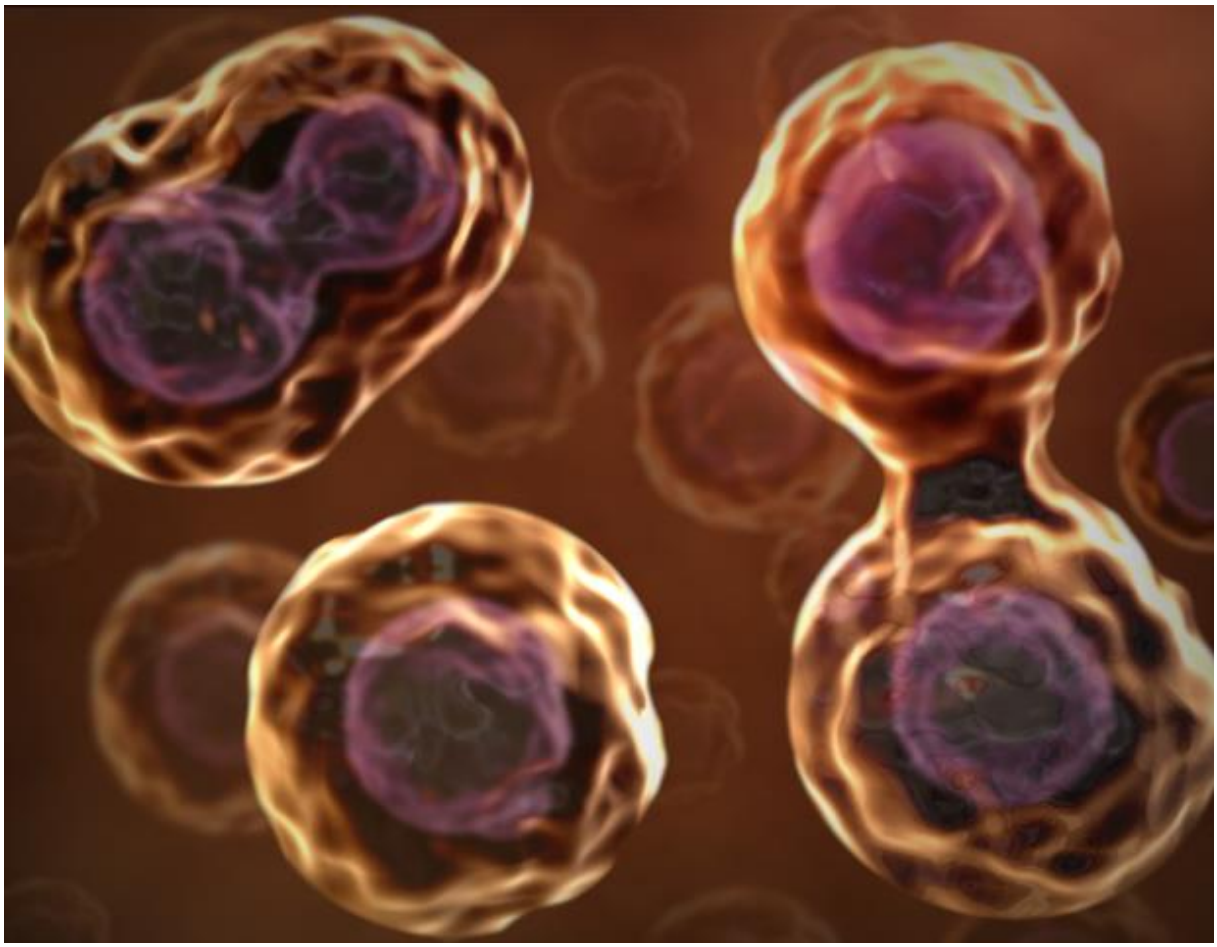
Is there an interaction between Wnt and cAMP signaling in cell proliferation?

Judith Muis



Is there an interaction between Wnt and cAMP signaling in cell proliferation?

Master essay

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Summary

The Wnt and cAMP signaling pathways are both known to stimulate cell proliferation. The aim of this review was to investigate whether there's an interaction between these two pathways in cell proliferation. There's already been a lot of research about the cooperation between cAMP/PKA and Wnt signaling at different levels. The first interaction between these two was PKA phosphorylation of β-catenin which stabilizes β-catenin and stimulates Wnt transcriptional activity. Besides that, PTH and PGE2, both stimulators of cAMP, can induce Wnt signaling at least in part through cAMP/PKA. When it comes to cAMP/Epac and Wnt signaling, there's not much known in literature. But recently there were some articles published about downstream effectors of Epac interacting with Wnt/β-catenin, for example Rap1 which can induce β-catenin-mediated transcription. Next to that, also PLD can be stimulated by β-catenin-mediated transcription and PLD can activate Wnt/β-catenin via a positive feedback loop. The Wnt pathway also interacts with H-Ras; when Wnt signaling is inactive, the destruction complex can bind to H-Ras and degrade it. In this way, it regulates the effect of Epac/H-Ras on cell proliferation. Finally, the ERK pathway can be stimulated by both PKA and Epac and this pathway is very important for cell proliferation. Two Wnt proteins, Axin and APC, can reduce β-catenin levels and this result in decreased Ras protein levels and ERK pathway components. So when β-catenin is stabilized by Wnt signaling, Ras protein levels and ERK pathway components are upregulated and can induce (tumorigenic) cell proliferation.
This review summarizes the connections between cAMP and Wnt signaling that are known in literature now.

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Introduction

The Wnt signaling pathway plays a very important role in cell proliferation and differentiation. The key role player in this pathway is β-catenin, and when Wnt signaling is activated, β-catenin can stimulate transcriptional activity of TCF/LEF. When the Wnt signaling pathway is inactive, β-catenin is bound by Axin, APC, CK1 and GSK3. These four proteins together are called the destruction complex, because it stimulates phosphorylation of β-catenin and thereby destruction of β-catenin by ubiquitylation. In this way, there's no transcriptional activity and no stimulation of cell proliferation and differentiation. Mutations in genes of components of the Wnt signaling pathway can lead to different types of cancer. For example, APC mutations can lead to FAP, Axin mutations to hepatocellular cancer and β-catenin mutations to several solid tumors

Next to the Wnt signaling pathway, there's another pathway which can stimulate cell proliferation; the cAMP signaling pathway. The stimulation of cell proliferation mainly occurs via activation of the ERK pathway by cAMP-stimulated B-RAF. The most well known effector of cAMP is PKA, and less well known is Epac, which is more recently discovered to be an important effector. cAMP does not only have stimulatory effects on cell proliferation, but it can also inhibit the growth of cells, mainly by reducing C-RAF activity by phosphorylation and that causes inhibition of activation of the ERK pathway. Whether cAMP stimulates or inhibits cell proliferation depends on the cell type.

Because both signaling pathways play a major role in cell proliferation, there is a possibility that these two cooperate to induce cell proliferation and maybe also induce tumorigenesis. So the question during the making of this review was: is there any interaction between the cAMP and Wnt signaling pathways in cell proliferation? And beside this major question, it was tried to create a hypothesis about the role of Epac in the interaction between cAMP and Wnt signaling pathways. The role of Epac in cell proliferation is still not entirely understood and also its role in cancer is unknown. This hypothesis may be used in a future study.

Chapter 1: Wnt signaling

Wnt (segment polarity gene Wingless and murine proto-oncogene INT-1) genes encode small secreted proteins. These proteins are found in the genomes of all animals. The Wnt signaling pathway is very important in cell proliferation and differentiation (reviewed by Logan and Nusse, 2004; Reya and Clevers, 2005; Clevers, 2006; Kang et al., 2011). In 1982, Nusse and Varmus identified the mouse Wnt1 proto-oncogene as being an important integration site for Mouse Mammary Tumor Virus in virally induced breast cancer. In 1993, the connection between the Wnt signaling pathway and human cancer was provided. The adenomatous polyposis coli (APC) gene was found to play a major role in familial adenomatous polyposis (FAP), a cancer syndrome. The APC protein seemed to interact with the Wnt signaling pathway protein β-catenin (Clevers, 2006).

Secretion

Wnt proteins are relatively insoluble. They have a high number of conserved cysteine residues that are palmitoylated in the ER by the enzyme encoded by the *porcupine (por)* gene. *Porcupine* is a *Drosophila* gene required in Wnt secreting cells (reviewed by Logan and Nusse, 2004; Clevers, 2006). The lipid modified Wnts are then moved to the Golgi apparatus. A seven-pass trans-membrane protein called Wntless resides primarily in the Golgi apparatus where it physically interacts with Wnts. Wnts can only be secreted when this Wntless protein is present. Eventually, Wntless leads Wnt to the outside of the cell. It is presumed that a multi-protein complex, called retromer, is involved in recycling Wntless between Golgi and Wnt secretion department (*see figure 1*) (Clevers, 2006).

