tight structural constraints upon the class II peptide. Only slight deviations from peptide to peptide occur in the backbone conformation of the central residues (see the figure). This seemingly general conformation for all class II peptides (6) is more restricted in nature than its class I counterpart (7).

In the new work on murine I-E^k MHC, Fremont *et al.* (1) examine why peptide loading is enhanced at the low pH of the endosomal-like compartment where peptide exchange takes place. Foreign peptide substrates are produced by proteolytic degradation of invading microbial pathogens by way of the endocytic pathway. An exchange is required so that the CLIP peptide (derived from the Invariant chain), acquired by MHC en route from the endoplasmic reticulum, is substituted by the foreign peptide antigen (8). A DR3-CLIP peptide structure (2) revealed that this "universal" peptide is bound like other peptides except that the specificity pockets for the central peptide side chains may not be optimally filled. Still, this complex is relatively stable even at low pH (2), so another MHC lookalike, HLA-DM, is required for timely catalysis of this exchange (9, 10).

Fremont *et al.* propose that a pair of conserved acidic residues in the DR1 and I-E MHC molecules at the bottom of the binding groove ensures that loading is favorable only at low pH, conditions under which protonation of these acidic residues is enhanced. However, these acidic residues also participate in a continuous hydrogen bonding network (1) that includes the same Asn and Gln residues that influence the polyproline II peptide conformation. This network could also orient the Asn and Gln side chains to guide proper polarity and orientation of the peptide in the binding site. SRC homology 3 domain (SH3) molecules also bind polyproline II peptide structures, but can do so in both directions (11). Because of their proline-rich nature, their backbone atoms are more restricted in their availability for hydrogen bonding, so they also make use of other interactions with peptide side chains to orient the peptide (11).

Do all class II molecules adopt the same peptide recognition strategy? Other class II families, such as human DQ and murine I-A, have substantially different α chains but retain the same hallmark peptide binding residues that include all of the key Asn's (12). Nonclassical class I molecules, such as H2-M3, which bind formylated hydrophobic peptides, have indeed provided interesting diversity to the class I recognition story (13). What is already clear is that these MHC class I and class II molecules use somewhat different strategies to form stable, high-affinity peptide complexes, but within the context of the same overall MHC fold. So what of CD1, another distantly related MHC class I which binds diverse homolog antigens, such as fatty acids and lipoglycans derived from mycobacterial cell walls (14, 15)? It will be interesting to see how the MHC fold has adapted to binding such disparate ligands.

References

1. D. H. Fremont, W. A. Hendrickson, P. Marrack, J. Kappler, *Science* **272**, 1001 (1996).
2. J. L. Stern *et al.*, *Nature* **368**, 215 (1994).
3. P. Ghosh, M. Amaya, E. Mellins, D. C. Wiley, *ibid.* **378**, 457 (1995).
4. R. L. Stanfield and I. A. Wilson, *Curr. Opin. Struct. Biol.* **5**, 103 (1996).
5. A. Rudensky, P. Preston-Hurlburt, S. C. Hong, A. Barlow, C. Janeway, *Nature* **353**, 622 (1991).
6. T. S. Jardetzky *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 734 (1996).
7. H. C. Guo *et al.*, *Nature* **360**, 364 (1992).
8. P. Cresswell, *Cell* **84**, 505 (1996).
9. L. K. Denzin and P. Cresswell, *ibid.* **82**, 155 (1995).
10. V. S. Sloan *et al.*, *Nature* **375**, 802 (1995).
11. S. Feng, J. K. Chen, H. Yu, J. A. Simon, S. L. Schreiber, *Science* **266**, 1241 (1994).
12. L. J. Stern and D. C. Wiley, *Structure* **2**, 245 (1994).
13. C. R. Wang *et al.*, *Cell* **82**, 655 (1995).
14. E. M. Beckman *et al.*, *Nature* **372**, 691 (1994).
15. P. A. Sieling, D. Chatterjee, S. A. Porcelli, T. I. Prigozy, *Science* **269**, 227 (1995).
16. H. Kozono, J. White, J. Clements, P. Marrack, J. W. Kappler, *Nature* **369**,151 (1994).
17. D. R. Madden, D. N. Garboczi, D. C. Wiley, *Cell* **75**, 693 (1993).
18. M. L. Silver, H.-C. Guo, J. L. Strominger, D. C. Wiley, *Nature* **360**, 367 (1992).
19. D. H. Fremont, M. Matsumura, E. A. Stura, P. A. Peterson, I. A. Wilson, *Science* **257**, 919 (1992).
20. D. H. Fremont, E. A. Stura, M. Matsumura, P. A. Peterson, I. A. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2479 (1995).

