overwhelmingly positive. Indeed, one student simply gave the feedback: 'More of these, please!'.

Future Potential of Adaptive e-Learning in Parasitology

The use of online adaptive learning as described in this article has been very successful in the training of veterinary students, supporting their growing clinical reasoning skills as they construct approaches to solving clinical problems. Further, we believe that these types of activities need not be restricted to clinical teaching programs. Skills that enable students to bring different types of knowledge and understanding together to solve real-world problems are needed throughout parasitology teaching programs, including in evolutionary biology, ecology, and molecular biology. The design considerations presented in this article aim to encourage further development of active and engaging online adaptive learning activities that can support students to achieve meaningful learning outcomes.

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Resources

[i]www.smartsparrow.com/

[ii]https://articulate.com/360/storyline

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References

1. Ben-Naim, D. et al. (2007) Virtual apparatus framework approach to constructing adaptive tutorials. In *Proceedings of the 2007 International Conference on E-Learning, E-Business, Enterprise Information Systems, and E-Government* (Arabnia, H.R. and Bahrami, A., eds), pp. 3–10, CSREA Press
2. Jabbar, A. *et al.* (2016) Can new digital technologies support parasitology teaching and learning? *Trends Parasitol.* 32, 522–530
3. Welsh, P.J.K. *et al.* (2009) Approaches to defining day-one competency: a framework for learning veterinary skills. *Rev. Sci. Tech. Off. Int. Epiz.* 28, 771–777
4. Delany, C. and Golding, C. (2014) Teaching clinical reasoning by making thinking visible: an action research project with allied health clinical educators. *BMC Med. Educ.* 14, 20
5. Krawthwohl, D.R. (2002) A revision of Bloom's taxonomy: an overview. *Theory Pract.* 41, 212–218
6. May, S.A. (2013) Clinical reasoning and case-based decision making: the fundamental challenge to veterinary educators. *J. Vet. Med. Educ.* 40, 200–209
7. Van Es, S.L. *et al.* (2016) Cytopathology whole slide images and adaptive tutorials for senior medical students: a randomized crossover trial. *Diagn. Pathol.* 11, 1
8. Kaw, A. et al. (2018) Adaptive lessons for pre-class preparation for flipped classroom. In *Proceedings of the 2018 American Society for Engineering Education – South-Eastern Section (ASEE-SE) Conference* (Brocato, J., ed.) pp. 14, American Society for Engineering Education
9. Weltman, H.R. *et al.* (2018) Evaluation of an adaptive tutorial supporting the teaching of mathematics, *Eur. J. Eng. Educ.* 43, 801–823

Forum

Sensing What's Out There – Kinetoplastid Parasites

Scott M. Landfear[1,*] and Dan Zilberstein[2]

Kinetoplastid parasites such as trypanosomes and *Leishmania* must adapt to their environments to survive within their hosts, yet they do not express many of the well established families of signal transduction receptors. Evidence suggests that other membrane proteins, including transporters and channels, play central roles in environmental sensing in these parasites.

Kinetoplastid parasites live in a variety of distinct environments in their mammalian hosts; *Trypanosoma brucei* is an extracellular parasite of the adipose tissue, skin, blood, and brain, whereas *Trypanosoma cruzi* lives in the cytosol of host cells in a variety of tissues, and *Leishmania* species live inside macrophage phagolysosomes. In both the vertebrate host and insect vector, and when transiting between them, parasites are exposed to dynamic physiological conditions, such as fluctuating nutrient levels, and they must sense the external environment to thrive within a hostile landscape. However, the signaling pathways that these evolutionarily ancient parasites employ to sense 'what's out there' are largely an enigma. Genome sequences for each of these parasites revealed that many of the classes of membrane proteins that mediate sensation (G-protein-coupled receptors, heterodimeric G-proteins, receptor tyrosine kinases) of the environment in most other eukaryotes are completely absent from these unicellular eukaryotes. How then is it that these pathogens sense and respond to pronounced environmental changes to support successful parasitism?