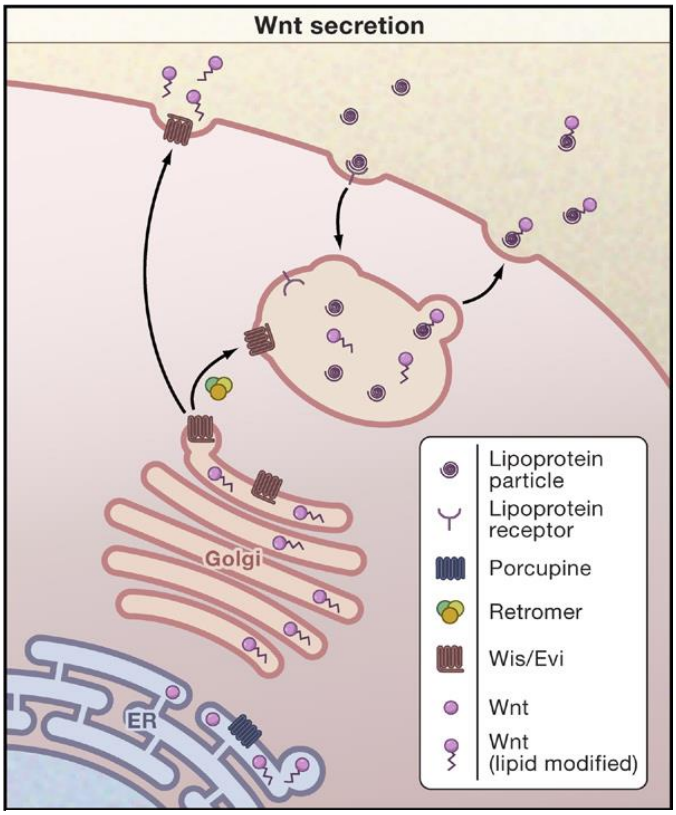

Figure 1: Wnt secretion. The Wnt proteins are being palmitoylated by Porcupine in the ER. Besides that, Wnt needs Wis/Evi to be secreted. Wnts also can be loaded onto lipoprotein particles to leave the cell by endo-/exocytose. (Adapted from Clevers, 2006)

Signaling Pathway

After Wnt is secreted, it binds Frizzled (Fz) proteins. These are seven-pass transmembrane receptors with a N-terminal cysteine-rich domain (CRD). When Wnt is bound to Fz, Fz cooperates with a single-pass transmembrane LRP molecule (LRP 5/6). The transport of LRP 5/6 to the cell surface is dependent on Mesd, a chaperone. The expression of both Fz and LRP 5/6 is required for canonical Wnt signaling initiation

(Clevers, 2006). Because of the activation of the co-receptor complex, the canonical Wnt signaling pathway can be activated. The most important protein in this pathway is β-catenin. When Fz and LRP 5/6 are not bound by Wnt, the destruction complex is activated to break down β-catenin. β-catenin is then bound by APC and Axin and β-catenin gets phosphor-rylated by the kinases CKI and GSK3 which are also part of the destruction complex. Once phosphorylated, β-catenin is proteasomally degraded by β-TrCP containing E3 ubiquitin ligase (Logan and Nusse, 2004; Reya and Clevers, 2005; Clevers, 2006). Wnt signaling leads to inhibition of β-catenin degradation by inhibition of the kinase activity of GSK3. The kinase activity inhibition is probably caused by direct interaction of Axin with LRP 5/6, and/or the actions of Dishevelled (Dsh), an Axin-binding molecule. Unphosphorylated β-catenin then travels into the nucleus where it interacts with the TCF/LEF family of transcription factors to control transcription (*see figure 2*).

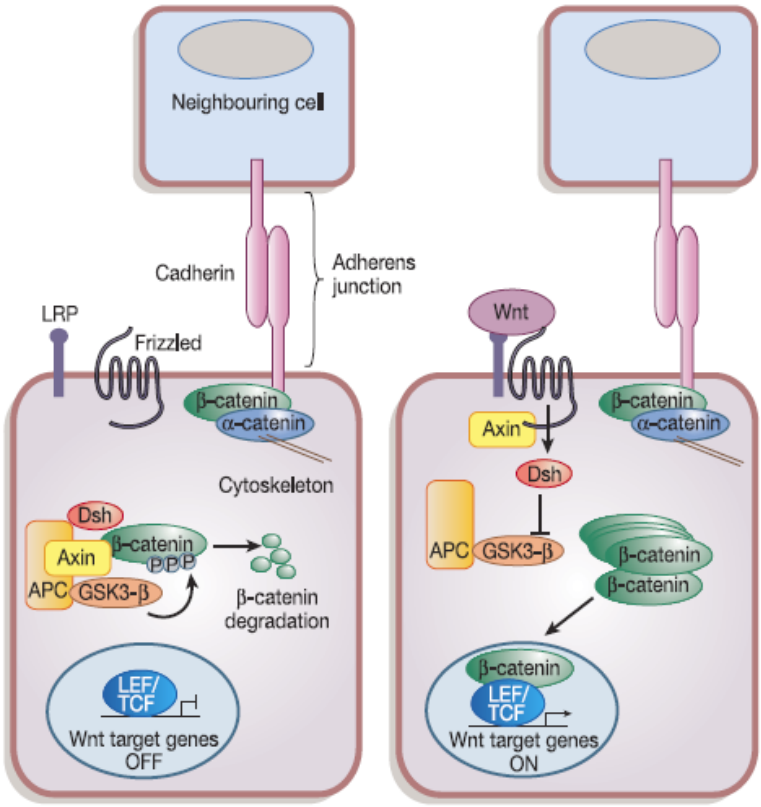

Figure 2: The canonical Wnt signaling pathway. In absence of Wnt (left panel), there's a complex formed including APC, Axin, Dsh, β-catenin and GSK3- β. β-catenin gets phosphorylated and degraded by β-TrCP. The Wnt target genes are off. When Wnt is present, GSK3 activity is inhibited and β-catenin can activate the Wnt target genes. (Adapted from Reya and Clevers, 2006)

β-Catenin can also be actively transported back to the cytoplasm, by either an intrinsic export signal or as a cargo of Axin or APC that shuttle between cytoplasm and nucleus. (Logan and Nusse, 2004; Reya and Clevers, 2005; Clevers, 2006).

When Wnt is absent, Tcf acts as a transcriptional repressor by forming a complex with Groucho/Grg/TLE proteins. The β-catenin/TCF interaction displaces Groucho from Tcf and this leads to transient transcription of Tcf target genes. In this way, Wnt signals promote cell proliferation (Logan and Nusse, 2004; Reya and Clevers, 2005; Clevers, 2006). Besides that, β-catenin also plays a role at the plasma membrane, where it is a component of adherens junctions. Newly synthesized β-catenin is thought to first saturate the pool of the adhesion junction and subsequently the excess free cytoplasmic β-catenin is degraded by the destruction complex (Clevers et al., 2006).

It can also regulate self-renewing tissues in adult mammals, for example the epithelium in the small intestine. Wnt proteins can stimulate crypt progenitor proliferation and promote terminal differentiation of Paneth cells, which are at the bottom of the crypt (Reya and Clevers, 2005; Clevers, 2006). Wnt signals are also required for establishment of the hair follicles. Wnt signaling in hair follicles can activate bulge stem cells, promote

entry into the hair lineage and recruit cells to the transit-amplifying matrix compartment. HSCs and bone marrow microenvironment can produce Wnt proteins. In vitro, soluble Wnt proteins promote the proliferation and inhibit the differentiation of murine hematopoietic progenitors. According to a genetic study it was found that osteoblastmaturation and activity is induced by the canonical Wnt pathway (Reya and Clevers, 2005; Clevers, 2006).

The β-catenin-independent noncanonical Wnt signaling pathways are called the Wnt/jun N-terminal kinase (JNK) or Wnt/calcium pathway. In the Wnt/JNK pathway, frizzled and dishevelled function in concert with other proteins to create cellular polarity by asymmetrical and polarized protein localization. Most of these proteins are involved in polarized cell movement, planar polarity of epithelial cells and they are also linked to ciliogenesis (reviewed in Rao and Kühl, 2010). The calcium-mediated pathway is linked to cardiac development. In this pathway, Wnt5a is able to activate calcium signaling in different cell culture models. It is a very rapid process that depends on heterotrimeric G proteins. Released Ca^{2+} activates calcium dependent enzymes like calcium/calmodulin-dependent kinase (CaMK)II, PKC or calcineurin. CaMKII can lead to activation of a nemo-like kinase (NLK), which interferes with β-catenin signaling. CaMKII suggests that the activity of histone deacetylase (HDAC) can be modified by noncanonical Wnt signaling.
The regulation of PKC by Wnt ligands is important for cardiac differentiation. There are different isoforms of PKC and detailed analysis suggested that PKCδ plays an important role during noncanonical Wnt signaling during cardiac differentiation.
The Wnt/calcium pathway connects to nuclear factor of activated T cells (NFAT) transcription factor and gene expression via calcineurin. Calcineurin an NFAT both play a role in cardiac hypertrophy (Rao and Kühl, 2010).
So the noncanonical Wnt pathways play important roles in cellular polarity and cardiac development, but they are less understood than the canonical pathways.

