New insights into Fat cadherins

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Summary

Cell-cell adhesion is fundamental to multicellular architecture. Several classes of adhesion molecule are used to achieve this, and cadherins represent a major family of such molecules. The cadherin family has multiple subfamilies. Members of the Fat cadherin subfamily, which is conserved across species, have an extraordinarily large extracellular region, comprising 34 repeated domains, making them the largest cadherin molecules. Classic Fat, identified in *Drosophila*, is known to regulate cell proliferation and planar cell polarity. Recent studies of one of its mammalian homologs, Fat1, have revealed novel functions of this molecule. Fat1 binds to Ena/VASP proteins and regulates actin dynamics at both cell-cell contacts and leading edges. These observations suggest that Fat1 is an important regulator of actin dynamics and controls cell-cell interactions through this activity.

Key words: Fat, Cadherin, Cell-cell interaction, Actin dynamics

Introduction

Animal morphogenesis requires cell-cell adhesion molecules, and many of these function not only as physical linkers between cells but also as signaling molecules. It is important to unravel the complex roles of these proteins if we are to understand how multicellular systems are generated and maintained. The cadherins are a large family of adhesion molecules. They have conserved repeat motifs in their extracellular domains, which are responsible for their Ca^{2+} dependence. The cadherin family can be divided into several subfamilies, including the classical cadherins, desmosomal cadherins, protocadherins, Flamingo/Celsr, and Dachsous and Fat cadherins (Gumbiner, 2000; Tepass et al., 2000; Hirano et al., 2003) (Fig. 1). Classical cadherins, the first cadherins identified, are well known to be essential for cell-cell adhesion, serving to organize the adherens junction. They have five cadherin repeats and a highly conserved cytoplasmic region to which p120-catenin and β-catenin bind. α-Catenin is believed to link the classical cadherins to the actin cytoskeleton by binding to β-catenin. Desmosomal cadherins, which are specific to the desmosome, also have five cadherin repeats, but they are linked with intermediate filaments through plakoglobin/desmoplakin (Garrod et al., 2002).

Protocadherins, by contrast, vary extensively in their primary sequences (Suzuki, 2000; Frank and Kemler, 2002; Hirano et al., 2003). They can have 5-27 cadherin repeats. Furthermore, their cytoplasmic sequences are quite divergent. Thus, protocadherins might play roles distinct from those of the classical and desmosomal cadherins. For example, CDH23, a large protocadherin molecule that has 27 cadherin repeats, is a component of the tip linker of stereocilia (Siemens et al., 2004; Sollner et al., 2004). Defects in CDH23 cause deafness and blindness in genetic forms of Usher type I syndrome (USH1) (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001), and loss of CDH23 results in disorganized stereocilia. PAPC, another protocadherin, plays a role in convergence and extension movements during gastrulation and is also a regulator of somite epithelialization associated with segmentation (Kim et al., 1998; Kim et al., 2000; Yamamoto et al., 1998; Rhee et al., 2003). Flamingo/Celsr is a well-studied non-classical cadherin. It is important for the regulation of planar cell polarity (PCP), such as in wing bristle patterning and ommatidial rotation in *Drosophila*, the development of a normal array of stereocilia in mammals, and neurite patterning (Chae et al., 1999; Usui et al., 1999; Gao et al., 2000; Curtin et al., 2003; Lee et al., 2003; Senti et al., 2003; Shima et al., 2004). Dachsous is a large cadherin molecule that has 27 cadherin repeats. Mutations in the *dachsous* gene of *Drosophila* show defects in morphogenesis of the thorax, wings and legs (Clark et al., 1995; Rodriguez, 2004). Recent studies in *Drosophila* have revealed that Dachsous also regulates the PCP pathway (Adler et al., 1998; Yang et al., 2002; Rawls et al., 2002; Casal et al., 2002).

The Fat subfamily of cadherins is conserved from flies to mammals (Fig. 2). The first gene identified, *Drosophila fat*, was cloned in 1991 and shown to encode an unusually large transmembrane molecule containing 34 cadherin repeats (Mahoney et al., 1991). In recent years, several other members of the Fat subfamily have been reported to exist in both invertebrates and vertebrates (Dunne et al., 1995; Ponassi et al., 1999; Cox et al., 2000; Mitsui et al., 2002; Nakayama et al., 2002). They commonly have 34 cadherin repeats, one or two laminin A-G domains and several epidermal growth factor (EGF) motifs in their extracellular regions. There are two Fat cadherins in *Drosophila*, Fat and Fat-like. Vertebrates have four Fat cadherins: Fat-J, Fat1, Fat2 and Fat3. From overall sequence similarity, Fat cadherins can be roughly divided into two subfamilies: one comprises Fat and Fat-J; and the other includes Fat-like, Fat1, Fat3 and Fat2 (Fig. 3). These may be the largest molecules regulating cell-cell interactions. Their huge molecular masses (about 500-600 kDa) have hindered studies of the molecular aspects of their roles. Nevertheless, recent efforts have shed light on their molecular and cellular functions.

