Adenomatous polyposis coli (*APC*): a multi-functional tumor suppressor gene

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Summary

The adenomatous polyposis coli (*APC*) gene is a key tumor suppressor gene. Mutations in the gene have been found not only in most colon cancers but also in some other cancers, such as those of the liver. The *APC* gene product is a 312 kDa protein that has multiple domains, through which it binds to various proteins, including β-catenin, axin, CtBP, Asefs, IQGAP1, EB1 and microtubules. Studies using mutant mice and cultured cells have demonstrated that APC suppresses canonical Wnt signalling, which is essential for tumorigenesis, development and homeostasis of a variety of cell types, such as epithelial and lymphoid cells. Further studies have suggested that APC plays roles in several other fundamental cellular processes. These include cell adhesion and migration, organization of the actin and microtubule networks, spindle formation and chromosome segregation. Deregulation of these processes caused by mutations in *APC* is implicated in the initiation and expansion of colon cancer.

Key words: APC, Wnt signalling pathway, Colon cancer, Chromosome instability, Cell migration, Cell adhesion, β-catenin, Asef, IQGAP1, Actin, EB1, Microtubules

Introduction

Germ-line mutations in the *APC* gene result in familial adenomatous polyposis (FAP), which is characterized by numerous polyps in the intestines (Groden et al., 1991; Kinzler et al., 1991). Mutations in *APC* have been found in ~60% of sporadic carcinomas and adenomas as well (Powell et al., 1992). Genetic studies using mutant mouse models have demonstrated that mutations in the *Apc* gene are responsible for intestinal tumorigenesis (Fodde et al., 1994; Oshima et al., 1995; Su et al., 1992). Homozygous *Apc* mutations in mice lead to embryonic lethality (Ishikawa et al., 2003; Moser et al., 1995; Oshima et al., 1995), and conditional deletion of the gene in the adult mouse disrupts homeostasis not only in the intestines but also in other tissues (Andreu et al., 2005; Gounari et al., 2005; Sansom et al., 2004). These pieces of evidence have indicated that APC is essential for development and homeostasis and that its inactivation facilitates tumorigenesis (Fig. 1, and Tables 1 and 2).

One of the most important issues in studies of APC is to identify the cellular pathways responsible for tumorigenesis when it is mutated. APC is a multi-domain protein that contains binding sites for numerous proteins, including microtubules, the Wnt/Wg pathway components β-catenin and axin, the cytoskeletal regulators EB1 and IQGAP1, and the Rac guanine-nucleotide-exchange factor (GEF) Asef1. Most (~60%) cancer-linked *APC* mutations occur in a region referred to as the mutation cluster region (MCR; Fig. 1) and result in C-terminal truncation of the protein (Beroud and Soussi, 1996). Because these truncations cause loss of the domains required for binding to β-catenin and to microtubules (Fig. 1), the interaction of APC with β-catenin or microtubules has been considered to be essential for its tumor suppressor activity. β-Catenin has a dual role in cells, functioning both in cell adhesion at adherens junctions and as a signal transducer that shuttles to the nucleus in response to Wnt signalling. Binding of APC to microtubules obviously has potential consequences for cytoskeletal architecture.

Loss of APC leads to accumulation of nuclear β-catenin, which activates the T-cell factor (TCF) and lymphoid enhancer factor (LEF) transcription factors (Barker and Clevers, 2006; Korinek et al., 1997; Morin et al., 1997; Reya and Clevers, 2005), the targets of the canonical Wnt signalling pathway. In a floxed β-catenin-knock-in mouse, Cre-mediated expression of stable β-catenin in the small intestine causes formation of numerous (700-3000/mouse) polyps morphologically similar to those found in the *Apc*-knockout mouse (Harada et al., 1999; Oshima et al., 1995). By contrast, suppression of canonical Wnt-dependent transcription by dominant-negative TCFs inhibits the proliferation of colon cancer cells that carry mutations in *APC* (Tetsu and McCormick, 1999; van de Wetering et al., 2002). Activation of the canonical Wnt pathway caused by *APC* inactivation is thus sufficient for intestinal tumorigenesis. However, it has been proposed that mutations in *APC* contribute to cancer development through processes other than Wnt signalling. Below, we discuss recent advances in this area, examining potential roles of both canonical Wnt targets and effects of APC on the cytoskeleton, cell migration and chromosome instability.