Wnt in cancer

In the hereditary cancer syndrome FAP, a germline APC mutation is the genetic cause. FAP patients develop a large number of polyps in early adulthood. Mutational inactivation of APC leads to the improper stabilization of β-catenin and TCF reporter constructs are inappropriately transcribed (Reya and Clevers, 2005; Clevers, 2006). In an early case report in 1983, it was observed the number of polyps was decreased when several FAP patients were given the NSAID sulindac. NSAIDs inhibit cyclooxygenase (COX) enzymes and COX enzymes normally induce synthesis of PGE2 (reviewed by Evans, 2009). Ten years later, this effect of sulindac was confirmed by a controlled clinical trial. Cancer researchers tried to find the link between APC and NSAIDs by focusing on the two major signaling pathways that are known: Wnts and PGE2 (PGE2 is an activator of the cAMP signaling pathway).
Besides these findings, loss-of-function mutations in Axin have been found in hepatocellular carcinomas and oncogenic β-catenin mutations are the cause of some solid tumors (Reya and Clevers, 2005; Clevers, 2006).
Mutations in β-catenin can also be the cause of some hair follicle tumors, such as pilomatricoma-like lesions and trichofolliculoma. Sebaceous tumors are caused by LEF1 mutations, impairing binding of β-catenin to TCF.
It was suggested that leukemic growth of myeloid and lymphoid lineages is dependent on Wnt signaling (Clevers, 2006).

Chapter 2: cAMP signaling

Cyclic adenosinemonophosphate (cAMP) is one of the most common and universal second messengers known and it plays a major role in controlling cellular events like cell proliferation, differentiation, migration, apoptosis and secretion (reviewed by Dumaz and Marais, 2005; Borland et al., 2009; Grandoch et al., 2010; Breckler et al., 2011). Because of all these effects, the cAMP signaling also pathway plays a major role in tumorigenesis. In the cell, cAMP is produced from ATP by the enzyme adenylyl cyclase (AC) and it can be reduced to AMP by the action of phosphodiesterases (PDEs). Adenylyl cyclases are membrane bound and activated by G-protein coupled receptors. After cAMP is produced, it can bind to effector molecules. The most well known cAMP effectors are protein kinase A (PKA) and exchange proteins directly activated by cAMP (Epac) (Dumaz and Marais, 2005).

Signaling pathway

PKA

PKA is a serine/threonine specific protein kinase that has two catalytic subunits (C) and two regulatory subunits (R). cAMP binds to the R subunits and thereby release the active C subunits, which are now able to phosphorylate substrates. In humans, three different catalytic subunits are known (Cα, Cβ and Cγ) and four different regulatory subunits (RIα, RIβ, RIIα and RIIβ). The RI subunits are mainly found in the cytoplasm whereas the RII isoforms associate with cell structures and organelles. A-kinase anchoring proteins (AKAP) lead RII subunits to specific subcellular structures, providing important regulation of cAMP signaling (Dumaz and Marais, 2005; Cheng et al., 2008). When PKA gets activated by cAMP signaling, it stimulates Ras which can induce activation of the ERK pathway via B-RAF and MEK (*see figure 3*). The MEK/ERK pathway is a very important stimulator of cell proliferation. So in this way, cAMP can induce cell proliferation via PKA stimulation.

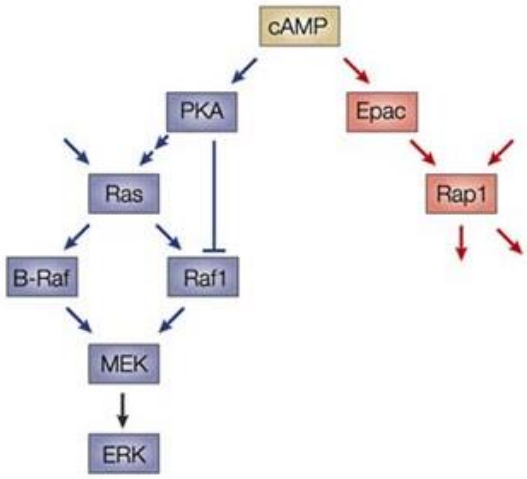

Figure 3: A model for the regulation of the Ras and Rap1 signaling pathways by cyclic AMP. In this picture, the pathway of Epac isn't complete. But Epac can also activate Ras proteins (*see figure 4*). (Adapted from Bos, 2003)

Epac

The exchange proteins directly activated by cAMP (Epacs) have two isoforms, Epac1 and Epac2, regulated by the microtubule network and H-Ras respectively. They catalyze the activation of Rap1 and Rap2 by acting as specific guanine nucleotide exchange factors (GEFs). Rap proteins are small G proteins of the Ras family which play a major role in cell attachment and migration. Epac proteins are also assumed to regulate different processes, including calcium handling and ion transport, cell proliferation and

differentiation, cell survival and apoptosis, gene transcription and chromosomal integrity, vesicle trafficking and secretion (Dumaz and Marais, 2005; Cheng et al., 2008; Grandoch et al., 2010; Breckler et al., 2011) (*see figure 4*). As is shown in figure 4, Epac proteins can activate Rap and R-Ras proteins, which in turn stimulate PLD and ERK pathways and in this way induce cell proliferation and survival.

Other cAMP effectors

Besides PKA and Epac, cAMP-gated membrane ion channels are also known as cAMP effectors. They regulate cation-influx into the cytosol in response to cAMP, a process that controls the activation of a large number of cellular proteins and functions. Besides these two actions, cAMP also regulates some of the phosphodiesterases. In this way, there's a feedback mechanism that controls the duration and intensity of cAMP signaling. This feedback is achieved through PKA that phosphorylates phosphordiesterase-4 (PDE-4) isoforms (Dumaz and Marais, 2005).

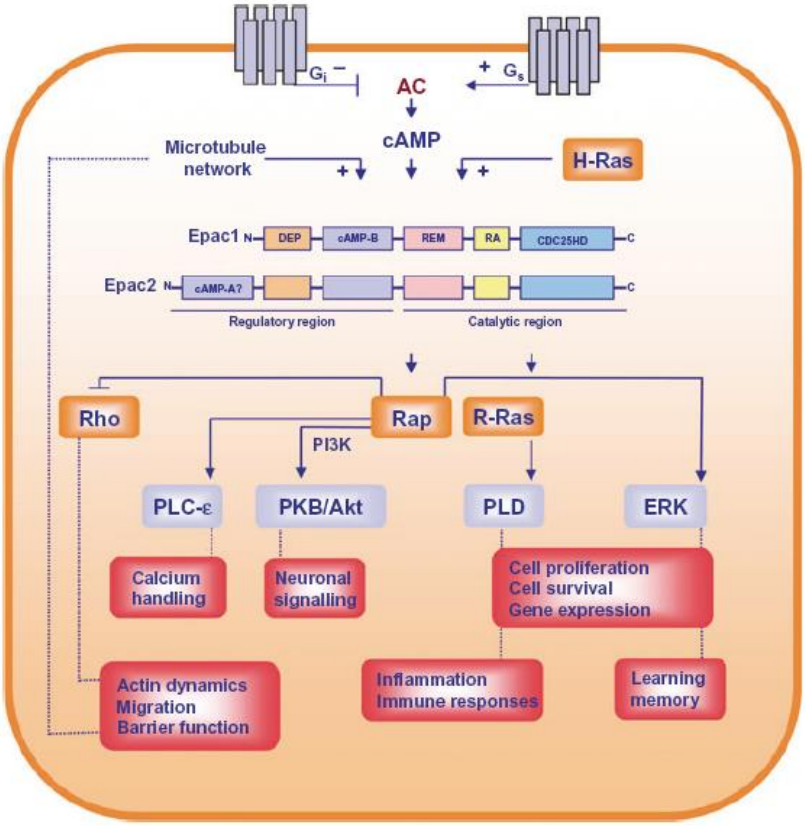

Figure 4: cAMP-Epac signaling pathway. The cAMP modulating receptors are presented in gray and small GTPases in orange; other effectors are depicted in blue and distinct cellular responses are shown in red. (Adapted from Grandoch et al., 2010)

cAMP and its effects on cell proliferation

cAMP has stimulatory and inhibitory effects on the cell proliferation and differentiation, depending on the cell type (Dumaz and Marais, 2005; Dumaz et al., 2006).

Inhibition of proliferation

In a number of cell lines, cAMP antagonizes the proliferative signals stimulated by growth factors and other activators of the ERK pathway. The inhibition of proliferation

is mainly attributed to this ability of cAMP to inhibit ERK signaling because ERK signaling is essential for the proliferation of many cells. Therefore ERK is often the target for cancer therapy. To inhibit ERK, cAMP uncouples C-RAF from Ras through direct phosphorylation of C-RAF by PKA. C-RAF is now inhibited and can't activate the ERK pathway anymore (*see figure 5*). cAMP also targets other proteins that control proliferation, like epidermal growth factor (EGF) receptor and the nonreceptor tyrosine kinase Src that are upstream of the ERK signaling pathway. This suggests that this pathway can be inhibited at several steps. And many downstream transcriptional targets of the ERK pathway are key cell regulatory proteins (such as cyclin A, cyclin D, cyclin dependent kinase 2 (CDK2) and Cdc25A) which expression are inhibited when ERK signaling is blocked by cAMP. Other targets of cAMP are nontranscriptional, like the ERK-induced degradation of cell-cycle inhibitory protein p27, which is blocked by cAMP. Finally, cAMP effects other pathways that are important for proliferation and which are downstream of Ras, such as the PI3K/PKB pathway, activating FOXO transcription factors which are also contribute to the antiproliferative effect of cAMP (Dumaz and Marais, 2005; Dumaz et al., 2006).

