Requirement of a Centrosomal Activity for Cell Cycle Progression Through $G_1$ into S Phase

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Centrosomes were microsurgically removed from BSC-1 African green monkey kidney cells before the completion of S phase. Karyoplasts (acentrosomal cells) entered and completed mitosis. However, postmitotic karyoplasts arrested before S phase, whereas adjacent control cells divided repeatedly. Postmitotic karyoplasts assembled a microtubule-organizing center containing $\gamma$-tubulin and pericentrin, but did not regenerate centrosomes. These observations reveal the existence of an activity associated with core centrosomal structures—distinct from elements of the microtubule-organizing center—that is required for the somatic cell cycle to progress through $G_1$ into S phase. Once the cell is in S phase, these core structures are not needed for the G2-M phase transition.

The centrosome in mammalian cells consists of a pair of centrioles associated with a cloud of pericentriolar material containing the $\gamma$-tubulin ring complexes that nucleate microtubules during interphase and mitosis (1). The centrioles, along with their associated structures, represent “core centrosomal structures” that determine the precise one-to-two duplication of the centrosome in preparation for mitosis (2). After removal of the centrosome, both somatic and embryonic cells can regenerate a microtubule-organizing center (MTOC) (3–5) but do not regenerate centrioles (2, 4), even though the cytoplasm (in the case of zygotes) contains enough subunits to assemble many complete centrosomes (6).

It has been generally understood that both the duplication of the centrosome and variations in its microtubule-nucleating capacity are driven by cell cycle–dependent changes in the cytoplasmic environment (7). The notion that the centrosome is a necessary participant in cell cycle progression through interphase was raised by a report that BSC-1 African green monkey karyoplasts (acentrosomal cells) do not enter mitosis even though they grow to larger than normal size (4). This finding, coupled with the observation that cyclin-dependent kinase 1–cyclin B (Cdk1-B) is concentrated at the centrosome (8), led to the proposals that the presence or duplication (or both) of an intact centrosome is required for the activation of Cdk1-B and entry into mitosis (4, 9). However, these proposals lacked direct experimental support because the karyoplasts were not continuously followed in vivo.

To investigate the role of the centrosome in cell cycle progression, we physically cut BSC-1 cells during interphase between the nucleus and the centrosome to form karyoplasts (4, 10) and continuously followed the karyoplasts for several days by time-lapse videomicroscopy (11). The fact that the centrosome is slightly separated from the nucleus and lies at the center of a mass of granules makes this cell type favorable for this microsurgery (12). We brought the microneedle down at the edge of the nucleus, which displaced the centrosome from the nucleus and segregated it into the anucleate cytoplasm as the needle approached the cover slip (Fig. 1A). In no case did we cut or fragment the nucleus. Although we cannot know at what point in the cell cycle the cells were cut, 5-bromo-2′-deoxyuridine (BrdU) incorporation experiments (13) revealed that they were cut before the completion of S phase (14), consistent with previous findings (4). None were cut in early $G_1$ or in prophase.

During the first 1 to 3 hours after the operation, the cytoplasmic granules became organized into a spherical mass at the center of the cytoplasm, indicative of the presence of the centrosome, while the granules in the karyoplast remained randomly distributed in the vicinity of the nucleus (Fig. 1B). Normally we removed the cytoplasm with the microneedle so that it would not interfere with observations of karyoplast behavior. Within an hour of the microsurgery, karyoplasts extended lamellipodia and resumed movement across the cover slip (Fig. 2A). Later, they grew in area and regenerated their Golgi apparatus to control levels, as judged by in vivo labeling with Bodipy FL C5-ceramide (4, 12, 15).

In 37 experiments, 32 karyoplasts entered mitosis (Fig. 2A), four remained in interphase until the recordings were terminated 24 hours after the microsurgery, and one died within 12 hours. The interval from the microsurgical operation to the onset of mitosis was on average 12.5 hours (range 4 to 24 hours), which is within the normal interphase duration for control cells in our preparations (average 15.5 hours, range 11 to 26 hours, $N = 25$). In mitosis, karyoplasts aligned chromosomes into a metaphase plate, separated two groups of chromosomes in anaphase, and formed a cleavage furrow (14). This indicates that karyoplasts organized a functional, albeit acentrosomal, bipolar spindle [see also (5, 16)]. Karyoplasts spent a longer and a more variable amount of time in mitosis (average 197 min, range 68 to 557 min) than did control cells (average 56 min, range 24 to 99 min; $N = 40$), presumably because of the need for extra time to organize an acentrosomal spindle. In telophase all karyoplasts initiated bipolar cleavage. However, in 13 of 32 cases (41%), the cleavage furrow regressed and the karyoplasts exited mitosis as a single cell with one or more nuclei (12, 17).

We unexpectedly found that in 28 of 32 experiments, the postmitotic karyoplasts—whether they divided or not—arrested in interphase for the duration of the observations, up to 60 hours after mitosis (Fig. 2A) (12). This was not attributable to loss of cell viability in our preparations, because the karyoplasts showed continuous lamellipod extension, cell motility, and movement of phase-dense granules toward...
the microtubule focus at the nucleus. In 32 experiments, only one postmitotic karyoplast underwent apoptosis. Control cells in the same preparations divided repeatedly until the observations were terminated.

