Volume 298, number 2,3, 182–184 FEBS 10737 February 1992

A family of laminin-related proteins controlling ectodermal differentiation in *Drosophila*

László Patthy

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest POB 7, H-1518, Hungary

Received 2 December 1991

It is shown that the proteins encoded by the tumor suppressor *fat* gene, the neurogenic *slit* gene and *crumbs* gene of *Drosophila* contain domains homologous with modules identified previously in laminin A. These proteins of *Drosophila* have a number of features in common: they have large extracellular regions containing laminin A modules linked to epidermal growth factor-like domains, and they are all involved in cell–cell interactions that are crucial for correct morphogenesis of ectodermal tissues (development of midline neuroepithelia, organization of epihelial tissues etc.). It is suggested that the laminin A-type modules of these proteins play important roles in the interactions that control ectodermal differentiation.

Tumor suppressor gene; Neurogenesis; Laminin; Epithelial polarization

1. INTRODUCTION

The *Drosophila* gene, *fat*, is one of the known tumor suppressor genes of *Drosophila*: recessive lethal (loss of function) mutations in this locus cause hyperplastic, tumor-like overgrowth of larval imaginal discs, defects in differentiation and morphogenesis [1]. In these mutants the cells of the imaginal discs continue to proliferate during the major part of the larval period, resulting in large discs containing convoluted, abnormal folds of epithelial cells. During metamorphosis these discs differentiate into defective structures with regions of reversed polarity [1], indicating that the product of *fat* is involved in interactions that control cell proliferation, morphogenesis and polarity in imaginal discs. The locus derives its name from the fat, broad thorax and abdomen of homozygous flies carrying recessive viable mutations in this gene. The *fat* gene is expressed on the surface of embryonic ectoderm and in the imaginal discs. The protein product of *fat* is a large transmembrane protein: its extracellular part contains 34 tandem copies of cadherin domains, five EGF-like domains, as well as three cysteine-poor regions that do not show homology with other proteins [1]. Since mutations in the *fat* gene lead to a cell-autonomous overgrowth phenotype [1] it is suggested that the *fat* gene product mediates cell–cell interactions in its cell-bound form (rather than as a diffusible protein) [2]. The specific roles of the individual domains of its extracellular part are unknown at present.

Correspondence address: L. Patthy, Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, PO Box 7, H-1518, Hungary. Fax: (36) (1) 1665-465.

The *Drosophila* gene, *slit*, is involved in embryonic development of the central nervous system: mutations in this locus result in the collapse of the regular scaffold of commissural and longitudinal axon tracts. From the blastoderm stage, expression of the *slit* gene is restricted to ectodermal tissues, and the protein is expressed at high levels on the surface of cells belonging to the midline neuroepithelium. The sequence of the protein product of *slit* was found to contain six tandem copies of the epidermal growth factor-like domain, a cysteine-poor region and a noncontiguous seventh EGF-like domain [2]. The product of *slit* is thought to correspond to an extracellular protein that mediates interactions between midline ectodermal cells and the growing axons [2]. Although nothing is known about the role of its domains in these interactions, it has been suggested that the EGF-like domains might play essential roles by mediating cell–cell or paracrine interactions [2].

The crb product of the *Drosophila* gene, *crumbs*, is essential for the organization of epithelia and establishment of polarity of epithelial cells. Mutations in *crumbs* lead to loss of polarized morphology and severe disorganization of epithelia but phenotypic abnormalities of crb$^-$ embryos also include neural hyperplasia [3,4]. Protein crb is a transmembrane protein; its extracellular part contains a total of thirty EGF-like domains in four clusters and it has been suggested that crb may function primarily via its EGF-like domains [4]. We have shown recently that the cysteine-poor regions separating the clusters of EGF-like domains of crb are homologous with domains of laminin A and merosin [5]. Since these domains of laminin A have been implicated in determining epithelial polarity we have suggested a similar role for the homologous modules of crb [5].

Published by Elsevier Science Publishers B.V.

