Functions of p120ctn isoforms in cell-cell adhesion and intracellular signaling

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1. ABSTRACT

The functions of many organs depend on the generation of an epithelium. The transition from a set of loosely connected nonpolarized cells to organized sheets of closely associated polarized epithelial cells requires the assembly of specialized cell junctions. In vertebrates, three major types of junctions are responsible for epithelial integrity: adherens junctions, tight junctions, and desmosomes. p120 catenin (p120ctn) is an Armadillo family member and a component of the cadherin–catenin complex in the adherens junction. It fulfils pleiotropic functions according to its subcellular localization: modulating the turnover rate of membrane-bound cadherins, regulating the activation of small RhoGTPases in the cytoplasm, and modulating nuclear transcription. Over the last two decades, knowledge of p120ctn obtained from *in vitro* experiments has been confirmed and extended by using different animal models. It has become clear that p120ctn is essential for normal development and homeostasis, at least in frog and mammals. p120ctn is a Src substrate that can be phosphorylated at different tyrosine, serine and threonine residues and can dock various kinases and phosphatases. Thereby, p120ctn regulates the phosphorylation status and the junctional stability of the cadherin–catenin complex. Multiple p120ctn isoforms are generated by alternative splicing, which allows the translation to be initiated from four start codons and enables the inclusion of four alternatively used exons. We will discuss the effects of different p120ctn isoforms on cadherin turnover and intracellular signaling, in particular RhoGTPase activity and phosphorylation events.

2. INTRODUCTION

Multicellular organisms rely on dynamic cell–cell adhesion systems to sustain the development and homeostasis of embryos and tissues. Several types of junctions have been identified in epithelial cells: tight junctions, desmosomes, gap junctions and adherens junctions. Tight junctions (reviewed in 1, 2) connect adjacent cells and create a barrier that is almost impermeable to fluids. In this way, tight junctions regulate movement of ions and solutes between cells. Tight junctions consist of the transmembrane proteins occludin and claudin, and the cytoplasmic scaffolding proteins ZO-1, -2, and -3, which link tight junctions to the actin cytoskeleton. Desmosomes (reviewed in 3, 4) are intercellular junctions that anchor membrane-associated plaque proteins to intermediate filaments. This anchoring provides mechanical strength. Desmosomes consist of desmosomal cadherins (desmoglein and desmocollin) and cytoplasmic plaque proteins (such as the Armadillo proteins plakoglobin and plakophilins), which bind to desmosomal cadherins, as well as to desmoplakin, which binds to intermediate filaments. Gap junctions (reviewed in 5) are aggregated channels that connect adjacent cells and permit the exchange of ions and small molecules. Gap junctions chemically couple cells and allow easy intercellular communication. Gap junction channels are composed of two end-to-end paired connexons, each of which consists of hexamers of connexins, which are the principle structural components of gap junctions. Adherens junctions (Figure 1A) consist of cadherin–catenin complexes in which single-span transmembrane cadherin molecules mediate calcium-dependent homophilic interactions *via* their extracellular domains. Mammalian genomes contain over 100 genes belonging to the cadherin superfamily (6). E-cadherin is the prototype of the classic cadherin family. This family is characterized by the presence of five tandem extracellular cadherin repeats (EC1-5) that function in calcium-dependent homophilic adhesion (7). The cytoplasmic domain of E-cadherin serves as a scaffold for catenins, which link cadherin-mediated cell adhesion to the actin cytoskeleton, either directly or indirectly (8). Two conserved catenin-binding domains are present, a membrane proximal domain (juxtamembrane domain, JMD) for binding p120catenin (p120ctn) and a beta-catenin binding domain (CBD). This review covers the structure and versatile functions of p120ctn.

p120ctn binds to various cadherins and regulates their stablility on the cell surface. We will discuss the mechanism of p120ctn-regulated cadherin turnover in Section 4. In addition, p120ctn plays a role in other subcellular compartments (Figure 1): it modulates RhoGTPase activity in the cytoplasm (Section 5) and regulates nuclear transcription (Section 6). The Armadillo protein p120ctn was first identified as a Src substrate (9). Other kinases and phosphatases can either interact with p120ctn or change its phosphorylation status (see Section 7). Depletion of p120ctn *in vivo* has shown that p120ctn is indispensable for normal vertebrate development, at least in frog and mammals (Section 8). On the other hand, deregulation of p120ctn is common in human cancer, and p120ctn influences several processes that are involved in tumor formation and progression (Section 9). During the previous decade, it has become clear that p120ctn is not translated as a single protein but as different isoforms (see Section 3). Although the existence of p120ctn isoforms has been known for a long time, their regulation and significance in p120ctn biology remains largely unresolved. We will review current knowledge of p120ctn, with special focus on the various isoforms, how they are expressed, and how they influence the many functions of p120ctn.

3. p120ctn ISOFORMS AND ALTERNATIVE SPLICING

The human p120ctn gene (*CTNND1*) is located on chromosome 11q11. Following its cloning in 1998, it was found that inter- and intra-exonic splicing events generate multiple p120ctn mRNA variants encoding different isoforms (10). Up to forty-eight putative p120ctn isoforms might be generated by using four different translation initiation sites (M1-4) combined with four alternatively spliced internal exons (A to D; Figure 2) (10, 11). Each p120ctn isoform consists of a large central Armadillo repeat domain flanked by an N-terminal and a C-terminal region (Figure 2). This central domain has nine Armadillo repeats, each of which consists of three helices (12, 13). The central domain is not affected much by alternative splicing. Only the six amino acid residues (AA) encoded by the alternatively spliced exon C, also called exon 11, are located in an insert loop between Armadillo repeats ARM5 and ARM6, but this probably does not cause conformational changes in the overall structure of the Armadillo repeat domain (12, 13). p120ctn 1ABC (968 AA) is the longest isoform, contains all the alternatively spliced internal exons, and has the longest N-terminal domain because it is translated from the first start codon (Figure 2). p120ctn isoform 3 has a shortened N-terminal domain because it lacks 100 AA containing a coiled-coil domain, whereas p120ctn isoform 4 lacks almost the entire N-terminal domain, including the phosphorylation domain (PD), which contains most of the phosphorylation sites.

In general, p120ctn is ubiquitously expressed during development and in adult organisms, except in several non-adherent hematopoetic cell lines and in loosely organized SW48 colon carcinoma cells, which exhibit only weak p120ctn expression (14, 15). p120ctn isoform 1 (long isoform) and p120ctn isoform 3 (short isoform) display tissue-specific and cell-specific expression patterns. Isoform 1 is predominantly expressed in highly motile cells, such as fibroblasts (*e.g.* NIH3T3 cells) and macrophages (14, 16, 17). In normal tissues, p120ctn isoform 1 is predominantly expressed in the central and peripheral nervous systems, heart, spleen, testis, ovary and endothelial cells (11, 18, 19). In contrast, isoform 3 is abundant in epithelial cell lines (14, 16, 17) and in epithelial structures of the skin and the gastro-intestinal lining, which have a rapid turnover, as well as in kidney, liver, pancreas, mammary gland and prostate (10, 11, 18, 19). These cells and tissues show bias for expression of certain p120ctn isoforms, but most cells and tissues express both long and short isoforms. Since long p120ctn isoforms

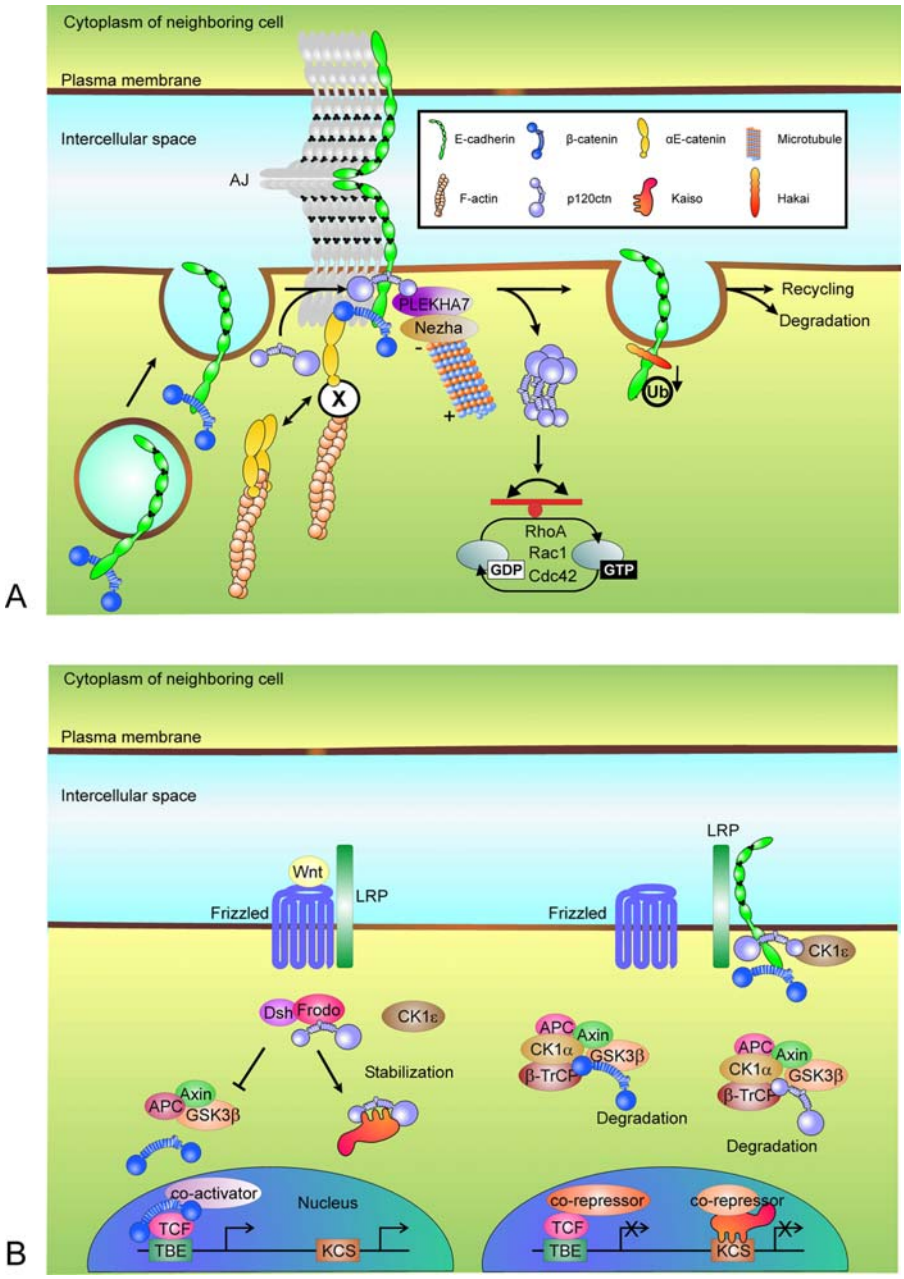

Figure 1. p120ctn performs different functions in different subcellular compartments. (A) Adherens junctions (AJ) in epithelial cells consist of transmembrane E-cadherins that bind to beta-catenin and p120ctn *via* their cytoplasmic tails. Beta-catenin binds to alphaE-catenin, which can link to the actin cytoskeleton *via* an adaptor (*e.g.* Eplin) or as a dimeric complex not coupled to the cadherins. p120ctn binds to the juxtamembrane domain of cadherins and prevents their endocytosis. Internalized cadherin molecules can be recycled or targeted for degradation. A p120ctn–PLEKHA7–Nezha chain forms a bridge between the cadherin–catenin complex and the minus ends of microtubuli. Cytoplasmic p120ctn can regulate the activity of RhoGTPase family members. (B) p120ctn is also involved in the Wnt signaling pathway. *Right:* In the absence of Wnt ligand, the beta-catenin destruction complex promotes the degradation of beta-catenin. This complex is composed of adenomatous polyposis coli (APC), glycogen synthase kinase-3beta (GSK3β), casein kinase-1alpha (CK1α), beta-transducin repeat-containing protein (β-TrCP) and axin. Also p120ctn is not available for the Wnt pathway because it binds to E-cadherin at the AJ or because it is degraded. When beta-catenin and p120ctn in signaling pools are reduced, TCF/LEF and Kaiso function as transcriptional repressors in the nucleus. *Left:* In the presence of Wnt ligand, both beta-catenin and p120ctn become available for the Wnt pathway. Beta-catenin enters the nucleus to relieve the repression by TCF–LEF. Simultaneously, cytoplasmic (and nuclear) p120-catenin becomes available to associate with Kaiso, resulting in derepression of Kaiso target genes, some of which are shared with beta-catenin/TCF–LEF. p120ctn also binds Frodo, which interacts with Dishevelled (Dsh), a Wnt pathway component. LRP, low-density lipoprotein receptor-related protein; TBE, TCF binding elements; KCS, Kaiso consensus (binding) sequence.

