belong to a group of genes implicated in hereditary spastic paraplegia (HSP) and are characterized by axonal degeneration of long corticospinal motor neurons (Salinas et al., 2008). Over half of the HSP mutations reside in three genes that include atlastin-1; spastin, a microtubule-severing ATPase that associates with atlastins; and REEP1, another member of the DP1 family of proteins. Therefore, defects in ER network formation appear to underlie most instances of HSP. These observations suggest that long spinal neurons are particularly dependent on elaborate ER networks for cell function. And while trafficking per se does not seem inhibited by these mutations, is may be that cortical ER networks that lie just beneath the plasma membrane play a critical role in axonal maintenance. This precise role is not known, although the observed degeneration of specific neuronal cells may provide an important clue to the cellular function of peripheral ER networks.

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Integrins Anchor the Invasive Machinery

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Cell invasion through the basement membrane, a process important for both development and disease pathogenesis, depends on an interplay of adhesive, force transducing, proteolytic, and chemotactic machineries. The mechanisms whereby these different processes are integrated on the cellular level have remained elusive. In this issue of *Developmental Cell*, Sherwood and coworkers now identify integrins as integration platforms for a specialized invasive membrane domain in *C. elegans*.

Basement membranes (BM) are dense sheets of extracellular matrix (ECM) that function as structural support for epithelial, endothelial, and mesenchymal tissues. They serve as barriers to separate tissues into compartments and provide signaling cues that regulate the behavior of these cells (Yurchenco et al., 2004). Crossing such tissue barriers is a hallmark of developmental programs such as migrating neural crest cells, the invading blastocyst, and endothelial cells during angiogenesis, but also of invasive growth and tumor metastasis (Rowe and Weiss, 2008). On a cellular level, traversing the BM requires multiple steps: an adhesive interaction of the cell with the BM, local degradation of the ECM, and finally migration through the tight and complex ECM sheet. Understanding this process on a molecular level requires identification of (1) genetic programs and secreted signals that induce invasive behavior of cells, (2) receptors that mediate cell-BM interactions, (3) mechanisms that allow focal proteolysis, and (4) mechanisms that facilitate transmigration through the locally degraded BM.

The *C. elegans* anchor cell (AC) is an elegant model for the study of invasive cell behavior (Sherwood and Sternberg, 2003). The AC is a single, specialized cell in the *C. elegans* gonad that initially resides in the uterus, but later invades the vulva through the BM that separates these two tissues during larval development (Sharma-Kishore et al., 1999). The invasive process is critical for specification of the vulvar precursor cells that will ultimately form a connection between the uterus and the vulva (Sharma-Kishore et al., 1999; Sherwood and Sternberg, 2003). Sherwood and coworkers showed in previous studies that AC invasion is initiated by a diffusible cue secreted by the vulval tissue and a second, less robust cue derived from the ventral nerve cord (VNC). The response of the AC to these cues is regulated by a developmentally timed intrinsic competence acquired by ACs (Sherwood and Sternberg, 2003). The VNC cue for AC invasion is UNC-6 (Ziel et al., 2009), the *C. elegans* ortholog of netrins, a family of secreted ECM-associated factors that act as guidance cues for cellular pathfinding (Cirulli and Yebra, 2007). UNC-6 acts through its receptor UNC-40 (Deleted in Colorectal Cancer; DCC in mammals) to establish an invasive membrane domain in the AC containing