Sensing of environmental changes in kinetoplastid parasites doubtless engages both intracellular and cell-surface sensors, as is the case with many biological systems. In this Forum article, we focus primarily on surface membrane sensing, although reference is made to how membrane proteins may be critical for the functioning of internal sensing systems. Indeed, probable roles for several membrane proteins have been suggested by recent research (Box 1). In particular, transporters and channels are likely to serve central functions in environmental sensing, perhaps to a significantly greater degree than in organisms that express a wide range of signal transduction receptors.

Some permeases or channels likely mediate sensing by allowing influx of metabolites or other solutes, thus enabling the activity of internal sensors by making their ligands available, even though the carriers are probably not sensors themselves. One such example is the PAD (proteins associated with differentiation) transporters [1]

Box 1. Cell Surface Receptors and Other Proteins Involved in Sensing

G-Protein-Coupled Receptors

This large family of integral membrane proteins can include hundreds of members in higher eukaryotes. These receptors typically bind small ligands that activate the receptor and transmit a signal via the heterotrimeric G-proteins (α, β, and γ subunits) that bind to the cytosolic surface of the receptor. Examples include the receptors for α-mating factor of *Saccharomyces cerevisiae*, for biogenic amines such as dopamine, epinephrine, and norepinephrine, hormones such as gonadotropin-releasing hormone, and olfactory receptors located in the cilia of olfactory neurons.

Receptor Tyrosine Kinases

These proteins areDimeric cell-surface receptors that contain an extracellular ligand-binding domain, a transmembrane domain, and a cytosolic tyrosine kinase domain that can phosphorylate this amino acid on various substrate proteins. They are typically activated by binding of a polypeptide growth factor (e.g., epidermal growth factor), cytokine, or hormone (e.g., insulin). Some 58 receptor tyrosine kinase genes have been identified in humans.

Ion Channels

Many families of ion channels exist that mediate uptake of cations or anions across the plasma membrane. They typically span the membrane multiple times, are usually multimeric, and form a hydrophilic pathway that allows flux of specific ions across the otherwise hydrophobic membrane. Some channels mediate uptake of noncharged hydrophilic ligands, such as the aquaglyceroporins that promote uptake of water, glycerol, or other small molecules. Ion channels are often 'gated' from closed to open configurations, some by cellular physiological changes including alterations in transmembrane voltage, such as voltage-gated potassium channels, and others by specific small-molecule ligands, such as acetylcholine or serotonin receptors. Ca^{2+} is a second messenger involved in many signal transduction pathways, and thus calcium channels can play roles in sensing.

Receptor-Adenylate Cyclases

These 'unusual' receptors are expressed by various kinetoplastid protozoa. They consist of a large extracellular domain, a transmembrane domain, and a cytosolic adenylate cyclase. They are distinct from more typical adenylate cyclases, which are cytosolic enzymes and whose activation is often linked to G-protein-coupled receptors in the plasma membrane to modulate levels of the second messenger cAMP. Some of these kinetoplastid proteins are expressed in the flagellar membrane, but most are more uniformly distributed across the plasma membrane. Putative ligands for these receptors are currently largely unknown.

Transporters or Permeases

These multispanning membrane proteins mediate uptake of solutes by allowing them to permeate the hydrophilic barrier of the membrane bilayer by passing through a transporter-internal, substrate-selective pore. These permeases may constitute part of a signaling pathway by allowing external solutes to access the cytosol, where they can engage cytosolic sensors.

