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COMMENTARY

The p120 catenin family: complex roles in adhesion, signaling and cancer

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SUMMARY

p120 catenin (p120) is the prototypic member of a growing subfamily of Armadillo-domain proteins found at cell-cell junctions and in nuclei. In contrast to the functions of the classical catenins (α-catenin, β-catenin, and γ-catenin/plakoglobin), which have been studied extensively, the first clues to p120's biological function have only recently emerged, and its role remains controversial. Nonetheless, it is now clear that p120 affects cell-cell adhesion through its interaction with the highly conserved juxtamembrane domain of classical cadherins, and is likely to have additional roles in the nucleus. Here, we summarize the data on the potential involvement of p120 both in promotion of and in prevension of adhesion, and propose models that attempt to reconcile some of the disparities in the literature. We also discuss the structural relationships and functions of several known p120 family members, as well as the potential roles of p120 in signaling and cancer.

Key words: Cell adhesion, p120ctn, ARVCF, δ-catenin/NPRAP/neurojungin, p0071, Plakophilin, Cadherin, Clustering, RhoA, Kaiso, Presenilin, Armadillo/β-catenin

INTRODUCTION

p120 (p120ctn, hereafter p120) was originally identified as a prominent substrate of the Src oncoprotein (Reynolds et al., 1989). cDNA cloning revealed that it contains an Armadillo-repeat domain (Arm domain) that shares 22% identity with that of the catenin Armadillo/β-catenin (Reynolds et al., 1992; Peifer et al., 1994) and led to experiments that demonstrated its direct interaction with cadherins (Reynolds et al., 1994; Daniel and Reynolds, 1995; Staddon et al., 1995; Shibamoto et al., 1995; see Fig. 1). Cadherins comprise a superfamily of transmembrane cell-cell adhesion receptors that link adjacent cells via calcium-dependent homophillic interactions between the cadherin extracellular domains. They regulate a variety of biological processes, including development, morphogenesis, and tumor metastasis (for review see Takeichi, 1995; Yap, 1998). During tumor progression to malignancy, the aberrant loss of expression of epithelial cadherin (E-cadherin), the major cell-cell adhesion molecule in epithelial cells, is widely believed to mediate the transition to metastasis (Perl et al., 1998; reviewed by Yap, 1998). Cadherin function is modulated by a group of cytoplasmic proteins called catenins (i.e. α-catenin, β-catenin, plakoglobin and p120), which interact with the cadherin intracellular domain. Defects in catenin expression or function have also been linked to metastasis. A major role of catenins is to anchor the cadherin complex to the actin cytoskeleton. β-Catenin and plakoglobin act as bridges connecting E-cadherin to α-catenin, which in turn associates with actin filaments either directly (Herrenknecht et al., 1991; Nagafuchi et al., 1991; Rimm et al., 1995) or indirectly (Knudsen et al., 1995; Nieset et al., 1997). β-Catenin also has signaling roles in the cytoplasm and nucleus that are important in development and cancer. For example, β-catenin/armadillo is a key player in the Wnt/Wg signaling pathway, directly mediating downstream events through transactivation of transcription factors of the Lef1/TCF family (Molenaar et al., 1996; Behrens et al., 1996; van de Wetering et al., 1997). In addition, β-catenin interacts directly with the tumor suppressor adenomatous polyposis coli (APC; Su et al., 1993; Rubinfeld et al., 1993). Inactivating mutations in APC, or activating mutations in β-catenin, cause the accumulation of β-catenin in the cytoplasm and nucleus, leading to constitutive signaling through interaction with Lef1/TCF (Munemitsu et al., 1995; Korinek et al., 1997; Rubinfeld et al., 1997). Collectively, defects in APC or β-catenin function are thought to account for initiation of the majority of human colon cancer (Powell et al., 1992; Morin et al., 1997) and a smaller percentage of melanoma (Rubinfeld et al., 1997). The well-characterized dual roles of β-catenin in adhesion and signaling establish an important paradigm for other Arm proteins, many of which are known to function as adhesion molecules (e.g. p120).

Unlike β-catenin, p120 does not interact with α-catenin or with APC (Daniel and Reynolds, 1995), which implies that p120 has a novel function in cadherin complexes. p120 apparently has both positive and negative affects on cadherin-mediated adhesion, depending on signaling cues that remain unspecified. Tyrosine and serine/threonine kinases are re-emerging as important candidates for the regulaton of p120

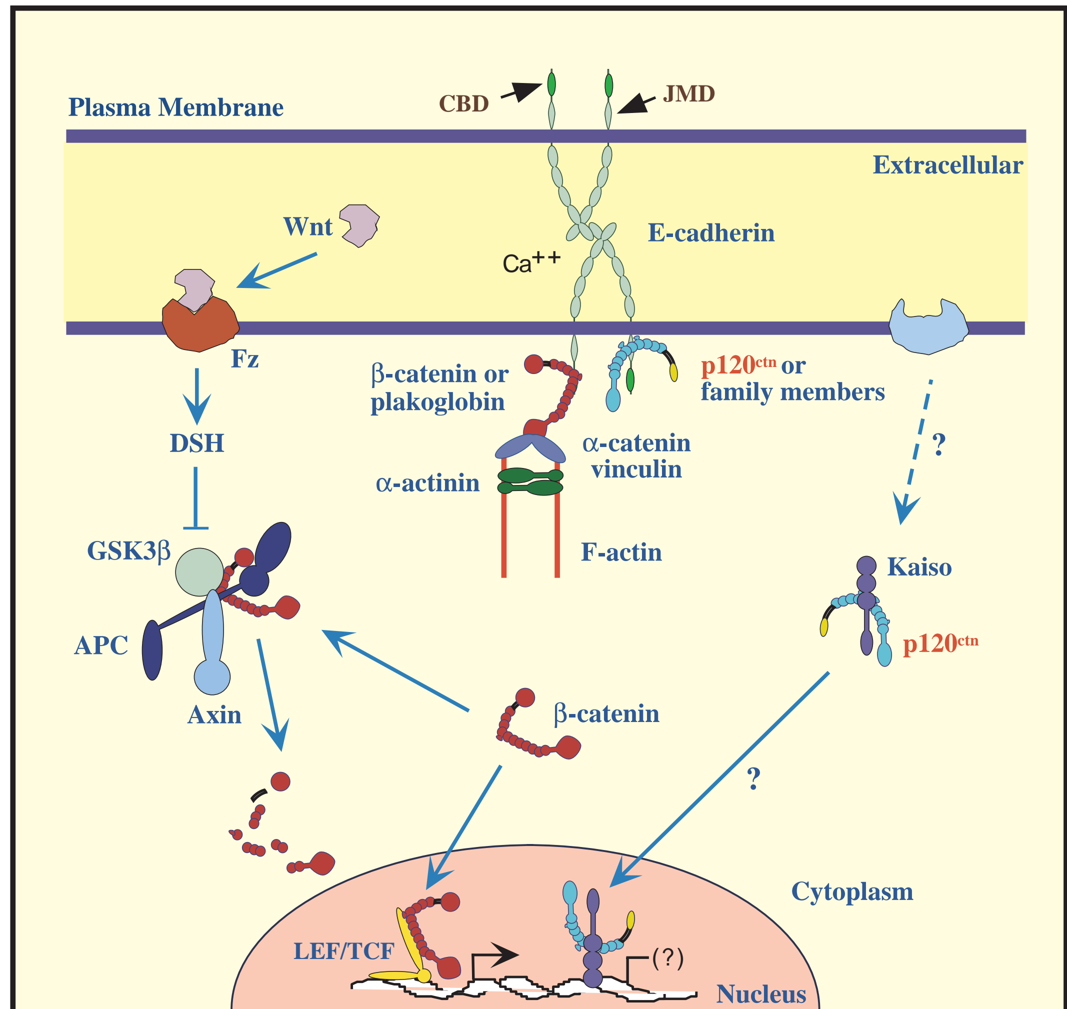

Fig. 1. The role of p120 in cell-cell adhesion and signaling. The catenins (p120^{ctn}, β-catenin, plakoglobin and α-catenin) bind to the cytoplasmic tails of classical cadherins. β-catenin and plakoglobin compete for binding to the so-called catenin-binding domain (CBD) and mediate the attachment of cadherins to the actin cytoskeleton via α-catenin. In contrast, p120 (including isoforms and probably other p120 subfamily members) associates with the cadherin juxtamembrane domain (JMD) and does not bind to α-catenin. p120 may act as a switch, either promoting or inhibiting cadherin-mediated adhesion in response to unspecified signaling cues. p120, like β-catenin, exhibits dual localization both at the membrane and in the nucleus. Wnt/Wg signaling during development, or APC mutations in human cancer, stabilize a normally transient cytoplasmic pool of β-catenin that translocates to the nucleus, where it participates in transcriptional regulation through interactions with transcription factors of the Lef1/TCF family. Kaiso is a novel transcription factor that interacts with p120. Although the biological significance of the p120-Kaiso interaction is not known, p120 may affect nuclear signaling by transactivating Kaiso, which is postulated to act as a transcriptional repressor. In contrast to β-catenin signaling, there is no mechanism to promote the degradation of p120 in metastatic cells that have downregulated cadherins. Thus, cadherin binding may be an in important factor in regulating the putative role of p120 in transcription through its sequestration at the cell membrane.

