hirose, S., Lavorgna, G., Suzuki, E. and Ueda, H. (2000). Temporally restricted expression of transcription factor betaFTZ-F1: significance for embryogenesis, molting and metamorphosis in *Drosophila melanogaster*. *Development* **127**, 5083-5092.

Yu, Y., Li, W., Su, K., Yussa, M., Han, W., Perrimon, N. and Pick, L. (1997). The nuclear hormone receptor Ftz-F1 is a cofactor for the *Drosophila* homeodomain protein Ftz. *Nature* **385**, 552-555.

Yussa, M., Lohr, U., Su, K. and Pick, L. (2001). The nuclear receptor Ftz-F1 and homeodomain protein Ftz interact through evolutionarily conserved protein domains. *Mech. Dev.* **107**, 39-53.