The Caenorhabditis elegans Gene lin-26 Can Trigger Epithelial Differentiation without Confering Tissue Specificity

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How epithelial cell fates become specified is poorly understood. We have previously shown that the putative C2H2 zinc-finger transcription factor LIN-26 is required for the differentiation of ectodermal and mesodermal epithelial cells in Caenorhabditis elegans. Here, we report that ectopic LIN-26 expression during early gastrulation transforms most blastomeres into epithelial-like cells. Specifically, LIN-26 induced the expression of three epithelial markers: the adherens junction protein JAM-1; DLG-1, which is essential for the assembly of JAM-1 at junctions; and CHE-14, which is involved in apical trafficking. Furthermore, ultrastructural studies revealed that ectopic LIN-26 expression induced the formation of adherens-like junctions. However, ectopic lin-26 expression did not confer any tissue-specific cell fate, such as the epidermal cell fate, as evidenced from the observation that several epidermal-specific genes were not induced. Conversely, we show that epidermal cells displayed some polarity defects in lin-26 mutants. We conclude that lin-26 can induce epithelial differentiation and that epitheliogenesis is not a default pathway in C. elegans. © 2001 Academic Press

Key Words: C. elegans; epithelial cell; cell fate specification; adherens junction; apical trafficking; cell polarity; epidermis; uterus; support cell; lin-26.

INTRODUCTION

Despite the importance and widespread occurrence of epithelial cells, the mechanisms that establish epithelial cell identity remain largely unknown. This contrasts for instance with the genetic programs that control myogenesis and neurogenesis, which have been described at the molecular level (Arnold and Braun, 2000; Brunet and Ghysen, 1999). Two key features make epithelial cells unique. First, they originate from all three embryonic germ layers, whereas muscles or neurons come from a single germ layer. Second, some cells only transiently acquire epithelial characteristics before they become mesenchymal (Birchmeier and Birchmeier, 1993), whereas myogenic and neuronal characteristics are stable. In addition, cells that permanently retain epithelial attributes (e.g., in the epidermis, kidney, and intestine) can become tumorigenic, in which case they generally lose their polarised and adhesive properties (Thiery et al., 1988). The epithelial-mesenchymal transition and the loss of adhesive properties following tumorigenesis highlight the plasticity of the epithelial phenotype, and may reflect that epithelial cell fate specification involves unusual differentiation pathway(s).

It is currently unclear whether the specification of epithelial cell fates requires a specific battery of regulatory genes, whether genes that specify the identity of tissues containing epithelial cells are sufficient to induce epithelial differentiation, or, as it has been suggested, whether epithelial differentiation is a default pathway (Frisch, 1997). The identification of genes that control epitheliogenesis has been hampered in part by the lack of markers unique to epithelial cells (Davies and Garrod, 1997). Although adherens junctions (AJs) are found predominantly in epithelia (Geiger et al., 1992; Gumbiner, 1996; Nose et al., 1988; Yeaman et al., 1999), E-cadherin and α- and β-catenins, which coassemble to form AJs, are not exclusively expressed in epithelial cells. So far, genes whose function would be devoted to the regulation of epithelial-specific factors have not been described. In support of the notion

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that epithelial differentiation is integrated within the genetic program that controls organ specification is the observation that the WT1 protein, which is essential for kidney development but also acts in a few nonepithelial tissues, can bind the E-cadherin promoter (Hosono et al., 2000). Likewise, the search for genes that control the expression of keratinocyte-specific intermediate filaments has identified several positive factors which do not act exclusively in epithelia (Sinha et al., 2000). The epithelial cell default-phenotype hypothesis, on the other hand, is also supported by several observations. First, many genes that control the expression of junction components or epithelial intermediate filament proteins in vertebrates appear to encode factors that act as negative regulators of epithelial differentiation. For instance, the adenovirus E1a protein indirectly turns on the expression of epithelial genes by counteracting the activity of the general repressors CtBP and p300/CPB (Frisch, 1994; Grootelaes and Frisch, 2000). Second, the zinc-finger protein Snail, which binds the E-cadherin promoter, is preferentially expressed in cancer cells and is up-regulated in carcinomas (Batlle et al., 2000; Cano et al., 2000). The related protein Slug can facilitate EMT by causing the dissociation of desmosomes (Savagner et al., 1997).

