Beyond 9+0: noncanonical axoneme structures characterize sensory cilia from protists to humans

Eva Gluenz,* Johanna L. Höög,*† Amy E. Smith,* Helen R. Dawe,*1 Michael K. Shaw,* and Keith Gull*2

*Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; and †Boulder Laboratory for 3D Electron Microscopy of Cells, Department of MCD Biology, University of Colorado, Boulder, Colorado, USA

ABSTRACT The intracellular amastigote stages of parasites such as Leishmania are often referred to as aflagellate. They do, however, possess a short axoneme of cryptic function. Here, our examination of the structure of this axoneme leads to a testable hypothesis of its role in the cell biology of pathogenicity. We show a striking similarity between the microtubule axoneme structure of the Leishmania mexicana parasite infecting a macrophage and vertebrate primary cilia. In both, the 9-fold microtubule doublet symmetry is broken by the incursion of one or more microtubule doublets into the axoneme core, giving rise to an architecture that we term here the 9v (variable) axoneme. Three-dimensional reconstructions revealed that no particular doublet initiated the symmetry break, and moreover it often involved 2 doublets. The tip of the L. mexicana flagellum was frequently intimately associated with the macrophage vacuole membrane. We propose that the main function of the amastigote flagellum is to act as a sensory organelle with important functions in host-parasite interactions and signalizing in the intracellular stage of the L. mexicana life cycle.—Gluenz, E., Höög, J. L., Smith, A. E., Dawe, H. R., Shaw, M. K., Gull, K. Beyond 9+0: noncanonical axoneme structures characterize sensory cilia from protists to humans. FASEB J. 24, 3117–3121 (2010). www.fasebj.org

Key Words: signaling · host-pathogen interaction · primary cilium · electron microscopy

Cilia are fundamentally important in cell morphogenesis, embryonic development, and tissue function. A general view has emerged that classifies axonemes into canonical, motile, 9 + 2, and noncanonical, immotile sensory 9 + 0 structures. The 9 + 2 cilia and flagella move fluids and particles across epithelia and facilitate protist and sperm motility. The link between 9 + 0 cilia and inherited disease energized the field again and emphasized the sensory function of 9 + 0 cilia (for a recent review see ref. 1). We show here that this general view is overly simplistic, and additional axonemal architectures associated with sensory structures should be incorporated into the prevailing models. Leishmania parasites cause a spectrum of debilitating human diseases. In their sand fly vector, the Leishmania promastigote 9 + 2 flagellum functions in motility to mouth parts and attachment to surfaces. The intracellular amastigote form that replicates in mammalian host macrophages is often described as “aflagellate.” A short flagellum is clearly present and likely to be of importance to the parasite (2), but there is confusion in the literature as to its architecture. Although the majority of studies report a 9 + 2 structure (3), some electron microscope images suggest a different architecture (4, 5). Using mouse macrophages infected with Leishmania mexicana, we analyzed the flagellum ultrastructure by serial thin section transmission electron microscopy (TEM) and electron tomography (Fig. 1; Supplemental Movies 1 and 2).

The amastigote flagellum is short (~1.5 μm), spanning the flagellar pocket, and only a small bulbous tip is exposed to the parasitophorous vacuole environment. Serial sectioning and tomography revealed that the axoneme microtubules were arranged as follows (Fig. 1A–E, G, and Supplemental Movies 1 and 2): 9 triplet microtubules in the basal body, a ring of 9 doublet microtubules in the transition zone with associated projections connecting to the flagellar membrane, 9 doublet microtubules surrounding a basal plate, 9 doublets surrounding a central electron dense core that displayed cell-to-cell variation, with 1 or 2 central singlet microtubules occasionally observed that terminated before the neck region. This basal structure was connected to a 9 + 0 doublet ring with occasional outer dynein arm-like structures. There was no extraaxonemal paraflagellar rod (PFR). More distally, the 9-fold symmetry was broken by microtubule doublets progressively occupying a more central position (Fig. 1G, H). This symmetry break occurred distal to the basal plate, but before the narrowing of the flagellar pocket neck (Fig. 1H). Serial section examination of doublet displacement in 22 axonemes showed that in 10 axonemes 1 doublet was displaced, while 2 doublets were displaced simultaneously in 12 axonemes. Thus,
we conclude that the structure of the amastigote flagellum is distinct from the promastigote flagellum (a canonical 9+2 structure); however, it is strikingly reminiscent of early reports of the structure of the vertebrate primary cilium.

