Centrioles reveal their secrets

Eric Karsenti

Centrioles, organelles that organize the microtubule spindle required for cell division, duplicate at almost exactly the same time that DNA replicates during the cell cycle. New research points to how these replication processes may be coordinated.

For many years, the function and exact mechanism of reproduction of centrioles, organelles involved in organizing the microtubular cytoskeleton, have fascinated cell biologists. It is not difficult to understand why: they are beautiful objects with an unusual geometry. Moreover, they seem to know how to count: there are only two per cell, and they duplicate at almost exactly the same time that DNA replicates during the cell-division cycle. It still isn't clear how centrioles duplicate themselves at the right time, or how they regulate their numbers. However, research by several groups has begun to dissipate the mysterious haze that surrounds these organelles, and in last month's *Nature Cell Biology* Meraldi *et al.* reported new findings about the transcriptional events involved in the temporal control of centriole duplication[1].

Centriole cylinders are made up of nine microtubule triplets, which are attached to each other by a fibrogranular material. The two centrioles together with their associated pericentriolar material constitute the 'centrosome'. In motile animal cells, such as fibroblasts or macrophages, the centrosome typically nucleates the aster of microtubules that radiates in the cytoplasm, and, as shown last year[2], the centrosome is organized by its pair of centrioles, which concentrate microtubule-nucleating material in their vicinity. Therefore, the number of centrosomes determines the number of astral arrays.

During the S (DNA-replication) phase of the cell cycle, each centriole cylinder duplicates through the budding of a new cylinder, which grows orthogonally to the old one. During the remainder of S phase and G2 phase, the two pairs of centrioles remain together; they are separated slightly, but still form one centrosome. The two centriole pairs split at the onset of mitosis (M phase), in early prophase, thus forming two centrosomes; the two resulting microtubule asters then determine the bipolarity of the mitotic spindle and participate in the orientation of the spindle axis[3].

Centrioles have counterparts in ciliated and flagellated cells in both multicellular organisms and unicellular protists. These counterparts are called 'basal bodies', because they are found at the base of cilia and flagella — motile, whip-like structures that grow out from the cell surface. In ciliated protists, rows of basal bodies duplicate synchronously during the cell cycle, and, as for centrioles, duplication occurs by budding of a new basal body off the wall of the older one[4].

But exactly how do the centrioles (or basal bodies) duplicate? There are several things we need to know if we want to answer this question. Which molecules are involved in the initial and late steps of centriole assembly? What are the key regulators of the duplication process? Finally, when are the regulators expressed, and how do they affect the expression or activity of the structural components of the centriole? On the basis of recent results, it seems that both the transcription of essential genes and the post-translational regulation of structural components required for centriole duplication are temporally regulated, by means of phosphorylation (Figs 1, 2).

The role of phosphorylation in post-translationally regulating structural components of the centriole-duplicating machinery has been discovered by looking at the early stages of embryogenesis that follow fertilization of an oocyte.

During oogenesis, which takes place over several months, the oocyte is blocked in the G2 phase of the cell cycle — it does not mul-

