

## Dictyostelium centrin B localization during cell cycle progression

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**R**ecently, we have reported the initial characterization of a novel centrin from *Dictyostelium discoideum* (DdCenB).<sup>1</sup> Sequence and phylogenetic analyses clearly establish DdCenB as a centrin, yet further characterization revealed some interesting peculiarities about this novel centrin. Figure 1 depicts the localization of DdCenB at three points in the cell cycle: interphase, mitosis and cytokinesis. In interphase DdCenB primarily localizes to the nuclear envelope (NE). Although the NE remains intact during mitosis and cytokinesis in *Dictyostelium*, DdCenB disappears from the NE at these two stages of the cell cycle. In addition to localization at the NE, we also see weak localization in the nucleoplasm and cytoplasm (weakest). Although the nucleoplasmic concentration appears constant throughout the cell cycle, the very faint localization in the cytoplasm does appear to increase to the level of the nucleoplasm during mitosis and cytokinesis. Unlike most centrins characterized to date, we found no evidence of DdCenB at the centrosome at any point in the cell cycle. Here we examine the importance of DdCenB localization in cell cycle progression, as well as several other roles.

To further explore DdCenB we generated a knock-out strain. One of the first phenotypes we observed was a difficulty in completing cytokinesis. Unable to synchronize cells, we relied on time-lapse photomicroscopy to observe cells for prolonged periods of time. We commonly observed dividing cells that remained interconnected by structures resembling cytoplasmic bridges. These bridges often persisted for over 15 minutes (Fig. 2A), even though *Dictyostelium* cells can

typically use traction to complete cytokinesis.<sup>2</sup> This strongly contrasted with wild-type cells, which divide relatively rapidly (Fig. 2B) and without the formation of a true midbody.<sup>3</sup> Mitotic defects can often times lead to aberrations in cytokinesis. Consequently, we also examined DdCenB knock-out cells in mitosis. Indicative of mitotic defects, we observed aberrant mitotic spindles and mislocalization of the microtubule binding protein DdCP224, a centrosome protein that also localizes to the mitotic spindle.<sup>4</sup> Interestingly, altered expression of DdCP224 also generates mitotic and centrosome defects<sup>5</sup> similar to those observed with DdCenB knock-out. In some cases, cells delayed in cytokinesis retained microtubule bundles in the connecting cytoplasmic bridges (presumably remaining from the mitotic spindle) and chromosomes delayed in reaching the poles (Fig. 2C and D). These persistent microtubules and delayed chromosomes are hallmarks of spindle defects and it may be because of these defects that cytokinesis is impaired.

How can it be that defects in mitosis and cytokinesis result from the disruption (either through knock-out or overexpression) of a protein that apparently disappears at these stages of the cell cycle? Part of the answer may lie in what happens to the centrosome when DdCenB function is compromised. Immuno-staining of DdCenB knock-out cells with NE and centrosome markers revealed changes in the normal centrosome-nucleus association. In wild-type *Dictyostelium* cells the centrosome is closely tethered to the NE during interphase.<sup>6</sup> The lack of DdCenB led to a protrusion of the NE towards a displaced centrosome. This phenotype is similar to that obtained upon overexpression of full length or truncated DdSun1,

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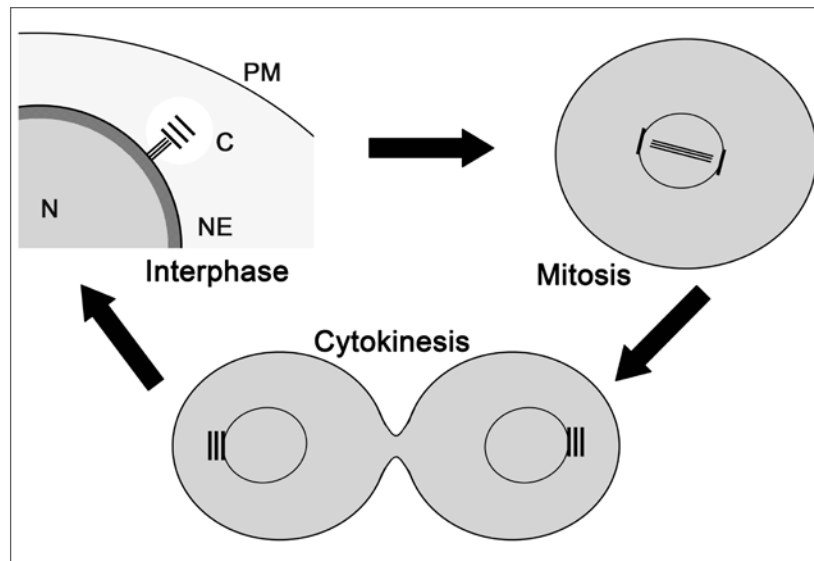
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**Figure 1.** Localization of DdCenB at three stages of the cell cycle. DdCenB is shown as gray shading, the darkness of which corresponds to the amount of DdCenB. N, nucleus; NE, nuclear envelope; C, centrosome; PM, plasma membrane.

