



Published in final edited form as:

*Gastroenterology*. 2002 September ; 123(3): 935–939. doi:10.1053/gast.2002.35773.

## APC as a Checkpoint Gene: The Beginning or the End?

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Colorectal cancer (CRC) is one of the most common malignancies in the United States. In 2002, it is estimated that 148,300 new cases of CRC will be diagnosed, resulting in approximately 56,600 deaths.<sup>1</sup> The latter renders CRC the second leading cause of deaths from cancer in the United States. An individual's life-time risk of developing CRC is 6%, with greater than 90% of cases occurring after age 50.<sup>2</sup> Together, these facts indicate that CRC is an important health concern.

It has been recognized for some time that cancer is the result of sequential mutations in key genes, such as oncogenes and tumor-suppressor genes. The existence of hereditary predisposition to certain cancers including CRC often paves the foundation for identifying genes whose mutations are involved in the formation of that particular cancer. Thus, the identification of the adenomatous polyposis coli (*APC*) gene as the gene mutated in the germline DNA of patients affected by familial adenomatous polyposis (FAP) was heralded as a major breakthrough in understanding the molecular pathogenesis of CRC.<sup>3,4</sup> Indeed, since its identification in 1991, there have been over 2000 studies published on *APC*. These studies demonstrate that mutations in the *APC* gene are not only important for the pathogenesis of FAP, but are responsible for the majority (>80%) of sporadic cases of CRC.<sup>5</sup> They also indicate that mutations in *APC* occur early in the tumorigenic process, netting it the name "gatekeeper."<sup>6</sup>

*APC* turns out to be a remarkably fascinating gene. It encodes a large protein consisting of 2843 amino acids and has a predicted molecular weight of greater than 300,000 kilodaltons. It is present in a variety of epithelial tissues, usually in cells that are postmitotic.<sup>7</sup> Immunocytochemical studies show that *APC* is often diffusely distributed in the cytoplasm, although it can sometimes be found in the apical or lateral regions of epithelial cells.<sup>7</sup> Studies indicate that *APC* participates in a variety of cellular functions including proliferation, differentiation, apoptosis, adhesion, migration, and chromosomal segregation.<sup>8</sup> To date, the question remains: Which of these functions is critically involved in the tumorigenic process when *APC* is mutated?

A glimpse to the answer may be provided by knowing which proteins interact with *APC*. Figure 1 shows the various domains within *APC* that interact with other proteins. The armadillo repeat in the N terminal portion, for example, binds to the B56 regulatory subunit of protein phosphatase 2A and *APC*-stimulated guanine nucleotide exchange factor.<sup>8</sup> These 2 proteins may be involved in the Wnt signaling pathway, of which *APC* is a component.<sup>9,10</sup> Another important domain includes three 15-amino acid repeats that bind  $\beta$ -catenin and seven 20-amino acid repeats that are required for the down-regulation of  $\beta$ -catenin.<sup>11,12</sup> Sites in *APC* have

also been identified that interact with axin and conductin,<sup>13,14</sup> 2 inhibitory proteins of the Wnt signaling pathway. The C terminal portion of the protein is involved in binding to microtubules and a tubulin-binding protein, EB1.<sup>15</sup> Lastly, *APC* contains both nuclear export and import signals,<sup>16,17</sup> suggesting that shuttling protein partners between the nucleus and cytoplasm may be a function of *APC*.

