ess of apoptosis. If, for example, the *dCsk* cells in these patches proliferate more slowly than their neighbors, they might be eliminated by competition. This is the process by which rapidly growing cells eliminate more slowly growing neighbors by JNK-dependent apoptosis (Gallant, 2005); in this process the apoptotic cells are also basally extruded. It would thus be interesting to determine whether basal migration still occurs when apoptosis is suppressed. An alternative possibility is that the apoptosis is a consequence of basal migration. Epithelial cells are dependent on cell-cell and cell-matrix signals for their survival. Cells that migrate basally from their normal position might therefore undergo apoptosis because they are deprived of antiapoptotic signals present in their normal niche. Thus, on this view the death of the migratory cells might be secondary to their movement out of a protective niche within the intact epithelium. The finding that loss of MMP2 blocks both basal migration and cell death is consistent with this latter view.

Irrespective of the precise model invoked to explain these observations, it seems clear that tissue context can determine the outcome of Src activation. Some of the same signaling pathways and molecules that act to promote mammalian tumor development and metastasis can, at least in the fly, function to promote cell death when activated in small groups of cells. Clearly the question now is: does discrete activation of Src have similar effects in mammalian epithelia, and does this account for the failure of mutationally activated Src to initiate tumor formation in man? Introduction of a conditionally expressed allele of activated Src into the mouse genome might provide the answer.

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Connecting COX-2 and Wnt in cancer

Both the cyclooxygenase-2 (COX-2) and Wnt signaling cascades are active in the majority of colorectal cancers. Nevertheless, a direct link between these two key pathways has remained elusive. Recent reports show that one of the bioactive products of COX-2, prostaglandin E_2, activates components of the canonical Wnt signaling system. The findings reviewed below reveal important crosstalk between these pathways, which may provide opportunities for the development of new drugs for treatment and/or prevention of colorectal cancer.

Colorectal cancer is a global concern that accounts for over 50,000 cancer-related deaths each year in the United States alone (Jemal et al., 2005). Colorectal cancer develops following mutations of key oncogenes such as Ras or disruption of tumor suppressor genes such as APC (adenomatous polyposis coli) and p53. The loss of function of DNA repair genes coupled with genomic instability also leads to the development of colorectal cancer. Hereditary predisposition for colorectal polyps and cancer occurs in people with familial adenomatous polyposis (FAP). These patients harbor germline mutations in one allele of the *APC* gene. Upon loss of function of the wild-type *APC* allele, intestinal adenomas develop that eventually progress into colorectal cancer. Interestingly, administration of Celecoxib (Celebrex), which selectively inhibits COX-2, significantly reduces polyp burden in FAP patients. In a murine model for FAP, mice with a germline *APC* mutation (APC^{min}) also develop intestinal polyps. Either treatment with COX-2-selective inhibitors or disruption of the *COX-1* or *COX-2* genes significantly reduces the number and size of intestinal polyps that develop in these mice.

Although there is a temporal association between the loss of APC function and the activity of COX-2 in vivo, there has been little evidence showing a direct connection between these pathways (Shao et al., 2005; Fujino et al., 2002). In a recent report, Castellone et al. identified a direct link between COX-2 and Wnt and have begun to dissect precisely how these signaling cascades are intertwined (Castellone et al., 2005). Using colorectal carcinoma cells in vitro, they show that prostaglandin E_2 (PGE_2) increased the

