**Int. J. Dev. Biol. 51**: 173-176 (2007)
doi: 10.1387/ijdb.062221tg

*Developmental Expression Pattern*

# The expression of *Fat-1* cadherin during chick limb development

TERENCE G. SMITH[1,*], NICK VAN HATEREN[2], CHERYLL TICKLE[1] and STUART A. WILSON[2]

*[1]Division of Cell and Developmental Biology, University of Dundee, U.K. and [2]Department of Molecular Biology and Biotechnology, University of Sheffield, U.K.*

**ABSTRACT Cellular adhesion is fundamental to the behaviour of cell populations during embryonic development and serves to establish correct tissue pattern and architecture. The cadherin superfamily of cell adhesion proteins regulates cellular organization and additionally influences intracellular signalling cascades. Here we present for the first time a detailed account of chick *Fat-1* gene expression during embryogenesis visualised by whole-mount *in situ* hybridisation. In part, we focus on the expression pattern in limb buds that has not been accurately documented. While *Fat-1* is generally expressed in epithelial tissues and its *Drosophila* counterpart *Fat-like* regulates formation of ectodermally-derived organs, in limb buds we have found that chick *Fat-1* is uniquely restricted to mesenchyme. This *Fat-1* expression pattern is remarkably dynamic throughout tissue differentiation, limb maturation and pattern formation. Diffuse expression of *Fat-1* begins at stage HH17 as the limb bud is forming. It then becomes more proximal as the limb bud grows and is expressed within both tendon and muscle progenitors in the dorsal and ventral subectodermal mesenchyme. Later, *Fat-1* transcripts were more abundant in anterior and posterior domains of the limb bud. During hand plate formation, *Fat-1* transcripts were expressed in the mesenchyme adjacent to the wrist joint zone and in the interdigit mesenchyme.**

KEY WORDS: *Fat-1, cadherin, limb bud, cell adhesion, tendon*

The cadherin superfamily consists of cell adhesion proteins that are highly conserved, contain multiple modular extracellular repeat motifs and homodimerise in a calcium-dependent manner (Tanoue and Takeichi, 2005). They constitute one of the many superfamilies of related genes that encode proteins that serve not only to tie cells together, but also to regulate intracellular signalling cascades. The Fat cadherins represent a distinct sub-group within the cadherin superfamily and are its largest members, generally containing 34 extracellular cadherin repeats, one or two laminin A-G motifs, 5 EGF domains and cytoplasmic EVH-1 and PDZ binding sites, giving them a molecular size of up to 600kDa (Tanoue and Takeichi, 2005). The *Drosophila* tumour suppressor *Fat* gene was discovered as a lethal mutation causing hyperplastic growth and altered cell-cell adhesion of larval imaginal discs, including those of the wing and leg (Bryant *et al.*, 1988, Jaiswal *et al.*, 2006). Studies have shown that *Drosophila Fat* genetically interacts with the *wingless (wg)* planar cell polarity pathway and in the eye contributes to dorsal-ventral patterning of ommatidia (Yang *et al.*, 2002, Fanto and McNeill, 2004, Jaiswal *et al.*, 2006).

There are 4 vertebrate *Fat* genes that closely resemble *Drosophila Fat* (Tanoue and Takeichi, 2005). Recent analyses have indicated however that *Fat 1-3* are the true orthologues of the second *Fat* gene to be identified in *Drosophila*, *Fat-like* (Castillejo-Lopez *et al.*, 2004, Rock *et al.*, 2005, Tanoue and Takeichi, 2005). *Fat 1-3* and *Fat-like* all encode proteins that contain a single laminin A-G motif, whereas the original *Drosophila Fat* has two of these motifs. A phylogenic tree shows that within this subfamily *Fat-1* and *Fat-3* are closely related to each other, while *Fat-2* is somewhat distinct (Tanoue and Takeichi, 2005). *Fat-J* is related to the original *Drosophila Fat* gene and is the most recently discovered fourth vertebrate ortholog (Hong *et al.*, 2004). Where *Drosophila Fat* is ubiquitously expressed in some tissues, its ortholog *Fat-J* is restricted to mesenchyme while *Fat-1* is reciprocally expressed in epithelium (Rock *et al.*, 2005). Thus, even though *Fat-1* has diverged from *Drosophila Fat*, it seems to have taken over some of the

*Abbreviations used in this paper:* EGF, epidermal growth factor; PDZ, post-synaptic density protein, Drosophila disc large tumor suppressor and zo-1 protein; EVH-1, Ena/Vasp homology domain 1; wnt is the vertebrate homolog of wg (wingless).