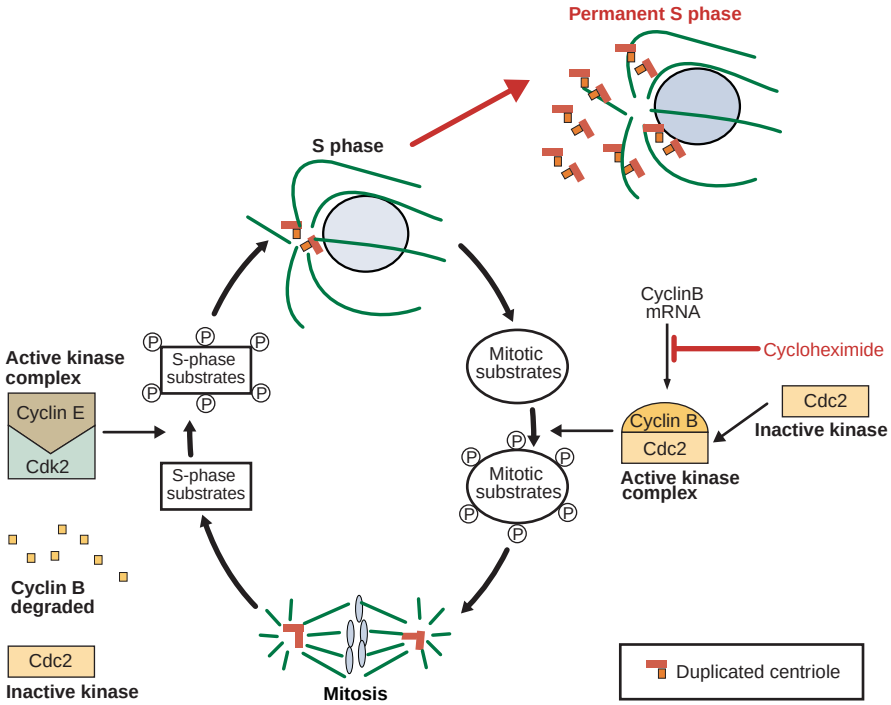

Figure 1 **Coupling centriole duplication to the cell-division cycle in early embryos. In early embryos, the cell cycle is uncoupled from growth and cells proceed rapidly from S phase to mitosis, without intervening G1 and G2 phases. Kinase complexes consisting of cyclin-dependent kinase 2 (Cdk2) and its regulatory cyclin, cyclin E, are constitutively active in such cells, and their phosphorylation of (as-yet-unknown) S-phase substrates leads to duplication of both DNA and centrioles. During the stages preceeding early embryogenesis, cyclin B is stockpiled in the form of messenger RNA, and, after S phase, is translated into protein and forms a complex with the kinase Cdc2; cyclin B–Cdc2 complexes phosphorylate mitotic substrates, leading to progression into mitosis. When the translation of cyclin B mRNA into protein is inhibited experimentally by the addition of cyclohexmide, permanent S phase and centriole duplication occur (red). It thus seems likely that overduplication of centrioles is blocked during the normal embryonic cell cycle by the activity of cyclin B–Cdc2 during each mitosis.**

tiply its DNA or divide into two, but it does stockpile, in the form of messenger RNA or proteins, all of the components necessary for progression through S and M phases, including essential cell-cycle regulators such as cyclin-dependent kinases (CDKs), which are needed for DNA replication and mitosis. Because no cell division is taking place, oogenesis results in the formation of a monstrous oocyte of 1 mm in diameter. After fertilization of the oocyte, the first 12 ensuing cell cycles are uncoupled from growth and alternate rapidly between S and M phases without intervening G1 and G2 phases (during which the cell normally grows before cell division occurs). This uncoupling is possible because of the earlier stockpiling of the essential cell-cycle components. Because there are no G1 and G2 phases, cell size decreases during these early stages of embryogenesis (these cell divisions are in fact called 'segmentation'), until the cells of the embryo reach the size of normal somatic cells.

During this period, S phase may be considered to be a 'default state': in the absence of the synthesis of proteins from the stored mRNAs (for example, when cells are cultured in the presence of the protein-synthesis inhibitor cycloheximide), DNA synthesis is permanently turned on. It turns out that this state allows also permanent centriole duplication (Fig. 1)[5]. In these early embryos, centriole duplication and DNA replication both depend on the presence of the protein kinase complex consisting of Cdk2 and cyclin E[5]. In eggs, cyclin E, unlike the B-type cyclins, is not degraded periodically at the end of mitosis (Fig. 1). As all of the components required for centriole duplication are stored in the egg, initiation of duplication must depend on the phosphorylation of some embryonic components by cyclin E–Cdk2. Interestingly, when protein synthesis is not inhibited, and the early-embryonic cell cycle runs normally, centrioles duplicate not anarchically but only at the end of each mitosis, so perhaps the initiation of duplication is usually inhibited during mitosis, possibly directly by the activity of cyclin B–Cdc2.

