PERSPECTIVES: CANCER

A CINtillating New Job for the APC Tumor Suppressor

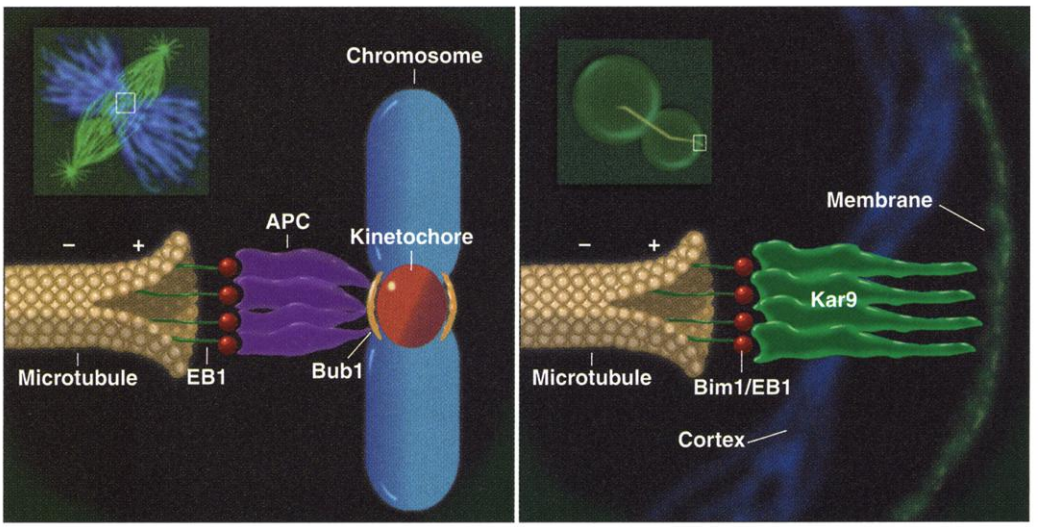
David Pellman

The search for new cancer treatments hinges on finding differences between cancer cells and the normal cells from which they originate. One striking difference, readily visualized under the microscope, is the abnormal number of chromosomes found in many cancer cells. This phenomenon, called aneuploidy, is thought to arise through defects in cell division (mitosis) that generate chromosomal instability (CIN). The molecular basis for CIN in most cancers is unknown but is the subject of intense investigation because even general inhibitors of mitosis, such as taxol, are important anticancer agents. A pair of recent *Nature Cell Biology* papers from Fodde *et al.* (*1*) and Kaplan *et al.* (*2*) report that chromosomal instability in colon cancer cells may be due, at least in part, to mutations in the adenomatous polyposis coli (APC) tumor suppressor protein (*1, 2*).

The development of colon cancer is accompanied by the progressive accumulation of mutations in different genes (*3*). About 15% of colon cancers have defects in one or more of the proteins that repair damaged DNA; the remaining 85% display chromosomal instability. Cell lines derived from colon cancers with CIN have an accelerated rate of chromosome missegregation during cell division (*4*). A number of CIN cancers appear to have defects in a cell cycle checkpoint that monitors the fidelity of mitosis. Although a few of these tumors have mutations in the genes encoding the mitotic checkpoint kinases Bub1 and BubR1, the molecular defects in the remainder are not known (*5*).

Now, the lead actor in colon cancer, APC, once again takes center stage, this time as a potential culprit in the generation of CIN. It is well established that APC regulates the transcription of target genes by promoting the destruction of β-catenin (*6*), a key component of a multiprotein transcription factor complex. Mutations in β-catenin that stabilize this protein and prevent its destruction are known to cause colon cancer. Despite APC's clearcut importance in β-catenin regulation, another more surprising idea has been smoldering in the literature for years—that APC also regulates the assembly of microtubules, the fibers that make up the mitotic spindle (*7*). APC appears to interact with microtubules in at least three ways (*8–10*). At its carboxyl terminus, APC has one domain that directly binds to microtubules and an adjacent domain that binds to EB1, a protein that interacts with the plus (distal) ends of microtubules (see the figure). Yet there is a mutant form of APC lacking both of these domains that is still somehow transported out to the plus ends of microtubules by an undefined energy-dependent transport system. Thus, it seems that the cell is ardently committed to getting APC out to the ends of microtubules. But the question is why.

