Co-ordination of cytokinesis with chromosome segregation

Manuel Mendoza and Yves Barral
ETH Zurich, Institute of Biochemistry, HPM D14, Schafmattstrasse 18, 8093 Zurich, Switzerland

Abstract
During anaphase, the spindle pulls the sister kinetochores apart until the sister chromatids are fully separated from each other. Subsequently, cytokinesis cleaves between the two separated chromosome masses to form two nucleated cells. Results from Schizosaccharomyces pombe suggested that cytokinesis and chromosome segregation are not co-ordinated with each other. However, recent studies indicate that, at least in budding yeast, a checkpoint called NoCut prevents abscission when spindle elongation is impaired, and might delay cytokinesis until all chromosomes are pulled out of the cleavage plane. Here, we discuss this possibility and summarize evidence suggesting that such a checkpoint is likely to be conserved in higher eukaryotes.

Introduction
During division of animal and fungal cells, ingestion of the cleavage furrow partitions the cytoplasm and prepares for the final cleavage of the cell at abscission [1,2]. In most cell types, furrow ingression is concomitant with chromosome segregation, while abscission must take place only after the full separation of the sister chromatids. The orderly repetition of chromosome segregation and cytokinesis is key to ensuring genome stability within tissues and across generations. Indeed, failures in either cytokinesis or chromosome segregation lead to aneuploidy, with often disastrous consequences for the cell [3]. Cytokinesis failure leads to the doubling of chromosome and centrosome numbers. This in turn can result in the formation of multipolar spindles, and unequal chromosome partitioning in the subsequent divisions [4]. The relationship between abortive cytokinesis, aneuploidy and tumorigenesis has thus received considerable attention over the last years [5]. On the other hand, premature cytokinesis (that is, before all chromosomes have migrated away from the division site) might also have deleterious consequences, which may have been relatively overlooked. Indeed, such events may cause the cleavage machinery to trap, damage or even cut the chromosome arms that are still lying in the way of the cleavage machinery. Such events might cause cell death, if essential genes are thus lost, and the generation of highly recombinogenic chromosome fragments in the surviving cells. Such fragments would be likely to trigger gross chromosomal rearrangements such as translocations, inversions and deletions, as are frequently observed in human cancers [3]. These considerations suggest that mechanisms might have evolved to co-ordinate chromosome segregation and cytokinesis with each other during mitosis. It would be remarkable indeed that these same cells that survey with such considerable precision the bipolar attachment of sister chromatids during metaphase [6,7] would leave the timing of cytokinesis to chance. Indeed, proper co-ordination of cytokinesis and chromosome segregation might well be as important for the maintenance of genome stability as the bipolar attachment of sister chromatids.

Co-ordination of chromosome segregation and cytokinesis

In spite of the above considerations, classic studies in the fission yeast Schizosaccharomyces pombe suggested that no such co-ordination would in fact exist. Indeed, in a hunt for mutants affecting spindle elongation and chromosome segregation, Yanagida and co-workers (reviewed in [8]) identified a plethora of so-called ‘cut’ mutants, which completed cytokinesis very much as wild-type, despite their inability to properly divide the nuclear mass. In these mutants, cytokinesis proceeds despite the presence of the undivided nucleus in the cleavage plane. The random and deadly cutting through the unsorted mass of genetic material observed in these many mutants, the ‘cut’ phenotype, effectively illustrated how blind cytokinesis can be. These observations contributed greatly to the widely held view that no mechanism co-ordinates cytokinesis with proper chromosome segregation. A potential problem with this interpretation, however, is that cut mutants were selected for their phenotype. Thus, these observations tell us that it is possible to identify mutants in which cytokinesis proceeds despite major anaphase defects. They do not necessarily demonstrate that cytokinesis is not co-ordinated with chromosome segregation in wild-type cells.

