Leukocytes are more likely to grab onto a vascular surface when under flow than in the absence of flow. This counterintuitive behavior relies on bonds between transmembrane selectins on the blood cell and their vascular ligands. These so-called “catch bonds” get stronger because flow flips open selectin into a binding-ready conformation, Lou et al. show on page 1107.

The authors have solved the structure of L-selectin, which can now be added to previous structures of P- and E-selectin. The extracellular domains of all three have an N-terminal lectin domain and an EGF-like domain that are separated by a hinge. In L- and E-selectin, the two sides of this hinge are connected by a hydrogen bond between a tyrosine at residue 37 and an asparagine at residue 138.

In the absence of force, the lectin domain of L-selectin flipped between closed and open angles relative to the EGF domain, flexing at the hinge. But with increasing force, L-selectin was more often in an open conformation. A mutant L-selectin without the hydrogen bond between Y37 and N138 took less than half the force to be opened than did the wild type, suggesting that the hinge is held shut by this hydrogen bond.

Maximal tethering of microspheres with this mutant L-selectin also occurred at a lower flow. The change was due to increased rotational freedom of the mutant’s hinge, which increased the likelihood of protein–ligand contact.

After the L-selectin bound its ligand, the authors found, dissociation also slowed as force increased (up to an optimal level, at which point the selectins are fully open).

The group proposes that an open hinge, which aligns selectin’s binding interface with the direction of flow, might allow a ligand that would otherwise let go to slide along its selectin until it can rebind to a new site.

P-selectin has a glycine at position 138 and thus does not form a hinge-closing hydrogen bond. The increased flexibility of the P-selectin hinge is consistent with its ability to form catch bonds under smaller shear forces than those of L-selectin. 

Cooperative motors

Two kinesin-2 motors, OSM-3 and kinesin-II, drive the intraflagellar transport (IFT) of cargo toward the tips of cilia. Although OSM-3 is quicker than kinesin-II, Pan et al. (page 1035) show that these motors cooperate to move things together. In a related paper, Imanishi et al. (page 931) find that OSM-3 folds up to slow down.

OSM-3 moves at approximately twice the speed of kinesin-II. Pan et al. found that, when mixed together in an in vitro microtubule gliding assay, both motors moved at an intermediate speed, suggesting that they work together rather than independently to pull cargo along microtubules.

Mathematical modeling suggested that, to produce an overall intermediate speed, the motors either take turns pulling a cargo or work in a concerted but competitive fashion, with OSM-3 hurrying kinesin-II along and kinesin-II holding OSM-3 back.

To distinguish between these possibilities, Pan et al. separated the two motors by fragmenting their shared IFT cargo particles. They then saw that one portion move at the rate of OSM-3 and the other at the rate of kinesin-II. Thus, the motors work together, tempering each others’ speed along the cilium. At the very distal tip of the cilium, where IFT is faster, OSM-3 is known to work alone.

Meanwhile, Imanishi et al. found that OSM-3, like kinesin-1, folds over on itself such that the head and tail of the protein are in contact. Disruption of this interaction relieved autoinhibition of the motor domain and allowed for processive movement, as also occurs when cargo is bound.

Outside of their motor domains, kinesin-1 and OSM-3 are not homologous. The researchers thus hypothesize that this type of nonsequence-specific intramolecular fold might be a common mechanism by which motors remain still until they find a reason to move—the appearance of cargo. 

There’s a catch

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In the absence of force, the lectin domain of L-selectin flipped between closed and open angles relative to the EGF domain, flexing at the hinge. But with increasing force, L-selectin was more often in an open conformation. A mutant L-selectin without the hydrogen bond between Y37 and N138 took less than half the force to be opened than did the wild type, suggesting that the hinge is held shut by this hydrogen bond.

Maximal tethering of microspheres with this mutant L-selectin also occurred at a lower flow. The change was due to increased rotational freedom of the mutant’s hinge, which increased the likelihood of protein–ligand contact.

After the L-selectin bound its ligand, the authors found, dissociation also slowed as force increased (up to an optimal level, at which point the selectins are fully open).

