to observe that the PG insertion occurs in a helical pattern. Of course, if this pattern was fixed within a cell, a uniform covering of the cell surface by PG could never occur. Remarkably, the authors show that the Mbl (*MreB-l*ike) protein, another bacterial homolog of actin which forms helical cables around the bacterial cell near the cell surface (Jones et al., 2001), is responsible for the observed pattern of PG synthesis. This provides an elegant model in which the Mbl cables in a growing cell sweep across the entire cell surface, generating a stiff PG layer as the cell surface is traversed by the Mbl cables. The coupling of PG synthesis to an actin-like system should not be completely surprising, as we have known for many years that different enzymes important in the glycolytic pathway of intermediate metabolism in eukaryotic cells (such as aldolase and phosphofructokinase) bind tightly to F-actin (Fulgenzi et al., 2001), raising the possibility still to be proven that certain basic enzymatic processes occur physically associated with the eukaryotic cytoskeleton, rather than as freely soluble molecules in the cytoplasm.

While the results on the link between Mbl, cell wall generation, and the control of cell shape appear quite convincing in *B. subtilis*, an obvious question arises about nonspherical bacteria that lack the MreB-Mbl cytoskeleton. Among these are the Mycoplasmas, which do not have a cell wall, but have cytoskeletal proteins controlling cell shape that appear rather different from those found in other bacteria (Trachtenberg, 1998). Daniel and Errington (2003) have examined other rod-shaped bacteria that have a cell wall but lack the MreB-Mbl system, and postulate a mechanism for the control of cell form based upon polar growth zones in these bacteria. The mechanism involves a modulation of zones of wall growth coupled to cell division. Obviously, cellular regulation, both temporally and spatially, of wall growth is key to cellular control of morphology, differentiating such processes from polymerization reactions that might be studied in vitro.

These new observations provide important leads into the different roles played by the two bacterial actin-like proteins, MreB and Mbl. While both form helical cables around the cell circumference, and both are required for the control of cell shape in many bacteria, it is only Mbl that is required for the helical pattern of PG insertion in *B. subtilis*. We can think of this as possibly parallel to the "division of labor" in eukaryotic cells between different actin isoforms. If one compares all existing eukaryotic actin sequences, most of the variation that exists is between different tissue-specific isoforms of actin (such as α-muscle versus β-cytoplasmic), rather than between species. Naively, one might expect that the relatively small sequence differences between these actin isoforms (different isoforms have $\sim$90% sequence identity) could be explained by the existence of actin binding proteins that bind to only one isoform of actin, providing a simple mechanism for the cell to regulate where these actin binding proteins localize. However, such proteins have not been found, and an actin binding protein that can bind one isoform of actin can bind all other isoforms tested in vitro. The elucidation of functional differences between the MreB and Mbl cables in *B. subtilis* is the first step in searching for the partners of these proteins (that share much less sequence identity than the actin isoforms do), as there are probably bacterial proteins that do bind to one but not the other.

Despite the assumptions that have been made in the past about bacteria being unstructured, it is now clear that both eukaryotic and eubacterial cells have a dynamic cytoskeleton. The new findings on cytoskeletal organization in bacteria serve to raise many more questions, such as: what are the proteins in bacteria that bind MreB and Mbl? Do the enzymes involved in PG synthesis directly bind Mbl, or are there other mechanisms of coupling? Answering such questions should lead to many new insights into areas as diverse as bacterial physiology, bacterial pathogenesis, and eukaryotic evolution.

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How Does a Cell Anchor and Invade an Organ?

In multicellular organisms, most cells are confined to a particular tissue. However, some cells invade organs during normal development and in diseases (e.g., angiogenesis and cancer). Recent studies reveal a fascinating step-by-step process in which specific vulval cells induce and attract a single gonadal cell to invade an epithelial tubular organ in order to connect the uterus to the vulva in *C. elegans*.

An invasion usually has negative connotations, involving armies, tumor cells, and pathogens. However, there are also "good" biological invasions, concerning normal cells that play positive functions in development such as in stem cell migration, gastrulation, and neurulation. Often the good and bad invaders use similar strategies;