APC and APC2 in mammals and flies

Both mammals and *Drosophila* carry two *APC* genes: *APC* and *APC2* (*APCL*) in mammals, and *dAPC1* and *dAPC2/E-APC* in *Drosophila* (Bienz, 2002; Polakis, 1997; van Es et al., 2001). Note, however, that *Drosophila dAPC1* and *dAPC2* are not direct orthologues of mammalian *APC* and *APC2*, respectively (Fig. 1). Human APC has 2843 residues whereas APC2

contains 2303 residues (Fig. 1). dAPC1 and dAPC2 have 2416 and 1067 residues, respectively. Mammalian APC has an oligomerization domain, an armadillo repeat-domain, a 15- or 20-residue repeat domain important for binding to β-catenin,

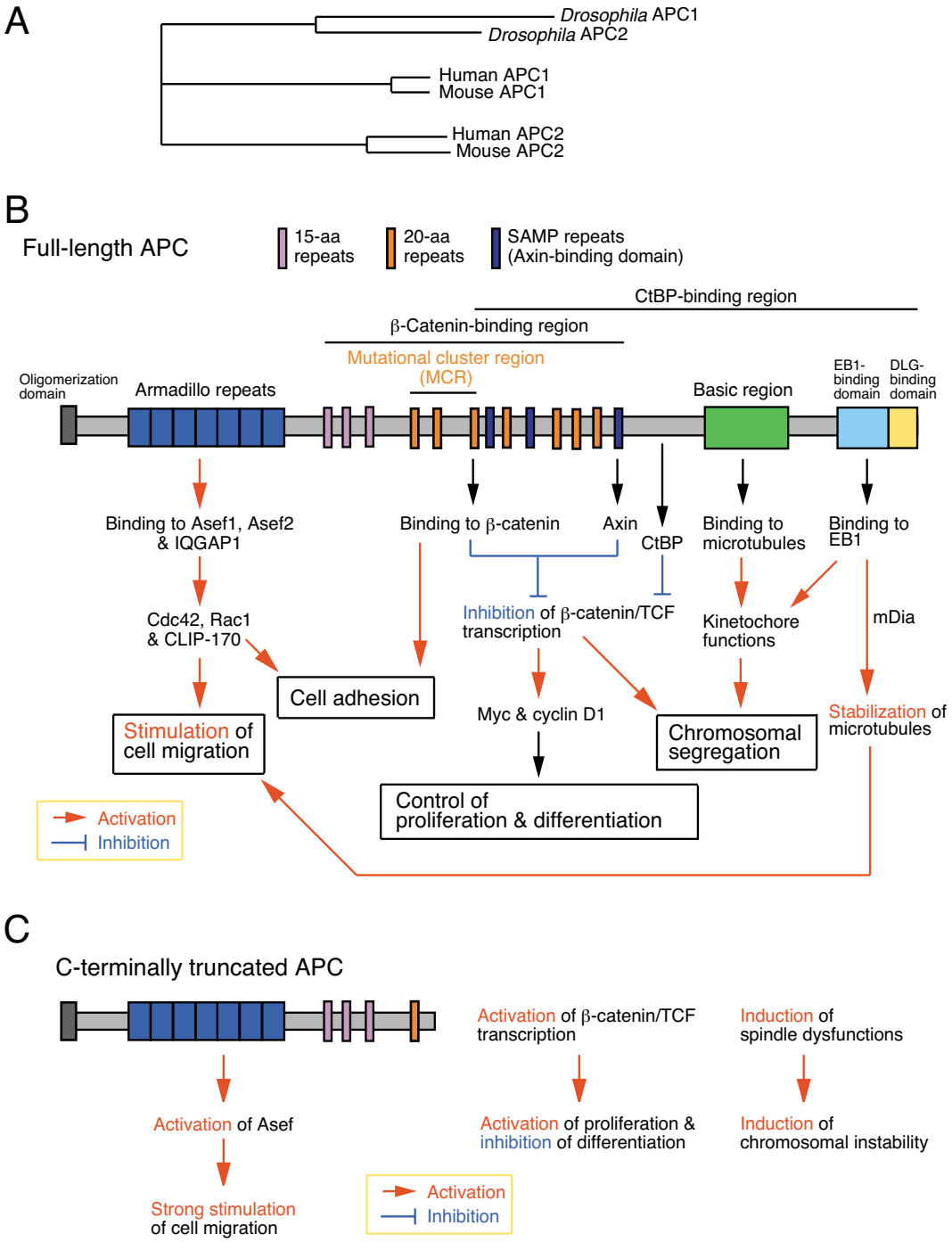

Fig. 1. Multiple domains and functions of APC, and its truncated mutants found in colon cancer. (A) An evolutionary tree of the fly, mouse and human APC1 and APC2 proteins drawn by a Vector NTI 7 software (Invitrogen Corporation). Note that the two fly *APC* genes are not direct orthologues of the two mammalian genes. (B) Schematic diagram that shows multiple domains of the full-length APC and their functions. APC stimulates cell migration through interactions with Asef, IQGAP1 or mDia. APC is involved in cell adhesion through controlling β-catenin distribution between the nucleus/cytoplasm and the plasma membrane. APC inhibits β-catenin/TCF transcription through interactions with β-catenin or CtBP. APC regulates chromosome segregation through kinetochore binding, and through suppression of the canonical Wnt signalling. Red arrows and blue bars indicate 'activation' and 'inhibition', respectively. (C) Schematic diagram that shows one of the C-terminally truncated APC proteins and its role in tumorigenesis. Truncated APC stimulates cell migration more strongly than the full-length APC does, whereas loss of its C-terminal domains causes activation of the Wnt signalling pathway and induction of chromosome instability (CIN).

SAMP repeats for axin binding, a basic domain for microtubule binding and C-terminal domains that bind to EB1 and DLG proteins (Fig. 1) (Polakis, 1997).

In mammals, *APC* is expressed in most fetal tissues and in adult epithelial cells (Midgley et al., 1997), whereas *APC2* is expressed ubiquitously and at high levels in the brain (van Es et al., 1999). Embryonic stem (ES) cells that lack *Apc* lose their ability to differentiate (Kielman et al., 2002), and the homozygous *Apc*-knockout mutation is embryonic lethal (Ishikawa et al., 2003; Moser et al., 1995; Oshima et al., 1995). An *Apc2* knockout mutation has not been reported. Although the heterozygous *Apc* mutant mice are viable, they develop a number of intestinal polyps that resemble those in the FAP patients (Fodde et al., 1994; Oshima et al., 