***Address correspondence to:** Dr. Terence Gordon Smith. School of Life Sciences, Division of Cell and Developmental Biology, University of Dundee, Dow Street, Dundee, DD1 5EH, U.K. e-mail: tel_smith@yahoo.co.uk

0214-6282/2007/$30.00
© UBC Press
Printed in Spain
www.intjdevbiol.com

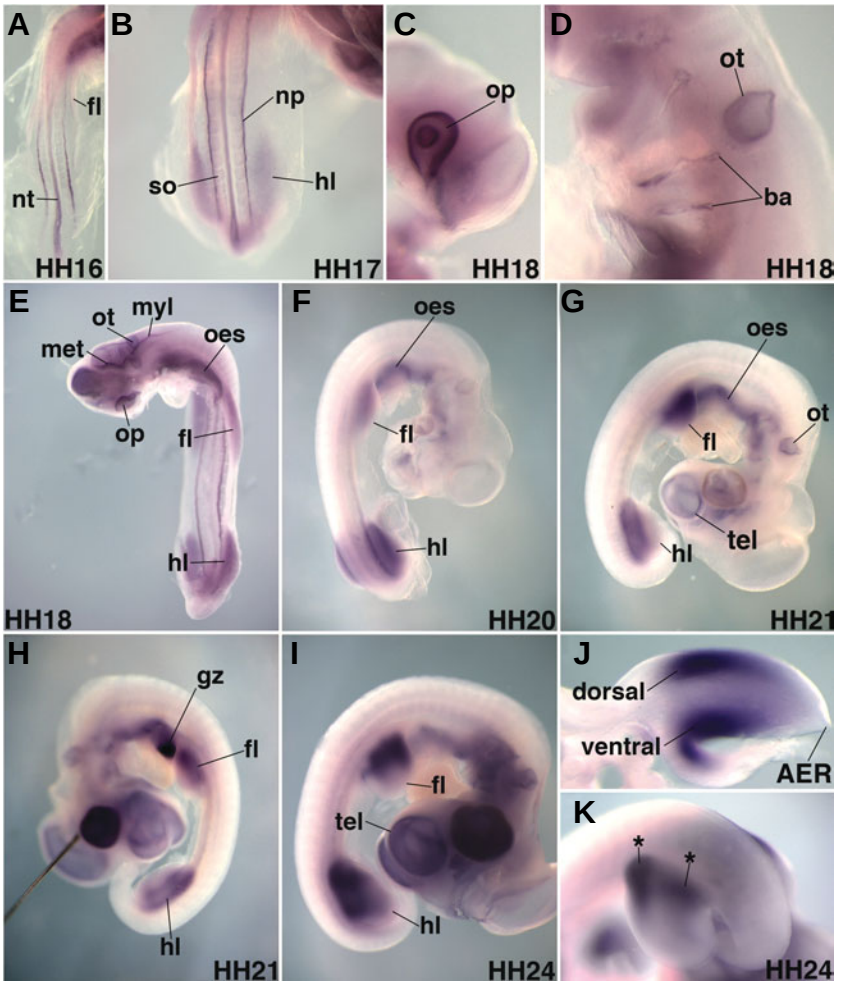


**Fig. 1. Expression of *Fat-1* during early limb bud formation. (A)** Fat-1 *expression in a stage HH16 chicken embryo. While expression is strong within the neural tube, it was not yet detected in the forming forelimb bud.* **(B)** *At HH17,* Fat-1 *is present in the hindlimb and robustly expressed in the nephritic primordium. Very weak expression was detected in epithelial somites.* **(C)** Fat-1 *transcripts within the optic vesicle at HH18.* **(D)** *The epithelium lining the otic vesicle and branchial arches expressed* Fat-1. **(E)** *A ventral view of* Fat-1 *expression at stage HH18 in hindbrain, limb buds and oesophagus.* **(F)** *Limb bud expression at stage HH20 is just starting to become proximally restricted.* **(G)** *Transcription pattern in limb buds at HH21 is now more proximal, especially in the hindlimb bud.* Fat-1 *transcripts were detected within the telencephalon of the developing forebrain.* **(H)** *A left-handed view of a HH21 embryo showing the prominent expression of* Fat-1 *in the presumptive gizzard.* **(I)** *Proximal* Fat-1 *expression in limb buds at HH24 and in lateral telencephalic vesicles.* **(J)** *Transverse section of a HH23 forelimb bud showing* Fat-1 *transcripts segregated into dorsal and ventral domains. These regions correspond to tendon and muscle primordia.* **(K)** *Close up view of a stage HH24 hindlimb showing the separation along the anteroposterior axis in the limb, marked by asterisks. Anterior is to the left, posterior the right. AER; apical ectodermal ridge. ba; branchial arches. fl; forelimb bud. gz; gizzard. hl; hindlimb bud. met; metencephalon. myl; myelencephalon. np; nephritic primordium. nt; neural tube. oes; oesophagus. op; optic vesicle. ot; otic vesicle. so; epithelial somites. tel; telencephalon.*

