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Geminin is bound to chromatin in G2/M phase to promote proper cytokinesis

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Abstract

Previous studies suggested that geminin plays a vital role in both origin assembly and DNA re-replication during S-phase; however, no data to support a role for geminin in G2/M cells have been described. Here it is shown that in G2/M-phase, geminin participates in the promotion of proper cytokinesis. This claim can be supported through a series of observations. First, geminin in G2/M is loaded onto chromatin after it is tyrosine phosphorylated. It is unlike S-phase geminin that resides in the nuclear soluble fraction, where it is exclusively S/T phosphorylated. Secondly, on chromatin, geminin gets S/T phosphorylated in late G1; this modification causes the release of geminin from the chromatin. Cyclins bind and phosphorylate geminin in a sequential, cell cycle-dependent manner. These modifications correlated well with geminin departure from the chromatin. This suggests that cyclin functions to either release geminin from chromatin or at least keep it at bay until late S-phase. Thirdly, depletion of geminin from a diploid mammary epithelial cell line (HME) causes cells to arrest in late G2/M-phase. Massive serine-10 phosphorylated histone H3 staining and survivin localization to mid-body were observed; this suggests that they could be arrested in either mitosis or at cytokinesis. Finally, while in the absence of geminin, cyclin B1, chk1 and cdc7 are all over expressed. This paper will demonstrate that only cdc7 is important in maintaining the cytokinesis arrest in the absence of geminin. Only double depletion of geminin and cdc7 induce apoptosis. Our results taken together show, for the first time, that phosphorylation–induction activates oscillation of geminin between both nuclear soluble and chromatin compartments. Chromatin-bound geminin species functions to initiate or maintain proper cytokineses. In the absence of geminin, cells arrest in cytokinesis; this defines a novel checkpoint, monitored by cdc7, rather than cyclin B1 or chk1.
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1. Introduction

Normal cells progress through a cycle that contains a series of intricate steps. Each stage can only advance after the proper completion of steps in the previous stage. In normal mitotic cells, cdks induce phosphorylation events that control whether or not the cell enters the M- or S-phase. Cyclin A/cdk1 (Diederichs et al., 2004; Muller-Tidow et al., 2002) and cyclin B/cdk1 (Azzam et al., 2004; Kramer et al., 2004) complexes mediate

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M-phase control (Erenpreisa et al., 2004; Kristjansdottir & Rudolph, 2004). S-phase is controlled by circumstances that include cyclin E/cdk2, cyclin A/cdk2, cyclin D/cdk4 or cdk6 (Keenan, Lents, & Baldassare, 2004). Some of these occurrences are negatively regulated by the cdk inhibitor p27Kip1 (Brandts, Bilanges, Hare, McCormick, & Stokoe, 2005).

Cyclin A synthesis starts at the G1/S transition. Cyclin B1 synthesis begins at the end of S-phase. While it is accepted that cyclin A protein is localized exclusively in the nucleus during interphase (Girard, Strausfeld, Fernandez, & Lamb, 1991), some studies show an accumulation of cyclin B in cytoplasmic compartment during interphase (Bailly, Pines, Hunter, & Bornens, 1992). It is not yet known whether a fraction exists in the nucleus during interphase.

Mitotic cyclin stability varies dramatically between different organisms and also between cell lines. For instance, Girard, Fernandez, & Lamb, 1995 has shown a delayed cyclin degradation in non-transformed mammalian cells from either fibroblastic or epithelial origin. This contrasts reports in the following: HeLa cells (Bailly et al., 1992; Pines & Hunter, 1990), clam or *Xenopus* embryos (Minshull, Golsteyn, Hill & Hunt, 1990; Minshull et al., 1989) and cellularized *Drosophila* embryos (Lehner & O'Farrell, 1990; Lehner, Yakubovich, & O'Farrell, 1991), which shows complete destruction of both proteins at metaphase. It is important to note that it is unclear whether these proteins localize to the chromatin. Cyclin A and B are associated with the condensed chromosomes at both metaphase and early prophase in both fibroblasts and HeLa cells (Maldonado-Codina & Glover, 1992). Finally, Girard et al. (1995) has shown that most, if not all of cyclin proteins are excluded from chromatin at this stage.