activity, which suggests that the story will eventually come full circle back to Src. In addition, given the β-catenin paradigm, it is perhaps not surprising that p120 also enters the nucleus (Van Hengel et al., 1999, and, in this issue, Mariner et al., 2000), where it interacts with a novel transcription factor, Kaiso (Daniel and Reynolds, 1999; Fig. 1). In fact, p120 is the prototypic member of a growing gene family, whose protein products localize both at junctions and in the nucleus. Elucidation of the potential signaling pathways implied by these observations promises to reveal important new information about cell-cell communication, differentiation and cancer.

p120 ISOFORMS AND NOMENCLATURE

Initially designated p120CAS (for cadherin-associated Src substrate), p120 was renamed p120^{ctn} (catenin; Reynolds and Daniel, 1997) to avoid confusion with a different Src substrate, p130CAS (Crk-associated substrate; Sakai et al., 1994). In addition, it is now evident that most cell types express multiple isoforms of p120, which are derived by alternative splicing of a single gene (Reynolds et al., 1994; Staddon et al., 1995; Mo and Reynolds, 1996; Keirsebilck et al., 1998). Thus, the nomenclature has been further refined to account for these multiple forms (Fig. 2). N-terminal splicing events lead to the use of four different ATGs (Keirsebilck et al., 1998), resulting in the expression of p120 isoform type 1, 2, 3 or 4, according to the respective ATG used as the translation start site. Furthermore, alternative splicing of the C-terminal end leads to use of exon A, exon B, both exons A and B, or none of them. On rare occasions, p120 contains a sequence encoded by an additional exon (exon C), which is inserted within Arm repeat 6. Various combinations of these N- and C-terminal exons generate the different p120 bands observed in various cell types. The current, and definitive nomenclature, is shown in Fig. 2 (for comparison with older 'CAS' nomenclature, see Reynolds and Daniel, 1997).

The existence of cell-type-specific expression patterns implies functional differences between the p120 isoforms. For example, macrophages and fibroblasts make N-cadherin and

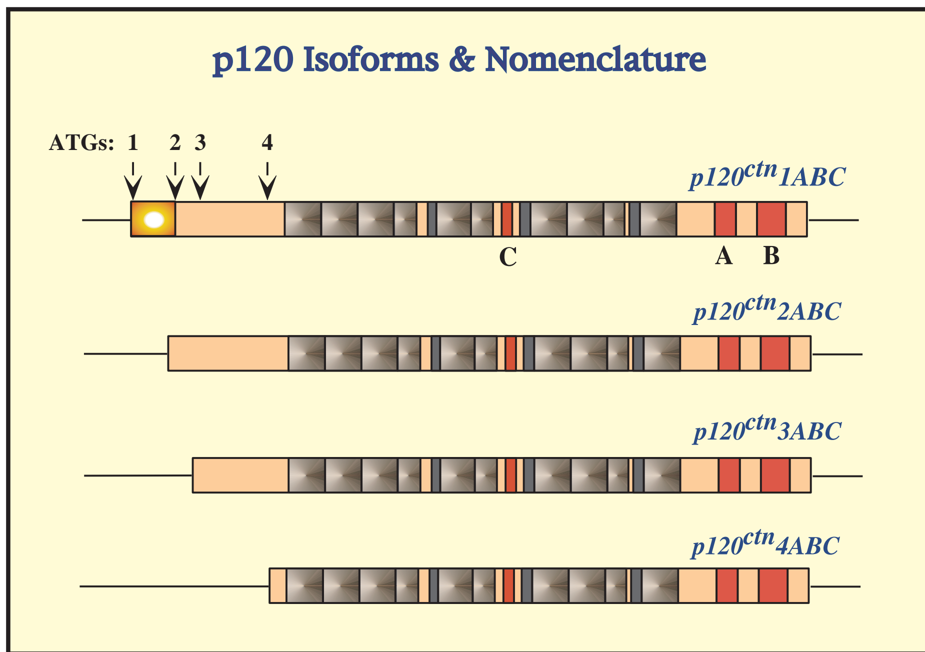

Fig. 2. p120 nomenclature and alternative splicing. p120 contains a central Armadillo domain that has ten Arm repeats (depicted by gray boxes) interrupted by short loops in repeats 4, 6 and 9. Cell-type-specific alternative splicing events result in multiple isoforms. Four N-terminal ATG start sites generate p120 isoforms 1, 2, 3, and 4. p120 isoform 1 contains a putative coiled-coil domain (yellow box), which is absent from isoforms initiating at internal ATGs 2-4 and is conserved among p120's close relatives (see Fig. 4). Additional alternative splicing results in alternative usage of exons in the C-terminal end. Exons A and B are often used, whereas exon C is used rarely. The splicing complicates the nomenclature. Isoforms are designated p120ctn 1-4, depending on the N-terminal start site. The A, B, and/or C designations are included if the exon is present (e.g. p120ctn1A, p120ctn1BC). The letter N (for none) is used if it is known that none of the C-terminal exons is present (e.g. p120ctn1N).

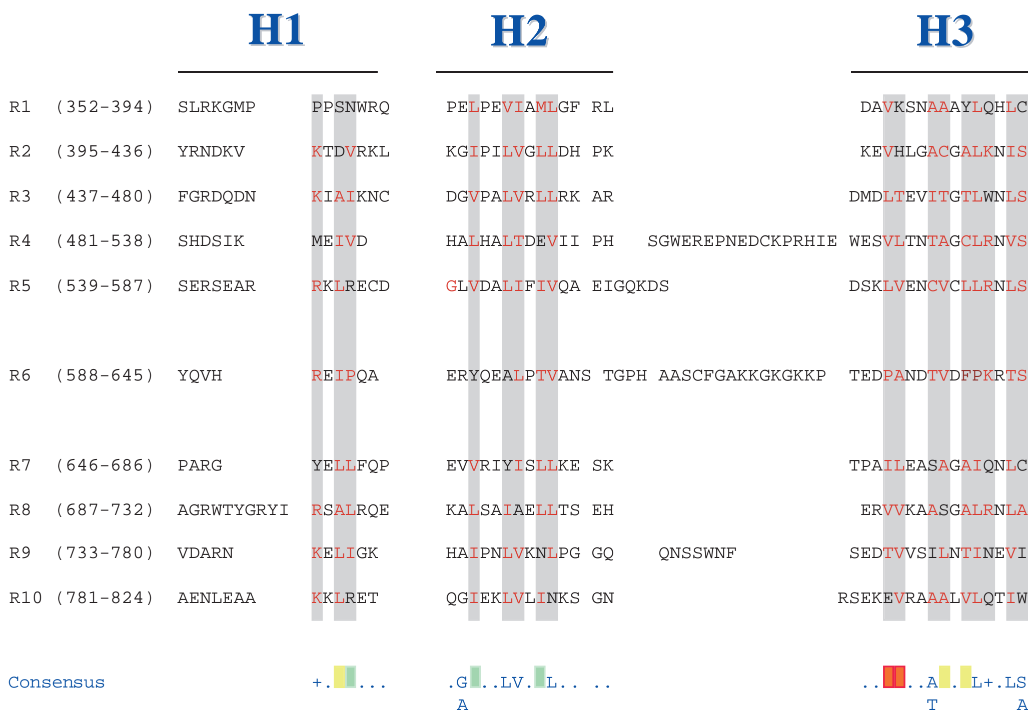

Fig. 3. Sequence alignment and organization of the p120 Arm repeats. The recently elucidated crystal structure of the β-catenin Arm repeats (Huber et al., 1997) makes it possible to realign the p120 Arm domain in the context of structural data, and is important for mutational analysis. According to this alignment, p120 has ten contiguous Arm repeats, each of which consists of three helices (H1, H2 and H3). Repeats 4, 6 and 9 contain looped-out structures located between helices H2 and H3. The basic motif (KKGKGKK) is thus embedded within repeat 6. Conserved residues are highlighted in red. In the consensus motif (bottom), + denotes basic residues (H,K,R), yellow boxes denote small hydrophobic residues (A,C,P,V,T), green boxes denote large hydrophobic residues (F,I,L,M,W), whereas orange boxes denote general hydrophobic residues (A,C,F,I,L,M,P,T,V, or W).

preferentially express p120ctn1A isoforms, whereas epithelial cells make E-cadherin and preferentially express smaller isoforms, such as p120ctn3A. Because alternative splicing usually affects only the N- and C-terminal domains, the Arm domain is left intact and free to interact with cadherins. Different isoforms may affect cadherin function by recruiting distinct binding partners to the cadherin complex. Other members of the p120 family are also expressed as multiple isoforms, which for the most part have not been characterized. The regulated expression of multiple p120 family members and their isoforms, might provide an important mechanism for fine-tuning the activities of the various cadherins in different cells.