Regulating Cell Proliferation: As Easy as APC

Mark Peifer

Animal cells communicate by an array of signals that travel from cell to cell, each activating its attendant signal transduction pathway. These pathways are critical for normal development and physiology; when they malfunction, cancer often results. Two reports in this week's issue (1, 2) describe new partners for the tumor suppressor APC (product of the adenomatous polyposis coli gene *APC*), which when mutated can cause cancer. One report (1) places APC firmly in the WINGLESS (WG) and WNT signal transduction pathways (of *Drosophila* and mouse, respectively). The other report (2) identifies a new target for APC, another tumor suppressor *Drosophila discs large* (*dlg*).

WG is a cell-to-cell signal in the fruit fly *Drosophila* that triggers many key developmental processes; WNT is the analogous molecule in mice. Many components of their signal transduction pathway were identified in genetic screens of *Drosophila* for gene products that control embryonic pattern formation (3). In addition to *wingless*, these screens yielded mutations in *porcupine*, *dishevelled*, *zeste white 3*, and *armadillo*, all encoding components of the WG pathway. Their order of action in the pathway has been defined by genetic and molecular studies (4): PORCUPINE is required for production and secretion of WG, whereas DISHEVELLED (DSH), ZESTE WHITE 3 (ZW3), and ARMADILLO (ARM) are required sequentially for signal transduction in the receiving cell. Vertebrates also use this pathway (5). In *Xenopus*, homologs of DSH, ZW3 [glycogen synthase kinase 3β (GSK3β)] and ARM (β-catenin) mediate WNT signaling during dorsal-ventral patterning.

Biochemical and cell biological studies supplement the genetic picture (6). WG recruits DSH to the membrane, presumably through an as yet uncharacterized transmembrane WG receptor. DSH negatively regulates the kinase ZW3, which normally promotes instability of ARM protein in the cytoplasm and nucleus. The WG signal thus stabilizes intracellular ARM, which is thought to act with as yet unknown partners to ultimately alter the expression of target genes like *engrailed*. ARM (and its vertebrate homolog β-catenin) are also key components of cell-cell adherens junctions (7), and β-catenin (and likely ARM) are found in a complex containing the tumor suppressor protein APC (8). In the report on APC in this issue, by Rubinfeld *et al.* (1), the role of the APC–β-catenin interaction is clarified.

APC was not initially found as a member of a signal transduction pathway, but rather as a culprit in cancer. Inheritance of one mutant *APC* gene results in predisposition to colon cancer; *APC* mutations also occur in sporadic colon tumors. These mutations result in benign overproliferation of the colon epithelium, forming a polyp, the first step in tumor development. Data from both patients and a mouse model of colon cancer suggest that both *APC* genes are mutated in polyps; one usually encodes a truncated APC protein lacking its COOH-