Activation of proliferation

In contrast to C-RAF, B-RAF can stimulate the proliferation by cAMP. Dumaz and Marais mention three studies in their review with each a different cell-line where it's showed that cAMP can induce B-RAF with possible help from, for example, Ras, Rap-1 or PKA (*see figure 5*). In this way, cell proliferation is stimulated.

Also the PLD and ERK pathways stimulated by R-Ras and Rap can induce cell proliferation and survival. PLD upregulation at protein and/or activity levels is common in various cancer types like breast, colon, gastric, kidney and thyroid cancer. PLD isozyme overexpression stimulates tumor cell growth and invasion, and the formation of metastases in syngeneic mice. Several PLD2 gene variations are associated with colorectal carcinoma and breast cancer. PLD1 overexpression gives high levels of markers of basal-like tumors, which are mostly associated with poor prognosis (Kang et al., 2011). The ERK pathway is a major transforming pathway and different activation of this pathway by oncogenic mutation(s) such as Ras mutation results in human cancer. The extracellular signal regulated kinase (ERK) is a major component of the ERK pathway. It plays a key role in transmitting Ras-mediated signals for proliferation and transformation (Jeon et al., 2007; Park et al., 2006)

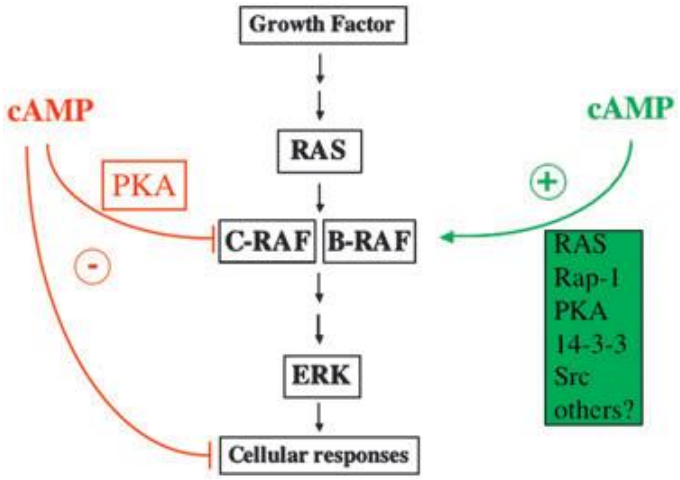

Figure 5: Crosstalk between cAMP and the RAS/RAF/MEK/ERK patwhay. The action of cAMP depends on the responses of C-RAF and B-RAF. When PKA is stimulated by cAMP to phosphorylate C-RAF, C-RAF is inactivated and thereby ERK is inhibited. ERK activation is less understood but it's linked to B-RAF activation by cAMP and its help from RAS, Rap-1, PKA, 14-3-3, Src and maybe some others. (Adapted from Dumaz and Marais, 2005)

Chapter 3: The interaction between Wnt and cAMP signaling pathway

Because Wnt and cAMP signaling both have effects on cell proliferation, there is a possibility that there's an interaction between these two during proliferation. In this chapter are the most important connections between Wnt and cAMP signaling in cell proliferation mentioned which are now known.
Two articles about the phosphorylation of β-catenin by cAMP-dependent PKA were published in 2005 and 2006 respectively (Hino et al., 2005; Taurin et al., 2006). In both studies they investigated the site of phosporylation on β-catenin and its effects on β-catenin-dependent transcription. For their studies, Taurin et al. and Hino et al. both used kidney cells of African green monkey (COS) and humen embryonic kidney (HEK)293 cell lines and Hino et al. also used a mouse fibroblast cell line (L cells). They both showed that β-catenin can be phosphorylated on the Ser675 site by PKA, and in this way stimulate the β-catenin dependent transcriptional activity. Next to Ser675, Taurin et al. also found Ser552 being a phosphorylation site. According to Hino et al., phosphorylation of Ser675 leads to stabilization of β-catenin and prevents β-catenin from being ubiquitinated. Also the transcriptional activity of Wnt is activated by β-catenin stabilization. Taurin et al. found in their study that β-catenin was not stabilized and could still be degraded after being phosphorylated at the Ser675 site, but they also found that the transcriptional activity of Wnt is stimulated after β-catenin phosphorylation. Taurin et al. mentioned the results of Hino et al. to be an additional possible mechanism by which PKA promotes the transcriptional activity of β-catenin, namely by facilitating its interaction with CREB-binding protein (CBP). This latter is also shown by Taurin et al. in their study; they showed that phosphorylation of Ser675 facilitates the interaction between CBP and β-catenin.
After that, Taurin et al. published in 2008 another article about β-catenin phosphorylation by PKA, but now about ATP-induced phosphorylation. They found that also ATP can stimulate PKA-dependent phosphorylation of endogenous β-catenin at Ser552 or Ser675 sites without affecting its expression levels in vascular smooth muscle cells (VSMC). By this phosphorylation, β-catenin can associate stronger with TCF and thereby increase the transcriptional activity. Next to that, ATP also stimulates cyclin D1 expression and proliferation of VSMC by TCF activities and in a PKA-dependent way.

Kulkarni et al. published an article in 2005 about the effects of parathyroid hormone on Wnt signaling pathway in bone (Kulkarni et al., 2005). Parathyroid hormone (PTH) is a hormone that plays a role in regulation of bone and mineral metabolism. It increases osteoblast activity, osteoblast-precursors differentiation, lining cell recruitment and osteoblast survival. After PTH has bound to its receptor PTH1R, the receptor interacts with G-proteins and adenylyl cyclase leading to the production of cAMP and activation of PKA. PTH can also activate PKC through Gq-coupled signaling. The Wnt signaling pathway was found to be an important regulator of bone mass. In analysis by microarrays, they've already found that PTH in vivo increases frizzled-1 (FZD1), a receptor for Wnt. Besides that, activating mutations in FZDs co-receptor lipoprotein-receptor-related protein 5 (LRP5) were found to give a increase in bone mass with increased bone formation and inactivating mutations in LRP5 result in osteoporosis-pseudoglioma syndrome (OPPG). So to investigate the role of PTH on the canonical Wnt signaling pathway, they examined the effect of PTH (1-38) on FZD-1, co-receptor LRP5/6, secreted antagonist Dickkopf-1 (Dkk-1) and the Dkk-1 receptor Kremen-1 (KRM-1). Next to that, they also investigated whether the β-catenin level was affected by

PTH, and further downstream response of PTH on TBE (TCF-binding element) reporter gen activity in the Wnt signaling pathway. They used two rat osteosarcoma cell lines and rat primary osteoblast cultures. In both bone and osteosarcoma cell lines were low FZD-1 and high DKK-1 levels found, and in primary osteoblast cells they found high FZD-1 and low DKK-1 levels. These findings suggest a negative correlation between FZD-1 and the antagonist DKK-1. LRP5/6 and KRM-1 were both found in bone, osteosarcoma cells and primary osteoblasts. The effect of PTH on the Wnt signaling components was examined in PTH infused rats and in osteosarcoma cell line. The PTH infused rats were sacrificed at 1, 3, 6 or 24 hours after infusion. The levels of mRNA for FZD-1, LRP6 and KRM-1 were increased at 6 and 24 hours. LRP5 and Dkk-1 showed a decrease in a time-dependent manner. The results in the osteosarcoma cell lines were similar to these *in vivo* observations. This suggests that the differential expression of Wnt signaling components can influence the PTH effects on bone and bone cells. After treating osteosarcoma cells with forskolin (a direct activator of adenylyl cyclase) and disbutyryl cAMP (a stable analog of cAMP), the levels of Dkk-1 were decreased in the same way as to cells with PTH. Stimulation of these cells with IL-1α had no effect on the Dkk-1 expression so it can be concluded that the PTH effects on the Wnt signaling pathway might be mediated through cAMP. But foskolin and disbutyryl cAMP didn't influence the expression of either LRP5 or LRP6. This might mean that PTH has less effect on LRP5/6 than on Dkk-1. After that, they demonstrated the elevation of β-catenin levels in cells stimulated with PTH. After only 1 hour, an increased level of β-catenin was observed (*see figure 6*). Also the TBE-luciferase reporter gene activity was stimulated by PTH in a dose dependent manner. These findings suggest that PTH may activate the canonical Wnt signaling pathway in osteosarcoma cells. Besides that, forskolin increases the TBE-reporter gene expression in a manner similar to PTH. There was no stimulation of the TCF-reporter gene activity when cells were transfected with PTH analogs, PTH (3-34) and PTH (7-34), which do not induce cAMP. The PTH-increased TCF-reporter gene activity in osteosarcoma cells transfected with TBE-luciferase reporter gene was decreased in the presence of PKA inhibitor H89. These results suggest that the PTH effects on Wnt signaling are mediated at least in part through the cAMP/PKA pathway (Kulkarni et al., 2005). In 2006, Tobimatsu et al. confirmed the findings about increased levels of β-catenin stimulated by PTH via cAMP/PKA pathways (Tobimatsu et al., 2006). Next to that, they demonstrated the involvement of the PKC pathway. They also found that the PTH-induced increase in β-catenin levels was antagonized when Smad3 was inactivated. Smad3 is a crucial TGF-β-signaling molecule which promotes markers of osteoblast differentiation. So their findings indicate that PTH increases the level of β-catenin in osteoblasts through both PKA and PKC signaling pathways. Moreover, Smad3 is directly involved in this upregulation of β-catenin levels. They also showed that both PTH and Wnt/β-catenin activation reverses osteoblast apoptosis. They suggest that these antiapoptotic actions are due to PTH stimulating Wnt/β-catenin signaling in osteoblasts (Tobimatsu et al., 2006). In 2010, Romero et al. showed that that PTH can also act via