C. elegans has only recently been used as a model to investigate epithelial biology. The intestine and the epidermis (also called hypodermis) are the two epithelial tissues that have so far been the most extensively studied in the embryo. Genetic analysis has established that generation of these tissues involves two classes of genes (for a review, see Labouesse and Mango, 1999). In the epidermis, the gene elt-1, which encodes a GATA factor, specifies epithelial cell identity. (Note that there are two classes of epidermal cells: cells from the so-called “major epidermis” which cover the body, and cells from the “minor epidermis” which cover the tip of the head and tail; elt-1 acts only in the former.) elt-1 is first expressed at the onset of gastrulation and its inactivation leads to a complete absence of the major epidermis (Page et al., 1997). Two likely elt-1 targets are elt-3, which encodes another GATA factor almost exclusively found in a subset of epidermal cells, and lin-26, which encodes a putative zinc-finger transcription factor expressed in ectodermal and mesodermal epithelial cells (Chanal and Labouesse, 1997; Gilleard and McGhee, 2001; Gilleard et al., 1999, 1994, 1996; Page et al., 1997). Inactivation of elt-3 does not appear to impair epidermal development (Gilleard and McGhee, 2001; Gilleard et al., 1999). In contrast, mutations in lin-26 lead to embryonic lethality due to the degeneration of ectodermal epithelial cells, and to sterility due to malformation of the somatic gonad epithelium (den Boer et al., 1998; Labouesse et al., 1994, 1996). How lin-26 acts is not known. For example, in the epidermis, lin-26 could act by repressing the expression of neuronal genes or by activating the expression of epidermal genes.

To address this issue, we analysed the consequences of ectopic lin-26 expression during neuronal differentiation and in noncommitted cells by monitoring the expression of several epithelial-specific and epidermal-specific markers. In particular, we examined the expression of the genes jam-1,dlg-1, and che-14, which are exclusively expressed in epithelial cells in C. elegans and characterise two aspects of the epithelial polarised phenotype (junction and polarised trafficking). The gene jam-1 encodes a protein of unknown function which is associated with adherens junctions (Francis and Waterston, 1991; Mohler et al., 1998). dlg-1 is required to aggregate JAM-1 at adherens junctions and encodes a Lethal Discs Large homologue (McMahan et al., submitted), che-14 encodes an apical multipass transmembrane protein that has recently been implicated in apical trafficking (Mchaux et al., 2000). In contrast to jam-1 and dlg-1, which are found in all epithelial cells, che-14 expression is restricted to the nonneuronal ectoderm (epidermal cells and support cells). We show that ectopic lin-26 expression during a specific time window in embryogenesis can confer a nonepidermal epithelial-like identity to all blastomeres. In parallel, we analyse the expression and subcellular localisation of the JAM-1, DLG-1, and CHE-14 proteins in lin-26-deficient embryos. We propose that lin-26 functions to promote epithelial differentiation and acts, directly or indirectly, on some of the genes that play a critical role in organising structures or processes that contribute to the polarised epithelial phenotype.

**MATERIALS AND METHODS**

**Constructs and Transgenic Animals**

The lin-26B cDNA from pML500 (Dufourcq et al., 1999) was cloned between the KpnI and SacI sites of the vector pPD49.78 carrying the hsp16 heat-shock promoter (gift from A. Fire). This has::lin-26 construct (pML501) was co-injected into wild-type animals (N2) at a concentration of 50 ng/μl with the dominant marker pRF4 (Mello and Fire, 1995). One extrachromosomal array was integrated by x-ray irradiation to generate the alleles mcls22 and mcls23, which were outcrossed four times against N2. Similarly, the lin-26B cDNA was cloned between the AccI and SacI sites of the vector pDS57.56 carrying the mec-3 promoter (gift from A. Fire). Several control constructs were made. The lin-26C spliced variant, which lacks the second LIN-26 Zn-finger (Dufourcq et al., 1999), was cloned into pPD49.78. Two stop codons were introduced after the initiation codon of lin-26B in pML501 to generate a protein with the predicted sequence MLSKFVVVEVSN-traffic (Michaux et al., 1999) was crossed with elt-1::gfp and elt-1::gfp (McMahon et al., 1998) and elt-1::gfp (vps12; gift from J. Gilleard), and established arrays carrying dpy-7::gfp or let-502::gfp (Wissmann et al., 1999) were crossed with mcls22 or mcls23 animals.
To examine whether elt-1 (Page et al., 1997) was required for jam-1 induction after ectopic LIN-26 expression, we generated a mcs23;elt-1(zu180) strain or performed RNA interference against elt-1 in mcs23 animals. A double-stranded elt-1 RNA preparation corresponding to exons 1–3 (625 nucleotides) was injected into hermaphrodites. The phenotype of elt-1(RNAi) embryos was identical to that of elt-1(zu180) mutants by Nomarski or by staining with antibodies against LIN-26 or JAM-1. To examine the distribution of epithelial markers in lin-26-deficient embryos, we performed RNA interference against lin-26 (exons 3–5) (Bosher et al., 1999) in animals carrying che-14::gfp, dI1-g::gfp, or jam-1-g::gfp transgenes, or stained embryos carrying the null mutation lin-26B (mc15) (den Boer et al., 1998) with the antibody MH27 (very similar results were obtained with both methods).

