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Patterning of cell assemblies regulated by adhesion receptors of the cadherin superfamily

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During morphogenesis, cell–cell association patterns are dynamically altered. We are interested in how cell adhesion molecules can regulate the patterning of cellular assemblies. Cadherins, a group of cell–cell adhesion receptors, are crucial for the organized assembly of many cell types, but they also regulate dynamic aspects of cell association. For example, during neural crest emigration from the neural tube, the cadherin subtypes expressed by crest cells are switched from one subtype to another. Artificial perturbation of this switch results in blocking of their escape from the neural tube. Intracellular modulations of cadherin activity also seem to play a role in regulation of cell adhesion. We identified p120^{ctn} as a regulator of cadherin function in carcinoma cells. With such regulators, cells may make a choice as to whether they should maintain stable cell contacts or disrupt their association. Finally, we found another type of cadherin-mediated cell patterning: Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity in *Drosophila* imaginal discs. Thus, the cadherin superfamily receptors control the patterning of cell assemblies through a variety of mechanisms.

Keywords: adhesion molecule; cadherin; Flamingo; neural crest; p120^{ctn}

1. INTRODUCTION

The regulation of intercellular adhesion is thought to be critical for organized cell assemblies. For example, cell adhesion must be regulated to allow the sequential rearrangement of cells that is necessary for a wide variety of morphogenetic events. Among many classes of cell adhesion molecules, cadherins play a central role in cell–cell adhesion (Takeichi 1995), and therefore it is important to know their regulatory mechanisms. We can consider at least two different mechanisms for the regulation of cadherin-mediated adhesion. One is based upon the specific binding properties of the cadherin molecules. Cadherins are a family of homophilic adhesion receptors, and in their interactions, each subtype of the cadherins preferentially binds to the like subtype (Takeichi 1995). This property can cause sorting of heterogeneous cells when they express different cadherins, and thus may play a role in dynamic cell rearrangement. It should also be stressed that, in some situations, even a quantitative difference in cadherin activity between cells can induce their sorting (Steinberg & Takeichi 1993; Godt & Tepass 1998).

The other mechanism is physiological regulation of cadherin activity. Cadherin-mediated adhesion is believed to be controlled by catenins associated with the cytoplasmic domain of cadherins. The carboxy-half region of the cadherin cytoplasmic domain binds to β-catenin, which in turn associates with α-catenin; and then the α-catenin interacts with the actin-based cytoskeleton directly or indirectly (Barth *et al.* 1997). These molecular interactions are essential for this adhesion system to exert its normal activity (Hirano *et al.* 1992; Watabe-Uchida *et al.* 1998; Ozawa & Kemler 1998). Another catenin, p120^{ctn}, which is a member of the Armadillo/β-catenin gene family (Peifer *et al.* 1994) also binds to cadherins, but at the juxtamembrane portion of the cadherin cytoplasmic domain, which is different from the β-catenin-binding region (Yap *et al.* 1998). Phosphorylation of β-catenins has been implicated in the instability of cell–cell adhesion, but this idea lacks compelling evidence (Daniel & Reynolds 1997). Regarding p120^{ctn}, on the other hand, we have provided evidence that this protein has the ability to regulate cadherin-mediated adhesion through a physiological mechanism (Aono *et al.* 1999).

Cadherin–cadherin interactions could generate some signals into cells, and this may also control cell arrangement patterns. A number of signalling molecules are actually associated with cadherin or catenins, including members of the epidermal growth factor receptor family (Jawhari *et al.* 1999) and phosphatases (Burden-Gulley & Brady-Kalnay 1999); and also β-catenin is a key mediator of the Wnt signalling cascade (Barth *et al.* 1997). It is even known that cadherin deficiency causes tumour-like tissue growth (Radice *et al.* 1997) and human cancers (Guilford *et al.* 1998). Thus the cadherin–catenin junctions look like intercellular signalling sites. However, little is known about whether the cadherin system indeed mediates the above-mentioned signals. Recently, we demonstrated that a member of the cadherin superfamily, but not a 'classic'

