RESEARCH: CELL CYCLE

Mitotic Arrest: Mad2 Prevents Sleepy from Waking Up the APC

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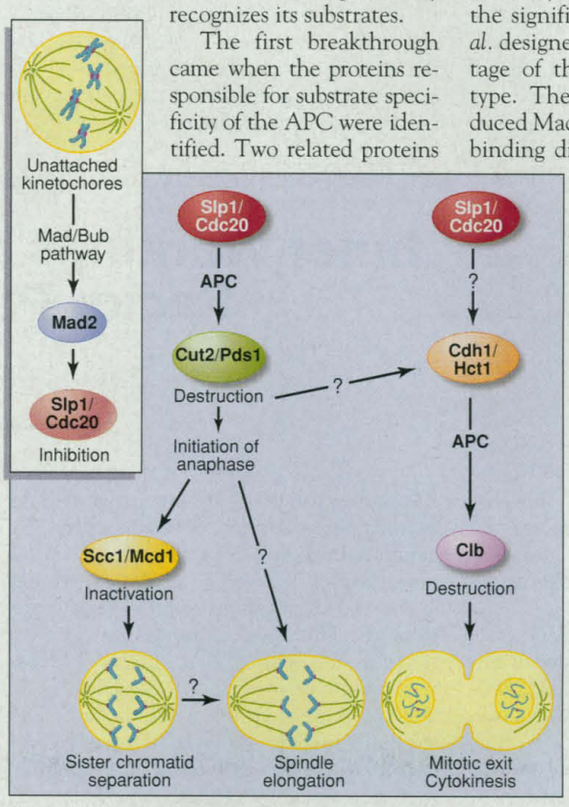
For plants and animals to grow, their cells must duplicate themselves. These cells must accurately copy their chromosomes and, through the process of mitosis, segregate them to daughter cells. Failure to deliver the new chromosomes successfully to offspring is disastrous, producing cells with too few or too many chromosomes (aneuploidy), resulting in cell death, birth defects, or cancer. One way that cells avoid this calamity is through a surveillance mechanism called the spindle assembly checkpoint (*1*). This pathway monitors the mitotic spindle, a bipolar array of microtubules that attach to a specialized region of the chromosome (the kinetochore), eventually pulling apart the replicated chromosomes (sister chromatids). The checkpoint blocks sister chromatid separation (anaphase) until the two sisters are attached to opposite poles of the spindle and thus ensures equal distribution of chromosomes into daughter cells. (See the related News story on page 477 of the 23 January issue of *Science*.)

This pathway gained a molecular footing in 1991 with the identification of the *MAD* (mitotis arrest deficient) and *BUB* (budding uninhibited by benzimidazole) genes in budding yeast (*2*, *3*). The Mad and Bub proteins sense chromosome position and spindle attachment and transduce this information to the basic cell cycle machinery. Mad2 and Bub1 are found on unattached kinetochores, providing a molecular link to cell biological experiments that suggest that the checkpoint detects kinetochores that have not yet interacted with microtubules (*4*–*6*).

Sister chromatids start to separate at the onset of anaphase when anaphase inhibitors (Pds1 in *Saccharomyces cerevisiae* and Cut2 in *Schizosaccharomyces pombe)* are destroyed by proteolysis. These proteins are marked for degradation with ubiquitin tags by the cyclosome or anaphase-promoting complex (APC), an E3 ubiquitin ligase. The APC also controls the degradation of several other mitotic proteins, but action on Pds1 is the only APC-dependent step in anaphase (*7*). Pds1 remains stable in cells arrested via the spindle assembly checkpoint, and cells without Pds1 cannot maintain chromatid cohesion during this arrest (*7*). Now a recent flurry of papers provide much-needed insight into how the Mad/Bub pathway controls the APC and how the APC specifically recognizes its substrates.