Hints in favour of cytokinesis and chromosome segregation being in fact co-ordinated with each other can indeed be gathered from other cell types. For example, time-lapse analysis of dividing PtK cells in culture [9] show that single kinetochores are occasionally captured simultaneously by microtubules originating from opposite spindle poles (Figure 1A). Such merotelic attachments are not detected by

Key words: abscission, Aurora, chromosome segregation, cytokinesis, NoCut.
Abbreviations used: CPC, chromosome passenger complex.
1email manuel.mendoza@bc.biol.ethz.ch or yves.barral@bc.biol.ethz.ch
Abscission (represented by triangular blades) is inhibited by the presence of a lagging chromosome in the vicinity of the spindle midzone. Once the lagging chromosome is segregated away from the cleavage plane, abscission proceeds. (B) Anaphase spindle breakage, as caused by depletion of midzone components, triggers a delay in both chromosome segregation and abscission. (C) Inactivation of the NoCut pathway impairs the co-ordination between completion of cytokinesis and chromosome segregation. Cells with midzone defects undergo abscission with incompletely segregated chromatids. Chromosome breakage ensues.

The spindle attachment checkpoint and therefore anaphase is allowed to proceed. The mis-attached chromosomes are consequently stranded in the cleavage plane until the tug-of-war between the opposite spindle poles is resolved, and the lagging chromosome is finally pulled to one side of the cleavage furrow. While the furrow ingressed normally in such cells, abscission was significantly delayed and did not proceed to completion as long as a chromosome was still present in the cleavage plane. Similarly, lagging chromosomes are observed with high frequency in HeLa cells with a defective spindle checkpoint, and this correlates with defects in completion of cytokinesis [10]. Also, formation of chromosome bridges and failure in chromosome disjunction are frequently followed by abortion of abscission and furrow retraction [11]. Thus, lagging chromosomes do affect the completion of cytokinesis in mammalian cell types. However, these hints are not conclusive. They might simply indicate that in animal cells, chromatin lingering in the cleavage plane is a potent mechanical impediment to abscission.

More demonstrative data, perhaps, were recently obtained from budding yeast. In this organism, chromosome segregation is mainly achieved through spindle elongation during anaphase B [12]. Remarkably, in yeast cells with anaphase spindle defects, such as mutants lacking the spindle midzone components Ase1 and Ndc10, cytokinesis is significantly delayed or even aborted [13] (Figure 1B). In these cells, actomyosin ring contraction is unperturbed and the cleavage furrow ingresses properly during cytokinesis. Abscission, however, is impaired. The microtubule-bundling protein Ase1 (called PRC1 in humans) and the kinetochore protein Ndc10 both contribute to the stabilization of the anaphase spindles. The fact that abscission is specifically affected in such midzone mutants is very reminiscent of what is observed in animal cells with lagging chromosomes. This phenotype is also very similar to what has been reported for midzone mutants like PRC1 in Drosophila and mammalian cells [14,15]. In metazoans, this requirement for midzone components suggested that the spindle midzone could be directly required for cytokinesis. Although the molecular mechanism is unknown, one hypothesis is that the spindle midzone is required for the assembly of the midbody, a microtubule-based structure closely associated with the late furrow that may directly regulate abscission [16]. However, this interpretation cannot be applied to yeast, which has no midbody: the entire spindle, which is intranuclear in this organism, disassembles before the onset of furrow ingression. Thus, the cytokinetic defect of yeast cells with spindle midzone defects cannot be due to a direct implication of the spindle in the cytokinetic process.