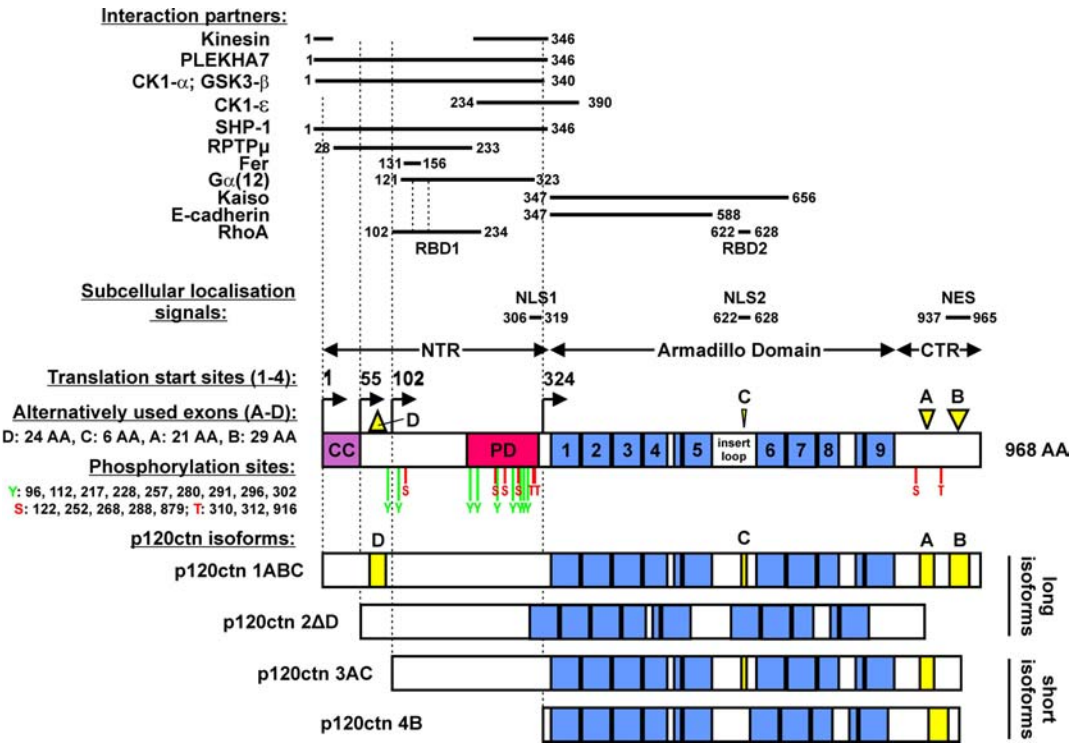

Figure 2. Structure of p120ctn isoforms. The structural features depicted include interaction domains, subcellular localization signals, phosphorylation sites, and alternative splice forms. The p120ctn domains for binding different interaction partners are shown at the top. p120ctn contains two conventional nuclear localization signals (NLS) and a nuclear export sequence (NES). Armadillo repeats (ARM) 3, 5 and 8 have also been implicated in nuclear trafficking. Due to alternative splicing, four translation start sites (1 to 4) can be used. Alternatively used exons A, B and C, encoding 21, 29 and 6 AA, respectively, can be included, whereas exon D (encoding 24 AA) is rarely excluded. p120ctn isoform 1ABC is the longest isoform, employs the first start codon, and contains all alternatively spliced exons. CC, coiled coil domain; CTR, carboxy-terminal (non-Armadillo) region; NTR, amino-terminal (non-Armadillo) region; PD, phosphorylation domain, comprising many Ser, Thr and Tyr residues that are phosphorylated under particular conditions; RBD, RhoA-binding domain.

are expressed in endothelial cells, which are present in all organs, tissue preparations from any organ might contain long isoforms derived from endothelial cells. Interestingly, a switch from short to long p120ctn isoforms is seen during epithelial-to-mesenchymal transition (EMT), which can be induced by expression of c-Fos (20), Snail (21), SIP1/ZEB2 (22, 23), E47 (23), Slug (23), Twist (24) or Zeppo1 (25).

Two epithelial splicing regulatory proteins (ESRP1 and ESRP2) favor the expression of epithelial isoforms of various proteins, including the short 'epithelial' p120ctn isoform (24). Reduction of ESRP1 and ESRP2 levels by RNAi-mediated knockdown or by Twist-induced EMT resulted in a switch from short to long p120ctn isoforms. Reciprocally, expression of 'epithelial' p120ctn in mesenchymal cells could be induced by ectopic expression of ESRP1, which causes a switch toward the epithelial splicing pathway (24). Some expression changes characteristic of EMT (*e.g.* up-regulation of vimentin protein) were also observed upon silencing of ESRPs, whereas others (*e.g.* downregulation of E-cadherin protein) were not (26). Nonetheless, immunofluorescence analysis of E-cadherin showed that ESRP knockdown led to loss of E-cadherin from cell–cell contacts. These observations suggest that ESRP down-regulation contributes to loss of epithelial properties, although 'full' EMT obviously requires additional changes in gene expression.

The diversity of p120ctn transcripts in man and mouse is increased by alternative splicing of internal exons. Exon A encodes a 21-AA sequence (Figure 2) that is ubiquitously expressed in various human cell lines and tissues (10). Exon B encodes a putative nuclear export signal (NES) with a characteristic leucine motif that counteracts the nuclear localization of p120ctn isoform 3A (27). Exon C encodes six AA that interrupt the second nuclear localization signal (NLS2) of p120ctn, which coincides with the second RhoA-binding domain (RBD2) (Figure 2). Expression of exon C indeed blocks translocation to the nucleus and at the same time inhibits dendritic-like branching (Pieters *et al.*, in preparation) (see also Section 5). Exon C is strongly expressed in brain (10) (Pieters *et al.*, in preparation). A rare deletion of a fourth alternatively spliced internal exon, exon D, was reported in fetal and adult brain tissue (11). In conclusion, the alternative usage of p120ctn exons is tissue-specific, and it might regulate the subcellular localization of p120ctn isoforms and direct their functionality.

4. p120ctn REGULATES CADHERIN TRAFFICKING AND STABILITY

4.1. p120ctn and cadherin biogenesis

Adhesive structures have to be highly dynamic so that cells can maintain homeostasis by adapting to internal and external signals. Consequently, cadherin–catenin complexes are highly dynamic and are constantly assembled and disassembled (Figure 1A). Cadherin trafficking involves cadherin synthesis in the Golgi, transport to the cell surface, stabilization at the cell surface or internalization, and recycling or proteasomal or lysosomal degradation (28, 29). After its synthesis, E-cadherin binds to beta-catenin, and together they are transported from the Golgi complex to the plasma membrane (30, 31). Unlike beta-catenin, p120ctn does not interact with E-cadherin during its biogenesis (32). Indeed, p120ctn-uncoupled E-cadherin is still properly targeted to the plasma membrane (33). On the other hand, cadherin-binding is essential for localizing p120ctn at the membrane: introducing exogenous E-cadherin in E-cadherin-negative cells relocalized p120ctn from the cytoplasm to the membrane (33). In contrast, p120ctn has been reported to associate with N-cadherin early during its biogenesis (34). Both N-cadherin and p120ctn move along microtubule tracks towards cell–cell contacts (35, 36). p120ctn isoform 1 colocalizes and interacts with microtubules and the motor protein kinesin, which transports cargo towards the plus ends of microtubules (towards the plasma membrane) (36-38). The N-terminal domain of p120ctn binds to the heavy chain of kinesin, but an N-terminal deletion mutant and the naturally occuring p120ctn isoform 4 do not (36, 37, 39). p120ctn isoform 1, which contains the entire N-terminal domain, binds to kinesin with a higher affinity than p120ctn isoform 3, which lacks 100 AA of the N-terminus (37). p120ctn, and in particular its isoform 1, might therefore play a role in delivering N-cadherin to the plasma membrane. Indeed, disassembly of the N-cadherin–p120ctn–kinesin complex delayed the delivery of N-cadherin to cell–cell contacts (36).

4.2. p120ctn stabilizes cell surface cadherin

Mature adherens junctions are formed after the synthesis and transport of cadherins and catenins to the membrane (Figure 1A). p120ctn plays an important role in the binding and stabilization of membrane-localized cadherins. The juxtamembrane domain (JMD) of classical cadherin interacts with the Armadillo repeat domain of p120ctn (17, 40), and Armadillo repeats 1 to 5 of p120ctn are essential for this interaction (13, 15). Both isoform 1 (long) and isoform 3 (short) of p120ctn can bind E-cadherin (16). The crystal structure of p120 isoform 4A in complex with the core region of the E-cadherin JMD (mouse E-cadherin AA 758–775) has been determined (13). The Armadillo repeat domain of p120ctn contains modular binding pockets that are complementary to the electrostatic and hydrophobic properties of the JMD core. Single-residue mutations within the JMD-binding site of p120 abolished the interaction of mutant p120ctn with E- and N-cadherins *in vitro* (13) and interfered with cell–cell adhesion (33). These findings indicate that p120ctn serves as a gatekeeper of cadherin turnover (41). We will discuss the effect of Ser/Thr phosphorylation of p120ctn on the stablility of cadherins in junctions in Section 7.

The first clue that p120ctn is critical for cadherin function emerged from analysis of SW48 colon carcinoma cells bearing mutations in the p120ctn gene (15). Due to p120ctn insufficiency, these poorly differentiated cells failed to form compact colonies and displayed less E-cadherin protein but not reduced E-cadherin mRNA (15). Epithelial morphology and cadherin levels could be rescued by expressing p120ctn isoform 1, 3 or 4, or by using a RhoA-uncoupled variant of p120ctn isoform 1 (*delta*662-628), but not by using an E-cadherin-uncoupled p120ctn mutant. Pulse chase experiments revealed that p120ctn expression increased the E-cadherin half-life (15). Stable knockdown of p120ctn in mammalian cells resulted in a drastic and dose-dependent decrease in cadherins, such as E-, N-, P-, VE-cadherin and cadherin-11 (42-45). Loss of p120ctn also resulted in decreased expression of beta- and alpha-E-catenin due to decreased cadherin levels (42, 43). Human cells depleted of p120ctn became dispersed, but introduction of a murine p120ctn cDNA rescued both morphology and cadherin levels (42).

