Vale measured diffusion coefficients between various signaling proteins. In their system, T cells were activated by plating on glass coverslips coated with antibodies to the T cell receptor. These immobilized antibodies not only initiate T cell activation but also hold the T cell receptor in clusters at the cell surface where they can be visualized. One hypothesis tested is that if the property of detergent insolubility regulated the clustering of signaling molecules, their lateral mobility should be constrained. As a "raft" marker, they fused the N terminus of Lck (which is acylated) to GFP. Thus, if activated T cell receptors recruit lipid rafts, they expected to find recruitment of the "raft" marker to clusters of T cell receptors. They found that their "raft" marker was highly mobile, suggesting that it was not confined to a lipid-based microdomain and presumably randomly distributed in the plasma membrane, which confirmed previous results from Kenworthy and Edidin (Kenworthy and Edidin, 1998).

In contrast to their raft marker, which was always highly mobile, lateral mobility measurements of Lck and LAT showed that these molecules could undergo an abrupt transition from a highly mobile to a highly immobile state. This transition in mobility required the ability of Lck and LAT to interact with other proteins. Because of these observations, they proposed that highly mobile signaling molecules diffusing in the plasma membrane randomly encounter activated signaling complexes. When the proper protein-protein interaction domain is present, this encounter results in the capture of the signaling molecule by the nascent signaling complex. Photobleaching experiments further supported their idea that signaling clusters were not fixed but rather constantly exchanging molecules, presumably through the formation and dissociation of protein-protein interactions. This exchange of molecules into and out of signaling clusters was observed directly using dual-color imaging where the trajectory of a single GFP-tagged protein could be imaged relative to a more immobile signaling cluster marked with RFP. Using this approach, Douglass and Vale found that immobilization of a single molecule occurred more frequently when it spatially overlapped with a signaling cluster; however, this immobilization was generally transient. Upon leaving the signaling cluster, the molecule could return to a highly mobile state rapidly diffusing into other areas of the plasma membrane. Thus, Douglass and Vale were able to visualize for the first time the assembly of signaling complexes mediated by the stimulation through the T cell receptor.

This study by Douglass and Vale challenges many of our assumptions and models regarding plasma-membrane microdomains and their role in T cell signaling. While these results suggest that detergent insolubility does not serve as the organizing principle for the assembly of signaling complexes at the plasma membrane, they also do not invalidate the existence of lipid rafts or their participation in T cell signaling. The results obtained here are compatible with the idea that lipid rafts are either extremely small and highly dynamic or that they constitute a significant proportion of the plasma membrane. Hopefully, the implications of this paradigm shift will begin to shed light upon the role these plasma-membrane microdomains play in mediating signals downstream of the TCR.

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Anchors Away! Fos Fosters Anchor-Cell Invasion

Invasion of cells through breakdown of the basement membrane is a crucial step during development and cancer metastasis. In this issue of *Cell*, Sherwood et al., (2005) describe a simple and genetically tractable cellular assay in the worm for elucidating the molecular processes that underlie cell invasion in vivo. They demonstrate that the transcription factor Fos is required for cell invasion and identify three of its downstream target genes (encoding a matrix metalloproteinase, hemicentin, and a fat-like protocadherin).

Tumor metastasis is an enormously complex phenomenon of critical biomedical importance. For tumors to form metastases, one of the first things the tumor cells must do is to break away from the tissue of origin and invade neighboring localities. Similar cell-invasion behaviors are a part of normal development in many organisms. In their new study, Sherwood et al., (2005) characterize a normal cell-invasion event in the worm *Caenorhabditis elegans* that is elegant in its simplicity, beautiful in the clarity with which it can be studied, and amenable to genetic analysis. With this cellular assay, they demonstrate a key role for the transcription factor Fos in cell invasion and identify three of its downstream target genes. In addition, the authors test the functional contributions of these target genes to cell invasion individually and in combination and arrive at results that one would not have entirely predicted.

In previous work, these authors described how a single cell in *C. elegans*, called the anchor cell, invades

the vulval epithelium during normal worm development (Sherwood and Sternberg, 2003). They found that specific ventral uterine precursor cells that develop directly beneath the anchor cell secrete an as-yet-unidentified chemoattractant. In response to this chemoattractant, the anchor cell extends a protrusion, which degrades the basement membrane precisely in between the anchor cell and the ventral uterine precursors. Thus, the anchor cell is able to move down in between the ventral uterine precursor cells leading to formation of the vulva.

