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The ABC of APC

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease characterized by the presence of adenomatous polyps in the colon and rectum, with inevitable development of colorectal cancer if left untreated. FAP is caused by germline mutations in the adenomatous polyposis coli (*APC*) gene. Somatic mutations in the *APC* gene are an early event in colorectal tumorigenesis, and can be detected in the majority of colorectal tumours. The *APC* gene encodes a large protein with multiple cellular functions and interactions, including roles in signal transduction in the wnt-signalling pathway, mediation of intercellular adhesion, stabilization of the cytoskeleton and possibly regulation of the cell cycle and apoptosis. The fact that APC is an integral part of so many different pathways makes it an ideal target for mutation in carcinogenesis. This review deals with our understanding to date of how mutations in the *APC* gene translate into changes at the protein level, which in turn contribute to the role of APC in tumorigenesis.

INTRODUCTION

The adenomatous polyposis coli (*APC*) gene encodes a large multidomain protein that plays an integral role in the wnt-signalling pathway and in intercellular adhesion. Germline mutations in the *APC* gene are responsible for the autosomal dominant inherited disease familial adenomatous polyposis (FAP), while somatic mutations in *APC* occur in ~80% of sporadic colorectal tumours. *APC* mutations almost always result in a truncated protein product with abnormal function. Recent research has concentrated on the interdependence of *APC* mutations in colorectal tumorigenesis, the contribution of *APC* missense variants to inherited risk of colorectal cancer and the biological interactions of the APC protein and its partners.

FAMILIAL ADENOMATOUS POLYPOSIS

Familial adenomatous polyposis (FAP) was first described in the literature as a disease with clear dominant inheritance by Lockhart-Mummery in 1925 (1). The clinical diagnosis of FAP depends upon the detection of hundreds to thousands of adenomatous polyps in the colon and rectum of affected individuals. The polyps usually appear by adolescence or the third decade of life. Untreated, colorectal cancer invariably develops by the early forties at the latest. The risk of cancer is generally considered to be related to polyp number (2). Annual colonoscopy is therefore indicated from adolescence, followed by prophylactic colectomy or proctocolectomy to eliminate the risk of developing colorectal cancer.

The incidence of FAP in the population is approximately 1 in 8000 (3). Despite the strong selective disadvantage of the disease, the incidence of FAP is maintained by the frequency of new mutations, which contribute about a quarter of all cases (3). The genetic basis for FAP lies in the germline (inherited) mutation of the *APC* gene (OMIM 175100). *APC* germline mutations achieve close to 100% penetrance, although there is marked variation in phenotypic expression of the disease (4–7).

Attenuated FAP (AFAP) is characterized by the presence of less than 100 adenomatous polyps but still carries a significantly increased risk of the development of colorectal cancer (8). It may occur in some or all of the affected individuals within a kindred (9). Full colonoscopy is often required to establish the diagnosis because polyps may not be seen in the rectosigmoid as in classical FAP.

There are a number of associated phenotypic features in FAP. Congenital hypertrophy of the retinal pigment epithelium (CHRPE) occurs in ~60% of families with FAP (10). The condition does not affect sight and has no malignant potential. CHRPE can be detected by ophthalmoscopy at any age, and thus can be used to identify at-risk family members well before the appearance of polyps (11).

Upper gastrointestinal tumours are commonly present in FAP patients (12,13) with periampullary carcinoma being the commonest cause of death in patients who have undergone prophylactic colectomy (14,15). Upper gastrointestinal endoscopy is therefore recommended in FAP patients.

Gardner's syndrome refers to the association of colonic polyps with epidermoid skin cysts and benign osteoid tumours of the mandible and long bones (16). It is now accepted that Gardner's syndrome is a variant of FAP. Desmoid tumours (benign fibromatosis) are a cause of significant morbidity and mortality in FAP patients (17,18). They usually arise in the abdominal wall or bowel mesentery, and can grow to considerable sizes. They commonly recur after surgical resection. Hereditary desmoid disease is characterized by autosomal dominant inheritance of multiple desmoid tumours in the absence of colonic polyposis, and this syndrome is also attributable to germline

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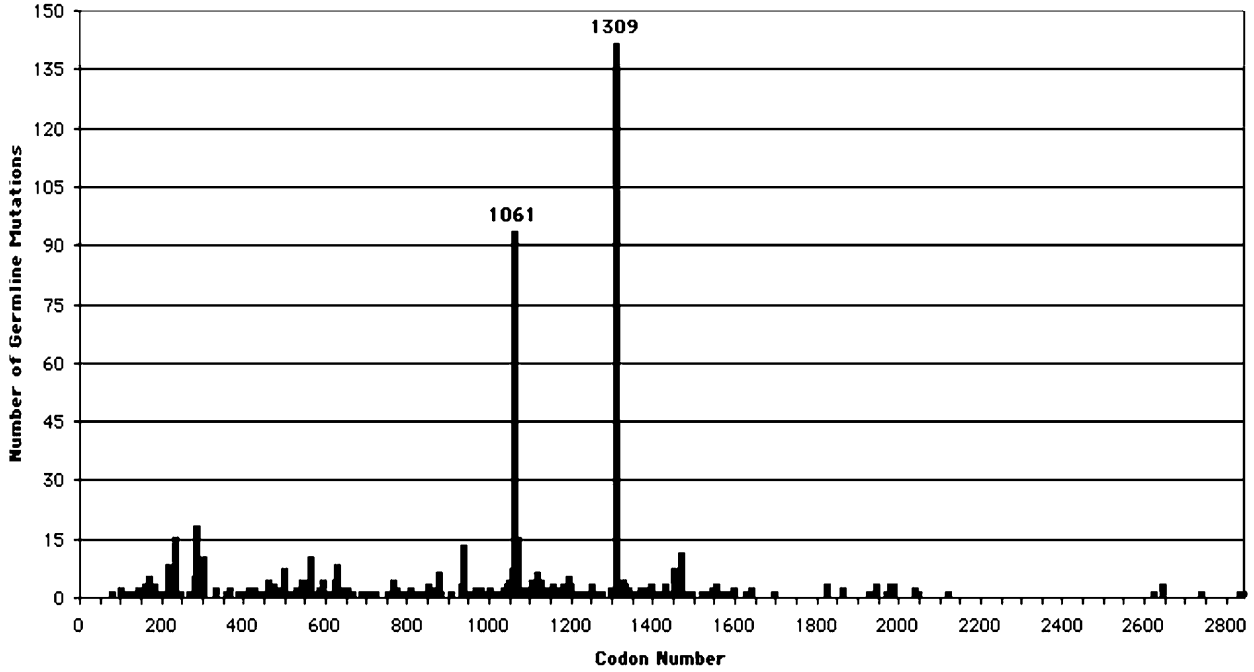