Morpolino-mediated depletion of p120ctn in *Xenopus* and its genetic depletion in mouse confirm that p120ctn can stabilize cadherins *in vivo*. Knockdown of p120ctn in *Xenopus* embryos reduces the levels of classical cadherins (46, 47). Moreover, gastrulation defects seen upon p120ctn depletion in *Xenopus* could be rescued by ectopic expression of C-cadherin (47, 48). Tissue-specific p120ctn depletion in mice decreases the levels of E-cadherin (49-52), N-cadherin (51, 53, 54), P-cadherin (49) and VE-cadherin (54). In conclusion, p120ctn regulates cadherin turnover at the cell membrane both *in vitro* and *in vivo*.

4.3. p120ctn prevents endocytosis of cadherins

p120ctn controls the expression levels of membrane-localized cadherins partly by preventing their endocytosis. Cell surface cadherins can be internalized *via* different pathways (reviewed in (29)), including clathrin-dependent endocytosis, caveolae-mediated endocytosis, lipid-raft-mediated endocytosis, and micropinocytosis. The decision to enter a certain endocytotic pathway is highly cell-specific but it also depends on the microenvironment. p120ctn blocks clathrin-mediated endocytosis of VE-cadherin, and this block depends on the binding of p120ctn to the JMD of VE-cadherin (55). Other studies showed increased E-cadherin endocytosis in cells expressing a p120ctn-uncoupled E-cadherin mutant as well as in cells in which p120ctn was depleted by RNAi (56). It is not clear how p120ctn prevents endocytosis, but the following possibilities have been proposed. First, p120ctn competes with presenilin-1 and Hakai for binding to the JMD of classical cadherin. Presenilin-1 favors E-cadherin degradation by proteolytic cleavage of the cadherin cytoplasmic tail (57, 58). Hakai is an E3 ubiquitin ligase that binds to the phosphorylated tyrosine motifs in the JMD of E-cadherin (but does not bind other classical cadherins). This leads to ubiquitination and endocytosis of E-cadherin (Figure 1A) (59, 60). Second, a dileucine motif in the

cytoplasmic tail of E-cadherin (close to the JMD) is responsible for clathrin-mediated internalization of E-cadherin (56). If the dileucine motif is mutated or if the cadherin tail is completely deleted, E-cadherin is not endocytosed (56, 61). p120ctn might regulate E-cadherin endocytosis by masking the dileucine motif to prevent its interaction with adaptor proteins, such as AP-2, which are required for clathrin-mediated endocytosis (62). In conclusion, it is conceivable that p120ctn acts as a "cap" that binds cadherin and prevents its endocytosis.

4.4. p120ctn localization at microtubules

In many cell types, microtubules (MTs) are organized in a radial array with their minus ends anchored at the centrosome and their plus ends extending toward the cell periphery. On the other hand, terminally differentiated cells might contain both centrosomal and noncentrosomal MTs. In polarized epithelial cells, noncentrosomal MTs are arranged along the apicobasal axis of the cell with their minus ends oriented apically and their plus ends basally. In both arrangements of MTs, p120ctn has been found at the minus ends.

A recent study revealed that the N-terminal domain of p120ctn interacts with PLEKHA7 (pleckstrin homology domain-containing, family A member 7) (63). In the adherens junction of polarized epithelial cells, a p120ctn–PLEKHA7–Nezha chain forms a bridge between the cadherin–catenin complex and the minus ends of microtubules (Figure 1A). The integrity of the adherens junction is dependent on this macromolecular complex, possibly because this complex recruits KIFC3, a minus-end directed microtubular motor, towards the junctions (63). Depletion of PLEKHA7, Nezha or KIFC3 resulted in disorganization of the adherens junction. Hence, that study revealed a novel p120ctn-dependent mechanism for regulation of cell–cell contacts.

It should be noted that p120ctn (39, 64, 65), PLEKHA7 (63), and KIFC3 (63) can localize in the centrosomes, which indicates that they perform functions there. We found that Kaiso, a p120ctn interaction partner, localizes at the mitotic spindle and is a constituent of the pericentriolar material (66). Chartier *et al.* (64) showed that overexpression of p120ctn isoform 3A leads to its accumulation at the centrosomes of mitotic HT29 cells. Interestingly, it has been proposed that isoform 3A rather than isoform 1A directly interacts with Kaiso (67). These observations require further investigation to elucidate the place of p120ctn in the regulation of MT minus ends.

5. p120ctn AND RHOGTPASES

5.1. RhoGTPases and cadherin-based junctions

The activities of the small GTPases RhoA, Cdc42 and Rac1 play a central role in regulation of cytoskeletal dynamics, formation and maintenance of adherens junctions, and migration of cells (68). Furthermore, cadherin recycling is important both during the formation of new contacts and in the maintenance of stable junctions (69). RhoGTPases regulate many stages of vesicular trafficking, but they affect cadherin-based junctions in ways that vary with the cellular environment (69-72). RhoGTPases have been shown to be important for cadherin-based adhesion *in vivo*. Rho1, the *Drosophila* RhoA homolog, colocalizes with DE-cadherin in embryos, and both the cadherins and catenins are mislocalized in Rho1 mutants (73). In conclusion, an intricate relationship exists between RhoGTPases and the formation and maintenance of cadherin-based junctions.

5.2. p120ctn and dendritic-like branching

The first hint about the role of p120ctn in altering RhoGTPase activity came from p120ctn overexpression studies. Expressing large amounts of exogenous p120ctn saturated cadherin-binding sites, and the excess p120ctn translocated to the cytoplasm and caused a neuron-like cellular morphology similar to dendritic branching (74). This phenotype was likely due to p120ctn-mediated RhoA inhibition because the phenotype could be mimicked by adding a RhoA inhibitor (C3 exotransferase) and by expressing p190RhoGAP (70, 75). On the other hand, the dendritic-like branching could be blocked by coexpressing a constitutively active (CA) RhoA variant (75-77) and by mutating one of the RhoA-binding sites of p120ctn (75, 78). Indeed, an N-terminal deletion that includes RBD1 diminishes p120ctn-induced branching (78), but branching is completely blocked by deleting a second RhoA-binding domain (containing AA 622-628; Figure 2) (75). Is this branching phenotype RhoA-specific or are there other RhoGTPases involved? Activation of Rac1 and Cdc42 also influences p120ctn-mediated branching because branching was blocked by dominant negative forms of Rac1 and Cdc42 in two studies (76, 77). However, this was not seen in another study (75).

5.3. p120ctn inhibits RhoA activity and activates both Rac1 and Cdc42

Compelling evidence that p120ctn modulates RhoA activity comes from two kinds of observations: p120ctn overexpression results in RhoA inhibition, whereas its knockdown or genetic ablation in mice results in increased RhoA activity. First, expression of p120ctn isoform 1A inhibits RhoA activation in 293T and CHO cells (75, 76). A deletion mutant of p120ctn isoform 1A, named *delta*622-628 and lacking the RBD2, failed to inhibit RhoA activity and to induce dendritic-like branching (75). Also, cells transfected with p120ctn isoform 1A do not form actin stress fibers due to weak RhoA activity (75-77). In a complementary approach, stable knockdown of endogenous p120ctn in different cell lines resulted in RhoA activation (43, 45, 79-81) and strongly enhanced formation of actin stress fibers (43, 79). These p120ctn-depleted cell lines are also suitable for testing the effect of various p120ctn isoforms on RhoA activity. These isoforms originate from another species and are therefore unaffected by the shRNA used to knock down the original p120ctn. The p120-mediated RhoA inhibition can be rescued by introducing p120ctn isoform 1, but not p120ctn isoform 4, in p120ctn-depleted cell lines (45, 80). Finally, modulation of RhoA activity by p120ctn is also observed *in vivo*, as genetic ablation of p120ctn in mouse

skin and dorsal forebrain result in increased RhoA activation (49, 53).

p120ctn expression also results in increased Rac1 and Cdc42 activity (76, 77). In line with this, knockdown or knockout of p120ctn results in decreased Rac1 activity (45, 53, 80). Re-expression of both p120ctn isoforms 1 and 4 in stable p120ctn-knockdown lines results in reactivation of Rac1 (80, 82). Remarkably, p120ctn isoform 4 can restore Rac1 activity while it fails to inhibit RhoA activity, indicating that a single p120ctn isoform can differentially regulate the activity of different RhoGTPases. Overall, p120ctn regulates RhoGTPase activity by inhibiting RhoA and activating Rac1 and Cdc42, which in turn alters cytoskeletal dynamics and increases cell migration (76).

5.4. Interaction between p120ctn and RhoA

Does p120ctn bind to RhoA or is it involved indirectly in regulating its activity? In *Drosophila*, p120ctn binds directly to Rho1, the RhoA homolog (73). Remarkably, also alpha-catenin binds to Rho1 in *Drosophila*, but alpha-catenin and p120ctn bind to distinct regions of the N-terminus of Rho1 (73). One RhoA binding domain (RBD1) has been identified in the N-terminus of mouse p120ctn (AA 102-234) (Figure 2) (83). Binding of this RBD1 domain to RhoA is regulated by tyrosine phosphorylation of RBD1 by Src family members. Fyn-mediated phosphorylation of Tyr residue Y112 or introduction of a phosphomimetic Y112E mutation inhibits the interaction of p120ctn with RhoA and prevents p120ctn-mediated dendritic-like branching and RhoA inhibition. On the other hand, Fer- or Src-mediated phosphorylation of Y217 and Y228 increases the affinity between p120ctn and RhoA, which leads to inhibition of RhoA activity (83). RhoA activity was also inhibited by expressing a Y112F mutant of p120ctn that cannot be phosphoryated by Fyn (83). RBD1 on its own does not have RhoGDI activity, indicating that additional sequences in p120ctn are required for RhoA inhibition and for RhoGDI functionality (83). The second RhoA binding domain (RBD2, AA622–628) coincides with NLS2 of p120ctn (Figure 2). The *delta*622–628 mutation of p120ctn isoform 1 fails to reduce intrinsic or GEF-induced RhoA activation but does not elicit dendritic-like branching (75). Deleting either RBD1 (like in isoform 4) or RBD2 (like in mutant delta622–628 of isoform 1) does not prevent GDP-RhoA from binding to p120ctn (80). However, deleting both RBDs of p120ctn, as in a *delta*622–628 mutant of isoform 4, completely abrogates the ability of p120ctn to bind GDP-RhoA *in vitro* (80).

5.5. Mechanism of p120ctn-mediated RhoA inhibition

The mechanism by which p120ctn inhibits RhoA is still unclear, but two mechanisms have been postulated: either p120ctn can bind to RhoA (see above) and act as a Rho guanine nucleotide dissociation inhibitor (GDI), or it can activate Rac1, which leads to RhoA inhibition mediated by p190RhoGTPase activating protein (p190RhoGAP) (Figure 3).