1995; Su et al., 1992). Accordingly, these mutant mice, often called 'Min' mice for multiple intestinal neoplasia (Moser et al., 1990; Su et al., 1992), have been widely used as a model for intestinal tumorigenesis (Boivin et al., 2003; Taketo, 2006). Conditional inactivation of APC in the adult mouse intestine leads to enlargement of the crypt compartment, which is associated with an increased cell population and apoptosis (Andreu et al., 2005; Sansom et al., 2004). It also perturbs differentiation and migration of epithelial cells. Expression of APC2 in colon cancer cells that lack wild-type APC inhibits Wnt signalling to a similar extent to that achieved by APC (van Es et al., 1999), although the roles of APC2 in tumorigenesis and homeostasis have been unclear. *Apc2* might be unable to compensate for loss of the tumor suppressor activity of *Apc*, given that loss of *Apc* in the intestine stimulates cell proliferation and polyp formation (Andreu et al., 2005; Fodde et al., 1994; Oshima et al., 1995; Sansom et al., 2004; Su et al., 1992).

In *Drosophila*, dAPC and dAPC2 function in a partially redundant manner (Ahmed et al., 2002; Akong et al., 2002a; Akong et al., 2002b) – both can inhibit Wnt signalling in human cancer cells (Hamada et al., 1999; Hayashi et al., 1997). The *dAPC1* gene is highly expressed in the central nervous system and the germ-line, whereas *dAPC2* is broadly expressed (Ahmed et al., 1998). Homozygous *dAPC1* mutants are viable, although they show neuronal degeneration and pigment cell hypertrophy of the retina (Ahmed et al., 1998; Hayashi et al., 1997). Because maternal/zygotic *dAPC2* mutants die during embryogenesis owing to excessive Wg signalling (McCartney et al., 1999), *dAPC2* must be essential for embryogenesis. Zygotic mutants of *dAPC2* are viable, however, and *dAPC1* therefore appears to compensate for lack of *dAPC2* in regulation of Wg signalling in many tissues. Both dAPC1 and dAPC2 are thus required for normal development and homeostasis in *Drosophila*.

APC in canonical Wnt signalling

APC plays a central role suppressing the canonical Wnt signalling pathway that controls cell proliferation and differentiation in the intestine (van de Wetering et al., 2002), skin (Gat et al., 1998; Huelsken et al., 2001; Nguyen et al., 2006), immune system (Cobas et al., 2004; Gounari et al., 2005; Kirstetter et al., 2006; Scheller et al., 2006), bone (Glass, 2nd et al., 2005; Hill et al., 2005) and brain (Brault et al., 2001; Kleber et al., 2005). When this pathway is stimulated, β-catenin activates TCF-dependent transcription of Wnt-target genes such as *CCND1* (Tetsu and McCormick, 1999), *MYC* (He et al., 1998) and *EphB* (Batlle et al., 2002).

