REVIEW: Multiple forms of cytokinesis in *D. discoideum*

Summary

Cell division is an important feature of life, which enables the continuity of a species. In the process of cell division two things need to be accomplished: division of the previously duplicated nuclei (mitosis), and the division of the cell itself (cytokinesis). This review will address four known mechanism by which the social amoebae D. discoideum divides, which contribute to a better understanding of cellular division in general. Up until the late '80's of the previous century cell division was believed to be executed in a quite linear fashion. Cell growth is followed by duplication of the genome, segregation of the nuclei and to finish division, the actual cell itself is cleaved. It was regarded to be a highly conserved process with but only small differences among distant organisms (e.g. unequal division in the baker's yeast S. cerevisiae). However it was shown that D. discoideum is capable of completing cytokinesis by a secondary adhesion dependent mechanism (cytokinesis B) upon dysfunction of the classic adhesion independent form of cytokinesis (cytokinesis A). Two more mechanisms of cellular division were discovered and named cytokinesis C and D. This review will focus on cytokinesis B, although the other mechanisms (A,C,D) will be quickly addressed to enable comparison between the different mechanisms. The SCAR complex, a protein complex involved in the regulation of the actin skeleton within a cell, showed to be of major importance of cytokinesis B. Where myosin II, a non muscle motor protein, is a key player in cytokinesis A. Cytokinesis C is not cell cycle dependent making it a completely different mechanism, which is not in the scope of this review. Finally in cytokinesis D a chemo attractant is responsible for the recruitment of neighboring cells to help with the division, the state of the neighboring cell as well as the distance to the dividing cell showed to be of high importance. Together these mechanisms show the diversity nature has to tackle a single problem like cellular division.

Keywords: cytokinesis, cell division, adhesion, myosin II, SCAR, actin

Introduction

Cell cycle

For any species reproduction is a necessity to survive. This can be sexual by means of meiosis for higher organisms (animals, fungi, plants), or asexual which is called mitosis, leading to the development of a complete new organism (Wilkins and Holliday 2009). One of the differences between meiosis and mitosis is the amount of genetic diversity they bring about. Meiosis is the

reproduction process resulting in germ cells. The mother cell divides into four gametes each with only half the amount of chromosomes of the mother cell. In the case of mitosis no genetic alterations are promoted resulting in a more or less exact copy of the mother cell, with the same amount of chromosomes as the mother cell. This form of cellular reproduction is found in all known organisms (Wilkins and Holliday 2009). In general it can be said that both mitosis and meiosis are mechanisms by which the cell segregates the genomic material after duplication. Since *D. discoideum*, our species of interest, duplicates only by means of mitosis, meiosis will not be further discussed.

After division of the nucleus the rest of the cell needs to be (equally) divided, this is called cytokinesis. Mitosis together with cytokinesis are the mitotic phase of cell proliferation. The mitotic phase is part of the cell cycle (Fig. 1), so to be able to get a good understanding of the two a short introduction of the cycle as a whole will be given. The cell cycle in general can be roughly divided in four phases: Gap_1-phase (G_1), Synthesis-phase (S), Gap_2-phase (G_2) and the mitotic phase (M). G_1 is considered to be a revalidation period for cell growth. In the end of G_1 a point of no return is passed, triggering advancement into the cycle. After genome duplication in the S-phase, a second gap phase (G_2) is needed for cell growth and protein production. After G_2 the cell cycle will be completed by mitosis and cytokinesis in the M-phase (Bruce, Johnson et al. 2007). In contrary to higher eukaryotic systems *D. discoideum* shows no G_1-phase of significant length (Weijer, Duschl et al. 1984). Thus although *D. discoideum* is haploid, it spends most of its lifetime in a genome duplicated state.

The M-phase itself is split into five stages: prophase, prometaphase, metaphase, anaphase and telophase. During prophase the chromatin (the combination of DNA and its associated proteins) condenses into pairs of chromosomes, called sister chromatids. The sister chromatids are attached to each other at the centromere, a DNA element each chromosome possesses involved in chromatid separation. The nucleolus (proteins and nucleic acids for the transcription of ribosomal RNA) dissolves and the centrosomes (nucleation centre of spindle associated microtubules) move to opposite poles, with the mitotic spindle in between them. In the prometaphase the nuclear envelope (partly) dissociates and the chromosomes form protein structures called kinetochores, to which kinetochore microtubules from the mitotic spindle attach (Moens