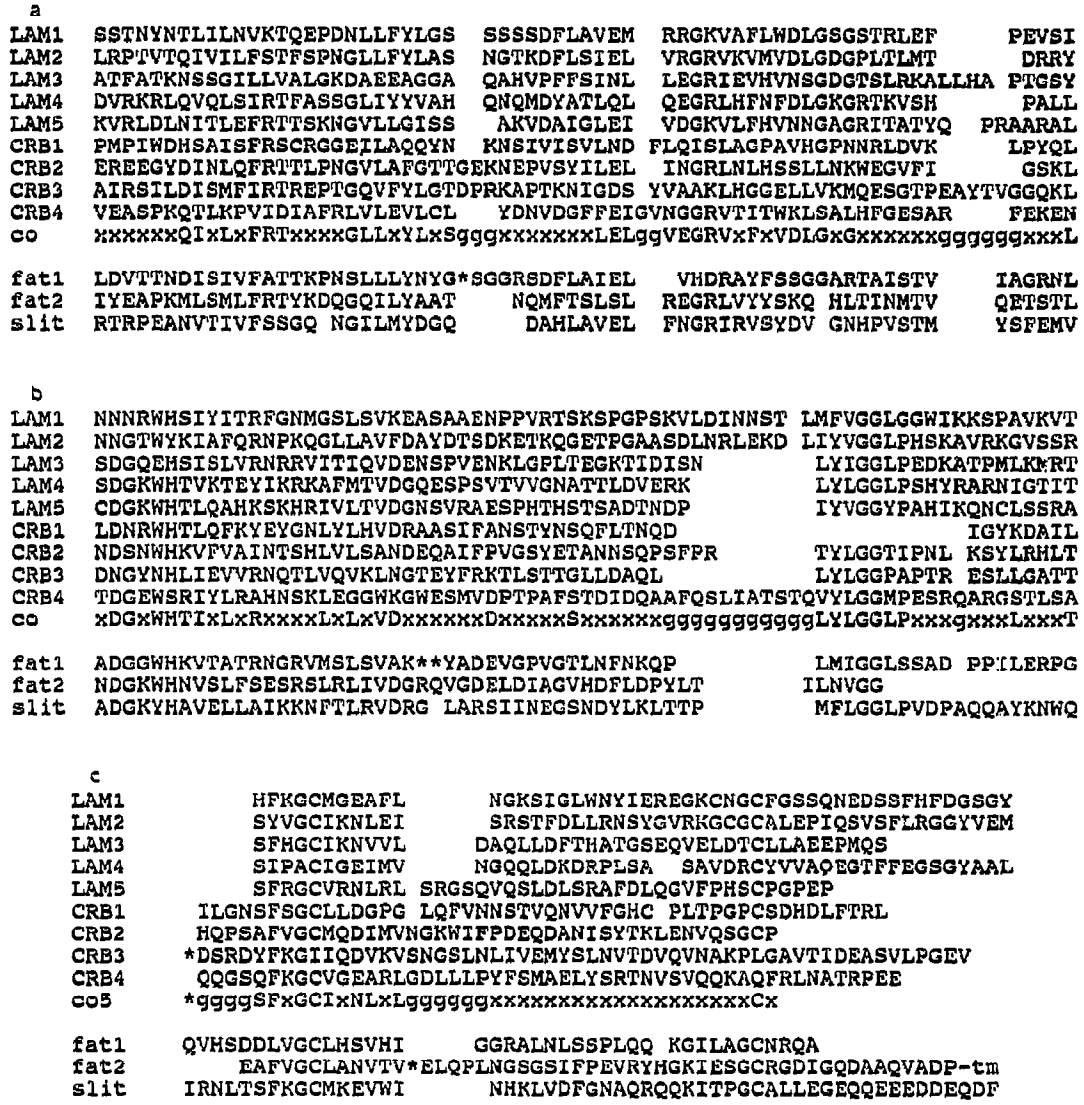

Fig. 1. Homology of *fat* protein (fat1–fat2) and *slit* protein (slit) with the laminin A-type modules of laminin A-chain (LAM1–LAM5) and crb protein of *Drosophila* (CRB1–CRB4). The consensus sequence (co) constructed for the LAM and CRB sequences were used in the homology search. For typographical reasons some insertions have been omitted from the multiple alignment (*).

Here we show that the cysteine poor regions of the proteins encoded by *slit* and *fat* genes of *Drosophila* are also homologous with laminin A and the laminin A-type modules of the crb protein of *Drosophila*. We suggest that the proteins encoded by *fat*, *slit* and *crumbs* genes of *Drosophila* constitute a family of laminin-related proteins controlling organization of ectodermal tissues, and that the laminin A-type modules of these *Drosophila* proteins (in addition to or rather than their EGF-like domains) may participate in the interactions that control morphogenesis.