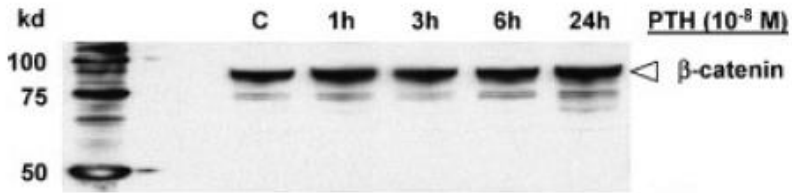

Figure 6: Western blot analysis of β-catenin from osteosarcoma cell line treated with human PTH (1-38) at different time points. Cells were treated for 1, 3, 6 and 24 h at a concentration of 10^{-8} M. Blots were immunostained using anti-β-catenin monoclonal antibody at 1:1000 dilution. (Adapted Kulkarni et al., 2005)

directly recruitment of Dishevelled (Dvl) by its receptor PTH1R and in this way activate the β-catenin pathway. So this indicates that PTH can affect β-catenin in a Wnt-independent manner. But activation of β-catenin by Wnt inhibits osteoclastogenesis and β-catenin activation via PTH stimulates osteoclastogenesis. But the divergent actions of the Fzd receptor and PTH1R are due to the PKA-dependent phosphorylation of β-catenin and GSK3β and the activation of CREB (*see figure 7*).

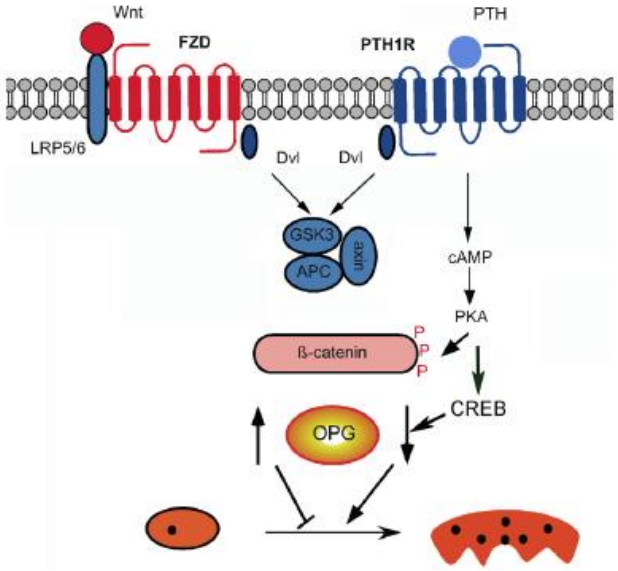

Figure 7: The divergent effects of PTHR1 and FZD signaling and action on osteoclastogenesis. Activation of both FZD and PTH1R can stimulate β-catenin. In case of FZD, this leads to inhibition of osteoclastogenesis, whereas PTH1R increases osteoclastogenesis. This may be due to the PKA-dependent phosphorylation of β-catenin and GSK3β and the activation of CREB. Adapted from Romero et al., 2010

In 2009, Goessling et al. found a genetic interaction between PGE2 (cAMP signaling pathway activator) and Wnt signaling which regulates the developmental specification of stem cells and regeneration (Goessling et al., 2009). PGE2 regulates vertebrate hematopoietic stem cells (HSC) and Wnt is known to control HSC self-renewal and bone marrow repopulation. Clinical evidence from colon cancer patients and cancer cell lines suggested a close association between PGE2 and Wnt signaling pathways. To verify this, they used zebrafish HSCs in their *in vivo* studie for demonstrating a direct interaction between PGE2 and Wnt and they did an *in vitro* study in hematopoietic embryonic stem cells followed by an *in vivo* transplantation experiment showing the interaction can regulate murine stem and progenitor population. Injecting PGE2 in β-catenin-responsive reporter zebrafish embryos caused an increase in reporter activity throughout the embryo 36 hours after fertilization. When PGE2 production in these embryos was suppressed by the non-selective cox inhibitor indomethacin, they showed by mass spectroscopy that Wnt activity in these embryos was decreased. With qPCR analysis they confirmed that PGE2 can modulate Wnt activity significantly. Then they used Wnt labeled HSCs and endothelial cells to determine whether Wnt activity was localized within the HSCs. These data demonstrated that Wnt activity within HSC/endothelial cell population and the hematopoietic niche during embryonic development is regulated by PGE2. They then found that PGE2 modifies Wnt-mediated regulation of HSC formation through cAMP/PKA activity and through alteration in β-catenin availability. So PGE2 and Wnt signaling interact at the level of the β-catenin destruction complex and regulate subsequent protein available for transcriptional activation. But PGE2 did not affect β-catenin transcription so it influences the β-catenin stability via non-transcriptional mechanisms. Finally, the PGE2/Wnt interaction was found to affect cell survival and proliferation of HSCs, it is functionally conserved in HSC regeneration, mammalian

hematopoietic stem and progenitor populations and it's also a central regulator of organ regeneration. Al these findings taken together comprise the first *in vivo* evidence that Wnt activation in stem cells requires PGE2, and that the interaction between these two is possible a master regulator of regeneration and recovery in vertebrate stem cells.
This article is reviewed by Evans who mentions that there are some mysteries to be solved (Evans T., 2009). In first place, β-catenin is not required for hematopoiesis. But it is not known if this is also true for tissue regeneration. He also mentions that PGE2 and Wnt signaling both can decrease the activity of stem cells (Goessling et al., 2009).
Another article about the cooperation between Wnt and PGE2 was published in 2010 by Kaur and Sanyal (Kaur and Sanyal, 2010). They investigated the association between PI3-kinase/Wnt and COX-2/PGE2 pathways to inhibit apoptosis in early stages of colon tumorigenesis. The PI3-kinase pathway was already demonstrated to mediate Wnt-induced growth and proliferation of fibroblast cells (Kim, Lee and Choi, 2007). In colon cancer ratmodels, COX-2 mRNA expression and PGE2 levels were elevated, however, COX-1 mRNA expression was unaltered. Also PI3-kinase, Akt, Wnt and β-catenin were upregulated and GSK-3β was reduced. When diclofenac (an NSAID which inhibits COX and thereby cell proliferation by inducing apoptosis) was injected into these rats, levels of GSK-3β were increased while PI3-kinase, Akt, Wnt and β-catenin were inactivated. These findings suggest that activation of PI3-kinase and Wnt signaling is associated with COX-2/PGE2 production and thereby inhibit apoptosis in colon cancer (Kaur and Sanyal, 2010).

In a recent study, Goto et al. investigated whether Rap1 can stabilize β-catenin and enhance invasion and β-catenin-dependent transcription in HNSCC (head and neck squamous cell carcinoma) (Goto et al., 2011). They previously demonstrated that Rap1 plays a role in tumor growth and invasion in malignant oral keratinocytes. In HNSCC, Rap1 shuttles between nucleus and cytoplasm. It is known as a key player in cell adhesion and migration. Rap can also be activated by cAMP/Epac signaling (*see figure 3 and 4*). It has two isoforms, Rap1A and Rap1B. Rap1 switches from an inactive GDP-bound form to an active GTP-bound form and this switch is regulated by several guanine nucleotide exchange factors (GEFs), including Epac. In their study they first demonstrated the presence of free β-catenin in human HNSCC using immunohistochemical studies and a pull-down assay analyzed by immunoblotting. Free β-catenin was observed but represented only a small fraction of the total β-catenin in whole cell lysates. They also demonstrated that β-catenin can bind to active Rap1 according to immunoblotting and immunoprecipitation. Then they investigated whether Rap1 enhances β-catenin mediated transcription. HNSCC cells were co-transfected with active Rap1A and β-catenin and with a lusiferase-based transcription assay they could see that Rap1A induced a two-fold increase in β-catenin-mediated transcription. A dominant negative mutant form of TCF4 (DNTCF) was added to the cells to determine if Rap1A induces β-catenin-dependent transcription via TCF4/LEF transcription factor, which is the transcription factor that's normally bound by β-catenin. Rap1A-induced, β-catenin-dependent transcription was totally blocked by DNTCF, so Rap1A-induced, β-catenin-dependent transcription occurs via a TCF-dependent pathway.
They were also interested whether Rap1 promotes nuclear translocation of β-catenin and they used immunofluorescence to verify this. Cells transfected with β-catenin and Rap1 showed an increase of 35% in β-catenin nuclear localization compared to cells transfected with only β-catenin and vector control. And to investigate whether Rap1 promotes the functional effects of β-catenin in HNSCC, they determined if Rap1 can