Heat-Shock Experiments and Animal Manipulations

Embryos or larvae were placed at 33°C for 25 min, then returned to 20°C for a minimum of 4 h. In many cases, embryos were collected after dissection of transgenic mothers and their stage was determined under the microscope before the heat-shock (HS). To examine the consequences of ectopic lin-26 expression, we also recorded the embryonic lineage of heat-shocked embryos using a 4-D microscope (Horner et al., 1998), using a microscope equipped with a temperature-control device (running water around the objective and under the slide). Transgenic hs::lin-26 embryos were constantly recorded for 7 h starting at the 4-cell stage and through the heat shock (30 min at 33°C), which was applied when embryos reached the 28-cell stage. To test whether the progeny of the first four blastomeres can respond to the effect of ectopic LIN-26, we eliminated three cells at the 4-cell stage using a laser microbeam (Laser Science) in the jcls1;mcs2 and in the jcls1 background. After the operation, embryos were allowed to recover for 1 h at 20°C before HS, and jam-1-g::expression was examined at least 10 h after the HS.

Touch response of animals carrying the mec-3::lin-26 construct was tested after gentle touch with an eyelash (Way and Chalfie, 1989).

Antibody Staining and Electron Microscopy

Immunostaining with polyclonal antibodies against LIN-26 (Labouesse et al., 1996), PHA-4 (pharynx and intestine; Horner et al., 1998), or with the monoclonal antibodies MH27 (adherens junctions; Francis and Waterston, 1991), NE8:4C6 (body wall muscles; Schnabel, 1994), 1CB4 (intestinal cells; Okamoto and Thomson, 1985), and 3N B12 (subset of pharyngeal muscles; Priess and Thomson, 1987) were performed as described (Chanal and Labouesse, 1997). Electron microscopy was performed as described (Legouis et al., 2000) on mcs23 embryos that had been heat shocked 5–7 h prior to embryo processing or on lin-26B (mc15) mutants that had reached midembryogenesis.

RESULTS

Early Ectopic LIN-26 Expression Prevents Organ Formation

To determine whether lin-26 is a negative or positive regulator, we expressed the major lin-26 cDNA isoform lin-26B under the control of the neuronal-specific promoter mec-3 (mec-3::lin-26 construct), which is an early-acting gene in touch neurons (Way and Chalfie, 1989), and under the control of the heat-shock responsive promoter hsp16 (hs::lin-26 construct) (Stringham et al., 1992). The effects of ectopic lin-26 expression were analysed by assaying the touch response of transgenic mec-3::lin-26 animals, or by examining the development of animals in which expression of the hs::lin-26 construct had been induced (see Materials and Methods).

Two lines of evidence suggest that ectopic expression of lin-26 in neurons does not affect neuronal differentiation. First, animals carrying the mec-3::lin-26 construct were still touch-sensitive (data not shown), indicating that touch neuron differentiation was unaffected. Second, inducing hs::lin-26 expression at the time of neuronal differentiation in embryos (350-cell to 550-cell stages) or in larvae (L1 stage) resulted in viable animals that could move normally despite robust LIN-26 expression in neuroblasts and neurons (data not shown). We conclude that lin-26 is unlikely to function as a repressor of neuronal differentiation.

Using the hs::lin-26 construct, we observed that ectopic LIN-26 affects development when it is induced prior to the 100-cell stage. C. elegans embryogenesis can schematically be divided into four periods (for a review, see Labouesse and Mango, 1999). From fertilisation until the 28-cell stage, which corresponds to the onset of gastrulation, maternal genes control patterning and blastomere fate specification. Between the 28- and 100-cell stages, a few zygotic genes specify organ and tissue identities. Between the 100- and 550-cell stages, cell differentiation takes place under the control of zygotic genes. Lastly, morphogenesis takes place once the embryo has reached the 550-cell stage (Figs. 1A and 1B). We observed three phenotypic classes among heat-shocked embryos (Fig. 1 and Table 1). The most severely affected embryos were observed when LIN-26 expression was induced between the 20- and 50-cell stages. These embryos (denoted class II) arrested with approximately 200 cells and lacked most tissues, as judged by Nomarski microscopy (Fig. 1D; class II embryos will be the main focus of this work). Embryos heat shocked prior to the 20-cell stage (Fig. 1C) were less severely affected than class II embryos, arresting with about 500 cells. Embryos heat shocked between the 50- and 100-cell stages (class III) failed to elongate beyond the comma stage and showed a strongly reduced pharynx (Fig. 1E). Beyond the 100-cell stage, embryos were not affected (Fig. 1F).

As shown in Table 1, none of the phenotypes described above were detected in control experiments, and, more generally, our heat-shock conditions did not affect the development of most control embryos. Specifically, we submitted wild-type embryos to the same heat-shock treatment, we introduced two stop codons close to the lin-26 AUG, and finally we expressed under the same heat-shock promoter the minor isoform lin-26C that lacks the second predicted zinc-finger of the LIN-26 protein. In this last
control experiment, we could detect protein expression using polyclonal antibodies recognising all isoforms (data not shown). In parallel, we also showed that ectopic expression of lin-26, which encodes proteins with two zinc fingers closely related to those of LIN-26 (Dufourcq et al., 1999), does not affect embryonic development (Table 1). These controls demonstrate that the effects observed in hs::lin-26 embryos are entirely specific to LIN-26 activity, and furthermore that they require the presence of both predicted LIN-26 zinc-fingers.