Transceptors

Transceptors are related in structure and sequence to functional transporters but function as signal transduction receptors. Some transceptors, such as the *S. cerevisiae* Snf3 and Rgt2 glucose sensors, have only ligand binding and sensory activity and do not transport glucose, although they are related in structure to other glucose transporters expressed by this yeast. These glucose transceptors bind to specific cytosolic proteins via their elongated C terminal hydrophilic domains, and upon glucose binding they regulate transcription of the genes for bona fide glucose transporters via a kinase-mediated signal transduction cascade. Other transceptors, such as Gap1 of *S. cerevisiae*, transport many amino acids but *also* activate a protein kinase A signaling pathway by a mechanism that does not require transport of the ligand.

expressed exclusively in stumpy bloodstream forms (SFs) of *T. brucei*, nondividing life cycle forms that are specialized for development into radically different procyclic forms (PFs) once the SFs have been taken up into the gut of the tsetse vector. These permeases mediate the uptake of carboxylates such as citrate and *cis*-aconitate that induce the transformation of SFs to PFs and thus constitute clear examples of transporters involved in environmental sensing that is critical for parasite development. A second elegant example of a transporter playing a critical role in transmitting an extracellular signal in *T. brucei* has emerged recently [2,3]. The SFs described above differentiate from long slender (LS) dividing forms, in the blood or interstitial tissue spaces, when parasite density reaches a sufficiently high level. Hence LS to SF development depends upon quorum sensing and is mediated by stumpy inducing factor (SIF), a molecule of unknown composition with MW <500 Da thought to be secreted from LS parasites, but the details of this signaling pathway have remained elusive for decades. Rojas *et al*. [3] discovered that a 9-transmembrane domain protein, which they call TbGRP89, and which has structural similarity to oligopeptide transporters (POTs), is expressed in LS parasites and is essential for sensing the long-sought SIF. TbGRP89 is able to transport di- and tripeptides of various structures when heterologously expressed in *Escherichia coli*, confirming that it is a functional oligopeptide transporter. Ectopic expression of TbGRP89 from an inducible promoter triggered growth arrest and differentiation of LS to SF parasites at low parasite density, as opposed to high parasite density where growth arrest and differentiation normally occur. These results imply that oligopeptides could represent SIF, and that TbGRP89 is essential to transport these signaling peptides into the cytosol of the trypanosome, via its permeation pore, where they induce differentiation by a still unknown mechanism. The model put forth suggests that increasing concentrations of

oligopeptides, generated by secreted parasite oligopeptidases acting upon host proteins, accumulate in the parasite's microenvironment as it achieves higher density, and that uptake of these peptides through TbGRP89 is essential to trigger LS to SF differentiation.

One point concerning the mechanism of membrane transporters such as PADs and TbGRP89 is worth emphasizing. Such proteins are sometimes loosely referred to as 'receptors' for their ligands. We suggest that this moniker should be avoided for these components of the signaling pathway because this term invites confusion regarding their mechanism of action. All current data suggest that these permeases transmit the sensory signal via their ability to vectorially transport the ligands from the cell exterior to the cytosol, and that other cytosolic components of the signaling pathway, typically not yet identified, take over to 'sense' the now intracellular signal. Designating such transporters as 'receptors' incorrectly implies that they are the ligand sensors, much along the line of G-protein-coupled receptors or 'transceptors' (see below and Box 1).

