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Research paper

CP91 is a component of the *Dictyostelium* centrosome involved in centrosome biogenesis

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ABSTRACT

The *Dictyostelium* centrosome is a model for acentriolar centrosomes and it consists of a three-layered core structure surrounded by a corona harboring microtubule nucleation complexes. Its core structure duplicates once per cell cycle at the G2/M transition. Through proteomic analysis of isolated centrosomes we have identified CP91, a 91-kDa coiled coil protein that was localized at the centrosomal core structure. While GFP-CP91 showed almost no mobility in FRAP experiments during interphase, both GFP-CP91 and endogenous CP91 dissociated during mitosis and were absent from spindle poles from late prophase to anaphase. Since this behavior correlates with the disappearance of the central layer upon centrosome duplication, CP91 is a putative component of this layer. When expressed as GFP-fusions, CP91 fragments corresponding to the central coiled coil domain and the preceding N-terminal part (GFP-CP91cc and GFP-CP91N, respectively) also localized to the centrosome but did not show the mitotic redistribution of the full length protein suggesting a regulatory role of the C-terminal domain. Expression of all GFP-fusion proteins suppressed expression of endogenous CP91 and elicited supernumerary centrosomes. This was also very prominent upon depletion of CP91 by RNAi. Additionally, CP91-RNAi cells exhibited heavily increased ploidy due to severe defects in chromosome segregation along with increased cell size and defects in the abscission process during cytokinesis. Our results indicate that CP91 is a central centrosomal core component required for centrosomal integrity, proper centrosome biogenesis and, independently, for abscission during cytokinesis.

1. Introduction

In many extant eukaryotes, microtubule organization is achieved by structurally highly organized organelles called centrosomes. After their duplication, centrosomes participate in formation of the bipolar mitotic spindle. Eukaryotic evolution has engendered different types of centrosomes (Gräf et al., 2015). In animals and several other organisms, the centrosome is characterized by centrioles mainly consisting of a nine-fold symmetrical, cylindrical arrangement of short microtubules embedded in a pericentriolar matrix (PCM). The latter consists of scaffolding proteins, which bind microtubule nucleation complexes. Yet, there are also acentriolar centrosomes such as the spindle pole body (SPB) in yeasts or the so-called nucleus-associated body (NAB) in amoebozoans. In vegetative cells centrosomes generally are tightly coupled to the nuclear envelope through so-called LINC complexes (linker of nucleoskeleton and cytoskeleton) consisting of a SUN protein in the inner nuclear membrane, and a KASH-domain protein in the outer nuclear membrane (Crisp et al., 2006).

We use *Dictyostelium discoideum* as an amoebozoan model to study structure and function of an acentriolar centrosome (Gräf, 2015). The *Dictyostelium* centrosome contains no centrioles but consists of a three-layered core structure surrounded by a so-called corona, which contains electron-dense microtubule nucleation complexes (called “nodules”) at the base of microtubules. The whole spheroid organelle has a diameter of approximately 600 nm. While the corona is the functional homologue of the pericentriolar matrix of higher cells, the layered core structure represents the duplicating unit of this centrosome type. As in vegetative animal cells, the *Dictyostelium* centrosome organizes a radial microtubule cytoskeleton. However, due to their amoeboid locomotion and the absence of ciliated gametes, these cells have lost centrioles/basal bodies during evolution (Carvalho-Santos et al., 2011; Gräf et al., 2015). Since the *Dictyostelium* centrosome appears to fulfill all