The new results (*1, 2*) suggest that APC is involved in linking the ends of microtubules to their attachment sites on chromosomes (called kinetochores). Both groups located APC at the ends of spindle microtubules, at the point where they insert into the kinetochore. If the microtubules became depolymerized, then this localization was lost, suggesting that APC is physically attached to the ends of microtubules. Intriguingly, Kaplan *et al.* found that APC directly binds to a kinetochore protein—none other than the checkpoint kinase Bub1, previously implicated in CIN—and that APC is an avid in vitro substrate of Bub1. Thus, APC could promote the growth of spindle microtubules toward the kinetochore, or attach the kinetochore to the spindle via Bub1, or send a signal that attachment was successful.



A stabilizing influence. Protein complexes at the plus ends of microtubules are adaptors that attach spindle microtubules to the kinetochores of chromosomes in animal cells or microtubules to the interior of yeast cells. **(Right)** In yeast, the Bim1p/Kar9p protein complex connects microtubules to actin-rich structures in the daughter cell. These interactions are important for establishing the position of the mitotic spindle within the dividing cell. **(Left)** In animal cells, the EB1-APC protein complex may connect the ends of spindle microtubules to the kinetochores of chromosomes. Both protein complexes may also regulate the dynamics of microtubule dynamics and stability.

Compellingly, mutations in the *Apc* gene produce defects in chromosome segregation. Both groups found that embryonic stem (ES) cells homozygous for the Apc^{min} allele become aneuploid with progressive passage in culture. The Apc^{min} allele encodes an aberrant APC protein that is missing a large section of its carboxyl terminus and thus cannot bind to microtubules, EB1, or several components of the β-catenin destruction machinery. The Apc^{min} cells also exhibit spindle defects, such as chromosomes that are left behind during the final stages of cell division. Fodde *et al.* discovered that in Apc^{min} cells the ends of microtubules (visualized by EB1 staining) fail to properly connect to the kinetochore. In addition, Kaplan *et al.* found

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that the mitotic checkpoint is intact in Apc^{min}/Apc^{min} ES cells, supporting the view that APC's function at the kinetochore is structural. Thus, at least in ES cells, APC is required for chromosome segregation.

One important complicating issue is that Apc^{min} cells cannot degrade β-catenin. This raises the possibility that the mitotic defect could be a secondary consequence of altered transcription. Fodde *et al.* address this point by characterizing a targeted *Apc* mutation (Apc^{1638T}) that encodes an APC protein with a less extensive carboxyl-terminal truncation than Apc^{min} and that still contains critical sites for binding components of the β-catenin destruction complex (*11*). It is already known that Apc^{1638T} cells degrade β-catenin as efficiently as their wild-type counterparts. Consistent with an absolute requirement for β-catenin stabilization in the development of colon cancer, Apc^{1638T} homozygous mice are viable and do not get colon cancer (*11*). Now, however, Fodde *et al.* show that Apc^{1638T} ES cells, like Apc^{min} cells, have defects in chromosome segregation and mitosis. Results with the Apc^{1638T} mutant cells provide a strong argument that the functions of APC in β-catenin degradation and mitosis are independent.

There are interesting parallels between APC's proposed involvement in the attachment of microtubules to kinetochores and recent work on the attachment of microtubules to the region below the plasma membrane of yeast cells known as the cortex (see the figure). In yeast, the attachment of microtubules to the cortex is necessary for the correct alignment of the mitotic spindle with the axis of cell division. A key mediator in this process is a protein complex composed of the yeast EB1 ortholog, Bim1p, and a cortical protein, Kar9p (see the figure). Once attached to the ends of microtubules, the Bim1p/EB1-Kar9p complex can link microtubule ends to actin-rich structures at the yeast cell periphery and to actin cables in the daughter cell (*12–15*).