Figure 1. Distribution of germline mutations in the *APC* gene (n = 826) according to codon number. Data derived from Thierry Soussi *APC* database at http://perso.curie.fr/Thierry.Soussi/APC.html. Note mutational hotspots at 1061 and 1309.

mutations in the *APC* gene (19–22). Although rare in the general population, patients with FAP have a significantly increased risk of hepatoblastoma (23–26). Turcot syndrome refers to the association between multiple colorectal polyps and cerebellar medulloblastoma; it too is the consequence of a germline defect in *APC* (27,28). Other occasional manifestations of FAP include papillary carcinoma of the thyroid (29) and adrenocortical tumours (30,31).

THE APC GENE

The identification of a patient with colorectal polyposis in association with mental retardation and other abnormalities was the first clue to localizing the position of *APC*; a deletion of the chromosomal band 5q21 was observed (32). Linkage analysis of families with FAP led to the subsequent mapping of the *APC* gene to 5q21 (33). The *APC* gene was then cloned, identified and characterized (34,35).

The *APC* gene consists of 8535 bp spanning 21 exons (36), and encodes a 2843-amino acid protein in its commonest isoform (37). Exon 10A, located downstream of exon 10, is the subject of alternative splicing and adds an additional 18 amino acids to the APC protein when transcribed (38,39). Exon 15 comprises >75% of the coding sequence of *APC* and is the most common target for both germline and somatic mutations (40).

GERMLINE MUTATIONS IN *APC*

Germline mutations in *APC* have been demonstrated in the majority of FAP patients (41–43). The majority (95%) of these are nonsense or frameshift mutations that result in a truncated protein product with abnormal function. In accordance with Knudson's two-hit hypothesis, colorectal tumours from FAP patients carry additional somatic *APC* mutations or loss of heterozygosity (LOH) at this locus in addition to the original germline mutation (44–48).

The most common germline mutations occur at codons 1061 and 1309 (Fig. 1), which between them account for a third of all germline mutations (40,49). Apart from these peaks, germline mutations in *APC* are spread fairly uniformly between codons 200 and 1600, but rarely occur beyond codon 1600.

The type of germline mutation in *APC* appears to determine the nature of the second hit to *APC*. If the germline mutation occurs between codons 1194 and 1392, then there is strong selection for allelic loss of *APC* as the second hit in the development of a colorectal adenoma. If the germline mutation lies outside this region, the second hit in tumorigenesis is most likely to produce a truncating mutation in the mutation cluster region (MCR) (48).

GENOTYPE–PHENOTYPE CORRELATIONS IN FAP

The risk of developing specific manifestations of FAP is often correlated with the position of the inherited *APC* mutation (Fig. 2). Severe polyposis (more than 5000 colorectal polyps) is usually seen in patients with mutations between codons 1250 and 1464 (50), although some patients with a similar phenotype have been found to have *APC* mutations at codon 233 in exon 6 (5) and at codons 486 and 499 in exon 11 (51). Mutations in codon 1309 and immediately 3′ of it tend to cause a particularly severe phenotype with an earlier onset of disease (5,52,53). Attenuated polyposis, in contrast, is usually attributed to mutations at the extreme 5′ (8,54–56) or 3′ (21,56,57) ends of the *APC* gene, or in the alternatively spliced region of exon 9 (7,56,58,59). CHRPE is only present in patients with