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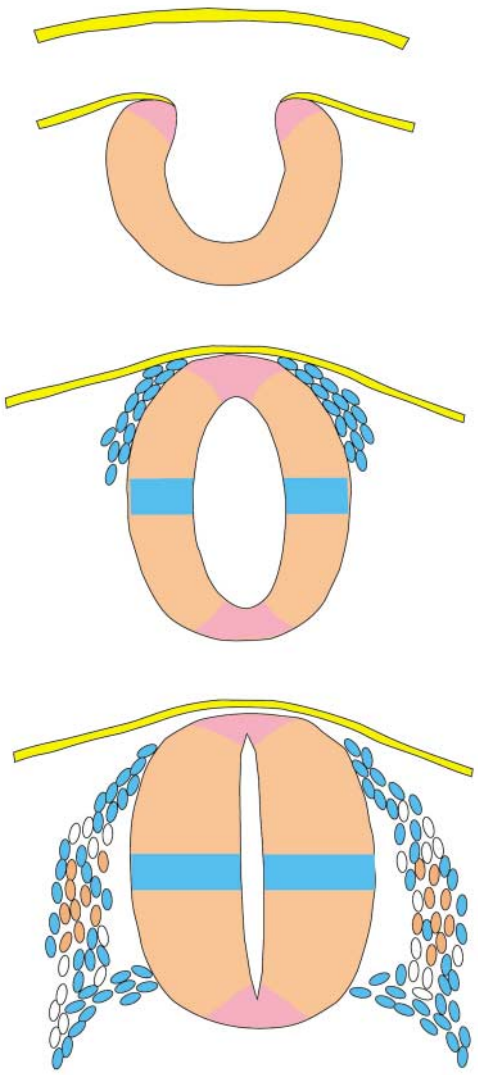

Figure 1. Cadherin expression during neural tube and crest development in the chick embryo. L-CAM is denoted by yellow, cad6B by pink, N-cadherin by orange and cad7 by blue.

cadherin, plays a peculiar signalling role in cell patterning (Usui *et al.* 1999); and this cadherin, Flamingo (Fmi), represents another type of mechanism for cadherin-mediated regulation of cell–cell interactions. In this review we summarize three different biological phenomena, corresponding to each of the above three mechanisms, and discuss the roles of cadherin superfamily members in the patterning of cell assemblies.

2. CADHERIN-TYPE SWITCHING DURING NEURAL CREST EMIGRATION

Neural crest cells escape from the neural tube, which indicates a need for a regulation of cell–cell adhesion. Neural crest precursors are born in the neural fold, and during and after the closure of the neural fold, neural crest cells detach from the future neural tube and undergo epithelial–mesenchymal transformation. Multiple subtypes of cadherin are dynamically expressed during these processes. For example, in the chick embryo (figure 1), the ectoderm expresses liver cell adhesion molecule (L-CAM) (Thiery *et al.* 1984), and during neural plate invagination, the L-CAM expression is replaced by that of N-cadherin

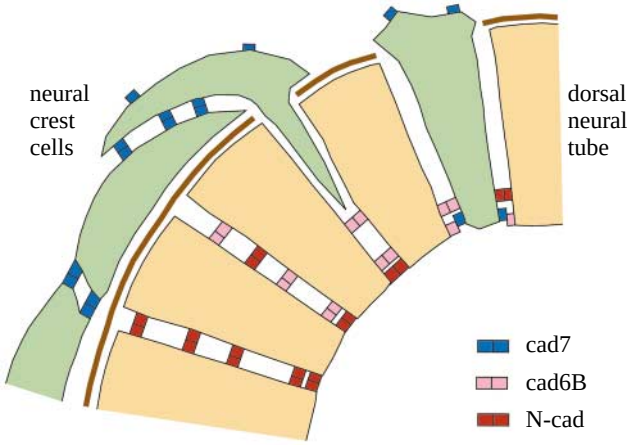

Figure 2. Switching of cadherin expression during neural crest emigration from the neural tube.