In their new study, Sherwood et al. (2005) describe how the transcription factor Fos and three of its target genes promote anchor-cell invasion during *C. elegans* development. The authors demonstrate that a mutation causing a premature stop codon in one of two alternative transcripts from the *fos* gene causes a failure of anchor-cell invasion. The phenotype can be rescued by expression of the affected transcript and protein in the anchor cell. The Fos-1a transcript and protein are normally expressed at highest levels in the anchor cell, whereas the alternative Fos product is more widely expressed. The authors show convincingly that the anchor cell of *fos-1a* worm mutants is still able to extend a cellular protrusion toward the ventral uterine precursor cells but that the defect lies in the basement membrane, which remains intact.

Isoforms of worm Fos belong to an evolutionarily conserved family of transcriptional regulators that contain a basic region-leucine zipper (bZIP; Chinenov and Kerppola, 2001). Fos was originally identified as the product of a viral oncogene that causes osteosarcoma in the mouse. Its normal cellular counterpart, *c-fos*, can be induced in response to a variety of extracellular stimuli. There are four normal cellular Fos proteins in mammals: Fos, FosB, Fra-1, and Fra-2. Fos proteins form heterodimers with Jun and bind to AP-1 sites in the promoters of target genes. But they can also associate with numerous other bZIP transcription factors in a variety of different complexes.

The finding that Fos is important in anchor-cell invasion in the worm validates this system as a general model because Fos has also been implicated in several types of cell invasion in mammals. For example, Fos and Jun are expressed at the appropriate time and place in mammalian trophoblast cells to stimulate trophoblast invasion during development of the placenta (Bischof, 2001). In addition, Fos-transformed rat fibroblasts are highly invasive (Reichmann et al., 1992). Fos also appears to contribute to tumor metastasis in at least some types of cancer. For example, *c-fos* deficiency prevents the progression to malignancy of benign papillomas in a mouse model of skin cancer (Saez et al., 1995). In addition, expression of *c-fos* correlates with poor prognosis in squamous-cell lung carcinomas (Volm et al., 1993), and *c-fos* is expressed at a higher level in malignant prostate cancer than in benign prostatic hyperplasia (Aoyagi et al., 1998). Thus, Fos is likely to contribute to cell invasion during both normal development and pathological processes. The new work by Sherwood et al., (2005), however, demonstrates this in vivo with unprecedented single-cell resolution and crystal clarity.

These authors also identified three likely downstream targets of Fos in the anchor cell of *C. elegans*. In so doing, they reveal some expected results as well as some surprises. They identified the three target genes as *zmp-1*, *him-4*, and *cdh-3*. The *zmp-1* gene encodes a matrix metalloprotease. *him-4* encodes hemicentin, which is a fibulin-like matrix component that accumulates specifically under the anchor cell. The *cdh-3* gene encodes a Fat-like protocadherin. Individually, mutations affecting *zmp-1* or *cdh-3* cause little discernible effect on anchor-cell invasion, whereas mutations in *him-4* cause delayed anchor-cell invasion in a small percentage of animals. However, when all three genes carry mutations, the phenotype is more severe than that seen in the *him-4* single mutant.

The identification of downstream targets of Fos in the anchor cell, together with the ability to test the functions of these target genes, both individually and in combination, demonstrate how the anchor-cell invasion assay extends our understanding of the role of Fos and its targets during cell invasion. Surprisingly, whereas matrix metalloproteases have been implicated as key targets of Fos during cell invasion in other systems, during anchor-cell invasion in the worm, matrix metalloproteases play at best a subtle role in degrading the basement membrane. In contrast, hemicentin, a member of the fibulin family, which is thought to organize the extracellular matrix and stabilize cell adhesion, contributes more significantly to promoting anchor-cell invasion. Many questions remain to be answered. What is the nature of the chemoattractant that initiates anchor-cell invasion? What controls the protrusive activity of the anchor cell? How, precisely, does hemicentin promote anchor-cell invasion? Does Fos-1a form dimers with the *C. elegans* Jun homolog in the anchor cell? What other Fos target genes contribute to anchor-cell invasion? Perhaps the identification of additional Fos targets in the worm anchor cell will shed light on target genes switched on by Fos in trophoblasts during placental development and in metastatic tumor cells.

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