To confirm the absence of certain tissues in class II and class III embryos, we stained them with several tissue-specific antibodies. We found that class II embryos had no body wall muscles (Fig. 2D), no pharynx, no rectum (Fig. 2F), and no intestine (Fig. 2H). Likewise, we observed that class III embryos had fewer pharyngeal cells (Fig. 2J). These observations indicate that cells that should become endodermal or mesodermal in class II embryos and pharyngeal in class III embryos have adopted another fate.

In summary, LIN-26 induced the strongest effects if ectopically expressed during early gastrulation, when organ or tissue precursors become assigned to their identities.

**LIN-26 Expression Promotes an Epithelial Differentiation Program**

To determine the fates adopted by cells in class II embryos, we examined whether they could express markers normally present in tissues that express lin-26 (e.g., support cells, epidermis, somatic gonad; Table 2). Strikingly, we could detect the adherens junction protein JAM-1 around most LIN-26-positive cells (Figs. 3E-3H) in a pattern that was generally more irregular and punctate than in wild-type epithelial cells (Figs. 3A-3D). Similarly, we detected ectopic DLG-1::GFP expression in class II embryos in a pattern that was quite similar to that observed for JAM-1 (Fig. 3N), and ectopic CHE-14::GFP expression at the membrane in many cells in class II embryos (Fig. 3P, Table 2). Ectopic JAM-1 expression was also present in class I embryos but in fewer cells (data not shown), suggesting that heat-shock effects are weaker at an early stage. In class III embryos, the JAM-1 pattern in the pharynx was discontinuous and abnormal (data not shown), often forming a small clump. It was often difficult to determine with certainty whether this staining resulted from partial suppression of pharyngeal development or from an ectopic jam-1 induction. We note, however, that, in pha-4 mutant embryos or in embryos homozygous for a complete deletion of the pha-4 locus, a similar MH27 pattern is observed (Chanal and Labouesse, 1997; Horner et al., 1998). Consistent with the observation that embryonic patterning and viability are affected only if hs::lin-26 expression is induced prior to the 100-cell stage, we did not observe ectopic expression of epithelial markers when the heat-shock treatment was applied after the 100-cell stage, despite strong LIN-26 induction (data not shown).

The simultaneous overexpression of che-14,dlg-1, and jam-1 after ectopic lin-26 expression strongly suggests that most cells in class II embryos acquired epithelial characters. To confirm this hypothesis, we examined the ultrastructure of individual cells in class II embryos by electron microscopy. Indeed, we could recognise distinct junctions both in external and internal cells (Figs. 4B and 4C), which were often present at multiple positions along the membrane instead of a unique subapical position. This feature, together with the fact that CHE-14::GFP was detected in a rather uniform pattern around cells, indicates that cells in class II embryos might have an enlarged apical domain.

The previous observations suggest that most cells have adopted an epithelial-like cell fate in class II hs::lin-26 embryos. To test whether all blastomeres can equally respond to LIN-26, we asked whether this reprogramming still occurs when each of the first four blastomeres (ABa, ABd, ABp, and ABm)
Expression of the Full-Length LIN-26B Protein Is Essential to Observe a Reprogramming of Early Blastomeres

<table>
<thead>
<tr>
<th>Construct</th>
<th>Heat-shock conditions</th>
<th>% embryonic lethality</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>no (N2 strain)</td>
<td>no</td>
<td>&lt;1</td>
<td>500</td>
</tr>
<tr>
<td>no (N2 strain)</td>
<td>Within 1 h after egg laying</td>
<td>6’</td>
<td>397</td>
</tr>
<tr>
<td>hs::lin-26B(stop)</td>
<td>Within 1 h after egg laying</td>
<td>15’</td>
<td>91</td>
</tr>
<tr>
<td>hs::lin-26B</td>
<td>Within 1 h after egg laying</td>
<td>8’</td>
<td>149</td>
</tr>
<tr>
<td>hs::lin-26B</td>
<td>Embryos with &lt;20 cells</td>
<td>87</td>
<td>95</td>
</tr>
<tr>
<td>hs::lin-26B</td>
<td>Embryos with &gt;20 cells, &lt;50 cells</td>
<td>81</td>
<td>91</td>
</tr>
<tr>
<td>hs::lin-26B</td>
<td>Embryos with &gt;50 cells, &lt;100 cells</td>
<td>55</td>
<td>85</td>
</tr>
<tr>
<td>hs::lin-26B</td>
<td>Embryos with &gt;100 cells</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>hs::lir-1A</td>
<td>Within 1 h after egg laying</td>
<td>9</td>
<td>223</td>
</tr>
</tbody>
</table>

At least two independent transgenic lines carrying extrachromosomal arrays were examined. Result for only one line is given; the other behaved similarly.

Animals were allowed to lay eggs for 1 h, then transferred to a new plate. Eggs were submitted to heat-shock treatment immediately after removal of mothers. Most embryos had on average between 20 and 50 cells.