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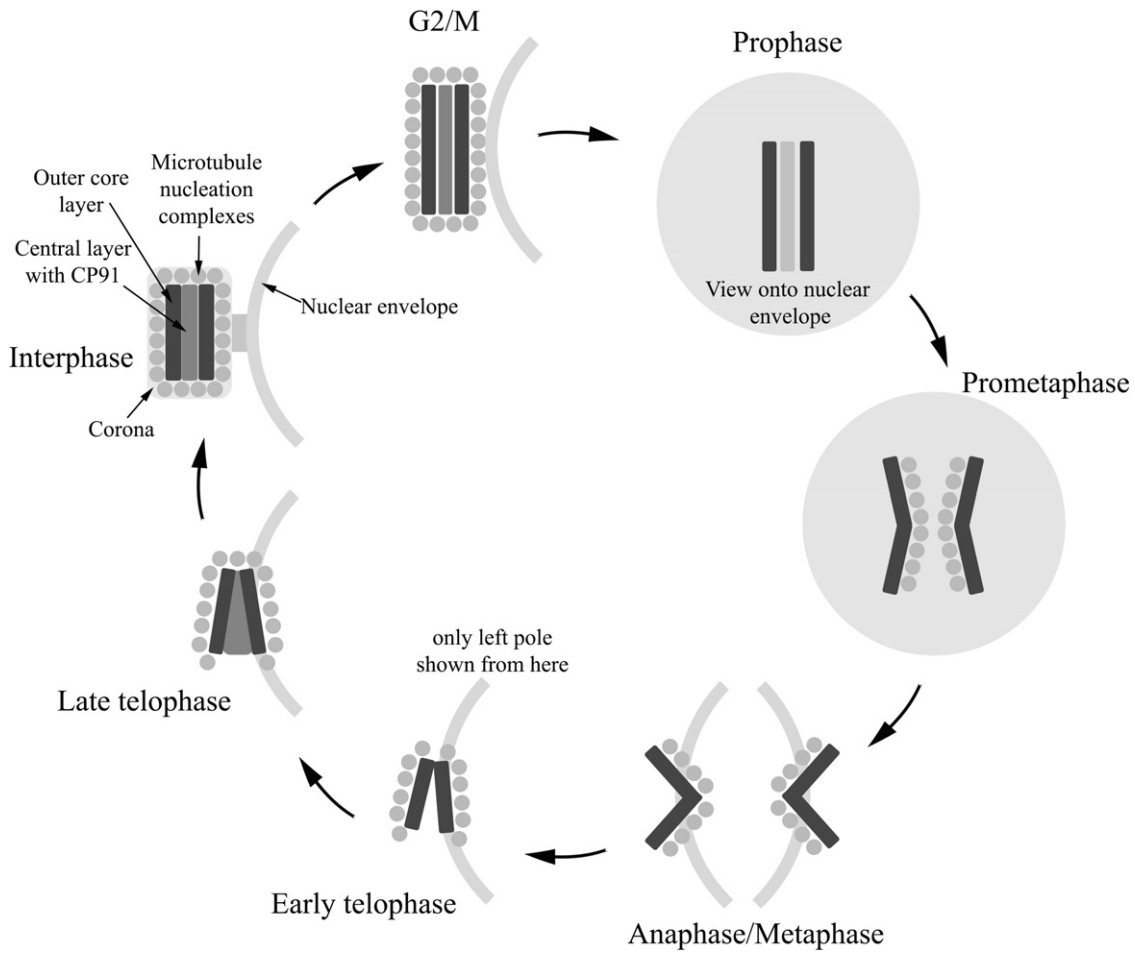

Fig. 1. Overview of *Dictyostelium* centrosome duplication. A key of depicted structures is given at the interphase stage. Only one pole is shown in the telophase stages. Microtubules emanating from microtubule nucleation complexes are omitted for the sake of clarity.
Adapted from: This figure is newly drawn for this paper and it is based on Ueda et al. (1999).

known functions of centrosomes except cilia formation, this organism offers the possibility to use comparative biology to identify those proteins essential for all centrosomal functions unrelated to cilia formation, such as microtubule organization, duplication and cytokinesis. The *Dictyostelium* centrosome starts its duplication at the G2/M transition with growth of the core structure and shedding of the whole corona with its attached microtubules (Fig. 1; Ueda et al., 1999). Thus, early prophase is characterized by a complete lack of microtubules at the centrosome. Next, the core structure enters a fenestra in the nuclear envelope and the two outer layers of the core structure peel apart from each other while the central layer disappears. In prometaphase microtubules are nucleated from the former inner surfaces of the two remaining layers and form a central spindle, whose elongation drives the two spindle poles apart until they have reached opposing poles in metaphase. At the transition to anaphase, the pole-forming layers start to fold with their edges pointing towards the cytosolic face of the nucleus and astral microtubules projecting towards the cell cortex become visible. In telophase the folding process of the poles is completed and both initially plaque-like layers have folded back onto themselves with their formerly inner surfaces now facing freshly recruited corona material. In late telophase the central layer re-appears. From prophase up to telophase there is no indication for a discontinuity of the nuclear envelope except from the fenestrae in which the two mitotic centrosomes reside. In late telophase the two daughter nuclei undergo karyokinesis, i.e. the thin tube of nuclear envelope connecting both nuclei ruptures prior to disassembly of the central spindle and cytokinesis (McIntosh et al., 1985).

There are still many open questions how the intriguing centrosome biogenesis process is orchestrated on the protein level. Most of the 33 known centrosome-associated proteins were characterized in our lab (Gräf, 2015). Analysis of the centrosomal proteome identified several novel centrosomal candidate proteins (Reinders et al., 2006), whereby nine of them were localized at the centrosome as overexpressed GFP-fusion proteins (Schulz et al., 2009). In this work we present a functional analysis of one of these proteins, CP91. We present evidence that endogenous CP91 is part of the centrosomal core structure, where it is presumably a part of the central layer, and that it is essential for proper centrosome duplication and chromosome segregation.

2. Results

2.1. CP91 is a component of the layered centrosomal core structure that disappears upon centrosome duplication in mitosis

In our previous survey of novel centrosomal proteins we have shown that GFP-CP91 localizes to the core structure of the *Dictyostelium* centrosome and that it is absent from mitotic centrosomes in metaphase (Schulz et al., 2009). In order to study the behavior of the endogenous protein we have raised polyclonal antibodies against the recombinant protein expressed in *E. coli*. In Western blots of centrosome/nucleus extracts the affinity puri-