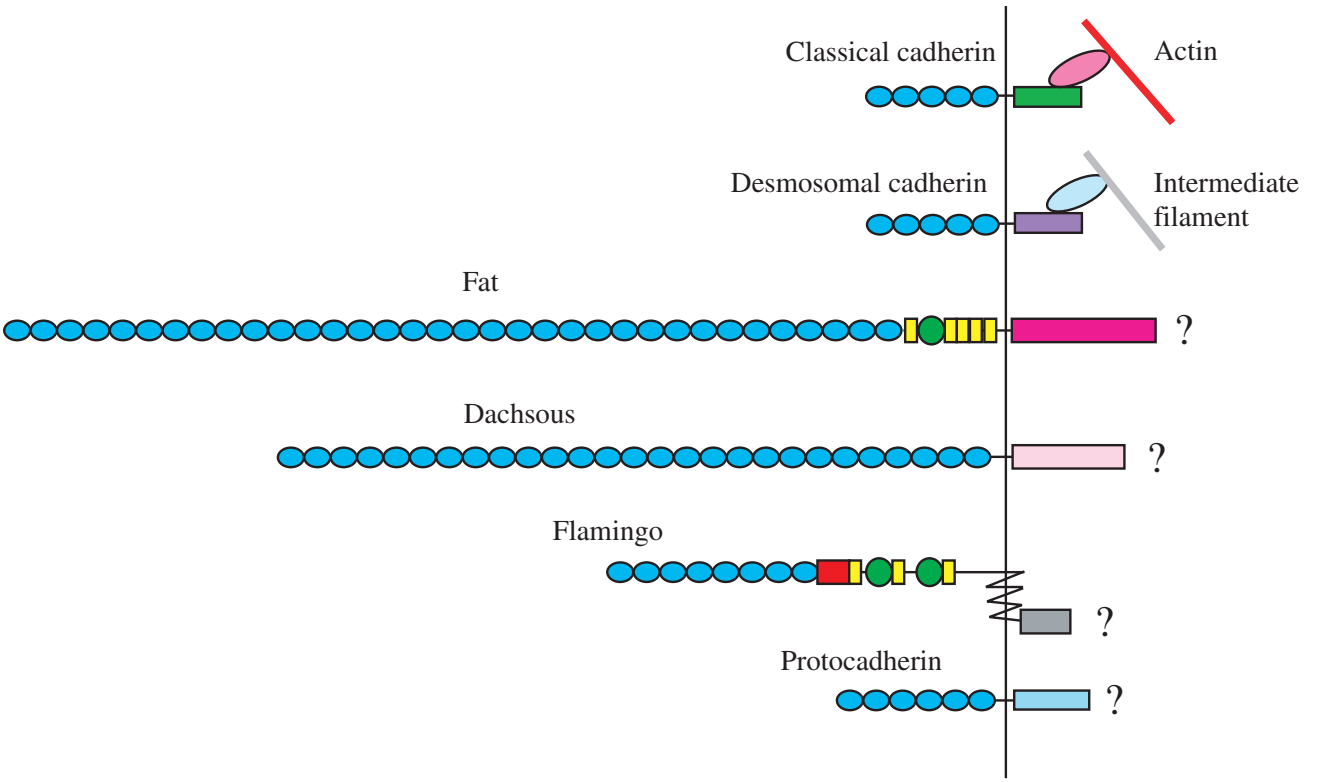

Fig. 1. Schematic representation of the cadherin superfamily. Fat cadherin is the largest cadherin, with 34 cadherin repeats. Blue ovals and green circles indicate cadherin repeats and laminin A-G motifs, respectively. Yellow boxes indicate EGF motifs. The red box in Flamingo indicates the flamingo box. Classical cadherin is believed to associate with the actin cytoskeleton through the β-catenin and α-catenin complex (pink oval). Desmosomal cadherins associate with intermediate filaments through plakoglobin/desmoplakin (light blue oval). The variously colored rectangles indicate the intracellular domains of the cadherins.

Fat cadherins in *Drosophila*

Drosophila fat was first described in the early 1920s as a lethal mutation (Mohr, 1923). Fat mutations cause hyperplastic overgrowth of all larval imaginal discs, including wing, leg, eye-antenna, haltere and genital imaginal discs (Bryant et al., 1988). This phenotype led to the proposal that Fat functions as a tumor suppressor protein, although how it regulates cell proliferation is not yet fully understood. Recently, a clue to the molecular roles of Fat was reported (Cho and Irvine, 2004). In strong *fat* mutants, wing imaginal discs become enlarged, displaying extra folds and outgrowth in the proximal wing. The outgrowth of the proximal wing discs in *fat* mutants is suppressed by a *wingless* mutation. Furthermore, in *fat* mutant clones, Wingless expression becomes upregulated. However, the *wingless* mutation does not suppress the overall enlargement of the imaginal disc. Thus, it was concluded that two distinct processes contribute to the overgrowth of wing imaginal discs in *fat* mutants: a broad-range process that results in enlargement of the entire disc, which is Wingless independent; and a local upregulation of Wingless expression in the proximal wing.

Recent studies have focused on the role of Fat in the regulation of PCP (Fanto and McNeill, 2004) (Fig. 4). Some classes of epithelial cell display a polarity in the plane of epithelium, a feature referred to as PCP. PCP is observed in many organs across species, including wing hairs and ommatidia of *Drosophila*, and hairs and stereocilia of mammals. In *Drosophila* eyes, the ommatidia in *fat* mutant clones exhibit reversed dorsal-ventral (D/V) polarity (Yang et al., 2002). PCP in the *Drosophila* eye is achieved by groups of cells – the ommatidial clusters – that must act as a single unit. Specification of R3 and R4 cells in the ommatidial cluster determines the orientation of ommatidial rotation in the plane of the epithelium. In contrast to mutations in core PCP components, such as Frizzled, a *fat* mutation does not affect the specification of R3 and R4 cells, indicating that Fat is not a core PCP component. In PCP regulation, Fat is proposed to be upstream of Frizzled and downstream of Four-jointed and Dachsous (Strutt and Strutt, 2002; Yang et al., 2002; Ma et al., 2003; Matakatsu and Blair, 2004; Strutt et al., 2004) (Fig. 4). How Fat regulates Frizzled activity is currently unknown. Four-jointed is a type II transmembrane protein mainly localized in the Golgi apparatus (Strutt et al., 2004) and is thought to modulate the activity of Fat and Dachsous in this organelle (Clark et al., 1995). Dachsous regulates Fat activity through its cadherin repeats (Matakatsu and Blair, 2004). Although Dachsous and Four-jointed might regulate Fat in the PCP pathway, its function as a tumor suppressor might be independent of them, because both *Dachsous* and *Four-jointed* mutants show no defect in proliferation of imaginal discs (Clark et al., 1995; Villano and Katz, 1995).