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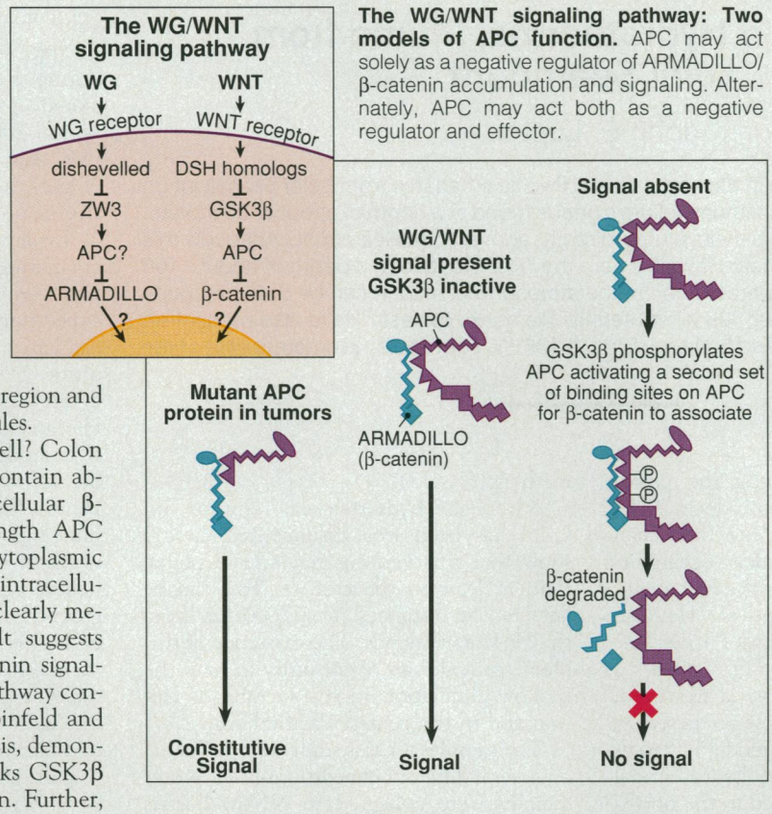
terminal half (9). APC is a large protein (over 300 kilodaltons) with multiple interesting domains. Its NH_2-terminal third contains an oligomerization domain followed by multiple Arm repeats (protein-protein interaction motifs also found in ARM). The middle third of APC mediates β-catenin binding; multiple copies of two related but distinct sequences constitute separate β-catenin binding sites. The COOH-terminus contains a basic region and a domain that can bind microtubules.



The WG/WNT signaling pathway: Two models of APC function. APC may act solely as a negative regulator of ARMADILLO/β-catenin accumulation and signaling. Alternately, APC may act both as a negative regulator and effector.

What does APC do in the cell? Colon cancer cells with mutant APC contain abnormally high levels of intracellular β-catenin; the addition of full-length APC destabilizes and eliminates this cytoplasmic β-catenin pool (*10*). Given that intracellular ARM, β-catenin's homolog, clearly mediates WG signaling, this result suggests that APC regulates ARM–β-catenin signaling and that dysfunction of this pathway contributes to polyp formation. Rubinfeld and co-workers (*1*) extend this analysis, demonstrating that APC physically links GSK3β (homolog of ZW3) and β-catenin. Further, GSK3β phosphorylates APC and thus regulates its interaction with β-catenin. Together, these data provide a biochemical correlate of the genetic data in flies and suggest that APC is part of the WG/WNT signaling pathway. APC, when phosphorylated by GSK3β, may down-regulate intracellular β-catenin, keeping signaling off. WG/WNT antagonizes GSK3β/ZW3 action, stabilizing intracellular β-catenin and activating signaling.

Is APC solely a negative regulator, or could it play a dual role as both a negative regulator and effector of WG/WNT signaling? As a negative regulator, APC would regulate intracellular pools of ARM/β-catenin (see figure inset). If it has a dual role, however, the APC–β-catenin complex would exist in two states. With ZW3/GSK3β kinase active, APC would degrade ARM/β-catenin, but if ZW3/GSK3β kinase was inactive (for example, in the presence of the WG/WNT signal), the APC-catenin complex would generate signals (see the figure). The mutated APC proteins in colon tumors, which lack the region required for β-catenin degradation but retain the ability to bind β-catenin, may be locked in the signaling mode. Other evidence also implicates WG/WNT signaling in carcinogenesis: WNT signals cause cell proliferation in certain tissues, and an NH_2-terminal–deleted β-catenin transforms cells (*11*).