Several lines of evidence indicate that APC inhibits β-catenin/TCF-dependent transcription through multiple mechanisms. First, APC provides a scaffold for a destruction complex together with GSK3β and axin that promotes phosphorylation and consequent ubiquitin-dependent degradation of β-catenin (Rubinfeld et al., 1996). Second, APC promotes export of β-catenin from the nucleus, which reduces the amount of nuclear β-catenin/TCF (Henderson and Fagotto, 2002; Neufeld et al., 2000; Rosin-Arbesfeld et al., 2003). Third, APC binds to β-catenin, blocking the interaction with TCF (Neufeld et al., 2000; Rosin-Arbesfeld et al., 2003).

Finally, recent studies show that APC can inhibit β-catenin/TCF-dependent transcription through a direct interaction with a repressor complex. APC binds to βTrCP and transcriptional repressor C-terminal binding protein (CtBP) and forms a stable complex with additional co-repressors TLE-1 and HDAC1 (Hamada and Bienz, 2004; Sierra et al., 2006). Because APC interacts with βTrCP at the *MYC* enhancer, it may facilitate CtBP-mediated repression of Wnt-target genes (Hamada and Bienz, 2004; Sierra et al., 2006). Because the C-terminal half of APC mediates binding to CtBP, truncated APC mutants found in cancer cannot stimulate the CtBP-mediated transcriptional repression (Hamada and Bienz, 2004; Sierra et al., 2006). Such mutations thus activate β-catenin/TCF-dependent transcription by both increasing levels of β-catenin-TCF complexes in the nucleus and alleviating CtBP-mediated inhibition of the complex (Fig. 1 and Table 1).

APC in cell adhesion

Inactivation of APC has also been proposed to promote tumorigenesis through loss of cell adhesion (Bienz and Hamada, 2004; Birchmeier et al., 1995). APC is found at the lateral plasma membrane of mammalian epithelial cells both in vivo and in culture (Miyashiro et al., 1995; Nathke et al., 1996), and it interacts with β-catenin, which links E-cadherin to α-catenin and the actin cytoskeleton (Kemler, 1993; Rubinfeld et al., 1993; Su et al., 1993). A mutation in *Apc* in mouse intestinal epithelial cells can decrease the level of E-cadherin at the cell membrane and association between β-catenin and E-cadherin (Carothers et al., 2001). Moreover, expression of the full-length APC leads to increased levels of E-cadherin at the cell membrane, which enhances cell adhesion in colon cancer cells in which truncated APC is expressed (Faux et al., 2004). In addition, expression of the full-length APC in the cancer cells also causes nuclear and cytoplasmic β-catenin to translocate to the cell periphery (Faux et al., 2004). APC thus appears to affect cell adhesion by controlling the distributions of β-catenin and E-cadherin between the cytoplasm and the cell membrane (Tables 1 and 2).

In *Drosophila*, dAPC2 is also found in apical adherens junctions, and an inactivating mutation in *dAPC2* causes mislocalization of oocytes in the egg chambers due to failure of cadherin-based adhesion (Hamada and Bienz, 2002). Although these results are consistent with the studies of mammalian cultured cells mentioned above (Faux et al., 2004), *Drosophila* that lack both dAPC1 and dAPC2 yield strikingly different results (McCartney et al., 2006). Complete loss of the two dAPC proteins elevates the levels of cytoplasmic β-catenin in the germ and follicle cells of ovaries, and embryonic epithelial cells. However, loss of both dAPC proteins in these tissues does not substantially affect the cortical localization of

