Figure 1. Hummingbird co-pollinator visiting morning-opening flowers of wild tobacco. (Photo courtesy of Danny Kessler.)

co-pollinators can recognize the more rewarding morning-opening flowers.

While, at first glance, the means by which wild tobacco copes with hawkmoth pollinating herbivores may seem rather novel and system specific, that may not be the case given that this system is similar to many others. Foremost, the plant hormone jasmonic acid (JA), which drives the herbivore-induced changes in flower-opening time, is a common signal transducer in plants and is vital for the production of a suite of chemical defenses. Experiments by Kessler *et al.* [5] revealed that while wounding alone did not change flower-opening time in JA-deficient plants, the application of JA to wounds could change flower opening, raising the possibility that a mechanism like this might occur in other plant species. Second, many plants that appear to specialize on particular types of pollinators may gain a significant portion of their pollination success from co-pollinators under some ecological contexts [12]. Third, pollinators often show strong preference for rewarding flowers by using correlated cues or honest signals that alert them to reward content. Hummingbird pollinators in particular, with their high energy demands for flight and hovering, often exhibit the ability to assess reward content prior to tasting flowers [13]. Taken together, these factors suggest that herbivore-induced changes in floral and flowering traits may be widespread and could positively affect plant fitness in the midst of co-pollinators that can cue in on reward content.

Like many studies that are the first to document novel phenomena, this work raises as many questions as it answers. In particular, it is unclear why wild tobacco don't always produce morning-opening flowers if they reduce herbivore loads and can effectively be pollinated by hummingbird co-pollinators. While this study provides no concrete answer, the authors speculate that hummingbird co-pollinators may be less reliable than hawkmoth pollinators for the fire-adapted wild tobacco that blooms in large, almost monoculture populations. Hawkmoths are often attracted to these blooms due to volatiles emitted by tobacco, whereas hummingbirds, which typically don't rely on volatile cues to locate host plants, may only be important co-pollinators in areas where wild tobacco is blooming in proximity to hummingbird nest sites or foraging territories. The validity of this speculation requires more experimental work in the natural system. Also of interest is how common these JA-induced changes in floral and flowering traits may be, their ecological consequences in other plant-herbivore–pollinator systems, and how the costs versus benefits tabulate for plant fitness and patterns of natural selection.

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DOI: 10.1016/j.cub.2009.12.005

Cell Cycle: The Art of Multi-Tasking

Separase is the protease that cleaves the cohesive link between sister chromatids to trigger chromosome segregation in mitosis and meiosis. This enzyme is known to orchestrate additional mitotic events and we now gain new insight into how it promotes cytokinesis in the nematode *Caenorhabditis elegans*.

Sandra López-Avilés and Frank Uhlmann

Since its identification [1], the role of separase has been intensively studied in different model organisms, from yeast to mammals. If it is clear that separase is essential for chromosome segregation during both mitotic and meiotic divisions through the cleavage of cohesin, other functions of separase are still under examination. In a report in this issue of *Current Biology*, Bembenek *et al.* [2] propose a function for separase in the regulation of vesicle trafficking associated with cytokinesis during *Caenorhabditis elegans* early embryonic cell divisions.

Cells depleted for separase (SEP-1 in *C. elegans*) using RNA interference (RNAi) show abortive cell division due

to regression of the cytokinetic furrow. The same is observed using the temperature sensitive *sep-1(e2406)* allele. This phenotype had been previously reported [3], but was attributed to defective eggshell formation and consequent cytokinesis defects in osmotically unbalanced medium. After revisiting these results, it appears that a vesicle trafficking defect in the absence of separase may cause both eggshell and cytokinesis defects.

As seen before, loss of *SEP-1* leads to chromosome non-disjunction and anaphase bridges. Recent studies in yeast and mammalian cells have shown that impaired sister chromatid resolution delays completion of cytokinesis in an Aurora B kinase-dependent pathway [4,5]. Therefore, could the presence of chromatin at the cleavage site be the reason for furrow regression? This is probably not the only explanation, as anaphase bridges resulting from depletion of topoisomerase II did not affect cytokinesis in the same way. More importantly, the *sep-1(e2406)* allele shows an interesting separation of function. Chromosome segregation is affected less severely than by RNAi-mediated separase depletion, but the cytokinesis defect is even greater.