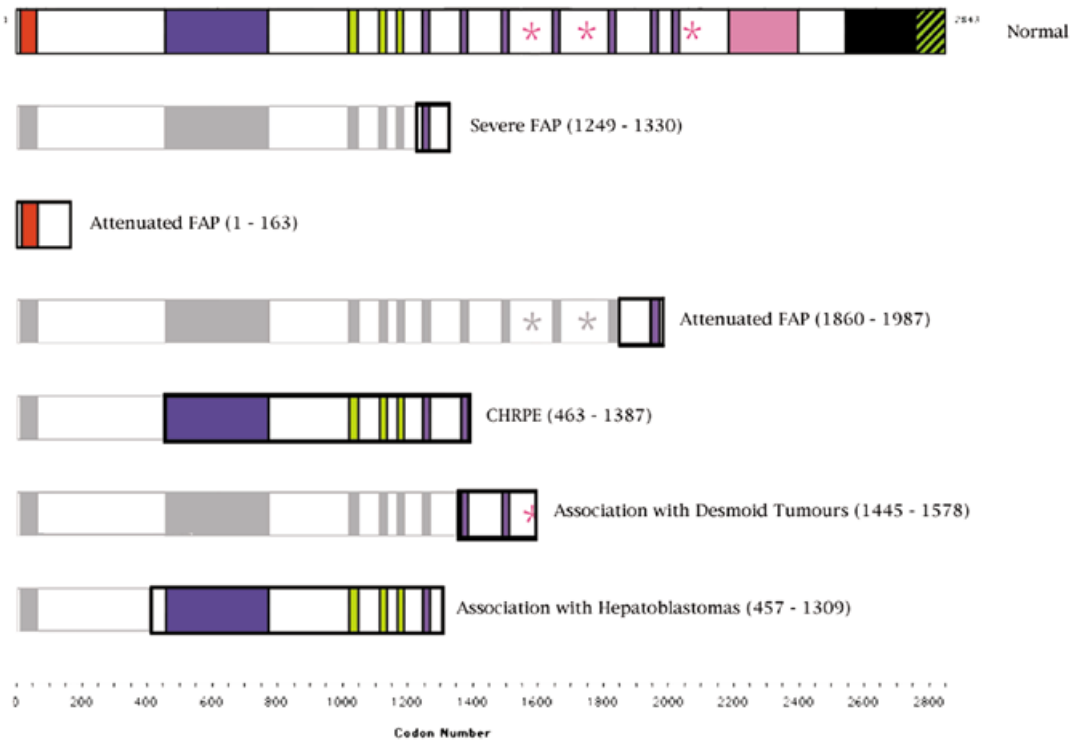

Figure 2. Diagram showing the association between FAP phenotype and position of the APC mutation. The grey area indicates translated region of protein. The coloured area indicates region of mutation in associated FAP phenotype. Compare colour coding with that in Figure 5 for protein domains. Truncated APC products would include both grey and coloured areas.

mutations between codons 457 and 1444 (8,55,60–64). Desmoid tumours appear to be limited to patients with mutations between codons 1403 and 1578 (62,63) although they have also been described with more 5′ mutations (5). Extracolonic manifestations (desmoids, osteomas, epidermoid cysts and upper gastrointestinal polyps) have been noted to occur most commonly in FAP patients with mutations between codons 1445 and 1578 (62) or between codons 1395 and 1493 (65). Hepatoblastoma appears to cluster in patients with mutations at the 5′ end of the gene (65,66). It is highly likely that this correlation between genotype and phenotype will become increasingly important in the future as a means of targeting genetic testing in FAP to the most likely regions, rather than screening the whole of such a large gene. The great variation in phenotypic appearance of germline *APC* mutations has led to the search for modifier genes that may influence the severity of FAP (67).

APC MISSENSE VARIANTS

Missense germline variants of *APC* have been described in non-FAP patients with multiple adenomas or a carcinoma developing at a young age. One particular missense variant, I1307K, is found in Ashkenazi Jews, and carriers of this allele are at several-fold higher risk of developing multiple adenomas and colorectal cancer (68–73). As the I1307K variant consists of a T→A substitution, producing a poly(A) tract, it was assumed that the variant precipitated polymerization errors during DNA replication, and thus indirectly predisposed to cancer (68). However, subsequent analysis has clearly indicated that I1307K acts because of a selective effect, probably due to dominant negative influence of mutations in this critical region of the *APC* gene.

Another germline *APC* variant, detected by single-strand conformational polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) analysis, is E1317Q. This mutation has been detected in patients with colorectal polyps or cancer, as well as in normal controls (69,74–76). E1317Q codes for a mutation in *APC* in the MCR that lies between the first and second 20-amino acid β-catenin binding sites. This mutation most probably acts, in a similar way to I1307K, through a dominant negative effect on the APC/β-catenin pathway, thus predisposing to adenoma formation (69).

Seemingly benign missense germline variants in APC may thus confer a growth advantage, and so carry a significant risk of developing colorectal tumours. Missense mutations have also been found in other candidate genes in the adenoma–carcinoma sequence, such as *MLH1*, *MSH2* and *CDH1* (E-cadherin). It has been suggested that such missense variants may contribute to multifactorial disease inheritance, possibly at low penetrance (77). As they have only a slight selective disadvantage for the individual, they may occasionally ‘drift’ up in frequency by chance. It is those that reach polymorphic or sub-polymorphic frequencies (namely significantly higher than rare mutations maintained by mutation selection balance) that are ascertained.

It seems very likely that this source of genetic variability will make a substantially larger contribution to the population load

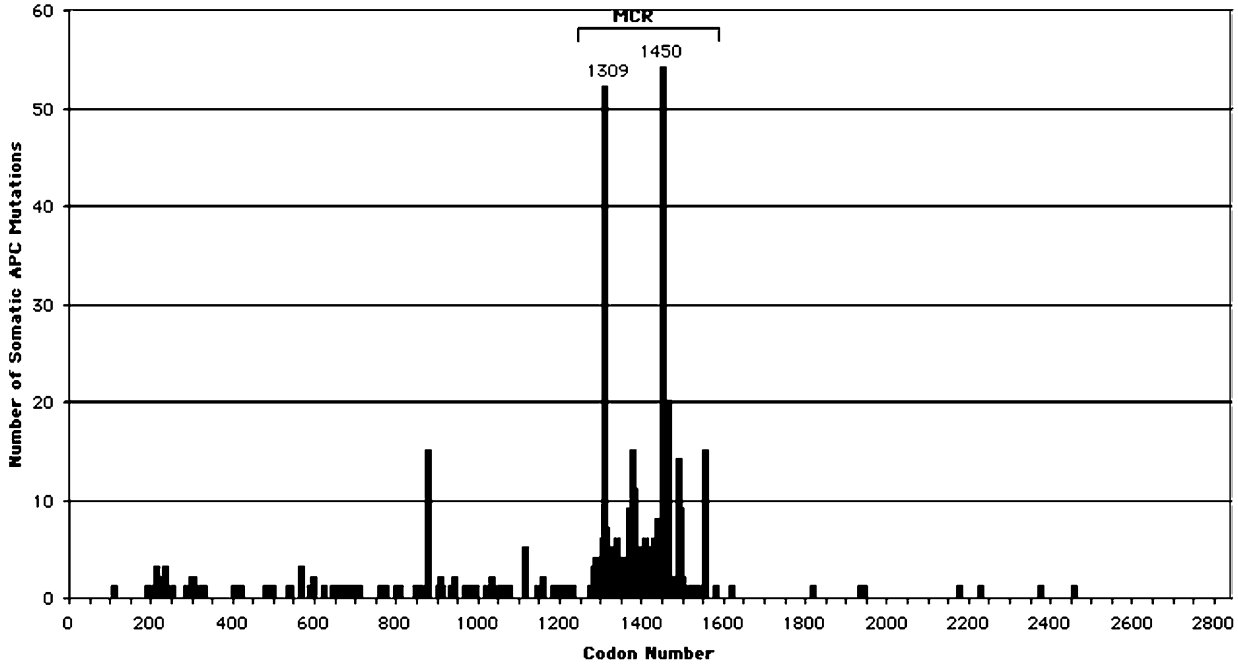

Figure 3. Distribution of somatic mutations found in the *APC* gene (n = 650) in colorectal tumours. Data derived from Thierry Soussi *APC* database at http://perso.curie.fr/Thierry.Soussi/APC.html. The MCR ranges between codons 1250 and 1550.

of cancer susceptibility than classical rare *APC* or mismatch repair gene missense variants, and may be as important collectively as the occasional more frequent polymorphic susceptibility. Further research into missense susceptibility variants may give important clues to gene function in colorectal cancer.