THE p120 FAMILY

Two groups with different functions

Members of the p120 family share a characteristic structural

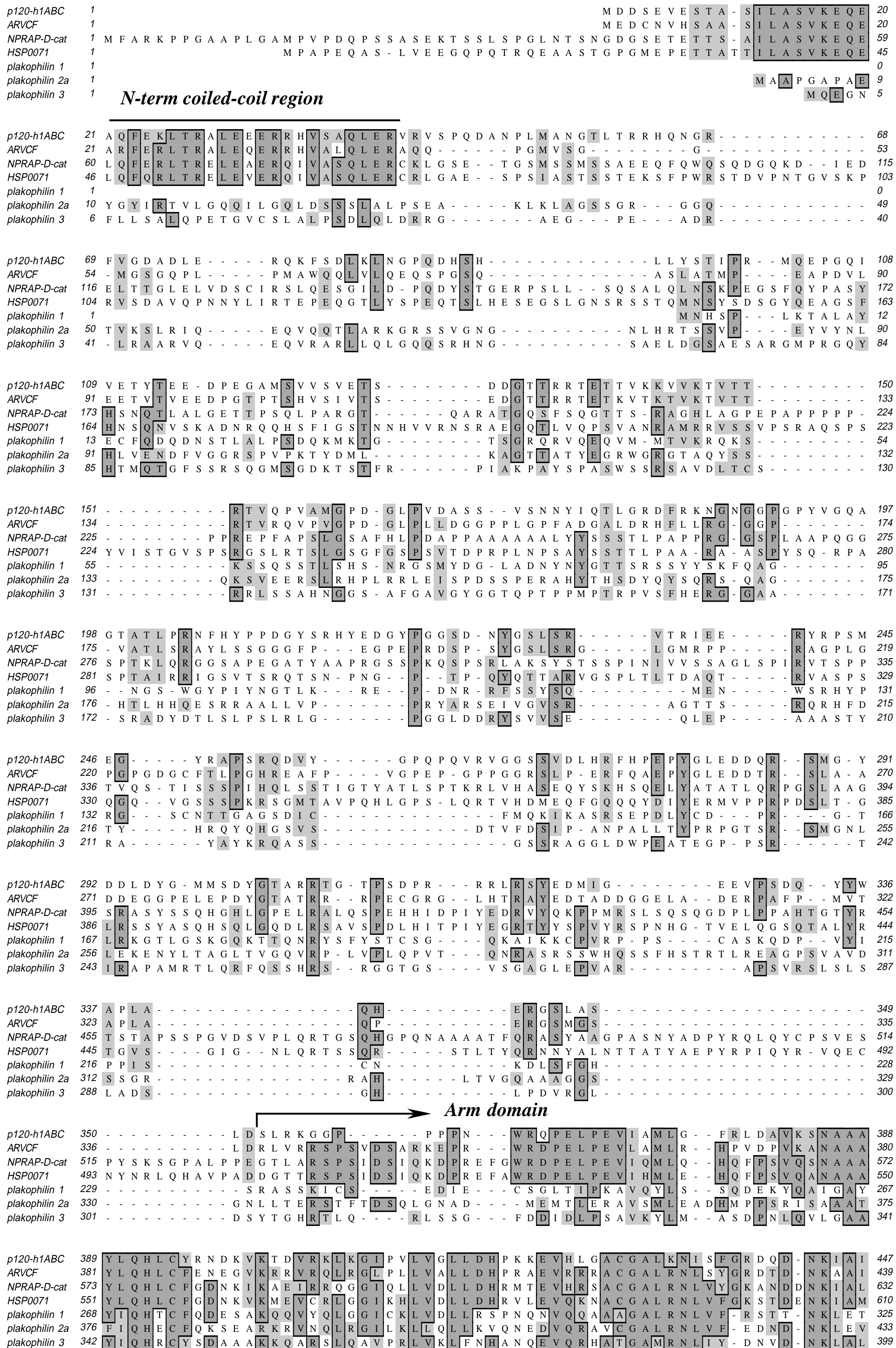

Fig. 4. For legend see p. 1324.

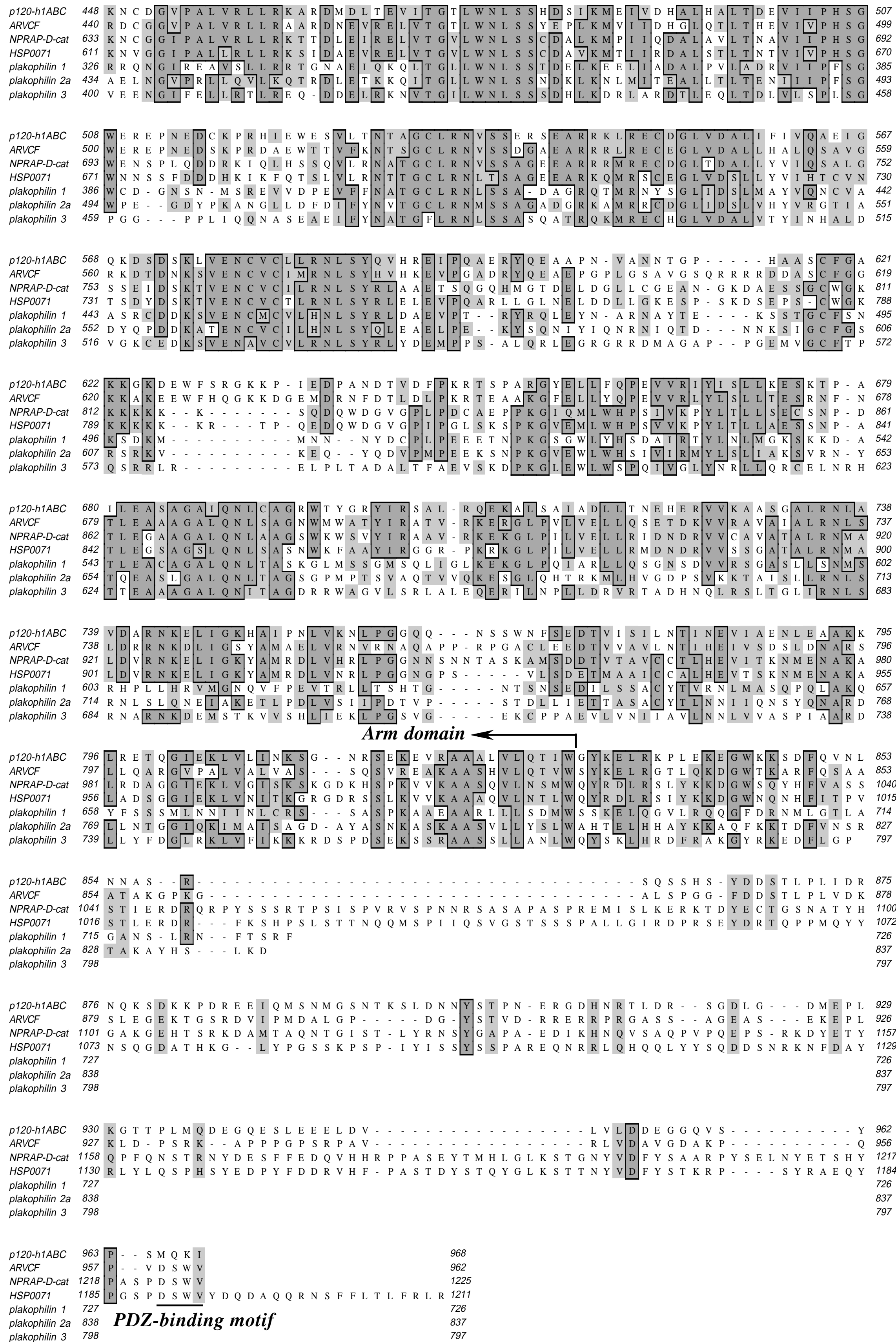

Fig. 4. For legend see p. 1324.

Fig. 4. CLUSTAL W alignment of human p120ctn1ABC, ARVCF, δ-catenin/NPRAP, p0071 and plakophilins 1a, 2a and 3. Dark-boxed areas designate amino acid identity between at least 4 out of the seven sequences. Light gray regions show similarity rather than identity. The conservation between family members in the central Arm domain is clear, whereas divergence is evident in the N- and C-terminal ends. A conserved coiled-coil domain in the extreme N-terminal domain of p120, ARVCF, δ-catenin/NPRAP and p0071 is depicted. A putative PDZ-binding domain at the extreme C-terminal of ARVCF, δ-catenin/NPRAP and p0071 (but not p120) is also shown. Closer inspection, coupled with functional data, justifies further division into two subgroups consisting of the top four sequences (the p120 family) and the bottom three sequences (the plakophilin family). Database accession numbers: p120ctn1ABC: AF062341; ARVCF: U51269; δ-catenin/NPRAP: U96136; p0071: X81889, plakophilin 1a: Z34974; plakophilin 2a: X97675; plakophilin 3: AF053719.

organization of the Arm-repeat domain (see Figs 2, 3), which suggests an ancient evolutionary relationship. However, two groups have emerged that differ both in the degree of similarity that they share with p120, and in their subcellular localizations. The first group includes ARVCF (Armadillo repeat gene deleted in Velo-Cardio-Facial syndrome; Sirotkin et al., 1997), δ-catenin/NPRAP/neurojungin (neural plakophilin-related Armadillo protein; Paffenholz and Franke, 1997; Zhou et al., 1997) and p0071 (Hatzfeld and Nachtsheim, 1996). These proteins share >45% identity with p120 in their Arm domains and, with the possible exception of p0071 (Hatzfeld and Nachtsheim, 1996), bind to and colocalize with classical cadherins at adherens junctions (Lu et al., 1999; Paffenholz et al., 1999; Mariner et al., 2000). The intron-exon boundaries of p120 and ARVCF are nearly identical (Keirsebilck et al., 1998), further highlighting the similarity between these proteins. The second group, the plakophilins, have ~30% identity to p120 in their Arm domains, which are otherwise organized similarly to that of p120. Unlike the first group, which interact with cadherins through their Arm domains, plakophilins localize to desmosomes through interactions mediated by their head (N-terminal) domain, and coordinate intermediate filaments (Hatzfeld et al., 1994; Heid et al., 1994; Mertens et al., 1996; Schmidt et al., 1999; Bonne et al., 1999; Kowalczyk et al., 1999; Schmidt et al., 1997; Smith and Fuchs, 1998). They exhibit significantly higher similarity to one another than to p120 (Bonne et al., 1999). Because of the significant differences between these subgroups, we will hereafter refer to them separately as the p120 subfamily (which includes ARVCF, δ-catenin/NPRAP and p0071), and the plakophilin subfamily (which includes plakophilins 1, 2 and 3). All of these proteins also localize to the nucleus (Bonne et al., 1999; Schmidt et al., 1997; Mertens et al., 1996; Van Hengel et al., 1999; Mariner et al., 2000); this interesting phenomenon has obvious implications for signaling between cell junctions and the nucleus.

