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In Silico Identification of Radial Spoke Proteins and Prediction of their Arrangement Patterns towards Assembly of the Flagellar Axoneme in Leishmania spp.

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Short Abstract: We present sequence and structural comparisons of radial spoke proteins (RSPs) in order to refine predictions that, if correct, suggest RSPs as part of the emerging group of flagellar proteins that are localized in specific axonemal compartments to carry out diversified activities related to flagellar maintenance in Leishmania spp.

Long Abstract:
BACKGROUND. Leishmania protozoa are responsible for a group of diseases, collectively known as leishmaniases. They are trypanosomatid members of the order Kinetoplastida, which contains other important uniflagellate pathogens such as Trypanosoma cruzi. As motile organelles, flagella are highly conserved across most eukaryotic phyla, being complex protein machines in which arrays of motor proteins must be precisely assembled along microtubule-based structures. Flagellar assembly is highly polarized, with all new growth ocurring at the tip. This requires specific transport processes to bring subunits out to the site of assembly. The flagellum contains around 250 proteins, and these are arranged into sub-assemblies, such as dynein arms and radial spoke proteins (RSPs). The radial spoke is a conserved, ubiquitous, macromolecular complex required for regulation of bending in motile 9 + 2 cilia and flagella. The T-shaped structure anchors to the nine outer doublets with a thin stalk, while its bulbous head contacts central pair apparatus periodically during the oscillatory beating. It is hypothesized that this intermittent interaction with central pair (CP) apparatus enables the radial spokes to distribute "signals," originating from CP apparatus, to subsets of outer doublets for localized control of dynein-driven microtubule sliding. There are at least 23 RSPs, most of them which have been recently characterized through mass spectrometry, and that are predicted to contain domains associated with signal transduction. Each of the nine MTDs has a row of RSPs that are arranged in triplet groups with a repeat of ~96 nm. The two spokes in each pair are uniquely positioned relative to inner row dyneins and to a dynein regulatory complex (DCR). RSPs appear to attach to CP projections and tilt as a result of dynein-induced sliding displacement of the MTDs. Because RSPs are associated with all nine outer doublets, but dynein activity must be limited to a subset of doublets at any one location along a flagellum, specific rows of CP projections are thought to interact with overlying RSPs in a doublet-specific pattern to modulate spoke regulation of

these kinases and phosphatases. At present, however, it is still unknown which specific radial spoke proteins interact with the CP, or which specific CP structures are involved in those interactions. Recently, Mitchell (2005) completed a detailed structural analysis of the CP and its orientation during the flagellar beat cycle which formed the basis for experiments that clarified the relationship between dynein activity patterns and orientation of the CP. The central apparatus and RSPs are thought to form a complex (CP/RSP) that regulates the activity of dynein arms. The rotation of the CP during ciliary and flagellar beating suggests that the CP might act as a `distributor' to regulate the activity of dynein arms. METHODS. Using a combination of integrated bioinformatics tools applied to genomic and proteomic findings on flagellar studies, here we present sequence and structural comparisons of RSPs in order to refine structural predictions that might be further used in a pathogenomics approach in Leishmania spp. As source of experimental data, we have used our own expressed sequence tag (EST) database on L. chagasi (Northeast Brazilian Genome Program), plus all publicly available datasets of individual or clusters of gene/protein sequences on Leishmania spp. It includes projects of whole genome shotgun (WGS) strategy on L. Major, L. infantum and L. braziliensis; proteome analyses on L. major; plus data from T. cruzi, T. brucei and T. gambiensi); data from other eukaryotes, including Chlamydomonas reinhardtii, among others, for specific comparisons on genes of interest. For databases searches, programs such as the variants of BLAST, and GlimmerHMM were widely used for sequence similarity searches and comparisons, while resulting data were built into a local database suitable for subsequent searches. Our survey included local BLASTP searches of the NCBI non-redundant protein database and the GeneDB database (accession numbers are those used in these two databases), searched against numerous collections of protein motifs and families, such as UniProt/Swiss-Prot, SMART, PROSITE, Pfam/iPfam, and Clusters of Orthologous Groups of Proteins (COGs). For global analysis of RSP sequences, we used MUSCLE, Cn3D (NCBI) and STING. We employed Modeller 8v2 for three-dimensional protein modeling and physical tangible models for predictions of macromolecular assemblies, both in a Silicon Graphics Fuel™ workstation running IRIX™ 6.5.25. RESULTS. Considering the "distributor" model of RSPs, where CP is asymmetric in structure and, in some organisms, it rotates once per beat cycle, our in silico analyses of RSPs in Leishmania spp. provided some interesting findings as the first report, in these trypanosomatid parasites, to tentatively describe a role for units of the complex of 23 polypeptides known to resist axonemal disassembly. For instance, since RSPs are thought to be relatively rigid to endure physical force arising from the interaction with CP, as well chemical signaling through calcium or nucleotide binding, it has been postulated that RSPs operate as both mechano- and chemo-transducers. Together, our predictions, based on identified sequences and modeled structures of RSPs in Leishmania spp., seem to suggest that: 1) the radial spokes are consistently heterogeneous and the proteins in the head end of spokes might be assembled differentially; 2) the radial spokes can be located either near the basal end of the stalk or toward the head end of the stalk; and 3) there is a higher ratio of head proteins to the stalk proteins. If our predictions are correct, RSPs will join the emerging group of flagellar proteins that are localized in specific axonemal compartments to carry out diversified activities related to flagellar maintenance in Leishmania spp. We speculate that these stalk proteins are not required to link head proteins to the base of stalk in a strict stoichiometric manner and may play additional roles other than as connectors of head and stalk of the radial spoke. In such case, the role might be the assembly of other relevant structural complexes within the axoneme.