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Article Addendum

Evolutionary conservation of the WASH complex

An actin polymerization machine involved in endosomal fission

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Abstract

WASH is the Arp2/3 activating protein that is localized at the surface of endosomes, where it induces the formation of branched actin networks. This activity of WASH favors, in collaboration with dynamin, the fission of transport intermediates from endosomes, and hence regulates endosomal trafficking of several cargos. We have purified a novel stable multiprotein complex containing WASH, the WASH complex, and we exam diverse eukaryot WASH complex oZ α/β heterodi e yeasts S. cere ubunits of the WA generated give a vi ization into domains nt subunits to prevent future confusions in the field.

The WASH complex has been purified from human cells by tandem affinity purification.[1] WASH was found associated with VPEF, KIAA1033, Strumpellin, Ccdc53, CapZ α and β. The native WASH protein is found in a large complex characterized by a Stokes' radius of 88 Å and a sedimentation coefficient of 12.5 S.[1] Moreover, depletion of any of the abovementioned proteins associated with WASH destabilizes WASH. Together these properties are compatible with a stable complex containing one molecule of each of these proteins. One expects to find all the genes encoding the 7 subunits of this molecular machine in species where this organization into a multiprotein complex is conserved. So using the BLAST algorithm, we looked into public databases for proteins homologous to the ones we had purified. Genes encoding subunits of the WASH complex were detected in genomes of animal, fungi and amoeba species. But none of the subunits were detected in bacterial and plant genomes. The orthologous subunits were retrieved and compared to the mouse subunits chosen as a reference instead of the human ones, to avoid the complexity of the human WASH family.[2,3]

The two subunits forming the CP cap actin filaments and hence blocks filament elongation. The 2 CP subunits, but no other subunits of the WASH complex, were detected in the S. cerevisiae and S. pombe yeasts. The same was true for other sequenced fungi. Since the amoeba D. discoideum possesses most WASH complex subunits, the ancestor of fungi and amoeba likely possessed these genes, suggesting that the WASH complex has been subsequently lost in fungi. In fungi genomes, the occurrence of CP without the WASH complex is in line with a free heterodimer representing the major pool of CP independently of the WASH complex.[1,4] The CP heterodimer is also an integral component of the Dynactin complex.[1,4] The many interactions of CP with actin, the WASH complex and the dynactin complex must exert a strong s enes encodin es.

The W its C-term ott-Aldrich Syndrom itself and replaced ery similar to the ones on this analogy, the N-terminal WAHD domain covering the first 300 amino-acids, is likely to be

integral to the multiprotein complex similar to the SCAR/WAVE N-terminal domain in the pentameric SCAR/WAVE complex and to the N-terminal domain of N-WASP, which complexes N-WASP with WIP family proteins.5 The rest of WASH, including the VCA domain, is predicted to be unstructured (Suppl. Fig. 2).

The VPEF subunit is of major interest, but confusing. VPEF was first characterized as a factor required for Vaccinia virus entry into mammalian cells, hence its name Vaccinia PEnetration Factor.6 Unfortunately, this publication has been overlooked in the two recent publications reporting VPEF association with WASH. We used the name of the cDNA, KIAA0592, to refer to this protein.1 Gomez and Billadeau used the name FAM21, standing for FAMily number 21 coined in automatic annotations of protein families.7 We recommend to use the name VPEF, which is anterior and descriptive. The major pool of the WASH complex and hence of VPEF is associated with endosomes.1,7 Nonetheless, the WASH complex is recruited to the plasma membrane upon infection with pathogens. WASH is detected at attachment sites of Salmonella, and is involved in the entry of this pathogenic bacterium into the cell.8 Similarly, VPEF downregulation impairs the entry of Vaccinia virus.6 A surprising result, however, was that entry of Vaccinia is also blocked by extracellular antibodies directed against VPEF or by soluble VPEF in the culture medium, as if VPEF played the role of an extracellular receptor for the virus.6 These observations remain to be explained, because the WASH complex is cytosolic and no transmembrane domain is predicted in the VPEF protein. VPEF is overall poorly conserved, and the percentage of identity drops so rapidly in vertebrates that invertebrate orthologs are not detected (Fig. 1). The N-terminal 350 amino-acids of VPEF are necessary and sufficient for building the WASH complex. 7 This result is in line with the conservation concentrated in the N-terminal domain (Fig. 2). Right after this domain, VPEF is predicted to be disordered with high probability (Suppl. Fig. 2). This lack of intrinsic structure for the most part of the protein might explain why VPEF is highly sensitive to proteases and does not migrate at its expected size after purification of the W

The Stru c paraph e disease more in unrelated families. omplex, and these m t alleles of Strumpe not sufficient to maintain a WT phenotype or that mutant Strumpellin blocks the assembly

of functional WASH complexes despite the presence of WT Strumpellin in a dominant negative manner.

The KIAA1033 subunit is also well conserved, similarly to Strumpellin. The last 100 amino-acids, however, correspond to a poorly conserved region, which is predicted to be intrinsically unstructured (Suppl. Figs. 2 and 4).

