APC at a glance

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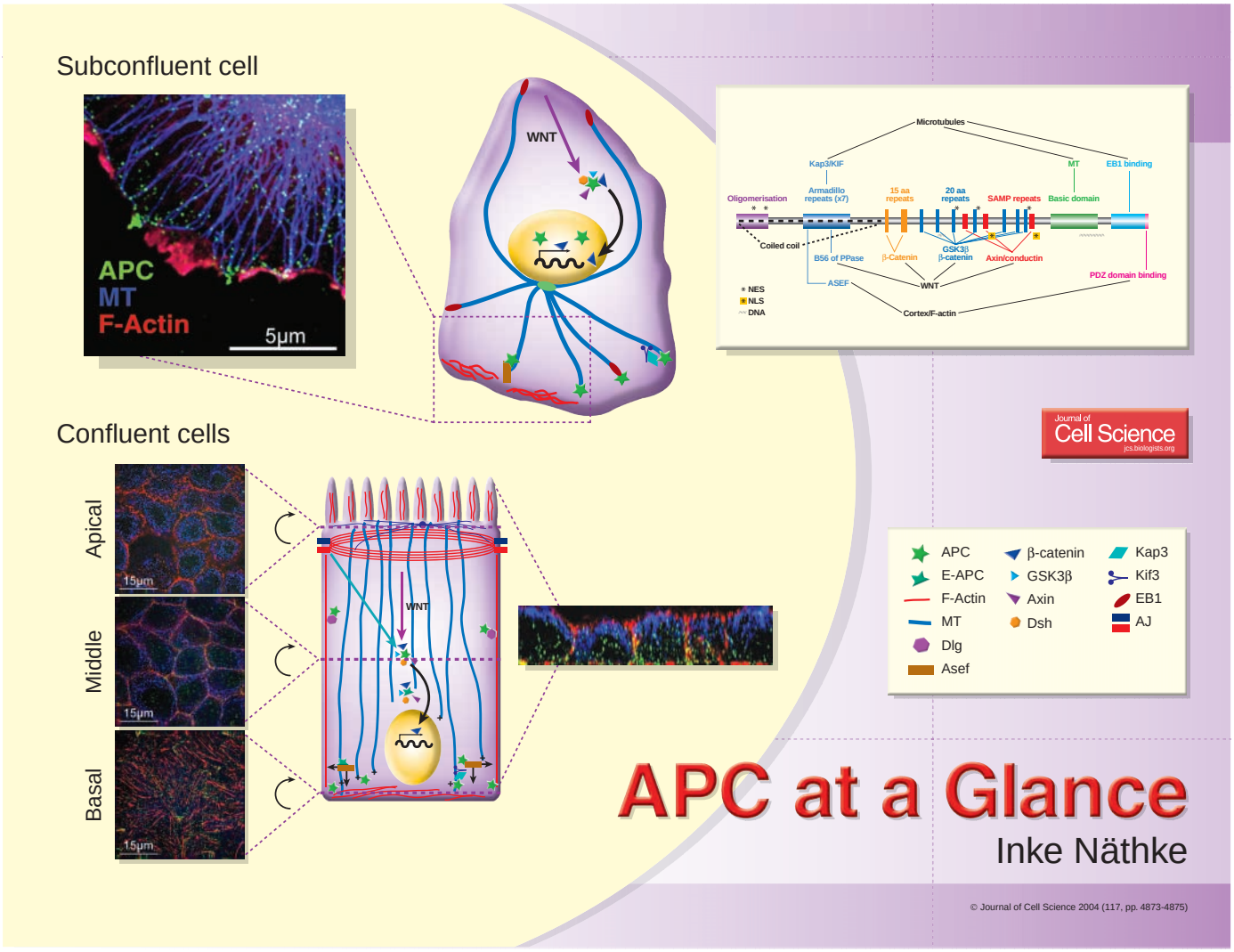
The gene encoding the adenomatous polyposis coli protein (APC) is mutated in most colon cancers. The major role of APC is thought to be as a scaffold for a protein complex that regulates the phosphorylation and thus degradation of β-catenin in the WNT signalling pathway (Huelsken and Behrens, 2002). However, there is increasing evidence that dysregulation of β-catenin is not the only effect of mutations in APC relevant to the development of cancer and that interactions between APC and cytoskeletal proteins are also important.