p120 family structure

The p120 relatives share several structural features. Each has ten Arm repeats, which are organized identically and contain interruptions within repeats 4, 6 and 9 (see Figs 2 and 3). Fig. 3 shows the sequence and predicted boundaries of each p120 Arm repeat according to the recently elucidated β-catenin crystal structure (Huber et al., 1997). An alignment of all known p120 family members is shown in Fig. 4 and highlights the sequence conservation shared by these proteins. The interruptions noted above are thought to represent looped-out structures positioned between helices H2 and H3 – as occurs in β-catenin (Huber et al., 1997). In the loop within Arm repeat 6, all of the p120 relatives contain motifs postulated to act as nuclear localization sequences. In ARVCF, there appears to be a bipartite nuclear-localization signal (NLS; Imamura et al., 1998), whereas p120, p0071 and δ-catenin/NPRAP have a highly conserved basic motif (CW/FGxKKxKxKK). Interestingly, the presence of p120 exon C alters this basic motif (Keirsebilck et al., 1998). Although it is suggested that these basic motifs play a role in nuclear localization (Lu et al., 1999), indirect data suggest otherwise (Mariner et al., 2000). The plakophilins also contain basic motifs within this loop, and several lines of evidence suggest that they also are not required

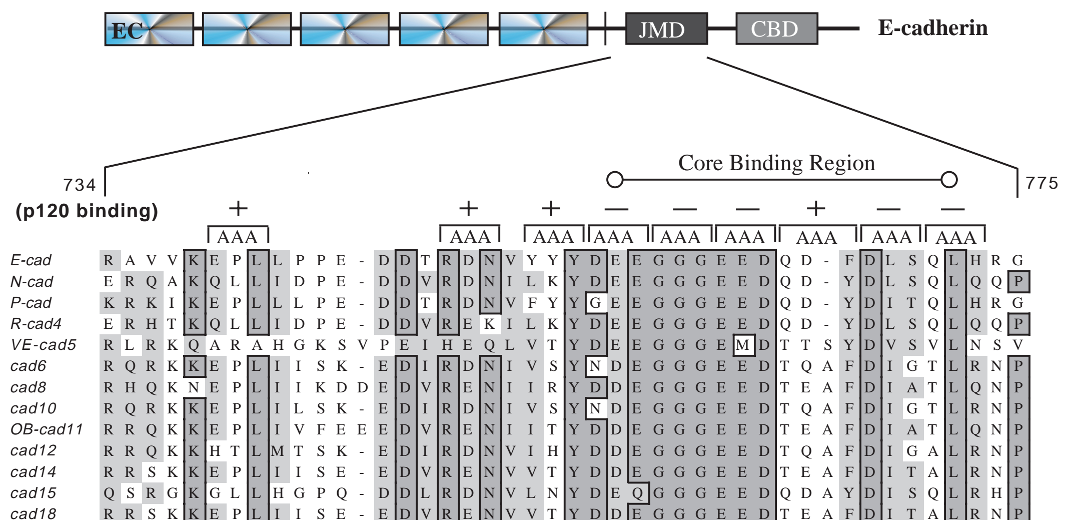

Fig. 5. p120 binds to the conserved cadherin juxtamembrane domain (JMD), a region highly conserved among type I (classical) and type II cadherins. β-catenin or plakoglobin binds to the so-called catenin-binding domain (CBD). Triple-alanine (AAA) substitution mutations (indicated) spanning the E-cadherin JMD define a core region (the p120-binding core) within the JMD that is required for interaction with p120 in yeast two-hybrid assays (Thoreson et al., 2000; + denotes normal p120 binding; – denotes loss of p120 binding). The triple substitution mutant 764 (EED/AAA) has been tested in mammalian cells and shown to uncouple p120 binding in vivo.

for nuclear translocation (Klymkowsky, 1999; Schmidt et al., 1997).

A highly conserved N-terminal motif in p120, ARVCF, p0071 and δ-catenin/NPRAP encodes a putative coiled-coil domain. Coiled-coil motifs are typically involved in protein-protein interactions. This region is spliced out in p120 isoforms 2, 3 and 4, whose translation begins at downstream ATGs. Given that p120 type 1 isoforms are prevalent in motile cell types (e.g. fibroblasts), they might be important for promoting more dynamic cadherin interactions. Roles in homo- or hetero-dimerization can also be envisioned, although such a mechanism would pertain selectively to type 1 isoforms. Alternatively, this domain could be responsible for the recruitment of novel effectors to the cadherin complexes. A different conserved region is present in the N terminus of all plakophilins (Bonne et al., 1999) and might be involved in desmosomal localization.

In the C-terminal end, ARVCF, δ-catenin/NPRAP and p0071, but not p120, share a third region of similarity. The conserved sequence DSWV is a PDZ-binding motif (type I; Fanning and Anderson, 1999), which in the case of δ-catenin/NPRAP binds to S-SCAM, a PDZ-domain-containing scaffold protein (Ide et al., 1999). Interactions between p120 family members and scaffold proteins could play a role in the recruitment of signaling molecules to cadherin junctions, and/or in promoting cadherin clustering – an event important in cadherin-mediated adhesion, which we discuss in detail later.

REGULATION OF THE p120-CADHERIN INTERACTION

Stoichiometry of the cadherin-p120 interaction

The relatively weak interaction between cadherins and p120 in detergent cell lysates led investigators to estimate that only 5-20% of total cellular p120 is in complex with cadherins (Ozawa and Kemler, 1998; Shibamoto et al., 1995; Papkoff, 1997; Staddon et al., 1995). In contrast, immunofluorescence analysis generally indicates precise colocalization of p120 with endogenous cadherins (Reynolds et al., 1994; Thoreson et al., 2000), which suggests a significantly higher stoichiometry. Recent evidence strongly favors the latter view. In cadherin-deficient cell lines (e.g. A431D, MDA-231 and L-cells), p120 is stranded in the cytosol, which is consistent with the hypothesis that cadherins are the only p120 receptor in the membrane (Thoreson et al., 2000; Ohkubo and Ozawa, 1999). Expression of intact E-cadherin, but not of an E-cadherin mutant containing minimal substitutions that uncouple p120 association, results in efficient recruitment of p120 to cell-cell junctions. Moreover, biochemical fractionation of these transfected cell lines in the absence of detergents shows that >90% of the p120 fractionates with membranes in the presence of wild-type E-cadherin (Thoreson et al., 2000). In contrast, p120 fractionates with the cytosol when the same experiment is carried out with p120-uncoupling E-cadherin mutants. Therefore, cadherins are both necessary and sufficient for recruitment of p120 to membranes, and the in vivo stoichiometry of p120 in cadherin complexes is likely to be high and similar to that of β-catenin (Nathke et al., 1994; Papkoff, 1997).

p120 binds to the cadherin juxtamembrane domain

Early work showed that immunoprecipitates of p120 contained β-catenin and vice versa, suggesting that the two proteins bind simultaneously to different regions of the cadherin cytoplasmic tail (Reynolds et al., 1994; Daniel and Reynolds, 1995). Subsequent studies demonstrate that p120 associates with the cadherin juxtamembrane domain (JMD; Finnemann et al., 1997; Lampugnani et al., 1997; Ozawa and Kemler, 1998; Yap et al., 1998; Aono et al., 1999; Ohkubo and Ozawa, 1999; Thoreson et al., 2000), whereas β-catenin and plakoglobin bind to the so-called catenin-binding domain (CBD; Nagafuchi and Takeichi, 1989; Ozawa et al., 1990; Stappert and Kemler, 1994; see Fig. 5). Yeast two-hybrid data and mutational analyses point to a stretch of ~10 residues that makes up the core of the JMD and constitutes the critical p120-binding sequence (Thoreson et al., 2000; Ohkubo and Ozawa, 1999). An engineered triple-alanine substitution, termed 764 EED/AAA, in this region of E-cadherin effectively uncouples the cadherin-p120 interaction in mammalian cells (Thoreson et al., 2000). The core p120-binding domain is the most highly conserved region among classical cadherins, which is consistent with the concept that p120 plays a fundamental role in regulation of these proteins.