SOMATIC MUTATIONS IN *APC*

Even prior to the identification of the *APC* gene, it had been implicated in sporadic colorectal tumorigenesis by LOH studies (45). Somatic mutations result in loss of the wild-type *APC* allele in the majority of sporadic colorectal cancers (41,44). *APC* mutations occur early during colorectal tumorigenesis. Somatic mutations are found in the majority of colorectal adenomas and carcinomas, including adenomas <5 mm in size (78). In fact, inactivation of both alleles of *APC* occurs very commonly in colorectal cancers (42).

Over 60% of all somatic mutations in *APC* occur within <10% of the coding sequence of the gene between codons 1286 and 1513; this region is termed the MCR (44). Within the MCR, there are two hotspots for somatic mutations at codons 1309 and 1450 (40) (Fig. 3). *APC* mutation within the MCR results in a truncated APC protein that lacks all of the axin binding sites and all but one or two of its 20-amino acid β-catenin binding sites (see below).

Examination of the *APC* mutations present in a panel of colorectal cell lines has indicated that there is interdependence of the two hits on *APC* in sporadic colorectal cancer as well as in FAP-associated tumours (Fig. 4). *APC* mutations in the MCR are associated with allelic loss (LOH) while tumours with non-MCR mutations are coupled with truncating mutations (79). These data indicate that there is strong selective pressure for a more advantageous mutant than the *APC* mutant that first occurs.

The number of G:C→T:A transversions contributes <15% of all the somatic mutations in *APC* (Thierry Soussi database). This fact strongly indicates that the early stages of colorectal tumorigenesis do not occur in response to the presence of a mutagen. Any putative environmental causative agents are, at most, promotional (rather than mutagenic) in colorectal tumorigenesis (80).

APC PROMOTER METHYLATION

The *APC* gene has two promoter regions, 1A and 1B (81). Promoter 1A is most commonly active. Hypermethylation of the APC promoter region has been postulated as a possible second-hit mechanism in colorectal tumours where only one *APC* mutation is present. Initial work in *Min* mice with an overall reduction in DNA methyltransferase activity demonstrated a dramatic reduction in the development of intestinal tumours (82). The 1A promoter region of *APC* has been shown to be heavily methylated in colorectal cancers, but not in adenomas (83). More recent research, however, has indicated that hypermethylation of the *APC* promoter occurs in both colorectal adenomas and carcinomas, but not in adjacent normal colonic mucosa. Tumours with promoter hypermethylation also failed to express transcripts of APC (84). *APC* promoter 1A hypermethylation has been reported in a number of other human gastrointestinal tumours, including oesophageal, gastric, pancreatic and hepatic cancers (84,85), but also occurs in normal gastric mucosa (85). There is no evidence for such an epigenetic mechanism with the *APC* 1B promoter. These findings suggest that *APC* promoter 1A hypermethylation may provide an alternative mechanism of *APC* inactivation in the early

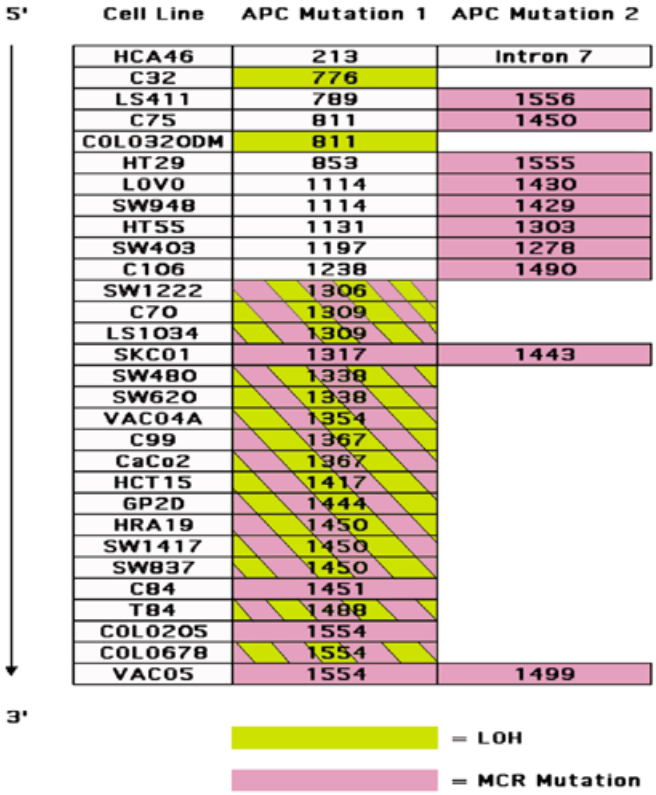

Figure 4. Diagram showing the interdependence of the two hits on *APC* in colorectal cancer cell lines. MCR mutations are shaded in pink and LOH in yellow. Where the first mutation lies within the MCR, the second hit results in allelic loss (LOH). Where the first mutation occurs outside the MCR, the second falls within it.

stages of colorectal tumorigenesis, but may be a normal event in gastric mucosa. Nevertheless, the preponderance of *APC* mutations and LOH at the *APC* locus in colorectal tumours precludes hypermethylation from being a major event in this process.