The first breakthrough came when the proteins responsible for substrate specificity of the APC were identified. Two related proteins containing WD repeats are each required for the degradation of different APC substrates (*8*, *9*). One of these, Cdc20, primarily controls Pds1 degradation (*8*), and another, Hct1/Cdh1, controls degradation of two other proteins—Clb2 and Ase1 (*8*, *9*). Neither Cdc20 nor Hct1/Cdh1 is bound tightly to the APC; their activities and association with the APC are likely to be tightly regulated. These specificity factors have not yet been shown to directly associate with the ubiquitination substrates either, although this is a distinct possibility.



A model for regulation of the anaphase-promoting complex. In response to a spindle assembly checkpoint signal, the Mad/Bub pathway inhibits Slp1/Cdc20 through Mad2 (**inset**). When the checkpoint signal is released, Slp1/Cdc20 allows APC-dependent ubiquitination and destruction of Pds1 (or Cut2). This initiates anaphase by inactivating sister chromatid cohesion proteins such as Scc1/Mcd1.

A second breakthrough came from the works of Kim *et al.* (*10*) and Hwang *et al.* (*11*), reported on pages 1041 and 1045 of this issue. Kim *et al.* were studying the function of the *S. pombe CDC20* homolog *slp1*$^+$ (sleepy) (*10*). With a yeast two-hybrid assay, they found an interaction between *slp1*$^+$ and the *S. pombe* homolog of *MAD2*, *mad2*$^+$, a conserved component of the spindle assembly checkpoint (*4*, *5*). In addition, Mad2 and Slp1 physically associate with each other in vitro and in vivo. *mad2*$^+$ can arrest *S. pombe* cells in mitosis when it is overproduced and is required for the spindle assembly checkpoint (*12*). To determine the significance of this interaction, Kim *et al.* designed a clever screen that took advantage of the Mad2 overproduction phenotype. They reasoned that if the overproduced Mad2 was inhibiting Slp1 function by binding directly to Slp1, then they should be able to detect Slp1 mutants that are resistant to Mad2 overproduction. They mutagenized Slp1 and selected Mad2-resistant mutants and one, *slpl-63*, no longer bound Mad2 in vivo or in vitro, suggesting the association between the proteins is likely direct. Furthermore, replacement of the wild-type *slp1*$^+$ allele with the *slp1-63* allele produced a strain defective in the spindle assembly checkpoint, thereby establishing the significance of the interaction to the signal transduction pathway.

Motivated in part by the Slp1-Mad2 interaction in *S. pombe*, Hwang *et al.* (*11*) discovered in budding yeast that not only Mad2 but also Mad1 and Mad3 associate with Cdc20. They also used a clever screen that gratuitously activates the checkpoint to identify dominant spindle checkpoint–defective alleles of *CDC20* (*11*), also recently identified by others (*13*). Together, these results lead to an elegant model whereby activation of the spindle assembly checkpoint results in the Mad2-dependent inhibition of Cdc20/Slp1's ability to promote entry into anaphase by degrading Cut2 (or Pds1) (see the figure). Whether this inhibition is achieved by blocking access of Cdc20/Slp1 to ubiquitination substrates or its ability to associate with the APC remains to be determined.

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Although pleasing, this model contains several unresolved but important details. For example, is the spindle checkpoint transiently activated in every cell cycle or only in response to spindle perturbation? Does Mad2 inhibit Cdc20/Slp1 by simple binding as inferred from the arrest caused by Mad2 overproduction? If preliminary experiments suggesting that Mad2 is bound to Cdc20 constitutively are correct (*11*), then how might Mad2 inhibit Cdc20? One possibility is that in response to the checkpoint signal additional Mad2 molecules bind and inhibit Cdc20 or that Mad2 facilitates an inhibitory modification of the Cdc20 pool.