Other examples from recent research include a number of flagellar membrane channels or transporters. Cilia and flagella are now recognized as sensory organelles in many eukaryotic cells [4], and signal transduction receptors are often concentrated in this organelle. A distinct example of a flagellar permease was a glucose transporter, designated GT1 [5], expressed in the promastigote or insect vector stage of *Leishmania mexicana*. This hexose carrier is upregulated ~50-fold by glucose deprivation, indicating that its expression is linked to the level of its ligands. Null mutants in which the *GT1* gene has been deleted undergo a rapid population collapse when they exhaust glucose in the medium, suggesting that this transporter may be involved in sensing that nutrient and allowing transition into stationary phase. Another flagellar membrane protein likely involved in a sensing pathway, albeit probably not functioning directly as a sensor, is the aquaglyceroporin, AQP1 [6], from *Leishmania major*. Knockdowns of this channel are impaired in osmoregulation and osmotaxis in response to changes in osmolarity, an environmental alteration that these parasites encounter during their life cycle. The restoration of osmolar homeostasis is mediated by other carriers, such as the AAP24 proline/alanine permease that fluxes these osmolytes across the plasma membrane [7]. A third example is the arginine transporter, AAP3 [8], from *Leishmania donovani*, a permease that is essential for viability of the disease-causing amastigote forms that live in parasitophorous vacuoles inside host macrophages. Deprivation of arginine within the phagolysosome appears to signal through this transporter by engaging a mitogen-activated protein kinase-2-dependent signal transduction cascade. This cascade upregulates expression of the AAP3 transporter itself, as well as other proteins, and thus allows the parasites to survive under arginine limiting conditions. Notably, the host macrophage also expresses an arginine transporter/sensor (transceptor), SLC38A9, that senses the arginine levels inside the phagolysosome [9]. Under conditions of arginine sufficiency within the organelle, this transceptor activates the mTORC1 signaling pathway in macrophages, which triggers the cytotoxic Th1 response [10]. It is hypothesized that when arginine levels are low inside the phagolysosome, this transceptor fails to activate the Th1 pathway and thus allows survival of the parasite. Hence, regulated uptake of arginine by the parasite via AAP3 not only fulfills an essential nutritional function but also likely dampens the host immune response by depleting intraphagolysosomal arginine and enabling parasite development within phagolysosomes.

In addition, a putative Ca^{2+} channel is restricted to the flagellar membrane of bloodstream form (BF) trypanosomes [11], and although the precise function of this channel is not clear, it is essential for trypanosome viability, and its probable activity in calcium influx into the flagellum suggests a likely role in sensing. Finally, several receptor-adenylate cyclases from PF trypanosomes, kinetoplastid-specific proteins with large extracellular domains and an intracellular adenylate cyclase component, are localized to distinct domains of the flagellar membrane [12], and they are important for quorum sensing-like social motility (SOMO) [13].

Much of the discussion above focuses upon *T. brucei* and *Leishmania* species because those systems have predominated in the experimental studies accomplished to date. However, we anticipate that similar sensory mechanisms apply to *T. cruzi* as well. All of these parasites express multiple receptor-adenylate cyclases, suggesting that this family of putative receptors is involved in environmental sensing in each of these protozoa. Some membrane proteins involved in sensing the environment are specific to *T. brucei*, such as the PADs and TbGRP89, or to *Leishmania*, such as AAP3 and the flagellar glucose transporter isoform GT1, but proteins that work by parallel mechanisms could exist in *T. cruzi*.

It is perhaps not surprising that transporters and unconventional receptors are emerging as significant players in environmental sensing in kinetoplastid protozoa, given the absence of many of the better recognized signal transduction receptors. Indeed, some transporters have been recognized as mediators of environmental sensing in a variety of eukaryotes, from yeast to plants, and the term 'transceptors' has been coined to designate their distinct functions in signal transduction as separate from transport [14]. Many transceptors share common

features, such as extended hydrophilic domains at either their amino- or carboxy-termini that are involved in interactions with other proteins that mediate signal transduction. Transceptors are usually expressed at relatively low levels, compared to other members of the same transporter family that may not mediate sensing, and their levels of expression are typically induced by restriction of their ligand concentrations. These features are shared by the GT1 and AAP3 permeases described above, adding further plausibility for their roles in sensing. While many details of the signaling pathways mediated through transporters, transceptors, ion channels, or receptor-adenylate cyclases in these protozoa remain to be elucidated, it is likely that such 'less conventional' sensory pathways will emerge as central players in environmental sensation among this remarkable and evolutionarily divergent group of organisms.