Although the vertebrate primary cilium is generally described as 9+0, early TEM studies from 40 yr ago indicated that the 9+0 symmetry is broken soon on exit of the cilium from the cell in diverse tissues and organisms (for example, see refs. 6–8). This feature of primary cilia has not been widely acknowledged recently, and its significance remains unknown. We revisited this issue using serial thin sections of the kidney primary cilium (Fig. 2A, B) and found that the 9+0 doublet structure distal to the triplet basal body (with associated procentriole) persisted for <500 nm (n=18 series). The first sign of symmetry break was a flattening of the axoneme (Fig. 2A7, black lines) followed by repositioning of the doublets at this position (Fig. 2A8, arrows). Progressive cilium tapering occurred; ultimately the axoneme contained only 1 doublet and 4 singlets. Rather than 9+0, we suggest that such axonemes be referred to as 9v axonemes, 9v standing for 9 variable. Variation comes in 3 particular areas (in addition to lack of central pair microtubules): bundling of the axonemal doublets, eventual reduction in doublet numbers to many less than the original 9, and eventually, in some cases, to singlet microtubules.

Interestingly, connections between the microtubule doublets and the ciliary membrane (generally indicative of the transition zone) persisted for much of the cilium length (e.g., Fig. 2A16, inset). These were also observed in the distal portion of the L. mexicana amastigote flagellum (Fig. 1G), raising the intriguing possibility that portions of the primary cilium and possibly the amastigote axoneme should be considered as extended transition zones.

We then asked whether a specific microtubule doublet became displaced in breaking the 9-fold symmetry. This is difficult to address in mammalian systems because the lack of an extra-axonemal reference point precludes absolute doublet numbering. We assigned each doublet a letter, A–I, based on its position relative to the procentriole (Fig. 2C), and used this nomenclature to look at doublet displacement. In serial sections of 18 axonemes, 4 showed displacement of a single doublet; in the remaining 14, 2 doublets were simultaneously displaced (Fig. 2A8, B7; arrows). These doublets were always separated by ≥2 other doublets (Fig. 2A, B). No particular doublet was consistently displaced, but there was a striking preference for doublets D, E, H, and I (Fig. 2F).

In Leishmania 9+2 promastigote axonemes, the singular position of the PFR and the fixed position of the 2 central microtubules provide reference points for absolute doublet and triplet numbering (Fig. 2D; ref. 14)}
Consequently, the position of the probasal body relative to the basal body was mapped by serial TEM of promastigote flagella. We found that the probasal body is always positioned adjacent to triplet 7 (Fig. 2D), an observation that has implications for basal body morphogenesis and inheritance. Assuming an identical positioning of the probasal body in the amastigote, this enabled us to determine which specific doublets were displaced via serial section TEM and tomography from the basal body to the flagellum tip in 11 flagella (Fig. 2D, F and Supplemental Fig. 1). Displacement was not restricted to a specific doublet; 8 of the 9 doublets were found to be displaced (Fig. 2F), suggesting essentially stochastic behavior.

Axoneme collapse occurs early, even during elongating flagellum in a dividing amastigote (Fig. 1H). Almost all 9 + 2 cilia and flagella are built and maintained by intraflagellar transport (IFT; ref. 10). In the L. mexicana amastigote, we observed IFT-like particles throughout the flagellum (Fig. 1B; Supplemental Fig. 1, arrowheads). Although we did not observe IFT particles along the collapsed primary cilium axoneme, we know from studies of ciliary disease that the primary cilium is also built by IFT (1, 10, 11). Thus a collapsed axoneme is no impediment to IFT.

