Interactions and functions of the adenomatous polyposis coli (APC) protein at a glance

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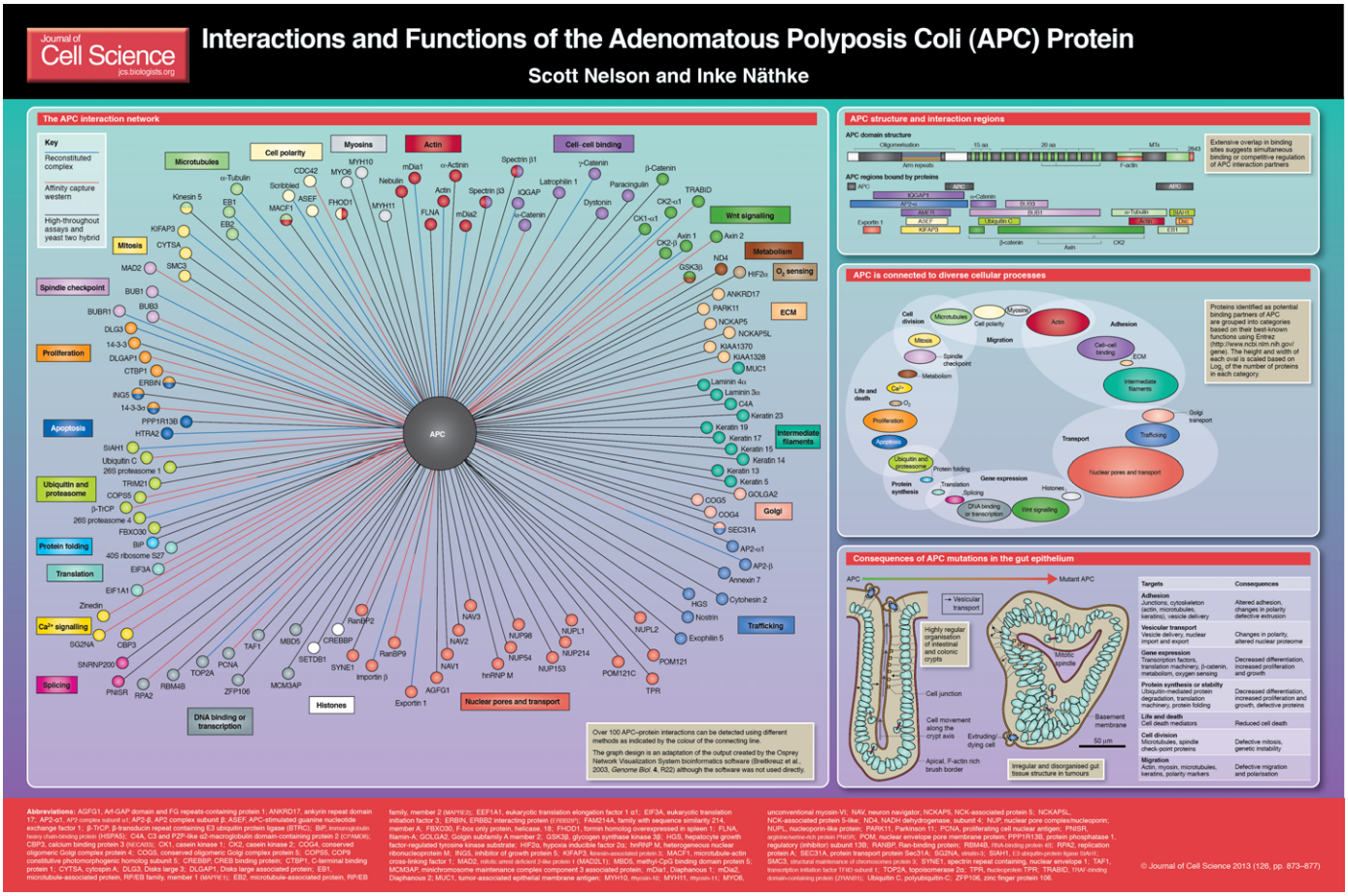
Since its discovery as the major tumour suppressor in colorectal cancer, the adenomatous polyposis coli (APC) protein has emerged as a multi-functional protein that directly or indirectly regulates the cellular processes that govern epithelial tissues (McCartney and Näthke, 2008). Today, the list of potential functions for APC and the knowledge with regard to how mutations in *APC* contribute to the second most common cause of cancer deaths continues to expand. In this Cell Science at a Glance article, we provide an overview of the different interactions that have been reported for the APC protein, the functions this suggests APC has, and how these functions might contribute to the role of APC in gut epithelium. Some of the protein interactions that underpin these functions of APC are well established. For instance, the role of APC in assembling a protein complex that also contains axin, glycogen synthase kinase 3β (GSK3β), and casein kinase has been studied and reviewed extensively (McCartney and Näthke, 2008; Moon et al., 2002; Polakis, 2007; Reya and Clevers, 2005). This complex targets β-catenin for degradation by the proteasome and is regulated by Wnt signalling. The loss of this particular function of APC has a major role in tumorigenesis, as the resulting increased availability of β-catenin causes changes in transcriptional programmes to decrease the amount of cell differentiation (Polakis, 2012). Similarly, the contribution of APC to the regulation of cytoskeletal proteins, particularly microtubules and actin, is well established (Moseley et al., 2007; Munemitsu et al., 1994; Näthke et al., 1996; Okada et al., 2010; Smith et al., 1994). The changes in cytoskeletal organization and processes associated with microtubules and actin, including migration and cell division that accompany APC loss, have been described by many research groups (Caldwell et al., 2007; Dikovskaya et al., 2010; Dikovskaya et al., 2004; Dikovskaya et al., 2007; Green et al., 2005; Green and Kaplan, 2003; Kita et al., 2006; Kroboth et al., 2007; Marshall et al., 2011). In the context of cancer, changes in these interactions of APC cause cells to migrate less efficiently and to increase their residence time in the challenging environment of the intestinal tract (Nelson



