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Commentary OMICS International

MYC-Binding Protein-1 is an R2D2 Protein

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Commentary

'9+2' cilia/flagella

The eukaryotic cilia/flagella have a '9+2' structure that is highly conserved in structure with 9 pair of outer microtubule doublets; one of these filaments is complete, and is known as the A microtubule; while the other microtubule is incomplete and is known as the B microtubule. These are present in the periphery and surround the two centrally placed microtubule singlets known as the central pair apparatus. These hair-like extracellular organelles can propel cells through an aqueous environment or also circulate the fluid surrounding them. This biological nano-machine without the membranous covering is known as the axoneme. With heterogeneity in their sizes (few µmm to few mm), these flexible extensions are conserved across evolution. A cross-section of the flagellum/cilium reveals the following sub-ciliary structures, the Outer Dynein Arms (ODA), Inner Dynein Arms (IDA), Dynein Regulatory Complex (DRC), Radial Spokes (RS) and the Central Pair (CP) apparatus that harbor several proteinaceous projections. These and other less evident structures are essentially protein complexes within the axoneme that operate in synchrony to generate the periodic beating that is typical of the flagella waves.

It is known that the second messenger, cyclic adenosine monophosphate (cAMP) regulates flagellar movement. Further, the use of pharmacological inhibitors on isolated axonemes of various flagellar mutants that have been mutated in the ODA, IDA, DRC, RS and CP have implicated cAMP-dependent protein kinase (PKA) and other phosphoenzymes in the dynein-driven microtubule sliding [1-6]. The PKA holoenzyme is made up of two regulatory (R) and two catalytic (C) subunits. Inside the cell, it is always found anchored to a scaffold protein; namely, the A-Kinase Anchoring Protein (AKAP) *via* its R subunit. The high-affinity binding partners of the R subunit of PKA [7] that were first discovered in 1982 are now known to be present in several organisms. With the RII subunit as bait, AKAPs are conveniently identified using the classical overlay assay [8].

A-Kinase Anchoring Proteins

All known AKAPs share little sequence homology; however, their common features include - a region for targeting it to a particular cellular location, an Amphipathic helix (AH) that binds to the Dimerization/Docking domain (D/D; or, R2D2 domain) of the PKA R subunit, and motifs other than these two for the recruitment of an array of molecules involved in signaling [9]. It is this AH that offers as one of the most important parameters for a protein to be designated as an AKAP. In fact, a recently conducted study used an *in silico* approach to determine AH-containing proteins that led to the identification of candidate AKAPs [10]. And, when RII overlays were performed in ciliated cells, a number of AKAPs were revealed; at least 7 were present in the fibrous sheath surrounding the mammalian sperm axonemes [11], one was found in the human respiratory tract cilia [12] and two (AKAP97 and AKAP240) in the *Chlamydomonas* flagellar axonemes [13]. The analysis of flagellar mutants lacking specific axonemal complexes revealed that AKAP97 is the radial spoke protein, RSP3; whereas, AKAP240 is localized in the CP. This finding indicates that both the RS and CP have a role in regulating dynein motors. Nevertheless, of the 23 proteins found to be present in the RS of the *Chlamydomonas* flagella, there is no resemblance whatsoever to the PKA C subunits [14]. And, the entire RS protein complex harbors features that are related to the PKA-AKAP signaling module. For example, (i) the N-terminus of RSP3 functions to anchor the entire RS to specific sites in the axoneme; (ii) RSP3 is known to form homodimer [15], and each of these monomers contains an AH for interacting with RSP7 or RSP11 [16], both containing the RII domain. Both these proteins lack any features required for cAMP signaling [17,18]. Therefore, it is believed that the *Chlamydomonas* flagella RS utilizes the AKAP-PKA signaling module to tether different molecules for its functioning; but, may not utilize cAMP in its transduction. Figure 1 describes this basic signaling module.

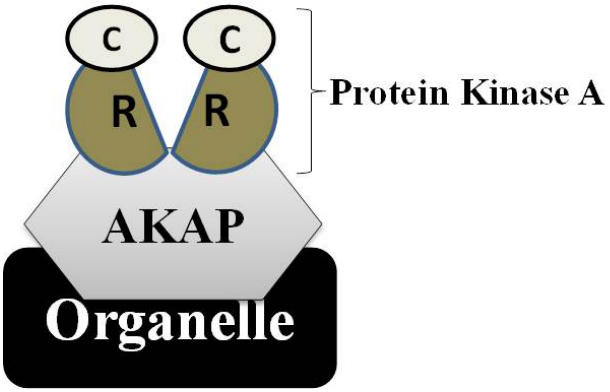

Figure 1: The basic signaling module (AKAP-R2D2 proteins). This module is found all over cells, including the cilia and flagella of eukaryotes. Note that protein kinase A consists of 2 Regulatory and 2 catalytic subunits (R2D2). Domains on AKAP for tethering signaling molecules other than R2D2 exist. This entire module is normally anchored on to some organelle inside the cell.