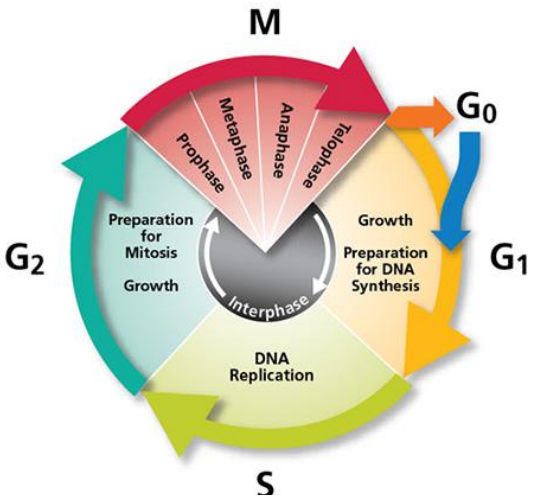

Fig. 1 The cell cycle

G_1 is the first gap phase for cell proliferation. Followed by DNA replication in the S-phase. Another period for growth in the G_2-phase and finally cell division during the M-phase. The M-phase is divided in four subphases (prophase, metaphase, anaphase, telophase) which are involved in genome segregation. Cytokinesis is not shown, but starts during the anaphase and is completed at the end of the M-phase. G_0 is a resting stationary phase in which the cell can arrest when environmental conditions are not favorable.

1976). During metaphase sister chromatids are aligned at the equatorial plate ready to be separated. In the anaphase actual separation of the chromosomes to the opposite poles occurs, and telophase concludes genomic division by reversing the prophase and prometaphase. Cytokinesis is started roughly around the same time as telophase, its role is the division of the cytoplasm and the organelles over the daughter cells.

Cytokinesis

Cytokinesis is the final part of mitosis (it is also said to be an independent process occurring next to mitosis) in which the cytoplasm is divided over the two daughter cells. For quite a while it was thought that cytokinesis was a single mechanism conserved throughout all life, the so called 'purse string model' from now on called cytokinesis A, which is regulated in a cell cycle dependent manner. However *D. discoideum* showed multiple other mechanisms to finish cell division named cytokinesis B,C,D (De Lozanne and Spudich 1987). Cytokinesis B however needs the presence of an adhesion plane and cannot be completed in suspension. Cytokinesis B is like cytokinesis A, cell cycle dependent. Cytokinesis B was also found in normal rat kidney (NRK) cells, suggesting a function in higher eukaryotes (O'Connell, Wheatley et al. 1999). A third mechanism was discovered, when multinucleate *D. discoideum* cells grown in suspension are placed on a substrate, called cytokinesis C (traction-mediated, cell cycle independent cytofission) (Spudich 1989). This third mechanism however seems not cell cycle dependent and leads to uneven division (Uyeda and Yumura 2000). Thus cytokinesis C might rather be more of a safe mechanism, than a well regulated manner of cytokinesis. Next to the three cytokinetic mechanisms mentioned above is a completely different method to finish cytokinesis called midwifery (cytokinesis D). In midwifery a second cell assists in the process of cytokinesis by physically interacting with the cleft of the dividing cell (Nagasaki and Uyeda 2008).

These different mechanisms of cytokinesis bring about the following questions. (i) How do these mechanisms tackle the physical barriers associated with cytokinesis (e.g. the force needed for furrowing). (ii) What are the main proteins involved in the different mechanisms of cytokinesis. (iii) Which elements play a role in choosing between these mechanisms. In this review the primary focus will be on cytokinesis B, although the other mechanisms will be reviewed for comparison.

Cytokinesis A

Cytokinesis A is the well known myosin II dependent type of cytokinesis. It is also known as the 'purse string model'. However a proper functional description of cytokinesis A is: a contractile-ring dependent, adhesion-independent and cell cycle dependent form of cytokinesis.

During the mitotic phase three things are of major importance for any of the cytokinetic mechanisms. First the two nuclei need to be separated (genomic segregation). Next the cytoplasm as well as the organelles need to be equally distributed on both sides of the equatorial region, and last is the actual cleaving of the cytokinetic bridge of the cell. During these steps the cell's morphology is

highly regulated, and varies for each step. When comparing the different kinds of cytokinesis, I will focus on the actual furrowing process of the membrane.

