

Special Report

Evidence That the Yeast Spindle Assembly Checkpoint Has a Target Other Than the Anaphase Promoting Complex

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KEY WORDS

anaphase promoting complex, cyclosome, APC, anaphase, spindle checkpoint, Pds1, securin, Cdc20, Cdh1, nocodazole, Clb5, Sic1, Mad2, Bub1

ABBREVIATIONS

APC anaphase promoting complex
MCC mitotic checkpoint complex

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ABSTRACT

The spindle assembly checkpoint monitors biorientation of chromosomes on the metaphase spindle and inhibits the Anaphase Promoting Complex (APC) specificity factor Cdc20. If APC-Cdc20 is the sole target of the spindle checkpoint, then cells lacking APC and its targets, B-type cyclin and securin, would lack spindle checkpoint function. We tested this hypothesis in yeast cells that are APC-null. Surprisingly, we find that such yeast cells are able to activate the spindle assembly checkpoint, delaying cell cycle progression in G₂/M phase. These data suggest that the spindle checkpoint has a non-APC target that can restrain anaphase onset.

MATERIALS AND METHODS

Yeast strains used in the cohesion assays were derived from BF264-15DU MATa ade1 his2 leu2-3, 112 trp1-1^{ura3} Dns¹¹ using LacO sequences integrated as previously described.¹ The APC-null cells and relevant controls were previously described.^{2,3} Cells were grown in YEP media containing 2% dextrose. Gene disruption, strain construction and DAPI staining were done using standard procedures.⁴⁻⁶ Nocodazole was used at 0.15 micrograms per ml.

RESULTS AND DISCUSSION

During mitosis in eukaryotes a pair of microtubule asters interact with each other to form a structure, the amphiaster, first described in the late 1870s. Upon mammalian nuclear envelope break down, microtubules of the amphiaster become enabled to interact with kinetochores of the newly condensed chromosomes. Once all of the chromosomes reach the equatorial (metaphase) plate, anaphase, or chromatid separation and chromosome segregation, are induced to occur after a short lag-time of about 20 minutes. These events must be tightly regulated if genomes are to be transmitted precisely.

It was first hypothesized by Murray and Kirschner that progression through mitosis is dependent on proper assembly of the mitotic spindle apparatus (or amphiaster).⁷ This idea presumably stemmed from earlier genetic experiments indicating that biochemical feedback pathways (or checkpoints) are able to delay cell cycle progression until certain criteria have been met.^{8,9} Studies using mammalian cells treated with chemicals that disrupt mitotic spindle formation did indeed reveal mitotic delays, these delays being more robust in human cells than in rodent cells.¹⁰ Soon after, experiments with budding yeast provided genetic evidence that a checkpoint system delays mitotic progression when spindle assembly is perturbed, and this checkpoint became known as the spindle assembly checkpoint.^{11,12}

However, it was not before elegantly performed phenomenological studies using video microscopy that our current definition of this checkpoint came in to being.¹³ That is, the spindle checkpoint monitors biorientation of chromosomes on the mitotic spindle and prevents the initiation of anaphase until the last chromosome congresses to the equatorial plate.¹³

Much work has since provided a model for the mechanistic basis of spindle checkpoint control (Fig. 1A). Until biorientation of the last chromosome, an anaphase inhibitor named Pds1/securin is present in cells; it keeps dormant a key mitotic protein known as Esp1/separase that has the unique ability to cleave a chromosomal cohesion factor, Rad21/Mcd1/Scc1, that seems to glue sisters together. Thus, to initiate anaphase, Pds1 would have to be degraded. This occurs upon Pds1 ubiquitination which is performed by the anaphase promoting complex (APC) along with its specificity factor, Cdc20. The spindle assembly checkpoint is thought to prevent anaphase initiation through the action of a mitotic checkpoint complex (MCC), containing proteins such as Mad2, that inhibits APC^{Cdc20}.