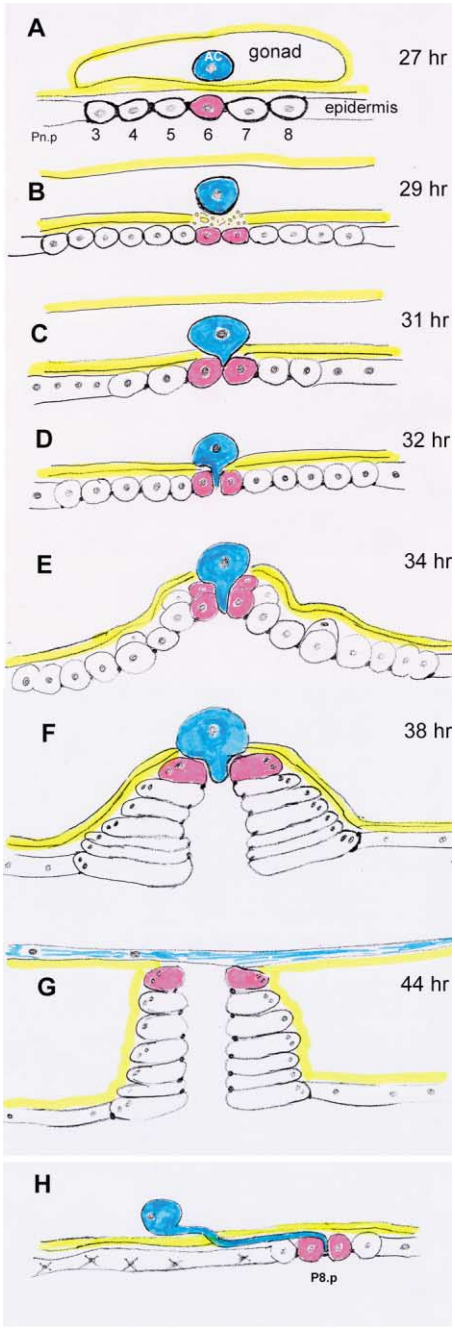

Figure 1. How Does a Cell Get In and Out of an Organ?

Schematic representation of AC invasion of the developing vulva.
(A) AC before invading, vulval precursor cells (P[3–8].p), and epidermis.
(B) Degradation of the laminae between AC and P6.p daughters.
(C) Initial AC filopodium gets in between the daughter cells of P6.p.
(D) AC invasion between the central granddaughters of P6.p.
(E) AC finger-like projection penetrates the apex of invaginating vulva.
(F) AC anchored inside the central and dorsal vulval ring (vulF).
(G) AC gets out of the vulva by fusing to eight uterine cells forming a hole and a thin membrane (hymen).
(H) AC invasion after cell ablation of all vulval cells except for P8.p. The descendents of P8.p induce the AC to send a long projection that is attracted and invades through the laminae and between these cells.
AC (blue); basement membranes (yellow); inducers and invaded cells (pink). Times given are hours from hatching at 20°C. Diagrams are longitudinal cross-sections. Anterior of the worm is on the left.

thus, biologists can learn the tricks of bad invaders by studying the mechanisms used by good invaders.

Cell-cell recognition and several physical barriers maintain the borders that constrain cells to a given tissue or organ. Basal laminae (basement membranes) underlying epithelial sheets and muscles are barriers keeping these cells separated from other tissues. In the normal human female reproductive system, the invasive extraembryonic cells break cell-cell contacts, reduce constraints on cell movement, and penetrate the uterine wall during implantation. Other examples where normal cells invade tissues during development include border cell migration in the ovary of *Drosophila*, white blood cell emigration during inflammatory response, neural crest cell migration, and epithelial-mesenchymal interactions.

Like "good" cells that invade during normal development, "malignant" tumor cells must locally break down the basal lamina using proteases to invade surrounding tissues. Many tumors also induce invasive behaviors in normal cells that will form new blood vessels that in turn invade the tumor to nourish it. This process requires degradation of the basal lamina surrounding a capillary followed by migration and proliferation of endothelial cells and formation of new basement membrane surrounding the elongated capillary.

In this issue of *Developmental Cell*, David Sherwood and Paul Sternberg highlight another elegant example of "benign" cell invasion in development (Sherwood and Sternberg, 2003). The single-cell invader is the mighty anchor cell (AC), a somatic gonadal cell in the *C. elegans* hermaphrodite (Delattre and Felix, 1999). The invaded organ is the vulva primordium and the reason for this conquest is to connect the uterus to the vulva by poking a hole between the two organs (Newman et al., 1996; Sharma-Kishore et al., 1999). John White described this cell invasion as a bizarre plumber who connects two tubes (the vulva and the uterus) without turning off the water in an actively moving house, the *C. elegans* feeding and developing larva. The life history of the invader AC is well documented. The wild-type hermaphrodite has a single AC that is induced to become the vulva organizer after cell-cell interactions between two equivalent cells using LIN-12/Notch signaling. The AC then induces the uterine and vulval precursor cells via LIN-3/EGF-LET-23/RTK-Ras-MAPK and LIN-12/Notch signaling pathways (Delattre and Felix, 1999). The morphogenetic role of the AC during invasion of the vulva was first discovered at nanoscale resolution. Using three-dimensional models of EM serial sectioned vulvae it is possible to see how the AC penetrates the newly formed vulval ring on the center of the invaginating organ (Sharma-Kishore et al., 1999). The final fate of the AC is to fuse to eight uterine cells, making a hole on the apex of the vulva and forming a thin syncytial membrane (hymen; Figure 1G) that prevents the spilling of the uterus (Newman et al., 1996).