Starting with the retraction of the cellular protrusions due to silencing of the actin cytoskeleton the cell becomes more or less spherical (King, Veltman et al. 2010). As a result of the retraction of protrusions the cell becomes partly or completely unattached to the substrate. At this point the only cellular forces on the membrane are: the outside directed force due to cellular pressure, and the uniformly distributed force directed inwards caused by the solvent pressure. Within minutes a third (inward) force is generated at the equatorial region which leads to furrowing (Fig. 2). This furrowing is caused by contraction of myosin II filaments inside the cell at the equatorial region. Myosin II accumulation at the equatorial region is highly regulated, with its assembly starting during anaphase (Matsumura 2005). Actual contraction of the myosin II actin ring starts in late telophase. The myosin II filaments are tightly attached to the membrane cortex pulling the membrane inwards as the ring tightens. Since the volume to surface ratio is at its maximum for a perfect sphere (which the cell more or less is at this moment, although it might be stretched a little), extra membrane vesicles need to be located at the furrowing region to provide membrane relaxation. A midbody is formed as the furrowing continues at the intercellular bridge, which is usually not longer than 10 µm (Moens 1976). The furrow turns into a cleft and cytokinesis can be completed by cleaving the midbody which is mediated by the Ste20-like kinase SvkA. Although the actual cleavage of the midbody is part of cytokinesis, its precise mechanisms are out of scope in this review.

As said earlier the cell adopts a spherical shape just before the furrowing begins at the equatorial region. However, to determine the equator of a sphere two poles are needed. Thus although the outside of the cell looks more or less unpolarized (Fig. 2 Cytokinesis A during anaphase), the opposite needs to be true for the inside of the cell. One of the first steps of internal polarization is the

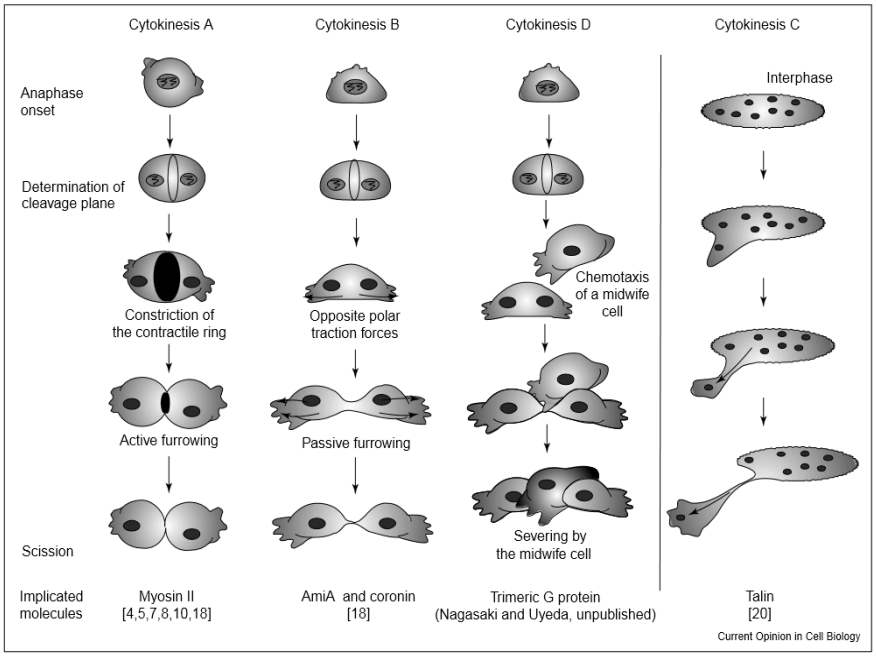

Fig. 2 An overview of the four different mechanisms of cytokninesis

A schematic depiction of the morphology of a dividing *D. Discoideum* during cytokinesis. All four mechanisms are shown (A,B, midwifery, C).

division of the centrosome, which in contrary to most vertebrates, is stalled up until early prophase in *Dictyostelium discoideum* (Ueda, Schliwa et al. 1999). Kinetochore microtubules from both centrosomes meet at the center and attach to the chromosomes, aligning sister chromatids for segregation during metaphase. While polar microtubules attach to each other creating the mitotic spindle, astral microtubules reach for the cell cortex. This positioning of the centrosomes by the astral microtubules is important for defining and stabilizing the orientation of the mitotic spindle (King, Veltman et al. 2010). The centrosomes were thought to be regulating the equatorial region protein assembly, but it seems the central spindle is of equal or greater importance for regulation at the equatorial region (Glotzer 2009). Altough Glotzer is focusing on higher eukaryotic systems, and exact signaling seems to differ in *D. discoideum*, it is likely that the central spindle is just as important for regulation.