Potential roles of the cadherin juxtamembrane domain

Increasing evidence indicates that the JMD and the CBD of cadherins contribute different functions. Early deletion studies indicate that the CBD and its association with β-catenin, α-catenin and the actin cytoskeleton, are required for cadherin-mediated adhesion (Nagafuchi and Takeichi, 1989; Ozawa et al., 1990; Fujimori and Takeichi, 1993; Barth et al., 1997; Kawanishi et al., 1995; Watabe et al., 1994). Subsequently, Kintner (1992) demonstrated that overexpression of a membrane-tethered N-cadherin JMD construct inhibits ectodermal cell-cell adhesion in *Xenopus* embryos. Dominant negative constructs lacking either the JMD or the CBD independently blocked adhesion, which suggests that these domains have separate but indispensable roles. Interestingly, in the JMD construct used by Kintner in these studies, a 'proximal' JMD sequence was deleted; the recently identified 'core' p120-binding region was left intact (Thoreson et al., 2000). Thus, the observed effects may not be due to the failure of the JMD deletion to selectively eliminate binding to p120. Interestingly, a peptide mimicking the 'proximal' JMD region (and lacking the core JMD p120-binding site) reportedly binds to endogenous N-cadherin and apparently displaces the tyrosine kinase Fer from the N-cadherin complex (Balsamo et al., 1999; Lilien et al., 1999). These data suggest that the JMD contains two binding motifs, a 'distal' one that binds p120 and a 'proximal' one that may be involved in cadherin dimerization and/or binding to Fer. Although the full spectrum of molecular interactions supported by these domains and their function in adhesion remain to be elucidated, the reported interaction between Fer and p120 (Kim and Wong, 1995; Rosato et al., 1998) suggests that these proteins may act in concert to modulate adhesion. For clarity subsequent references to the JMD in this review will refer to the p120-binding region.

In other work, the JMD has been implicated in the ability of ectopically expressed cadherin to suppress invasion and motility. Chen et al. (1997) have shown that introduction of a

JMD-deleted E-cadherin in WC5 cells (which lack cadherin expression) induces aggregation, but is unable to suppress motility. The authors suggest that the adhesion and motility functions of the cadherin cytoplasmic domain can be functionally uncoupled. As these studies were conducted in cells expressing a temperature-sensitive Src mutant, it is not yet clear whether the data are broadly relevant.

The JMD also appears to be required for neuronal outgrowth mediated by N-cadherin attachment, since overexpression of dominant negative constructs containing the JMD, but not the CMD, were inhibitory (Riehl et al., 1996). N-cadherin homophilic binding induces neurite extension in a manner dependent on intracellular signaling by the FGF receptor (Saffell et al., 1997), which is thought to bind to the N-cadherin extracellular domain. One possible interpretation is that N-cadherin binding between adjacent cells promotes the activation of FGF receptor tyrosine kinase signaling by inducing clustering of the receptors at areas of cell-cell contact. Further work in these systems to clarify the role of p120 is warranted.

Two recent reports appear to contradict the idea that the JMD is involved in cell-cell adhesion: using dominant negative JMD constructs, Zhu and Watt (1996), and Nieman et al. (1999) did not detect an inhibition of adhesion measured by aggregation assays. A potentially unifying hypothesis is that the JMD modulates the transition from weak to strong adhesion – these two processes relate to the ability of cadherin-dependent cell-cell aggregates to withstand distracting forces (e.g. shear forces, such as pipetting through a narrow aperture). Indeed, if overexpression of membrane-tethered JMD constructs block strong, but not weak adhesion, the aggregation assays used in the later studies would not be predicted to show an inhibitory effect, because they do not discriminate between these adhesive states.

Early studies suggested that dominant negative cadherins that contain both the JMD and the CBD but lack the extracellular domain block adhesion by sequestering catenins. However, several reports indicate that the dominant negative effect of the CBD is not due to sequestration of classical catenins (i.e. α-catenin, β-catenin and plakoglobin; Fujimori and Takeichi, 1993; Zhu and Watt, 1996; Nieman et al., 1999; Troxell et al., 1999) but rather to downregulation of endogenous cadherin expression (Zhu and Watt, 1996; Nieman et al., 1999; Troxell et al., 1999). Nonetheless, dominant negative effects of cadherin cytoplasmic domains containing both the JMD and the CBD cannot always be ascribed to downregulation of endogenous cadherin expression (Kintner, 1992; Fujimori and Takeichi, 1993; Troxell et al., 1999). In some cases, the dominant negative effect of these constructs might be mediated by the JMD and its ability to competitively uncouple p120 from endogenous cadherins. β-Catenin and α-catenin are stabilized by cadherin binding; thus, their levels in most normal cells are strongly dependent on binding to cadherins. Dominant negative cytoplasmic cadherin constructs may simply stabilize increased levels of these proteins. By contrast, the amount of p120 is not significantly changed by the presence or absence of ectopic E-cadherin (Thoreson et al., 2000; Papkoff, 1997). Therefore, dominant negative constructs may inhibit adhesion largely through sequestration of the limited amount of available p120.

Interestingly, membrane-tethered cadherin cytoplasmic domains expressed at levels that do not affect endogenous cadherin expression localize at cell-cell junctions and inhibit adhesion in a manner consistent with inhibition of endogenous cadherin clustering (Fujimori and Takeichi, 1993; Troxell et al., 1999). It is not yet clear whether the adhesive defect is due to direct inhibition of cadherin dimerization or clustering or, alternatively, to the inhibition of cadherin function through dominant negative interactions with p120. Nonetheless, the data suggest that the cadherin cytosolic domain (Fujimori and Takeichi, 1993; Amagai et al., 1995; Katz et al., 1998), and specifically the JMD (Navarro et al., 1998; see below), is responsible for the selective recruitment of membrane-associated cadherins into areas of cell-cell contact.

CONTROVERSIES REGARDING THE ROLE OF p120 IN ADHESION

Experiments focused specifically on the role of p120 in mediating JMD function have led to two results that appear initially to be diametrically opposed. The first strongly implicates the JMD and p120 in cadherin clustering and the induction of strong adhesion independently of the CBD, whereas the second implicates p120 in negative regulation of cell-cell adhesion.

'Activation' of p120 and positive effects on cell-cell adhesion

Stable expression in cadherin-deficient CHO cells of a C-cadherin construct lacking the CBD induced strong adhesion, despite the lack of any detectable association of the protein with the actin cytoskeleton (Yap et al., 1998). This strong adhesion was dependent on the clustering of cadherins mediated by contact with immobilized extracellular cadherin fragments. A construct lacking the JMD did not induce clustering, despite evidence that the mutated cadherin could associate with the cytoskeleton. Interestingly, the clustering potential of the JMD appeared to be activated by the homophilic interaction between cadherin extracellular domains (formation of adhesive or *trans* dimers) and was not observed in the absence of cadherin *trans* association (Yap et al., 1998). Thus, cadherin clustering in vivo might be secondary to the formation of adhesive cadherin dimers, and requires the JMD but not the CBD (or the interaction with the actin cytoskeleton that the CBD mediates). Coupled with the demonstration that p120 associates with the JMD construct that was used in these experiments, these data strongly implicate p120 in ligand-dependent activation of cadherin clustering. An active p120-mediated clustering process is also consistent with laser-trapping experiments measuring the kinetics of cadherin lateral diffusion. The kinetic data argue that cadherin clustering is an active process occurring too rapidly to be dependent on passive diffusion alone (Kusumi et al., 1999).

Overexpression in CHO cells of a VE-cadherin construct lacking the CBD also induces aggregation independently of cytoskeletal association (Navarro et al., 1995). This CBD-negative protein localizes appropriately to cell-cell junctions and recruits p120 (Navarro et al., 1995; Lampugnani et al., 1997). In related experiments, Navarro et al. (1998) show that VE-cadherin selectively excludes N-cadherin from cell-cell junctions when co-expressed in cadherin-deficient CHO cells,

and provide evidence that VE-cadherin binds to p120 with greater affinity than does N-cadherin. Interestingly, VE-cadherin constructs lacking the JMD (and therefore unable to bind to p120) cannot exclude N-cadherin (Navarro et al., 1998), which suggests that p120 plays a role in this phenomenon. An implication is that high-affinity binding of p120 to VE-cadherin, and/or low-affinity binding of p120 to N-cadherin, results in differential recruitment of these cadherins to the adherens junction. High-affinity interactions with p120 may favor selective clustering of VE-cadherin at cell-cell contacts, and affinity modulation might be further regulated by post-translational modification. The data generated in this model system reflect the natural scenario in endothelial cells, in which both cadherins are present, and VE- but not N-cadherin is found at the adherens junction.

In two separate cadherin-deficient cell model systems, p120-uncoupled E-cadherin mutants failed to induce the strong adhesion observed in cells transfected with wild-type E-cadherin (Thoreson et al., 2000), which is consistent with the observations reviewed above. Minimal triple-alanine mutants (e.g. E-cad 764 EED/AAA, see Fig. 5) within the p120-binding region were used to reduce the chance that the observed effects are due to conformational artifacts or disruption of other cadherin-protein interactions, such as the above-noted potential association between N-cadherin and Fer. In aggregation assays, the p120-uncoupled cadherins failed to induce compaction even after overnight incubation, and aggregates of cells expressing p120-uncoupled cadherins readily dissociated to single cells upon pipetting, unlike their wild-type-cadherin-expressing counterparts. Correlating with the loss of compaction, colonies of cells expressing the mutant cadherins exhibited a disruption of the cortical actin cytoskeleton. This suggests that p120-induced events (presumably clustering) are prerequisite for the organization of the actin cytoskeleton in epithelial cells.

The above evidence suggests that the clustering of cadherins at cell-cell junctions requires the JMD and, by implication, p120. On the basis of these studies, we have defined the apparent activity of p120 that is associated with clustering (and the strengthening of adhesion) as ‘activation’ of p120.