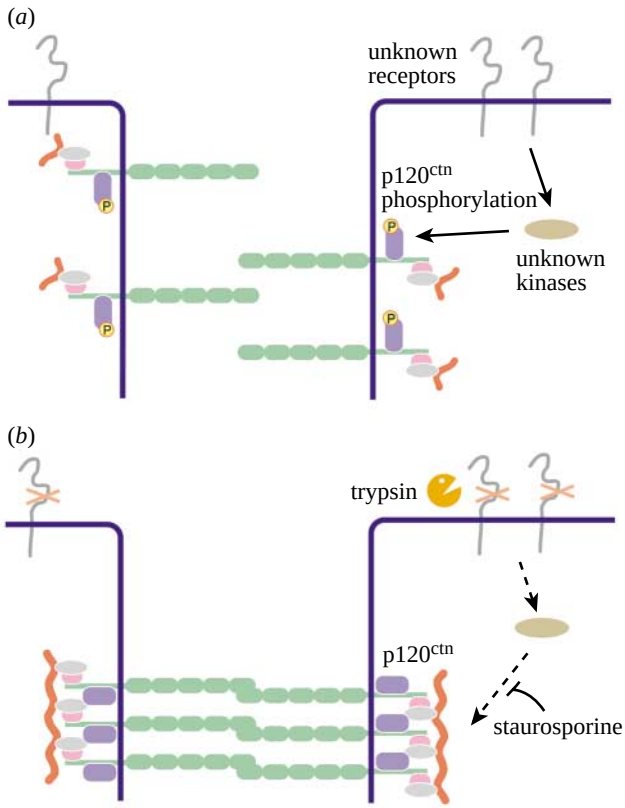

Figure 3. A model for the signalling system to regulate p120^{ctn} function. (*a*) Inhibition and (*b*) activation of the cadherin system.

(Hatta & Takeichi 1986). At the same time, cadherin-6B (cad6B) begins to be expressed in the invaginating neural plate, most strongly at the neural crest-generating area (Nakagawa & Takeichi 1995). In the neural tube that has just closed, N-cadherin and cad6B are co-expressed at the dorsal portion. When neural crest cells emerge from the neural tube, these cadherins become undetectable, and cadherin-7 (cad7) appears instead (Nakagawa & Takeichi 1995). The cad7 expression persists during migration of the crest cells. Based on these observations, we proposed that changes in cadherin expression during neural crest development may play a role in the segregation of neural crest cells from the neural tube.

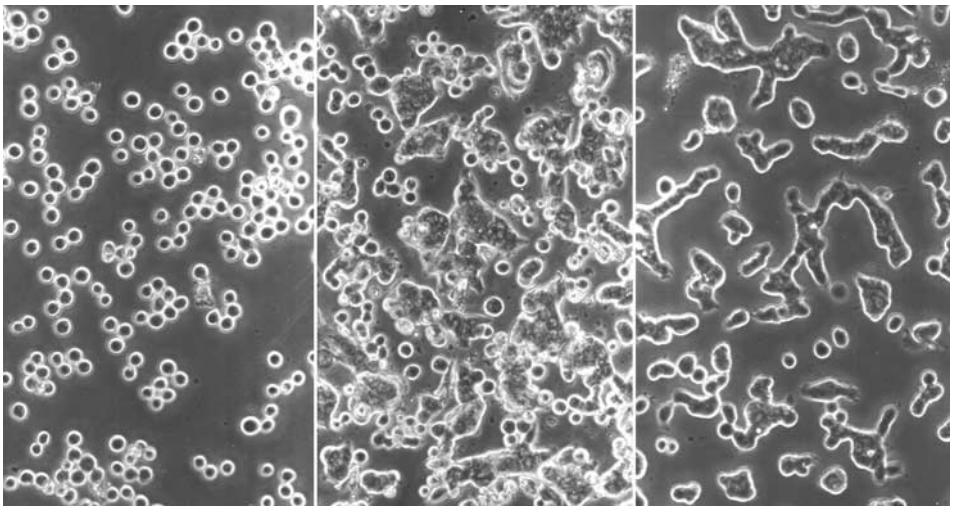

Figure 4. Induction of cadherin-dependent aggregation of Colo 205 cells by (*b*) staurosporine or (*c*) trypsin. (*a*) Indicates the control.