THE DOMAINS AND FUNCTIONS OF THE APC PROTEIN

The APC protein consists of an oligomerization domain and an armadillo region in the N-terminus, a number of 15- and 20-amino acid repeats in its central portion, and a C-terminus that contains a basic domain and binding sites for EB1 and the human disc large (HDLG) protein (Fig. 5). The multiple domains of the APC protein allow it to interact with numerous protein partners. The APC protein is an integral part of the wnt-signalling mechanism, but also plays a role in cell–cell adhesion, stability of the microtubular cytoskeleton, cell cycle regulation and possibly apoptosis (Fig. 6). Each domain of APC is described in conjunction with its functional importance.

THE OLIGOMERIZATION DOMAIN

The heptad repeats that occur within the oligomerization domain at the N-terminus of the APC protein allow APC to form homo-dimers (86). Retention of amino acids 6–57 in APC is essential for this oligomerization (87). The presence of an oligomerization domain at the N-terminus means that wild-type APC may form dimers with both wild-type and truncated mutant APC proteins (88). If the amount of available wild-type APC is reduced, not just by the presence of a mutant protein, but also by dimerization of the remaining wild-type APC with mutant protein, it is feasible that APC mutants may elicit a dominant negative effect in reducing the tumour suppressor function of APC (41,77,86,89).

THE ARMADILLO REGION

The armadillo region consists of seven repeats and shows a high degree of homology to a similar area in β-catenin and its *Drosophila* homologue, the segment polarity protein armadillo (35). This domain is highly conserved and invariably retained in mutant APC proteins (44). The armadillo region of APC has been shown to bind to the regulatory B56 subunit of protein phosphatase 2A (PP2A) (90), an enzyme that also binds axin via its catalytic subunit (91). This finding has led to the sugges-

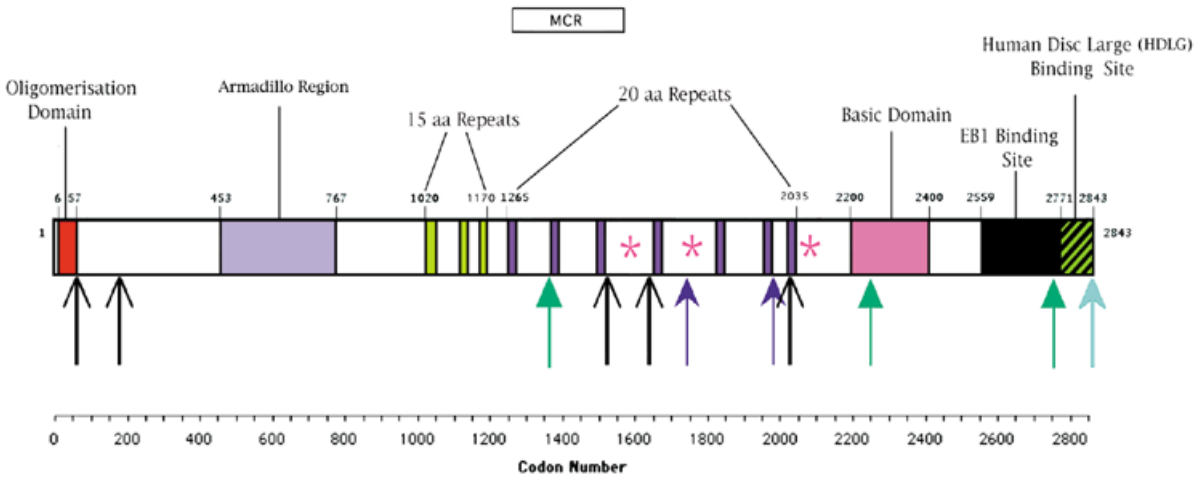

Figure 5. Functional domains of APC protein. Pink asterisks indicate SAMP repeats of axin binding sites. Black arrows mark active NESs. Purple arrows indicate sites of NLSs. Green arrows mark the positions of putative DNA binding sequences. The pale blue arrow indicates the PTP-BL binding site. Note that the 3′ end of the MCR falls before the first axin binding site.