Experiments in which heat-shock was performed on precisely staged embryos indicate that wild-type embryos with less than 10 cells are the most sensitive to heat-shock treatment. Among all embryos examined, 1 arrested at the comma stage, 30 developed beyond the 2-fold stage, and the others hatched and developed to normal fertile adults.

Among the 149 embryos examined, 1 arrested prior to the comma stage, 13 arrested after the 2-fold stage, and the others hatched.

Among the 91 embryos examined, 1 arrested prior to the comma stage, 13 arrested after the 2-fold stage, and the others hatched.

These animals carried the integrated array mcIs23. Animals carrying the integrated array mcIs22 behaved similarly in all our tests.

Experiments in which heat-shock was performed on precisely staged embryos in the integrated mcIs23 background (see Fig. 1).

None of the other lir-1 isoforms gave a phenotype.

ABp, EMS, P2) is isolated. Using a laser microbeam, we eliminated three blastomeres at the 4-cell stage in a strain carrying integrated jam-1::gfp and hs::lin-26 constructs, and subsequently induced lin-26 expression by a heat-shock at a time corresponding to the normal 20- to 50-cell stage. As shown in Fig. 5, we observed that each of the first four blastomeres was competent to generate ectopic jam-1-expressing cells upon ectopic lin-26 expression. The extra-GFP seen in these partial embryos was scattered and present in a large proportion of cells, whereas it was only seen in a restricted area in corresponding controls. In addition, it was always more similar to the JAM-1 distribution observed in normal embryonic cells (see Fig. 3A) than in normal intestinal cells (see Fig. 3C), even in isolated EMS embryos which normally produce pharynx and gut. We noticed that the P2 blastomere did not respond as strongly to ectopic LIN-26, since only 53% of isolated P2 embryos presented ectopic JAM-1::GFP, compared to 92% concerning EMS embryos (Fig. 5). This could be due to specific properties of P2, which generates the germline and is protected against somatic transcription by the transcriptional repressor pie-1 (Seydoux et al., 1996). Zhu and coworkers had noticed a similar phenomenon for the P2 blastomere when attempting to ectopically express end-1, which specifies intestine identity (Zhu et al., 1998). We conclude that all blastomeres at the 4-cell stage can be reprogrammed to express epithelial markers after induction of LIN-26 at the gastrulation stage.

In summary, ectopic lin-26 expression between the 20- and 50-cell stages transforms most cells into epithelial-like cells.

LIN-26 Does Not Confer Epidermal Identity

The apparent epithelial transformation that was observed in class II embryos and the observation that the JAM-1 pattern in isolated EMS embryos was epithelial-like raise the possibility that early blastomeres have adopted the fates of epidermal precursors. To assay this possibility, we first examined whether the class II phenotype depends on the activity of elt-1, which specifies epidermis identity (Page et al., 1997). When elt-1 is inactive, embryos fail to express jam-1 and lin-26 in their “major epidermis” (Fig. 3I). We still observed ectopic JAM-1 expression after ectopic LIN-26 expression, both in internal and external cells of elt-1-deficient embryos (Fig. 3J). Thus, ectopic LIN-26 expression can bypass the requirement for elt-1 to induce jam-1 expression.

To test more directly whether ectopic lin-26 confers epidermal identity, we examined the expression of markers that are exclusively expressed in the epidermis, namely the cuticle collagen dpy-7 (Gilleard et al., 1997), and the muscle attachment antigens recognised by the antibodies MH4 and MH5 (Francis and Waterston, 1991). We also examined the expression of let-502, which controls actin reorganisation in the epidermis and the somatic gonad (Wissmann et al.,
of elt-1. We found that these markers were expressed in approximately the same number of cells relative to controls and in much fewer cells than jam-1 (Table 2, Fig. 6, and data not shown).

Together, our results demonstrate that lin-26 does not induce epidermal differentiation. Furthermore, they rule out an alternative explanation for the effects observed after ectopic lin-26 expression, which would be that lin-26 up-regulates the major regulator of epidermal development, elt-1.

### Partial Polarity Defects in lin-26 Mutant Embryos

If ectopic lin-26 expression can induce ectopic expression of che-14, dlg-1, and jam-1, an expectation would be that their expression is impaired in lin-26 mutants. We reported earlier that jam-1 expression is strongly down-regulated and that the uterus does not form a lumen in animals specifically lacking lin-26 in the somatic gonad but not in the ectoderm (den Boer et al., 1998). We thus examined whether this is also the case in lin-26-deficient embryos. Surprisingly, che-14, dlg-1, and jam-1 were still expressed at apparently normal levels in these embryos (Figs. 3L and 3R, and data not shown). However, we noticed more subtle defects in the subcellular distribution of JAM-1 and CHE-14. For instance, the JAM-1 pattern showed internal clumps in 45% of the embryos (n = 59; arrowheads in Fig. 3L) or less frequently breaks (arrows in Fig. 3L), and CHE-14::GFP was detected along the lateral membrane as well as the apical membrane in 42% of the embryos (n = 79; arrows in Fig. 3R), raising the possibility of epithelial polarity defects.