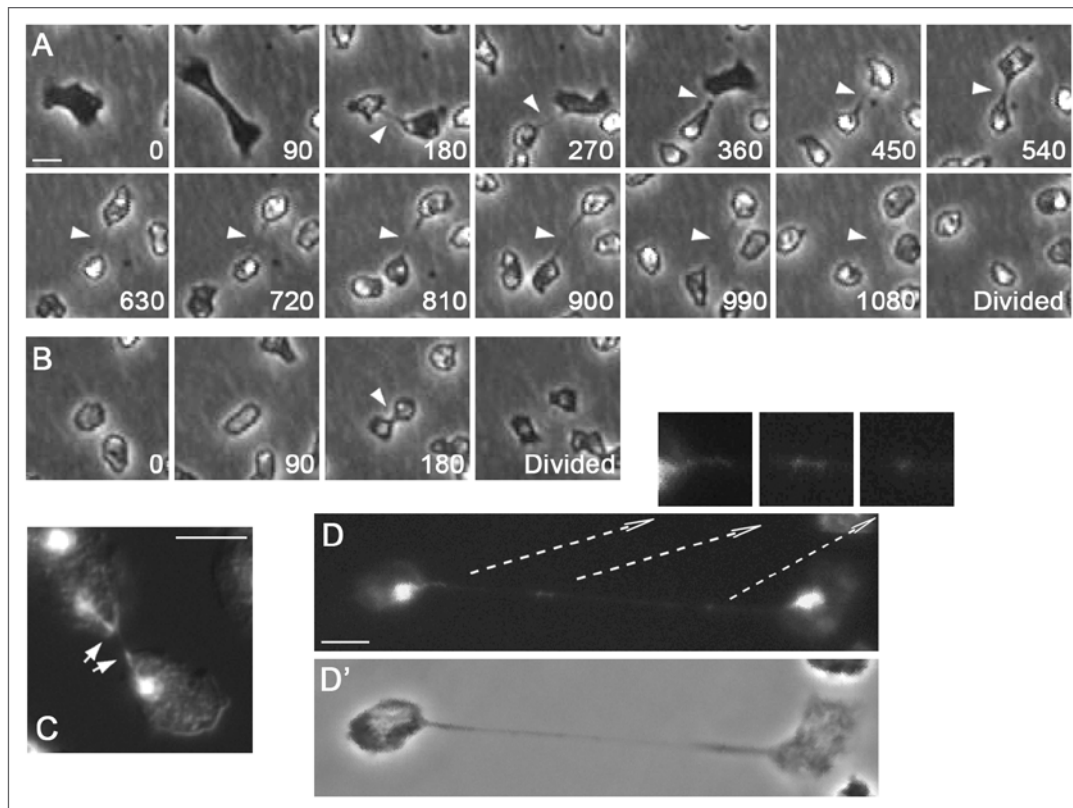
a SUN domain protein with an established role in anchoring the centrosome to the nucleus.<sup>7,8</sup> SUN domain proteins span the NE and ultimately link centrosome-interacting KASH domain proteins to lamins (metazoans) or DNA (yeast, *Dictyostelium*).<sup>9,10</sup> The similarity of phenotype between DdCenB knock-out and DdSun1 overexpressing cells suggests that DdCenB might also function in tethering the centrosome to the NE. In addition to defects in the centrosome-NE link, or perhaps because of this, we have also observed multiple centrosomes and centrosome-related bodies in DdCenB knock-out cells. The presence of altered centrosomes would certainly have profound effects on the ability of those cells to establish a normal mitotic spindle and proceed through cell division in a normal fashion. Alternatively, DdCenB (before delocalizing from the NE) may aid in the transition of the centrosome into the NE in a similar fashion to yeast Cdc31 and the nascent spindle pole body (SPB).<sup>11</sup> Disruption of this process would release the centrosomes from

the NE and allow for aberrant rounds of centrosome duplication.

Regarding cytokinesis, in addition to the possibility that these defects are simply a result of defects in mitosis, it is also possible that DdCenB actively participates in the regulation of cytokinesis. The mechanism by which DdCenB is lost from the NE as cells transition into mitosis has not been determined. It is possible that DdCenB, released from the NE at this time, becomes cytoplasmic and more directly involved in cytokinesis. Centrioles are known to be calcium sensors and there is evidence for calcium in cytokinesis.<sup>12</sup> Since we have not observed a distinct subcellular localization for DdCenB in dividing cells (e.g., the cleavage furrow) it remains feasible that DdCenB acts more globally at this stage in the cell cycle. For example racE, a small GTP-binding protein required for cytokinesis in *Dictyostelium*, is necessary for maintaining proper cortical tension, essential for furrow completion.<sup>13</sup> Considering its involvement with the NE, DdCenB may

play a role in membrane trafficking during cytokinesis.

The multiplicity of phenotypes observed following DdCenB knock out or overexpression, coupled with its peculiar cell cycle dependent localization suggests multiple roles for DdCenB. In addition to localization at the NE, there is faint localization in the nucleoplasm (see Fig. 1). Unlike DdCenB at the nuclear envelope, the nucleoplasmic DdCenB remains there throughout the cell cycle. This suggests that nucleoplasmic DdCenB might constitute a different protein population with different roles. Reports have shown that centrioles are required to stimulate DNA repair mechanisms. For example, yeast CDC31 and human centriole 2 bind and stimulate the NEF2 complex and XPC, respectively.<sup>14</sup> A homolog to human XPC has been identified in *Dictyostelium*. Consequently, nucleoplasmic DdCenB may play a similar role to yeast and human centrioles in the stimulation of nucleotide excision repair mechanisms.



**Figure 2.** Cytokinesis defects as a result of DdCenB knock-out. (A) consists of images captured from time-lapse microscopy. The time of capture (minutes) for each frame is indicated in the lower right corners. The arrowheads point to the persistent cytoplasmic bridges observed in dividing cells. In (B) we see a wild-type cell dividing. Here the arrowhead points to a typical cleavage furrow. (C) is a dividing mutant cell stained with DAPI (DNA) and visualized by fluorescence/DIC microscopy. Arrows point to distorted nuclei in the furrow region. (D) shows a mutant cell, with a long cytoplasmic bridge, stained with DAPI. The arrows point to higher magnifications of the respective areas, and highlight the presence of DNA in the cytoplasmic bridge. D, fluorescence; D', phase contrast. Bars in all cases correspond to 5  $\mu$ m.

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