

Generally Physiological

Of cytokine receptor scissors, grasping bacterial hands, and movement across the blood–brain barrier



This month's installment of *Generally Physiological* focuses on movement across membranes, discussing how the human growth hormone (hGH) receptor transmits the ligand-binding signal across the cell membrane to activate intracellular signaling pathways, how the bacterial outer membrane transport protein FepA reaches out to grasp its substrate, and the identification of two disparate functions for an orphan transporter at the blood–brain barrier.

Activating JAK

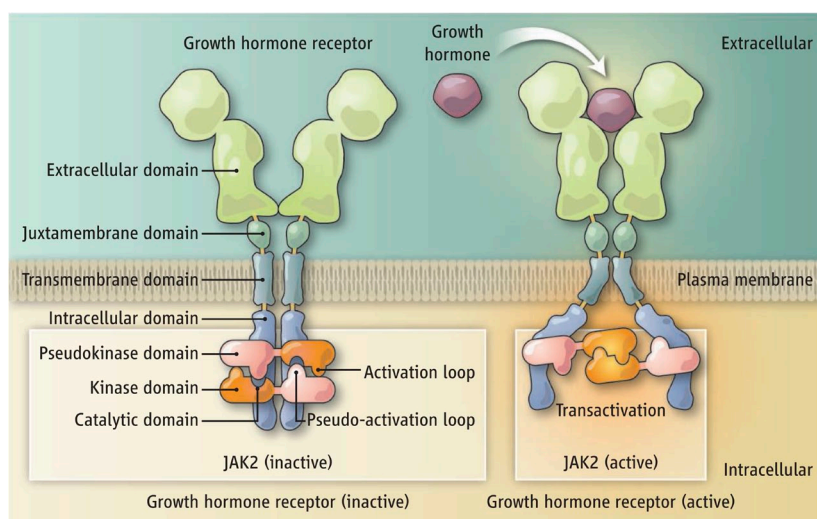
The human growth hormone (hGH) receptor functions as a dimer: a single molecule of the hGH polypeptide binds sequentially to the extracellular domains of identical single-transmembrane domain monomeric sub-

units, initiating the intracellular JAK-STAT signaling pathway, however, has been unclear. Early models postulating that hGH binding drew together the two monomers, and thereby their intracellular domains, were cast into doubt by data indicating that the unliganded receptor exists as a dimer (see Wells and Kossiakoff, 2014). Using a combination of Förster resonance energy transfer (FRET) analysis, cysteine cross-linking, mutagenesis, and molecular dynamic simulations, Brooks et al. (2014) developed a “scissor-like” molecular model for JAK2 activation in which the two JAK2 molecules associated with a resting hGH receptor dimer are oriented so as to mutually inhibit each

other through the separation of the membrane-proximal JAK2-binding regions in the receptor intracellular domain; this leads to the movement apart of the JAK2 molecules, relieving their mutual inhibition and juxtaposing their kinase domains, enabling them to activate each other and initiate the downstream signaling pathway. Thus, in marked contrast to the notion that hGH binding draws together the receptor intracellular domains, it appears to promote their separation.

Accumulating iron

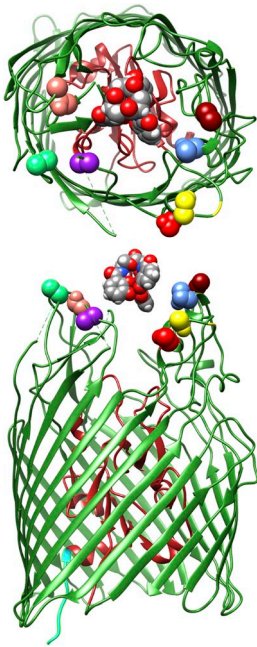
Gram-negative bacteria like *Escherichia coli* accumulate the essential micronutrient iron bound to siderophores such as ferric enterobactin (FeEnt), transporting the complex through the selectively permeable bacterial outer membrane by means of transporters such as FepA, a 22-stranded transmembrane β -barrel protein. Ligand transport by FepA depends on an initial step of high-affinity binding by surface loops, followed by a second step involving energy-dependent interactions with an additional protein, TonB; both of these steps depend on conformational changes in FepA. In this issue, Smallwood et al. used site-directed spectroscopic analysis of fluorophore-labeled *E. coli* FepA to characterize the movement of seven individual surface loops as they interacted with FeEnt. They found that the FepA loops moved at different rates during FeEnt adsorption, reaching out to adsorb and grasp the ligand, capturing it like the fingers of two hands in preparation for its transport into the periplasm.