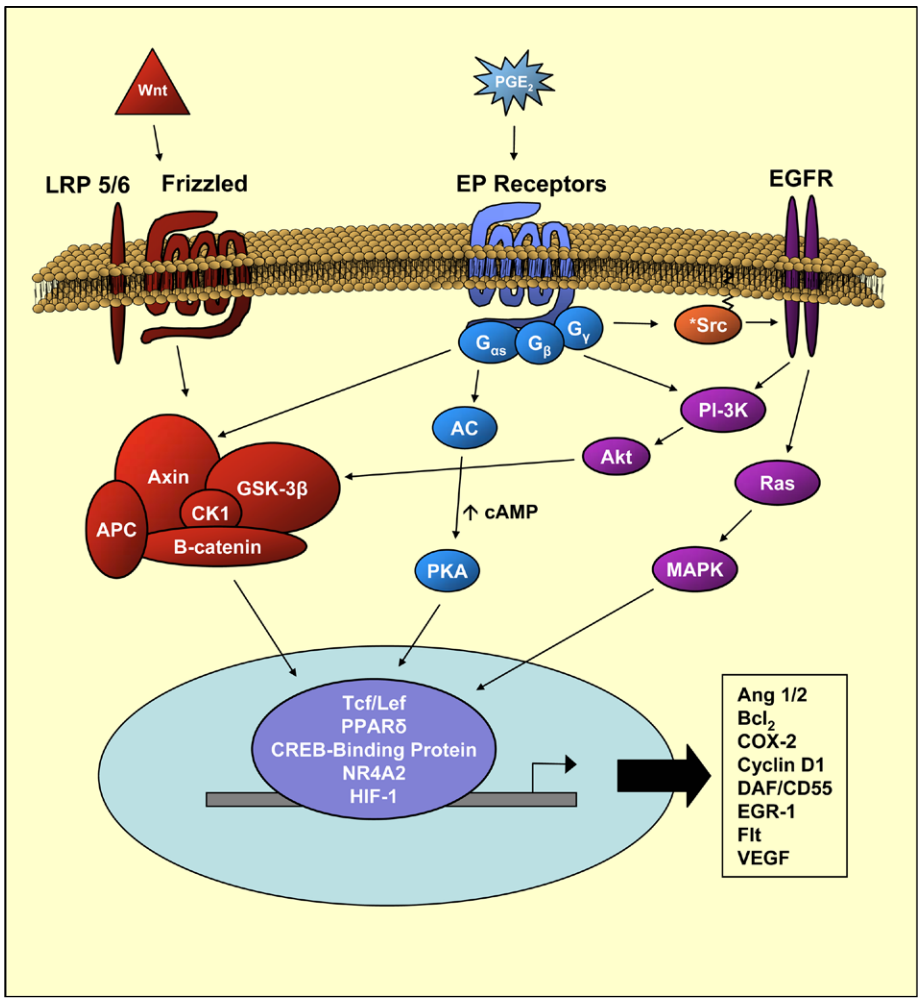

Figure 1. PGE_2-induced transactivation of the canonical Wnt and EGFR signaling pathways.

Cross-talk between G protein-coupled receptors and growth factor receptors can lead to the amplification of many commonly used signaling cascades as well as the activation of signaling components once believed to be monogamously associated with a receptor. PGE_2 induces the activation of G proteins which classically activate PKA-mediated transcriptional activation via an increase in cAMP. PGE_2 can also transactivate the EGFR pathway leading to the activation of Akt and MAPK which induce transcriptional activation. Activation of Akt and Gα subunits can also result in the accumulation of β-catenin of the canonical Wnt pathway in the nucleus which also leads to transcriptional activation. The ability of PGE_2 to stimulate a diverse set of transcription factors gives rise the induction of many different gene products.

activation of Tcf/Lef transcription factors and activated components of the canonical Wnt signaling cascade (Figure 1). PGE_2 induced the loss of phosphorylation of β-catenin and increased its nuclear accumulation. Furthermore, nuclear translocation of β-catenin was required for Tcf/Lef activation and increased proliferation. In resting cells, β-catenin forms a complex with axin, CK1, GSK-3β, and APC. CK1 and GSK-3β phosphorylate β-catenin, which results in its ubiquitin-dependent degradation. Loss of β-catenin phosphorylation can arise from the following: inactivation of CK1 and GSK-3β; the inability of APC to enhance the association of axin and β-catenin; or mutations in the phosphorylation sites on β-catenin.