functions from the corresponding *Fat* ortholog. In fact, vertebrate *Fat-1* is widely expressed in many epithelial tissues (Tanoue and Takeichi, 2005) and is necessary for the development of epithelial organs. For instance, deletion of *Fat-1* in mice resulted in perinatal fatality due to lack of glomerular slit junction formation between the foot processes of podocytes in the developing kidney (Ciani *et al.*, 2003). At lower penetrance, holoprosencephaly and anophthalmia resulted in *Fat-1*$^{-/-}$ mutant embryos.

The expression of *Fat-2* transcripts and of Fat-2 protein have been detected within the cerebellum of rat embryos (Nakayama *et al.*, 2002). Similarly, *Fat-3* is expressed in the rat spinal cord and central nervous system (Mitsui *et al.*, 2002). In mouse, *Fat-J* is expressed in the neural tube, inner ear and intervertebral discs (Rock *et al.*, 2005). Of all these *Fat* genes, *Fat-1* shows the most complex and interesting expression pattern. However there are no current reports on the expression of *Fat-1* in chick embryos. Furthermore, although the fact that *Fat-1* expression in the mouse limb has been mentioned, the details of the expression pattern at different stages of development have not been described (Cox *et al.*, 2000). Therefore, we have analysed chick *Fat-1* expression throughout limb development and also report its expression in other regions of the chick embryo.

Beginning at stage HH16, *Fat-1* transcripts were expressed prominently in the neural tube, but were absent from the emerging forelimb bud (Fig. 1A). At HH16 and HH17, the nephritic primordium strongly expressed *Fat-1* (Fig. 1A, B) and a diffuse expression now became apparent in the forelimb (not shown) and hindlimb (Fig. 1B). Epithelial somites displayed a very weak expression in both stages (Fig. 1A, B). *Fat-1* transcripts were evident around the optic vesicle and lined the branchial arches (Fig. 1C, D, E). Persistent expression was maintained within the otic vesicle (Fig. 1D, E, G, Fig. 2H). We found *Fat-1* transcripts expressed in the neuroepithelium lining the metacoele and myelocoele of the hindbrain and in the developing oesophagus (Fig. 1E, F, G).

During limb bud outgrowth, diffuse expression throughout the limb persisted at stage HH18 (Fig. 1E), which then became more proximal as development preceded through stage HH20 (Fig. 1F), HH21 (Fig. 1G) and HH24 (Fig. 1I, K). Cross section of a stage HH23 limb bud showed that *Fat-1* transcripts had separated into two exclusive domains along the subectodermal mesenchyme (Fig. 1J). The similarity of the limb *Fat-1* pattern in cross section to the expression of *scleraxis*, a marker of tendons (Schweitzer *et al.*, 2001), suggests that tendon progenitors within the limb express *Fat-1* cadherin. This region additionally contains migrating muscle progenitors (Schweitzer *et al.*, 2001). The proximal restriction of *Fat-1* in the early limb bud correlates well with the fact that only proximal and intermediate tendons expressed *Fat-1* transcripts at later stages of development (Fig. 2A, B, C, D). Unlike *scleraxis*, *Fat-1* expres-

sion became stronger along the anterior and posterior edges of the limb bud, but weaker in the core (Fig. 1K).