Fat is proposed to be involved in long-range propagation of polarity signaling (Rawls et al., 2002; Strutt and Strutt, 2002; Ma et al., 2003). Two experimental results support this notion.

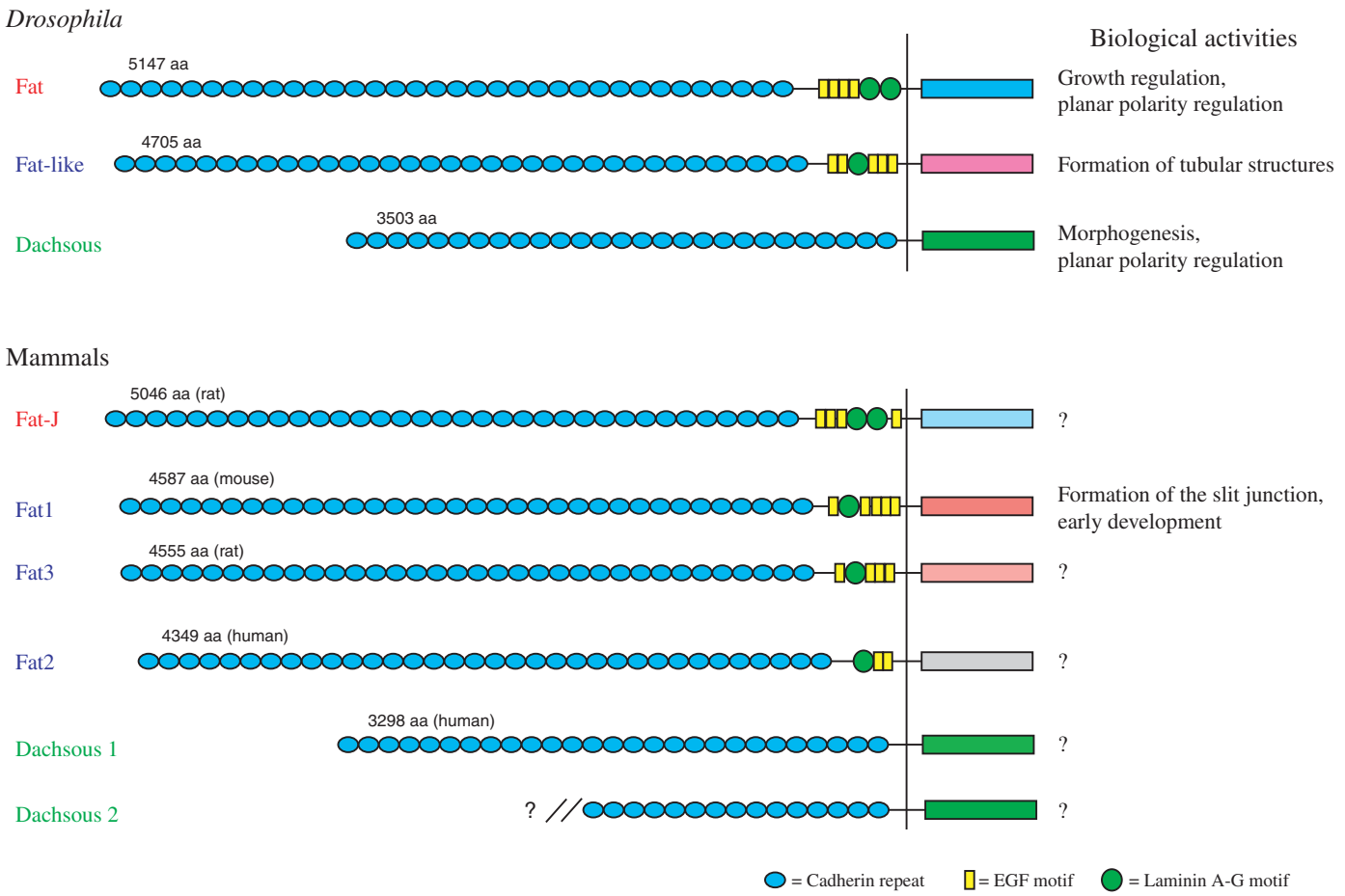

Fig. 2. Members of the Fat and Dachsous cadherin subfamilies. *Drosophila* has two Fat cadherins (Fat and Fat-like) and one Dachsous cadherin. In vertebrates, four Fat cadherins (Fat-J, Fat1, Fat2 and Fat3) and two Dachsous cadherins (Dachsous 1 and Dachsous 2) have been reported. Fat-J is probably the counterpart of *Drosophila* Fat. Fat1 and Fat3 resemble each other in their overall sequences, and they show weak similarity to *Drosophila* Fat-like. The precise length of Dachsous 2 has not been determined. The rectangular boxes depict the intracellular domains of the various Fats. The regions between the last cadherin repeat and the first EGF motif or the laminin A-G motif (for Fat2) of Fat cadherins show weak similarities to the Flamingo box, which is conserved in Flamingo cadherins (Usui et al., 1999).

First, in the eye, ectopic equators are generated in *fat* mutant clones, which suggests that Fat conveys D/V positional information to developing ommatidia for creating the D/V midline (Rawls et al., 2002). Second, in the wing, some *fat* mutant clones show polarity defects, whereas others do not. Only a subset of *fat* mutant clones in the central region of the wing exhibit severe polarity defects (Strutt and Strutt, 2002; Ma et al., 2003). This means that Fat activity is not required uniformly throughout the wing to establish polarity but instead is required to direct global orientation, such as a proximal-distal orientation of the hair. In the wing, Fat is apical to the adherens junctions (Ma et al., 2003). How Fat regulates the PCP pathway from these sites has not yet been fully answered. A possible interaction between Fat and atrophin, a transcriptional corepressor that is another regulator of the PCP pathway, has also been reported (Fanto et al., 2003). Further studies are obviously needed if we are to understand the molecular mechanism underlying Fat-dependent regulation of PCP.