Table 1. Functions of APC

Cellular processes	Effects by wild-type APC	Effects by loss of APC or truncated APC	APC domains	APC functions	Binding proteins	References
The canonical Wnt signal transcription	Inhibition	Activation (loss)	Armadillo repeats; 15- or 20-aa repeats	Stimulation of phosphorylation and degradation of β-catenin	β-catenin, GSK3β, Axin	Korinek et al., 1997; Morin et al., 1997
	Inhibition	Activation (loss)	Armadillo repeats; 15- or 20-aa repeats	Export of β-catenin from the nucleus	β-catenin	Henderson and Fagotto, 2002; Neufeld et al., 2000; Rosin-Arbesfeld et al., 2003
	Inhibition	Activation (loss)	Armadillo repeats; 15- or 20-aa repeats	Keeping β-catenin from the interaction with TCF	β-catenin	Neufeld et al., 2000; Rosin-Arbesfeld et al., 2003
	Inhibition	Activation (loss)	15-aa repeats	Recruitment of repression complex to the transcriptional machinery	CtBP	Hamada and Binez, 2004; Sierra et al., 2006
Cell adhesion	Stimulation	Weaken (loss)	Armadillo repeats; 15- or 20-aa repeats	Control of β-catenin distribution between in the nucleus/cytoplasm and the plasma membrane	β-catenin	Faux et al., 2004; Klingelhofer et al., 2003
	Stimulation	Weaken (loss)	Armadillo repeats; 15- or 20-aa repeats	Increase in E-cadherin levels at the plasma membrane	β-catenin	Faux et al., 2004
Cell migration	Stimulation	Stronger stimulation (truncated)	Armadillo repeats	Activation of Asef1 and Asef2; Activation of Cdc42	Asef1 and Asef2	Hamann et al., 2007; Kawasaki et al., 2003; Kawasaki et al., 2000; Mahmoud et al., 1997; Oshima et al., 1997; Wong et al., 1996
	Stimulation	Inhibition (loss)	Armadillo repeats	Recruitment of IQGAP1 and CLIP-170; Formation of a complex with IQGAP1, CLIP-170, and activated Rac1 and Cdc42; Formation of actin meshwork	IQGAP1	Watanabe et al., 2004
	Stimulation		EB1-binding domain	Functions downstream of Rho and mDia; Stabilization and polymerization of microtubules	EB1	Wen et al., 2004
Chromosomal segregation	Proper segregation	Mis-segregation (loss)	Basic domain	Regulation of kinetochore functions	Microtubules	Dikovskaya et al., 2007; Fodde et al., 2001; Kaplan et al., 2001
	Proper segregation	Mis-segregation (loss)	Armadillo repeats; 15- or 20-aa repeats	Inhibition of apoptosis at the G2/M checkpoint through Wnt signaling	β-catenin	Aoki et al., 2007; Hadjihannas et al., 2006
	Proper segregation	Dominant negative (truncated)	Not determined	Regulation of kinetochore functions	Not determined	Tighe et al., 2004; Tighe et al., 2001

β-catenin, levels of E-cadherin, or cadherin-based adhesion (McCartney et al., 2006). More recent studies show that simultaneous deletion of both dAPC proteins in the brain does not reduce the levels of cadherins (Hayden et al., 2007). Since these results cannot be explained in terms of the predicted functions of APC, further investigation will be needed to determine its contribution to cell adhesion.

APC and the actin network

APC has been shown to regulate cell polarity and migration through control of the actin cytoskeleton (Akiyama and Kawasaki, 2006). Forced expression of APC in the small intestine induces disordered migration of epithelial cells (Wong et al., 1996). By contrast, loss of APC slows down their migration (Andreu et al., 2005; Sansom et al., 2004) and causes formation of a nascent polyp that consists of a single layer of adenoma cells (Oshima et al., 1997). In addition, the C-terminally truncated mutant of APC decreases migration of intestinal epithelial cells (Mahmoud et al., 1997). These studies suggest that expression of APC or its truncated form affects cell migration.

APC interacts with Asef, a Rac-specific GEF (Kawasaki et al., 2003; Kawasaki et al., 2000) and IQ-motif-containing GTPase activation protein 1 (IQGAP1) (Noritake et al., 2005; Watanabe et al., 2004). The interaction between APC and Asef1 was initially shown to stimulate the GEF activity of Asef1 and thus lead to Rac1 activation, cell flattening, membrane ruffling, formation of lamellipodia and increased cell migration (Kawasaki et al., 2003; Kawasaki et al., 2000). A recent analysis has shown that APC also interacts with Asef1 relative Asef2 (Hamann et al., 2007). This work also shows that both Asef proteins have GEF activities towards lipid-modified recombinant Cdc42 (which mimics post-translationally modified Cdc42) but not its unmodified form. By contrast, they fail to promote detectable GEF activity of Asefs towards lipid-modified Rac1, despite the fact that both can bind to Rac1 (Hamann et al., 2007). These results suggest that APC activates Cdc42 rather than Rac1 through its interactions with Asef1 and Asef2. Because APC interacts with Asef1 through its armadillo repeat domain, which is N-terminal to the MCR (Fig. 1), truncated APCs in most colon cancer cells should still interact with Asef1 (Kawasaki et al., 2003). Interestingly, C-terminally truncated APC stimulates Asef1 more strongly than the full-length APC does (Kawasaki et al., 2003). Expression of the truncated APC mutants could thus induce aberrant migration and invasion of cancer cells through Asef1 and Asef2. However, mutations in the *Apc* gene alone do not lead to invasive carcinoma, although an additional mutation in *Smad4*, which encodes a TGF-β-responsive transcription factor, promotes invasion (Kitamura et al., 2007; Oshima et al., 1995; Takaku et al., 1998). Therefore, aberrant cell migration caused by truncated APC is probably not sufficient to cause progression to malignancy.