The APC protein

Cancer-associated mutations in APC usually lead to the expression of N-terminal fragments. This region of the APC protein contains heptad repeats that are predicted to form coiled-coil domains and might be involved in oligomerisation (Joslyn et al., 1993). The N-terminal region of APC also contains two nuclear export signals (NES) that are required for shuttling of APC between the nucleus and cytoplasm and may directly interact with exportin Crm-1 (Henderson and Fagotto, 2002).

Embedded in the heptad repeats of APC are armadillo repeats. Proteins that bind to this region of APC include the regulatory subunit of phosphatase 2a (PP2A), APC-stimulated guanine nucleotide exchange factor (ASEF) for Rho family proteins, and KAP3, which is a linker protein for kinesin motor proteins (Bienz, 2002; Dikovskaya et al., 2001). Whether all these interactions can occur simultaneously and how they are regulated is not known.

The middle of the APC molecule contains domains important for interactions with proteins in the WNT signalling pathway (Rubinfeld et al., 1996). This region includes three 15-residue repeats that constitutively bind to β-catenin and seven 20-residue repeats that also bind to β-catenin but are regulated by phosphorylation (Rubinfeld et al., 1996). In addition, it contains three stretches that are involved in binding to



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axin (Bienz, 2002). The N-terminus and middle region of APC are the most highly conserved regions in the sequences of APC proteins from different species (http://www.rzpd.de/cgi-bin/cards/carddisp?APC).

The C-terminal region of APC contains motifs that mediate interactions with a number of structural proteins: a basic stretch enriched in positively charged residues is similar to the microtubule-binding site of the tau protein and represents the major microtubule-binding site of APC; the terminal 170 residues can bind to EB1, a small microtubule-end-binding protein; the C-terminal 15 residues constitute a binding site for PDZ domains (Dikovskaya et al., 2001).

The C-terminal third of APC proteins is the least well conserved between species and the second, smaller APC proteins – E-APC and APC-2/L – do not contain a basic domain that is readily identifiable as a microtubule-binding site, an EB1-binding site or the C-terminal motif that mediates binding to PDZ domains (Dikovskaya et al., 2001). The role of these alternative APC proteins in human cancer remains elusive.

Functions of APC

The best-characterised function of APC is as a scaffolding protein in a multi-protein complex whose activity is modulated by WNT signalling (Fodde, 2002). This complex regulates the phosphorylation of β-catenin and thus controls the amount of β-catenin available for transcriptional activation via TCF/LEF transcriptions factors. Other proteins that are part of this complex include GSK3β, β-catenin, axin along with several kinases and phosphatases. A number of reviews describe the details of this pathway (Bienz, 2002; Fodde, 2002; Huelsken and Behrens, 2002), making an in-depth discussion here not necessary. Briefly, in the absence of extracellular WNT signals, GSK3β in the APC–β-catenin–axin complex is active, phosphorylating all three of these proteins, which increases their interaction. This phosphorylation of β-catenin creates a recognition site for ubiquitin ligases and leads to its destruction by the proteasome. In the presence of a WNT signal, the protein Dishevelled (Dsh) inactivates GSK3β. This results in a decrease in the amount of β-catenin targeted for degradation, thus increasing the amount available to activate TCF/LEF transcription factors.

Truncation mutations of APC that render it unable to bind and recruit β-catenin lead to an accumulation of β-catenin, which correlates with changes in transcriptional activation by TCF/LEF transcriptions factors and the expression of a variety of genes that can change the proliferation and differentiation state of cells, including those encoding MYC, cyclin D, ephrins and caspases (Chen et al., 2003; Fodde, 2002; van de Wetering et al., 2002). However, there are additional pathways that contribute to the regulation of β-catenin and are independent of APC and other proteins involved in the WNT pathway. These include the p53-inducible Siah-1 protein (Liu et al., 2001) and a retinoid X receptor (Xiao et al., 2003). The complicated relationships between these pathways make it difficult to establish exactly how the deregulation of β-catenin that result from mutations in APC contributes to the initiation of colon cancer. This in turn supports the idea that other functions of APC are involved in its role in cancer.