Which molecules involved in initiating centriole duplication are controlled by the cyclin–CDK complexes? Unfortunately, we still have very little information about these potential substrates. γ-Tubulin seems to be involved in the very early steps of centriole duplication[4,6], so it may be helpful to look at molecules that interact with this protein.

Studies of somatic cells have confirmed the importance of cyclins and their CDK partners in controlling centriole duplication, and also revealed the part played by phosphorylation in regulating the transcription of genes involved in the process.

Somatic cells have to reach a certain size before entering the mitotic cycle. Size is monitored by a checkpoint during G1 phase that is called the 'restriction point', after which the transcription of genes encoding enzymes, chromatin components and cell-cycle-regulatory molecules (cyclin A, cyclin E and their CDK partner, Cdk2) is activated. The synthesis of these proteins from newly transcribed RNAs leads the cells to progress irreversibly towards DNA replication (Fig. 2). Molecules required for centriole duplication also seem to be expressed after the restriction point. Indeed, there are drugs that block the cell cycle just before or just after this point: mimosine blocks the cell cycle in late G1 phase and hydroxyurea produces an S-phase arrest (Fig. 2). Cyclin-A- and cyclin-E-dependent kinase activities remain low, and centrioles do not duplicate, in mimosine-arrested cells[7]. In hydroxyurea-arrested cells, however, kinase activities dependent on cyclin A and cyclin E are high and centrioles duplicate repeatedly (Fig. 2), just as in embryonic cells. In hydroxyurea-arrested cells, Cdk2 activity is required for centriole duplication.

The decision to pass through the restriction point relies on the activity of a transcription factor of the E2F family that is repressed by the retinoblastoma protein (Rb) (Fig. 2). When the overall cell condition becomes permissive to enter S phase, cyclin D–Cdk4/6 becomes active and phosphorylates Rb. This results in the release from Rb, and activation, of the E2F transcription factor, allowing transcription of the genes encoding cyclin E, cyclin A and other molecules required for DNA replication. Meraldi *et al.*[1] reasoned that the initiation of centrosome duplication is probably under the control of the Rb pathway too.

To test this idea, Meraldi *et al.* blocked the activity of the cyclin D–Cdk4/6 complex before and after the cell cycle was arrested with hydroxyurea. Centrioles did not duplicate when this kinase complex was inhibited before the addition of hydroxyurea, whereas they did when the kinase was inhibited after the S-phase block. Further experiments showed that cyclin D–Cdk4/6 acted indirectly on centriole duplication through the phosphorylation of Rb, as expected. Therefore, the cytoplasmic state required for permanent centriole duplication in somatic cells is indeed the same as that required for DNA replication: it is generated by the transcription of genes governed by the cyclin D–Cdk4/6→Rb→E2F pathway (Fig. 2).

In Meraldi *et al.*'s experimental system, the kinase that seems to act through a direct post-translational event on the initiation of centriole duplication is not cyclin E–Cdk2 but rather cyclin A–Cdk2. This is in apparent contradiction with the results obtained in egg extracts (see above), where cyclin E is