Again the optimal force was lower for the hydrogen bond mutant than for wild-type L-selectin. The group proposes that an open hinge, which aligns selectin’s binding interface with the direction of flow, might allow a ligand that would otherwise let go to slide along its selectin until it can rebind to a new site.

P-selectin has a glycine at position 138 and thus does not form a hinge-closing hydrogen bond. The increased flexibility of the P-selectin hinge is consistent with its ability to form catch bonds under smaller shear forces than those of L-selectin. JCB
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Spaced just right

Mitochondria and ER membranes are directly tethered to one another, Csordás et al. report on page 915. Changes in tether length make the cell more or less vulnerable to apoptotic triggers.

Indirect evidence suggested that the mitochondria and ER were physically tied to one another, but what such a connection might be was obscure. Using electron tomography, Csordás et al. saw thin threads that ran between the organelles, ranging in size from 6 to 15 nm at the smooth ER, and 19 to 30 nm at the rough ER.

Limited protease digestion lengthened the ties and made the mitochondria less sensitive to Ca\(^{2+}\) release from the ER. By contrast, when the group engineered a 5-nm linker to narrow the gap between the organelles, mitochondria took in apoptosis-inducing amounts of Ca\(^{2+}\).

When wild-type cells were exposed to ER stress, the interorganelle tethers appeared to shorten before the cells entered apoptosis. The researchers speculate that the decreased distance enhances Ca\(^{2+}\) transfer from the ER to the mitochondria by keeping the organelles in immediate proximity. Whether other conditions push the organelles further apart—to safeguard mitochondria and prevent cell death—is unknown as yet.

It is also not yet clear what proteins comprise the tethers, but given their varied lengths, the team predicts that it will not be a single protein. In addition to organelle spacing, tether components might also help to control ER and mitochondria fusion and fission. JCB

A tether (gray) holds together mitochondria (red) and the ER (yellow).

Speeding nuclear import

Faster, better import is gained by increasing levels of the importin β nuclear transport receptor, according to Yang and Musser (page 951).

Current nuclear pore models do not consider the possibility that transport time might vary under different conditions. But that is just what Yang and Musser found when they increased the concentration of importin β in an in vitro system. Transport speed increased as much as sevenfold. Transport was also more efficient—more of the molecules that entered the pore passed through it successfully.

Structure-based studies suggest that long strands of phenylalanine–glycine (FG) repeats extend from the edge of the nuclear pore into the channel, creating a spaghetti-like network that molecules must wiggle through as they traverse the pore. A single importin β protein can bind to several of these FG repeats at the same time. Yang and Musser hypothesize that, as importin β moves through the channel, it may temporarily rearrange and open up the FG meshwork.

Higher importin β concentrations also increased the rate and efficiency of dextran movement through the pore, even though dextran is small enough to move through the pore without a transport receptor. The team is currently studying the effect of hyperactive import on the rate and efficiency of nuclear export. If excess importin β structurally disrupts the meshwork, it might facilitate movement in both directions. JCB

Trimming ERAD

Ubiquitins are zip codes for proteins heading to the proteasome. But misfolded ER proteins must be deubiquitinated before they are degraded, as shown by Wang et al. on page 963.

Misfolded ER proteins are sent back through the ER membrane into the cytosol by the p97 ATPase. Upon reaching the membrane’s cytosolic side, the substrates are modified by ubiquitin ligases. Wang et al. found that an enzyme that undoes the work of the ligases, a deubiquitinase called ataxin-3 (atx3), associates with p97 and the rest of this ER-associated degradation (ERAD) complex.

Overexpression of an atx3 mutant that lacks its deubiquitinase activity blocked degradation of at least two ERAD substrates and thus induced ER stress. The mutant also led to an accumulation of ubiquitinated substrates that were bound to p97, suggesting that substrates must shed ubiquitins before they can be moved to the proteasome. As a few ubiquitins are needed to target proteins to the proteasome, atx3 probably trims, rather than removes, the ubiquitin chains.

The atx3 protein is also compromised by polyQ expansions that cause spinocerebellar ataxia. Preliminary data from Wang et al., as well as data from Zhong and Pittman (Hum. Mol. Genet. 2006. 15:2409), show that these mutations effectively block degradation of some ERAD substrates. The resulting ER stress likely leads to cell death and may be the cause of neurodegeneration. JCB