Fodde *et al.* provide evidence that EB1 is also required for the APC-mediated attachment of microtubules to kinetochores. They created cell lines that expressed the EB1-binding domain of APC and, strikingly, these cells had a CIN phenotype. These results suggest unifying themes for how spindle microtubules form attachments. Protein complexes that bind to the ends of microtubules, such as Bim1p/EB1-Kar9 or EB1-APC, may mark the plus ends, distinguishing them from the rest of the microtubule. These complexes may regulate microtubule growth and also may serve as adaptors that link microtubule ends to target sites on kinetochores or at the plasma membrane.

How successfully do the ES cell experiments model the CIN phenotype of colon cancers? One issue is that development of colon cancer involves multiple genetic changes. Although the precise timing of chromosomal instability during the transformation of normal cells into tumor cells is not known, it may occur earlier than previously suspected (if there is a lag before changes in chromosome number become apparent). Fodde *et al.* report that blocking apoptosis in either Apc^{min} or Apc^{1638T} ES cells results in a greater number of aberrant chromosomes than observed in control cells that are able to undergo apoptosis. This is notable because it suggests that the chromosome segregation defect in *Apc* mutant cells could lie silent until an additional genetic "hit" suppresses the mitotic checkpoint or the apoptosis of defective cells. This neatly integrates the new work with the "multiple genetic hit" hypothesis of colon cancer development.

A second key issue is that CIN is a complex phenomenon—it not only involves missegregation of whole chromosomes, but also includes complex rearrangements between chromosomes. When apoptosis is suppressed in either Apc^{min} or Apc^{1638T} ES cells, they develop complex chromosomal rearrangements. It is well-established that defects in the enzyme telomerase (which maintains the ends of chromosomes) can generate chromosomal rearrangements in colon and other epithelial cancers (*16*). However, aberrant mitosis can also lead to broken chromosomes, potentially providing an alternate route for the development of CIN. Indeed, Kaplan *et al.* detected torn chromosomes in metaphase Apc^{min} ES cells. Thus, the *Apc* mutant ES cells recapitulate many characteristics of the CIN phenotype. Ultimately, the key test will come from animal models in which it should be possible to determine whether Apc^{1638T} and other more specific alleles promote CIN and accelerate tumor formation in vivo.

These studies open up new avenues for understanding chromosomal instability and cancer. The desired destination is a realization of how kinetochore attachment, chromosome movement, and cell cycle checkpoints are integrated to prevent CIN. The newly discovered protein complexes at the ends of microtubules may point us in the right direction.

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PERSPECTIVES: QUANTUM PHYSICS

Standing Room Only at the Quantum Scale

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A central tenet of quantum mechanics is that all matter exhibits both wave- and particle-like features. All particles are endowed with a de Broglie wavelength, which is inversely proportional to the particle's momentum and determines its effective "size." When a gas containing many identical particles is confined and cooled, the average momentum can be lowered so far that the typical de Broglie wavelength is larger than the average separation between the particles. In this case, the gas is said to be "degenerate," meaning that the wave functions of neighboring particles overlap. Degenerate gases exhibit two dramatically different types of behavior, depending on whether the identical particles are bosons (such as photons) or fermions (such as electrons). Even in the absence of interactions between the particles, bosons tend to clump together, whereas fermions must avoid one another. The latter thus occupy much more volume in the container.

On page 2570 of this issue, Truscott *et al.* report a direct observation of this remarkable feature of quantum mechanics (*1*). They simultaneously confine a mixture of lithium-6 atoms (which are fermions) and lithium-7 atoms (which are

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