Matrix Spacing Controls Adhesion

Circulating lymphocytes are floating in a sea of fibrinogen, fibronectin, and vitronectin, all potential binding partners for integrins on the cell surface. Somewhere adhesion is turned off until these matrix proteins leak out of the blood and into tissues at sites of inflammation and injury. Only then does cleavage by pro-coagulants induce polymerization, and the resulting polyvalency, according to Stupack et al. (page 777), results in integrin-mediated adhesion. “It gives the cell a middle ground, they can explore a situation without prior activation,” says senior author David Cheresh.

Spacing rather than average density is the key. Stupack et al. use as their model a pentavalent coat protein of adenovirus, which has presumably evolved for optimum interaction with integrins to allow virus uptake. Pentavalent protein distributed at a density of just 125 binding sites/μm is sufficient for integrin-mediated lymphocyte adherence, whereas a monovalent version of the protein does not work at a density of 800 sites/μm.

Adherence alone is sufficient to activate Syk kinase. Stimulation with antigen also turns on Syk, perhaps allowing fully activated cells to bind even unpolymerized matrix proteins or proteolyzed fragments. The convergence on Syk may explain how two stimuli, antigen and extracellular matrix proteins, cooperate in the activation of immune cells.

Sphingolipid Trafficking

Lipid transport has been the poor cousin of intracellular trafficking, so any advance in the field is welcome. On page 673, Fukasawa et al. characterize a cell line that is defective in the ATP-dependent transport of ceramide from the endoplasmic reticulum (ER) to the Golgi. Once ceramide is transported to the Golgi it is converted into sphingomyelin (SM) on the luminal side, or glucosylceramide (GlcCer) on the cytosolic side. Fukasawa et al. study this process using the lysenin-resistant LY-A cell line. Lysenin is a protein from the coelomic fluid of the earthworm Eisenia fetida that binds SM and lyses the cell, possibly by forming a channel. The LY-A cell line has low SM but normal levels of ceramide and GlcCer. All known enzymes involved in the metabolism of SM are normal in cell lysates, and SM levels are restored by treatment with brefeldin A, which merges the ER and the Golgi. The cells are defective in ATP-dependent transport of a fluorescent derivative of ceramide from the ER to the Golgi, whereas trafficking of proteins remains normal.

The ATP-independent trafficking of ceramide is sufficient to maintain production of GlcCer. The energy-dependent step may involve flipping ceramide to the luminal side, delivering this topologically-segregated ceramide to the Golgi, or targeting the trafficking machinery to the more distal area of the Golgi where SM is made.

A Hyaluronan Receptor in the Lymphatic System

Hyaluronan (HA) is a large glycosaminoglycan found in tissues and body fluids. In vitro it supports cell rolling, and it can promote cell motility by surrounding the cells and thus preventing tight cell adhesion. On page 789, Banerji et al. report the identification of LYVE-1, a new receptor for HA that is specifically expressed in endothelial cells lining lymph vessels. LYVE-1 is most similar to CD44, an HA receptor found on epithelial, mesenchymal, and lymphoid cells. The authors speculate that HA may act as a sandwich between LYVE-1 and CD44, either aiding the entry of lymphocytes into the lymphatics or promoting their movement along them. LYVE-1 is important for several reasons: it provides an entrée into the neglected area of lymph biology, it is the first useful marker for lymph vessels, and it may prove important for the spread of cancer cells through lymphatics.

Controlling Platelet Shape Change

The G protein G₁₂ is known to trigger platelet aggregation and degranulation. Using platelets from a G₁₂ knockout mouse, Klages et al. show that the cell shape changes that accompany normal platelet activation are directed by G₁₂ and G₁₃ (page 745). At the end of the coagulation cascade, thrombin activates platelets to aggregate, degranulate, and change shape, then thromboxane A₂ from activated platelets amplifies the message. By changing shape from discoid to spheroid with pseudopodia, platelets expose greater surface area, and presumably aggregate more easily. The aggregated platelets form a first line of defense which is strengthened by the polymerization of coagulation proteins.

Klages et al. find that inhibitors of Rho or Rho kinase prevent the shape change and the phosphorylation of myosin light chain (MLC). A guanine nucleotide exchange factor (GEF) for Rho is the only known effector for G₁₂ and G₁₃, so there is a plausible pathway from the G proteins to the GEF, Rho, Rho kinase, MLC, and finally to a change in cell shape. MLC phosphorylation, at least in muscle cells, causes contraction of the actin–myosin network. In platelets there may be other cascades that end with actin polymerization and changes in the microtubule cytoskeleton.

Gene-specific Association with Domains

It makes sense that splicing factors would cluster at highly
transcribed genes that need them. On page 617, Smith et al. suggest that the organization of splicing factors is not so simple. Of two genes with similar amounts of pre-mRNA in the nucleus, the one with more introns is not associated with the concentrations of splicing factors termed SC-35 domains.

Nuclear staining with an antibody to SC-35, a spliceosome-assembly factor, reveals up to 40 large spots and numerous smaller speckles. Smith et al. define the large spots as SC-35 domains (sometimes also referred to as interchromatin granule clusters). The Lawrence group has previously noted the association of several highly expressed genes with these domains. In this report they note that the myosin heavy chain (MyHC) gene follows this pattern, but the dystrophin gene does not.

Dystrophin transcription is initiated less frequently than MyHC transcription, but it takes far longer to complete thanks to the size of the gene. Therefore, the total amount of pre-mRNA accumulated in the nucleus is approximately equal for the two genes. Dystrophin pre-mRNA is distributed in a large patch along the length of the gene, presumably as a “tree” of nascent transcripts. The patch of MyHC is of a similar size but tails away from the gene in a “track” that may be involved in export from the nucleus.

How can we explain the difference in domain association? The dystrophin/MyHC difference might arise from the variation in track vs. tree organization or transcription initiation rate. But of four other genes that share similar sizes, complexity, and moderate expression levels, two are associated with the domains and two are not. There is no gene sequestration (at least for dystrophin) in the dorsal and peripheral regions of the nucleus where SC-35 domains do not form.

Two classes of mechanism might explain domain formation at specific loci: an active mechanism directed by specific DNA or RNA sequences, or a more passive agglomeration of splicing factors from one or several active genes. Smith et al. postulate that some highly active genes may nucleate the formation of domains, with other active genes in the “neighborhood” then associating with the domains. Swapping gene locations by targeted integrations should provide some answers.

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