If this model holds true, and if APC^{Cdc20} is the sole target of the spindle checkpoint, then cells lacking APC and its substrate, securin, would be expected to lack spindle checkpoint function. Yeast cells that lack the APC active site containing Apc2 and Apc11, and the specificity factors Cdc20 and Cdh1, have been made viable by removing the essential targets of APC-Pds1 and Clb5.^{2,3} Anaphase is thought to proceed with some fidelity (at least enough to maintain viability) in these “APC-null” cells owing to securin-independent regulation of separase. It is assumed, however, that spindle assembly checkpoint activation would be inconsequential to these yeast cells, because the target of the MCC (the APC) is absent.

Therefore, we asked if APC-null yeast cells arrest in metaphase in the presence of nocodazole which activates the spindle checkpoint (Fig. 1B,C). Wild type and APC-null budding yeast cells were grown to mid log-phase, then nocodazole was added to the liquid cultures. After 2–3 hrs with nocodazole the mutant cells, similar to wild type, accumulated with large buds indicating mitotic arrest. The presence of large budded cells indicated that cell growth had been uncoupled from cell cycle progression, an effect that checkpoint activation would promote. However, large budded cells might be arrested or delayed either in metaphase (with a single replicated nucleus) or in telophase (with divided nuclei) of the cell cycle.

We therefore stained the cells from the above experiment with DAPI to reveal nuclear DNA. The large budded APC-null cells mostly possessed undivided nuclei positioned at the bud neck, indicating an accumulation of cells in the G₂/M phase of the cell cycle. (G₂/M phase in budding yeast is cytologically equivalent to metaphase in other eukaryotes.) Telophase cells are normally abundant in the APC-null yeast strain due to inefficient mitotic exit that relies on inhibition of mitotic cyclin-dependent kinases by the Sic1 protein rather than being executed by APC-dependent proteolysis of the B-type cyclins. However, in the APC-null cells, telophase cells were depleted from the log-phase population after addition of nocodazole, with a timing that paralleled the accumulation of cells in the G₂/M phase. Therefore, the cell cycle response evoked by addition of nocodazole did not impede progression from telophase into G₁. This suggests that the APC-null cells did not activate the Bub2-checkpoint pathway in response to nocodazole treatment, since activated Bub2 would have further perturbed the telophase-to-G₁ transition.

By four hrs after addition of nocodazole, some re-budded cells emerged in the population of APC-null cells and the proportion of cells with divided nuclei increased. This also occurred in wild type cells, but after longer times of incubation with nocodazole (data not shown). Thus, as is the case in wild type cells, G₂/M arrest in the presence of nocodazole in APC-null yeast was transient. This is a typical feature of checkpoint controls, that cell cycle arrest is not absolute. However, it is interesting that the APC-null cells were able to maintain G₂/M arrest for a shorter time period than the APC-plus cells.