Inactivation of p120 and negative effects on cell-cell adhesion

A second group of studies appears to contradict the above results and suggests that p120 plays a crucial role in the disassembly of adherens junctions. In Colo205 cells, the known components of the E-cadherin complex are essentially intact, but the cells remain rounded and largely non-adherent in cell cultures (Aono et al., 1999). Interestingly, p120 is constitutively hyperphosphorylated in these cells. Brief trypsinization under conditions that protect cadherins, or treatment with the serine/threonine kinase inhibitor staurosporine, correlates with decreased p120 phosphorylation and restored adhesiveness (Aono et al., 1999). Moreover, expression of an N-terminally deleted p120 construct that lacks most of the phosphorylation sites also restores adhesion. One interpretation is that an aberrant constitutive signaling event in Colo205 cells leads to inactivation of p120’s ability to promote clustering and adhesion. Although Colo205 cells are clearly exceptional, experiments in K-562 leukemia cells and murine L-fibroblasts also suggest that p120 can block adhesion (Ozawa and Kemler, 1998; Ohkubo and Ozawa, 1999). In K-562 cells, expression of membrane-tethered E-cadherin extracellular domains promotes aggregation, whereas similar constructs containing the JMD, but lacking the CBD, block aggregation. Moreover, minimal mutation of the JMD in the latter construct restores adhesiveness (Ozawa and Kemler, 1998). Inhibition of adhesion again correlated with hyperphosphorylation of p120, and N-terminally deleted p120 restored adhesiveness (Ohkubo and Ozawa, 1999). These results implicate p120 in inhibition of cell-cell adhesion.

Mechanisms and models

The above data indicate that the JMD and p120 can mediate both positive and negative regulation of adhesion. One obvious problem is how to integrate the seemingly inconsistent results in one all-encompassing model. The most likely explanation is that *trans* binding between cadherins is somehow linked to the positive effect of the JMD (p120 activation; Yap et al., 1998; Navarro et al., 1998; Thoreson et al., 2000), whereas intracellular signaling can induce its negative function (Aono et al., 1999; Ohkubo and Ozawa, 1999). Rapid activation of adhesion in Colo205 cells by brief trypsinization might, for example, result from clipping of a receptor and subsequent loss of an aberrant constitutive signaling event acting ultimately on p120. Thus, p120 could act as a switch that induces cadherin clustering and strong adhesion when activated, and also mediate junction disassembly following signaling events leading to its inactivation. Although there is evidence that serine or tyrosine phosphorylation constitutes the switch between these forms (Aono et al., 1999; Ohkubo and Ozawa, 1999), this has not yet been proven, and the terms activation and inactivation cannot be assumed to reflect changes in phosphorylation status.

Fig. 6 illustrates a p120-centric view of cadherin-mediated adhesion that summarizes our interpretation of these data and possible mechanisms of p120 action. Cadherins are thought to exist both as monomers and as lateral (or *cis*) dimers in the plasma membrane. On the basis of the N-cadherin-repeat crystal structure and other data (Shapiro et al., 1995; Nagar et al., 1996; Pertz et al., 1999; Tamura et al., 1998), it has been proposed that the Ca^{2+}-dependent homophillic association of adhesive *trans* dimers (connecting adjacent cells) occurs after the association of lateral dimers. Strengthening of adhesion is correlated with both cadherin clustering and association with the actin cytoskeleton – experimentally separable events related to JMD and CBD functions, respectively. As mentioned earlier, the formation of adhesive dimers might activate the clustering potential of p120 and the JMD. The mechanism of this activation is not known, but it could involve simple conformational changes affecting p120 binding or an adhesion-sensitive signaling cascade (phosphorylation?) ultimately impacting on p120 function. Under normal conditions, p120 activation induces clustering and results in significant enhancement of adhesive strength. When p120 is constitutively inactivated, adhesive dimers cannot cluster and revert back to form lateral dimers, which are not adhesive. In this view, the CBD mediates subsequent or possibly concomitant immobilization of cadherin complexes by actin crosslinking. The latter might not be necessary for strong adhesion, per se, but is probably a key factor in compaction – a term used to

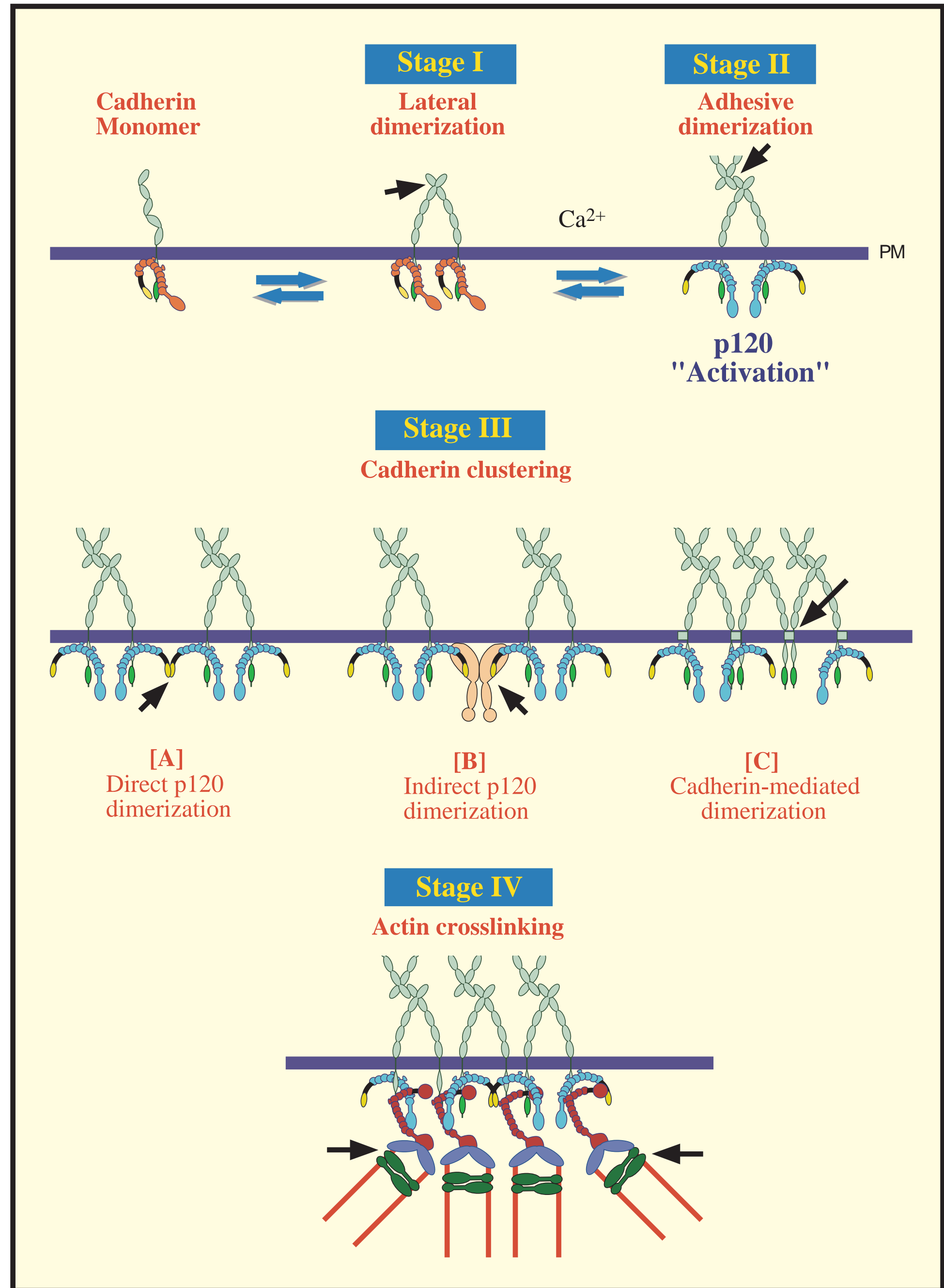

Fig. 6. A p120-centric model for cadherin-mediated adhesion. Both monomers and lateral (cis) dimers are thought to exist at the plasma membrane. Formation of lateral dimers (stage I) is believed to temporally precede the formation of adhesive (trans) dimers (stage II) between cadherins on adjacent cells. Adhesive dimers cluster (stage III) at areas of cell-cell contact, which results in the establishment of strong adhesion. Although diffusion alone might bring trans-dimers on adjacent cells into contact, several lines of evidence suggest that the cytoplasmic domain is required for proper targeting of these complexes to cell-cell junctions. Evidence suggests that cadherin clustering requires ligand binding (trans-adhesive dimerization), an intact JMD (presumably to coordinate p120 binding) and RhoA, but not the CBD and/or α-catenin-mediated interaction with the actin cytoskeleton (see text). In all of the models, formation of an adhesive dimer leads somehow to 'activation' of p120, which facilitates subsequent cadherin clustering. The switch that regulates the activated (blue) and inactivated (orange) states is not yet clear. Possible mechanisms include changes in p120 phosphorylation, conformation or binding-partner interactions. The interaction between cadherins and other catenins through the CBD (depicted in dark green) is omitted here for simplicity. Three possible models are proposed for p120-induced clustering. In the first (A; direct p120 dimerization), direct homodimerization of 120 is responsible for the clustering of adhesive dimers. Heterodimerization of p120 with other p120 family members is a related possibility. In the second model (B; indirect p120 dimerization), clustering is also mediated by a dimerization mechanism, but unknown crosslinking proteins are postulated. Finally, the third model (C; cadherin-mediated dimerization) predicts that binding of activated p120 to cadherin adhesive dimers induces conformational changes in the cadherin itself, which subsequently promotes clustering. This model is supported by evidence that the extracellular and/or transmembrane domains can in fact dimerize on their own but are regulated somehow by intracellular interactions. Finally, the last stage of cadherin-mediated adhesion, actin crosslinking (stage IV), is probably coordinated with clustering but further requires the CBD, Rac1 and Cdc42, and direct association with the actin cytoskeleton. The distinction between clustering (stage III) and actin crosslinking (stage IV), both of which result in so-called strong adhesion in aggregation assays, may be relevant for distinguishing the specific role of p120 in the complex.

reflect an even-tighter consolidation of cellular aggregates (Adams et al., 1996, 1998).