Recently, a second *Drosophila* Fat subfamily cadherin, Fat-like, was identified (Castillejo-Lopez et al., 2004). Fat-like is expressed in salivary glands and tracheal cells at the embryonic stage. The protein localizes to the apical portion of the epithelia. Knocking down of Fat-like by RNA interference (RNAi) results in abnormal development of tubular structures, such as small deletions or complete lack of the trachea, proventriculus, hindgut and salivary glands. Thus, Fat-like is required for morphogenesis and maintenance of tubular structures of ectodermal origin. In contrast to loss of Fat, loss of Fat-like does not result in overgrowth phenotypes, which indicates that its function might have diverged from that of Fat. No other Fat cadherins besides Fat and Fat-like exist in the *Drosophila* genome (Hill et al., 2001).

Fat cadherins in vertebrates

In mammals, only three members of the Fat subfamily, Fat1, Fat2 and Fat3 (Dunne et al., 1995; Ponassi et al., 1999; Cox et al., 2000; Mitsui et al., 2002; Nakayama et al., 2002), were known to exist until recently. Now a fourth member, Fat-J, has been added (Fig. 2) (Hong et al., 2004). Fat1 and Fat3 resemble each other significantly, whereas Fat2 is relatively distinct (Fig.

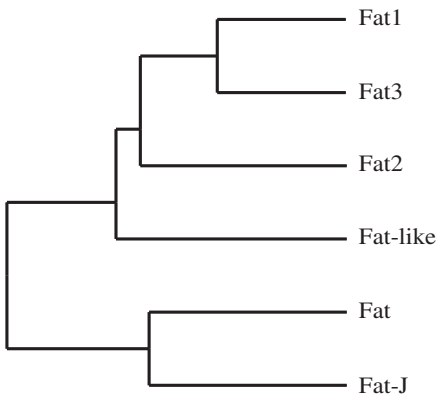

Fig. 3. A phylogenic tree of Fat cadherins. Full-length sequences of these molecules are compared.

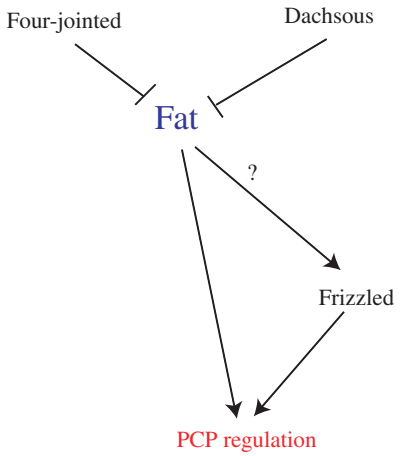

Fig. 4. Potential signaling cascades in Fat-dependent regulation of planar cell polarity (PCP). Four-jointed and Dachsous regulate (probably negatively) Fat. How Four-jointed and Dachsous regulate Fat and how Fat regulates the PCP pathway have not been currently clarified.

3). Fat-J shows modest sequence similarity to *Drosophila* Fat, and they may be orthologs. Fat1 and Fat3 show a low level of sequence similarity to *Drosophila* Fat-like, especially in the cytoplasmic regions. Although their overall sequence similarity indicates that Fat-like, Fat1, Fat3 and Fat2 form a subfamily (Fig. 3), Fat2 is relatively unique in the subfamily: the number and order of its laminin A-G and EGF motifs, and the sequence of its cytoplasmic region, are quite different from the other members.

Fat1 regulates actin dynamics and cell-cell contacts

Among the vertebrate Fat cadherins, only Fat1 has thus far been well studied. It is expressed mainly in proliferating epithelial tissues, such as neuroepithelium, lung, kidney and the basal layer of the skin (Dunne et al., 1995; Ponassi et al., 1999; Cox et al., 2000). Fat1 expression peaks in the embryonic stages and diminishes later in the adult. Reflecting the restricted expression pattern seen in vivo, Fat1 is expressed only in a limited number of cell lines, especially epithelial lines; among these, its expression level varies. For example, PAM212 cells, a transformed keratinocyte line, express a high level of Fat1, whereas MDCK cells, a kidney epithelial line, show a relatively low level (Tanoue and Takeichi, 2004). In such lines, Fat1 localizes to cell-cell contact sites as well as peripheral structures, such as lamellipodial edges and filopodial tips, in which it overlaps with actin filaments (Moeller et al., 2004; Tanoue and Takeichi, 2004) (Fig. 5). Its localization differs from that of β-catenin at cell-cell contact sites, indicating that Fat1 is not a component of the classical cadherin system. This difference is clearly observed in DLD-1 cells, a polarized colon carcinoma line, in which Fat1 is mainly localized at baso-lateral regions and present at much lower levels at the apical junctions, where classical cadherins are most highly concentrated. In some cell lines, Fat1 accumulates in protrusions of the basal portion of the cell where actin fibers converge, although these structures remain uncharacterized. Another interesting feature of some cell lines is that the expression level of Fat1 diminishes as the cells become confluent. This suggests that Fat1 has more important roles in the early phases of cell-cell contact.

During the early stages of cell-cell contact in PAM212 cells, actin filaments are recruited to junctional sites, and radial actin cables form. Knocking down Fat1 perturbs these processes: junctional actin filaments are poorly organized, and radial actin cables do not form. The Fat1 cytoplasmic tail can promote actin stress fiber formation in MDCK cells (Tanoue and Takeichi, 2004), and a mitochondrion-targeted form of this tail induces ectopic actin accumulation at the mitochondria (Moeller et al., 2004). These observations led to the conclusion that Fat1 plays an important role in actin dynamics. Knocking down Fat1 also results in loosening of cell-cell contacts, which suggests that this molecule takes part in cell-cell adhesion. However, whether this effect on cell adhesion can directly be ascribed to the lack of Fat1 itself remains unclear, because actin disorganization probably affects classical cadherin activities too. In experiments using NRK-52E cells (Moeller et al., 2004), Fat1 was shown to regulate actin dynamics at the leading edge. In wound-healing assays, Fat1-knockdown NRK-52E cells exhibit abnormal lamellipodial dynamics at wound margins, leading to a delayed wound closure; however, cell-cell adhesion is not affected. Thus, Fat1 might have cell-type-dependent actions.