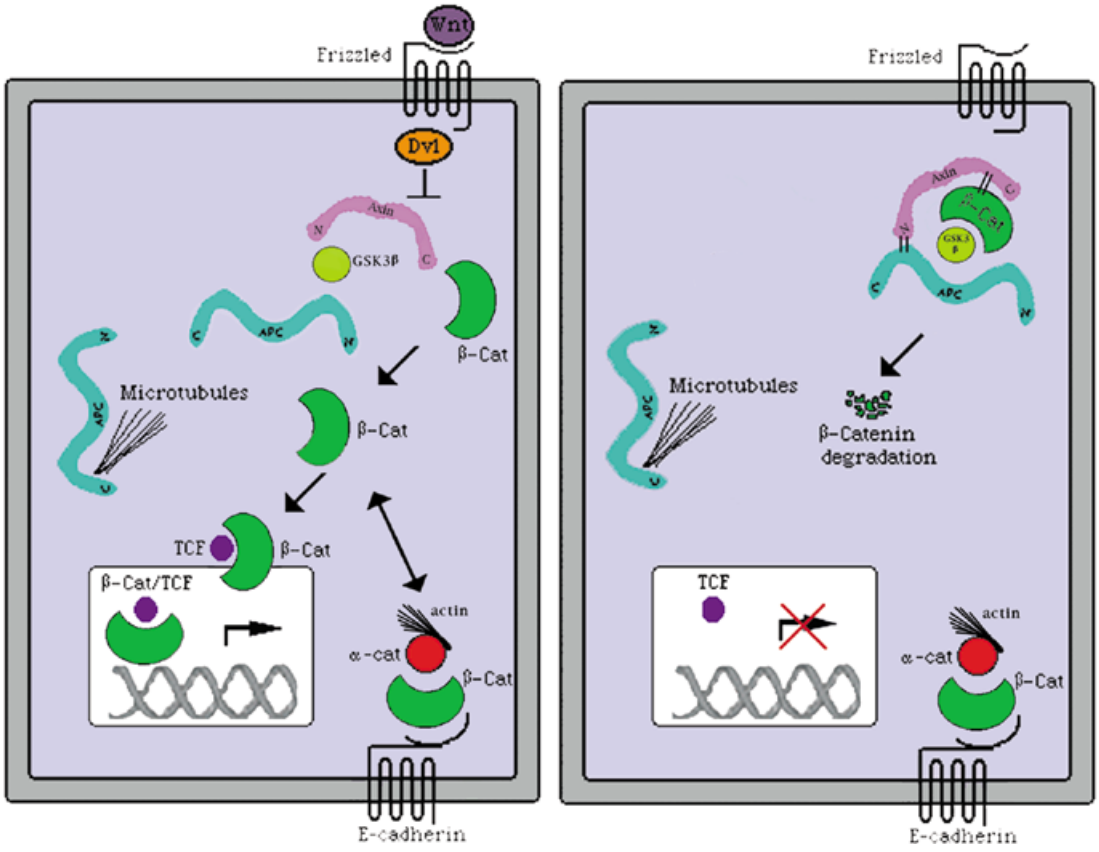

Figure 6. The interactions of APC in wnt signalling and cell adherence. The left-hand diagram shows that wnt signalling results in stabilization of β-catenin and subsequent transcription activation. The right-hand diagram illustrates β-catenin degradation in the absence of a wnt signal.

tion that PP2A may act as an antagonist to the glycogen synthase kinase 3β (GSK3β) phosphorylation of β-catenin that marks the latter for degradation (90). The armadillo region is also known to bind to the APC-stimulated guanine nucleotide exchange factor (Asef), which acts as a guanine nucleotide exchange factor (GEF) for the Rac and Rho GTP binding proteins. This finding suggests an alternative role for APC in stabilization and motility of the actin cytoskeleton network (92). While it is probable that the armadillo domain is essential for cellular survival, it is unlikely that it plays an integral role in the tumour suppressor function of APC.

THE 15-AMINO ACID REPEATS

Three 15-amino acid signature repeats occur between amino acids 1020 and 1169, providing binding sites for β-catenin (93,94). Binding of β-catenin to these 15-amino acid sites on APC does not mark β-catenin for subsequent downregulation (95) unlike binding at the 20-amino acid sites (see below). As with the armadillo region, the 15-amino acid repeats are retained in the majority of mutant APC proteins. This is borne out by the fact that both wild-type and mutant APC can bind β-catenin (93,94). The 15-amino acid β-catenin binding sites are unique to APC as they do not resemble the β-catenin binding sites in the cadherin family of proteins (96–98).

THE 20-AMINO ACID REPEATS

The central region of the APC protein contains a series of seven 20-amino acid repeat motifs, each of which carries the signature TPXXFSXXXSL (34). Fragments of APC containing these repeats bind β-catenin (98) but only a single 20-amino acid repeat is essential for such binding (99). β-catenin binding at these sites on APC only occurs after phosphorylation of each site by GSK3β, as with the 15-amino acid repeats (95,100). β-catenin binds to APC and axin in a complex (see below) that promotes GSK3β-mediated phosphorylation of its own serine and threonine residues (101,102). In this way, β-catenin is marked for subsequent degradation by ubiquitin-mediated proteolysis (103–107). Downregulation of β-catenin is dependent on the presence of at least three of the seven 20-amino acid repeats in APC (99). The 3′ limit of the somatic MCR in the *APC* gene falls at codon 1513 (44), which coincides with the 3′ end of the third 20-amino acid repeat. The majority of truncated mutant proteins lack all or most of the 20-amino acid repeats, suggesting that this area is a target for elimination during tumorigenesis (88).

THE ROLES OF APC AND β-CATENIN IN WNT SIGNALLING

If β-catenin is not phosphorylated and therefore not broken down, it accumulates within the cellular cytoplasm and nucleus

(108). Accumulation of β-catenin may occur as the result of wnt-signal (109,110) (see below), by inactivation of APC, or by direct mutation of β-catenin itself (111). Inside the nucleus, β-catenin associates with members of the T cell factor (TCF) and lymphoid enhancer factor (LEF) family of transcriptional activators (112). TCF4 is expressed in the nuclei of intestinal epithelial cells (113). β-catenin and TCF/LEF form a complex that activates transcription of target genes (114–116). Mutations in the *APC* or *CTNNB1* (β-catenin) genes that prevent GSK3β-mediated phosphorylation and subsequent β-catenin degradation ultimately result in β-catenin pooling (95,101,102,117) and activation of the β-catenin transcription (99,118). Introduction of wild-type APC protein into cell lines with truncating *APC* mutations reduces the available pool of cytoplasmic β-catenin (95,119) and therefore also reduces TCF4/β-catenin mediated transcriptional activity (113). β-catenin has at least two separate transcriptional activation domains (TADs), which probably act in collaboration (120,121). The TADs on β-catenin have been demonstrated to react directly and specifically with the TATA binding protein involved in transcriptional activation (121). Targets for transcriptional activation by β-catenin include the oncogene c-*myc* (122) and cyclin D1 (123,124), both of which regulate cell cycle progression. Other possible targets include the gap junctional protein connexin 43 (125) and the metalloproteinase matrilysin (126). Recent evidence suggests that TCF1 is also a transcriptional target of the TCF4/β-catenin complex and may thus provide a negative feedback mechanism for the complex (112).

AXIN BINDING SITES ON APC

Axin is the human homologue of the murine Fused protein (127,128). Axin binds to APC at binding sites present within the region of the β-catenin binding 20-amino acid repeats (129,130). Axin binds to APC via its regulator of the G protein signalling (RGS) domain (129,130). A similar domain on the axin homologue conductin also binds to the same region of APC (131). The axin binding sites on APC lie between the third and fourth, between the fourth and fifth and after the seventh 20-amino acid repeats. Each axin binding site contains the characteristic SAMP amino acid sequence, which if altered results in the abolition of axin binding to APC (131). The RGS domain of axin appears to have a tertiary structure distinct from that of other members of the RGS family (132). Both axin and conductin also have adjacent binding sites for GSK3β and β-catenin in their central regions (129,131,133–135), as well as a C-terminus Dix (Dishevelled and axin) domain for homodimerization (91,136,137). Overexpression of axin stimulates increased degradation of β-catenin (130,134,138).