Fig. 6 also shows three potential mechanisms by which p120 could affect adhesion. In the simplest scenario, activated p120 mediates the physical interaction between cadherins by direct homodimerization, or heterodimerization involving other p120 family members (Fig. 6A). Although this scenario is intuitively attractive, p120 does not interact with itself in yeast two-hybrid assays (Daniel and Reynolds, 1995) or in direct GST-pulldown assays (Yap et al., 1998). In the second scenario, p120 mediates cadherin clustering by interacting with unidentified crosslinking proteins (Fig. 6B). For example, in neuronal synapses the p120 relative δ-catenin/NPRAP interacts directly with S-SCAM (Ide et al., 1999), a scaffold protein implicated

in the clustering of membrane receptors. Crosslinking to scaffold proteins might therefore facilitate the clustering process. This scenario is necessarily vague because it invokes additional p120-binding partners that have not yet been identified. In the third scenario, association of adhesive dimers with activated p120 leads to conformational changes in the cadherins themselves, which in turn facilitate cadherin-clustering mechanisms involving the extracellular, transmembrane, or proximal juxtamembrane domains (Fig. 6C). Inside-out signaling analogous to that which occurs with integrins (for review see Kolanus and Zeitlmann, 1998; Faull and Ginsberg, 1996; Tozer et al., 1996) may be involved. The ability of the extracellular domains to support clustering has been proposed on the basis of structural data (Shapiro et al., 1995; Nagar et al., 1996) but is not supported by other evidence (Fujimori and Takeichi, 1993; Katz et al., 1998; Troyanovsky, 1999). However, Huber et al. (1999) recently showed that the cadherin transmembrane domain can homodimerize, and Balsamo et al. (1999) reported that peptides mimicking the proximal JMD bind cadherins. All of these models predict increased adhesion due to p120 activation and are not necessarily mutually exclusive.

Rho family GTPases and cadherin-mediated adhesion

An important consideration in all of these models is the incompletely understood role of the Rho family of GTPases in cadherin function (Braga et al., 1997; Takaishi et al., 1997; Braga et al., 1999; Jou and Nelson, 1998). RhoA transiently localizes to cell-cell junctions upon induction of calcium-dependent adhesion (Takaishi et al., 1995; Kotani et al., 1997), which raises the possibility that it is recruited to junctions and activated following the formation of adhesive dimers. In addition, several studies indicate that Rac1 and Cdc42 activities mediate the crosslinking of cadherins with the actin cytoskeleton (Braga et al., 1997; Jou and Nelson, 1998; Takaishi et al., 1997; Kodama et al., 1999). RhoA is thought to act at an earlier step, because inhibition of RhoA by C3 exotransferase expression blocks both cadherin localization at areas of cell-cell contact (Braga et al., 1997; Zhong et al., 1997; Takaishi et al., 1997) and Rac1-mediated actin recruitment (Braga et al., 1997). Furthermore, forced clustering of cadherins on antibody-coated beads abolishes the ability of C3 to inhibit Rac1-mediated actin recruitment (Braga et al., 1997), which suggests that RhoA is required for cadherin clustering. Together, these data suggest potential links between RhoA, p120 and the JMD in cadherin clustering.

p120 PHOSPHORYLATION

p120 is a superb Src substrate both in vivo and in vitro (reviewed by Daniel and Reynolds, 1997). Although constitutive tyrosine phosphorylation of p120 in cells expressing activated Src correlates with cell transformation (Reynolds et al., 1989), we cannot yet distinguish the direct effects of p120 phosphorylation from the many other events associated with Src activation. Multiple lines of evidence suggest that tyrosine phosphorylated p120 binds with increased affinity to various cadherins (Kinch et al., 1995; Skoudy et al., 1996b; Papkoff, 1997; Calautti et al., 1998). There is also evidence that transient tyrosine phosphorylation of p120 occurs in nascent cell-cell junctions (Calautti et al., 1998; Lampugnani et al., 1997; Kinch et al., 1997), although, under steady-state conditions, tyrosine phosphorylation of p120 is generally not detected. p120 is also tyrosine phosphorylated in response to EGF, PDGF, CSF-1, VEGF and NGF (Downing and Reynolds, 1991; Shibamoto et al., 1995; Daniel and Reynolds, 1997; Esser et al., 1998), but it is not clear whether the implicated receptors phosphorylate p120 directly or through the recruitment of associated tyrosine kinases, such as Src. Nonetheless, the numerous reports linking cadherin complexes with Src, receptor tyrosine kinases, and a variety of tyrosine phosphatases (reviewed by Daniel and Reynolds, 1997), argue strongly for an important role for tyrosine phosphorylation in the regulation of cadherin function. Discriminating the direct effects of tyrosine phosphorylation of p120 and β-catenin on cadherin function will probably require the mapping and subsequent mutation of the relevant sites.

In most cells, p120 is extensively phosphorylated on serine residues (and to a lesser extent on threonine residues; Downing and Reynolds, 1991; Ratcliffe et al., 1997, 1999; Aono et al., 1999; Ohkubo and Ozawa, 1999). In fact, even the receptor tyrosine kinase ligand EGF induces extensive serine phosphorylation of p120 (Downing and Reynolds, 1991). p120 isoforms generally migrate diffusely on polyacrylamide gels, mostly because of serine phosphorylation, and phosphatase treatment collapses the bands into sharply migrating species (Staddon et al., 1995; Aono et al., 1999; Ohkubo and Ozawa, 1999; Thoreson et al., 2000). Early work demonstrated that only membrane-associated Src can phosphorylate p120 (Reynolds et al., 1989). The observation that tyrosine phosphorylation and serine/threonine phosphorylation of p120 require recruitment of p120 to cell membranes through association with cadherins is consistent with this (Reynolds et al., 1994; Ohkubo and Ozawa, 1999; Thoreson et al., 2000). Conversely, when p120 is stranded in the cytoplasm (e.g. in metastatic cell lines that have lost cadherin expression), it is virtually unphosphorylated (Ohkubo and Ozawa, 1999; Thoreson et al., 2000) and probably unavailable to receive membrane-associated signals. The level of serine phosphorylation of p120 can be reduced by the kinase inhibitor staurosporine (Ratcliffe et al., 1997, 1999; Aono et al., 1999), and by either inhibition or activation of protein kinase C, which implicates a PKC-modulated protein kinase (Ratcliffe et al., 1997, 1999). As mentioned earlier, two important studies now implicate serine dephosphorylation as a mechanism for the activation of p120's adhesive function (Aono et al., 1999; Ohkubo and Ozawa, 1999). However, given that concrete evidence of the relevant phosphorylation events is lacking, other events mediated by the N-terminal domain of p120 might also account for these effects. The mapping of specific serine phosphorylation sites and identification of respective kinases will be crucial for an understanding of how these events modulate p120 function.

NUCLEAR SIGNALING

p120 was recently added to the list of Arm-repeat proteins that have dual localization at cell-cell junctions and in the nucleus (Van Hengel et al., 1999; Mariner et al., 2000). Perhaps the

best-known example is β-catenin, which plays a key role in both adhesion and signaling (reviewed by Ben-Ze'ev and Geiger, 1998, and references therein). A novel transcription factor, Kaiso, binds directly to the p120 Arm domain, providing the first candidate for a nuclear target of a p120 family member (Daniel and Reynolds, 1999). Kaiso belongs to a growing POZ/ZF (Pox virus and zinc finger) superfamily of transcription factors (reviewed by Bardwell and Treisman, 1994; Albagli et al., 1995), members of which are characterized by a conserved N-terminal hydrophobic domain of ~120 residues (the POZ domain) and C-terminal C_2H_2-type zinc-finger motifs (the ZF domain). Many POZ/ZF proteins are sequence-specific transcriptional repressors that apparently recruit histone-deacetylase complexes via their POZ domains (Grignani et al., 1998; Lin et al., 1998; David et al., 1998). Although the functional significance of the Kaiso-p120 interaction is currently speculative, the known activities of the other POZ/ZF proteins in development and cancer make Kaiso an attractive signaling candidate for a conveyor of information related to cell-cell adhesion. Van Hengel et al. (1999) have demonstrated that p120 enters the nucleus. Moreover, a nuclear-exclusion signal that appears to mediate p120 export is present in alternatively spliced forms of p120 containing exon B. The simplest hypothesis, therefore, is that p120 interacts with Kaiso in the nucleus, thereby modifying its predicted transcriptional activity. Further analysis of this interaction awaits the characterization of Kaiso transcriptional targets, which will also allow elucidation of the roles of p120 and cadherin in Kaiso-mediated effects. It is likely that other members of the p120 subfamily also have nuclear partners. An interesting observation is that the nuclear localization of p120 is enhanced in cadherin-deficient cells (Van Hengel et al., 1999). Thus, aberrant regulation of Kaiso by p120 might contribute in previously unsuspected ways to the pleiotropic effects of cadherin loss in metastatic cells.