We and others have identified Ena/VASP proteins as binding partners of Fat1 (Moeller et al., 2004; Tanoue and Takeichi, 2004). This family of proteins regulates the actin cytoskeleton by antagonizing capping proteins and/or by increasing the rate of dissociation of the branched junctions on actin filaments at the cell periphery, focal adhesions or cell-cell contact sites (Bear et al., 2002; Krause et al., 2002; Renfranz and Beckerle, 2002; Samarin et al., 2003). There are three members of the Ena/VASP family in mammals: Mena, VASP and Ena-VASP-like (Evl) (Reinhard et al., 1992; Gertler et al., 1996). These are thought to play redundant roles in regulating actin dynamics. Each comprises three domains: an N-terminal EVH1 domain; a proline-rich central domain; and a C-terminal EVH2 domain. Through the EVH1 domain, Ena/VASP proteins interact with several cell-surface receptors and adaptor molecules, such as Sema6A, Robo, vinculin, zyxin, Fyb/slap, lamellipodin and RIAM, all of which regulate the actin cytoskeleton (Krause et al., 2002; Renfranz and Beckerle, 2002; Krause et al., 2004; Lafuente et al., 2004). Several Src-homology 3 (SH3)-domain-

containing proteins bind to the central proline-rich domain, and Ena/VASP proteins associate with actin through the EVH2 domain.

Fat1 has three potential EVH1-binding sites in its cytoplasmic portion and binds to Ena/VASP proteins through these sites (Moeller et al., 2004; Tanoue and Takeichi, 2004). Moreover, it colocalizes with Ena/VASP proteins at early cell-cell contact sites, lamellipodial edges and filopodial tips. This Fat1-Ena/VASP system appears to be involved in formation of the junctional actin cytoskeleton at early cell-cell contacts: in Fat1-knockdown PAM212 cells, VASP no longer accumulates at these sites, and the junctional actin cytoskeleton does not form properly. Similarly, in Fat1-knockdown NRK-52E cells, the amount of VASP protein accumulating at the leading edges is significantly reduced. The Fat1-Ena/VASP system thus seems to have an important role in regulation of actin dynamics by Fat1 (Fig. 5). However, the phenotypes of Fat1-knockdown cells cannot be explained solely in terms of dysfunction of Ena/VASP proteins. There might be other signaling pathways downstream of Fat1. Fat1 has several proline-rich sequences and a PDZ-domain-binding motif. Identification of molecules interacting with these sites should help us to understand all of the functions of Fat1. Ena/VASP-binding sequences are not found in other Fat family members, which suggests that the regulation of Ena/VASP proteins might be a unique function of Fat1.

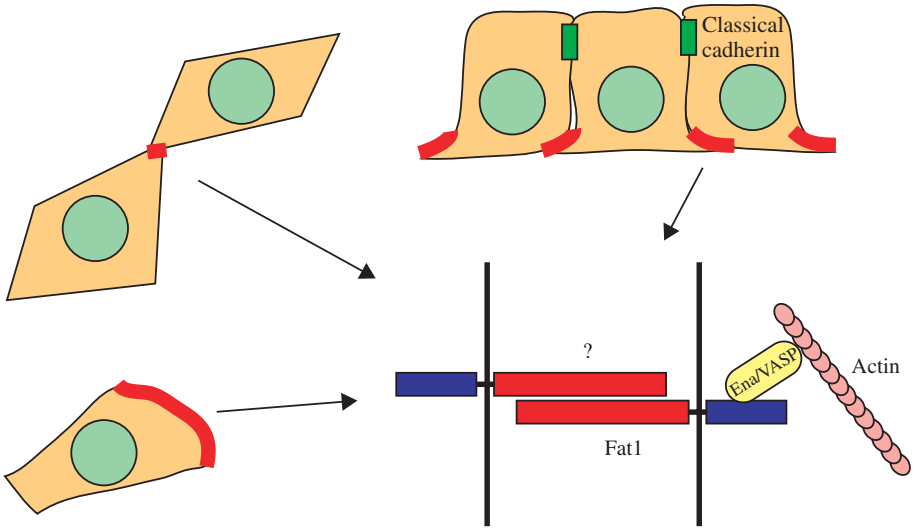

Fig. 5. Fat1 appears to be involved in cell-cell contacts and lamellipodial dynamics at the leading edge. Fat1 localizes to early cell-cell contact sites, lamellipodial edges and the basolateral portion of polarized epithelia (red). Fat1 regulates actin dynamics by binding to Ena/VASP proteins at these sites. Whether Fat1 cadherins undergo homophilic interactions remains unknown.

Fat1-knockout mice exhibit perinatal lethality, probably caused by loss of glomerular slit junctions (Ciani et al., 2003). Glomerular epithelial cells (podocytes) show an interesting profile of development. At the early embryonic stage, the cells have a polarized morphology, in which ZO-1 protein, a component of the tight junction, is apically localized. The apical junctions then move to the basal portion of the cells and, from these portions, actin-rich cellular processes called foot processes form. Between the foot processes of adjacent cells, a 40-50 nm cell-cell junction called the slit junction, is organized. This functions as the filter of the kidney. Fat1 localizes to slit junctions (Inoue et al., 2001); indeed, in the Fat1-knockout glomeruli, the slit junctions are absent. In addition, holoprosencephaly and anopthalmia are observed in Fat1-knockout mice (Ciani et al., 2003). In rare cases, cyclopia is also found. These abnormalities, generally called midline defects, might be caused by defective cell-cell interactions in early developmental stages, but the precise roles of Fat1 in these processes remain to be investigated. Partial redundancy with Fat3 might explain the relatively mild phenotypes of the Fat1-knockout mice.