The Ccdc53 subunit is the second less conserved subunit after VPEF. It cannot be detected in D. discoideum, even though a functional ortholog is likely to exist in this species, where 5 out of 7 subunits are detected (Fig. 1). Its name comes from Coiled-coil domain containing protein 53, because of a short and conserved coiledcoil predicted in the N-terminal domain. In this protein, two blocks of conserved residues alternate with two variable regions, predicted to be highly disordered (Suppl. Fig. 2).

Understanding how these 7 subunits are assembled into a functional actin polymerization machine is of major fundamental importance and might also provide an explanation for why hereditary spastic paraplegia arises in patients affected by Strumpellin mutations.

Figures and Tables

Figure 1 (A) Percentage of identity of orthologous subunits of the WASH complex in different species. More than a dozen of WASH genes are present in Homo sapiens and the exact number varies between individuals.[2,3] Reference has thus been set to the Mus musculus ortholog. The number of CapZ α is indicated in parentheses when more than one. The reference was set to murine CapZ α1, and the % of identity was calculated from the closest homolog in species possessing more than one paralogous CapZ α g… ND : Not Detected. Accession numbers of the genes can be found in Supplem… elected species …

A

Name alternative	VPEF (KIAA0592, FAM21)	KIAA1033	Strumpellin (KIAA0196)	WASH (Orf19)	CapZ α	CapZ β	Ccdc53
M. musculus	100	100	100	100	100 (3)	100	100
H. sapiens	72,5 (3)	96,8	95,6	84,2 (12-20)	96,5 (3)	90,2	89,6
X. tropicalis	43,9	82,7	87,6	69,3	88,1 (2)	88,8	68,1
D. rerio	37,9	83,8	87,6	67	79,3 (2)	87,7	61,8
D. melanogaster	ND	25,1	42,3	27,9	61,4	77,6	31,7
C. elegans	ND	17,5	19,2	23,6	52,3	64,7	22,8
Capitella sp	ND	61,9	63,4	43,9	56,5	76,4	47,8
L. gigantea	ND	60,6	66,6	42,8	61,1	80,1	44,5
M. brevicollis	ND	42,8	52,1	33,3	54,5	70,1	33,1
S. cerevisiae	ND	ND	ND	ND	25,8	44,4	ND
S. pombe	ND	ND	ND	ND	25	46,8	ND
D. discoideum	ND	41,1	48,6	26	35,1	57,1	ND

B

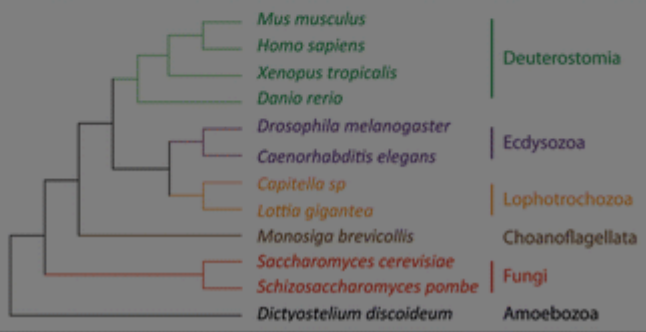

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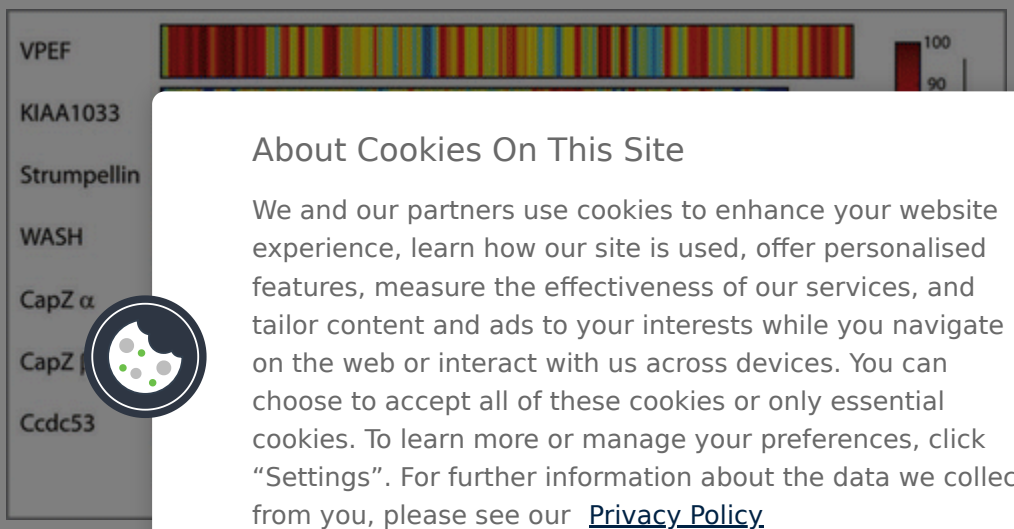
Figure 2 Percentage of similarity among orthologous subunits of the WASH complex displayed in a color-coded manner to highlight domain organization. Hot colors represent the highest level of conservation. The similarity score at each position was calculated from each multiple alignment including all subunit orthologs described in Figure 1 using Jalview. These scores were then averaged using a gliding window of 10 residues and colorcoded using the MAT LAB software. This representation gives an overview of the organization of the subunits into domains, but cannot be used to compare the conservation between subunits since the number of detected orthologous genes is variable. The VPEF plot, for example, is derived from only four orthologous genes.



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Addendum to:

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