Mutational analysis of the *APC* gene indicates that the majority of germline mutations found in FAP patients are nonsense mutations, leading to the formation of a truncated protein.<sup>4</sup> More than 60% of the *APC* mutations are found in the central region (between codons 1284–1580) of the protein, which is called the mutation cluster region (MCR).<sup>18</sup> The fact that MCR coincides with the region in *APC* important for down-regulation of  $\beta$ -catenin suggests that this function is important for the pathogenesis of CRC. Subsequent studies demonstrate that *APC* and  $\beta$ -catenin are important parts of the Wnt signaling pathway. As seen in Figure 2A, cells are quiescent in the absence of Wnt signaling. This is caused by the interaction among axin/conductin, *APC* and glycogen synthase kinase 3 $\beta$  (GSK) targets  $\beta$ -catenin for degradation by the ubiquitination pathway.<sup>19</sup> In the absence of free  $\beta$ -catenin, gene transcription directed by the T-cell factors (TCFs) is silenced by repressors such as CtBP and Gro. Upon binding of Wnt to its member receptor frizzled, a protein called “disheveled” is activated.<sup>20</sup> This leads to the dissociation of the complex formed by axin/conductin, *APC* and GSK, resulting in the accumulation of free cytoplasmic  $\beta$ -catenin (Figure 2B).  $\beta$ -catenin is then translocated to the nucleus where it forms a complex with TCFs,<sup>21</sup> resulting in the activation of gene transcription and subsequent cell proliferation. Mutations in *APC* have the same effect as Wnt signaling in destabilizing the axin/*APC*/GSK complex. Consequently, mutations in axin or conductin have been found in cancers.<sup>22,23</sup> Alternatively, mutations resulting in the constitutive activation of  $\beta$ -catenin no longer subject to the inhibitory effect of axin/*APC*/GSK have also been identified in CRC in the absence of *APC* mutation.<sup>24</sup>

How does the  $\beta$ -catenin/TCF complex induce cell proliferation? This, in large part, depends on the target genes regulated by this transcriptional complex. In this issue of *GASTROENTEROLOGY*, Heinen et al.<sup>25</sup> reports that a mechanism by which *APC* inhibits cell proliferation is to control entry into the S phase of the cell cycle by regulating  $\beta$ -catenin/TCF. This study stems from the previous observations that the oncogene *c-MYC* and the cell cycle regulator *cyclin D1* are 2 direct targets of the  $\beta$ -catenin/TCF complex.<sup>26,27</sup> Both *c-MYC* and cyclin D1 are involved in the transition between the G1-S checkpoint of the cell cycle and do so by influencing the activity of the retinoblastoma tumor-suppressor pRB.<sup>28</sup> Using primarily transient transfection, Heinen et al.<sup>25</sup> showed that colon cancer cells transfected with wild-type *APC* became arrested in the G1 phase of the cell cycle. This cell cycle arrest was abrogated by cotransfection with either a constitutively activated  $\beta$ -catenin or *c-MYC* and cyclin D1 combined. Moreover, they showed that *APC* inhibits pRB phosphorylation (thus inhibiting the release of E2F from pRB) and decreases levels of cyclin D1. They conclude that *APC* functions upstream of pRB and that both *c-MYC* and cyclin D1 are involved in *APC*-mediated regulation of the G1/S boundary of the cell cycle.

Is the cell cycle effect of *APC* reported by Heinen et al.<sup>25</sup> sufficient in providing an explanation for the formation of CRC when *APC* is mutated? To address this question, one needs to consider what steps a cell must take in order for it to become cancerous. There is a minimum of 2 requirements: the cell must acquire a selective advantage to allow for its initial clonal expansion and genetic instability to allow for mutations in other genes responsible for the process of malignant transformation. The latter behavior constitutes a common phenotype in CRC called chromosomal instability (CIN). CIN tumors have a defect in chromosomal segregation, which is manifested by both qualitative and quantitative variations in chromosome numbers.<sup>29</sup> Recent studies have shown that *APC* mutations can give rise to chromosomal instability<sup>30, 31</sup> and may elicit the CIN phenotype in synergy with other potent oncoproteins or tumor

suppressors. This effect is likely dependent on the interaction between *APC* and EB1 and possibly between *APC* and microtubules. Combining the results of study by Heinen et al.<sup>25</sup> and the CIN studies, *APC* seems to fulfill both requirements cited above for malignant transformation.