Sherwood and Sternberg show that the AC and the vulval primordium remain separated by a double basal membrane until the central vulval precursor cell (P6.p) divides (Figure 1A). Then the basement membranes between the AC and the central daughters of the P6.p cell disappear, probably degraded by unidentified proteases and glycosaminoglycanases derived from the AC and/

or P6.p descendants (Figure 1B). Once the integrity of the physical barrier between the AC and the vulva is compromised, the AC starts invading by extending a finger-like projection between the central vulval cells (Figures 1C–1F). The invasion starts before the central vulval cells complete their divisions, and when vulval proliferation is blocked by hydroxyurea, AC invasion is only delayed, confirming that the invaded vulval cells induce AC invasion independently of the cell lineage (Sherwood and Sternberg, 2003). This is consistent with the recent finding that vulval cells continue differentiating even if they fail to divide (Shemer and Podbilewicz, 2002). Probably, if the AC failed to invade, then the vulva would have no hole in its apex and no connection would form to the uterus. Removal of the vulval inducers by microsurgery or mutations results in the formation of long directed AC-derived filopodia that find and invade vulval tissue (Figure 1H). Thus, the descendants of P6.p or P6.p-like cells attract and induce AC invasion at a distance. However, in 24% of the operated worms and in 20% of the mutant animals that lack vulval inducer cells there was invasion of nonvulval epidermal cells, suggesting that there is also a vulva-independent signal. This abnormal AC behavior may be the result of a cell-autonomous activity or it may reflect a weaker signal from the epidermis or muscles. This second signal is proposed to be independent of the strong diffusible signal derived from the P6.p descendants (Sherwood and Sternberg, 2003). An alternative explanation is that the putative nonvulval cells may be able to send the primary inducing cue even when they appear morphologically to be epidermal.

Sherwood and Sternberg accomplish a fascinating description of the spatial and temporal behavior of the AC invader that will allow the answering of the following questions:

What is the molecular nature of the cues that attract and induce AC invasion?

How is the competence of the AC to respond to cell invasion cues regulated?

How are the gonadal and vulval basement membranes degraded?

What are the receptors and ligands responsible for the AC-matrix interactions?

How does the AC interact with the vulval P6.p cell and its descendants?

Is anchor cell fusion essential to end the invasion and to form the connection?

Genetic approaches have identified mutants with defects in the connection of gonad (Cog) to the vulva including *cog-1*, *cog-2*, *lin-11*, and *lin-29* (Hanna-Rose and Han, 1999; Palmer et al., 2002). In addition, morphogenesis mutants with everted (Evl), squashed (Sqv), or protruding (Pvl) vulvae may identify the armaments or molecular signals and motives used by the invader and the invaded cells (Eisenmann and Kim, 2000; Herman et al., 1999; Seydoux et al., 1993). Sherwood and Sternberg have established a new model system to visualize and manipulate developmental cell invasion in vivo (Sherwood and Sternberg, 2003). As in the case of apoptosis, future cellular, molecular, and genetic studies on the invasion of the anchor cell will certainly connect not only the nematode vulva and uterus but will also reveal novel universal cell invasive mechanisms used by good and bad invaders and conserved from worms to humans.

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Getting (Chromosomes) Loaded—A New Role for Timeless

A recent study in *C. elegans* reveals an unanticipated link between sister chromatid cohesion and the TIM-1 protein, a homolog of the *Drosophila* circadian rhythm protein TIMELESS. The phenotypes of *tim-1* mutants suggest that cohesin subunits load onto chromosomes in a stepwise manner. Whether TIM-1 is also involved in circadian rhythms is discussed.

It is rare that research fields as diverse as chromosome segregation and circadian rhythms converge, but a recent study published in *Nature* stumbled upon a possible connection between the two processes. In an attempt to learn more about sister chromatid cohesion in *C. elegans*, Chan et al. (2003) discovered that the TIM-1 protein, which is homologous to the *Drosophila* circadian rhythm protein TIMELESS, physically associates with the cohesin complex that links the two sister chromatids. In the past few years, sister chromatid cohesion has emerged as a crucial component of many processes, including chromosome segregation, recombination, and repair (reviewed in Jessberger, 2002). In meiosis, the cohesin complex is required for proper