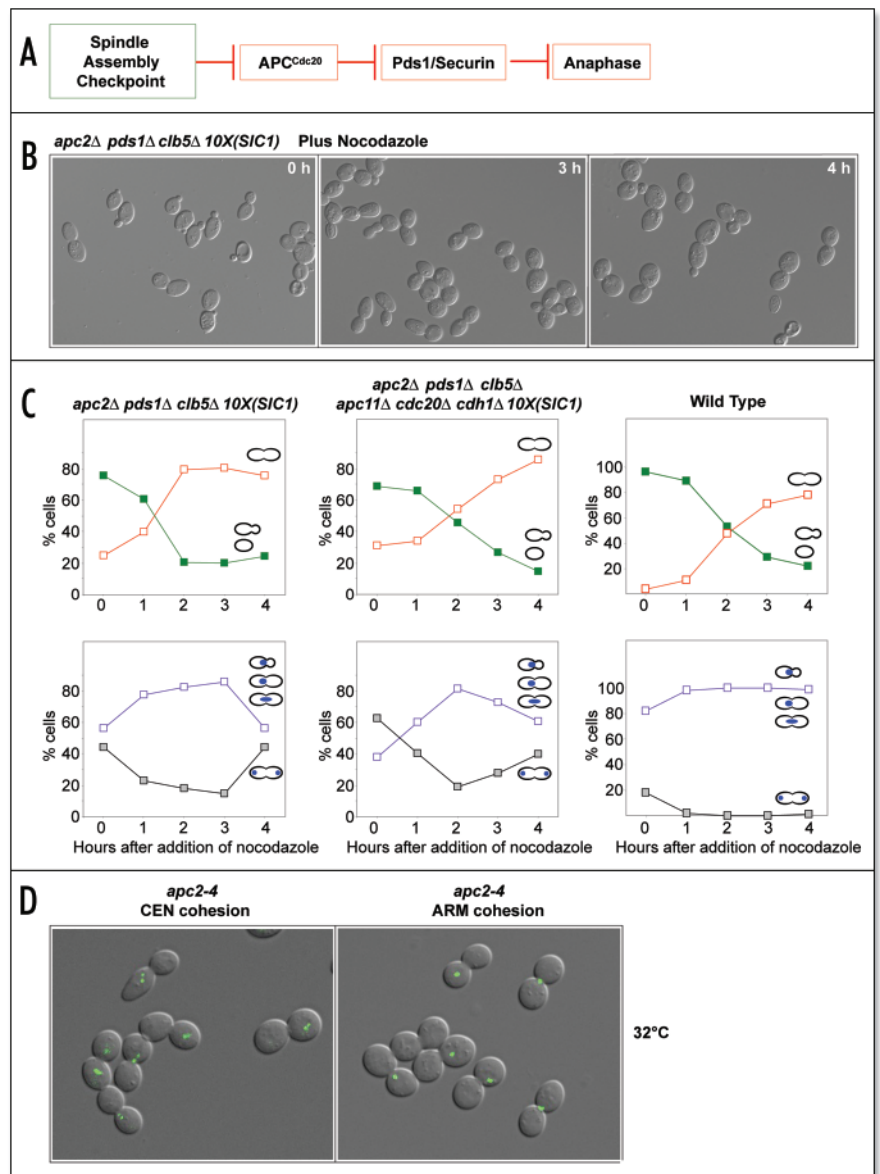


Figure 1. G₂/M arrest with nocodazole in yeast *apc* mutants. (A) Model of anaphase control. Spindle checkpoint inhibits Cdc20, promoting securin activity that in turn maintains centromere cohesion by inhibiting separase. (B,C) Yeast cells lacking APC, Clb5 and Pds1 arrest with large buds (B,C) and single nuclei (C) with nocodazole. Morphologies of the DAPI stained cells that were scored are indicated on the graphs. (D) *apc2* mutants arrest with separated centromeres but cohered arms. Cells contained LacO sequences integrated at CEN4 (CEN) or *TRP1* (ARM) and expressed a LacR-GFP fusion. Cells were grown at the restrictive temperature for 2 hrs before analysis.

To determine unequivocally if this cellular response to nocodazole treatment was indeed checkpoint-induced, we attempted to show that deletion of a spindle checkpoint gene causes bypass of the G₂/M delay. However, deletion of *MAD2* did not bypass the delay and we were unable to derive *bub1*-null APC-null mutants (data not shown). The latter result does, however, leave open the possibility that the G₂/M delay might require Bub1, and moreover that Bub1 is likely essential for accurate chromosome segregation in APC-null cells.

In conclusion, these data demonstrate a transient G₂/M cell cycle delay induced by nocodazole; a cellular response, presumably a function of the spindle checkpoint, that could not have involved inhibition of APC^{Cdc20} or stabilization of Pds1 since these factors were absent from the APC-null strain. Therefore, the data indicate

that a second target of the spindle assembly checkpoint contributes to the metaphase delay seen in the absence of a mitotic spindle. Conversely, evidence from mammalian cells has indicated that anaphase is able to proceed, albeit aberrantly, in the absence of APC activity.¹⁴ In human and mouse cells that lacked *Apc2*, sister centromeres were able to separate, after a short metaphase delay. Chromosome arm separation was perturbed more strongly during anaphase, but nevertheless full sister chromatid separation was ultimately achieved.