Another key link between APC and the cytoskeleton is IQGAP1 (Watanabe et al., 2004), an effector of Rac1 and Cdc42 (Briggs and Sacks, 2003). IQGAP1 is a scaffold protein that controls actin filaments and microtubules (Briggs and Sacks, 2003). It recruits APC to specific sites in migrating cells, at which they form a complex with the microtubule-stabilizing protein CLIP-170, and with activated Rac1 or Cdc42 (Watanabe et al., 2004). Depletion of either APC or IQGAP1 inhibits formation of the actin meshwork and polarized migration, and also causes mislocalization of CLIP-170 (Watanabe et al., 2004). Because these results have been obtained mainly in a cell line, Vero, that was isolated from monkey kidney epithelial cells, further investigation will clarify the significance of the interaction in cells of other tissues.

Note that APC might also bind to actin directly through its C-terminal domain, which mediates binding to microtubules via EB1 (see below) (Moseley et al., 2007; Polakis, 1997). Because the interaction of APC with EB1 can disrupt its interaction with actin, EB1 could control the APC-mediated regulation of actin and microtubules (Moseley et al., 2007). Because these data are based on overexpression experiments, however, additional studies such as loss-of-function experiments are needed to establish whether there is indeed a direct link between APC and actin.

APC and the microtubule network

APC is found at the ends of microtubules (Nathke et al., 1996) and can bind to and stabilize them (Munemitsu et al., 1994; Smith et al., 1994). These results indicate that APC regulates the microtubule network and may play roles in microtubule-mediated processes such as cell migration and spindle formation (Nathke, 2006; Nathke, 2004). Indeed, recent studies using siRNA targeting *APC* have shown that depletion of APC inhibits cell migration and protrusion formation (Kroboth et al., 2007). Knocking down *APC* decreases overall microtubule stability and the level of post-translationally modified microtubules at the migrating edge of the cell (Kroboth et al., 2007). APC thus appears to contribute to cell migration by regulating microtubules as well as the actin cytoskeleton (Fig. 1).

Stabilization and polymerization of microtubules by APC are mediated also by interaction with EB1 (Morrison et al., 1998; Nakamura et al., 2001; Su et al., 1995). Recent results have shown that APC and EB1 function downstream of Rho and the formin family protein mDia in microtubule stabilization (Wen et al., 2004). Because APC may form a complex with EB1 and mDia at stable microtubule ends, the involvement of APC in cell migration could encompass cooperation with mDia as well (Wen et al., 2004). APC therefore seems to stimulate cell migration through several pathways (Fig. 1).

APC in chromosome segregation

Chromosome instability (CIN) is considered to be one of the driving forces that stimulate tumorigenesis (Sotillo et al., 2007; Weaver et al., 2007). Some studies have shown that loss of APC induces mis-segregation of chromosomes (Tables 1 and 2) (Alberici et al., 2007; Aoki et al., 2007; Dikovskaya et al., 2004; Dikovskaya et al., 2007; Fodde et al., 2001; Green and Kaplan, 2003; Green et al., 2005; Hadjihannas et al., 2006; Kaplan et al., 2001; Tighe et al., 2001; Tighe et al., 2004); others have produced conflicting results (Draviam et al., 2006; McCartney et al., 2006; Sieber et al., 2002).