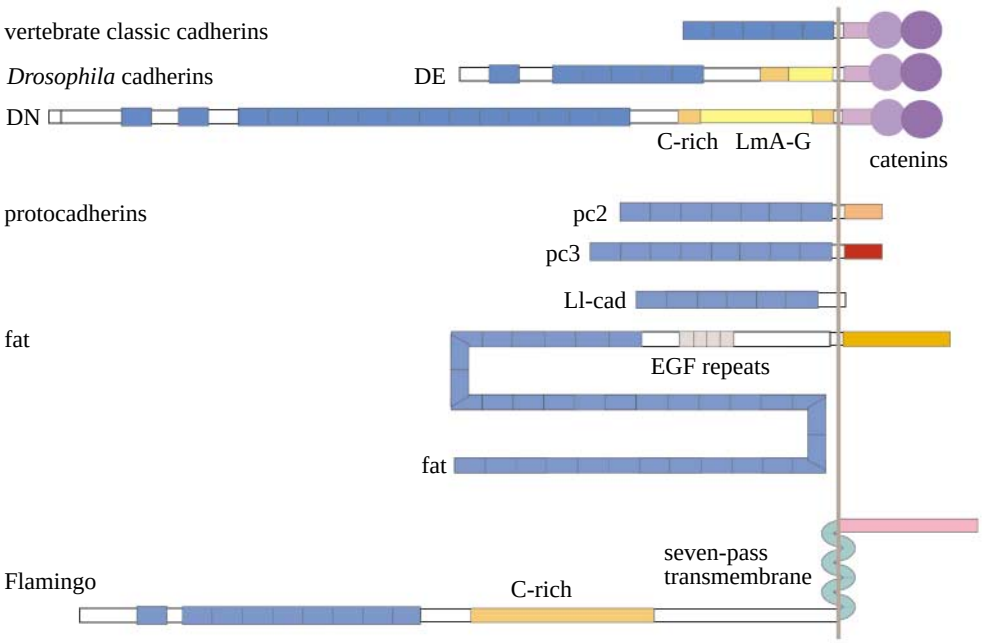

Figure 5. Examples of cadherin superfamily members.

We tested this idea by experiments that perturbed the cadherin expression in the neural crest-generating area. The experiments were designed to express N-cadherin or cad7 constitutively in cells of the dorsal neural tube (Nakagawa & Takeichi 1998). In these embryos, the above cadherin-type switches cannot normally take place; that is, cells cannot turn off the N-cadherin expression upon their escape from the neural tube or they prematurely express cad7. We found that, under these experimental conditions, the escape of crest cells from the neural tube was dramatically suppressed.

The neural tube cells are connected to one another along their lateral surfaces. At the luminal side of the lateral cell–cell contacts, the adherens junction (AJ) develops, which probably represents the major site for the interconnection of neuroepithelial cells. Cadherin adhesion molecules are localized throughout the lateral contact sites between these cells, being highly concentrated in the AJs (figure 2). For the neural crest cell escape, these cell–cell contacts must be disrupted. We can hypothesize that, in the normal situation, the switching of the expression of N-cadherin and cad6B to that of cad7 permits cells to detach from the tube, because of the distinct binding specificities of these molecules. Under the above experimental conditions, however, this switching was perturbed; i.e. a single type of cadherin, either N-cadherin or cad7, was always expressed during the emergence of crest cells from the neural tube. This presumably prevented them from separating. Thus, our findings support the hypothesis that the cadherin-type switching plays a role in neural crest emigration from the neural tube. Similar mechanisms may function for other processes of cell separation.