Two critical issues remain: How are Cdc20/Slp1 activation and anaphase timing normally regulated in the absence of checkpoint activation? How do Cdc20/Slp1 and Cdh1/Hct1 regulate the substrate specificity of the APC? A clue to how these proteins function comes from an unanticipated source, the SCF (Skp/Cdc53-cullin/F-box protein) complex, an E3 ubiquitin ligase unrelated to the APC that controls the G_1–to–S phase transition in *S. cerevisiae*. The SCF complex promotes the degradation of proteins such as the cyclin-dependent kinase (Cdk) inhibitor Sic1. It has been hypothesized that the substrate specificity of the SCF complex is conferred by different F-box proteins (*14*). For Sic1, this protein is Cdc4, which has both an F-box motif and WD repeats. Sic1 association with and ubiquitination by the SCF requires Sic1 phosphorylation (*15*, *16*) and the WD repeats of Cdc4 (*15*), suggesting that WD repeats recognize phosphoproteins. Because WD proteins serve as specificity factors for both the APC and SCF, they may allow the APC to be indirectly regulated by phosphorylation, as they do in the SCF.

How does the cell control APC substrate selection to ensure the proper order of mitotic events? Although all known APC substrates contain a motif called a destruction box that is required for degradation, they disappear at different times during mitosis (*17*). The substrate timing problem is now the central mystery of mitosis, for it holds the key to the order of mitotic events. As with most cell cycle events, phosphorylation is a prime candidate for regulation of APC function, perhaps directly controlling substrate selection as in the SCF pathway or controlling Cdc20 and Cdh1/Hct1 association with APC.

In addition, Cdk activation correlates with APC inactivation in late G_1 (*18*), and Cdk inactivation in G_2 is sufficient to activate the APC (*19*). How might Cdk activity negatively regulate the APC? Cdks could phosphorylate protein inhibitors of the APC, perhaps through association with the WD repeat proteins Cdc20 and Cdh1/Hct1, or by direct phosphorylation and inhibition of the specificity factors themselves. Thus, Cdks may simultaneously set up the mitotic apparatus as well as the inhibitory barriers that must be overcome for APC activation and mitotic progression. APC activation could be achieved either by inhibitor destruction or reversal of inhibitory phosphorylation. This mode of APC regulation could temporally control substrate selection. If APC substrates include inhibitors of specificity factors required for ubiquitination of subsequent APC substrates, an ordering mechanism could operate in a domino fashion to sequentially activate the destruction of APC substrates. Checkpoints could prevent inhibitor destruction and arrest the cycle. Alternatively, the order of substrate selection could be set by a clock mechanism initiated by Pds1 or Cut2 destruction. Regardless of the outcome, the frenzied pace of activity in this field should provide enough excitement to keep all but the most severe narcoleptics wide awake.

References and Notes

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TRANSCRIPTION

Inner Workings of a Transcription Factor Partnership

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How do proteins that turn genes on and off recognize their sites of action within the genome? Lock-and-key type molecular complementarity between a regulatory protein and its DNA binding site provides the primary recognition. A constellation of electrostatic and hydrophobic interactions between matching surfaces of the DNA helix and the protein establish high-affinity and sequence-specific binding. The effectiveness of this macromolecular matchmaking, first elucidated by elegant experiments in prokaryotes, is challenged by the complexities of eukaryotes. There are hundreds of regulatory transcription factors that function by binding DNA sequences within their target genes. Almost all of these proteins are encoded by multigene families. Members of a family display the same structural fold for binding DNA and recognize similar DNA sequences. How can specificity be obtained within such a complex world?

Combinatorial arrays of multiple proteins add specificity and stability to DNA-protein interactions. Homodimers and heterodimers can be formed between members of the same gene family. Alternatively, partnerships can form between two proteins that belong to unrelated groupings. Wolberger and colleagues have studied one such partnership (*1*), and their report on page 1037 provides a snapshot of the molecular basis of combinatorial control of transcription. The report describes the crystal structure of the ternary complex of GABPα, a member of the *ets* gene family, with its heterotypic partner GABPβ on a DNA duplex.

The *ets* gene family dramatically illustrates the specificity problem (*2*). *ets* genes are present in all metazoan phyla, with more than 20 homologs in the human genome. The ETS domain, a highly conserved 85–amino acid region, defines the family and directs DNA binding. The DNA binding sites of all *ets* proteins include the core recognition sequence 5′-GGA-3′. Additional DNA contacts that also require conserved sequences extend the binding site to include at least nine base pairs. With such a high degree of conservation, how is specificity programmed into the family? For example,

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