Transporters, channels and receptors in flagella

Dayana Rodriguez-Contreras and Scott M Landfear
Department of Molecular Microbiology & Immunology; Oregon Health Sciences University; Portland, OR USA

Over the past decade or so, cilia and flagella have become appreciated as sensory organelles or ‘antennae’ that monitor the extracellular environment in a wide variety of organisms. Among the kinetoplastid protozoa, single cell parasites that cause a range of devastating diseases such as leishmaniasis, African sleeping sickness, and Chagas’ disease, there has been increasing interest in the biological role of flagella and their capacity as sensors. Notably, these parasites do not express many of the typical families of sensory membrane proteins such as G-protein coupled or tyrosine kinase receptors, increasing the puzzle about how they monitor their environments during life cycles in which they pass through startlingly different physiological milieus. Studies over the years have identified a cohort of integral membrane proteins that are selectively localized to the parasite flagella, and many of these proteins are related in sequence or apparent structure to transporters, ion channels, or possible receptors.

The first study to suggest that proteins important in sensing might be localized to the parasite flagella emerged from the discovery of integral membrane adenylate cyclases in *Trypanosoma brucei*, some of which were localized to the flagellar membrane.1 The large extracellular domain, transmembrane segment, and cytosolic adenylate cyclase domain suggested that they might serve as receptors that modulate adenylate cyclase activity, but connecting these proteins with possible ligands and biological functions has remained a challenge. Other examples of probable flagellar sensory proteins emerged subsequently, such as the calfla-gins, EF hand proteins that associate with the luminal surface of trypanosome flagellar membrane and undergo conformational changes upon binding Ca\(^{2+}\) ions.2 A landmark paper in 2011 identified some 158 probable flagellar membrane proteins in *T. brucei* bloodstream form parasites by surface labeling, flagellar isolation, and mass spectrometry.3 Notably, proteins confirmed by immunofluorescence to be in the flagella included receptor-type adenylate cyclases, a putative Ca\(^{2+}\) channel, and a putative Ca\(^{2+}\)-ATPase.

Separate investigations on several species of *Leishmania* parasite also identified transporters and channels with flagellar localization and suggested they could be involved in environmental sensing. Thus one of 3 glucose transporters expressed by *Leishmania* parasites was targeted selectively to the flagellar membrane (Fig. 1), whereas the other 2 glucose transporters were excluded from the flagellum and associated with the plasma membrane around the cell body.4,5 An aquaporin designated AQP1, which mediates flux of water and a variety of small solutes, was localized to the flagellum of *L. major* parasites, and subsequent studies suggested that AQP1 was critical for both osmotaxis and volume regulation.6 Nonetheless, to date there is no example among the kinetoplastid protozoa of a sensory receptor with a known ligand and a defined biological readout or physiological function.

The role of certain transporters or transporter-like proteins as sensors of the levels of extracellular ligands has been established in a variety of microbial systems, and such transporter-receptors have been designated ‘transceptors’.7 Indeed, the first such transceptors identified were 2 glucose transporter-like proteins from *Saccharomyces cerevisiae*.8 Recent studies from our laboratory have now provided...
evidence strongly suggesting that the flagellar glucose transporter from *L. mexicana*, GT1 (Fig. 1), likely also plays a role in glucose sensing. Growth curves of Δgt1 null mutants in which the *GT1* gene was deleted showed that these mutants initially overshot the density attained by wild type parasites but then underwent a catastrophic loss of cell viability, instead of entering stationary phase, as do wild type parasites. Notably, this loss of parasite viability occurred precisely when the culture medium became depleted of glucose. These and other results suggest that a major function for GT1 is to monitor extracellular glucose and let the parasite know when this important nutrient has been exhausted.

Many questions remain to be answered regarding this sensory function for GT1. Is this permease, which can mediate uptake of glucose and other hexoses, also a bona fide transceptor that binds glucose and sends a signal to the interior of the cell, possibly by interacting with associated proteins as occurs with the yeast glucose transceptors? If so, it would represent perhaps the first example of a known membrane protein that binds a specific ligand and relays a message to the interior of a kinetoplastid parasite. The identification of potential GT1-interacting partners will be important to elucidate how binding of ligands to GT1 may transduce the signal. Alternatively, it is possible that the sensory function of GT1 is to import glucose into the flagellar lumen where it or a metabolite binds to another protein that is the true sensor. In either case, GT1 seems to play a critical sensory role that allows the parasite to adapt to its changing environment. What specific pathways are activated downstream of GT1 that normally promote entry into stable stationary phase but are inoperative in the Δgt1 null mutant, resulting in catastrophic cell death? Are there other transporters that localize selectively to the flagellar membrane in *Leishmania* species and may play similar sensory roles? The targeting of a putative Ca^{2+}-ATPase and Ca^{2+} channel to the flagella of African trypanosomes suggests that flagellar association of transport proteins may be more common among kinetoplastid parasites than previously appreciated. Connecting such proteins with their potential roles as sensors and establishing their mechanisms of action represents the current challenge.

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