In the first proposed mechanism, p120ctn binds to RhoA and prevents its activation by Rho guanine exchange factors (GEFs). p120ctn has been shown to specifically inhibit the intrinsic GDP/GTP exchange activity of RhoA, which resembles the mode of action of GDIs (75, 83). Also, *in vitro* translated and purified *Drosophila* p120ctn preferentially binds to GDP-bound Rho1 (73). In addition, p120ctn binds dominant-negative (DN) Rho1 but not constitutively active (CA) Rho1 (73). The p120ctn-binding region of Rho1 adopts different conformations depending on which nucleotide is bound, and this might explain the binding preference of p120ctn for GDP-bound Rho1 (73). Although p120ctn has no sequence similarity to GDI, it interacts with the same face of RhoA protein as GDI does (73, 84).

The second proposed mechanism involves interplay between several RhoGTPases. Rac1 and Cdc42 can inhibit RhoA activity in NIH3T3 cells (85). p120ctn can interact with Vav2, a Rho family GEF, which might account for the p120ctn-mediated activation of Rac1 and Cdc42 (76). Therefore, p120ctn-mediated Rac1 activation might indirectly inhibit RhoA through a pathway that involves LMW-PTP and p190RhoGAP (Figure 3) (79, 86, 87).

5.6. Effect of p120ctn on RhoGTPase activity is cell-type specific

After the first reports on the dendritic-like branching induced by cytoplasmic p120ctn, it turned out that this effect varies with cell type, assay conditions, micro- and macro-environments, and types of cadherins expressed. Therefore, it is impossible to make general conclusions about dendritic-like branching and RhoGTPase activity, and it is necessary to keep an open mind to new and possibly unexpected results. For example, in one study the knockdown of p120ctn in DU145 prostate cancer cells did not affect the activities of RhoA and Rac1 (88). But in another study, cytoplasmatic p120ctn activated RhoA in primary cells derived from a mouse model of invasive lobular carcinoma, whereas knockdown of p120ctn in these cells resulted in RhoA inhibition (207). In contrast to the dogma stating that p120ctn isoform 1 inhibits RhoA activity and activates Rac1 and Cdc42 (see Section 5.3), in lung cancer cells p120ctn isoform 1 blocks Rac1 activity whereas isoform 3 activates RhoA and inhibits Cdc42 activity (89). Knockdown of p120ctn in lung cancer cell lines results in inhibition of RhoA and activation of Rac1 and Cdc42 (90).

RhoGTPase signaling is altered by cadherin expression. p120ctn interacts with cadherins, and dendritic-like branching can be blocked by co-expression of p120ctn with E- or C-cadherin (75, 76). Expression of C-cadherin constructs encoding the complete cytoplasmic domain or just the JMD can sequester overexpressed p120ctn to the cell membrane, but not if the construct encodes beta-catenin binding domain CBD alone without the JMD (76). Similar findings were reported by use of soluble fragments of the E-cadherin cytoplasmic domain (75). On the other hand, E-cadherin binding is dispensable for p120ctn-induced dendritic-like branching because E-cadherin-uncoupled p120ctn mutants lacking either Armadillo repeat 1 (ARM1) or Armadillo repeats 1-3 (ARM1-3) can still

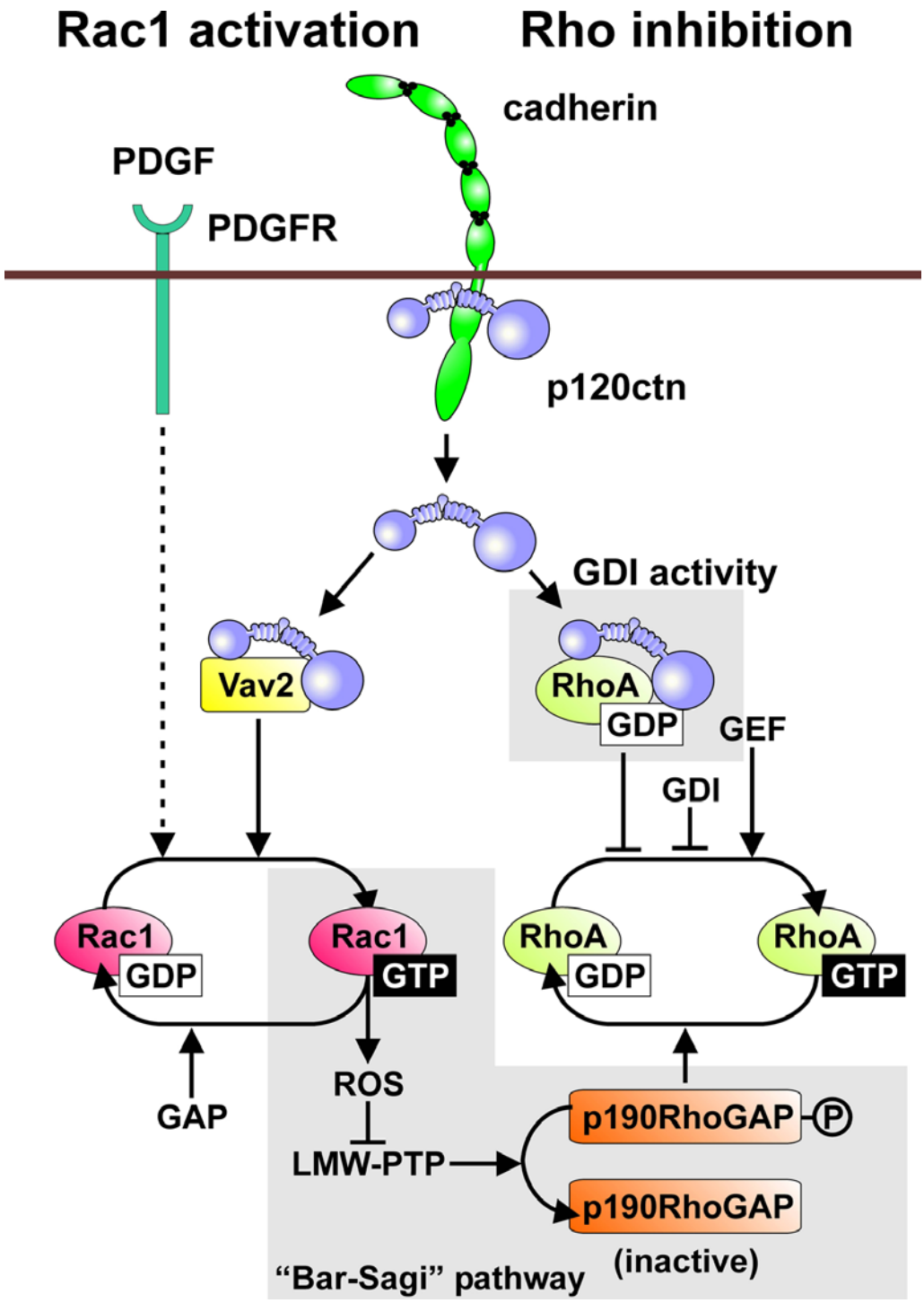

Figure 3. p120ctn regulates the activities of RhoGTPases, which function as molecular switches that alternate between an active (GTP-bound) and an inactive (GDP-bound) state. Guanine exchange factors (GEFs) promote RhoGTPase activation, whereas GTPase activating proteins (GAPs) and Rho guanine dissociation inhibitor (GDI) contribute to RhoGTPase inactivation. p120ctn has been shown to activate Rac1 (and Cdc42) and to inhibit RhoA activity. Indeed, p120ctn interacts with the Rho family GEF Vav2, which can directly activate Rac1. On the other hand, p120ctn can inhibit RhoA either by its RhoGDI activity or by Rac1-mediated activation of p190RhoGAP *via* the 'Bar-Sagi' pathway (depicted on a gray background). This 'simple' hypothesis has not been confirmed in all studies. ROS, reactive oxygen species; LMW-PTP, low molecular weight protein tyrosine phosphatases.

elicit a branched morphology (45, 74). Endogenous p120ctn activates Rac1 in E-cadherin-negative MDA-MB-231 cells, but this activation is lost upon stable expression of exogenous E-cadherin (82). In addition, p120ctn-mediated Rac1 activation is not observed in MCF7 cells, which express endogenous E-cadherin (82). Like cadherins, microtubules can tether p120ctn away from the cytoplasmic pool and thereby prevent p120ctn-induced dendritic-like branching. Coexpression of the kinesin heavy chain reduced the branching elicited by p120ctn isoform 1 but not the branching elicited by isoform 3; this means that isoform 1 has a higher affinity for kinesin (37).

5.7. p120ctn isoforms and dendritic-like branching

In an initial report by Reynolds *et al.* (17), p120ctn isoform 1A was shown to induce dendritic-like branching. A follow-up study revealed that p120ctn isoforms 2A and 3A, but not 4A, also elicit dendritic-like branching. Remarkably, there seems to be a correlation between nuclear localization of p120ctn and its ability to elicit branching. p120ctn isoform 1 can be imported into the nucleus and it induces dendritic-like branching, whereas isoform 4, which lacks the N-terminal domain and was never localized in the nucleus, did not induce branching (78). Because p120ctn isoform 4 lacks the phosphorylation domain, there might also be a link between phosphorylation and dendritic-like branching. In addition, both p120ctn isoforms 1AB and 3AB have 29 additional AA encoded by the alternatively spliced exon B and containing a NES (Figure 2). p120ctn isoforms 1AB and 3AB can block branching and promote nuclear export (27, 78). Mutational analysis revealed a second NES in Armadillo repeat 7 [new repeat numbering based on (12, 13)] (38). Full-length p120ctn contains two conventional nuclear localization sequences, NLS1 and NLS2 (Figure 2). Deleting NLS1 does not prevent branching (78). However, branching is abolished by mutation (91) or deletion (75) of NLS2. Interrupting NLS2 by expression of six AA encoded by the alternatively spliced exon C disrupts both dendritic-like branching and nuclear translocation (Pieters *et al.*, in preparation). NLS2 comprises AA 622–628, which coincide with the second RhoA binding domain, RBD2 (Figure 2). Remarkably, nuclear import is not fully blocked by mutating the two conventional NLSs, and an additional role has been proposed for the Armadillo repeat domain in nucleocytoplasmic shuttling. Indeed, Armadillo repeats 3 and 5 of p120ctn turned out to be essential for nuclear import (38). Deleting them both by removing either repeats ARM3-5 or repeats ARM3-9 blocks the neuron-like morphogenesis (74, 77). Interestingly, the p120ctn*delta*ARM3-5 construct still contains both RhoA-binding domains but nevertheless fails to induce branching (74, 77). Is nuclear import of p120ctn *per se* enough to induce branching? No, because forced nuclear translocation of p120ctn (following administration of Leptomycin B or coupling of an SV40 NLS) does not trigger dendritic-like branching (91). In conclusion, the p120ctn isoforms differ in their ability to elicit a branched morphology, and this is often correlated with their ability to translocate to the nucleus.

6. p120ctn MODULATES SIGNALING IN SEVERAL CELL COMPARTMENTS

6.1. p120ctn modulates signaling in the nucleus

In adherens junctions, E-cadherin interacts with beta-catenin and p120ctn. Both Armadillo proteins, when released from junctions, travel to the nucleus, where they act as transcriptional regulators (Figure 1B). In canonical Wnt signaling, beta-catenin is generally considered the key transmitter of Wnt signals to the nucleus (92-94). However, p120ctn can also localize in the nucleus (27, 38, 78, 91, 95). Nuclear trafficking of p120ctn depends on conventional NLS and NES signals (Figure 2) (27, 78, 91), as well as on the p120ctn Armadillo repeat domain (38) and on the microenvironment (96). p120ctn interacts with transcription factors Kaiso (67) and Glis2 (97). Kaiso also contains a functional NLS for its nuclear import (98). p120ctn might also associate with Kaiso in the cytoplasm, which would tether away Kaiso from the nucleus and thereby prevent it from acting in the nucleus (99).