Consistent with this possibility, analysis of the ultrastructure of epidermal cells in lin-26-null embryos revealed that adherens junctions were positioned at a more basal position than normal and were more extended than normal in 40% of the cells in lin-26(mc15) embryos (Fig. 4D). Therefore, using three different criteria, we observed cell polarity defects in lin-26-deficient embryos.

### TABLE 2

<table>
<thead>
<tr>
<th>Marker</th>
<th>Wild-type</th>
<th>mcIs22</th>
<th>n</th>
<th>Wild-type</th>
<th>mcIs22</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIN-26</td>
<td>129 ± 3</td>
<td>38</td>
<td>152 ± 24</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAPI</td>
<td>50 ± 3</td>
<td>5</td>
<td>216 ± 36</td>
<td>40</td>
<td></td>
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</tr>
<tr>
<td>JAM-1 (MH27)</td>
<td>153 ± 26</td>
<td>25</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CHE-14::GFP</td>
<td>7 ± 3</td>
<td>30</td>
<td>43 ± 29</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPY-7::GFP</td>
<td>56 ± 4</td>
<td>13</td>
<td>31 ± 13</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LET-502::GFP</td>
<td>39 ± 8</td>
<td>13</td>
<td>39 ± 15</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELT-1::GFP</td>
<td>61 ± 9</td>
<td>15</td>
<td>58 ± 7</td>
<td>15</td>
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</tbody>
</table>

* Data from Chanal and Labouesse, 1997 and Horner et al., 1998.
* Data from Den Boer et al., 1998.
* The strong reduction in cell number is not due to cell death, as recording the lineage of two hs::lin-26 embryos during a heat-shock experiment showed that cells from all early embryonic lineages divided much more slowly and stopped dividing earlier than normal, but did not die (see Materials and Methods).
* The nature of the JAM-1 pattern, it is difficult to count staining cells in the head and the digestive tract of wild-type embryos. The C. elegans lineage predicts that 85 epidermal cells, 40 support cells, 9 arcade and 9 marginal cells in the pharynx, 20 intestinal cells, 9 rectal cells, and 9 valve cells (total 181 out of 558 cells; compare to 153 out of 161) would be expected to express JAM-1 in wild-type embryos.
* The number of LIN-26-staining cells in the same set of embryos was 161 ± 26.
FIG. 3. Ectopic LIN-26 expression induces the expression of epithelial markers. Wild-type comma stage (A–D), class II hs::lin-26 (E–H), elt-1-deficient (I), elt-1-deficient hs::lin-26 class II (J), dlg-1::gfp transgenic 200-cell stage (K), or lin-26-deficient (L) embryos were stained with the antibodies recognizing the proteins indicated above or below the panels. (A–H) Pictures are shown in pairs showing the JAM-1 pattern alone (A, C, E, G) and after merging with LIN-26 (B, D, F, H). GFP autofluorescence of wild-type (M, O, Q), class II hs::lin-26 (N, P), or lin-26-deficient (R) embryos carrying either a dlg-1::gfp (M, N) or a che-14::gfp (O–R) construct. (A, B) External focal planes and (C, D) internal focal planes of a control embryo at midembryogenesis. (E, F) External focal planes and (G, H) internal focal planes of a class II hs::lin-26 embryo showing JAM-1 expression in most cells. (I, J) External focal plane of embryos after RNAi interference against elt-1. There was little JAM-1 left in the elt-1-deficient embryo (I, compare to A; the bracketed hazy staining corresponds to out of focus internal organs, while JAM-1 clumps marked with arrows corresponds to minor epidermal cells where elt-1 is normally inactive), but ectopic lin-26 expression could overcome this effect (J). (K) Control dlg-1::gfp embryo at the 200-cell costained with antibodies against GFP, LIN-26, and JAM-1. In contrast to class II embryos which arrest with approximately 200 cells, wild-type embryos with the same cell number express LIN-26 in about 40 epidermal precursors but not JAM-1 nor DLG-1 (compare to H, N); note that class II hs::lin-26 embryos are comparable with this control embryo for cell number but not for developmental age as we had to wait for at least 3 h after the heat-shock to see expression of jam-1, dlg-1::gfp, or che-14::gfp. (L) External focal plane of an embryo after RNAi interference against lin-26. There were internal aggregates (arrowheads) and less frequently breaks in the adherens belt (arrows). (M) External focal plane of a control dlg-1::gfp embryo (the pattern is identical to that of JAM-1 both externally and internally). (N) Internal focal plane of a class II dlg-1::gfp;hs::lin-26 embryo. DLG-1::GFP fluorescence was detected in most cells. (O) Most external (apical) focal plane and (Q) focal plane at the level of epidermal nuclei of the same control che-14::gfp embryo. At this stage, CHE-14::GFP is detected mainly at the apical surface (O) and in a punctate perinuclear pattern (Q). (P) Internal focal plane of a class II che-14::gfp;hs::lin-26 embryo showing CHE-14::GFP fluorescence in most cells (che-14 is normally not expressed in internal tissues). (R) Subapical focal plane after RNAi interference against lin-26. CHE-14::GFP was detected in lateral membranes (arrows), unlike in control embryos (compare to Q).
DISCUSSION