At HH27, *Fat-1* transcripts are seen as a V-shape in developing tendons of the stylopod and zeugopod (Fig. 2A, B, C, D). In contrast, the distal limb expression is not within tendons but is associated with forming cartilage elements. Likewise, the rat ortholog of *Fat-1* is expressed in condensing cartilage in limbs (Ponassi *et al.*, 1999). We noticed a progressive maturation of this distal *Fat-1* expression pattern. As the forelimb developed, it initially contained a distal crescent-shaped domain of expression (Fig. 2A, B), which later disappeared and was replaced by two small areas at the joint interzones of the wrist-forming region (Fig. 2C). Finally, at HH28 the forelimb contained two long stripes of *Fat-1* expression that extended along a narrow region between forming digits (Fig. 2D, E).

In mice, *Fat-1*$^{-/-}$ mutant embryos have forebrain defects (Ciani *et al.*, 2003) and throughout embryonic development we found prominent *Fat-1* transcription in the proliferating neuroepithelium lining the lateral telencephalic vesicles (Fig. 1G, I, Fig. 2G). Further *Fat-1* expression was noticed in the perineal area and anus (Fig. 2F). Lastly, we noted robust expression of *Fat-1* in the presumptive gizzard, positioned on the left side of the body wall (Fig. 1H). Later, *Fat-1* transcripts were still well maintained in the gizzard proper (Fig. 2I).

Expression of *Fat-1* transcripts in rat, mouse and zebrafish are developmentally restricted to mainly epithelial tissues such as the eye, kidney, lung, gut, branchial arches, dermomyotome and neural tube and its *Drosophila* counterpart *Fat-like* regulates the formation of tubular organs of ectodermal origin (Ponassi *et al.*, 1999, Cox *et al.*, 2000, Castillejo-Lopez *et al.*, 2004, Down *et al.*, 2005). In contrast to this well-reported epithelial cell expression profile, mesenchymal cells within the limb bud expressed *Fat-1* (Fig. 1J). The limb bud therefore represents a unique expression domain of the *Fat-1* gene.

*Fat-1* may have an important role in maintaining the separation of different cell lineages in limb bud mesenchyme, where no anatomical boundaries exist (Yajima *et al.*, 2002). Rather, fixed expression domains of cadherins could group cell populations together through cellular adhesion to maintain the pattern of the limb bud. For example, while *Fat-1* is proximally expressed (Fig. 1I, K), *cadherin-11* transcripts are expressed distally (Kimura *et al.*, 1995).

In *Drosophila*, *Fat* has multiple roles in the regulation of the conserved *wg/wnt* signalling pathway during wing development (Jaiswal *et al.*, 2006). It is known that *wg/wnt* expression in the proximal *Drosophila* wing is regulated by a different mechanism than that in the distal wing (Rodriguez Dd Ddel *et al.*, 2002). Somatic clones lacking *Fat* in proximal wing cells showed an increase in *wg/wnt* expression (Cho and Irvine, 2004, Jaiswal *et al.*, 2006). Therefore, in the early chick limb bud the proximal restriction of *Fat-1* (Fig. 1I, K) could inhibit *wnt* expression in those cells. Indeed, *wnt-5a* transcripts are excluded from the proximal limb bud cells at these stages (Loganathan *et al.*, 2005).

Conversely in the distal *Drosophila* wing, *Fat*$^{-/-}$ cells had no difference in *wg/wnt* transcription but increased levels of cytoplasmic β-catenin/armadillo and therefore had higher levels of wg/wnt signalling (Jaiswal *et al.*, 2006). We could therefore suppose that during digit formation in the chick distal limb, Fat-1 may regulate wnt signalling, rather than *wnt* expression. In the distal limb bud at these stages, the expression pattern of *wnt-4* is similar to the pattern of *Fat-1* in the wrist (Fig. 2C)