Growth hormone binding to its receptor leads to the separation of the intracellular domains of the two subunits, moving apart the associated JAK2 molecules to relieve their mutual inhibition. (From Wells and Kossiakoff. 2014. Science. <http://dx.doi.org/10.1126/science.12544799>. Reprinted with permission from AAAS.)

units to activate a pair of Janus kinase-2 (JAK2) protein kinases associated with the intracellular domains. The

other through pseudokinase domains. hGH binding elicits a conformational change in the receptor that



Structural models of FeEnt and FepA. (From Smallwood et al., 2014.)

Traversing the blood–brain barrier

The blood–brain barrier, made up of capillary endothelial cells connected by specialized tight junctions, keeps undesirable substances out of the brain but necessitates the existence of specialized mechanisms whereby desirable substances can be transported into the brain (see Betsholtz, 2014). Nguyen et al. (2014) identified Mfsd2a, an orphan member of the major facilitator superfamily of membrane transporters, as crucial for transport of the essential omega-3 fatty acid docosahexaenoic acid (DHA) into the brain. Mfsd2a was highly enriched in brain microvessels of wild-type mice, where it localized to endothelial cells. The brains of mice lacking Mfsd2a were smaller than those of wild-type mice and showed decreased numbers of cerebellar Purkinje cells and of neurons in the CA1 and CA3 regions of the hippocampus. Moreover, they showed cognitive and behavioral defects resembling those associated with omega-3 deficiency. Lipidomic analyses revealed decreased DHA, but not other fatty-acid species, in brain phospholipids, but not in those from heart or liver. Cell-based assays indicated that Mfsd2a mediated the

sodium-dependent transport of DHA in the form of lysophosphatidylcholine DHA, rather than as the unesterified fatty acid, as did analyses of brain uptake of radiolabeled DHA in vivo. The authors thus conclude that Mfsd2a represents the major transporter for DHA uptake into brain.

In a separate study, Ben-Zvi et al. (2014) found that Mfsd2a was essential for blood–brain barrier development in mice. Having used a novel tracer-injection method to determine that the blood–brain barrier became functional at embryonic day 15.5, these authors compared the gene expression profiles of cortical and lung epithelium during the period the barrier was developing (embryonic day 13.5) and identified *Mfsd2a* as selectively expressed in cortical endothelium. Moreover, the blood–brain barrier in mice lacking Mfsd2a was leaky at embryonic day 15.5 on into adulthood. Electron microscopic analysis revealed apparently normal endothelial tight junctions, but a marked increase in transcellular vesicular transport (transcytosis). Noting that mice lacking pericytes (cells that wrap around endothelial cells of the microvasculature) also show impaired blood–

brain barrier function and increased transcytosis, the authors determined that loss of pericyte coverage was associated with a decrease in Mfsd2a. They thus conclude that Mfsd2a represents a key regulator of blood–brain barrier integrity, perhaps by suppressing transcytosis. The relationship, if any, between these two apparently disparate functions for Mfsd2a at the blood–brain barrier remains to be elucidated.

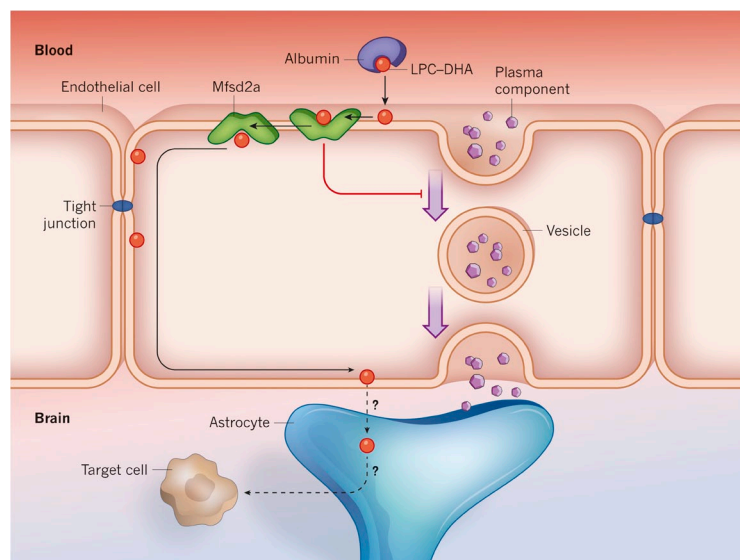
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Dual roles for Mfsd2a in brain capillary endothelial cells. (Reprinted by permission from Macmillan Publishers, Ltd. C. Betsholtz. 2014. *Nature*. <http://dx.doi.org/10.1038/nature13339>, copyright 2014.)