What mechanistic function could the 9v axoneme architecture serve? One has to recognize that a sensory function is a common attribute of flagella and cilia, including many with canonical 9 + 2 axonemes (12,
Remarkably, a close examination of amastigote from the flagellum of the green algae proposed, based on observations of vesicles derived signal. A secretory function for cilia has recently been for targeted secretion of vesicles or luminal content flagellum raises the possibility that it could also be used compartmentalization of proteins to the amastigote pocket, again implying specific sorting mechanisms, from that of the plasma membrane and flagellar protein composition of the flagellar membrane differs the flagellum. Moreover it is likely that lipid and also be sorted and targeted specifically to the lumen of and associated structures. Cytoplasmic proteins may IFT-dependent delivery for assembly into the axoneme specific targeting of proteins to the flagellum and established that building of the flagellum depends on sense environmental cues and signal or transport mol-
receptor or transport proteins could be located that might provide a specialized membrane surface where exposed to the vacuole contents. We propose that this uole of the macrophage, and the tip of the flagellum is The amastigote resides inside the parasitophorous vacuole membrane associated. No other study has looked at the amastigote flagellum tip in relation to parasite attachment. Generally the evidence in the literature addressing the issue of amastigote cell body region of attachment consists of single micrographs showing a range of attachments (see ref. 18). This may indicate variability or may be a reflection of fixation speed. More concerted studies have found that L. amazonensis amastigotes were attached to the vacuole membrane by their posterior pole (19). Indeed, the possibility has been rehearsed that MHC class II molecules could be internalized by the parasite via its

Figure 3. The amastigote flagellum is in intimate contact with the parasitophorous vacuole membrane. A) Thin section TEM view of L. mexicana amastigotes in a J774 macrophage vacuole. B) Higher-magnification view of the area delineated by the white box in A. Amastigote flagellum tip is closely associated with the vacuole membrane (black arrowhead). White arrow indicates doublet displacement. C) Further example of the flagellum-vacuole membrane junction. D) Tomographic slice from a reconstructed 3-D volume of an amastigote flagellum in close contact with the vacuole membrane. E, F) Tomography model views (23) of the flagellum-vacuole membrane junction. A, L. mexicana amastigote; M, macrophage; V, vacuole. Scale bars = 500 nm (A–E); 200 nm (F).
posterior pole (20), although there is no evidence of such a site being endocytic and much against. The difficulty with all of these studies is the lack of three-dimensional imaging to allow full analysis of the arrangement of the different Leishmania species in the parasitophorous vacuole. This will be a major undertaking for the future.

We propose here that receptors on the flagellum tip may bind ligands in the vacuole membrane, triggering signal transduction pathways in the parasite. The structures we observed may even indicate a higher-order organization: perhaps a parasite synapse? Engagement of the flagellum tip with the vacuole membrane could orient the parasite such that signaling and secretion of parasite molecules are precisely targeted to a focal point. In summary, based on structural studies, we propose a hypothesis that signaling and secretion of parasite molecules are precisely targeted to a focal point. In summary, based on structural studies, we propose a hypothesis that signaling and secretion of parasite molecules are precisely targeted to a focal point.

We thank Dick McIntosh for comments on the manuscript and David Vaux (University of Oxford, Oxford, UK) for the gift of J774 macrophages. This work was funded by the Wellcome Trust, the Beit Memorial Fellowships for Medical Research, the Jackson Scholarship Fund (Merton College Oxford, to A.E.S.), an EMBO long-term fellowship (to J.L.H., and the E. P. Abraham Trust. K.G. held a Wellcome Trust Principal Research Fellowship, H.R.D. is a Beit Memorial Fellow, and J.L.H. is a Sir Henry Wellcome Fellow. The Boulder Laboratory for 3D Electron Microscopy of Cells is supported by National Institutes of Health Biotechnology grant RR00592 to A. Hoenger (University of Colorado, Boulder, CO, USA).

REFERENCES


Received for publication December 22, 2009. Accepted for publication March 18, 2010.