Bembenek *et al.* [2] further characterize the cytokinetic defect and observe that separase depletion impairs trafficking of RAB11-positive vesicles that arrive from the cell interior. RAB11 marks vesicles destined for exocytosis, and without separase these vesicles accumulate at the cleavage furrow but fail to fuse with the target membrane. Previously, the same authors have shown that separase is necessary for exocytosis of cortical granules in response to fertilization during anaphase of meiosis I [6]. The secretion of vesicles containing proteoglycans and enzymes that form the eggshell at this time helps to prevent polyspermy. Therefore, a common role of separase in exocytosis seems to be important during both meiotic and mitotic divisions.

One could integrate these results into a neat model in which separase coordinates chromosome segregation and cytokinesis. However, the mechanism that separase uses to do so appears uncertain. The straightforward hypothesis suggested by Bembenek *et al.* [2] is that separase, in addition to cleaving cohesin, cleaves an unknown substrate that regulates vesicle trafficking. This suggestion has a precedence in budding yeast where separase, in addition to cohesin, cleaves the kinetochore component Slk19 [7]. The idea of additional proteolytic targets in human cells has gained support by the observation that separase's protease activity is required for disengagement of centriole pairs during mitotic exit to re-license centrosome duplication in the next cell cycle [8]. Slk19 cleavage occurs concomitant with cohesin cleavage at anaphase onset and centriole disengagement could reasonably be triggered at this time. Cytokinesis, however, should occur distinctly later, after chromosome segregation is complete. It is therefore unclear whether direct separase cleavage of an unknown target could provide the right timing.

There is an alternative possibility. In budding yeast, separase promotes activation of the mitotic exit phosphatase Cdc14. This occurs by an as yet incompletely understood mechanism, but notably is independent of separase's proteolytic activity [9,10]. The Cdc14 phosphatase in turn brings about many anaphase and mitotic exit events, from spindle elongation to subsequent spindle disassembly and cytokinesis. Cdc14 counteracts phosphorylation by mitotic cyclin dependent kinase (Cdk1) and contributes to Cdk1 downregulation. The Cdc14 substrate(s) that regulate cytokinesis remain to be identified but, clearly, budding yeast separase inactivation causes a delay to cytokinesis that is due to defective Cdc14 activation [11]. Does a similar mechanism operate in other organisms?

In fission yeast, mutations in the gene encoding separase, *cut1*$^{+}$, cause the expected chromosome segregation but no cytokinesis defect, thus leading to the characteristic 'cut' phenotype [12]. However, one must bear in mind that the allele used in this analysis may be defective in cohesin cleavage yet retain sufficient non-proteolytic activity to promote cytokinesis. An early analysis of a budding yeast separase mutation, *esp1-1*, also concluded that separase did not affect cell cycle progression and cytokinesis [13]. Only careful studies using improved loss of function alleles of separase using degron strategies revealed the full extent of separase's contribution to cell cycle progression [11].

Separase's contribution to mitotic progression has also been considered in higher eukaryotes. No cytokinesis defects have been reported in mouse embryonic fibroblasts after recombination-mediated deletion of parts of the separase gene [14,15]. Human cultured cells depleted for separase show complex cell cycle defects, but apparently normal cytokinesis [16]. In *Drosophila* embryos carrying separase mutations, only a small delay in cytokinesis has been documented [17]. It is difficult to make conclusions from these initial results. As the studies in budding yeast show, chromosome segregation can be severely affected by modest reduction in separase activity to levels that allow cytokinesis to progress normally, and it remains unknown how much residual separase persisted in the above experiments at the time of analysis.

While the above studies remain undecided, a contribution of separase to what could be called cytokinesis has been observed during vertebrate meiosis I [18,19]. Oocyte-specific separase gene deletion in mice, or separase antibody injection into frog oocytes, not only blocks meiosis I chromosome segregation but also extrusion of the first polar body. Whether this defect is caused by defective vesicle trafficking is not known. What is known is that the defect can be rescued by separase lacking its protease activity. Polar body extrusion in separase-inhibited oocytes can also be restored by simply reducing Cdk1 activity using a chemical Cdk inhibitor, arguing that polar body extrusion is regulated by separase via Cdk1 inhibition. Indeed, vertebrate separase directly binds to and inhibits Cdk1 [20].