Axin appears to act as a scaffold protein in the formation of a multiprotein complex with APC and β-catenin, which then facilitates phosphorylation of both APC (129,139) and β-catenin (133,136) by GSK3β. Phosphorylation of APC results in improved β-catenin binding (100) with the ultimate consequence of enhanced APC-mediated GSK3β phosphorylation and the subsequent degradation of β-catenin (140). Axin thus acts as a negative regulator within the wnt-signalling pathway by reducing the amount of β-catenin available for transcriptional activation (110,135,141). Absence of a wnt signal allows GSK3β-mediated phosphorylation of axin and efficient binding to APC and β-catenin. Activation of the wnt receptor (a member of the Frizzled gene family) by a wnt signal is transduced by the cytoplasmic protein Dishevelled (Dvl) (142–145). Dvl forms a complex with axin (146) and induces its dephosphorylation (147,148). This reduces the capacity of axin to form complexes with APC and β-catenin, and ultimately results in accumulation of β-catenin and transcriptional activation (149). Upstream modulation of this effect is achieved by the presence of a binding site for PP2A on axin (91). Axin complexed with PP2A has been shown to dephosphorylate APC at sites previously phosphorylated by GSK3β (139), and overexpression of the B56 subunit of PP2A results in reduced levels of β-catenin (90).

THE ROLES OF APC AND β-CATENIN IN INTERCELLULAR ADHESION

The fact that β-catenin binds to APC implies that APC may also have a role in epithelial cell adhesion. The catenins α-catenin, β-catenin and plakoglobin (γ-catenin) are all associated with cadherins that mediate intercellular adhesion (150,151). E-cadherin is responsible for cell–cell adhesion in epithelial cells, with its catenin binding cytoplasmic domain being essential for this function (96,97,152,153). The cytoplasmic domain in cadherins shares the SLSSL sequence found in four of the seven 20-amino acid repeats of APC (154). β-catenin localizes to the zonula adherens junction and interacts with E-cadherin to link the latter to α-catenin (154,155) and thence to the actin network (153,156,157). The interaction between E-cadherin and β-catenin is regulated by tyrosine phosphorylation of the latter (158). Cytoplasmic APC predominantly accumulates at the leading edges of cells (159–161), an observation that depends on the presence of an intact microtubule (but not actin) network (162). The interaction of APC with the microtubular cytoskeleton will be considered in more detail below. The *Drosophila* epithelial homologue of APC, E-APC, requires actin filaments to maintain its localization at the adherens junction (163,164). APC contributes to orderly migration of intestinal cells within the intestinal crypt and β-catenin plays a crucial role in this function (165). Indeed, if APC is overexpressed, migration of murine epithelial cells becomes disordered (166). APC and E-cadherin compete for binding sites in the region of the armadillo repeats on β-catenin (98,167,168) but, whereas binding one excludes the other, it appears that separate (though overlapping) regions of β-catenin mediate its roles in wnt signalling and cell adhesion (169). It is certainly likely that affecting one pathway will impact upon the other (117,170,171), but the precise nature of this interaction remains unclear (163,172).

In addition to APC and E-cadherin, the armadillo region of β-catenin interacts with the cytoplasmic domain of the epidermal growth factor receptor (EGFR) (173). Cellular junctional proteins are common targets for soluble growth factors and cytokines including epidermal growth factor (EGF) and the trefoil peptides (174). EGF binds to the extracellular domain of EGFR and thus effects a variety of changes within the cytoskeleton and in cell motility (175). Intestinal trefoil factor 3 appears to interact with both APC and E-cadherin in complexes that modulate epithelial cell adhesion, migration and survival (176). Insulin-like growth factor-1 (IGF1) has been shown to stabilize cytoplasmic β-catenin (177). It is thus

conceivable that changes in cytoplasmic β-catenin levels may also impact on cellular adhesion and motility by interaction with growth factors and their receptors, and vice versa.

THE BASIC DOMAIN IN APC

The basic domain lies within the C-terminus of APC between amino acids 2200 and 2400 (34). The domain derives its name from the large proportion of (basic) arginine and lysine residues in this region, but it also contains an unusually high percentage of proline residues. This combination suggests that the basic domain is probably a microtubule binding site, a theory that has been corroborated by observations that the C-terminal of APC binds microtubules and stimulates polymerization of tubulin *in vitro* (178,179). More precisely, an APC fragment containing amino acids 2219–2580 has been shown to bind to unassembled tubulin and promote tubulin assembly *in vitro* (180). Truncated APC proteins found in colorectal tumours seldom retain the basic domain.

THE EB1 BINDING DOMAIN OF APC

The C-terminus of APC also contains a binding site for the end-binding protein EB1 (181). EB1 has been found to be closely associated with the centromere, mitotic spindle and distal (plus) tips of microtubules at all stages of the cell cycle (182–184). Studies in yeast suggest that EB1 may be involved in a checkpoint mechanism in the cell cycle (185,186). The presence of EB1 at the plus end of the microtubule occurs independently of APC (182,183), as does its interaction with the dynactin complex (187). EB1 is thus in an ideal position for linkage with other structures within the cell (188). APC has also been shown to localize to the microtubule cytoskeleton (162) and, more specifically, to the plus ends of growing microtubules (189). If the EB1 binding domain is eliminated from the APC protein, the latter can still bind to microtubules (via its basic domain) but does so indiscriminately (190). These findings suggest that EB1 directs APC to the microtubule tips, and may thus facilitate the interaction of APC with other specific sites at the cell membrane (188). It is intriguing to note that aneuploidy is a significant feature of sporadic colorectal cancer and that the majority of such cancers carry APC mutations resulting in truncated APC proteins lacking the C-terminus (44). However it is unlikely that the breakdown in genomic stability during chromosome segregation arises from the elimination of the EB1 binding domain on APC, as a number of replication error positive (RER+) APC-mutant near-diploid colorectal cell lines exist (79). EB1 itself does not appear to play a direct role in tumorigenesis; somatic mutations in the *EB1* gene have not been found in colorectal tumours (191) and transgenic mice with a truncated version of APC lacking the EB1 binding site are not at increased risk of gastrointestinal tumours (192).