In mitosis, APC localizes to kinetochores, spindles and centrosomes (Dikovskaya et al., 2004; Kaplan et al., 2001; Louie et al., 2004; Olmeda et al., 2003). Truncating mutations in the *Apc* gene can cause CIN, which is evident from aneuploidy and chromosomal aberrations in mutant mouse

Table 2. Studies using *APC* knockouts or knockdowns

APC genes	Models	Methods	Tissues	Main phenotypes	Main mechanisms	References
APC	Knockout mouse	Homozygous knockout	Embryo	Embryonic lethality at 5.5 d.p.c	Activation of Wnt signaling pathway	Ishikawa et al., 2003; Moser et al., 1995; Oshima et al., 1995
	Knockout mouse	Conditional homozygous deletion	Intestines	Defects in cell migration, proliferation and differentiation	Activation of Wnt signaling pathway	Andreu et al., 2005; Sansom et al., 2004
	Knockout mouse	Conditional homozygous deletion	Immune system	Defects in differentiation	Activation of Wnt signaling pathway	Gounari et al., 2005
	Knockout mouse	Heterozygous knockout	Intestines	Formation of numerous polyps resembling FAP polyps	Activation of Wnt signaling pathway	Fodde et al., 1994; Oshima et al.,1995; Su et al., 1992
	Mouse ES cells	Homozygous knockout	Mouse cultured cells	Spindle aberrations; Chromosomal instability	Defects in kinetochore functions; Activation of Wnt signaling pathway	Aoki et al., 2007; Fodde et al., 2001; Kaplan et al., 2001
	Mouse ES cells	Homozygous knockout	Mouse cultured cells	Defects in differentiation	Activation of Wnt signaling pathway	Kielman et al., 2002
	U2OS cells, HCT116 cells	Knockdown using siRNA	Human cultured cancer cells	Chromosomal instability	Deregulated expression of spindle checkpoint proteins; Defects in kinetochore functions; Inhibition of apoptosis	Dikovskaya et al., 2007
	HeLa cells	Knockdown using siRNA	Human cultured cancer cells	No defects in expression of spindle checkpoint proteins; No notable chromosomal instability	Unchanged expression of spindle checkpoint proteins	Draviam et al., 2006
	SW480 cells, WiDr cells	Knockdown using siRNA	Human cultured cancer cells	Inhibition of cell migration	Inhibition of Asef and Rac activities	Kawasaki et al., 2003
	Vero cells	Knockdown using siRNA	Monkey cultured cells	Inhibition of polarized migration	Inhibition of actin meshwork and mislocalization of CLIP1	Watanabe et al., 2005
	U2OS cells MEF	Knockdown using siRNA and conditional deletion	Human and mouse cultured cells	Inhibition of cell migration and formation of cellular protrusions	Decrease in overall microtubule stability and decreased posttranslationally modified microtubules	Kroboth et al., 2007
APC2	No study reported					
dAPC1	*Drosophila*	Homozygous knockout	Eye	Viable; Neuronal degeneration and pigment cell hypertrophy of retina	Activation of Wnt signaling pathway	Ahmed et al., 1998; Hayashi et al., 1997
dAPC2	*Drosophila*	Homozygous knockout Homozygous knockout	Embryo Oocytes	Embryonic lethality Mislocalization of oocytes in egg chambers	Defects in Wnt signaling pathway Defects in cadherin-based adhesion	McCartney et al., 1999 Hamada and Bienz, 2002
dAPC1/dAPC2	*Drosophila*	Homozygous knockout	Ovary	No defects in cell adhesion, chromosomal segregation or spindle formation	No defects in cortical β-catenin localization or E-cadherin; No defects in spindle morphology or spindle orientation	McCartney et al., 2006

embryonic stem (ES) cells and intestinal polyps (Alberici et al., 2007; Aoki et al., 2007; Dikovskaya et al., 2007; Fodde et al., 2001; Kaplan et al., 2001). In addition, depletion of APC has been shown to compromise the formation of spindles in *Xenopus* extracts (Dikovskaya et al., 2004). Expression of a C-terminal fragment of APC (containing the microtubule-binding region) can also cause aneuploidy in a human colon cancer cell line (HCT116) that expresses the full-length APC (Fodde et al., 2001). In addition, expression of an N-terminal fragment of APC can weaken kinetochore-microtubule interactions and induce CIN in HCT116 cells (Tighe et al., 2001; Tighe et al., 2004). APC thus seems to be involved in kinetochore function and chromosome segregation and this probably involves its interaction with microtubules (Fodde et al., 2001). Truncated forms of APC may therefore have dominant-negative effects on chromosome segregation, although the mechanism is unclear.