What does this mean for separase regulation of vesicle trafficking in *C. elegans*? During meiosis I, like separase mutation, depletion of other cell cycle proteins, including Cdk1 regulators, impairs granule exocytosis [6]. Whether these regulators function in the activation of separase or work together with, or downstream of, separase to regulate exocytosis

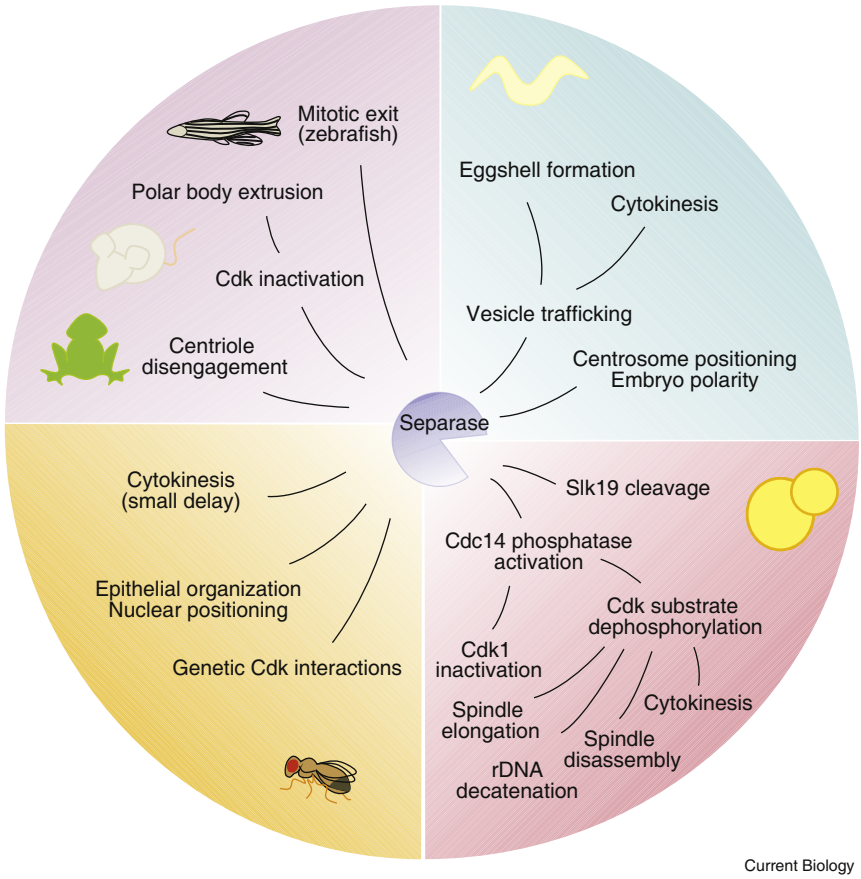

Figure 1. Multi-tasking during cell division: biological functions reported for separase.

In addition to the more widely appreciated role in cohesin cleavage to trigger chromosome segregation, separases in budding yeast (red), *Drosophila melanogaster* (yellow), vertebrates (purple) and *Caenorhabditis elegans* (blue) have been reported to play other roles, many related to processes occurring during cell division.

remains to be elucidated. The specific inhibition of cytokinesis, but less so of chromosome segregation, by the *sep-1(e2406)* allele is consistent with the idea that a proteolysis-independent function of separase is at play.

A common theme that emerges from comparing separase's diverse reported functions (Figure 1) is that of regulating cell cycle progression. Cdk activity must be downregulated, and many phospho-proteins dephosphorylated, for cells to divide. Direct downregulation of Cdk1 activity by separase (as in vertebrate oocytes) or activation of a Cdk-counteracting phosphatase (as in budding yeast) can contribute. It would not be surprising if separase, in addition to cleaving cohesin and other substrates, contributed to both downregulation of Cdk1 activity and activation of mitotic exit phosphatases. This would promote robust mitotic exit and could help to ensure that cytokinesis regulators are dephosphorylated at the appropriate time. Further work addressing these issues in more than one model organism will be instrumental for our understanding of the meiotic and mitotic cell division cycles.

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DOI: 10.1016/j.cub.2010.01.001