We previously established that lin-26 is normally expressed in three polarised cell types, epidermal cells, support cells, and uterine cells, where it is essential for normal differentiation (den Boer et al., 1998; Labouesse et al., 1994, 1996). However, its precise function in these cells had not been characterised. In the present study, our goal was first to determine for which specific aspect of cell differentiation lin-26 is required and second to identify its potential targets.

This new analysis shows that ectopic LIN-26 expression can reprogram the normal fates of all blastomeres to induce the expression of three markers that play different roles in maintaining epithelial cell polarity (jam-1, dlg-1, and che-14). Although the adherens junction pattern in embryos that overexpress lin-26 was looking epidermal-like, we have demonstrated that lin-26 does not turn on epidermal mark-
ers (five markers tested), strongly suggesting that lin-26 does not confer epidermal identity per se. We could not directly test whether lin-26 confers support cell identity because there are no support cell markers available. However, we do not think that this is the case, since cells overexpressing lin-26 did not send a projection as seen in the *che-14::gfp* background (support cells normally send a projection), which is a hallmark of support cells. Similarly, we do not think that lin-26 specifically turns on uterine differentiation, because it turned on *che-14* which is normally not expressed in uterine cells. Furthermore, ectopic lin-26 expression did not abolish *elt-1* and *dpy-7* expression (Table 2) in the same way as it repressed the expression of intestinal and muscle markers (Fig. 2); we would have expected no *dpy-7* or *elt-1* expression if the embryo had become uterine tissue.

An attractive interpretation of these data is that lin-26 promotes epithelial differentiation within the nonneuronal ectoderm and the somatic gonad. Genetic analysis in mice and flies has led to the notion of “master regulatory genes,” which are both necessary and sufficient to induce the formation of a particular tissue. Typical examples are the myogenic genes MyoD and Myf5 (Arnold and Braun, 2000) or the proneural genes achaete/scute (Brunet and Ghysen, 1999). We do not think that lin-26 qualifies as a “master epithelial regulatory gene.” Although lin-26 is sufficient to induce the expression of *jam-1*, *dlg-1*, and *che-14*, expression of these genes is not deficient in *lin-26-null* embryos. Nonetheless, several observations are consistent with lin-26 playing an essential role in maintaining the expression of *jam-1*, *dlg-1*, *che-14*, and other genes required for achieving epithelial cell polarity. First, we document the existence of polarity defects in the epidermis of *lin-26*-deficient embryos (Fig. 4), which can easily account for the degeneration of epidermal cells in *lin-26*-null embryos. Second, animals that are engineered to prevent lin-26 expression in the somatic gonad generally fail to form a uterine lumen and strongly down-regulate *jam-1* in the uterus (den Boer et al., 1998). Finally, *che-14* mutants display the same specific ultrastructural defects as the weak allele *lin-26(n156)* (Labouesse et al., 1994, 1996; Michaux et al., 2000), raising the possibility that *che-14* could be a downstream target of *lin-26*.

In addition to its maintenance function, lin-26 could act in a redundant manner to induce *jam-1*, *dlg-1*, and *che-14* expression during epithelial differentiation. Interestingly, it

![FIG. 5](image-url)