(See poster insert)

et al., 2012). The ability of APC to bind to itself also contributes to its effect on cell migration (Li et al., 2008). Changes in cytoskeletal interaction of APC underpin defects in mitotic spindles and mitotic checkpoints to promote genetic instability, a major contributing factor to tumorigenesis (Caldwell and Kaplan, 2008; Caldwell and Kaplan, 2009; Dikovskaya et al., 2007). Moreover, changes in microtubules in cells with mutant APC contribute to changes in how cells extrude during the process of their elimination from epithelia. Instead of extruding apically, which ensures the removal from the body, cells with mutant APC tend to extrude basally, which could cause their inappropriate retention (Marshall et al., 2011). Whether the interactions between APC and microtubules or other cytoskeletal proteins also have a role in the increased compaction of mitotic chromatin in APC-deficient systems is not clear (Dikovskaya et al., 2012).

The picture that emerges for this large protein (2843 amino acids for human APC) is that of a scaffold that has its finger in every epithelial biology pie. In most cases, APC is not absolutely required for a particular process; instead, APC contributes to the fidelity of processes, such as migration, differentiation, death and mitosis (McCartney and Näthke, 2008). This means that loss of APC, which has been found in almost all colorectal tumours and occurs extremely early in the process of tumour development, causes an increase in the residence time of cells in tissue. Loss of APC also leads to an increase in defective chromosome segregation and increases the tolerance of cells and tissues for such mistakes, thereby allowing the accumulation of cells that are unable to respond appropriately to cues for their elimination. In the rapidly turning over tissue of the gut (humans lose and replace between 20 and 50 million cells every minute for their entire life), this is particularly detrimental as the occurrence of, for example, chromosome segregation errors and the accumulation of cells with such errors is more likely. This is consistent with the fact that loss of APC invariably causes tumours in this tissue (Barker et al., 2009; Kim et al., 1993).

New interaction partners of APC

In addition, to the well-known interactions and their functional relevance described above, potential links between many other cellular processes and APC have been reported. To illustrate the complexity of APC biology, we searched for reported interactions of the APC protein to gain an insight into both the well-established and the emerging roles this large protein is known to play.