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References

1. Dean, S. *et al.* (2009) A surface transporter family conveys the trypanosome differentiation signal. *Nature* 459, 213–217
2. Sollelis, L. and Marti, M. (2018) A major step towards defining the elusive stumpy inducing factor in *Trypanosoma brucei*. *Trends Parasitol.* Published online December 13, 2018. http://dx.doi.org/10.1016/j.pt.2018.11.009
3. Rojas, F. *et al.* (2019) Oligopeptide signaling through *Tb*GPR89 drives trypanosome quorum sensing. *Cell* 176, 1–12
4. Singla, V. and Reiter, J.F. (2006) The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* 313, 629–633
5. Rodriguez-Contreras, D. *et al.* (2015) Regulation and biological function of a flagellar glucose transporter in *Leishmania mexicana*: a potential glucose sensor. *FASEB J.* 29, 11–24
6. Figarella, K. *et al.* (2007) Biochemical characterization of *Leishmania major* aquaglyceroporin LmAQP1: possible role in volume regulation and osmotaxis. *Mol. Microbiol.* 65, 1006–1017
7. Inbar, E. *et al.* (2013) A versatile proline/alanine transporter in the unicellular pathogen *Leishmania donovani* regulates amino acid homoeostasis and osmotic stress responses. *Biochem. J.* 449, 555–566
8. Goldman-Pinkovich, A. *et al.* (2016) An arginine deprivation response pathway is induced in *Leishmania* during macrophage invasion. *PLoS Pathog.* 12, e1005494
9. Rebsamen, M. *et al.* (2015) SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature* 519, 477–481
10. Wolfson, R.L. and Sabatini, D.M. (2017) The dawn of the age of amino acid aensors for the mTORC1 pathway. *Cell Metab.* 26, 301–309
11. Oberholzer, M. *et al.* (2011) Independent analysis of the flagellum surface and matrix proteomes provides insight into flagellum signaling in mammalian-infectious *Trypanosoma brucei*. *Mol. Cell. Proteom.* 10, M111.010538
12. Saada, E.A. *et al.* (2014) Insect stage-specific receptor adenylate cyclases are localized to distinct subdomains of the *Trypanosoma brucei* flagellar membrane. *Eukaryot. Cell* 13, 1064–1076
13. Oberholzer, M. *et al.* (2010) Social motility in african trypanosomes. *PLoS Pathog.* 6, e1000739
14. Thevelein, J.M. and Voordeckers, K. (2009) Functioning and evolutionary significance of nutrient transceptors. *Mol. Biol. Evol.* 26, 2407–2414

Forum

Biostatistics for Parasitologists – A Primer to Quantitative Parasitology

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The aggregated distributions of host–parasite systems require several different infection parameters to characterize them. We advise readers how to choose infection indices with clear and distinct biological interpretations, and recommend statistical tests to compare them across samples. A user-friendly and free software is available online to overcome technical difficulties.

> Women were frequent visitors: Humboldt counted the lice in their plaited hair.
>
> Bonpland . . . wanted to know what statistics about lice were good for.
>
> One wanted to know, said Humboldt, because one wanted to know.
>
> Daniel Kehlmann: *Measuring the World*, 2005

The Nature of Host–Parasite Distributions

When collecting a sample of parasites, host individuals typically act as natural sampling units. Consequently, collection is a two-step procedure: first we collect hosts, and then we collect parasite individuals from them. Thus, parasites are practically collected in groups, so-called infrapopulations [1], where group size, expressed as the number of parasite individuals, may be 0 or a positive integer. The occurrence of parasites across members of a host sample (or the whole host population) exhibits a complex pattern that cannot be adequately described by a single measurement or index of infection, but different indices need to be applied that capture more-or-less different aspects of infection.

To describe the distribution of conspecific parasites across host individuals, it is traditional (i) to create infection classes: such as the categories of hosts with 0 parasite, those with one parasite, those with two parasites etc., (ii) to classify each host individual into one of these categories, and then (iii) to draw a histogram to represent either the number or the proportion of hosts belonging to each of these classes. Such frequency distributions do not approximate a normal distribution, but they generally exhibit an aggregated distribution. This means that most hosts have no, or just a few, parasites, and a few hosts have many [2] (except for some strictly controlled experimental infections under laboratory conditions).

This results in two problems. First, unlike normal distributions that can be