Since PGE_2 serves as a ligand for G protein-coupled receptors (GPCRs) EP1–4, the investigators dissected the classical downstream activation pathways associated with GPCRs and found that the PGE_2-induced activation of Tcf/Lef was not due to signaling via the PKA-cAMP pathway. Rather, this process was dependent on the direct association of $G\alpha_s$ with axin. In an elegant set of binding studies utilizing the binding domains of axin and a mutant form of $G\alpha_s$ ($G\alpha_s$QL), which mimics the active GTP bound form of $G\alpha_s$, they demonstrated that the $G\alpha_s$ subunit interacts with the RGS (regulator of G protein signaling) domain of axin. Overexpression of the RGS domain of axin inhibited PGE_2-induced transcriptional activation of Tcf/Lef and cellular proliferation. Given that the RGS domain is also the site of APC binding to axin, one assumes that the binding of $G\alpha_s$ to axin results in displacement of APC and loss of phosphorylation, leading to increased nuclear accumulation of β-catenin. However, details concerning the interplay of APC, axin, and the $G\alpha_s$ subunit were not addressed in this article. Since the authors only used APC mutant cells such as DLD-1, SW-480, SW-620, and Caco2 to study the role of $G\alpha_s$ and axin following PGE_2 treatment, further experiments are needed to fully understand the role of APC in this setting. What effect would expression of wild-type APC in these cells have on PGE_2-induced activation of Tcf/Lef? Although PGE_2 was shown to induce Tcf activity in LS-174T colorectal cancer cells, which have wild-type APC (Shao et al., 2005), these cells express a mutant form of β-catenin, resulting in constitutive Tcf/Lef activation. It was reported over a year ago that treatment of APC mutant mice with PGE_2 leads to a marked acceleration of intestinal polyp growth (Wang et al., 2004) without any effect in wild-type mice. Since the current studies were restricted to cultured cells, it will be crucial to determine whether PGE_2 treatment leads to accumulation of nuclear β-catenin via this pathway in vivo.

Another important result of this study is the demonstration that PGE_2 induces the phosphorylation of GSK-3β in HEK293 T cells that were programmed to ectopically express the EP2 receptor. The phosphorylation of GSK-3β on serine 9 inhibits its kinase activity. Therefore, the phosphorylation of GSK-3β results in the net loss of phosphorylation of β-catenin. Interestingly, Castellone et al. show that Akt/PKB (protein kinase B) and not PKA is the kinase responsible for the phosphorylation of GSK-3β. The sequestration of Gβγ subunits following expression of βARK-C inhibited phosphorylation of Akt and GSK-3β. These results indicate that the regulation of β-catenin signaling by PGE_2 requires two independent components: (1) $G\alpha_s$ binding to axin and (2) the phosphorylation of GSK-3β via Akt. However, the roles of Akt and PKA in this process remain

unclear. While Castellone and coworkers report that PKA activity was not required for Tcf activity, a previous report indicated that PGE_2 induced the activation of Tcf in a PKA-dependent manner (Fujino et al., 2002). Furthermore, PGE_2 has been shown to activate Akt in other colorectal carcinoma cell lines (Buchanan et al., 2003) and in APC^{min} mice in vivo (Wang et al., 2004) via the EGFR, yet Castellone et al. also report that EGFR-specific inhibitors had no effect on PGE_2-induced proliferation in DLD-1 cells. Because the study by Castellone et al. investigated only the EP2 receptor, the role of EP1, EP3, and EP4 remains to be evaluated; all four EP receptors bind PGE_2 and may be important for intestinal polyp formation (Hansen-Petrik et al., 2002; Mutoh et al., 2002). Moreover, while both EP2 and EP4 couple to $G\alpha s$, Ep1 and EP3 couple with $G\alpha_i$ and $G\alpha_i/G\alpha_{13}$ subunits, respectively.

The underlying question Gutkind and colleagues attempt to address is the downstream effects of COX-2 inhibition in humans and/or mice that lack normal APC. Although another report was published prior to this one indicating a direct connection between PGE_2 and β-catenin signaling (Shao et al., 2005), Castellone et al. have provided a more detailed explanation for precisely how PGE_2 can induce nuclear transactivation of Tcf/Lef. These findings will improve our overall understanding for the role of PGE_2 in colorectal cancer if they can be shown to occur in vivo. PGE_2 is known to activate other transcription factors in addition to β-catenin, such as the peroxisome proliferator-activated receptor delta (PPARδ) and NR4A2 (Wang et al., 2004; Holla et al., 2005). A recent report also indicates that the effects of COX inhibition in vivo may be dependent upon the expression of PPARγ and RXRα (Lu et al., 2005). Understanding the role of the newly defined PGE_2-regulated transcription factors and gene products may reveal additional therapeutic targets. Although this study is the first or second of many investigations concerning the interplay between PGE_2 and the Wnt signaling cascade, several crucial questions still remain. For example, how does the inhibition of COX-2 in vivo reduce polyp formation in APC^{min} mice if APC lies downstream of the EP receptors? Presumably, following disruption of APC in vivo the β-catenin pathway is fully engaged and would not require further activation by PGE_2. Is there a role for EP1, EP3, and EP4 receptors in this process? These and other questions are currently being addressed during this exciting time as our understanding continues to evolve concerning the early events leading to the development of colorectal cancer.

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