The BioGRID is a curated database of protein–protein interactions in humans based on published data (Stark et al., 2006). Using database version 3.1 to identify physical interactors using ‘Human Adenomatous Polyposis Coli’ as ‘query’ revealed more than 100 interactions. The interactions were categorised based on the type of study (i.e. high or low throughput) and the experimental approach used to detect the interaction (see poster). About two thirds of these interactions are based on high-throughput studies and derive from a yeast two-hybrid screen (Bandyopadhyay et al., 2010), whereas about half of the interacting proteins were found by low-throughput studies. Many of the known interaction partners of APC, such as β-catenin, are accounted for. However, not all of the previously published protein–protein interactions of APC, including those with the Ras GTPase-activating-like protein IQGAP1, topoisomerase 2, and the APC membrane recruitment proteins AMER1 and AMER2, were included in the BioGRID database, suggesting that it is not complete (Pfister et al., 2012a; Pfister et al., 2012b; Wang et al., 2008; Wang et al., 2010; Watanabe et al., 2004). The entire set of the protein interactions of APC reported by BioGRID, plus the omissions we detected on the basis of published data, is shown on the accompanying poster grouped into colour-coded clusters showing the functions of the interactors (Breitkreutz et al., 2003). (A comprehensive list of all reported and substantiated APC interactions is not available and we apologise for any omissions in this analysis. A table listing the interactors is shown in supplementary material Table S1.)

Many of these reported interactions have not been yet substantiated by additional functional data, so it is possible that some of the members of this list represent false positives or experimental artefacts. It is also important to note that the included interactions are not necessarily direct. Nonetheless, a surprisingly complex picture emerges in which certain functional groups that are not usually considered to be linked to APC are strongly represented. For instance, 17 nucleoporins, as well as other proteins involved in nuclear transport, have been reported to associate with APC, as well as seven keratins. Proteins involved in membrane trafficking and metabolism or oxygen sensing have also been found. The functional consequences of these interactions have not been investigated, but they potentially link APC to processes that are fundamental for normal cell function but are not usually considered in the context of APC.

Functions of APC-interacting proteins

To illustrate the cellular processes the reported interactions of APC might impact on, we clustered the APC-interacting proteins into functional groups and represented them on the poster by ovals of a size that is proportional to the number of proteins detected in that category. The largest group that emerges from such an analysis are ‘nuclear pores’, which mostly contains nuclear pore complex proteins and proteins involved in nuclear pore transport, such as importin-β. Another function that is not usually considered in the context of APC is trafficking. A few proteins that participate in various aspects of vesicular trafficking were identified in our analysis. They range from lipid-associated proteins involved in general vesicle trafficking, to effectors of small GTPases that are involved in endosomal trafficking and filopodia formation, to structural proteins involved in Golgi transport. It is possible that the ability of many of these proteins to become ubiquitylated, and thus be recruited to the proteasome, means they come into proximity to APC-containing complexes, which in turn might lead to their detection in association with APC (Kim et al., 2011). Another surprising link was detected between APC and proteins that are involved in metabolism and oxygen sensing. Although an interaction between APC and hypoxia-inducible factors (HIFs) has not been detected biochemically, a functional link between APC and oxygen sensing has previously been suggested by the ability of HIF1α to directly repress transcription of *Apc* (Newton et al., 2010).

Many of the suggested links emerging from this analysis need to be corroborated by biological experiments. Nevertheless, the picture that emerges here is that the interactions of the APC protein impinge on most, if not all of, cell biology, suggesting that APC could contribute to cellular behaviour in many different ways.

Regions of APC involved in protein–protein interactions

The large size of the APC protein has made it difficult to obtain detailed information

about its structure. However, the regions within APC that interact with proteins involved in the regulation of β-catenin and regions involved in regulating microtubules or actin have been mapped in the linear sequence of the molecule (see poster) (Stamos and Weis, 2012). For instance, β-catenin can interact directly with 15-amino-acid repeats that are at the beginning of, and 20-amino-acid repeats that are interspersed through the middle of the APC molecule (Stamos and Weis, 2012). For microtubules, two direct binding sites have been identified in the C-terminal third of the protein (Dikovskaya et al., 2010; Munemitsu et al., 1994). The binding site in the APC molecule has been partially defined for at least 19 proteins. When examining the distribution of interaction sites with known binding partners and the functional groups they fall into, none of the groups map to a specific region of APC. For instance, interactions that contribute to migration or cytoskeletal regulation occur all along the sequence of the molecule (Li et al., 2008; Tickenbrock et al., 2002). However, at least two regions appear to contain binding sites for proteins that participate in diverse processes. Amino acids 1100–1200 can bind to α-catenin, β-catenin, BUB1, BUB3 and ubiquitin C; a similarly large number of interacting proteins bind to regions in the C-terminus of APC (see poster). This strongly suggests that different APC-binding partners compete with each other for APC binding and that mechanisms must exist to regulate the temporal and spatial availability of APC for different interaction partners and thus functions.