A second important equatorial associated complex is the chromosome passenger complex (CPC). It's main function is the localization and activation of Aurora B kinase, which regulates the chromosome congression during metaphase, and directs myosin II function during furrowing (Kitagawa, Fung et al. 2013). The CPC is a multi-subunit complex consisting of three helical bundles made of the following proteins: inner centromere protein (INCENP), Aurora B (the only aurora in *D. discoideum*), survivin and borealin. Although the CPC complex will not be further discussed in this review an article covering the CPC complex in high detail was recently placed in 'Molecular Cell Biology' (Carmena, Wheelock et al. 2012). The central spindle in combination with the CPC might be the major factors driving cytokinesis A in the cell, regulating the recruitment of proteins and their activities associated with equatorial furrowing.

Cytokinesis B

When a *mhc A*$^-$(myosin II heavy chain null) mutant of *D. discoideum* is grown in suspension a severe cytokinesis defect was observed, confirming the necessity of myosin II for cytokinesis in suspension (cytokinesis A) (De Lozanne and Spudich 1987). An interesting feature of these multinucleated cells is their behavior when placed on a substrate. They rapidly start dividing in a cell cycle independent way, more or less tearing the cell in several pieces. This was called cytokinesis C and will be discussed later on. However if the a *mhc A*$^-$ cells are grown under the same conditions as the cells in suspension, accept for the fact that they are placed on a substrate, cytokinesis is still occurring in a cell cycle dependent manner. Although cell division is somewhat slower compared to wild type division, the success rate is 90% which is remarkably high (Nagasaki, de Hostos et al. 2002). This contractile-ring independent, substrate dependent and cell cycle coupled way of cytokinesis is named cytokinesis B. In contrary to cytokinesis A, cytokinesis B is myosin II independent. So how does a cell divide without the central ring driven furrowing? The answer seems to be linked to the poles of the dividing cell.

Although cytokinesis B is considered to be a secondary mechanism, wild type cells grown on

a substrate show a form of cytokinesis resembling both cytokinesis A and B. During anaphase wild type cells round up (Cytokinesis A like) to initiated myosin II assisted furrowing, but before cytokinesis is completed they reattach to finish cytokinesis (Cytokinesis B like). This suggests that the cytokinesis B mechanism is not an artifact from the past, but an actively used method for assisted cell division on a substrate.

During early cytokinesis B the polar cortices start to ruffle in both wild type and *mhc A*$^-$ *D. discoideum* cells on a substrate. This activity of the actin skeleton persists as these ruffles become the leading edges of the daughter cells, which migrate away from the equatorial region. It is now thought that these traction forces generated by the leading edges are passively causing ingression of the equatorial region (Fig 2 Cytokinesis B). A morphologically distinct feature of cytokinesis B is the flat shape of the cell during furrow ingression. The shape of the furrow itself is also more 'U'-like in the case of cytokinesis B compared to a more 'V'-like shape of the furrow in cytokinesis A. To verify the hypothesis of actin having a key role at the poles during cytokinesis, inhibition experiments of actin in the poles were conducted and showed severe cytokinetic defects for *mhc A*$^-$ cells on a substrate. The inhibition of actin at the furrow however seemed to be of little importance, supporting the suggested involvement of actin at the poles during cytokinesis B (O'Connell, Warner et al. 2001).

As cell adhesion is of major importance during cytokinesis B, a deeper insight of the underlying mechanism(s) is essential. Inhibition experiments of adhesion associated proteins such as talin (TalA), vinculin (VinA), and paxillin (PaxB) showed to partially block cytokinesis B. Suggesting their involvement in adhesion during cytokinesis B. All three of these proteins are part of so called focal adhesion points, linking the actin skeleton to the cytosolic domain of integrins (proteins involved in intercellular adhesion). Fluorescent microscopic analysis of *mhc A*$^-$ *D.* discoideum cells during cytokinesis, showed a strong localization of these proteins at the poles, which was not observed for cells in the interphase (Hibi, Nagasaki et al. 2004) (Nagasaki, Kanada et al. 2009). The combined observation of partly blocked cytokinesis on a substrate in *mhc A*$^-$ /(*TalA*$^-$, *VinA*$^-$, *PaxB*$^-$), and their localization during cytokinesis on a substrate shows their involvement in adhesion-dependent cytokinesis B.