3. p120^{ctn} REGULATES CADHERIN ACTIVITY

A physiological mechanism for the regulation of cadherin activity has been identified by use of colon carcinoma cells. We happened to find a unique aggregation property of Colo 205 colon carcinoma cells (Aono *et al.*

1999). They grow as dispersed cells, despite the expression of all general components of the E-cadherin–catenin complex. In these cells, however, typical E-cadherin-dependent aggregation can be induced by treatment with staurosporine, a kinase inhibitor, or with trypsin (figure 4). These treatments concomitantly induced an electrophoretic mobility shift of p120^{ctn}, which seems to be due to partial dephosphorylation of this molecule. We assumed that p120^{ctn} plays a role in this cadherin reactivation process, and tested this idea by transfecting Colo 205 cells with a series of p120^{ctn} deletion constructs. Interestingly, when N-terminally deleted p120^{ctn} was expressed, the Colo 205 cells aggregated strongly. This result suggests that the original p120^{ctn} molecule may serve to inhibit the cadherin system; that is, if its N-terminal deletion mutants lost this activity, they could compete with the endogenous p120^{ctn} to abolish its adhesion-blocking action. To corroborate this idea, we introduced a mutant cadherin lacking the p120^{ctn}-binding site into Colo 205 cells, and found that this mutant molecule also induced cell aggregation, indicating that cadherins are able to function normally in these cells, if they do not bind to p120^{ctn}.

Based on the above findings, we proposed that there exists a signalling mechanism to modify a biochemical state of p120^{ctn}, which originates in the cell surface, and that biochemically modified p120^{ctn} molecules block the cadherin system (figure 3). Trypsin probably digests some cell surface proteins involved in this signalling system, leading to an activation or suppression of their downstream cascade. The effect of staurosporine may be involved in an intermediate step of this hypothetical signalling cascade via its inhibitory action on certain kinases. The correlation between the dephosphorylation of p120^{ctn} and adhesion induction suggests that the inhibitory activity of this molecule may be elicited by hyperphosphorylation. How does p120^{ctn} inhibit cadherin function? The juxtamembrane domain of the cadherin cytoplasmic region, where p120^{ctn} binds, is required for clustering of cadherin molecules (Yap *et al.* 1998), which is thought to be essential for the normal activity of cadherins. It is possible that p120^{ctn} regulates the lateral clustering of cadherins; e.g. it may inhibit this process when hyperphosphorylated, with this action being suppressed in the default state.

We thus demonstrated a p120^{ctn}-dependent inhibition of the cadherin system in carcinoma cells. Many tumour cells maintain strong cadherin-mediated adhesion; nevertheless they can still metastasize. Such tumour cells must have a mechanism to destabilize their own cell–cell adhesion, at least transiently, for detaching themselves from the original tumour mass. The p120^{ctn}-dependent process is one such mechanism. Perhaps the inhibitory state of p120^{ctn} could fluctuate, because of unstable physiological conditions in cancer cells, and this could cause a temporary disruption of cadherin-mediated cell–cell adhesions. Colo 205 cells may be an extreme case in which the p120^{ctn}-dependent inhibitory mechanism is constitutively turned on. Although the inhibitory activity of p120^{ctn} was discovered in tumour cells, we believe that the p120^{ctn}-dependent mechanisms are used for morphogenetic regulation of cell adhesion in embryos. Actually, the juxtamembrane domain of the cadherin cytoplasmic domain to which p120^{ctn} binds has been implicated in cell motility (Chen *et al.* 1997) and in axon outgrowth (Riehl *et al.* 1996). Overexpression of p120^{ctn} in *Xenopus* embryos perturbs gastrulation (Geis *et al.* 1998; Paulson *et al.* 1999). As proposed above, there must be a signalling cascade, originating in the cell surface, to regulate the p120^{ctn} activity. Identification of such cascades in various developmental systems should help us to uncover further morphoregulatory roles of cadherins.

4. REGULATION OF PLANAR CELL POLARITY BY Fmi, A CADHERIN SUPERFAMILY MOLECULE

In many organs, epithelial cells are polarized within their plane. In the *Drosophila* wing, each cell of the epithelial layer produces a single hair that points distally (Mitchell *et al.* 1983; Eaton *et al.* 1996; Turner & Adler 1998), and exhibits proximal–distal (P–D) polarity. A number of genes regulate tissue polarity, and among them *frizzled* (*fz*) is well known. It is believed that Fz activates the polarity pathway via Dishevelled (Dsh) and that Fz and Dsh are required to restrict prehair formation to the distal edge of the cell (Wong & Adler 1993). We now find that a member of the cadherin superfamily, designated as Fmi, regulates planar cell polarity under the control of Fz.