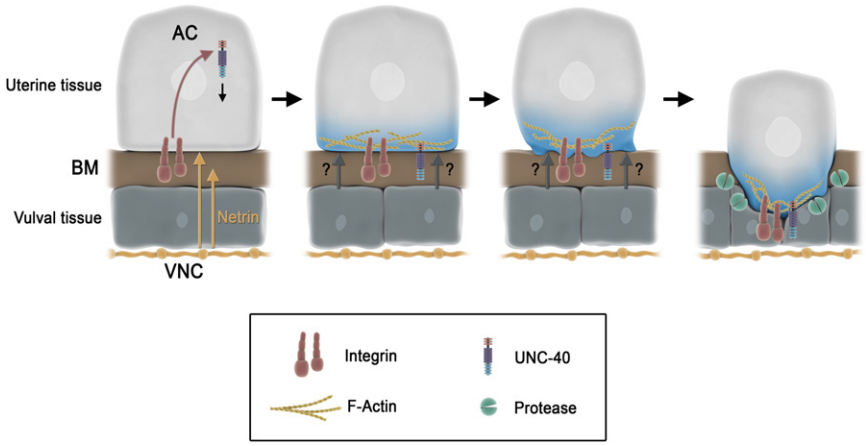

Figure 1. Model for Integrin and UNC-40 Cooperation in Promoting Anchor Cell Invasion
The anchor cell (AC) attaches to the underlying basement membrane (BM), which separates the uterine and vulval tissues. Binding of INA-1/PAT-3 integrins to their BM ligands induces the recruitment and/or stabilization of the netrin receptor UNC-40 at the basal plasma membrane as well as the recruitment of the F-actin cytoskeleton together with actin modulatory proteins and phospholipids to form a plasma membrane-associated actin scaffold. Netrin, secreted by the ventral nerve cord (VNC), is now able to signal through UNC-40, resulting in the rearrangement of the actin scaffold to form a protrusive membrane structure facing the BM. The formation of the invasive membrane scaffold together with an unknown stimulatory signal from the vulval tissue facilitates the invasion of the AC through the locally degraded BM.

F-actin, actin modulators such as the Rac GTPase MIG-2, and the phospholipid PI(4,5)P_2, which is involved in anchoring cortical actin to the plasma membrane (Ziel et al., 2009).

The new study by the Sherwood group in this issue of *Developmental Cell* identifies integrins as the initial organizers of the invasive signaling platform, targeting platform components including UNC-40/DCC to the basal membrane of the AC (Hagedorn et al., 2009). Integrins are a major class of cell surface receptors that bind to ECM and BM proteins. Mammalian integrins exist on the plasma membrane as 24 distinct heterodimers composed of α and β subunits that can assemble in different combinations with cell-type-specific expression patterns, enabling specificity for substrate recognition and signal transduction (Hynes, 2002). The use of *C. elegans* as a model organism allows analysis of integrin function in a simplified system, as worms possess only a single β subunit, βPAT-3, and two α subunits, INA-1 and PAT-2. Disruption of βPAT-3 or PAT-2 expression results in contraction-induced muscle detachment from the body wall, whereas disruption of INA-1 results in defective neuronal and muscle cell migration, pointing to a crucial role for integrins in tissue morphogenesis during *C. elegans* development (Cox et al., 2004). In the current study Sherwood and coworkers show that the INA-1/PAT-3 heterodimer is also essential for the invasive capability of the AC. In this case INA-1/PAT-3 act in the AC to establish the invasive membrane domain. In the absence of an intact INA-1/PAT-3 heterodimer, the recruitment of F-actin and Rac GTPase MIG-2 to the basal plasma membrane and subsequent invasive behavior of the AC were severely impaired. This is not unexpected since the hallmark of integrin signaling is the assembly of adaptor proteins such as talin, vinculin, and integrin-linked kinase (PAT-4) onto their cytoplasmic tails, resulting in the engagement and organization of the actin cytoskeleton (Legate et al., 2009). Somewhat surprisingly, the integrin was not required for the attachment of the AC to the underlying BM, although it remains possible that the dominant-negative and hypomorphic alleles used in this study result only in a partial loss of integrin function and thus fail to uncover this function. The exciting finding of this study is the concept that integrin signaling is essential for the stable plasma membrane localization of the netrin receptor UNC-40/DCC, which subsequently acts to localize the invasive membrane structure adjacent to the BM. This finding suggests that integrins generate a plasma membrane-attached protein scaffold, consisting of F-actin, actin-modulatory proteins, and phospholipids, that functions to recruit UNC-40 to this part of the plasma membrane, which in turn creates a signaling platform for invasion. The precise molecular mechanism of this recruitment is left open for further study. Nevertheless, it is tempting to speculate that this mechanism could apply to other signaling receptors as well.