Each blastomere can respond to ectopic lin-26 expression. Transgenic *jcIs1* (middle left column) or *jcIs1;hs::lin-26* (middle right column) embryos were operated to leave intact only one blastomere (left column), and then heat-shock treated as detailed in Materials and Methods. GFP autofluorescence micrographs of representative embryos are shown. The right column gives the proportion of *jcIs1;hs::lin-26* embryos with clear evidence of ectopic GFP fluorescence compared to control embryos. We tested 10 isolated ABa, 10 isolated P2, 7 isolated EMS, and 6 isolated ABp in the *jcIs1* background (wt control), which all showed a reproducible pattern. Note that the GFP pattern in the control EMS embryo resembles the wild-type intestine JAM-1 pattern (narrow zigzag line), whereas in the *jcIs1;hs::lin-26* EMS embryo it is more similar to the normal JAM-1 epidermal pattern (large rings).
has recently been reported that forced expression of the essential GATA factor elt-1 and of the dispensable GATA factor elt-3 can turn on jam-1 expression in most cells, in a manner very similar to that observed with lin-26 (Gilleard and McGhee, 2001). A noteworthy difference between elt-1 and elt-3 on the one hand, and lin-26 on the other, is that forced expression of elt-1 or elt-3 also induces the expression of the epidermal collagen gene dpy-7 (Gilleard and McGhee, 2001). Thus, a plausible model for the formation of the epicardium in C. elegans is the following (Fig. 7). First, elt-1 specifies tissue identity and turns on lin-26, elt-3, as well possibly as the other epicardial-specific GATA factors elt-5 and elt-6 (K. Koh and J. Rothman, personal communication). In turn, elt-1, lin-26, and elt-3 act redundantly to induce epithelial-specific genes (jam-1, dlg-1, and che-14), while elt-1 and elt-3 act redundantly to switch on epithelial-specific genes (collagens). By extension, in other tissues where it is expressed, lin-26 could act redundantly to promote epithelial differentiation, while other regulatory genes would confer tissue identity and dictate the type of epithelium being made. A comparable scheme has been described for the genetic hierarchy controlling formation of the intestinal tube-shaped epithelium. First, the GATA factor end-1 together with at least one other gene specify intestine identity and turn on the GATA factor elt-2, which is essential for intestine differentiation, and for its function during postembryonic development (Fukushige et al., 1998; Zhu et al., 1997, 1998). Both end-1 and elt-2 then act in a redundant manner to turn on several intestinal genes, including jam-1 and the gut esterase gene ges-1 (Fukushige et al., 1998; Zhu et al., 1998). Although ELT-2 can bind the ges-1 promoter in vitro and induce ges-1 expression in vivo if expressed ectopically, ges-1 is normally expressed in elt-2-null mutants (Fukushige et al., 1998; Hawkins and McGhee, 1995).

Our results have also shown that blastomeres can be reprogrammed during a narrow time window of C. elegans embryogenesis, at the beginning of gastrulation (28- to 50-cell stages). In this respect, lin-26 behaves like other tissue-specific regulatory genes that have been tested using a similar approach, such as pha-4, end-1, elt-1, or elt-3 (Gilleard and McGhee, 2001; Horner et al., 1998; Zhu et al., 1998). This time window corresponds to a period when the pregastrula embryo has been patterned but organ/tissue identities have not yet been assigned (Labouesse and Mango, 1999). Interestingly, the pharynx remained susceptible to ectopic lin-26 expression between the 50- and 100-cell stages (class III embryos), and the phenotype of class III embryos was similar to that of pha-4 mutant embryos, pha-4 is first required between the 50- and 100-cell stages to specify the fates of pharyngeal precursors (Horner et al., 1998). In pha-4 mutants, the pharynx primordium is missing and there is an excess of LIN-26- and JAM-1-expressing cells where pharyngeal cells should be (Chanal and Labouesse, 1997; Horner et al., 1998). These observations are consistent with the suggestion that pha-4 is required to repress lin-26 expression in the pharynx (Horner et al., 1998).
So far, we have suggested that lin-26 promotes epithelial differentiation and might do so by inducing the expression of epithelial-specific genes. In theory, the LIN-26 protein could do so by directly binding to the promoters of epithelial-specific genes, by binding to the promoter of an intermediate transcription activator or by repressing a repressor. Our experiments do not address this issue; however, we do not favour at least one version of a double repressor model. We previously raised the possibility that lin-26 could act in the nonneuronal ectoderm by repressing the expression of neural-specific genes (Labouesse et al., 1996). The experiments presented in this study show that lin-26 expression in neuroblasts or neurons does not affect neuronal differentiation, suggesting that lin-26 does not repress a neuronal regulatory gene that would prevent epithelial differentiation in neurons. Future experiments will be aimed at determining whether LIN-26 can bind the promoters of jam-1, dlg-1, and che-14.

It has been speculated that epithelial differentiation could be a default pathway in vertebrate development (Frisch, 1997). In contrast, our experiments in C. elegans suggest that a specific gene can promote epithelial differentiation without conferring any tissue specificity. One possibility to account for these seemingly opposing conclusions could be that vertebrate studies were based on the analysis of tumours and may not reflect the situation encountered in all epithelial tissues. In addition, we examined the expression of genes that are required to organise junctions or control apical trafficking, whereas previous studies in vertebrates systems generally examined the expression of structural proteins.

ACKNOWLEDGMENTS

We thank Elise Camut who made the initial hs:lin-26 construct. We are grateful to M.-A. Félix for use of her laser microscope. We thank John Gilleard for sharing unpublished results, A. Fire, J. Gilleard, J. Hardin, I. Jonhstone, S. Mango, J. McGhee, B. Page, A. Wissmann, and Bob Waterston for reagents. We thank Julia Bosher for critical reading of the manuscript, J. L. Vonesch for confocal images, and J. M. Lafontaine and B. Boulay for photographs. This work was supported by funds from the CNRS, INSERM, and Hôpital Universitaire de Strasbourg and by grants from the EEC-TMR program and the Association pour la Recherche contre le Cancer (to M.L.).

REFERENCES

E-cadherin is a WT1 target gene. J. Biol. Chem. 275, 10943–10953.


Submitted for publication March 5, 2001
Revised April 10, 2001
Accepted April 10, 2001
Published online June 13, 2001