Members of the cadherin superfamily are defined as proteins having a tandem array of repeated unique sequences, called cadherin repeat or motif, in their extracellular domain (figure 5); this motif is considered to be essential for their ability to bind Ca^{2+} (Johnson *et al.* 1999). This superfamily consists of many subfamilies: one of them is the classic cadherin subfamily, whose members interact with catenins and function as adhesion molecules, as focused on above. Fmi belongs to a completely new subfamily (Uemura 1998). The structure of Fmi is unique (figure 5), as it has a seven-pass transmembrane with similarity to the secretin receptor (Usui *et al.* 1999). Fmi is localized in cell–cell boundaries, and its ectodomains interact homophilically, as do those of the classic-type cadherins. However, before morphological polarization of wing cells along the P–D axis, Fmi becomes redistributed to the proximal and distal cell boundaries (P–D boundaries). Importantly, in the absence or overexpression of Fmi, the P–D polarity in hair growth is dramatically altered (figure 6). When Fz was ectopically expressed in a pattern to form an activity gradient vertical to the P–D axis, the Fmi distribution was altered so as to cross the Fz gradient. As a consequence, the direction of hair growth was modified; i.e. they followed the Fz gradient. These results suggest that the restricted Fmi distribution is a prerequisite for the polarized hair development and that Fz signalling determines the Fmi distribution towards P–D cell boundaries. In the experiment for gradient expression of Fz, wing hairs point down the Fz slope. This result can be interpreted to suggest that, in normal wing development, the proximal side of the P–D boundary has a stronger Fz activity (Adler *et al.* 1997). Cells would monitor this bias of the Fz activity, and may recruit Fmi molecules to the distal edge where Fz activity is stronger. Then, the homophilic binding property of Fmi would probably induce the assembly of Fmi on the other side of the P–D boundary, leading to formation of an 'Fmi zipper'. In this way, the