Fat2, Fat3 and Fat-J

Fat2 is unique in its restricted expression pattern: Fat2 mRNA is only apparent in granule cells of the cerebellum (Nakayama et al., 2002). Interestingly, granule cells in the inner external germinal layer and migrating granule cells produce the Fat2 mRNA, whereas proliferating cells in the outer external germinal layer do not. Fat2 protein localizes to the parallel fibers of cerebellar granule cells. Its level peaks during the third postnatal week and remains high in adults. The cytoplasmic region of Fat2 shares no sequence similarity with other Fat cadherins, which suggests that its intracellular signal transduction mechanisms, if any, are unique. Importantly, Fat2 has been reported to engage in homophilic interactions. Fat2 might be involved in mediating the developmental organization of parallel fibers through such interactions. No other vertebrate Fat cadherin has been tested for its ability to undergo homophilic or heterophilic interaction.

Fat3 is mainly expressed in the central nervous system, especially in the spinal cord (Mitsui et al., 2002). Its sequence most resembles that of Fat1, especially in the cytoplasmic portion, which suggests that its function overlaps that of Fat1. Human Fat-J was identified in a computer-based search for unidentified cadherin molecules (Hong et al., 2004). It was predicted to have 17 rather than 34 cadherin repeats. However, rat Fat-J is predicted to have 34 cadherin repeats. The true number of cadherin repeats of Fat-J should therefore be determined in future studies. Because Fat-J most resembles *Drosophila* Fat, whether it regulates cell proliferation and PCP, as *Drosophila* Fat does, is an interesting question. Mammalian orthologs of Dachsous and Four-jointed have recently been identified (Ashery-Padan et al., 1999; Nakajima et al., 2001; Hong et al., 2004). We must therefore clarify the relationship between the three molecules in mammals.

Fat1, Fat2, Fat3 and Fat-J are present in the genomes of chick, zebrafish and Fugu. The sequence of each of these is well conserved among vertebrate species, which suggests their functions are also conserved. However, as mentioned above, the degree of similarity shared by the vertebrate and

invertebrate Fat cadherins is low; therefore, it is possible that the vertebrate molecules have acquired roles different from those of invertebrate Fat cadherins.

Conclusions and perspectives

The majority of studies on Fat cadherins have been based on *Drosophila* genetics. Although these studies have revealed that Fat has intriguing biological functions, they have not disclosed the molecular mechanisms by which this protein functions. Cell biological studies of vertebrate orthologs of Fat cadherins are now complementing the genetic studies and allowing more-detailed study of this subfamily. The finding that Fat1 interacts with Ena/VASP proteins has for the first time linked this membrane protein to cytoplasmic elements. However, it appears that Fat1 is not the ortholog of the classic *Drosophila* Fat and that the functions of Fat cadherins have diverged during evolution. Thus, the study of Fat1 is just the first step towards a deeper understanding of the molecular roles of Fat cadherins, and many questions must be answered. For example, why must these molecules be such a large size? What is the significance of the 34 cadherin repeats conserved among the Fat cadherins? How are such large cell-surface molecules accommodated in intercellular spaces? Do all Fat cadherins interact homophilically or do some bind to other ligands? Are Fat cadherins primarily adhesion molecules or signaling molecules? How does the classic fly Fat protein regulate cell proliferation and polarity? Is this activity conserved in vertebrates? Unraveling the mysteries of this intriguing molecular family should help us to understand how complex architecture forms in animals.

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References

Adler, P. N., Charlton, J. and Liu, J. (1998). Mutations in the cadherin superfamily member gene dachsous cause a tissue polarity phenotype by altering frizzled signaling. *Development* **125**, 959-968.

Ashery-Padan, R., Alvarez-Bolado, G., Klamt, B., Gessler, M. and Gruss, P. (1999). Fjx1, the murine homologue of the *Drosophila* four-jointed gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain. *Mech. Dev.* **80**, 213-217.

Bear, J. E., Svitkina, T. M., Krause, M., Schafer, D. A., Loureiro, J. J., Strasser, G. A., Maly, I. V., Chaga, O. Y., Cooper, J. A., Borisy, G. G. et al. (2002). Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* **109**, 509-521.

Bolz, H., von Brederlow, B., Ramirez, A., Bryda, E. C., Kutsche, K., Nothwang, H. G., Seeliger, M., del C-Salcedo Cabrera, M., Vila, M. C., Molina, O. P. et al. (2001). Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat. Genet.* **27**, 108-112.

Bork, J. M., Peters, L. M., Riazuddin, S., Bernstein, S. L., Ahmed, Z. M., Ness, S. L., Polomeno, R., Ramesh, A., Schloss, M., Srisailpathy, C. R. et al. (2001). Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *Am. J. Hum. Genet.* **68**, 26-37.

Bryant, P. J., Huettner, B., Held, L. I., Jr, Ryerse, J. and Szidonya, J. (1988). Mutations at the fat locus interfere with cell proliferation control and epithelial morphogenesis in *Drosophila*. *Dev. Biol.* **129**, 541-554.

Casal, J., Struhl, G. and Lawrence, P. A. (2002). Developmental compartments and planar polarity in *Drosophila*. *Curr. Biol.* **12**, 1189-1198.

Castillejo-Lopez, C., Arias, W. M. and Baumgartner, S. (2004). The fat-like gene of *Drosophila* is the true orthologue of vertebrate fat cadherins and is involved in the formation of tubular organs. *J. Biol. Chem.* **279**, 24034-24043.

Chae, J., Kim, M. J., Goo, J. H., Collier, S., Gubb, D., Charlton, J., Adler, P. N. and Park, W. J. (1999). The *Drosophila* tissue polarity gene starry night encodes a member of the protocadherin family. *Development* **126**, 5421-5429.