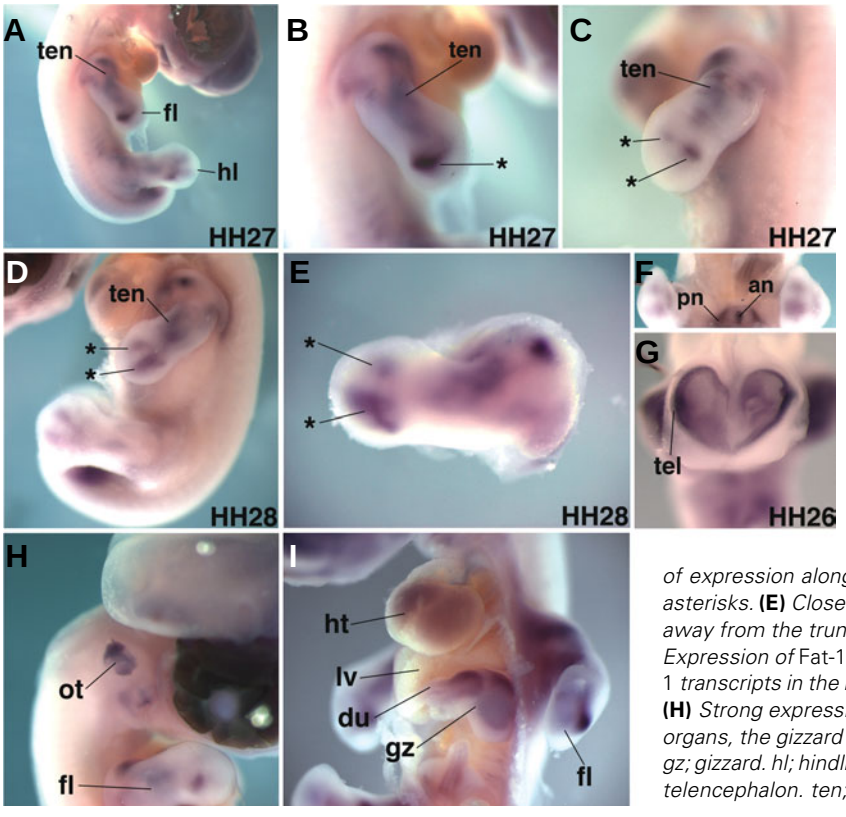


**Fig. 2. Expression of *Fat-1* during late limb formation. (A)** *HH27 embryo showing* Fat-1 *expression in fore- and hindlimbs. Note how the distal limb pattern differs between forelimb and hindlimb buds. The forelimb has a crescent-shaped domain, whereas the hindlimb contains a residual crescent domain overlapping two stripes of expression that extend along the interdigit mesenchyme.* **(B)** *Close up view of a stage HH27 forelimb showing the crescent domain marked by an asterisk. In the stylopod and zeugopod,* Fat-1 *transcripts are expressed by proximal and intermediate tendon progenitors in a V-shape.* **(C)** *Close up view of a HH27 forelimb showing the transition of expression from crescent to two small marks, shown with asterisks. These marks delineate the interzone wrist-forming joint* **(D)** *At HH28 the forelimb has matured to the same stage as the hindlimb as both have extended stretches of expression along the interdigit mesenchyme, marked in the forelimb with asterisks.* **(E)** *Close view of a stage HH28 forelimb bud that has been dissected away from the trunk. Two asterisks mark the hand-plate* Fat-1 *expression.* **(F)** *Expression of* Fat-1 *in the perineal area and anus of the chick embryo.* **(G)** Fat-1 *transcripts in the neuroepithelium of the left and right telencephalic vesicles.* **(H)** *Strong expression is seen in the developing otic vesicle.* **(I)** *Of the internal organs, the gizzard expressed* Fat-1. *an; anus. du; duodenum. fl; forelimb bud. gz; gizzard. hl; hindlimb bud. ht; heart. lv; liver. ot; otic vesicle. pn; perineum. tel; telencephalon. ten; tendon primordia.*

(Loganathan *et al.*, 2005). In addition, *wnt-5a* is expressed along the interdigit mesenchyme and both of these signals could be regulated by Fat-1 (Fig. 2D, E) (Loganathan *et al.*, 2005).

However it must be taken into account that during evolution, the sequences encoding *Drosophila Fat* and its vertebrate homologues have diverged significantly, with alterations in key motifs such as the PDZ domain and in the arrangement of EGF and laminin A-G repeats (Ponassi *et al.*, 1999, Rock *et al.*, 2005). Thus, vertebrate Fat proteins may have acquired different cellular functions to those employed in *Drosophila*. It would be interesting to know how the properties conferred upon Fat proteins by their various protein domains could further influence pattern formation and gene expression. The chicken limb bud represents an ideal model system to test such properties (Tickle, 2003).