Kaiso is composed of an N-terminal poxvirus and zinc finger (POZ) domain and three carboxy-terminal zinc fingers. The POZ domain also enables Kaiso to homodimerize (67, 100), whereas its C-terminal zinc finger domain can bind to DNA. Kaiso has a dual specificity for DNA: it can bind to sequence-specific Kaiso consensus (binding) sites (KCS) and to methylated CpG-dinucleotides (101, 102). Remarkably, the Kaiso function involving binding to sequence-specific KCSs is dispensable in *Xenopus laevis* (103). However, the binding of Kaiso to methylated DNA is evolutionarily conserved (103) and allows histone-deacetylase-dependent transcriptional repression. This involves the recruitment of chromatin co-repressor components, such as nuclear co-repressor 1 (NCOR) (104), to the N-terminal POZ domain of Kaiso. On the other hand, the Armadillo repeat domain of p120ctn can bind to the C-terminal zinc finger domain of Kaiso and thereby inhibit Kaiso's interaction with DNA (67). Kaiso acts as a transcriptional repressor. The binding of p120ctn to Kaiso blocks this repressor activity, which therefore results in activation of Kaiso target genes, such as *Siamois*, *c-Fos*, *Myc*, *Ccnd1* (encodes Cyclin D1) (105), *xWnt11* (106), and *Mmp7* (encodes Matrilysin) (107).

There is significant overlap between the target genes of the p120ctn–Kaiso and beta-catenin–TCF signaling pathways, and cross-talk between these pathways has been reported. This synergism has been observed both in cell lines (107) and in *Xenopus* embryos (105). The promoter of the matrilysin gene contains two KCSs, and Kaiso can repress beta-catenin-induced activation of the *Mmp7* gene. This Kaiso-mediated transcriptional repression of Mmp7 has been found to be reversed by p120ctn expression (107). Several other beta-catenin target genes contain KCSs, and in *Xenopus* embryos they can be either repressed by Kaiso or activated by either beta-catenin activity or Kaiso depletion (105). p120ctn relieves the Kaiso-mediated repression of beta-catenin target genes, whereas its ablation in *Xenopus* embryos increases their repression (105).

Table 1. Overview of kinases and phosphatases, which bind cadherins and catenins and/or modify their phosphorylation status

Subtrates	Protein kinases[1]		Phosphoprotein phosphatases[1]	
	Non-receptor kinases	Receptor tyrosine kinases	Non-receptor phosphatases	Receptor-associated phosphatases
Cadherins				
E-cadherin	v-Src (172)	*EGFR* (189)	*PTP1B* (190), *PP2A* (S/T) (191)	*PTPmu* (192)
N-cadherin	v-Src (193)	*FGFR-4* (194)	*PTP1B* (195)	*PTPmu* (192)
VE-cadherin	c-Src (196)	VEGFR-2 (140, 196, 197)	PTP1B (198)	VE-PTP (199)
R-cadherin				*PTPmu* (192)
Catenins				
alpha-catenin	?	?	?	?
beta-catenin	Fer (119), Fyn (119), Yes (119), c-Src (119), v-Src (17, 146, 172)	EGFR (189), TrkA (139), VEGFR-2 (140), c-Met (132)	PTP1B (190, 195), SHP2 (200), PP2A (S/T) (191)	*DEP1* (143, 144), PTPkappa (201), PTP-LAR (202, 203), *PTPlabda* (204), PCP-2 (205), PTPbeta/zeta (206)
plakoglobin	v-Src (17)	EGFR (189), VEGFR-2 (140), c-Met (138)	SHP2 (200), *PP2A* (S/T) (191)	*DEP1* (143, 144), *PTPkappa* (201), *PTP-LAR* (202)
p120ctn	v-Src (17, 121), Fer (119, 135, 136), *Fyn* (119), *Yes* (119), GSK3beta (S/T) (125), CK1epsilon (S/T) (261), CK1alpha (S/T) (262)	TrkA (139), c-Met (138, 139), EGFR (137), PDGFR (137), CSFR (137), VEGFR-2 (140)	SHP2 (200), PKC[2] (S/T) (133)	DEP1 (143, 144), VEFGR-2[2] (S/T) (132), RPTPmu (145)

[1] Normal font denotes only phosphorylation or dephosphorylation; underlined enzymes denote both physical binding and (de)phosphorylation; italic denotes binding only; (S/T) denotes (de)phosphorylation on Ser or Thr residues. [2] denote kinases that activate phosphatases, most probably in an indirect way.

Morpholino-mediated depletion of Kaiso in *Xenopus* embryos results in severe defects in both gastrulation and convergent extension, and these phenotypes can be rescued by re-expression of Kaiso (105, 106). In contrast, Kaiso-deficient mice are viable (108). Although Kaiso acts as a genome wide transcriptional repressor in *Xenopus* embryos (109), gene expression is not increased in Kaiso-deficient mice (108). Kaiso is a member of a small protein family that contains two other Kaiso-like proteins, namely ZBTB4 (Kaiso-like 1) and ZBTB38 (ZENON), which bind methylated DNA and act as transcriptional repressors (110). The discrepancy between the findings in mice and in *Xenopus* concerning the developmental requirement for Kaiso and its gene regulatory activity might be explained by functional redundancy of these Kaiso-like family members (99). Indeed, no ZBTB4 homolog could be indentified in *Xenopus*, which indicates that ZBTP4 might substitute for Kaiso in mouse but not in frog.

6.2. p120ctn modulates signaling in the cytoplasm

The work described above has outlined the role of p120ctn in the nucleus, which is to interfere with Wnt signaling. Two recent reports point at interplay between p120ctn and the Wnt signaling pathway at cell junctions or in the cytoplasm (111, 112) (Figure 1B). A report by the McCrea group showed that p120ctn is targeted for proteasomal degradation *via* the same pathway as beta-catenin (112). p120ctn degration involves a series of steps, including its association with axin and APC proteins, its phosphorylation by kinases of the CK1 family and by GSK3beta, and eventually its ubiquitination and proteasomal degradation. Only isoform 1 of p120ctn can associate with CK1alpha and GSK3beta, and it is prone to phosphorylation by these kinases, as well as to axin binding, ubiquitination, and proteasomal degradation (112). In the other report, p120ctn was shown to bind to CK1epsilon *via* an N-terminal domain (Figure 2) (111). This interaction domain is present in isoforms 1 and 3. CK1alpha can inhibit canonical Wnt signaling by destabilizing cytoplasmic beta-catenin, whereas CK1epsilon is activated upon Wnt triggering and contributes to stabilizing beta-catenin. In this model, p120ctn is required for the activation of CK1epsilon as it functions as an anchor protein to mediate the interaction of CK1epsilon with the Wnt receptor complex. Earlier, Frodo was found to physically link the p120ctn–Kaiso and beta-catenin–TCF pathways by binding to both p120ctn and Dishevelled (Dsh) in the cytoplasm (113) (Figure 1B). Frodo acts upstream of both signaling pathways and stabilizes p120ctn (113).

7. p120ctn AND PHOSPHORYLATION

7.1. Phosphorylation of the cadherin–catenin complex

Several lines of evidence suggest that cadherin-based junctions, like integrin-mediated adhesion plaques, are regulated by phosphorylation (Figure 4). In fact, all members of the cadherin–catenin complex, except alpha-catenin, are prone to phosphorylation on tyrosine (Tyr), serine (Ser) and threonine (Thr) by receptor tyrosine kinases (RTKs) and non-receptor protein kinases (Table 1). On the other hand, protein tyrosine phosphatases (PTPs) reverse this phosphorylation and allow dynamic protein modification in response to internal and external cues (reviewed in 114). The presence of multiple

phosphorylation sites in cadherins and catenins and the multitude of different kinases and phosphatases located at the membrane reveal the complexity of protein phosphorylation and its effects on adhesion. Tyr phosphorylation of cadherins and catenins has been studied extensively and is thought to modulate adhesive strength. The underlying mechanism is not clear and there is evidence for both a positive role and a negative role for Tyr phosphorylation.

Phosphorylation affects the affinity between the components of the cadherin–catenin complex. Phosphorylation of Tyr residue Y654 in beta-catenin is important for binding to E-cadherin. Src-induced phosphorylation of this residue or introduction of a phosphomimetic mutation (Y654E) decreased the affinity between beta-catenin and E-cadherin and disrupted the cadherin-based junctions (Figure 4C, D) (115). Like E-cadherin, N-cadherin does not bind Tyr-phosphorylated beta-catenin (116). The presence of p120ctn does not influence the binding of phosphorylated or unphosphorylated beta-catenin to E-cadherin (115). Beta-catenin binds the acidic E-cadherin sequence at a long, positively charged groove; adding a negative charge in this groove by phosphorylation might disrupt intermolecular binding (117).

Binding of beta-catenin to alpha-catenin involves 29 AA in beta-catenin, only one of which is a Tyr, Y142 (118). Alpha-catenin becomes uncoupled from beta-catenin upon phosphorylation of Y142 by the non-receptor Tyr kinase Fer or Fyn (but not by Src or Yes) as well as by using the tyrosine phosphatase inhibitor pervanadate (Fig 4B, C) (119-121). Therefore, keeping Tyr residues Y142 and Y654 of beta-catenin unphosphorylated is critical for the integrity of cadherin-based adhesion.

PTP1B, a non-receptor tyrosine phosphatase, associates with N-cadherin and can dephosphorylate beta-catenin; it can thereby stabilize cadherin-mediated adhesion (reviewed in 122). The binding of PTP1B to E-, VE- and N-cadherin (Table 1) requires phosphorylation of PTP1B on Tyr residue Y152 by Fer, which is recruited to the cadherin–catenin complex by binding to p120ctn (Figure 4B) (123). Cell-permeable peptides that disrupt the Fer–p120ctn interaction cause Fer to dissociate from N-cadherin complexes, after which PTP1B, beta-catenin and eventually p120ctn are lost (123).

Tyr phosphorylation not only affects the binding efficacy of catenins but also determines the amount of membrane-localized E-cadherin. Src-mediated disruption of cell–cell contacts is accompanied by increased ubiquitination of E-cadherin, which causes its endocytosis (59). Src- or HGF-mediated Tyr phosphorylation of E-cadherin on residues Y755 and Y756 (which are situated in the JMD) is a prerequisite for the binding of Hakai, an E3 ubiquitin ligase. p120ctn binds to phosphorylated and unphosphorylated E-cadherin equally, but it has to compete with Hakai for binding to Tyr-phosphorylated E-cadherin (Figure 4D) (59).