Cho, E. and Irvine, K. D. (2004). Action of fat, four-jointed, dachsous and dachs in distal-to-proximal wing signaling. *Development* **131**, 4489-4500.

Ciani, L., Patel, A., Allen, N. D. and ffrench-Constant, C. (2003). Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype. *Mol. Cell. Biol.* **23**, 3575-3582.

Clark, H. F., Brentrup, D., Schneitz, K., Bieber, A., Goodman, C. and Noll, M. (1995). Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*. *Genes Dev.* **9**, 1530-1542.

Cox, B., Hadjantonakis, A. K., Collins, J. E. and Magee, A. I. (2000). Cloning and expression throughout mouse development of mfat1, a homologue of the *Drosophila* tumour suppressor gene fat. *Dev. Dyn.* **217**, 233-240.

Curtin, J. A., Quint, E., Tsipouri, V., Arkell, R. M., Cattanach, B., Copp, A. J., Henderson, D. J., Spurr, N., Stanier, P., Fisher, E. M. et al. (2003). Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr. Biol.* **13**, 1129-1133.

Di Palma, F., Holme, R. H., Bryda, E. C., Belyantseva, I. A., Pellegrino, R., Kachar, B., Steel, K. P. and Noben-Trauth, K. (2001). Mutations in Cdh23, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. *Nat. Genet.* **27**, 103-107.

Dunne, J., Hanby, A. M., Poulsom, R., Jones, T. A., Sheer, D., Chin, W. G., Da, S. M., Zhao, Q., Beverley, P. C. and Owen, M. J. (1995). Molecular cloning and tissue expression of FAT, the human homologue of the *Drosophila* fat gene that is located on chromosome 4q34-q35 and encodes a putative adhesion molecule. *Genomics* **30**, 207-223.

Fanto, M. and McNeill, H. (2004). Planar polarity from flies to vertebrates. *J. Cell Sci.* **117**, 527-533.

Fanto, M., Clayton, L., Meredith, J., Hardiman, K., Charroux, B., Kerridge, S. and McNeill, H. (2003). The tumor-suppressor and cell adhesion molecule Fat controls planar polarity via physical interactions with Atrophin, a transcriptional co-repressor. *Development* **130**, 763-774.

Frank, M. and Kemler, R. (2002). Protocadherins. *Curr. Opin. Cell Biol.* **14**, 557-562.

Gao, F. B., Kohwi, M., Brenman, J. E., Jan, L. Y. and Jan, Y. N. (2000). Control of dendritic field formation in *Drosophila*: the roles of flamingo and competition between homologous neurons. *Neuron* **28**, 91-101.

Garrod, D. R., Merritt, A. J. and Nie, Z. (2002). Desmosomal cadherins. *Curr. Opin. Cell Biol.* **14**, 537-545.

Gertler, F. B., Niebuhr, K., Reinhard, M., Wehland, J. and Soriano, P. (1996). Mena, a relative of VASP and *Drosophila* Enabled, is implicated in the control of microfilament dynamics. *Cell* **87**, 227-239.

Gumbiner, B. M. (2000). Regulation of cadherin adhesive activity. *J. Cell Biol.* **148**, 399-404.

Hill, E., Broadbent, I. D., Chothia, C. and Pettitt, J. (2001). Cadherin superfamily proteins in *Caenorhabditis elegans* and *Drosophila melanogaster*. *J. Mol. Biol.* **305**, 1011-1024.

Hirano, S., Suzuki, S. T. and Redies, C. M. (2003). The cadherin superfamily in neural development: diversity, function and interaction with other molecules. *Front. Biosci.* **8**, D306-D355.

Hong, J. C., Ivanov, N. V., Hodor, P., Xia, M., Wei, N., Blevins, R., Gerhold, D., Borodovsky, M. and Liu, Y. (2004). Identification of new human cadherin genes using a combination of protein motif search and gene finding methods. *J. Mol. Biol.* **337**, 307-317.

Inoue, T., Yaoita, E., Kurihara, H., Shimizu, F., Sakai, T., Kobayashi, T., Ohshiro, K., Kawachi, H., Okada, H., Suzuki, H. et al. (2001). FAT is a component of glomerular slit diaphragms. *Kidney Int.* **59**, 1003-1012.

Kim, S. H., Yamamoto, A., Bouwmeester, T., Agius, E. and Robertis, E. M. (1998). The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation. *Development* **125**, 4681-4690.

Kim, S. H., Jen, W. C., de Robertis, E. M. and Kintner, C. (2000). The

protocadherin PAPC establishes segmental boundaries during somitogenesis in *Xenopus* embryos. *Curr. Biol.* **10**, 821-830.

Krause, M., Bear, J. E., Loureiro, J. J. and Gertler, F. B. (2002). The Ena/VASP enigma. *J. Cell Sci.* **115**, 4721-4726.

Krause, M., Leslie, J. D., Stewart, M., Lafuente, E. M., Valderrama, F., Jagannathan, R., Strasser, G. A., Rubinson, D. A., Liu, H., Way, M. et al. (2004). Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. *Dev. Cell.* **7**, 571-583.

Lafuente, E. M., van Puijenbroek, A. A., Krause, M., Carman, C. V., Freeman, G. J., Berezovskaya, A., Constantine, E., Springer, T. A., Gertler, F. B. and Boussiotis, V. A. (2004). RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. *Dev. Cell.* **7**, 585-595.

Lee, R. C., Clandinin, T. R., Lee, C. H., Chen, P. L., Meinertzhagen, I. A. and Zipursky, S. L. (2003). The protocadherin Flamingo is required for axon target selection in the *Drosophila* visual system. *Nat. Neurosci.* **6**, 557-563.

Ma, D., Yang, C. H., McNeill, H., Simon, M. A. and Axelrod, J. D. (2003). Fidelity in planar cell polarity signalling. *Nature* **421**, 543-547.