## Experimental Procedures

### Fat-1 *cadherin probe synthesis and* in situ *hybridisation*

The *Fat-1* cadherin cDNA used to produce a DIG-labelled probe was obtained from the Chicken EST database, ChEST533a5 and sequence verified (Boardman *et al.*, 2002). Not1 and T3 enzymes were used to linearize the plasmid and transcribe the RNA probe. *In situ* hybridisation was performed as previously described (Smith *et al.*, 2005), with a small modification. For stages HH24-26 and HH27-28, embryos were incubated with 10μg/ml and 15μg/ml respectively of Proteinase K at room temperature for 20mins. Embryos were then post-fixed in 4% PFA for a further 20mins.

*Acknowledgements*

*TGS was funded by The Royal Society and the MRC. NH, CT and SAW were funded by a joint BBSRC grant with K. Storey and M. Placzek.*

## References

BOARDMAN, P.E., SANZ-EZQUERRO, J., OVERTON, I.M., BURT, D.W., BOSCH, E., FONG, W.T., TICKLE, C., BROWN, W.R., WILSON, S.A. and HUBBARD, S.J. (2002). A comprehensive collection of chicken cdnas. *Curr Biol* 12: 1965-9.

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: 541-54.

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: 24034-43.

CHO, E. and IRVINE, K.D. (2004). Action of fat, four-jointed, dachsous and dachs in distal-to-proximal wing signaling. *Development* 131: 4489-500.

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: 3575-82.

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: 233-40.

DOWN, M., POWER, M., SMITH, S.I., RALSTON, K., SPANEVELLO, M., BURNS, G.F. and BOYD, A.W. (2005). Cloning and expression of the large zebrafish protocadherin gene, fat. *Gene Expr Patterns* 5: 483-90.

FANTO, M. and MCNEILL, H. (2004). Planar polarity from flies to vertebrates. *J Cell Sci* 117: 527-33.

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: 307-17.

JAISWAL, M., AGRAWAL, N. and SINHA, P. (2006). Fat and wingless signaling oppositely regulate epithelial cell-cell adhesion and distal wing development in Drosophila. *Development* 133: 925-35.

KIMURA, Y., MATSUNAMI, H., INOUE, T., SHIMAMURA, K., UCHIDA, N., UENO, T., MIYAZAKI, T. and TAKEICHI, M. (1995). Cadherin-11 expressed in association with mesenchymal morphogenesis in the head, somite and limb bud of early mouse embryos. *Dev Biol* 169: 347-58.

LOGANATHAN, P.G., NIMMAGADDA, S., HUANG, R., SCAAL, M. and CHRIST, B. (2005). Comparative analysis of the expression patterns of wnts during chick limb development. *Histochem Cell Biol* 123: 195-201.

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: 1260-6.

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: 563-78.

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: 207-12.

ROCK, R., SCHRAUTH, S. and GESSLER, M. (2005). Expression of mouse dchs1, fjx1 and fat-j suggests conservation of the planar cell polarity pathway identified in Drosophila. *Dev Dyn* 234: 747-55.

RODRIGUEZ DD DDEL, A., TERRIENTE, J., GALINDO, M.I., COUSO, J.P. and DIAZ-BENJUMEA, F.J. (2002). Different mechanisms initiate and maintain wingless expression in the Drosophila wing hinge. *Development* 129: 3995-4004.

SCHWEITZER, R., CHYUNG, J.H., MURTAUGH, L.C., BRENT, A.E., ROSEN, V., OLSON, E.N., LASSAR, A. and TABIN, C.J. (2001). Analysis of the tendon cell fate using scleraxis, a specific marker for tendons and ligaments. *Development* 128: 3855-66.

SMITH, T.G., SWEETMAN, D., PATTERSON, M., KEYSE, S.M. and MUNSTERBERG, A. (2005). Feedback interactions between mkp3 and erk map kinase control scleraxis expression and the specification of rib progenitors in the developing chick somite. *Development* 132: 1305-14.

TANOUE, T. and TAKEICHI, M. (2005). New insights into fat cadherins. *J Cell Sci* 118: 2347-53.

TICKLE, C. (2003). Patterning systems - from one end of the limb to the other. *Dev Cell* 4: 449-58.

YAJIMA, H., HARA, K., IDE, H. and TAMURA, K. (2002). Cell adhesiveness and affinity for limb pattern formation. *Int J Dev Biol* 46: 897-904.

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: 675-88.

*Received: 15th September 2006*
*Reviewed by Referees: 9th November 2006*
*Modified by Authors and Accepted for Publication: 20th December 2006*
*Published Online: 31st January 2007*