7.2. Phosphorylation of p120ctn

Over the previous decade, the laboratory of Reynolds has been using two approaches to decipher how phosphorylation affects the diverse functions of p120ctn. In one approach, two-dimensional tryptic mapping allowed the identification of eight Tyr and eight Ser/Thr phosphorylation sites in p120ctn (Figure 2) (124, 125). A ninth Tyr phosphorylation site was identified by mutational analysis. In comparison with L-cells transformed with v-Src and expressing wild type p120ctn and exogenous E-cadherin, the Y217F mutation of p120ctn increased cell aggregation (121). All Tyr sites and most of the Ser and Thr sites are located in the N-terminal domain. Two phosphorylation sites, S879 and T916, flank the sequence encoded by the alternatively spliced exon A nearby the C-terminus. S879 and T916 correspond to the previously reported S873 and T910, respectively (the new numbering is due to the inclusion of the six AA encoded by the alternatively used exon C). Remarkably, no phosphorylation sites are present in the Armadillo repeat domain. In the other aproach, panels of p120ctn phospho-specific monoclonal antibodies were generated against specific phosphorylated Tyr, Ser and Thr residues in the p120ctn sequence context: Y228 (126), S879 (127), S268, S288, T310, and T916 (128). These antibodies have been helpful in identifying the p120ctn residues that are phosphorylated in response to EGF (126) or PDGF (129), namely Y228 and S879, respectively.

In cells that were not transformed by Src, p120ctn was mainly phosphorylated on Ser, and to a minor extent on Thr and Tyr (124, 130, 131). Src-transformed cells have higher phosphotyrosine content, and mutating all eight Src-phosphorylation sites to Phe (p120ctn-8F) prevents Tyr phosphorylation of p120ctn and abolishes its binding to SHP-1 (124). Physiological Tyr phosphorylation of p120 occurs transiently in response to growth factor signaling and is rapidly terminated (126). In contrast, all Ser and Thr phosphorylation of p120ctn appears to be constitutive, with the exception of S879, which is phosphorylated by phorbol-ester-activated PKC (125) or PDGF-activated PKC-alpha (129). Basal Ser/Thr phosphorylation of p120ctn is transiently down-regulated in response to several cellular stimuli, such as VEGF (132) and PKC (133), and the inflammatory stimuli histamine (H1), thrombin and lysophosphatidic acid (LPA) (134). This downregulation is thought to modulate a variety of cadherin-associated activities, such as vascular permeability and leukocyte transcytosis.

p120ctn, which was initially identified as a substrate for the non-receptor tyrosine kinase Src (9), is also phosphorylated by the Src-family members Fyn and Yes. Fyn interacts equally well with tyrosine-phosphorylated and -unphosphorylated p120ctn (119). p120ctn is also a good substrate for the tyrosine kinase Yes, which interacts with p120ctn in an activation-dependent manner and subsequently activates Fer and Fyn (119) (Figure 4C). Tyrosine kinase Fer is constitutively associated with p120ctn (135, 136). The N-terminus of p120ctn serves as a docking protein facilitating interaction of Fer (and Fyn) with the cadherin–catenin complex

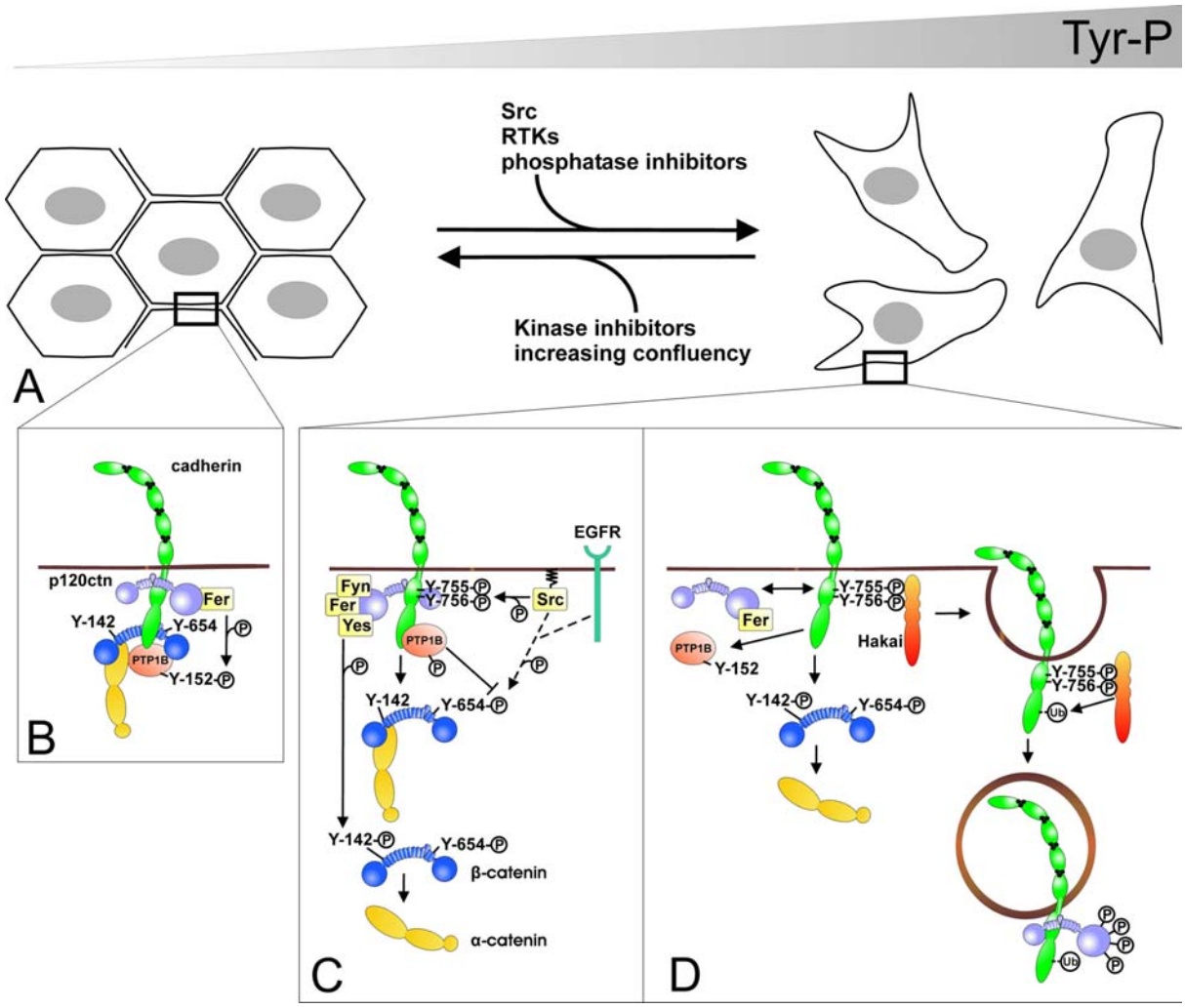

Figure 4. Constitutive tyrosine (Tyr) phosphorylation of the cadherin–catenin complex results in disassembly of junctions. (A) Src transformation, receptor Tyr kinase (RTK) signaling and protein phosphatase inhibition result in Tyr phosphorylation of cadherins and catenins, which leads to dissociation of adherent cell layers *(left, blowup in B)* into non-adherent spindle-shaped cells *(right, blowup in C, D)*. Tyr phosphorylation in cadherin-based structures is abolished by kinase inhibition and upon junctional maturation and formation of confluent layers. (B) In adherent cells, p120ctn recruits the non-receptor Tyr kinase Fer to the cadherin–catenin complex, which activates the protein Tyr phosphatase PTP1B. Dephosphorylation of key Tyr residues Y142 and Y654 in beta-catenin by PTP1B promotes junctional integrity. (C) The phosphorylation status of Y142 and Y654 in beta-catenin can be shifted by growth factor signaling and by the activity of Src family members, which can be recruited by p120ctn. Phosphorylation of Y142 or Y654 in beta-catenin results in disassembly of, respectively, the beta-catenin–alpha-catenin and cadherin–beta-catenin complexes. (D) Dissociation of p120ctn from the cadherin tail results in removal of PTP1B and subsequent dissociation of cadherin-based junctions. The E3 ubiquitin ligase Hakai can now bind to phosphorylated Tyr residues Y755 and Y756 in the tail of E-cadherin, which results in its ubiquitination and internalization. EGFR: epidermal growth factor receptor.

(Figure 4B). p120ctn-bound Fer can phosphorylate PTP1B on Y152, and this phosphorylation is essential for the binding of PTP1B to cadherin (123) (Figure 4D). In addition, p120ctn is phosphorylated in response to PDGF, CSF-1, EGF, NGF, HGF and VEGF (Table 1) (137-140).

Tyr phosphorylation of p120ctn is transient, so a feedback mechanism must exist for reversing this phosphorylation. Indeed, several phosphatases have been reported to bind to and dephosphorylate p120ctn (Table 1). Src homology 2 (SH2) domain-containing protein tyrosine phosphatase 1 (SHP-1) binds to p120ctn following EGFR activation (141). SHP-1 is activated by Src-mediated phosphorylation at its C-terminus, and it very effectively dephosphorylates Src substrates, including phosphorylated Y296 of p120ctn (142). Experiments on substrate trapping mutants of receptor protein tyrosine phosphatase DEP1 revealed that it interacts with p120ctn, as well as with beta-catenin and plakoglobin (143, 144). p120ctn might interact with DEP1 in a phosphorylation-dependent manner, whereas beta-catenin and plakoglobin might interact with DEP1 constitutively (144). The receptor-like protein

tyrosine phosphatase RPTPmu specifically binds and dephosphorylates p120ctn (but not beta-catenin) independently of cadherins and of the phosphorylation status of p120ctn (145) (Table 1). The RPTPmu binding site in p120ctn is in the N-terminal domain, and deletion of AA 28–233 of p120ctn abrogates its interaction with RPTPmu (145). In conclusion, the N-terminus of p120ctn acts as a scaffold for many kinases and phosphatases that can interact with cadherin-bound p120ctn. In that way, p120ctn enables the modulation of the cadherin–catenin complex by phosphorylation.

How does phosphorylation of p120ctn affect cadherin-based adhesion? In Src-transformed MDCK and L-cells, constitutive Tyr phosphorylation of p120ctn and beta-catenin weakens cadherin-based junctions (121, 146). Phosphorylation of p120ctn is dependent on its association with E-cadherin, as L-cells expressing an E-cadherin mutant lacking the cytoplasmic domain failed to phosphorylate p120ctn. Remarkably, membrane localization of p120ctn, but not *via* E-cadherin ligation *per se*, is essential for its susceptibility to phosphorylation on Ser or Thr residues. Chimeric cadherin-containing extracellular part of the IL-2R fused to the cytoplasmic cadherin part as well as CAAX anchoring of p120ctn localize p120ctn to the membrane and allow its phosphorylation in the absence of E-cadherin (147). Ser/Thr phosphorylation of p120ctn does not affect the p120ctn–cadherin binding affinity or cadherin complex stability, because mutating either of the Ser/Thr phosphorylation sites to Ala or mutating all six major Ser/Thr sites to Ala does not interfere with the capacity of this mutated p120ctn to rescue the phenotypes of p120ctn-deficient cells (147). On the other hand, the presence of cadherin-bound beta-catenin is not essential for p120ctn phosphorylation, as a fusion of E-cadherin and alpha-catenin is equally potent in phosphorylating p120ctn junctions (121, 146). In conclusion, increased Tyr phosphorylation of p120ctn leads to disruption of junctions, while constitutive Ser/Thr phosphorylation of p120ctn does not affect the integrity of adherens junctions.