In late mitosis/early G1-phase of a cell cycle, replication origins are 'licensed' for replication (Blow, 1993; Diffley, 1996; Lei et al., 1997). The origin of replication complex (ORC) first binds to each replication origin, and then recruits two other proteins, Cdc6 and Cdt1 during G1 (Bell & Dutta, 2002). Subsequently, the MCM2-7 complex is bound at replication origins to license the start for replication fork initiation at the onset of S-phase (Diffley, 1996; Labib, Diffley, & Kearsey, 1999; Labib, Tercero, & Diffley, 2000). Cdks and geminin play a critical role in preventing re-licensing by inhibiting the loading of new MCM complexes onto the origins. This is accomplished during S-, G2- and early M-phase (Blow, 1993; Diffley, 1996; Lei et al., 1997).

Previously, geminin was shown to be a substrate for the anaphase-promoting complex (APC) because it is degraded in mitotic *Xenopus* egg extracts (McGarry & Kirschner, 1998). Geminin is absent from cells during G1-phase; its accumulation is observed during S-, G2- and M-phases (McGarry & Kirschner, 1998). In *Xenopus* embryos, geminin induces uncommitted embryonic cells to differentiate as neurons (Kroll, Salic, Evans, & Kirschner, 1998). Eliminating geminin mRNA by an antisense technique arrests the embryos in G2 immediately after the midblastula transition. This indicates that geminin plays an essential role in these cells. Loss of this function prevents entry of cells into mitosis. This is due to a mechanism that depends on Chk1, the effector kinase of the DNA damage checkpoint pathway (McGarry, 2002).

The role of geminin as an inhibitor of origin assembly and re-replication in multicellular eukaryotes is consistent with tumor suppressor function (Quinn, Herr, McGarry, & Richardson, 2001; Tada, Li, Maiorano, Mechali, & Blow, 2001). Immunohistochemistry and immunoblotting for geminin shows that this protein is expressed specifically in proliferating lymphocytes and epithelial cells (Wohlschlegel et al., 2000). Moreover, geminin appears to be expressed at high levels in several malignancies. Its upregulation correlates with the high rate of tumor cells proliferation (Wohlschlegel et al., 2000). This suggests that geminin may have additional function(s) in cells, perhaps as a proto-oncogene. However, how geminin could execute such a function is not yet known.

Here, we take a closer look at geminin expression throughout the cell cycle. It is evident that while consistent with published data, geminin is absent from the soluble fraction of nuclear extracts in G2/M- and G1-phase cells; it is located on chromatin. We also studied its phosphorylation pattern and found that geminin is in the soluble fraction in late S-phase is tyrosine phosphorylated. This is an event that coincides with its loading onto chromatin. Furthermore, in late G1, chromatin-bound geminin loses tyrosine phosphorylation and/or becomes S/T phosphorylated, most likely through cyclin/cdk-dependent events; and is released from chromatin. We also studied the effect of depletion of geminin in diploid cells. We found that in the absence of geminin, cells arrest at cytokinesis. This function most likely attributes to the chromatin-bound species of geminin. Finally, we discovered that neither cyclin B1 nor chk1 (two proteins important for G2/M checkpoint) is needed to maintain arrest in cytokinesis in the absence of geminin. Instead, the kinase cdc7 plays a major role in maintaining this checkpoint. This suggests that geminin and cdc7 collaborate to execute proper cytokinesis. Depletion of both proteins from cells enhances in cell death, most likely, when they attempt to initiate unscheduled cytokenesis imposed by geminin loss in the absence of cdc7.

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