Mahoney, P. A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P. J. and Goodman, C. S. (1991). The fat tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. *Cell* **67**, 853-868.

Matakatsu, H. and Blair, S. S. (2004). Interactions between Fat and Dachsous and the regulation of planar cell polarity in the *Drosophila* wing. *Development* **131**, 3785-3794.

Mitsui, K., Nakajima, D., Ohara, O. and Nakayama, M. (2002). Mammalian fat3: a large protein that contains multiple cadherin and EGF-like motifs. *Biochem. Biophys. Res. Commun.* **290**, 1260-1266.

Moeller, M. J., Soofi, A., Braun, G. S., Li, X., Watzl, C., Kriz, W. and Holzman, L. B. (2004). Protocadherin FAT1 binds Ena/VASP proteins and is necessary for actin dynamics and cell polarization. *EMBO J.* **23**, 3769-3779.

Mohr, O. (1923). Modifications of the sex-ratio through a sex-linked semi-lethal in *Drosophila melanogaster* (besides notes on an autosomal section deficiency). In *Studia Mendelina: Ad Centesimum Diem Natalem Gregorii Mendelii a Grata Patria Celebrandum*, pp. 266-287. *Apud Typos, Brunn, Czechoslovakia.*

Nakajima, D., Nakayama, M., Kikuno, R., Hirosawa, M., Nagase, T. and Ohara, O. (2001). Identification of three novel non-classical cadherin genes through comprehensive analysis of large cDNAs. *Brain Res. Mol. Brain Res.* **94**, 85-95.

Nakayama, M., Nakajima, D., Yoshimura, R., Endo, Y. and Ohara, O. (2002). MEGF1/fat2 proteins containing extraordinarily large extracellular domains are localized to thin parallel fibers of cerebellar granule cells. *Mol. Cell Neurosci.* **20**, 563-578.

Ponassi, M., Jacques, T. S., Ciani, L. and ffrench Constant, C. (1999). Expression of the rat homologue of the *Drosophila* fat tumour suppressor gene. *Mech. Dev.* **80**, 207-212.

Rawls, A. S., Guinto, J. B. and Wolff, T. (2002). The cadherins fat and dachsous regulate dorsal/ventral signaling in the *Drosophila* eye. *Curr. Biol.* **12**, 1021-1026.

Reinhard, M., Halbrugge, M., Scheer, U., Wiegand, C., Jockusch, B. M. and Walter, U. (1992). The 46/50 kDa phosphoprotein VASP purified from human platelets is a novel protein associated with actin filaments and focal contacts. *EMBO J.* **11**, 2063-2070.

Renfranz, P. J. and Beckerle, M. C. (2002). Doing (F/L)PPPPs: EVH1 domains and their proline-rich partners in cell polarity and migration. *Curr. Opin. Cell Biol.* **14**, 88-103.

Rhee, J., Takahashi, Y., Saga, Y., Wilson-Rawls, J. and Rawls, A. (2003). The protocadherin papc is involved in the organization of the epithelium along the segmental border during mouse somitogenesis. *Dev. Biol.* **254**, 248-261.

Rodriguez, I. (2004). The dachsous gene, a member of the cadherin family, is required for Wg-dependent pattern formation in the *Drosophila* wing disc. *Development* **131**, 3195-3206.

Samarin, S., Romero, S., Kocks, C., Didry, D., Pantaloni, D. and Carlier, M. F. (2003). How VASP enhances actin-based motility. *J. Cell Biol.* **163**, 131-142.

Senti, K. A., Usui, T., Boucke, K., Greber, U., Uemura, T. and Dickson, B. J. (2003). Flamingo regulates r8 axon-axon and axon-target interactions in the *Drosophila* visual system. *Curr. Biol.* **13**, 828-832.

Shima, Y., Kengaku, M., Hirano, T., Takeichi, M. and Uemura, T. (2004). Regulation of dendritic maintenance and growth by a mammalian 7-pass transmembrane cadherin. *Dev. Cell.* **7**, 205-216.

Siemens, J., Lillo, C., Dumont, R. A., Reynolds, A., Williams, D. S., Gillespie, P. G. and Muller, U. (2004). Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature* **428**, 950-955.

Sollner, C., Rauch, G. J., Siemens, J., Geisler, R., Schuster, S. C., Muller, U. and Nicolson, T. (2004). Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. *Nature* **428**, 955-959.

Strutt, H. and Strutt, D. (2002). Nonautonomous planar polarity patterning in *Drosophila*: dishevelled-independent functions of frizzled. *Dev Cell.* **3**, 851-863.

Strutt, H., Mundy, J., Hofstra, K. and Strutt, D. (2004). Cleavage and secretion is not required for Four-jointed function in *Drosophila* patterning. *Development* **131**, 881-890.

Suzuki, S. T. (2000). Recent progress in protocadherin research. *Exp. Cell Res.* **261**, 13-18.

Tanoue, T. and Takeichi, M. (2004). Mammalian Fat1 cadherin regulates actin dynamics and cell-cell contact. *J. Cell Biol.* **165**, 517-528.

Tepass, U., Truong, K., Godt, D., Ikura, M. and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. *Nat. Rev. Mol. Cell. Biol.* **1**, 91-100.

Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R. W., Schwarz, T. L., Takeichi, M. and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* **98**, 585-595.

Villano, J. L. and Katz, F. N. (1995). Four-jointed is required for intermediate growth in the proximal-distal axis in *Drosophila*. *Development* **121**, 2767-2777.

Yamamoto, A., Amacher, S. L., Kim, S. H., Geissert, D., Kimmel, C. B. and de Robertis, E. M. (1998). Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. *Development* **125**, 3389-3397.

Yang, C. H., Axelrod, J. D. and Simon, M. A. (2002). Regulation of Frizzled by fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. *Cell* **108**, 675-688.