Another known regulatory complex of the actin cytoskeleton is the SCAR/WAVE complex. It is associated with actin branching in lamellipodia and the formation of mitosis-specific dynamin actin structures (MiDASes) during mitosis. These MiDASes are involved in cellular adhesion, during cytokinesis they are positioned underneath the nuclei and the mitotic spindle (Itoh and Yumura 2007). Disruption of the SCAR complex leads to multiple dysfunctions in cytokinesis. (a) A reduced membrane strength resulting in a weak adhesion to the substrate, causing at least one daughter cell to detach during cytokinesis. (b) During cytokinesis parts of the membrane bleb off due to lack of membrane strength. (c) *SCAR*$^-$ cells are not capable to correct for drifting of the mitotic spindle, resulting in division non-parallel to the substrate. (d) Movement of the daughter cells became interphase-like during cytokinesis, instead of the observed directional movement away from the

equatorial region of wild type cells (King, Veltman et al. 2010). So although *SCAR*$^-$ is not essential for cytokinesis, its involvement is indisputable.

So what does the SCAR complex do during mitosis? Earlier work showed that SCAR is an activator of the ARP2/3 complex which is involved in f-actin branching at the cortex (Machesky and Insall 1998). TIRF microscopy localization of the SCAR complex using HSP300-GFP (one of its subunits to track SCAR), mRFP-ARPC4 (used to track the ARP2/3 complex) and mRFP-actin showed a strong interaction (King, Veltman et al. 2010). Within seconds of SCAR localization at the edge of cellular protrusions at the poles the ARP2/3 complex co-localizes followed by a broad band of actin. During interphase the SCAR complex showed weak localization at the small vegetative pseudopodia (Pollitt, Blagg et al. 2006). Hinting that the SCAR complex has a migrational regulatory function during adhesion mediated cytokinesis, this cell movement might passively contribute to the furrowing at the equatorial region.

An interesting observation is the measured area of dividing wild type cells which is clearly separated in two groups. Cells that formed MiDASes had an alomost twofold increase in their area, compared to non-MiDASes assisted dividing cells. These cells also showed an increased actin level in the cortex, which is also SCAR regulated and part of cyokinesis B. This distinct difference in dividing wild type cells suggest the existence of a switch, which pushes cell division in a more myosin II or SCAR dependent manner (King, Veltman et al. 2010).

If SCAR is indeed as important for cytokinesis B as is postulated above, the deactivation of the SCAR complex in myosin-II-deficient cells should result in heavy cytokinesis defects, which has indeed been found. Double mutants of SCAR and myosin II quickly become multinucleated due to cytokinetic defects both in suspension and on a substrate.

As SCAR seems to regulate important processes required for the successful completion of cytokinesis B, some knowledge of its regulators might give extra insight in the regulation of cytokinesis B as a whole. In the search for SCAR regulators a *SCAR*$^-$ resembling phenotype was found upon knockout of EB1, which is binding to the growing end of microtubules involved in astral microtubule localization (Berrueta, Kraeft et al. 1998). *EB1*$^-$ lead to a uniform distribution of SCAR in the cytosol of the knockout cells, proposing it is an upstream regulator of the SCAR complex involved in its localization to the leading edges of the cell. Since EB1 is an astral microtubule binding protein, a regulatory role of microtubules during cytokinesis is thinkable. Thus microtubule localization might be an upstream regulator of the SCAR complex.

Cytokinesis C

Myosin-II-deficient *D. discoideum* cells grown in suspension become large and multinucleated. When these multinucleated are place on a substrate they rapidly start a adhesion dependent, cell cycle independent form of cytokinesis (cytokinesis C) (De Lozanne and Spudich 1987).

Although cytokinesis B and C are both adhesion dependent, some major morphological differences between these two mechanisms makes them very distinct. When cytokinesis C is triggered (underlying mechanism is not known), a leading edge of the multinucleated cell starts migrating away from the center. This migration leads to passive furrowing of the connecting cytoplasm, creating a cytokinetic bridge. In contrary to cytokinesis A and B which rarely have cytokinetic bridges longer than ten micrometer, the cytokinetic bridge of cytokinesis C can become tenths of micrometers long. This creates a tension on both parts of the cell which most of the time cannot be surpassed. Causing the two parts to merge back together (Fig. 2 Cytokinesis C) (Uyeda and Nagasaki 2004).

Although wild type cells are capable of utilizing cytokinesis C when both cytokinesis A and B are blocked, no evidence is found for its involvement in natural cell division. Some speculations are made, suggesting cytokinesis C is a remnant of a more primordial cytokinesis. Although convincing proof does not seem easy to find.