It is not clear how phosphorylation of p120ctn affects its binding to E-cadherin. Several groups reported that Tyr phosphorylation of p120ctn increases its binding affinity for E-cadherin (115, 135, 148-150). However, others found no change in binding affinity between E-cadherin and p120ctn upon Src-mediated phosphorylation (17, 121). Increased binding of phosphorylated p120ctn to E-cadherin during junctional disassemby in Src-transformed cells implies that p120ctn blocks the key residues for Hakai-binding (Y755 and Y756) (Figure 4C and D) and therefore that E-cadherin internalization is not mediated by ubiquitination. In contrast, phosphorylated p120ctn has also been reported to dissociate from E-cadherin, and in this way it allows Hakai-mediated ubiquitination and endocytosis of E-cadherin (Figure 4D) (151). A recent report shows that CK1epsilon can be recruited to the cadherin–catenin complex, where it phosphorylates E-cadherin, resulting in the dissociation of p120ctn and beta-catenin from membrane-localized cadherins (111).

7.3. p120ctn isoforms and phosphorylation

Alternative splicing determines the extent of phosphorylation of different p120ctn isoforms by restricting the number of available phosphorylation sites or by allowing isoform-specific binding of kinases and phosphatases. The N-terminal domain of p120ctn is essential for its phosphorylation, and different p120ctn isoforms that can be generated from the four translation initiation sites exhibit different truncations of this N-terminal domain (Figure 2). p120ctn isoform 1 has the longest N-terminal domain and contains all the Tyr, Ser and Thr sites that can be phosphorylated. p120ctn isoform 3 lacks the first Tyr residue (Y96), which is present only in the long isoforms. p120ctn isoform 4 is translated from the fourth translation initiation site and lacks almost the entire N-terminal domain, including all nine Tyr sites and six of the Ser and Thr sites. Hence, p120ctn isoform 4 is not regulated by phosphorylation and can be viewed as a dominant-negative p120ctn variant for several functions.

Besides the role of isoforms in determining the potential phosphorylation sites, some kinases and phosphatases preferentially bind to certain p120ctn isoforms. The Fer binding domain in p120ctn is confined to a 26-AA region (131 to 156) present in both long and short isoforms (123). p120ctn interacts with tyrosine kinase Fer in Rat-2 embryonic fibroblasts and in epithelial A431 cells. Although both cell types express long and short isoforms, Fer predominantly binds and phosphorylates long isoforms (135, 136). Different isoforms of p120ctn interact to different extents with the tyrosine phosphatase SHP-1, and this seems to be partly related to their susceptibility to EGF-dependent phosphorylation (141). p120ctn isoform 3A is efficiently phosphorylated on Tyr in response to EGF stimulation and binds SHP-1 strongly. Although p120ctn isoform 1A contains all Tyr phosphorylation sites, including the EGF-specific phosphorylation site Y228, it is less efficiently phosphorylated upon EGF stimulation and interacts more weakly with SHP-1. As both p120ctn isoforms 1A and 3A contain Y228, it is strange that they are phosphorylated to different degrees upon EGF triggering. p120ctn isoform 1A can probably dock an additional phosphatase *via* its exclusive Y96 site, which would diminish its overall Tyr phosphorylation. p120ctn isoform 4A has no Tyr phosphorylation sites at all and interacts with SHP-1 very weakly. Remarkably, the presence of six AA encoded by the alternatively spliced exon C leads to a strong reduction in both Tyr phosphorylation and SHP-1 binding (141). RPTPmu interacts with p120ctn isoform 1A but fails to bind to a mutant lacking much of the N-terminus (145). However, whether the natural long and short p120ctn isoforms have different binding capacities for RPTPmu has not been tested.

8. p120ctn IN DEVELOPMENT

The role of p120ctn has been investigated in several animal model systems (reviewed in 152, 153). Although *C. elegans* and *Drosophila* have each only one p120ctn subfamily member (JAC-1 and *Drosphila* p120ctn), genetic or RNAi-mediated reduction of p120ctn

levels in *C. elegans* and *Drosophila* does not affect normal development (154-156). In contrast, RNAi-mediated depletion of both zygotic and maternal p120ctn in *Drosophila* embryos results in severe morphogenetic defects (73). Except for the findings in the latter report, it seems that p120ctn is not required for normal development in invertebrates.

In frog, proper p120ctn levels need to be maintained throughout development, because overexpression or knockdown of p120ctn in *Xenopus* embryos leads to severe embryonal abnormalities. Induced expression of murine p120ctn isoform 1A or 1N during *Xenopus* development leads to gastrulation defects and head malformation (157, 158). On the other hand, morpholino-mediated p120ctn knockdown in *Xenopus* results in severe developmental defects; the nature of the defects depended on which cells of early cleavage embryos were injected with morpholinos (46, 47).

Also in mice, proper p120ctn levels are a prerequisite for normal development, and embryos with total knockout of p120ctn die *in utero* (mentioned in 50, 54). In addition, several tissue-specific p120ctn knockouts generated by using the Cre/LoxP system have been reported. Two types of floxed p120ctn alleles have been generated. In one of them, p120ctn exons 3 to 8, encoding all four possible start codons (M1-4), were flanked by LoxP sites to prevent any natural initiation of translation after Cre-mediated recombination (50). In the other approach, exon 7 of p120ctn was floxed, and Cre-mediated recombination resulted in a frameshift leading to degradation of mRNA by non-sense mediated decay (53). Crossing these p120ctn floxed mice with mice in which the Cre-recombinase is controlled by a tissue-specific promoter resulted in specific depletion of p120ctn in salivary gland (50), skin (49, 159), teeth (51), dorsal forbrain (53), intestine (52), endothelial cells (54), oral cavity, esophagus and forestomach (160). Though these conditional p120ctn knockout mice have a wide range of tissue-specific phenotypes (reviewed in 152, 153), reduction of cadherin levels seems to be a common underlying mechanism. In these studies, the requirement for p120ctn during development turned out to depend to a large degree on the time at which the Cre recombinase is expressed. For instance, Cre-mediated ablation of p120ctn in endothelial tissues at 7.5 dpc causes death at 11.5 dpc (54). In other studies, developmental defects in tissue-specific p120ctn knockouts are mostly avoided by employing Cre-lines that are expressed near the end of embryonic development. Nevertheless, ablating p120ctn after midgestation in salivary gland and intestine can still result in perinatal death (50, 52), whereas its ablation in the skin, teeth or dorsal forebrain does not affect viability (49, 51, 53).

9. p120ctn AND CANCER

9.1. Altered expression of p120ctn in tumors

The expression pattern of p120ctn is abberant in a wide range of human tumors (reviewed in 161, 162). In general, p120ctn is either absent or altered in most human tumors, and its derangement is often correlated with poor prognosis. Alterations in p120ctn expression include decreased levels and translocation to the cytoplasm, and occasionally to the nucleus. p120ctn can act as a proto-oncogene or as an invasion suppressor, depending on the order in which p120ctn and E-cadherin are down-regulated. Loss of p120ctn results in decreased E-cadherin levels, and E-cadherin is indeed frequently down-regulated in epithelial cancers, in which it acts as a tumor-suppressor (163, 164). Downregulation of p120ctn in tumors cannot be fully explained by genetic inactivation because so far only a few mutations in the *CTNND1* gene have been reported and only in breast cancer (165) and in the SW48 colon carcinoma cell line (15). Perhaps other mechanisms are involved in p120ctn downregulation in tumors, such as transcriptional downregulation, epigenetic modification, or microRNA-mediated silencing, but this has not been properly documented. One study reported that p120ctn is transcriptionally downregulated by FOXC2 in non-small cell lung cancer cells (NSCLC) (166). FOXC2 binds to the p120ctn promoter and reduces its activity. RNAi-mediated silencing of FOXC2 increases p120ctn promoter activity as well as p120ctn and E-cadherin levels (166). On the other hand, E-cadherin loss results in translocation of p120ctn to the cytoplasm, where it modulates RhoGTPases in a way that favors cell motility.

Cytoplasmic p120ctn can regulate several processes involved in tumorigenesis, such as cell proliferation, motility, invasion, anchorage-independent growth, and inflammation. p120ctn can either stimulate or inhibit cell proliferation and tumor growth. Expression of p120ctn promotes transformed growth of both E-cadherin-negative and -positive cells (43, 82). On the other hand, knockdown of p120ctn in E-cadherin-negative cells reduces the growth rate, as evidenced by a reduction in the proportion of cells in the S-phase and by decreased MAPK signaling (82). In E-cadherin-positive breast cancer cells, p120ctn depletion results in increased proliferation and activation of Ras-MAPK signaling (82). In addition, expression of p120ctn isoform 3 in E-cadherin-positive cells blocks cell proliferation, DNA synthesis and *in vivo* tumor growth (64, 89).

Several p120ctn knockdown studies have illustrated that p120ctn is important for cell migration and invasion. Cells devoid of p120ctn failed to repopulate the wounded area in a scratch assay (79). On the other hand, stable knockdown of p120ctn in E-cadherin-deficient cells resulted in decreased migration and invasiveness (45). In clear renal cell carcinomas, E-cadherin is downregulated and p120ctn is translocated to the cytoplasm. Furthermore, p120ctn undergoes an isoform switch from predominantly short to long p120ctn isoforms. This switch is correlated with micrometastasis, which indicates that p120ctn isoform 1 simulates invasiveness *in vivo*, at least in this type of cancer (80). In contrast, p120ctn can also block invasion, because p120ctn depletion in lung cancer cell lines enhances invasion and metastasis due to differential regulation of RhoGTPase activity (90). p120ctn also acts as a proto-oncogene by relieving the Kaiso-mediated repression of beta-catenin target genes, such as *c-Fos*, *Myc*, *Ccnd1* (encodes Cyclin D1) and *Mmp7* (encodes

Matrylisin) (105, 107), and this favors tumor formation and invasion.

The role of p120ctn in cancer was further investigated in animal models. Ablation of p120ctn in salivary gland resulted in morphological abnormalities closely resembling high-grade intraepithelial neoplasia, but it did not result in cancer (50). Ablation of p120ctn in skin caused hyperproliferation (49) and p120ctn-deficient skin grafts displayed signs of epidermal hyperkeratosis and dysplastic keratinocytes (159). Recently, p120ctn was deleted from the oral cavity, esophagus and forestomach (160). The mutant animals survived, and by 4 to 6 months of age most of them had developed precancerous lesions. By the age of 9 to 12 months, 70% of them had developed tumors. None of the control littermates developed cancer. This confirmed that p120ctn is a tumor suppressor gene. The tumors in this mouse model closely resemble human oral squamous cell cancers and esopahgeal cell cancers, and this model might thus be useful for development of early detection strategies and novel therapies.

9.2. p120ctn in inflammation and cancer

The signaling pathways involved in cancer and inflammation are interconnected (167). Indeed, poorly differentiated human squamous cell carcinomas also showed nuclear NF-κB and perturbed expression and/or localization of p120ctn (159). p120ctn depletion in mouse skin caused hyperproliferation and chronic inflammation due to increased NF-κB activation (49). Both general immunosuppresive drugs (dexamethasone) and a NF-κB inhibitior (IKK2 inhibitor) reduced the hyperproliferation in p120ctn-deficient skin grafts (49, 159). NF-κB activation in p120ctn-depleted keratinocytes is dependent on RhoA activity. Constitutively active RhoA and ROCK mutations result in nuclear translocation of NF-κB in wild-type keratinocytes, whereas nuclear NF-κB expression can be reverted in p120ctn-deficient keratinocytes by expression of dominant-negative RhoA and by treatment with a ROCK inhibitor (49). Moreover, nuclear NF-κB can also be reverted by introducing a cadherin-uncoupled p120ctn mutant, but not by a RhoA-uncoupled mutant (*delta*622-628) (49). Finally, genetic ablation of intestinal p120ctn also results in increased infiltration of COX-2-positive neutrophils, which is commonly seen in inflammatory bowel disease (52). This disease predisposes intestinal tissue to cancer (168). The tumor cells that develop in mice with conditional loss of p120ctn in the oral cavity, esophagus and squamous forestomach secrete inflammatory signals that lead to immune cell infiltration (160). Blocking the recruitment of immune cells with dexamethasone resulted in hyperplasia but not in desmoplasia or inflammation.

