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A Centrin3-dependent, Transient, Appendage of the Mother Basal Body Guides the Positioning of the Daughter Basal Body in *Paramecium*

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Basal bodies are tightly controlled not only for their time of duplication but also for their movements, which ensure proper division and morphogenesis. However, the mechanisms underlying these movements only begin to be explored. We describe here a novel basal body appendage in *Paramecium*, the anterior left filament (ALF), which develops transiently from the mother basal body before duplication and disassembles once the new basal body is docked at the surface. By comparing the ultrastructure of dividing wild type cells to that of cells defective in basal body duplication, either by depletion of conserved proteins required for basal body assembly, or by mutation, we showed 1) that assembly of the ALF requires PtCen3p, one of the two basal body specific centrins and 2) that absence of the ALF correlates with a failure of the newly assembled basal bodies to tilt up to their docking site at the surface. This correlation suggests that the function of the ALF consists in anchoring centrin-containing contractile fibers which pull up the new basal body toward its site of docking. The presence in *T. thermophila* of an ALF-like appendage suggests the conservation of an ancestral mechanism ensuring the coupling of basal body duplication and cell morphogenesis.

Key words: Anterior-Left-Filament; basal body; centriole; cytotaxis; centrin.

Introduction

Centrioles and basal bodies share two remarkable features, their conserved nine-fold symmetry and their mode of duplication. Although de novo assembly of a centriolar structure can be achieved under particular physiological conditions or in particular cell types (for reviews: Beisson and Wright 2003; Song et al. 2008), a new basal body or centriole generally arises by the side of the mother organelle according to a precise geometry. In the case of the basal bodies present in unicellular organisms, ciliates or flagellates, in addition to the budding at right angles, the sites of assembly of a new basal body and of its anchoring at the cortex are precisely determined according to the polarity of the cell and of the mother basal body itself (Azimzadeh and

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Marshall 2010; Beisson and Jerka-Dziadosz 1999; Bornens 2008; Dutcher 2003; Feldman et al. 2007; Lacomble et al. 2010; Silflow and Lefebvre 2001). Over the last few years, the molecular dissection of basal body/centriole assembly in a wide range of organisms has led to the characterization of the major conserved constituents and of the basis of the nine-fold symmetry (reviews in: Azimzadeh and Bornens 2007; Azimzadeh and Marshall 2010; Brito et al. 2012; Pearson and Winey 2009; Strnad and Gönczy 2008). In contrast, the nature of the physico-chemical constraints which channel the movements of a new centriolar structure has only recently begun to be considered (Farnum and Wilsman 2011; Vaughan and Dawe 2011), although a specific role of centrin has been demonstrated (Geimer and Melkonian 2005; Koblenz et al. 2003; Ruiz et al. 2005; Stemm-Wolf et al. 2005; Yang et al. 2010). In addition, a breakthrough has recently been obtained by cryo-electron tomography of isolated, duplicating mammalian centrioles: Guichard et al. (2010) described a short stalk connecting a microtubule triplet of the mother centriole and the axis of the cartwheel of the developing pro-centriole. This is the first evidence of a physical mechanism ensuring the growth at right angle of the pro-centriole.

Among unicellular organisms, *Paramecium*, like other ciliates (Pearson and Winey 2009; Vonderfecht et al. 2011) is a most suitable model to follow the steps of basal body duplication, as their regular organization over the cell surface, the well-known spatio-temporal pattern of their duplication during division and the homogeneous polarities of the basal body appendages provide unambiguous landmarks in ultra-structural studies (Iftode et al. 1989; Jerka-Dziadosz et al. 1998; Ruiz et al. 1987). In addition, an efficient method of RNAi by feeding ensures a fast targeting of the genes of interest allowing detection of both the early and late effects of gene silencing (Galvani and Sperling 2002). These particularities have permitted us to identify, by standard ultra-structural studies, a previously overlooked transient filamentous appendage, the ALF (Anterior Left Filament), which is assembled at the onset of basal body duplication and develops from the left of the mother basal body, running anterior and along the ciliary row.

Although the ultra-structural study of basal body duplication in ciliates seemed to have little new information to reveal since the pioneer studies of Dippell (1968) and Allen (1969), it is by examining dividing cells under depletion of the basal body core proteins PtBald10p or PtSas6p (Jerka-Dziadosz et al. 2010), that the ALF was pinpointed: at the empty presumptive docking sites of new basal bodies. We then examined dividing cells in other mutational or physiological conditions affecting basal body duplication and could demonstrate that assembly of the ALF required PtCen3p, a centrin isotype previously shown to localize at the anterior edge of basal bodies and to be involved in new basal body positioning in *Paramecium* (Ruiz et al. 2005). Finally, we showed that this ALF is not a *Paramecium*-specific device and that a similar structure exists in *Tetrahymena*.

The general significance and interest of these observations with respect to the physico-chemical constraints at play in the duplication of centriolar structures and guidance of their movements will be discussed.

Results

Like centriole duplication (see Azimzadeh and Marshall 2010), basal body duplication in unicellular organisms presents distinct phases, which proceed from initiation, i.e. the canonical budding of the new basal body at right angles to the mother, to elongation of the microtubular shaft, and maturation with assembly of the transition zone. But basal body development presents an additional step: an upward tilting movement of the new basal body leading to its docking at the cell surface (Fig. 1 A) (see also Aubusson-Fleury et al. 2012). As in other unicellular organisms, these movements of the new basal bodies are precisely guided to orchestrate the complex morphogenetic processes of division. For each species, the pattern of basal body distribution on the cell surface is precisely determined and the migration of new basal bodies after budding strictly channeled (Beech et al. 1991; Cavalier-Smith 1974; Lacomble et al. 2010; Tucker 1971).

In *Paramecium*, the ciliature is arranged in parallel longitudinal rows forming an overall pattern faithfully reproduced through binary division and each new basal body aligns within the existing ciliary row, with the same antero-posterior and circumferential polarities (Fig. 2) as the mother. Ciliary rows comprise two types of cortical units that contain either one (1-bb) or two (2-bb) basal bodies (Fig. 1B, C). In all units, the basal bodies are flanked by specific appendages, microtubular ribbons and striated rootlets (Iftode et al. 1989, 1996). In addition, in 2- bb units, the two members of the pair are connected by filamentous linkers, a situation similar to connectors observed between centriole pairs in animal cells (Vladar and Stearns 2007) or basal bodies in flagellates (Geimer and Melkonian 2004).