A more effective demonstration that the spindle midzone is not directly involved in abscission is that its role in yeast cytokinesis can be bypassed when midzone defects are combined with additional mutations. Indeed, when the Aurora kinase Ipl1 is inactivated in ndc10-1 and ase1Δ mutant strains, cytokinesis is restored although spindle elongation is still defective [13]. Ipl1 and its cofactors Sli15 and Bir1 [homologous to animal INCENP (inner centromere protein) and Survivin] form the so-called CPC (chromosome passenger complex) [17]. During metaphase, the CPC localizes to kinetochores where it regulates microtubule attachments and the spindle assembly checkpoint [18,19]. During anaphase, the CPC relocates to the spindle midzone, where its function is unclear [20,21]. The observation that inactivation of the CPC restores abscission in the ndc10-1 mutant cells indicates that the yeast Aurora kinase inhibits abscission in cells with a spindle midzone defect. Aurora fulfills this function at least in part through the anillin-related proteins Boi1 and Boi2 [13]. In wild-type cells, these two proteins accumulate at the site of division, the bud neck in yeast, during anaphase and disappear from this location during cytokinesis. In cells with a midzone defect, they remain at the bud neck where they appear to prevent abscission, since their inactivation restores the completion and timing of cytokinesis as efficiently as Aurora inactivation. Furthermore, in the absence of Ipl1 function, Boi1 and Boi2 fail to translocate to the bud neck during anaphase. They instead accumulate in the nucleus. Together, these data establish several important conclusions. First, yeast cells do not directly require the spindle midzone to proceed through a complete and successful cytokinesis. Instead, the abscission defect observed in cells with a defective spindle midzone is due to a secondary response of the cell. Secondly, this secondary response depends on the Aurora kinase Ipl1, and the two abscission inhibitors Boi1 and Boi2, which together form the ‘NoCut pathway’ [13,22]. In this pathway, Aurora acts upstream of Boi1 and Boi2 and controls their translocation to the bud neck during anaphase. These observations raise further questions. What is the physiological relevance of inhibiting abscission in cells with spindle defects? What is the event that triggers the NoCut signal in cells with spindle defects? How does Ipl1
control Boi1 and Boi2 localization and function? Finally, by which mechanism do Boi1 and Boi2 inhibit abscission?

Some experimental data allow the first question to be answered already. The inactivation of NoCut is disastrous for cells with spindle midzone defects. Ddc1 is a DNA damage checkpoint protein, which accumulates at sites of double strand breaks, and hence Ddc1–GFP forms fluorescent foci upon chromosome breakage, providing an excellent reporter for such damages [23]. Using this reporter, it appears that some chromosome breaks spontaneously occur during S-phase in wild-type and Ase1-depleted cells, but not later during the cycle. In contrast, Ase1 depletion results in DNA damage during cytokinesis, specifically when NoCut is inactivated by deletion of BOI1 and BOI2 [13] (Figure 1C). As a consequence, the ase1Δboi1Δboi2Δ triple mutant is not viable. Thus, delays in chromosome segregation need to be matched by a corresponding delay in abscission; in the absence of this co-ordination, lagging chromosomes are trapped and broken by the abscission process. Thus, NoCut functions as a checkpoint that prevents abscission in response to spindle defects during anaphase, protecting the genetic material from being injured by cell cleavage.

Interestingly, the boi1Δboi2Δ double disruption is lethal on its own in some yeast genetic backgrounds, but the precise event leading to death in these cells has not been determined. In viable boi1Δboi2Δ strains, abscission is advanced relative to wild-type [13]. Cytokinesis-induced chromosome breakage may be the reason why these cells are not viable. Thus, different genetic make-ups might render the NoCut checkpoint more or less crucial for cell survival.

We do not yet know whether NoCut responds to midzone defects or to the presence of chromosomes lagging in the cleavage plane. Premature completion of cytokinesis in boi1Δboi2Δ cells correlates with an increased incidence of chromosome damage during cytokinesis, although spindle elongation is normal [13]. This suggests that NoCut is activated in every cell cycle, independently of midzone damage. Thus chromosome segregation might be the process monitored by NoCut, rather than the integrity of the spindle midzone. If this is the case, it could indicate that the segregation of chromosome arms is more stochastic than anticipated. Indeed, this would explain why inactivation of NoCut increases the frequency of chromosome breakage during cytokinesis, and would provide a strong rationale for the very existence of the NoCut checkpoint. But why should the separation of chromosome arms be variable from cell to cell? One possibility is that the length of chromosome arms is variable enough to introduce a strong irregularity in chromosome segregation. Repetitive chromosomal regions, such as the rDNA (ribosomal DNA) loci, undergo many recombination events that stochastically increase and decrease their length [24]. It would be interesting to see whether an increase in the length of the corresponding chromosomes sensitizes cells to NoCut defects. In any case, further investigations should elucidate whether NoCut directly responds to the status of chromosome separation, or to the clearance of the spindle midzone from chromatin. For example, we have detected late-segregating chromosomes in yeast under certain conditions, and it will be important to know whether abscission is delayed in these cases, and whether NoCut plays a role in this process.