The importance of interactions between APC and the cytoskeleton are reflected by the distribution of the endogenous APC protein: in sub-confluent, migrating epithelial cells, APC predominantly localises to the peripheral ends of a subset of microtubules and accumulates in distinct clusters near the plasma membrane that coincide with areas of active cell migration, although the F-actin content at these sites is low (Näthke et al., 1996). In polarised cells, the highest concentration of APC is detected at the basal surface where the plus ends of microtubules terminate (Mogensen et al., 2002).

Although the ability of APC to interact with KAP3 is at least partially responsible for its distribution, fragments of APC that can bind to KAP3 but lack the direct microtubule-binding site do not localise to microtubule plus ends efficiently. This suggests that a combination of direct and indirect interactions of APC with microtubules determine its peculiar distribution (Barth et al., 2002; Zumbrunn et al., 2001). To complicate matters further, the same region of APC that binds to KAP3 also stimulates ASEF (Kawasaki et al., 2000), which suggests that APC can also stimulate actin polymerisation locally. This may explain why APC can be detected in association with actin-rich structures (Rosin-Arbesfeld et al., 2001). The ability to bind to other actin-associated proteins, including PDZ-domain-containing proteins such as Dlg, may further contribute to the interaction.

The intricate interplay of different protein interactions involving APC suggests that the distribution of APC between specific intracellular networks is determined by a variety of signals that depend on the cellular environment. For instance, in polarised epithelial cells, APC appears to be weakly associated with F-actin structures but is mostly concentrated at the basal region of the plasma membrane, where the plus ends of the microtubules that form large parallel arrays in fully polarised epithelial cells terminate (Mogensen, 2002). In fact, APC may be involved in establishing such large microtubule arrays since in $APC^{+/-}$ mice the number of microtubules in such arrays is significantly reduced (Mogensen, 2002).

A lack of APC leads to defects in the structure of mitotic spindles that can only be rescued by APC containing the direct microtubule-binding site; this is consistent with a role for APC in establishing microtubule arrays (Dikovskaya et al., 2004). Furthermore, in early mitosis, APC localises to the ends of microtubules that are embedded at kinetochores, but also decorates spindle microtubules and centrosomes (Dikovskaya et al., 2004; Fodde et al., 2001; Kaplan et al., 2001; Louie et al., 2004). Loss of APC leads to chromosome mis-segregation, suggesting that mutations in APC contribute to cancer by causing aneuploidy (Dikovskaya et al., 2004; Fodde et al., 2001; Kaplan et al., 2001). Interestingly, N-terminal fragments that result from mutations in APC are not only unable to support the formation of

normal mitotic spindles (because they do not bind to and stabilise microtubules) but also appear to exert a dominant effect on mitotic spindles (Green and Kaplan, 2003).

APC is also found in the nucleus and contains several nuclear import and export signals (Henderson and Fagotto, 2002). One function suggested for nuclear APC is the support of nuclear shuttling of β-catenin. However, nuclear-cytoplasmic shuttling of β-catenin can proceed independently of APC (Henderson and Fagotto, 2002). Other potential functions for APC in the nucleus include a contribution to transcriptional regulation via its ability to bind DNA directly (Deka et al., 1999).

The relative amount of APC in the nucleus may be related to cell cycle stage since cells at low confluency exhibit more staining for APC in the nucleus compared with densely growing cells (Fagman et al., 2003; Zhang et al., 2001); however, nuclear staining observed using many APC antibodies has to be interpreted carefully because antibody cross-reactivity can contribute significantly to staining of APC in the nucleus. Indeed, at least one commercially available APC antibody reacts strongly with the DNA-binding protein Ku80 and only very weakly with APC (Mogensen et al., 2002; Roberts et al., 2003).

In summary, by interacting with a complex set of cellular proteins and pathways, APC contributes to differentiation, cell migration, proliferation and adhesion. The toxic environment of the gut lumen means that all cells other than stem cells have a short life span in this tissue. Active migration accompanies cell differentiation to ensure that epithelial cells in the gut are usually exfoliated within 3-5 days. As a consequence, normal gut maintenance requires that cell-cell and cell-substrate adhesion along with migration, proliferation and differentiation are balanced and maintained at all times. Mutations in *APC* are likely to affect all of these processes, which may explain why mutations in this single gene are sufficient to initiate the development of cancer.

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