stimulate β-catenin-induced invasion. Transfected cells were seeded on Matrigel and invasion was assayed. Invasion was upregulated by roughly 50% in cells with Rap1A overexpression compared to cells without Rap1A. To verify whether inhibition of endogenous Rap1 can downregulate the β-catenin-mediated invasion, cells were nucleofected with siRNAs to knockdown Rap1. This led to significantly reduced levels of free β-catenin. Also the increase of free β-catenin induced by respectively lithium chloride and Wnt3A was reduced in cells transduced with siRNA Rap1A. Besides that there was an increased expression level of MMP7, a matrix-metalloproteinase which is a transcriptional target of β-catenin/TCF, in cells without siRNA Rap1 and the expression levels were reduced in presence of siRNA Rap1. Taking the results of all these studies togheter, at least 30% of free β-catenin was decreased in cells transduced with siRNA Rap1A. And immunohistochemical studies showed that increased β-catenin intensity was correlated with higher tumor stage in tissues of primary oropharyngeal squamous cell carcinoma (SSC). When the β-catenin expression was high, higher Rap1 intensity was associated with more advanced N-stage (lymph node involvement) and when β-catenin expression is low, lower activity Rap1 intensity was associated with more advanced N-stage. These data are consistent with their in vitro data showing high β-catenin and high Rap1 were more invasive in HNSCC cells than cells expressing high β-catenin and low Rap1. Also the findings of low β-catenin and low Rap1 being more invasive than cells expressing low β-catenin and high Rap1 are consistent with their in vitro findings. Goto et al. finally proposed a model for interaction between Rap1, β-catenin and MMP7 in SCC progression. Free β-catenin bound by Rap1 increases TCF/LEF transcriptional activity which stimulates MMP7 secretion and MMP7 promotes invasion. Rap1 inhibits invasion via inhibition of MMP9 and MMP (*see figure 8*) (Goto et al., 2011).

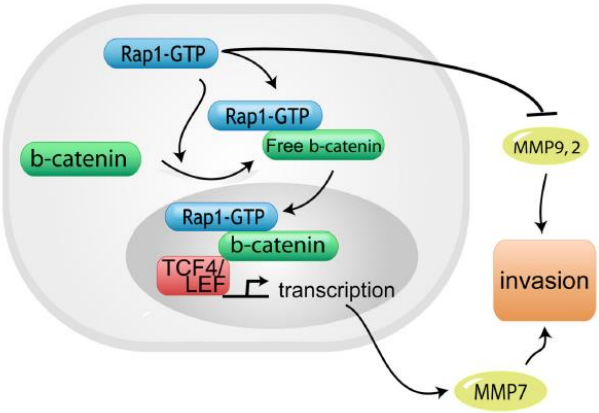

Figure 8: Proposed model for interaction between Rap1, β-catenin and MMP7 in SCC progression. Rap1 promotes invasion via β-catenin mediated effects and inhibits invasion via inhibition of MMP9 and MMP2. Adapted from Goto et al., 2011

In 2006 and 2007, one group released two articles about the interaction between the Wnt/β-catenin signaling pathway and ERK pathway during tumorigenesis (Park et al., 2006; Jeon et al., 2007). In their studies, they investigated the relationship between those two pathways by measuring the effects of Axin and APC. These two proteins were already known as a negative regulator of Wnt/β-catenin signaling, and they were also found in several types of cancer. They demonstrated reduction of ERK pathway components (Raf-1, MEK and ERK kinase) in cells in which the β-catenin level was reduced by APC or Axin overexpression. The proteins can also reduce Ras protein levels, and the levels of both Ras protein levels and ERK pathway components were simultaneously decreased by siRNA-mediated reduction of β-catenin. Therefore, β-catenin protein level regulation is essential for the regulation of Ras and ERK pathway components. Also involvement of β-catenin/TCF-mediated gene transcription in ERK pathway regulation was indicated because ERK activation by Ras or β-catenin was

lowered by dominant negative TCF. In summary, they showed that the Ras-ERK pathway, cell proliferation and celltransformation are regulated by Wnt/β-catenin signaling involving Axin and APC and their effector β-catenin. This is evidence for the role of the Ras-ERK pathway in tumorigenisis induced by abnormalities in Wnt/β-catenin signaling (Park et al., 2006; Juon et al., 2007).
In 2005 and 2007 another article was published about the interaction between Wnt/β-catenin signaling and the ERK pathway (Yun et al., 2005; Kim and Choi, 2007). Their results indicated that Wnt stimulates proliferation and motility of NIH3T3 fibroblasts via (EGFR-mediated) ERK pathway activation. Their results also indicated that Wnt might act upstream of Ras. These results are consistent with those of Park et al. and Juon et al..

Recently a review was released about the connection between Wnt signaling pathway and Phospholipase D (PLD) (Kang et al., 2011). The PLD pathway can be stimulated by cAMP/Epac/R-Ras signaling and it induces cell proliferation and survival (*see figure 4*). PLD genes are transcriptional targets of Wnt/β-catenin-signaling (*see figure 9*). So when β-catenin binds to TCF, PLD genes are transcribed and phosphatidic acid (PA) is generated by hydrolysis of phosphatidylcholine (PC). PA can induce several mechanisms that lead to (tumorigenic) cell proliferation and invasion. PA can activate Ras, which leads to cell transformation. PA can also bind to Raf, and in this way it can stimulate the Raf-Map/ERK kinase (MEK)-extracellular signal regulated kinase (ERK) pathway. The most critical target of PA is mTOR, a key regulator of cell growth. Kang et al. recently demonstrated transcriptional activation of PLD by Wnt3a, and activation of Wnt/β-catenin-signaling target genes via β-catenin/TCF transcription complex stimulation by the second messenger PA produced by PLD (Kang et al., 2010). They found that the expression levels of β-catenin and PLD in colorectal cancer tissues are significantly correlated and this demonstrates the strong link between the Wnt/β-catenin pathway and PLD *in vivo*. They also found that PLD activates Wnt/β-catenin signaling via a positive feedback loop. PLD1 provides selective downregulation of ICAT (an inhibitor of β-catenin-interaction protein) which normally inhibits Wnt/β-catenin signaling (*see figure 9*). When PLD1 was inhibited, it suppressed the association of β-catenin with TCF and enhanced expression of ICAT, but it did not enhance expression of other β-catenin/TCF-binding inhibitors (like Chibby, PParγ, FOXO1, FOXO3 or plakoglobin). So this suggests that ICAT is a selective molecular target of the PLD1-mediated Wnt/β-catenin signaling pathway. Overall, they identified a bidirectional cross-talk between PLD and Wnt/β-catenin signaling pathway which induces (tumorigenic) cell proliferation (Kang et al., 2011).

Another evidence for the interaction between cAMP/Epac and Wnt was supplied by Ahmed et al. in their study to investigate the cooperation between Ras mutation and β-catenin in bladder tumorigenesis (Ahmed et al., 2011). The gene H-Ras was the first human oncogene that was isolated in human urothelial cell carcinoma (UCC). A mutation in this gene leads to constitutive activity and can also result in overexpression of the protein. H-Ras mutation occurs in approximately 30-40% of low-grade papillary UCC and up to 10% of muscle-invasive UCC. But a mutation in H-Ras alone fails to fully induce bladder tumors. It was also already clear that Wnt signaling pathway is activated in a proportion of UCCs, but just like H-Ras was the activation of this pathway alone not enough to drive UCC, however it strongly cooperates with PTEN loss to drive tumorigenesis. Because of these findings, Ahmed et al. were curious about whether Ras

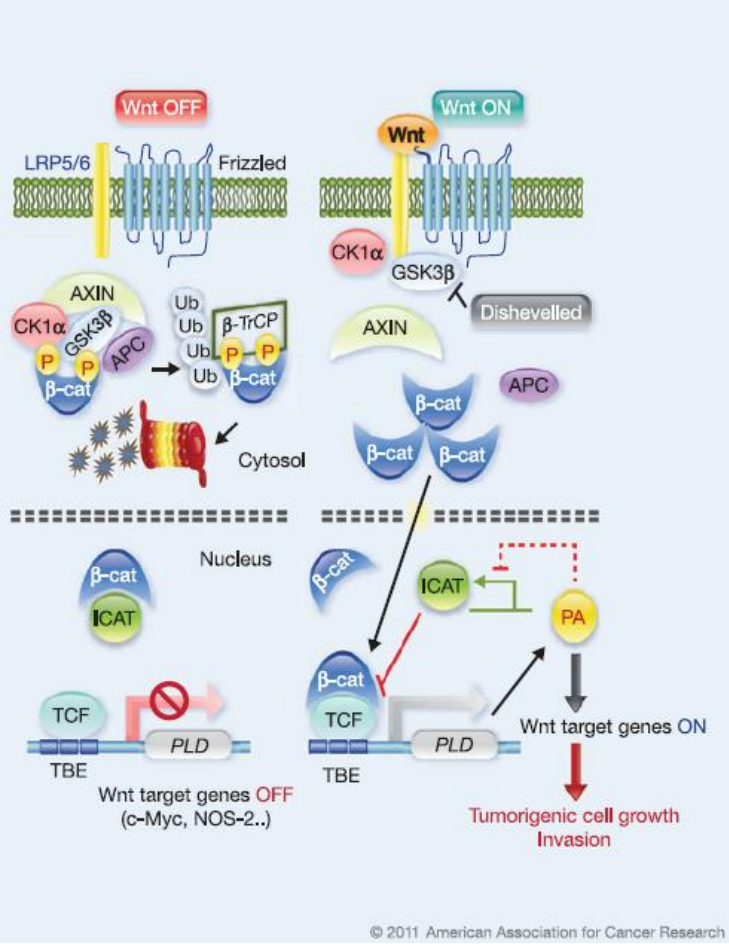

Figure 9: the canonical Wnt signaling pathway including PLD. PLD1 en PLD2 genes are transcriptionally activated via binding of β-catenin/TCF to the TBE of its promoters. PLD-generated PA suppresses the ICAT expression, which inhibits the interaction of β-catenin/ TCF to TBE, followed by promotion of Wnt/β-catenin/ TCF signaling. This way, transcription of the target genes are upregulated and tumorigenic cell growth is ultimately stimulated. (Adapted from Kang et al., 2011)