ECTOPIC p120 OVEREXPRESSION

Overexpression studies offer an obvious avenue for testing the biological functions of novel proteins. p120-overexpression studies have been hindered because of unexplained problems in generating stable cell lines. Interestingly, transient overexpression of p120 in fibroblasts induces severe branching of cellular processes, resulting in a so-called dendritic phenotype (Reynolds et al., 1996). Morphological changes, albeit less dramatic, are also induced in other cell types. Overexpression of other Arm-domain proteins, including the closest relative of p120, ARVCF (Mariner et al., 2000), has little effect in fibroblasts. Moreover, deletions in the Arm-repeat domain completely block the effect (Reynolds et al., 1996). A similar phenotype is observed under a variety of conditions that disrupt RhoA-mediated contractility (Jalink et al., 1994; Kranenburg et al., 1999), further suggesting a functional link between p120 and Rho signaling.

Ventral overexpression of p120-1A or 1N in *Xenopus* embryos does not induce Wnt signaling or the duplicate axis formation associated with ventral overexpression of β-catenin. In contrast, overexpression of p120 in dorsal blastomeres perturbs gastrulation (Geis et al., 1998; Paulson et al., 1999), apparently because of reduced cell motility, reduced adhesion between blastomeres, and ectodermal defects. These data suggest that p120 affects morphogenetic events, which is consistent with a role for p120 in cell-cell adhesion.

p120 EXPRESSION IN TUMORS

Although knockout data are not yet available, analysis of p120 expression in human tumors suggests that p120 plays an important role in malignancy. Loss of p120 expression has been demonstrated in a variety of human tumors and in some cases is statistically linked to an aggressive tumor phenotype (Dillon et al., 1998; Gold et al., 1998; Shimazui et al., 1996; Skoudy et al., 1996a; Syrigos et al., 1998; Valizadeh et al., 1997). By contrast, Jewhari et al. (1999) report striking upregulation of p120 expression in ~66% of gastric carcinomas. In addition, expression of p120 isoforms is remarkably heterogeneous in human tumor cell lines (Wu et al., 1998; Skoudy et al., 1996a; Keirsebilck et al., 1998), but the significance of this observation is unknown. One possibility is that misexpressed p120 isoforms promote tumor progression by dysregulating cadherin-mediated adhesion, or promoting cell motility and invasion. Finally, the correlation of constitutive p120 phosphorylation with defects in Colo205 cell adhesion (Aono et al., 1999) suggests that signaling pathways contribute to malignancy through direct modification of p120 function.

FUNCTIONAL ROLES OF p120 FAMILY MEMBERS

Competition among p120 relatives for cadherin binding

A largely unexplored issue involves the potential functional crosstalk between p120 and other closely related family members. The existence of p120 relatives suggests that they have functional similarities and differences that play out in different cell types. For example, p120 family members may compete for cadherin binding. δ-catenin/NPRAP, like p120, interacts directly with the JMD (Lu et al., 1999), and the binding of ARVCF and p120 to E-cadherin is apparently mutually exclusive (Mariner et al., 2000). One clue comes from the selective expression of δ-Catenin/NPRAP/neurojungin in neuronal tissues, which suggests that it has cell-type specific functions. In addition, although ubiquitously expressed, ARVCF appears to be present in most adult tissues at extremely low levels (Mariner et al., 2000). Thus, it is unlikely to act as a direct p120 competitor in vivo. Thus far, p120 appears to be the most abundant member of the family in most cell types, which suggests that it is the dominant player in cadherin function. However, p0071 is also believed to be abundant and is a potential, although largely unstudied, rival for this role (Hatzfeld and Nachtsheim, 1996). An important area of future research is to determine whether these family members bind to the same or different cadherins and whether (and if so how) competition for the JMD imparts functional diversity on cadherin-mediated adhesion.

Specific functional roles of individual p120 relatives

Table 1 summarizes the expression patterns, cellular localizations, binding partners and chromosomal locations of all known p120 family members. Specific functions have been

Table 1. p120 family expression and localization

	Expression	Subcellular localization	Binding partners	Chromosomal localization
p120 ctn	Ubiquitous, high level expression. Not in B/T cells	Adherens junctions, nucleus	Classical cadherins, Kaiso, BP180*, FER kinase‡	11q11‖
ARVCF	Ubiquitous, low level expression	Adherens junctions, nucleus	Classical cadherins	22q11 (region deleted in Velo-cardio-facial syndrome)
δ-Catenin/NPRAP	Neuroepithelium, neuroendocrine	Adherens junctions, nucleus tissues	Classical cadherins, presinilin 1, S-SCAM	5p15 (region involved in Cri-du-chat Syndrome
P0071	Ubiquitous expression	Desmosomes and adherens junctions§	Presinilin 1¶	2q23-q31
PKP 1	Epithelial tissues, especially stratified epithelia	Desmosomes, nucleus	Desmoplakin, cytokeratins, Dsg 1	1q32 (linked to ectodermal dysplasia/skin fragility syndrome)
PKP 2	Ubiquitous expression	Nucleus, desmosomes	Desmoplakin, cytokeratins	12p11, 12p13 (pseudogene)
PKP 3	Stratified and single layered epithelia. Not hepatocytes, foreskin fibroblasts, sarcoma-derived cells	Desmosomes, nucleus	Not identified	11p15

*p120^{ctn} reportedly interacts with the hemidesmosomal protein BP180 (Aho et al., 1999) but the significance of this interaction is unknown.
‡p120^{ctn} interacts with FER kinase either directly or indirectly (Kim and Wong, 1995).
§It is not known whether p0071 is also found in the nucleus.
¶Direct association of p0071 with components of the desmosomes or adherens junctions has not been reported to date.
‖For chromosomal localization of p120 family members see Bonne et al. (1998) and references therein.

suggested for particular p120 relatives and plakophilins. For example, it is possible that p0071 has dual roles in both adherens junctions and desmosomes (Hatzfeld and Nachtsheim, 1996). Furthermore, p0071 (Stahl et al., 1999; Tanahashi and Tabira, 1999) and δ-catenin/NPRAP (Levesque et al., 1999; Zhou et al., 1997; Tanahashi and Tabira, 1999) interact with presenilin 1, a protein that when mutated leads to familial Alzheimer's disease (Nishimura et al., 1999); this raises the possibility that p120 family members are involved in neurodegeneration. In addition, Sirotkin et al. (1997) have linked the gene that encodes ARVCF to Velo-Cardio-Facial syndrome, whereas δ-catenin/NPRAP maps to a region involved in Cri-du-chat syndrome (cat cry; Bonne et al., 1998). Although the involvement of ARVCF or δ-catenin/NPRAP in these syndromes has not been confirmed, it is interesting that both syndromes exhibit developmental facial and heart defects.

As mentioned earlier, p120 induces a striking branching phenotype when overexpressed in fibroblasts. δ-Catenin/NPRAP induces morphological changes in MDCK cells (increased spreading, and formation of lamelipodia and filopodia), negatively regulates cadherin adhesiveness and increases motility in response to HGF (Lu et al., 1999). Although these phenotypic changes are similar to those induced by p120 in MDCK cells (unpublished observation), δ-catenin/NPRAP does not induce branching in fibroblasts (Q. Lu and K. Kosik, personal communication). Interestingly, overexpression of the plakophilin 1 Arm domain but not overexpression of full-length plakophilin 1 induces a similar phenotype in epithelial cells (M. Hatzfeld, personal communication), whereas overexpression of ARVCF in fibroblasts or epithelial cells does not alter cell morphology (Mariner et al., 2000). The significance of the observation that p120 is particularly difficult to express stably in cells is unclear: stable expression of both ARVCF (Mariner et al., 2000) and δ-catenin/NPRAP (Lu et al., 1999) has been accomplished in a variety of cell lines. Clearly, there are important differences between these proteins, and each is likely to contribute unique functions at cell junctions and in the nucleus.

CONCLUSIONS

It is now clear that p120 is a major cadherin-binding partner and the likely mediator of JMD function. Further elucidation of the mechanism of p120 action in cadherin-mediated adhesion will undoubtedly clarify our understanding of processes involved in cadherin-dependent tissue morphogenesis and related issues in metastatic cells. It is likely that p120 has both positive and negative roles in cadherin adhesion, functions that are probably regulated by post-translational modifications. Genetic studies in mice and other organisms are likely to provide further insight on the specific functions of p120 and family members in cell-cell adhesion and morphogenesis. Detailed mapping of p120 phosphorylation sites and identification of new p120-binding partners and effectors are also important goals for future progress. Another interesting and largely unexplored issue is the significance of the apparently universal dual localization of p120 family members in junctions and nuclei. The relative stability of p120 and its cytoplasmic/nuclear localization in cadherin-deficient cells raises the possibility that aberrant p120/Kaiso signaling contributes to the pleiotropic effects of cadherin loss in metastatic cells. An important possibility is that the interplay between oncogenes, catenins and nuclear signaling is responsible for tumor-cell defects associated with the contact inhibition of cell growth, a hallmark of malignancy that remains unexplained at the molecular level.

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