The taxanes, including taxol, produce unattached kinetochores by inhibiting microtubule assembly and spindle formation, which leads to activation of the mitotic spindle checkpoint and accumulation of cells at prometaphase of the cell cycle (Weaver and Cleveland, 2005). Because treatment of homozygous *Apc*-mutant ES cells with taxol leads to accumulation of cells in G2/M phase, the spindle checkpoint seems to work properly, which suggests that the checkpoint itself is unaffected by inactivation of APC (Fodde et al., 2001; Kaplan et al., 2001). However, further investigation has indicated that APC does have a role in the spindle checkpoint (Tables 1 and 2). Studies using siRNA directed against *APC* have shown that knocking down *APC* in U2OS and HCT116 cells induces polyploidy (Dikovskaya et al., 2007). Depletion of APC in these cells reduces levels of checkpoint proteins Bub1 and BubR1 at the kinetochores and promotes mitotic progression despite spindle damage caused by the depletion (Dikovskaya et al., 2007). Because loss of APC in these cells inhibits apoptosis as well, Dikovskaya et al. have proposed that kinetochore dysfunction and inhibition of apoptosis collaborate to promote CIN in APC-mutant cells (Dikovskaya et al., 2007).

Another analysis using siRNA to knock down *APC* mRNA, however, found that inhibition of APC in HeLa cells does not affect chromosome instability significantly (Draviam et al., 2006). In most of the APC-depleted cells, chromosomes congress correctly at the spindle equator and anaphase ensues with little or no delay. In addition, high levels of the mitotic spindle checkpoint proteins Mad1, Mad2, Bub1 and BubR1 bind to the kinetochore (Draviam et al., 2006). These results suggest that inactivation of APC does not interfere with the operation of the spindle checkpoint or cause dramatic changes in the extent or stability of kinetochore-microtubule interactions (Draviam et al., 2006), contradicting the results above (Dikovskaya et al., 2007). Although further studies will be needed to settle this issue, it is conceivable that the observed effects of reduced levels of APC on chromosome segregation depend on the cell type and/or the method of detection.

A role for APC in chromosome segregation has also been suggested in *Drosophila*. dAPC2, armadillo (fly β-catenin) and α-catenin localize to the sites of cortical spindle attachment, and a mutation in either *dAPC2* or β-catenin results in the loss of nuclei (McCartney et al., 2001). However, recent studies have shown that deletion of both APC1 and APC2 has no effect on spindle assembly, spindle orientation or asymmetric division in the ectoderm during gastrulation (McCartney et al., 2006), which is consistent with the studies in HeLa cells discussed above (Draviam et al., 2006). Moreover, segregation of chromosomes is unaffected by expression of truncated APC in *Drosophila* (Draviam et al., 2006). Its truncated form therefore seems not to play a dominant-negative role in chromosome segregation, which also argues against the results obtained with human cancer cells (Tighe et al., 2001; Tighe et al., 2004).

We and others have found recently that activation of the canonical Wnt signalling pathway can cause CIN in mouse ES and human colon cancer cells (Aoki et al., 2007; Hadjihannas et al., 2006). Expression of stable β-catenin induces CIN in both ES cells and mouse intestinal polyps, whereas expression of dominant-negative TCF in these cells reduces the anaphase bridge index, a marker for CIN (Aoki et al., 2007; Gisselsson et al., 2000). Mutations in *APC* could therefore contribute to CIN through activation of the canonical Wnt signalling pathway (Fig. 1). Wnt signalling in mouse ES cells increases the fraction of surviving cells upon stimulation of the G2/M checkpoint by exposure of cells to nocodazole or colcemid. In addition, Wnt signalling suppresses the Cdc2 kinase activity that induces apoptosis at the G2/M checkpoint (Aoki et al., 2007; Tan et al., 2002). It is thus possible, through suppression of cyclin-B–Cdc2, that activation of canonical Wnt-signal-dependent transcription can inhibit apoptosis of cells even when they carry chromosomal aberrations. Hadjihannas et al. also showed that expression of conductin/axin2, a target of TCF/β-catenin-dependent transcription, induces CIN by compromising the spindle checkpoint (Hadjihannas et al., 2006). Wnt signalling could thus also induce CIN through increased levels of conductin.

Conclusions and perspectives

Inactivation of APC has been recognized as one of the most important genetic alterations in colon carcinogenesis, and activation of the canonical Wnt signal plays an essential role in tumorigenesis caused by *APC* mutations. However, APC plays additional roles in other cellular processes, such as cell migration, adhesion, and chromosome segregation. Although elevated cell migration, chromosome instability and loss of cell adhesion are possible contributing factors to tumorigenesis and progression, it is still unclear whether mutations in *APC* accelerate tumorigenesis through such mechanisms. To understand the precise mechanisms of colon cancer progression, further studies are needed to delineate the effects of canonical Wnt signal activation and those of other APC-dependent mechanisms.

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