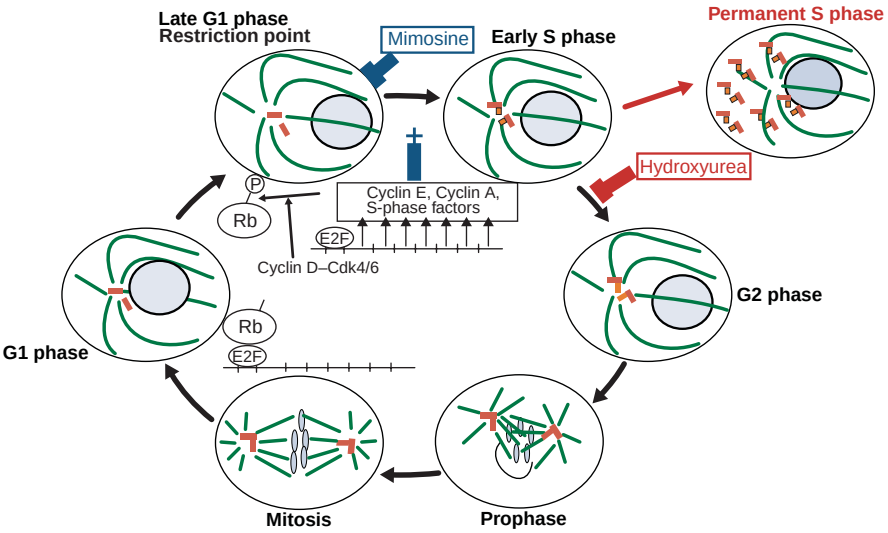

Figure 2 **In somatic cells, which undergo G1 and G2 phases, centriole duplication starts only after cells have entered early S phase. This corresponds to the time at which genes under transcriptional control of retinoblastoma protein (Rb)–E2F complexes have been activated: after cells pass through the restriction point of the cell cycle, complexes consisting of cyclin D and cyclin-dependent kinase 4/6 (Cdk4/6) phosphorylate Rb, which releases E2F and thus allows it to activate transcription. Activated genes include those encoding cyclin A and cyclin E. Mimosine blocks cells in late G1 phase, before the restriction point (blue); kinase activities dependent on cyclins A and E remain low in such cells, and centrioles do not duplicate. Hydroxyurea blocks cells in S phase; in these cells, centrioles duplicate permanently (red). Thus factors required for centriole duplication in somatic cells probably include cyclins A and E and their CDKs.**

required[5]. However, in Meraldi *et al.*'s studies the requirement for cyclins was tested in cells in which E2F-mediated transcription was blocked by a non-phosphorylatable Rb. This means that most of the components required for DNA replication, and probably some of the components involved in centriole duplication, that rely on E2F-dependent transcription were not synthesized in these cells. Therefore, cyclin A–Cdk2 may trigger centriole duplication directly, whereas cyclin E–Cdk2 may do so only when other components are also present. These components are probably stored up in *Xenopus* eggs, explaining the discrepancy between these two sets of results.

These findings are important because they provide an extra, strong piece of evidence that both centrosome duplication and DNA replication are regulated through the same pathway. This is provocative: could it be that centrioles contain nucleic acid, the duplication of which is controlled by the same machinery that controls DNA replication? This idea has been around for a long time, but all investigations so far have failed to reveal the existence of such a molecule. Perhaps there is one after all that has escaped close scrutiny! Or perhaps the protein complex that initiates the assembly of centrioles contains some structural molecules that respond to the same signals as those involved in the formation of DNA-replication complexes. Clearly, the centriole still has a lot of surprises in store for those who are fascinated by these little objects.□

Eric Karsenti is in the European Molecular Biology Laboratory, Cell Biology Program, Postfach 10.2209, Meyerhofstrasse 1, 69012 Heidelberg, Germany.
e-mail: eric.karsenti@EMBL-heidelberg.de

1. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J. & Nigg, E. A. *Nature Cell Biol.* **1**, 88–93 (1999).
2. Bobinnec, Y. *et al. J. Cell Biol.* **143**, 1575–1589 (1998).
3. Heald, R., Tournebize, R., Habermann, A., Karsenti, E. & Hyman, A. *J. Cell Biol.* **138**, 615–628 (1997).
4. Ruiz, F., Beisson, J., Rossier, J. & Dupuis-Williams, P. *Curr. Biol.* **9**, 43–46 (1999).
5. Hinchcliffe, E. H., Li, C., Thompson, E. A., Maller, J. L. & Sluder, G. *Science* **283**, 851–854 (1999).
6. Fuller, S. *et al. Curr. Biol.* **5**, 1384–1393 (1995).
7. Matsumoto, Y., Hayashi, K. & Nishida, E. *Curr. Biol.* **9**, 429–432 (1999).

Stress fibres take shape

Anne J. Ridley

The GTPase Rho is well known for its ability to induce the assembly of stress fibres. Two interacting targets of Rho, ROCK and Dia1, have been found to act in concert to mediate this response to Rho.