Regulation of β-catenin signaling may be only one of the functions of the large and complex APC protein. The domains dedicated to binding β-catenin make up a small part of APC—other regions likely are docking sites for different proteins. The COOH-terminus binds microtubules and also to the novel EB1 protein, isolated as an interactor in a two-hybrid screen (*12*). In another paper on APC in this issue, Matsumine and co-workers report the results of a yeast two-hybrid screen for additional APC interactors (*2*). Using APC's COOH-terminus as bait, they isolated a rather surprising partner, the human homolog of the *Drosophila discs large* (*dlg*) tumor suppressor. DLG protein is a component of *Drosophila* septate junctions, analogs of vertebrate tight junctions, and *dlg* mutations result in tumor development (*13*). DLG is the progenitor of the membrane-associated guanylate kinase (MAGUK) protein family (*14*), which includes the vertebrate tight junction proteins Z0-1 and Z0-2 and neuronal proteins like PS95, among others. All share a similar domain organization. Several PDZ domains (protein-protein interaction motifs) are followed by an SRC homology 3 (SH3) domain (a distinct protein-protein interaction motif) and a domain with sequence similarity to guanylate kinase. The biochemical function of MAGUKs remains mysterious, although they may help to organize membrane proteins into complexes (*15*) and participate in signaling.

This intriguing interaction of APC with DLG, although far from understood, underscores APC's participation in numerous signaling pathways. APC may act as a nexus integrating different inputs and generating multiple outputs. Indeed, APC's tasks may go beyond proliferation, as suggested by APC immunolocalization in cultured cells. In isolated cells or in small colonies, APC is concentrated in puncta at the leading edge of membrane protrusions (*16*). These puncta often cluster at the ends of microtubule bundles. This localization led to the suggestion that APC regulates the balance between cell migration and cell adhesion (*16*).

APC is a jewel with many facets, only some of which we have glimpsed. The Arm repeats are likely docking sites for other protein partners, which remain to be identified. The new partners for APC point us in two of the many directions that must be pursued to come to a full understanding of APC's role in regulating cell behavior.

References and Notes

1. B. Rubinfeld *et al.*, *Science* **272**, 1023 (1996).
2. A. Matsumine *et al.*, *ibid.*, p. 1020.
3. C. Nüsslein-Volhard and E. Wieschaus, *Nature* **287**, 795 (1980); N. Perrimon, L. Engstrom, A. P. Mahowald, *Genetics* **121**, 333 (1989).
4. N. Perrimon, *Cell* **76**, 781 (1994).
5. M. W. Klymkowsky and B. Parr, *ibid.* **83**, 5 (1995).
6. M. Peifer, D. Sweeton, M. Casey, E. Wieschaus, *Development* **120**, 369 (1994); F. van Leeuwen, C. Harryman-Samos, R. Nusse, *Nature* **368**, 342 (1994); S. Yanagawa, F. van Leeuwen, A. Wodarz, J. Klingensmith, R. Nusse, *Genes Dev.* **9**, 1087 (1995).
7. M. Peifer, *Trends Cell Biol.* **5**, 224 (1995); B. M. Gumbiner, *Cell* **84**, 345 (1996).
8. L.-K. Su, B. Vogelstein, K. W. Kinzler, *Science* **262**, 1734 (1993); B. Rubinfeld *et al.*, *ibid.* **262**, 1731 (1993).
9. P. Polakis, *Curr. Opin. Gen. Dev.* **5**, 66 (1995).
10. S. Munemitsu, B. Souza, I. Albert, B. Rubinfeld, P. Polakis, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3046 (1995); B. Rubinfeld, B. Souza, I. Albert, S. Munemitsu, P. Polakis, *J. Biol. Chem.* **270**, 5549 (1995).
11. R. Nusse and H. E. Varmus, *Cell* **31**, 99 (1982); M. E. Dickinson, R. Krumlauf, A. P. McMahon, *Development* **120**, 1453 (1994); I. Whitehead, H. Kirk, R. Kay, *Mol. Cell. Biol.* **15**, 704 (1995).
12. K. J. Smith *et al.*, *Cancer Res.* **54**, 3672 (1994); S. Munemitsu *et al.*, *ibid.*, p. 3676; L. K. Su *et al.*, *ibid.* **55**, 2972 (1995).
13. D. F. Woods and P. J. Bryant, *Cell* **66**, 451 (1991).
14. S. K. Kim, *Curr. Opin. Cell Biol.* **7**, 641 (1995).
15. D. F. Woods and P. J. Bryant, *J. Cell Sci. Suppl.* **17**, 171 (1993); S. N. Gomperts, *Cell* **84**, 659 (1996).
16. I. S. Näthke, C. L. Adams, P. Polakis, J. H. Sellin, W. J. Nelson, *J. Cell Biol.*, in press.

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