The importance of the cell cycle checkpoint function of *APC* during tumor formation can also be revealed by studying mouse models of CRC in which mice with various germline mutations in the *APC* gene have been generated. The classic mouse model is the *APC<sup>Min</sup>* mouse.<sup>32</sup> *APC<sup>Min</sup>* carries a nonsense mutation in codon 850 of the *APC* gene and no longer contains the region required to regulate  $\beta$ -catenin. Heterozygous *APC<sup>+/Min</sup>* animals develop numerous adenomas in their intestinal tract. A second model has since been developed that involves a truncating mutation at codon 1638 of the *APC* gene (*APC<sup>1638T</sup>*).<sup>33</sup> The *APC<sup>1638T</sup>* mutation truncates the C terminal portion of *APC* responsible for CIN-related functions but retains the  $\beta$ -catenin regulatory domain. Consequently, embryonic stem (ES) cells isolated from homozygous *APC<sup>1638T</sup>* animals are CIN.<sup>31</sup> However, the corresponding mice are viable and tumorfree. These observations underscore the importance of selective advantage provided by the loss of  $\beta$ -catenin control in tumor formation and argues against the ability of chromosomal instability to initiate the transformation process.

The findings by Heinen et al.<sup>25</sup> also carry potential therapeutic implications. If the checkpoint function of *APC* is involved in the initiation of CRC, therapies directed toward restoring the  $\beta$ -catenin down-regulatory activity of *APC* or inhibiting the overactive  $\beta$ -catenin may be used in the prevention of CRC, especially in high-risk individuals such as FAP patients. In contrast, one may wish to target the CIN-related function of *APC* in later stages of tumorigenesis. Additional studies will help differentiate the effectiveness of these 2 strategies.