Midwifery

Cytokinesis is the last horde for dividing cells to overcome, resulting in two completely separated daughter cells. Besides from the well known purse string model, multiple different mechanisms to accomplish cytokinesis were found during the late 80's in the social amoeba *D. discoideum*. This extra mechanism of cytokinesis gave researchers a tool to study cell proliferation in ways not possible before. Cytokinesis quickly became a hot topic in the scientific world and research groups published articles covering the subject like wildfire. Although many difference were found in the different cytokinetic mechanisms *D. discoideum* and other organisms showed, the involved forces needed for successful cytokinesis always were provided by the dividing cell (Fig. 2 Midwifery (Cytokinesis D)). Whether myosin II or build-up tension due to adhesion of the poles caused furrowing of the equatorial region, both were processes regulated from inside the cell. However a totally different mechanism for cytokinesis was observed in the parasitic amoeba *E. invadens*. This organism showed a social approach for completing cytokinesis (Biron, Libros et al. 2001). Later on the same social cytokinetic mechanism was shown for *D. discoideum*.

In contrary to the other known cytokinetic mechanisms these amoebae use some form of chemotaxis signal radiated from the cleavage furrow. Adjacent cells migrate to the dividing cell and form a protrusion which physically helps with the segregation. Although the exact chemo attractant is still unknown aspired medium from the furrow region of a dividing cell showed to be attracting 50% of the exposed neighboring cells (Biron, Libros et al. 2003). An important factor for midwifery is the population density. For if a neighboring cell is needed for help, the signal must first diffuse to the midwife. When the midwife receives the signal it needs to migrate to the dividing cell. If the cell is too far away division is either already successfully completed or aborted before the midwife reaches the cell in need.

It would be interesting to examine the importance of midwifery in multi-cellular organism, for there is always a in the neighboring area. Although the chemotaxis is not possible for all cell types, assistance can be much broader than just the protrusion interaction displayed by *E. invadens* and *D. discoideum*. Although much is still unclear about midwifery, its existence alone is a beautiful example of the diversity and beauty nature has to solve a problem.

Discussion

The view scientists have on cytokinesis is not as linear as it was thirty years ago. De Lozanne *et al.* showed a substrate dependent character of cytokinesis for *D.discoideum* which was completely new. Multiple theories tried to postulate a mechanism by which myosin II independent cytokinesis should be possible. The main point of dispute being active or passive contraction of the furrow. Nowadays the latter is supposed to be correct, for a myosin II like protein causing active ingression has not been found. Next to the absence of a motor protein taking over the myosin II function, computer simulations have shown that the passive furrowing caused by polar adhesion can explain the equatorial furrowing seen in cytokinesis B (Poirier, Ng et al. 2012). These models really help clarifying the overall forces acting on a dividing cell, however no deeper insight in what is causing these forces can be analyzed in detail based on these 'simplified' simulations.

For a better molecular understanding of cytokinesis a lot of screening has been done to identify involved proteins. More and more single proteins and complexes are found to be related to either one of the cytokinetic mechanisms of *D. discoideum*, or cytokinesis as a whole. At this point it should be clear that each cytokinetic path (A,B,C,Midwifery) has its own key players as well as general factors. For cytokinesis A myosin II and actin filaments are important factors for the equatorial furrowing. Cytokinesis B is more dependent on the actin skeleton and focal adhesion points, with its main furrow inducing elements at the poles. Cytokinesis C and midwifery still remain in the dark, although chemotaxing involved in midwifery showed to be trimeric G protein dependent. Other proteins like coronin, cortexillin I and II showed to be affecting multiple cytokinesis mechanisms in a broader sense (de Hostos, Rehfuess et al. 1993, Weber, Gerisch et al. 1999).

Some good progress has been made in mammalian cells regarding the regulation of cytokinesis, which is probably very related to cytokinesis A in *D. discoideum*. However none of these mechanisms seem to be one to one translatable for *D. discoideum*, with important GEFs and GAPs missing. But one way or the other, the importance of small GTPases in regulating cytokinesis, and more general structural cell morphology is indisputable (Spiering and Hodgson 2011). How these small GTPases act on cytokinesis A might be even quite preserved with RacE as an important upstream regulator of dynacortin (a actin bundling protein) and coronin, as well as it being important for cortical tension (Zhou, Kee et al. 2010). The homology for cytokinesis B, or even C and midwifery unfortunately is much smaller leaving these cytokinetic mechanisms to remain in the dark.

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Fig 1 (The cell cycle)
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