THE HDLG BINDING SITE ON APC

HDLG is the human homologue of the *Drosophila* discs large tumour suppressor protein. The C-terminus of APC binds to HDLG, an association that is abolished by deletion of the final 72 amino acids of APC, whereas an APC fragment containing only these 72 amino acids associates strongly with HDLG (193). The presence of the characteristic PDZ binding motif S/TXV within the C-terminus gives further indirect evidence of an HDLG binding site in this region (193). Overexpression of APC suppresses cell cycle progression from the G_0/G_1 to S phase (194) with recent evidence suggesting that the APC–HDLG complex is responsible for this effect in a manner independent of the effect of β-catenin on the cell cycle (195). The VTSV motif at the extreme C-terminus of APC also binds the protein tyrosine phosphatase PTP-BL via its PDZ2a domain with some evidence that APC may modulate tyrosine phosphorylation on proteins interacting with it, such as β-catenin and GSK3β (196).

FUNCTIONAL ROLE OF APC IN THE NUCLEUS

It has been recognized for some time that APC is found within the nucleus, as well as in the cytoplasm (161,166,176). More recent evidence suggests that APC may shuttle β-catenin between the nucleus and the cytoplasm (197–199). These studies differ, however, in the precise location of the pertinent nuclear export signals (NESs). Henderson (197) identified three putative NESs at amino acids 68–77, 165–174 and 1472–1481, each of which carried the motif LXXXLXXLXL or VXXXVXXVXV. Only the first two NESs were shown to be functionally active with the 68–77 NES showing stronger export activity than the 165–174. Deletion of these two motifs prevented CRM1-dependent nuclear export of APC, and inhibition of CRM1 with leptomycin B (LMB) led to nuclear accumulation of APC. Nuclear localization of β-catenin was also regulated by CRM1, but was dependent on the presence of wild-type APC. Neufeld *et al.* (200) provided corroboratory evidence for the presence and functional importance of the two N-terminal NESs in APC. Rosin-Arbesfeld *et al.* (198) confirmed that the APC shuttling of β-catenin from the nucleus was dependent on CRM1, but also noted that C-terminal fragments of APC appeared to be excluded from the nucleus, suggesting that this region contained an active NES. They demonstrated three functional NESs in human APC, each of which contained the repeat motif LXXLXL/I/M/V. These NESs corresponded precisely with the third, fourth and seventh 20-amino acid repeats in APC. Two further putative NES sites were noted, one in the N-terminal and the other immediately after the armadillo region. It is worth noting that the 3′ end of the somatic MCR coincides with the NES in the third 20-amino acid repeat, suggesting a strong selective pressure to eliminate these NESs during tumorigenesis.

It has been demonstrated that β-catenin may enter the nucleus independently of a nuclear import signal by binding directly to the nuclear pore machinery (201). APC, on the other hand, is too large to enter the nucleus without the presence of a nuclear localization signal (NLS), which can be recognized by the importins that mediate nuclear translocation in an energy-dependent manner. Zhang *et al.* (199) have identified two NLSs spanning amino acids 1767–1772 and 2048–2053 in APC; both of these were found to be necessary for optimal nuclear import of wild-type APC. Their findings also suggested that phosphorylation of the NLS may inhibit nuclear import of APC, thus providing a regulatory mechanism for nucleocytoplasmic shuttling. It is nevertheless likely that other NLSs exist in APC, as mutant APC proteins lacking both the above NLSs have been demonstrated in the nucleus (192,197).

Consistent with its presence within the nucleus, APC can interact directly with DNA. Three potential DNA binding sites have been mapped, each containing a cluster of between three and five S/TPXX repeat sequences. The first DNA binding site overlaps the proximal part of the second 20-amino acid repeat, the second falls within the basic domain and the last lies close to the C-terminus of APC. These domains bind preferentially to A/T-rich DNA sequences and may directly or indirectly regulate transcription (202). If APC were shown to interact simultaneously with microtubules and with DNA, this would indicate a direct role in cell division.

APC AND APOPTOSIS

In normal human colon, APC expression is limited to cells in the luminal part of the crypt (159,203). Cells are shed from the luminal surface after undergoing apoptosis (programmed cell death). Intercellular contact and specific cytokines provide protection against apoptosis in colonic epithelial cells (204). Adenomatous epithelial cells show disordered patterns of apoptosis but remain susceptible to apoptotic signals (205). It is possible that APC may also play an indirect role in regulation of apoptosis, as the induction of expression of wild-type APC in a cancer cell line with mutant APC increased cell death through apoptosis (206). There is preliminary evidence that the region of APC that may be involved in regulation of apoptosis coincides with the region that mediates β-catenin binding and degradation (207). As apoptosis is associated with release of cells from the extracellular matrix (ECM) or from cellular contacts (208,209) it is conceivable that APC may indirectly impact on intercellular adhesion and the ECM, thus providing the stimulus for apoptosis.

CONCLUSIONS

Mutation in the *APC* gene is the basis of inherited predisposition to colorectal cancer in FAP and is also the primary event in initiation of sporadic colorectal tumours. It is still an enigma as to why *APC* mutations have been found in so few other tumour types. It is most likely that the role of APC in wnt-signalling is responsible for its role in colorectal carcinogenesis. Mutant APC may also disrupt intercellular adhesion and stability of the cytoskeleton, both of which play a part in cancer progression. Further research into the contribution of low-penetrance *APC* mutations or missense variants to inherited risk of colorectal cancer is required. Improved understanding of both the genetics and biology of APC may, in time, culminate in preventative or therapeutic strategies specifically targeted at reducing the burden of colorectal cancer.

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