To ask if this might be the case also in yeast cells, we took advantage of the LacO/LacR-GFP system that allows visualization of specific chromosomal loci within living yeast cells. We used strains in which LacO repeats had been integrated either at the centromere of chromosome IV (CEN4) or 12 kb away from this centromere at the *TRP1* locus. We then introduced a temperature sensitive allele of *APC2* (*apc2-4*) that results in G₂/M arrest when the cells are grown at 32°C, and we asked if cohesion at these two loci can be maintained in the absence of APC activity. After 2 hrs at the non-permissive temperature, most *apc2* mutant cells had arrested in the G₂/M phase and the CEN4 loci were separated in the majority of the cells (Fig. 1D). This was the expected result because when budding yeast chromosomes become bioriented on the mitotic spindle, centromere regions are seen to become precociously separated (i.e. cohesion is not present at the centromeres in metaphase). In contrast, regions more than 2 kb away from the centromere (such as *TRP1*) retain high levels of cohesion until the onset of anaphase. In agreement, most cells containing LacO sequences at *TRP1* had a single fluorescence signal 2 hours after the temperature shift in the *apc2-4* mutant (Fig. 1D). However, a fraction of these cells did possess two *TRP1* signals and upon further incubation at the non-permissive temperature, the frequency of such cells increased (unpublished data). Thus it is possible that even in yeast cells, where APC is thought to be essential for anaphase, a limited amount of cohesin might be removed by an APC-independent mechanism.

In summary we have provided genetic evidence for the existence of a spindle checkpoint mechanism that operates independently of inhibiting the APC in budding yeast. Proof that this cell cycle response to nocodazole does indeed correspond to checkpoint activation will require identification of factors required for the G₂/M cell cycle delay in the absence of the APC-pathway. It will also be important to determine if APC-independent mechanisms can induce cohesin removal from budding yeast chromosomes.

References

1. Straight AF, Belmont AS, Robinett CC, Murray AW. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr Biol* 1996; 6:1599-608.
2. Thornton BR, Toczyski DP. Securin and B-cyclin/CDK are the only essential targets of the APC. *Nat Cell Biol* 2003; 5:1090-4.
3. Thornton BR, Chen KC, Cross FR, Tyson JJ, Toczyski DP. Cycling without the cyclosome: modeling a yeast strain lacking the APC. *Cell Cycle* 2004; 3:629-33.
4. Sherman F, Fink G, Hicks JB, *Methods in Yeast Genetics*. 1982, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
5. Wach A, Brachat A, Pohlmann R, Philippsen P. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* 1994; 10:1793-808.
6. Burke D, *Methods in yeast genetics*. 2000: Cold Spring Harbor Laboratory Press.
7. Murray AW, Kirschner MW. Dominoes and clocks: The union of two veils of the cell cycle. *Science* 1989; 246:614-21.
8. Hartwell LH, Weinert TA. Checkpoints: Controls that ensure the order of cell cycle events. *Science* 1989; 246:629-34.
9. Weinert TA, Hartwell LH. The RAD9 gene controls the cell cycle response to DNA damage in *S. cerevisiae*. *Science* 1988; 241:317-22.
10. Kung AL, Sherwood SW, Schimke RT. Cell line-specific differences in the control of cell cycle progression in the absence of mitosis. *Proc Natl Acad Sci U S A* 1990; 87:9553-7.
11. Li R, Murray AW. Feedback control of mitosis in budding yeast. *Cell* 1991; 66:519-31.
12. Hoyt MA, Totis L, Roberts BT. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 1991; 66:507-17.

13. Rieder CL, Schultz A, Cole R, Sluder G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol* 1994; 127:1301-10.
14. Giménez-Abián JF, Díaz-Martínez LA, Wirth KG, Andrews CA, Giménez-Martín G, Clarke DJ. Regulated Separation of Sister Centromeres depends on the Spindle Assembly Checkpoint but not on the Anaphase Promoting Complex/Cyclosome. *Cell Cycle* 2005; 4:1561-75.
15. Richardson HE, Wittenberg C, Cross FR, Reed SI. An essential G₁ function for cyclin-like proteins in yeast. *Cell* 1989; 59:1127-33.