Although the ‘cut’ mutants in fission yeast suggest that cytokinesis is not co-ordinated with chromosome segregation at least in this organism, it is interesting to note that many cut mutants are deficient in separase activity or in its regulation. Thus cut1 and cut2 are separase and its regulator securin; components of the anaphase-promoting complex, which activates separase, are encoded by cut4, 9, 20 and 23. In budding yeast, separase regulates localization of the CPC during anaphase [21], raising the possibility that both chromosome segregation and NoCut are under the control of separase. If this is the case, separase mutants should show a cut phenotype. Thus, the existence of cut mutants in fission yeast does not necessarily rule out that NoCut exists in this organism, and might rather suggest that at least some cut mutations might in fact inactivate a pathway similar to NoCut.

The observation that spindle midzone defects cause a NoCut-dependent abscission defect in budding yeast questions our classical interpretation of midzone function in other eukaryotes. Although earlier studies have focused on the potentially direct involvement of the midzone-derived midbody in the actual process of abscission [1,2], midzone defects might also influence cytokinesis via other pathways. Indeed, midzone mutants also show defects in chromosome segregation due to impairment of anaphase B and higher incidence of lagging chromosomes [25]. Since NoCut components are highly conserved, NoCut might also be operational in other organisms, and co-ordination of cytokinesis with chromosome segregation might be a widespread process.

If NoCut-like mechanisms exist also in animal cells, what is the role of Aurora B in this process? This question is not easy to address, since in animal cells, unlike in yeast, Aurora B is also required for cell cleavage [17]. For historical and practical reasons, reports on Aurora B function have focused on the effects of its inactivation before furrow ingression. To study its possible function in inhibition of cytokinesis, it would therefore be necessary to inactivate the protein specifically after furrow ingression but before abscission is complete. This is indeed possible thanks to the availability of potent and specific Aurora B inhibitors (discussed in [1]). If Aurora B is indeed required for inhibition of abscission, its inactivation within this reduced time window should result in premature cytokinesis. Although this has yet to be addressed directly, a recent study [26] has used the same technical approach to investigate the role of Aurora B in chromosome condensation during anaphase. Ellenberg and co-workers showed that treatment of cultured cells with Aurora inhibitors after anaphase onset causes a reduction in chromosome compaction and in the axial shortening of chromosome arms [26]. Similar results were observed when Aurora B localization to the anaphase spindle was perturbed by affecting microtubule dynamics. This function of Aurora kinases appears to be conserved, since anaphase chromosome condensation is also under the control of Aurora in yeast [27]. Interestingly, cells perturbed for chromosome segregation (by depletion of
condensin) showed a marked increase in segregation defects when this Aurora B function was inactivated [26]. Aurora B thus protects chromatin from the damaging action of the cytokinetic furrow in animal cells by facilitating the segregation of chromatin away from the cleavage plane. Whether it also controls the timing of abscission relative to chromosome segregation, as is the case in budding yeast, remains to be determined.

Conclusion
Although the underlying molecular mechanisms are still unclear, Aurora kinases might turn out to co-ordinate cytokinesis with chromosome segregation in different and complementary ways: by delaying abscission as long as chromosomes are present in the plane of furrowing, and by promoting chromatin clearance from the same area through chromosome condensation.

References