Toll-like receptors (TLRs) are key elements of the innate immune system. These receptors recognize bacterial lipopolysaccharide (LPS) and trigger the induction of immunoregulatory chemokines and cytokines. When TLR4 binds LPS, it activates the MyD88-dependent and MyD88-independent pathways. The MyD88-dependent pathway, which is triggered by all TLRs except TLR3, activates NF-κB and activator protein-1 (AP-1). This leads to the induction of proinflammatory chemokines and cytokines, such as Tumour-Necrosis-Factor α and interleukin-1β. Recently, Wang *et al.* (169) showed that p120ctn modulates innate immune function in the lung by disrupting the association of TLR4 with MyD88 and thereby blocking TLR4 signaling and NF-κB activation in endothelial cells.

9.3. p120ctn isoforms in EMT and cancer

A switch in p120ctn isoforms has been observed during epithelial-to-mesenchymal transition (EMT). EMT is an orchestrated series of events that allows epithelial sheets to dissociate, lose cell-cell interactions and cell-extracellular matrix interactions, and reorganize the cytoskeleton and transcriptional program in order to induce a mesenchymal phenotype (170). During EMT, E-cadherin downregulation in epithelial cells is accompanied by upregulation of mesenchymal cadherins (e.g. N-cadherin), a phenomenon called cadherin switching (171). Interestingly, a switch from short to long p120ctn isoforms has been observed during the EMT induced by expression of c-Fos (20), Snail (21), SIP1/ZEB2 (22, 23), E47 (23), Slug (23), Twist (24) or Zeppo1 (25). The downregulation of short 'epithelial' p120ctn isoforms during EMT is due to decreased expression of epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2), which favors skipping of exon 3 (encoding the first two translation initiation sites) and initiation of translation from the third start codon (encoded by exon 5) (24). Also, Src transformation of MDCK cells induces an EMT-like process (172) associated with a switch from short to long p120ctn isoforms (14). This switch during EMT is consistent with the expression of long and short p120ctn isoforms in fibroblasts and epithelial cell types, respectively. Re-expression of E-cadherin in Snail-induced mesenchymal cells failed to restore the epithelial morphology and the expression of 'epithelial' short p120ctn isoforms (21). This confirms that the abundance of the different p120ctn isoforms is regulated by cell type-specific splice factors and not necessarily by the expression of certain cadherin types (24). In contrast, the p120ctn isoform switch was not observed during EMT induced in highly differentiated colon cancer cells by TGF-beta and TNF-alpha (81). This discrepancy might be explained by the expression of long p120ctn isoforms in non-induced cells at high levels that cannot be augmented further during EMT (81).

During tumor progression, primary tumor cells also undergo an EMT-like process to break away from the primary tumor, enter blood or lymphatic vessels, and form metastises at secondary sites. EMT-like processes have been observed during progression of prostate carcinoma and in anaplastic thyroid carcinomas, and their occurrence coincides with a switch from short to long p120ctn isoforms and from E- to N-cadherin (173, 174). Forced expression of E-cadherin in the pancreatic carcinoma cell line MIA PaCa-2 restored the epithelial phenotype and suppressed cell migration and invasion, but forced expression of N-cadherin did not have the same effect (175). Interestingly, E-cadherin bound predominantly to unphosphorylated p120ctn isoform 3, whereas tyrosine-

phosphorylated p120ctn isoform 1 interacted exclusively with N-cadherin (175). However, no p120ctn isoform switch could be observed during tumor progression from benign (immortalized human keratinocytes) to malignant epithelial skin tumors (SCCs). In lung SCCs and adenocarcinomas, abnormal expression of p120ctn, including downregulation of both long and short isoforms, is associated with tumor progression and poor prognosis (176). E-cadherin and p120ctn isoform 3 are expressed in normal bronchial epithelium, but p120ctn isoform 1 is upregulated and localized in the cytoplasm of squamous cell lung cancers and lung adenocarcinomas (179). Further analysis revealed that overexpression of p120ctn isoform 1 mRNA correlates significantly with abnormal E-cadherin expression, lymph node metastasis, and poor differentiation (177). Another study confirmed a significant reduction of both long and short p120ctn isoforms in lung carcinomas compared to normal lung tissues (178). In summary, although EMT-like processes can occur during tumor progression in some tumor types, this is not a common feature in all cancers, as might be expected in view of the heterogenous nature of cancer.

Very little is known about the effects of the alternatively spliced internal exons of p120ctn during tumorigenesis. Exon B encodes a NES and is expressed in some human tissues, such as kidney, pancreas, colon, small intestine and prostate. Interestingly, expression of exon B is lost in the corresponding tumorigenic tissues (78), suggesting that during tumor progression p120ctn is shifted towards the nucleus, which might then relieve the Kaiso-mediated repression of oncogenic target genes.

10. THE p120ctn FAMILY

What complicates matters even more is that p120ctn does not act alone. In vertebrates, and particularly in mammals, the diversity of the p120ctn family has increased throughout evolution. This has led to the emergence of two evolutionarily conserved clusters: the p120ctn clade consisting of p120ctn and ARVCF (Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome), and the delta-catenin clade, consisting of p0071 (plakophilin-4) and delta-catenin (neural plakophilin-related armadillo protein or NPRAP or neurojungin) (152, 179). The four p120ctn family members have similar overall structures, with a central Armadillo repeat domain containing nine Armadillo repeats (12, 13). This central domain enables all p120ctn subfamily members to bind the JMD of classical cadherins (40, 180-184). We have recently reviewed the structures and functions of the other family members in comparison with p120ctn (153).

When ARVCF and delta-catenin are overexpressed in, respectively, MDCK and CWR22Rv-1 prostate cancer cells, they can compete with p120ctn for JMD binding (181, 185). Also they can restore cadherin-based junctions in p120ctn-depleted cells (42). However, Zhang *et al.* (186) showed in two lung cancer cell lines that overexpressed delta-catenin binds to the cytoplasmic domain of E-cadherin in a way that is non-competitive with p120ctn. Competitive binding to E-cadherin might thus occur only in certain cell types. Moreover, endogenous ARVCF and delta-catenin fail to stabilize classical cadherins in tissue-specific p120ctn knock-out mice (49, 50, 52). So, at endogenous levels, apparently none of the p120ctn family members can substitute for p120ctn. Of course, crossing mouse lines in which knock-out of individual p120ctn family members was not lethal should provide more information about redundancy. For example, mating mice with a total knock-out of ARVCF (mentioned in 152) with mice in which p120ctn is deleted specifically in the skin (49) might reveal whether ARVCF and p120ctn do not substitute at all for each other. Another approach to assess the extent of functional overlaps amongst p120ctn family members is to evaluate the consequence of knocking-in the coding region of one family member into the genetic locus of another.

11. CONCLUSIONS AND PERSPECTIVES

In this review we focused on the new insights into the molecular mechanisms of the diverse functions of p120ctn. Like beta-catenin, p120ctn plays key roles at cell–cell junctions and in intracellular signaling. At the cell membrane, p120ctn regulates cadherin stability and trafficking. p120ctn binds newly synthesized N-cadherin, and together they are transported to the membrane. In contrast, p120ctn binds to E-cadherin only after it is transported to the membrane. Thus, p120ctn is recruited to different cadherins at different time points in their biogenesis. What is even more intriguing is that p120ctn can stabilize both N-cadherin and E-cadherin to similar extents but seemingly by different mechanisms. For instance, p120ctn blocks two tyrosine phosphorylation sites in the JMD that are important for Hakai-mediated endocytosis of E-cadherin. This mechanism is specific for E-cadherin, because Hakai does not bind to N-cadherin.

Recent findings revealed a high degree of interconnectedness between p120ctn/Kaiso and beta-catenin/TCF signaling. In the cytoplasm, these pathways are physically linked to each other by a protein called Frodo. Also, p120ctn and beta-catenin are targeted for proteasomal degradation *via* the same pathway. In the nucleus, p120ctn can induce gene expression by inhibiting the transcriptional repression of Kaiso, whereas beta-catenin induces expression by acting as a co-activator. Moreover, in the nucleus there is cross-talk between the p120ctn/Kaiso and beta-catenin/TCF signaling pathways, which share some target genes. These commonly regulated genes can be activated by nuclear beta-catenin and repressed by Kaiso. p120ctn can block this Kaiso-mediated repression by an unknown mechanism. Live cell imaging in combination with fluorescence resonance energy transfer (FRET) might give some hints about where p120ctn and Kaiso interact. In addition, we need to gain insight into where and when the p120ctn/Kaiso pathway is activated. This would give us a better understanding of the importance of this pathway relative to the beta-catenin/TCF signaling pathway.

A heavily discussed function of p120ctn in the cytoplasm is its influence on the activity of RhoGTPases.

p120ctn mediates inhibition of RhoA and activation of both Rac1 and Cdc42, possibly *via* an interaction between p120ctn and RhoA or by one of the other mechanisms discussed in section 5. It is becoming increasingly clear that the effect of p120ctn on RhoGTPase activity is cell-type specific. Moreover, all that we know about p120ctn modulation of RhoGTPase signaling is based on the use of 'classic' Rho GTPase tools that do not faithfully reflect the degree of regulation (187). These tools include dominant-positive and -negative mutants of the three major Rho GTPases: Rac1, Cdc42 and RhoA. Another is the biochemical pulldown assay, which measures the average activation status of a given GTPase in a population of cells. However, new imaging technologies allow visualization of Rho GTPase signaling with high spatio-temporal resolution (187)**.** This kind of visualization is expected to resolve many controversial results reported in the literature and to provide the information needed to understand how p120ctn modulates RhoGTPase activity at cell–cell junctions and at intracellular locations.

The complexity of p120ctn research is increased by phosphorylation and alternative splicing events. Phosphorylation affects several functions of p120ctn, such as modulation of the strength and composition of cadherin-based junctions and determination of the binding affinity between p120ctn and RhoA. Resolving all phosphorylation events in time and space is nearly impossible, but it has become clear over recent years that p120ctn is important both as a substrate and as a scaffold for kinases and phosphatases. In addition, accumulating evidence indicates that the different splice variants of p120ctn have specific functions and can be targeted to different subcellular compartments.

p120ctn performs pleiotrophic functions in several cell compartments. This is clearly true for its effect on signaling. These varied functions lead us to believe that the p120ctn proteins are important during development and that disturbance of the levels of different isoforms can influence cancer development. It is fairly clear that p120ctn influences the initiation, growth and metastasis of cancer. Moreover, an association between the development of cancer and inflammation has been known for a long time (188). As targeted ablation of p120 results in a "cytokine storm" and intense inflammation, it is believed that p120ctn functions as a potent suppressor of inflammation. Recently, p120ctn has been described as a tumor suppressor gene (160). Future studies will have to unravel how the different functions of p120ctn participate in normal development and in disease.

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