Sherwood and coworkers' results, when taken together, shed light on how multiple molecular requirements of the invasive process are integrated at the cellular level. In their model, integrins bind to their BM ligands, and through engagement of the cytoskeleton and accessory proteins form a plasma membrane-associated scaffold. This permits the recruitment of UNC-40 to the plasma membrane, and subsequently allows netrin, secreted by the VNC, to bind to its receptor. Netrin signaling through UNC-40 localizes the actin scaffold to form the mature protrusive membrane structure, which together with an unknown signal from the vulval tissue and breakdown of the BM allows the cell to invade the underlying vulval tissue (Figure 1). However, several questions remain open. Perhaps the most important one is the nature of the cell-intrinsic program that converts the AC into an invasive cell. Understanding this signaling cascade might provide important insights into the mechanisms that convert benign carcinomas in situ into malignant invasive tumors. Furthermore, the identity of the factor secreted by the vulval cells that is essential for the translocation of the cell through the BM pore remains unknown. Finally, the proteases that execute the degradation of the BM, and the accessory molecules that facilitate the focal nature of this process, are yet to be identified. The authors of the current paper speculate that the invasion might occur in a protease-independent manner. This seems rather unlikely, given the dense structural nature of the BM sheet, which differs strikingly from that of the looser ECM. The BM provides a mechanical barrier and it is difficult to envision how inflexible cellular structures such as the nucleus could be pushed through it. Interestingly, the Sherwood group shows that loss of integrins also reduced the deposition of hemicentin, a matrix protein that enhances BM

degradation, suggesting that integrins also regulate localized degradation of the BM (Hagedorn et al., 2009). Future studies utilizing mammalian systems with greater integrin signaling complexity are now needed to build on and refine the current model, and, hopefully, to bridge the gap between mechanisms regulating controlled invasion during development and the complex signaling networks promoting human diseases such as cancer.

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LIN28 *lets* BLIMP1 Take the Right Course

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The transcription factor BLIMP1 is a master regulator of primordial germ cell (PGC) specification and is suppressed by the microRNA *let-7*. In a recent issue of *Nature*, West and colleagues use a unique in vitro ES cell differentiation strategy to show that LIN28 is an essential regulator of PGC formation through inhibition of *let-7* maturation and consequential induction of BLIMP1.

PGCs are the first cells in the mouse embryo to become committed to a specific fate. Cells in the early mouse embryo are pluripotent, but a transition occurs around embryonic day (E) 5.5–6.0 when signaling by bone morphogenetic proteins (BMP2, BMP4, and BMP8B) induces proximal epiblast cells to adopt a PGC fate. At about E6.25–6.5 founder PGCs emerge (Figure 1A), and by E7.5 about 40 PGCs can be identified by the expression of PGC-specific markers.

Identifying the factors required for PGC specification in the embryo has been challenging and so far only a few factors have been found. Early studies were rather laborious, relying on differential expression analyses, comparing gene expression in PGC and non-PGC cells, followed by gene knockout studies. These studies nevertheless were able to identify some important PGC regulators such as PRDM14 ([PRDI-BF1-RIZ]/SET domain containing 14) and BLIMP1 (B-Lymphocyte-induced maturation protein1, also known as PRDM1), which are expressed in PGCs starting at the founder stage and are essential for PGC specification (Ohinata, et al., 2005; Vincent, et al., 2005; Yamaji, et al., 2008). Two other proteins, TNAP (tissue nonspecific alkaline phosphatase) and STELLA (also known as DPPA3, developmental pluripotency-associated 3), are also expressed specifically in PGCs, starting at E7.5, but are not essential for PGC formation. West et al. (2009) build on this previous work to create, manipulate and study PGCs outside of the embryo, leading to the identification of LIN28 as a third major player in PGC specification.

To identify PGCs in vitro, West et al. (2009) and Wei et al. (2008) took advantage of 100% concordance of PGCs and STELLA expression. They created mouse ES cells that contain *Stella*-GFP reporter constructs. They then manipulated the cells (by formation of embryoid bodies) and found a small population that expressed GFP, indicating that founder PGCs were forming in vitro, and that the *Stella*-GFP constructs were working as expected (Figure 1B). West et al. (2009) were then able to identify PGC specification genes by testing the effects of lentivirus-delivered shRNA knockdown of 30 candidate genes, selected based on previous differential expression in PGCs versus somatic cells. Because TNAP more precisely marks true PGCs in vitro, the authors used formation of TNAP-positive colonies as their assays for the effects of each knockdown. As expected, knockdown of *Blimp1* dramatically reduced the number of TNAP-positive colonies. Equally effective was knockdown of *Lin28*, an evolutionarily conserved gene that was recently shown to play key roles in ES differentiation. Along with the three transcription factors, OCT4, SOX2, and NANOG, the RNA-binding protein LIN28 can reprogram human fibroblasts into induced pluripotent stem (iPS) cells that resemble human ES cells (Yu, et al., 2007).

Several recent studies are now starting to shed light on the mechanism by which LIN28 functions in ES and other cells. During microRNA biosynthesis, a primary miRNA (pri-miRNA) is processed to a