Some biochemical data for competition between APC-binding partners and their regulation are available already. For instance, importin-β and β-catenin bind to overlapping domains of APC and compete with each other for APC binding (Dikovskaya et al., 2010). Similarly, binding of β-catenin to APC prevents it from binding to microtubules (Dikovskaya et al., 2010; Penman et al., 2005). Reducing the self-association of APC that is mediated by residues 750–1000 (immediately preceding the 15-amino-acid repeats that bind to β-catenin) affects the ability of APC to form large peripheral microtubule-dependent clusters and results in reduced cell migration (Dikovskaya et al., 2010; Li et al., 2008; Penman et al., 2005). These data, together with the large number of overlapping interaction sites, suggest that an extensive network of competing and cooperating interactions exists that far exceeds the well-characterised examples discussed above. Therefore, an important challenge in the APC field is to elucidate how the binding of different partners to APC, and thus the participation of APC in different cellular functions, is achieved. Furthermore, to understand how individual mutations in APC lead to specific cellular changes, it will be important to determine which of the binding partners can bind simultaneously and which compete, and how a hierarchy of binding is established to suit the requirements of individual cells and tissues.

There is limited information about how individual interactions of APC are regulated. For instance, the binding of β-catenin to APC is enhanced by phosphorylation of APC by GSK3β. Conversely, binding to microtubules is inhibited by this phosphorylation, consistent with a mutually exclusive nature of these interactions (Dikovskaya et al., 2010; Penman et al., 2005; Zumbrunn et al., 2001). Phosphorylation of C-terminal regions, possibly by cyclin-dependent kinase 2 (CDK2) also modulates the binding of APC to EB1 (also known as MAPRE1) (Nakamura et al., 2001; Trzepacz et al., 1997). Binding of importin-β to APC is regulated by Ran-GTP (Dikovskaya et al., 2010).

The most common truncations in APC occur in the region between amino acid residues 1000–1300, in which mutations are clustered (Rowan et al., 2000). However, other truncation mutations have also been recorded in colorectal cancer patients and a strong correlation between genotype and phenotype is not always apparent (Rowan et al., 2000). The ability of retained truncated APC fragments to oligomerise, and the effect this has on other interactions, is likely to contribute to the overall effect of specific mutations. For instance, the binding of APC to itself via N-terminal and C-terminal domains affects its interaction with proteins that bind to the armadillo region (Li and Näthke, 2005). Heterozygous expression of such truncated fragments causes measurable changes in cell migration, and changes the ability of cells to extrude from epithelial tissue normally during homeostasis (Marshall et al., 2011; Nelson et al., 2012). However, the detailed molecular mechanisms that underpin these changes are not known. To allow accurate predictions of which cellular functions of APC are lost upon specific mutations will require understanding of the relationship between different interaction partners of APC, including their spatial and temporal regulation.

Furthermore, to distinguish direct consequences of APC mutations, which result from changes in the interaction with and thus function of a specific protein, from indirect changes, which occur because the interaction of one in a pair of competing binding partners has been altered so that another interaction is indirectly affected, will require more in-depth studies of how different interactions affect each other and are coordinated.

Predicted consequences of lack of APC in cells

APC contributes to a wide spectrum of protein functions and cellular processes. Many of the changes associated with tumorigenesis can be attributed to changes in these functions that are predicted to result from APC loss or mutations. The role of APC in targeting β-catenin for degradation is particularly important in this context (McCartney and Näthke, 2008; Moon et al., 2002; Polakis, 2007; Reya and Clevers, 2005). The increased availability of β-catenin that results after APC loss causes changes in transcriptional programmes and results in increased proliferation (Stamos and Weis, 2012). Changes in cytoskeletal regulation that result from loss of APC lead to decreased migration, which reduces the elimination of cells from the challenging environment of the intestinal tract (Nelson et al., 2012). In addition, changes in cytoskeletal regulation also underpin defects in mitotic spindles and mitotic checkpoints that can promote genetic instability, an important contributor to tumorigenesis (Caldwell and Kaplan, 2008; Caldwell and Kaplan, 2009; Dikovskaya et al., 2007).