In 1990, we found out that the small GTPase Rho can stimulate, in fibroblast cells, the formation of stress fibres — plasma-membrane-linked, long cytoskeletal cables that can contract and so exert tension (reviewed in ref. 1). After many more studies, the consensus opinion is that Rho induces increased stress-fibre assembly in cultured mammalian cells that can form stress fibres (such as fibroblasts) and increased actomyosin-based contractility in cells that cannot (such as neuronal cells).

At the same time, many direct downstream targets of Rho have been identified, on the basis of their ability to interact preferentially with the GTP-bound (active) rather than GDP-bound (inactive) conformation of Rho[2]. One unanswered question is which of these targets is responsible for the stress-fibre formation induced by Rho. Given the number of targets, it has always seemed unlikely that just one would be sufficient, and indeed mutational studies of Rho indicate that at least two are involved[3]. On page 136 of this issue[4], Watanabe *et al.* suggest that two targets, the serine/threonine kinase ROCK and the profilin-binding protein Dia1, could be all that are required for Rho-induced stress-fibre assembly. They show that an appropriate balance of ROCK and Dia1 activity can induce stress-fibre organization indistinguishable from that induced by activated Rho, suggesting that these two targets alone could account for Rho's effects on the actin cytoskeleton, at least in the cells studied here.

So what do these two Rho targets do to regulate stress-fibre assembly? Stress fibres are bundles of actin and myosin-II filaments that also contain many of the proteins found in smooth-muscle filaments. They can contract when cells are released from the substratum, and depend on the ATPase activity of myosin for their assembly and maintenance[1]. The activity of myosin and its interaction with actin filaments in non-muscle cells is regulated by phosphorylation of the myosin light chain (MLC), and so evidence that Rho, acting through ROCK (also known as Rho kinase or ROK), stimulates an increase in MLC phosphorylation was a breakthrough in our understanding of how Rho could induce stress-fibre assembly and/or increased contractility[1,5].

Two ROCK isoforms, ROCK-I and ROCK-II, have been identified so far. They can phosphorylate the myosin-binding subunit of MLC phosphatase, inactivating it and thereby preventing dephosphorylation of MLC[5]. If MLC is continuously cycling between its phosphorylated and dephosphorylated forms, then inactivation of the MLC phosphatase would be expected to lead to an increase in phosphorylated MLC in cells, and indeed this is observed in cells expressing activated Rho[2].

As constitutively active ROCK mutants can stimulate stress-fibre assembly[2], and as a ROCK inhibitor prevents Rho-induced stress-fibre formation[6], the effects of ROCK on MLC phosphorylation seemed at first to explain Rho's ability to induce stress-fibre assembly. As usual, however, things rapidly became more complicated with the (still ongoing) identification of several other ROCK targets that might contribute to Rho's effects on the actin cytoskeleton. For example, ROCK can stimulate phosphorylation of ezrin/radixin/moesin (ERM)-type proteins at a carboxy-terminal threonine residue, thereby inhibiting intramolecular head-to-tail interaction between the amino and C termini[7]. This promotes interaction of the ERM proteins with actin and transmembrane receptors, so ROCK-induced ERM-protein phosphorylation might explain their apparent requirement in Rho-induced stress-fibre formation[8]. ROCK also phosphorylates adducin[9], a membrane-associated cytoskeletal protein, and the Na–H exchanger NHE1 (ref. 10), both of which could contribute, in some as-yet-unknown way, to stress-fibre assembly.

Whatever the mode of ROCK action, the thickness, number and distribution of stress fibres induced by constitutively active ROCK mutants in cells is clearly not like that induced by activated Rho[3,4], indicating that another ingredient is necessary for the fully refined product. This is where Dia comes into the story. The two related mammalian Dia proteins, Dia1 and Dia2 (the 'm' in 'mDia1'[3] stands for 'mouse'), are both downstream Rho targets, and are part of a formin-related protein family that includes the *Drosophila* proteins Diaphanous and Cappuccino and the *Saccharomyces cerevisiae* protein Bni1. These all contain two formin-homology regions, FH1 and FH2, in their C-terminal halves[11].

The FH1 region consists of multiple polyproline sequences which interact with the actin-monomer-binding protein profi-