2. MATERIALS AND METHODS

The protocol suitable for the detection of distant homologies of mosaic proteins [6,7] has been applied to the proteins encoded by *fat* and *slit* genes of *Drosophila*. The principle of this procedure is that sequences of suspected modules of mosaic proteins are compared with a library of consensus sequences of modules identified previously. This protocol was shown to be able to detect distant homologies that are not detectable with conventional search programs [5–8]. The library of consensus sequences used in the present work differed from its earlier forms [7–10] inasmuch as it also contained the consensus sequence for the recently recognized laminin A-module [5].

3. RESULTS AND DISCUSSION

The presence of the class 1–1 EGF-like modules [9] in the products of *fat* and *slit* genes raised the possibility that these proteins also belong to the clan 1 group of mosaic proteins that were assembled from modules [9]. Therefore we suspected that the cysteine-poor regions

linked to EGF-like domains in these proteins could also correspond to some class 1–1 modules.

Comparison of the unassigned cysteine-poor sequences of the *fat* product with our library of consensus sequences identified two regions that showed remarkable similarity with the laminin A-type modules (Fig. 1). The first laminin A-type module (fat 1, amino acid residues 4140–4325) separates the fourth and fifth EGF-like domains of *fat* product; the second unit (fat 2, amino acid residues 4366–4580) is found between the fifth EGF-like domain and the transmembrane segment. The two laminin A-related domains of the *fat* product show the presence of most of the motifs (DGxWHxI, LYLGG and FxGCI etc.) which are characteristic of laminin A-type modules (Fig. 1).

Comparison of the cysteine-poor region that separates the sixth and seventh EGF-like domain of the *slit* product with our library of consensus sequences revealed that this region also corresponds to a laminin A-type module; this region contains all the typical motifs of this module family (Fig. 1).

The *Drosophila* proteins encoded by the *fat, slit* and *crumbs* genes are thus similar inasmuch as they contain laminin A-type modules linked to EGF-like domains in their extracellular parts and they are all involved in the control of differentiation of ectodermal tissues. These similarities raise the possibility that they are of common evolutionary origin and that their function/mode of action may also be related. There are indeed some functional similarities among these proteins: mutations in both *crumbs* and *fat* appear to lead to disorganization and loss of polarity of ectodermal tissues [1,4], mutations in both *slit* and *crumbs* lead to abnormalities in the development of neural tissues [2–4] and laminin A modules of laminin also appear to be involved in promoting neurite outgrowth and epithelial polarization [4,5,11]. It seems possible that the laminin A-type modules of these proteins may be intimately involved in the interactions that render these proteins indispensable for ectodermal differentiation.

If this assumption proves to be correct it will be interesting to see whether some of the motifs conserved in the laminin A modules of *fat, crumbs* and *slit* and laminin A may serve as recognition sites for interactions that control ectodermal differentiaton, or these motifs are conserved simply because they are essential for the structural integrity/folding of these domains.

REFERENCES

[1] Machoney, P.A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P.J. and Goodman, C.S. (1991) Cell 67, 853–868.
[2] Rothberg, J.M., Jacobs, J.R., Goodman, C.S. and Artavonis-Tsakonas, S. (1990) Genes Dev. 4, 2169–2187.
[3] Knust, E., Ditrich, U., Tepass, U., Bremer, K.A., Weigel, D., Vassin, H. and Campos-Ortega, J.A. (1987) EMBO J. 6, 761–766.
[4] Tepass, U., Theres, C. and Knust, E. (1990) Cell 61, 787–799.
[5] Patthy, L. (1991) FEBS Lett. 289, 99–101.
[6] Patthy, L. (1987) J. Mol. Biol. 198, 567–577.
[7] Patthy, L. (1988) J. Mol. Biol. 202, 689–696.
[8] Patthy, L. (1990) Cell 61, 13–14.
[9] Patthy, L. (1987) FEBS Lett. 214, 1–7.
[10] Patthy, L. (1991) Curr. Opinion Struct. Biol. 1, 351–361.
[11] Edgar, D., Timpl, R. and Thoenen, H. (1984) EMBO J. 3, 1463–1468.