However, little is known about the contribution of APC to the functions of nuclear pore complex proteins or cellular trafficking. Nuclear import and export is a key element of Wnt signal transduction (Krieghoff et al., 2006). For instance, the ability of β-catenin to act on the transcription factors it regulates requires its entry into the nucleus (Krieghoff et al., 2006). Similarly, APC is present in the nucleus and depleting it from the nucleus results in increased epithelial proliferation in the intestinal and colonic epithelium, although the molecular basis for this effect is not understood (Zeineldin et al., 2012). At present, it is not clear how the function of nuclear pore proteins is affected by their interaction with APC or whether mutations in APC change the dynamics of the nuclear trafficking of, for example, β-catenin by

modulating nuclear pore proteins. Existing data support a role of APC as a linker between the nuclear envelope and microtubules, which might contribute to the role of APC in directed migration (Collin et al., 2008; Murawala et al., 2009). In addition, the emergence of nuclear pore proteins as regulators of mitosis, and the ability of APC to bind to microtubules and kinetochore proteins, raises the possibility that APC acts as a physical link between nuclear pore proteins and mitotic spindles (Chatel and Fahrenkrog, 2012; Dikovskaya et al., 2007; Kaplan et al., 2001).

The idea that APC can bind to proteins involved in general cell trafficking has also not been investigated in any detail. Defects in vesicular trafficking have not been examined in APC-deficient cells making it difficult to know the potential functional relevance of their association with APC. It is possible that the interaction of APC with factors that are involved in vesicular trafficking and its ability to bind to different cytoskeletal proteins create a link between vesicles and the cytoskeleton. Even subtle changes in the delivery or uptake and transport of proteins, for instance growth factor receptors that are usually downregulated by endocytosis to prevent continuous signalling, could result in a more permissive background for tissue changes that occur during tumour formation (Avraham and Yarden, 2011).

The complexity of the overlapping protein interactions of APC and the crosstalk between the pathways that these interactions are involved in makes it extremely difficult to cleanly dissect whether any effects that are observed after APC loss or specific APC mutations are a direct result of changes in single or multiple protein interactions, or whether they are indirect consequences of concurrent changes in the dynamics of β-catenin and/or of the cytoskeleton. Nevertheless, the overall combined effect of the changes induced by of APC loss might explain why mutations in *APC* are so effective at producing tumours in gut tissue – all the changes that are either predicted or known to be the result of mutations in APC are among the hallmarks of oncogenic mutations (Hanahan and Weinberg, 2011).

A question that arises is why in the gut tissue a single molecule is in charge of so many processes? It is possible that the gut epithelium has a unique need for a central coordinator to facilitate a rapid and robust response to stress, which is particularly important in this tissue because of its chemically and mechanically challenging environment. The benefit that might be provided by relying on central coordination in this tissue might be sufficient to compensate for the disadvantages this creates, that is, increasing the likelihood for colorectal cancer with increasing age. However, significant experimental effort using different biological and biophysical approaches and systems will be required to substantiate this speculation.

Conclusions and perspectives

In summary, the APC protein has emerged as a multifunctional protein that functions at the intersection between many different cellular pathways that together govern cellular behaviour and function: differentiation, proliferation, cytoskeleton, adhesion, polarity, migration, genetic stability, and life and death decisions. As a consequence, loss or mutation of APC causes defective cells to accumulate and survive inappropriately. This explains the extremely high penetrance of APC mutations in the gut, where continued high turnover of tissue is part of normal maintenance and relies on the fidelity of all these processes and their co-ordination.

Many of the interactions revealed by various screens still require biological validation. Questions that emerge are how are the different interactions of APC and their input into distinct cellular functions regulated and coordinated? How do the fragments of APC frequently expressed in tumour cells contribute to cellular changes? How do different tissues vary in their use of APC as a central coordinator and how are such differences achieved? Ultimately, it will be important to establish how the position of APC at the crossroads of so many different important cellular processes can be exploited to selectively eliminate cells that lack proper functioning of this central coordinator.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.100479/-/DC2

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