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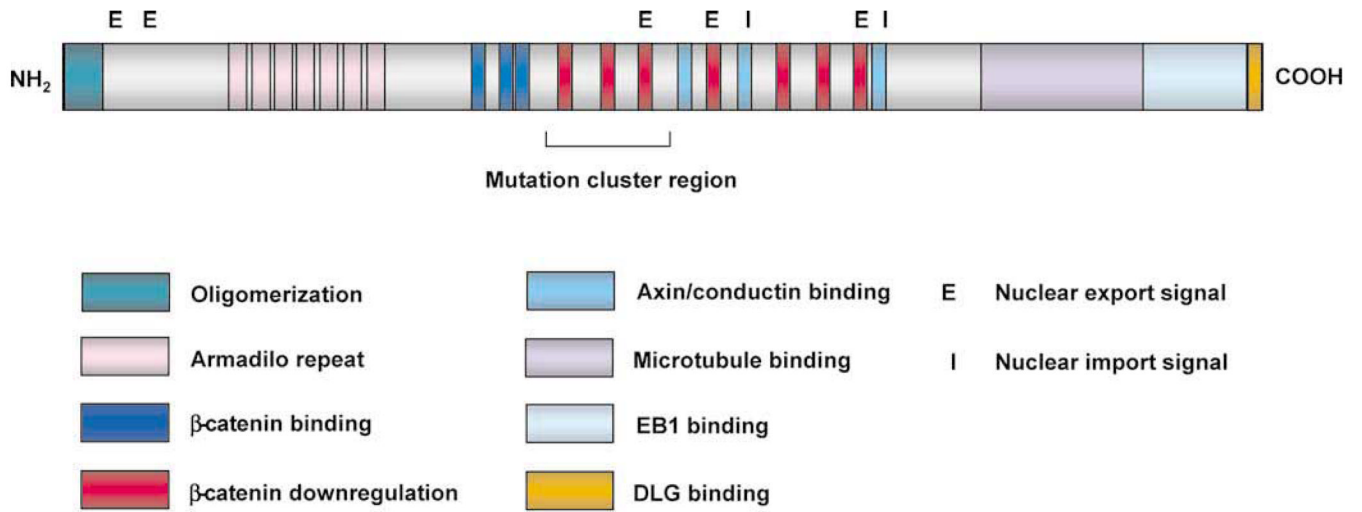
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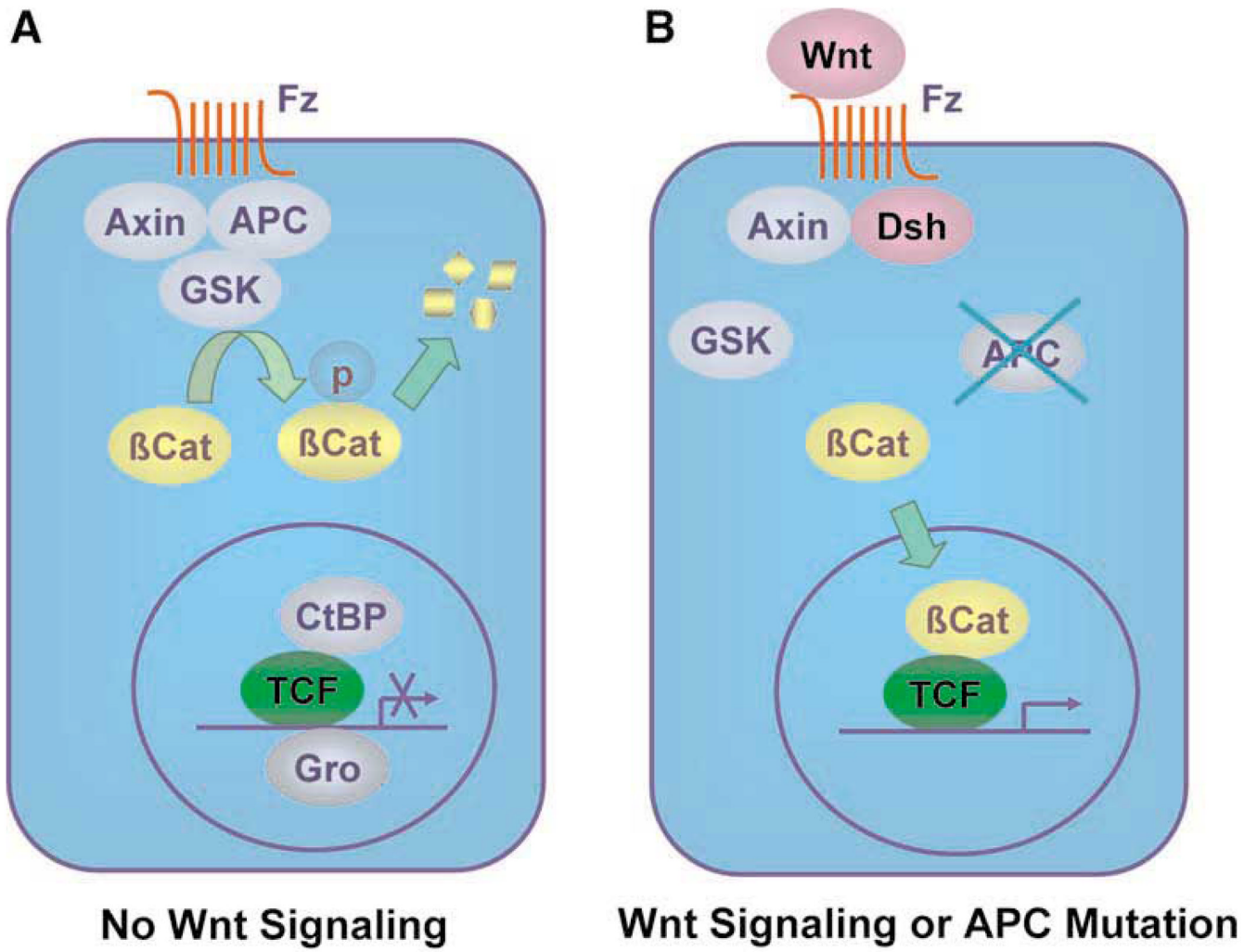
## Acknowledgments

Supported by grants from the National Institutes of Health (DK52230 and CA84197).



**Figure 1.**  
The different domains of the *APC* protein.





**Figure 2.**  
The signaling pathways in (A) the absence and (B) presence of Wnt.