To control for the microsurgical operation and loss of cytoplasm, we amputated equivalent areas of cytoplasm with the cut located in the granule mass on the side of the centrosome away from the nucleus. In all cases these control cells divided at least twice (Fig. 2B). The interval from control amputation to first mitosis was 9.5 hours on average ($N = 4$, range 7 to 13 hours), and the time from first to second mitosis for control cells. For six postmitotic karyoplasts fixed at 12 hours and three fixed at 28 hours after mitosis, we found no BrdU incorporation into any nuclei (Fig. 3), indicating that postmitotic karyoplasts arrest before S phase. Control cells on the same cover slips showed robust nuclear staining for BrdU (Fig. 3).

To test whether our karyoplasts arrested in interphase because they spent extra time in mitosis (19), we treated BSC-1 cells with low doses of Taxol (20), which prolongs mitosis in PtK (rat kangaroo kidney) cells to a variable extent but nonetheless allows them to divide in a normal fashion (21). We found that 79% of Taxol-treated cells, which spend at least as much time in mitosis as do the karyoplasts, divided two or more times. Also, seven karyoplasts went through mitosis in 68 to 97 min, which is within the normal range of mitotic duration, yet all arrested in interphase. Control cells that spent the same amount of time in mitosis continued to divide two or more times (12). Thus, the interphase arrest observed in karyoplasts appears not to result from extra time they spent in mitosis.

All postmitotic karyoplasts reformed a single microtubule focus next to the nucleus that collected phase-dense granules to the same extent as did the focus in normal cells (Fig. 4). Both $\gamma$-tubulin ($N = 4$) and pericentrin ($N = 3$) immunoreactivities in karyoplasts localized to the center of the microtubule focus (Fig. 4). However, karyoplasts do not contain complete centrosomes, because serial semi-thick
section ultrastructural reconstruction (22) of a postmitotic karyoplast revealed that there were no centrioles present in the MTOC 10 hours after mitosis (12). Also, all other karyoplasts behaved as if they lacked core centrosomal components; their MTOCs never doubled, either before or after mitosis. In addition, when a karyoplast divided into two, each daughter contained a single microtubule focus, whereas karyoplasts that failed to cleave organized only one microtubule focus, never two. Because centrosome number and ability to duplicate are determined by centrioles (2, 23), both behaviors are characteristic of the lack of centrioles.

Our finding that BSC-1 karyoplasts enter mitosis demonstrates that, once committed to the cell cycle, these cells do not require the presence or duplication of the intact centrosome for the G2-M transition, as had been proposed (4, 9). Our data also reveal that a heretofore unrecognized activity associated with the centrosome is required for primate somatic cells to progress through G1 into S phase. This activity is evidently not the microtubule-mediated accumulation (or dispersal) of cellular structures or molecules, because postmitotic karyoplasts reestablish a single MTOC of seemingly normal composition and function, yet arrest before S phase. The fact that centrioles do not regenerate in karyoplasts suggests that this activity is physically associated with core centrosomal structures, such as the centrioles and/or centriole-associated structures. The phenomena we observe here may be specific to animal somatic cells, because embryonic systems with abbreviated cell cycles—such as frog egg extracts and early mouse zygotes, as well as plant cells—enter the cell cycle without centrioles (24).

There are two possible explanations for why karyoplasts arrest before S phase. Perhaps BSC-1 cells have a checkpoint that monitors centrosome duplication, and this checkpoint remains activated in the absence of the core centrosomal components necessary for centrosome duplication (2). Alternatively, core centrosomal structures could bind cell cycle regulatory molecules in a way that activates their function or raises their local concentration to the point that essential reactions occur in a timely fashion.

References and Notes
As an organelle coupling nuclear and cytoplasmic divisions, the centrosome is essential to mitotic fidelity, and its inheritance could be critical to understanding cell transformation. Investigating the behavior of the centrosome in living mitotic cells, we documented a transient and remarkable postanaphase repositioning of this organelle, which apparently controls the release of central microtubules from the midbody and the completion of cell division. We also observed that the absence of the centrosome leads to defects in cytokinesis. Together with recent results in yeasts, our data point to a conserved centrosome-dependent pathway that integrates spatial controls into the decision of completing cell division, which requires the repositioning of the centrosome organelle.

The checkpoint concept (1, 2) was introduced to describe mechanisms controlling the progression of the cell division cycle at critical steps that impose delays to allow corrections or repairs, or even to trigger cell death. Many of the key genes involved in these processes are not essential but, when defective, can be oncogenic (3). In Saccharomyces cerevisiae, a checkpoint mechanism monitors the coupling between nuclear and cytoplasmic division and relies on the spatial proximity of interacting components. A guanosine triphosphatase (GTPase) is concentrated at the spindle pole body (SPB), whereas the corresponding exchange factor is concentrated in the bud. Therefore, the migration of one SPB to the neck is necessary to activate the GTPase and to trigger mitotic exit (4–7). This ensures that cytokinesis does not take place before the nucleus has entered the bud.

We investigated whether similar spatial controls involving the centrosome (the functional equivalent of the SPB) might operate in vertebrate cells, which are much larger than yeast cells. We made time-lapse recordings of cell division in cell lines stably expressing the centrin protein coupled to the green fluorescent protein (GFP). As an organelle coupling nuclear and cytoplasmic divisions, the centrosome is essential to mitotic fidelity, and its inheritance could be critical to understanding cell transformation. We observed that the absence of the centrosome leads to defects in cytokinesis. Together with recent results in yeasts, our data point to a conserved centrosome-dependent pathway that integrates spatial controls into the decision of completing cell division, which requires the repositioning of the centrosome organelle.