R2D2-like proteins

There is accumulating evidence for the presence of a number of proteins (ropporin, ropporin-1-like, CABYR and SPA17) that bind to AKAPs harboring the R2D2 domain have been discovered in mammalian cells harboring cilia or flagella [18,19]. In addition, several RII proteins have been discovered that invariably harbor the D/D domain. In fact, the flagellar proteins from *Chlamydomonas*, viz. the two RS proteins (RSP7 and RSP11) contain the R2D2 domain (RII fold) -what is also known as the DPY30 domain. These proteins share a similar secondary and tertiary structure like other R2D2 proteins and also bind to amphipathic helices of AKAPs [16,20,21]. In yet another study, an AKAP interactor, MYC-binding protein-1 (MYCBP-1) was found to bind to the AH of AKAPs. c-MYC is a transcription factor and when complexed with MYCBP-1 it forms and regulates transcription, thereby enhancing the transcription of genes controlled by the E-Box element and leading to erythrocyte differentiation [22,23]. MYCBP-1 was found to be present in the nucleus and also the membranous networks surrounding the nucleus. This probably assists c-MYC trafficking between both the compartments. Additionally, it forms a ternary complex with AKAPs and MYCBPAP in the nucleus. It probably uses its RII-like domain to bind to the AKAPs and its coiled-coil region to bind to other partners such as c-MYC and MYCBPAP [24]. The same group proposed that MYCBP-1, PKA and AKAP95 form a ternary complex in the nucleus to negatively regulate the kinase activity [25]. When outside the nucleus and especially during the interphase, MYCBP-1 interacts with other AKAPs, such as AKAP149 in the sperm mitochondria and its splice variant S-AKAP84 [26,27]; with BIG2, an AKAP in the trans-Golgi network [28].

A MYCBP-1 orthologue from the *Chlamydomonas* flagella was found to be a Flagella Associated Protein 174 (FAP174). When an *in silico* approach, was undertaken, FAP174 was compared with MYCBP-1 from several species of animals, fungi, plants, and protozoans. We found a significant sequence identity at the protein level; the N-terminal region was observed to be 43-87% similar; this region also spans the helix-turn-helix fold that is typical of all the RII clan of proteins (i.e. R2D2-like proteins such as RII and DPY-30). When a phylogenetic analysis was conducted with all these sequences, we found that FAP174 formed a cluster with those from Volvox and protozoans; it seemed to have branched from the mammalian lineage of MYCBP-1. When the secondary structure was predicted, the C-terminus helix exhibited a strong propensity to form a coiled-coil motif, the latter being known for protein-protein interaction. Therefore, it was speculated that FAP174 harbors two molecular modules, one for binding to the AH of an AKAP and the other for partnering with proteins [29]. One of these modules was tested for interaction with a flagellar protein containing AH, viz. AKAP240 and RSP3.

No.	Name of the protein	Sub-cellular location	Source	Reference
	Type I: AKAP			
1	S-AKAP84	cytoplasm	Human HeLa cell line	[25]
2	AKAP95	nucleus	Human HeLa cell line	[25]
3	AKAP97 (RSP3)	flagella	*Chlamydomonas reinhardtii*	[13,15]
4	AKAP189	sperm mitochondria	Mus musculus	[26,27]
5	AKAP240	flagella	*Chlamydomonas reinhardtii*	[13]
6	BIG2	trans-Golgi network	*Mus musculus*	[28]
	Type II: R2D2 domain-containing			
1	RII	various locations	All organism	[7]
2	DPY30 domain	flagella	*Chlamydomonas reinhardtii*	[21]
3	Ropporin	sperm flagella	*Mus musculus*	[18,19]
4	Ropporin-like	sperm flagella	*Mus musculus*	[18,19]
5	CABYR	sperm flagella	*Mus musculus*	[18,19]
6	SPA17	sperm flagella	*Mus musculus*	[18,19]
7	MYCBP-1 (AMY-1)	sperm mitochondria	Human HeLa cell line	[22-26]
8	FAP174	flagella	*Chlamydomonas reinhardtii*	[29]

Table 1: List of AKAPs and R2D2 domain-containing proteins mentioned in this text. This work emphasizes on the AKAPs and the R2D2 domain-containing proteins, both known to interact with each other.

Using conventional protein-protein interaction experiments, it was shown that FAP174 indeed interacts strongly with the CP AKAP240 and very mildly with RSP3. This indicated that FAP174 harbors an RII-like domain at the N-terminus and its dimerization property was tested. FAP174 dimerizes *via* a cysteine disulphide bridge and is being added to the growing list of R2D2 proteins. The findings essentially bring to light the roles of all those proteins that harbor the RII-like domain and form complex assemblies' partnering with AKAP and

other signaling molecules, thereby revealing a new insight in the composition as well as functioning of the CP apparatus. Our group found independently that FAP174 is a conserved structural part in a novel molecular complex present in the C2 microtubule of CP apparatus. We surmise that FAP174 is definitely not a physiological interactor of RSP3 [29]. Its presence in the CP, basal body or TZ, nucleus and cytoplasm could indicate that it is trafficking between the various locations of the *Chlamydomonas* vegetative cell after synthesis. In these locations, it might be binding to additional AKAPs and the same has not yet been determined. Circumstantially, AKAP450 was found to be present in the proteome of the human and fly centrosome [30,31]. Taken together, FAP174 is a versatile molecule and might be involved in the assembly of several protein molecular complexes in distinct cellular compartments (Table 1).

Conclusions

Emerging classes of proteins that harbor the D/D domain and possess an RII fold have been identified in sperms, flagella and several other cell types as well. These appear to be like RII but do not participate in cAMP signaling or phosphorylation. Currently, sperm proteins such as ropporin, ropporin-1-like, CABYR and SPA17 have been identified with the R2D2-like domains. We now add FAP174 as a new protein to this list.

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