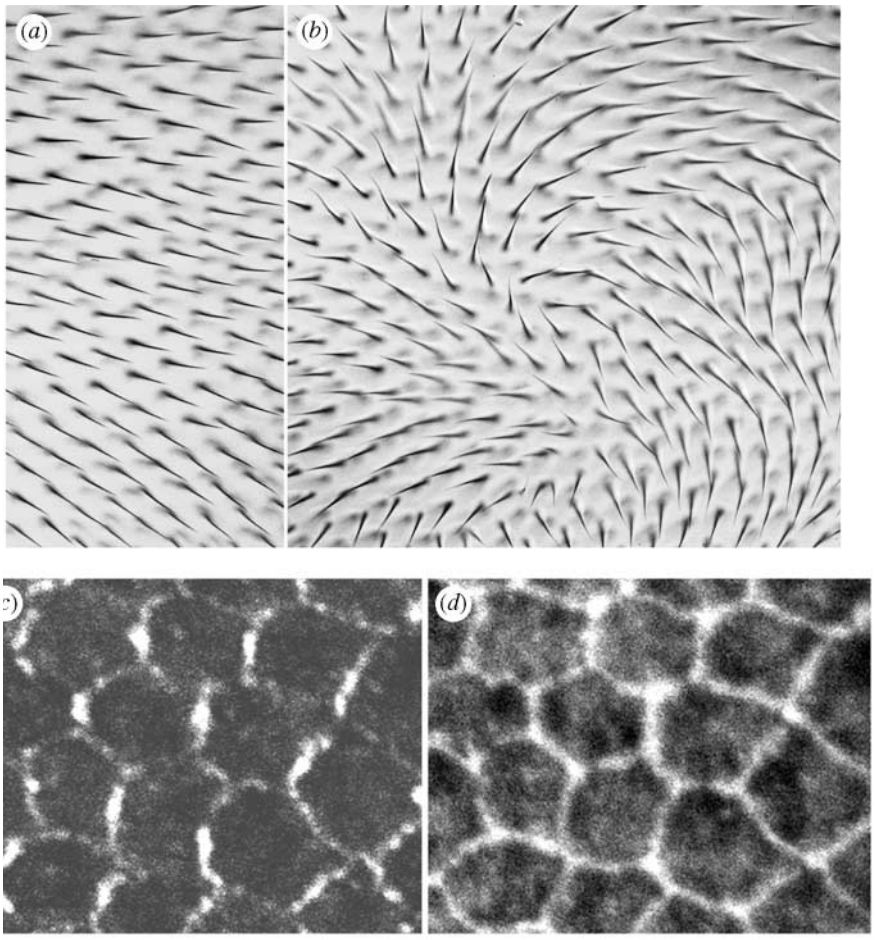

Figure 6. Fmi regulates planar cell polarity. (*a*) Hair distribution in a wild-type wing of *Drosophila*. (*b*) The effect of overexpression of Fmi on hair distribution. (*c*) Double-staining for Fmi and (*d*) DE-cadherin in a wing disc at the stage of prehair formation. Fmi is concentrated at cell–cell boundaries vertical to the P–D axis, whereas DE-cadherin is localized at every cell–cell boundary. The proximal end of the wing is located at the right in all panels.

asymmetrical Fz activity can be converted into the distribution of Fmi molecules that is apparently symmetrical, and determine the direction of hair growth. This model, however, does not explain why the hairs are pointed distally; this problem was interpreted in terms of unequal activity of Fmi across the P–D boundary, as detailed in our original paper (Usui *et al.* 1999). In summary, Fmi molecules seem to generate some signals that determine polarized hair growth, perhaps through their homophilic interactions. This finding provides a new insight into the signalling role of homophilic cadherin interactions in cellular patterning.

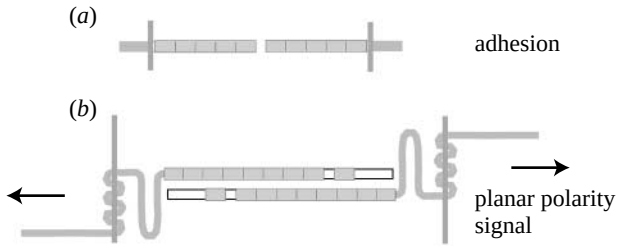

Figure 7. Homophilic interactions of different cadherins probably produce different types of signals. (*a*) Classic cadherins, (*b*) Fmi.

5. CONCLUDING REMARKS

We have discussed three different aspects of cadherin-dependent cellular patterning processes. Each process is based upon completely different functions of this molecular family. In the natural state, however, these mechanisms should be harmonized. For example, during the emigration of neural crest cells, not only cadherin-type switching but also a temporary inhibition of cadherin activity might be involved. The latter should be particularly important for the cell-separation events, which do not accompany cadherin-type switching, as in the case of tumour cell detachment.

The mechanism identified for Fmi appears to be unique, and is probably specific for this subfamily. Interestingly, even Fmi with such a peculiar structure seems to use homophilic interactions between its extracellular domains to generate its activities. This suggests that the homophilic interaction between the cadherin motifs is a general property of the members of this superfamily and that this molecular reaction is crucial for exerting their functions (figure 7). Nevertheless, each member of the superfamily shows a unique biological activity; e.g. the classic cadherins are adhesion molecules and Fmi is a signalling molecule that regulates planar cell polarity. This means that their homophilic interactions are not for the generation of a single kind of biological activity. The amino-acid sequences of the cytoplasmic domain considerably diverge within the superfamily. It can therefore be assumed that the biological function of each member is determined in part by these cytoplasmic sequences. Probably, the initial signals created by the homophilic interactions between the cadherin motifs are in turn converted

into diverse signals at the cytoplasmic side, depending on the structure there. The biological functions are still unknown for most of the cadherin superfamily members, and unravelling their functions might shed light on many unresolved problems related to cell assembly mechanisms.

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