overexpression or mutation cooperates with deregulated Wnt signaling to promote UCC in the murine urothelium. The results show that the combination of mutated H-Ras and Wnt signaling/β-catenin activation indeed can induce UCC. But these tumors remain non-invasive and do not metastasis despite extended follow-up. They have increased MAPK signaling but no p-AKT/p-mTOR activation, while tumors that have PTEN loss and β-catenin activation show little MAPK pathway and instead have high levels of p-AKT/p-mTOR. So these tumors have differential sensitivity to MEK and mTOR inhibition. But it can be concluded that Ras and Wnt can cooperate and promote non-metastatic bladder tumors. This cooperation was also found in hepatocellular carcinomas (HCC) investigated by Harada et al., who also didn't see tumorigenesis in cells with H-Ras mutation alone (Harada et al., 2004).

The reason that a mutation in H-Ras alone can't induce tumorigenesis is because Ras protein stability is regulated by β-TrCP-mediated polyubiquitylation and degradation via the proteasomal machinery which is controlled by Wnt/β-catenin signaling (Kim et al., 2009). When Wnt signaling was activated by Wnt3a, the binding affinity of β-TrCP to H-Ras was reduced and the level of H-Ras protein was increased. But Axin and APC induced β-TrCP-mediated ubiquitylation and degradation of H-Ras. Ras protein destabilization via Wnt/β-catenin signaling maybe acts as a safeguard against tumorigenesis that could be caused by aberrant activation of Ras or hyperstimulation of upstream Ras activators (such as the EGF receptor). The regulation of Ras protein stability via polyubiquitylation is a newly discovered Ras regulatory mechanism involved in the regulation of cellular transformation (Kim et al., 2009).

Discussion

This review focused on the question whether there is an interaction between the cAMP and Wnt signaling pathways in cell proliferation. This interaction can be accomplished at many different levels of these pathways. There is much known about the association between cAMP/PKA signaling and β-catenin stabilization. β-catenin can be phosphorylated at Ser675 by PKA and in this way, according to Hino et al., β-catenin is stabilized and can't be ubiquitylated anymore (Hino et al., 2005). This also leads to activation of β-catenin-mediated transcription. But according to Taurin et al., phosphorylation of Ser675 doesn't stabilize β-catenin and β-catenin can still be degraded (Taurin et al., 2006). But it does increase the transcriptional activity and it also it facilitates the interaction of β-catenin with cAMP response element-binding (CREB) binding protein (CBP). So whether β-catenin is stabilized by this phosphorylation or not, at least Wnt transcriptional activity is stimulated. Also ATP-induced phosphorylation of β-catenin by cAMP/PKA showed an increase of transcriptional activity because of a stronger association of β-catenin with TCF (Taurin et al., 2008).
The cAMP/PKA pathway can also be stimulated by PTH (Kulkarni et al., 2005). When PTH has bound to its receptor PTH1R, the receptor interacts with G-proteins and adenylyl cyclase which leads to cAMP production and PKA activation. They also showed upregulation of Wnt proteins and downregulation of Wnt antagonists. These same patterns were seen in cells without PTH and with high cAMP, so the PTH effects are probably mediated by cAMP signaling. PTH can also elevate the β-catenin levels in cells and thereby the transcriptional activity. In conclusion, PTH can stimulate the Wnt signaling pathway, at least in part, through cAMP/PKA signaling. In 2006, Tobimatsu et al. confirmed these results. They also showed that both PTH and Wnt/β-catenin activation can reverse osteoblast apoptosis. So anti-apoptotic actions are due to PTH stimulation of Wnt/β-catenin signaling in osteoblasts. Later, in 2010, Romero et al. demonstrated that PTH can also activate β-catenin directly via recruitment of Dishevelled by PTH1R.
Besides PTH, another cAMP signaling pathway activator, called PGE2, interacts with the Wnt signaling pathway. This interaction can regulate the developmental specification and regeneration of stem cells (Goessling et al., 2009). They showed that suppressed PGE2 production causes a decrease in Wnt activity in zebrafish embryos. They also found that PGE2 modifies Wnt-mediated regulation of HSC formation through cAMP/PKA activity and through alteration in β-catenin availability. So they associate at the level of the β-catenin destruction complex, but PGE2 didn't affect β-catenin transcription so it influences the β-catenin stability in a non-transcriptional manner. These findings suggest that PGE2 is required for Wnt activation in stem cells and that the interaction is a possible major regulator of regeneration, proliferation and survival in vertebrate stem cells. But in a review of this article it was mentioned that β-catenin isn't required for hematopoieses. But it might be for tissue regeneration. Besides that, PGE2 and Wnt signaling both can also decrease the stem cell activity (Evans, 2009).
Next to the effect on stem cells, the interaction between PGE2 and Wnt can also inhibit apoptosis in early stages of colon cancer (Kaur and Sanyal, 2010). They demonstrated the association between activation of PI3-kinase and Wnt signaling with COX-2/PGE2 production and due, to this association, apoptosis of colon cancer cells is inhibited.

When you search for studies about the interaction between Epac and Wnt signaling, there's not much known in literature. But there are some articles published this year

(and some of them little earlier) which expose the interaction between some Epac signaling pathway components and Wnt/β-catenin. Recently, Goto et al. investigated whether Rap1 can stabilize β-catenin and enhance invasion and β-catenin-dependent transcription in HNSCC (Goto et al., 2011). Rap1 is a downstream effector of Epac (*see figure 3 and 4*). They showed a two-fold increase of the β-catenin-mediated transcription, a 35% increase in β-catenin nuclear translocation and 50% upregulation of invasion in cells transfected with Rap1 and β-catenin (compared to cells transfected with β-catenin only). Besides that, levels of MMP7 (transcriptional target of β-catenin/TCF) and 30% of free β-catenin were decreased in cells transduced with siRNA Rap1A. Also increased β-catenin intensity was correlated with higher tumor stage in SCC. The conclusion which can be made out of this study is that Rap1 can bind to free β-catenin and thereby stimulates the β-catenin/TCF transcriptional activity. This upregulates the levels of MMP7 and this can stimulate invasion (*see figure 8*).
The ERK pathway is very important in cell signaling and this pathway can be activated by cAMP via both PKA and Epac (*see figure 3 and 4*). Research on interaction between the Wnt and ERK pathways in tumorigenisis include two studies which measured the effects of two Wnt proteins, Axin and APC (Park et al., 2006; Jeon et al., 2007). Both proteins can reduce the β-catenin level and simultaneously Ras protein levels and ERK pathway components were decreased. Also involvement of β-catenin/TCF-mediated gene transcription in ERK pathway regulation was indicated because ERK activation (by Ras or β-catenin) was suppressed by dominant negative TCF. The results of this study are evidence for the role of the Ras-ERK pathway in tumorigenesis induced by abnormalities in Wnt/β-catenin signaling.
A recently published review about the connection between the Wnt signaling pathway and the phospholipase D (PLD) pathway shows another connection between Epac and Wnt (Kang et al., 2011). The PLD pathway can be activated by cAMP/Epac/R-Ras signaling and its genes are transcriptional targets of Wnt/β-catenin signaling (*see figure 4 and 9*). When β-catenin binds to TCF, this stimulates PLD gene transcription which leads to generation of phosphatidic acid (PA) and this can induce (tumorigenic) cell proliferation and invasion via association with Ras, Raf and mTOR. In 2010, Kang et al. showed that there's strong link between Wnt/β-catenin and PLD *in vivo*. They also demonstrated that PLD activates Wnt/β-catenin via a positive feedback loop (*see figure 9*). In conclusion, (tumorigenic) cell proliferation is induced by bidirectional cross-talk between PLD and Wnt/β-catenin signaling.
Another recently published article found evidence for the connection between Epac and Wnt. Ahmed et al. investigated the cooperation between H-Ras and β-catenin in bladder tumorigenisis (Ahmed et al., 2011). H-Ras mutation and Wnt signaling activation both appear in urothelial cell carcinoma (UCC), but any of these two alone fails to fully induce bladder tumors. The results of their study show that combination of mutated H-Ras and Wnt signaling/β-catenin activation can induce non-invasive, non-metastatic UCC. The same results were obtained by Harada et al. in hepatocellular carcinomas (HCC) (Harada et al., 2004).
There's also a reason for H-Ras failing to induce tumorigenesis alone. In 2009, Kim et al. showed that the Ras protein stability is regulated by polyubiquitylation via Wnt/β-catenin signaling (Kim et al., 2009). When Wnt-signaling is inactivated, β-TrCP binds with H-Ras and induces degradation of H-Ras by polyubiquitylation. When Wnt3a activates the Wnt signaling pathway, the binding affinity of β-TrCP to H-Ras is reduced. This mechanism may be a safeguard against tumorigenesis caused by continuous H-Ras activation.

Finally, it can be concluded that there is indeed an interaction between the Wnt signaling pathway and cAMP signaling in cell proliferation. They can interact at different levels in the pathways, which are summarized in figure 10 and 11. In figure 10, you can see the interactions between Wnt and cAMP/PKA, and in figure 11 the interactions between Wnt and cAMP/Epac. The most important interaction would be those with MEK/ERK, because this is very important, even essential, for cell proliferation. It is stimulated by cAMP via both PKA and Epac and also by β-catenin and TCF/LEF. It can be concluded that Epac also has a major role in cell proliferation because it can activate ERK and PLD which induce cell proliferation. And it can also interact with the Wnt/β-catenin signaling to enhance the ERK and PLD activation.

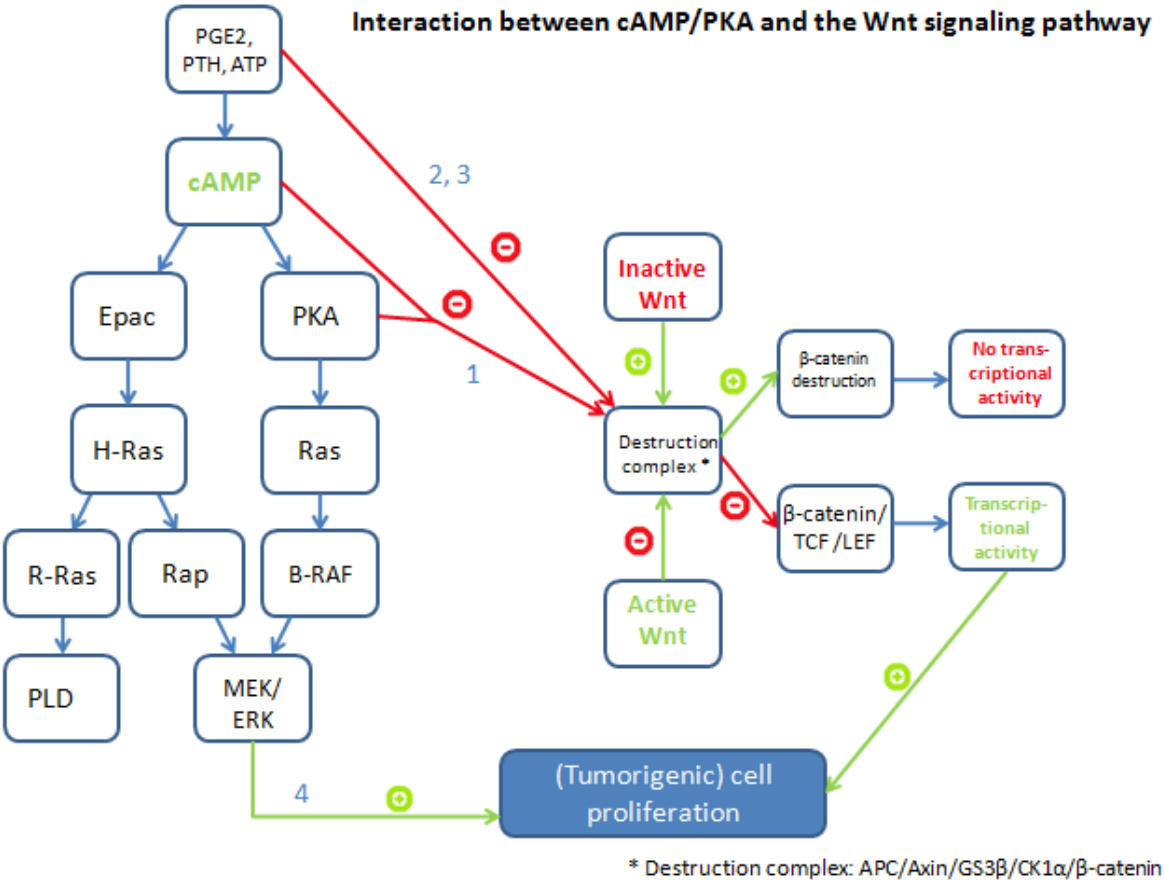

Figure 10: The interaction between cAMP/PKA signaling and the Wnt signaling pathway. PGE2, PTH and ATP both can activate the cAMP signaling pathway. They can also inhibit the destruction complex, thereby reducing β-catenin degradation and stimulate transcriptional activity. Besides that, both cAMP and PKA can also inhibit the destruction complex with the same consequences. cAMP/PKA signaling leads to the activation of the MEK/ERK pathway. Both this pathway and Wnt transcriptional activity induce cell proliferation and they may also cause tumorigenesis.

1: Hino et al., 2005
2 (PTH): Kulkarni et al., 2005; Tobimatsu et al., 2006; Romero et al., 2010
3 (PGE2): Goessling et al., 2009; Kaur&Sanyal, 2010
4: Park et al., 2006; Jeon et al., 2007; Yun et al., 2005; Kim&Choi, 2007

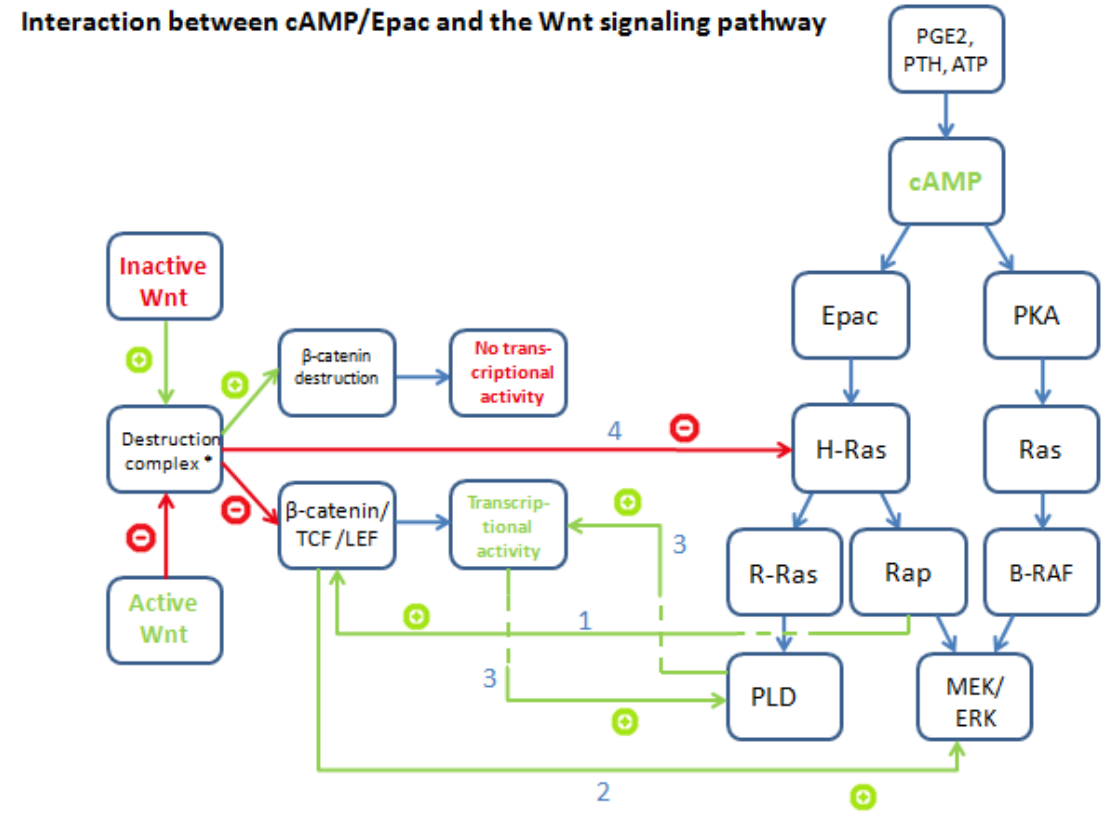

Figure 11: The interaction between cAMP/Epac signaling and the Wnt signaling pathway. Rap1, stimulated by Epac, can stabilize β-catenin and enhance its binding to TCF/LEF. Stabile β-catenin and β-catenin-mediated transcription both can activate the MEK/ERK pathway. PLD, another effector of Epac, can also be stimulated by β-catenin-mediated transcriptional activity and induce transcriptional activity via a positive feedback loop. But when Wnt signaling is inactivated, the β-catenin destruction complex can degrade H-Ras via polyubiquitylation and thereby inhibit PLD stimulation. The MEK/ERK pathway and Wnt transcriptional activity can both induce (tumorigenic) cell proliferation (as already shown in figure 10) but next to that, also PLD can induce cell proliferation.

1: Goto et al., 2011
2: Park et al., 2006; Yun et al., 2005; Kim&Choi 2005
3: Kang et al., 2011
4: Kim et al., 2009

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