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# Laminin A-related domains in crb protein of *Drosophila* and their possible role in epithelial polarization

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It is shown that four domains of the integral membrane protein crb of *Drosophila* are homologous with five C-terminal domains of the laminin A chain and merosin. Since the latter domains of laminin A have been implicated in determining epithelial polarity, the homology may suggest a similar function for these domains of crb. Such a function would be consistent with the known importance of crb for organization of epithelia in *Drosophila*.

Laminin; Epithelial polarization; Protein evolution

## 1. INTRODUCTION

The *Drosophila* gene *crumbs* is essential for the organization of epithelia and is involved in the establishment and/or maintenance of polarity of epithelial cells. Mutations of *crumbs* may disrupt the organization of epithelia and in some cases cause cell death in these tissues [1].

The product of *crumbs* is an integral membrane protein with a large N-terminal extracellular part and a short intracellular region [1]. The extracellular domain was found to contain a total of thirty EGF-like domains in four clusters (19, 1, 2 and 8 repeats) separated by cysteine-poor regions that did not show significant similarity with other proteins. Since developmental processes based on cellular interactions are frequently mediated by proteins structurally related to growth factors or their receptors, it has been suggested that crb may function primarily via its EGF-like domains [1]. In analogy with the EGF-precursor the authors hypothesized that some EGF-like domains of crb may be clipped off and act as diffusible growth factors.

Here we show that the four cysteine-poor regions of crb are distantly related to domains present in the C-terminal part of laminin A chains. Since the C-terminal part of laminin A has been implicated in determining the polarity of epithelial cells, this homology raises the possibility that the cysteine-poor regions of crb (rather than or in addition to its EGF-like domains) may be directly involved in interactions organizing epithelia.

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## 2. METHODS

The protocol suitable for detection of distant homologies of mosaic proteins [2,3] has been applied to crb protein. The essence of the procedure is that the test sequence (a suspected domain of a mosaic protein) is searched for homologies with a library of consensus sequences (rather than real sequences) of modules of other mosaic proteins. This protocol permits detection of distant homologies not detectable with conventional search programs [2–4].

## 3. RESULTS AND DISCUSSION

The presence of EGF-like domains in crb raised the possibility that this protein also belongs to clan 1 of mosaic proteins, i.e. proteins assembled from class 1-1 modules [3,5]. This assumption implied that the four cysteine-poor domains of crb (residues 91–266, 1023–1206, 1245–1480 and 1557–1758) linked to the EGF-like domains may also correspond to class 1-1 modules and were therefore analyzed for homology with modules of other clan 1 mosaic proteins.

Comparison of the amino acid sequences of the four 170–230 residue long cysteine-poor regions of crb with our library of motifs/consensus sequences revealed that they show significant similarity with the 180 residue modules present in the C-terminal domain of laminin A chains [6,7] and merosin [8]. Significantly, most of the motifs characteristic of the five domains of laminin A and the laminin-homologue merosin [6–8] was found to be present in the four cysteine-poor regions of crb (Fig. 1).

The homology of domains of crb with the domains of the C-terminal globular region of laminin A-chain raises the possibility that their function may also be related. A number of functions have been assigned to the C-terminal repeats of laminin A-chain. Laminin

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LAM1    SSTNYNTLILNVKTQEPDNLLFYLGS  SSSSDFLAVEM  RRGKVAFLWDLGSGSTRLEFPEVSI
LAM2    LRPTVTQIVILFSTFSPNGLLFYLAS  NGTKDFLSIEL  VRGRVKVMVDLGSGPLTLMTDRRY
LAM3    ATFATKNSSGILLVALGKDAEEAGGA  QAHVPFFSINL  LEGRIEVHVNSGDGTSLRKALLHAPTGSY
LAM4    DVRKRLQVQLSIRTFASSGLIYYVAH  QNQMDYATLQL  QEGRLHFNFDLGKGRTKVSHPALL
LAM5    KVRLDLNITLEFRTTSKNGVLLGISS   AKVDAIGLEI  VDGKVLFHVNNGAGRITATYQPRAARAL
co      xxxxxxQIxLxxxTxxxxxLLxxLxx  gxxxDFxxLEL  xxGRVxxxxDxGxGxxxxxxxxxxggggg

CRB1    PMPIWDHSAISFRSCRGGEILAQQY  NKNSIVISVLND FLQISLAGPAVHGPNNRLDVKLPYQL
CRB2    EREEGYDINLQFRTTLPNGVLAFGTTGEKNEPVSYILEL  INGRLNLHSSLLNKWEGVFIGSKL
CRB3    AIRSILDISMFIRTREPTGQVFYLGTDPRKAPTKNIGDS YVAAKLHGGELLVKMQESGTPEAYTVGGQKL
CRB4    VEASPKQTLKPVIDIAFRLVLEVLC   LYDNVDGFFEIGVNGGRVTITWKLSALHFGESARFEKEN

LAM1    NNNRWHSIYITRFGNMGSLSVKEASAAENPPVRTSKSPGPSKVLDINNST LMFVGGLGGQIKKSPAVKVT
LAM2    NNGTWYKIAFQRNPKQGLLAVFDAYDTSDKETKQGETPGAASDLNRLEKD LIYVGGLPHSKAVRKGVSSR
LAM3    SDGQEHSISLVRNRRVITIQVDENSPVENKLGPLTEG    KTIDISN    LYIGGLPEDK ATPMLKMRT
LAM4    SDGKWHTVKTEYIKRKAFMTVDGQESPSVTVVGNATT      LDVERK   LYLGGLPSHY RARNIGTIT
LAM5    CDGKWHTLQAHKSKHRIVLTVDGNSVRAESPHTHSTS      ADTNDP   IYVGGYPAHI KQNCLSSRA
co      xDGxWHTIxxxRxxxxxxLxVxxxxxxxxxxxxxxxTgggggglDxxDggggIYVGGLPxxxgxxxxLxxxg

CRB1    LDNRWHTLQFKYEYGNLYLHVDRAASIFANSTYNSQFLTNQD                        IGYKDAIL
CRB2    NDSNWHKVFVAINTSHLVLSANDEQAIFPVGSYETANNSQPSFPR       TYLGGTIPNL  KSYLRHLT
CRB3    DNGYNHLIEVVRNQTLVQVKLNGTEYFRKTLSTTGLLDAQL          LYLGGPAPTR  ESLLGATT
CRB4    TDGEWSRIYLRAHNSKLEGGWKGWESMVDPTPAFSTDIDQAAFQSLIATSTQVYLGGMPESR QARGSTLSA

LAM1            HFKGCMGEAFL      NGKSIGLWNYIEREGKCNGCFGSSQNEDSSFHFDGSGYAMVEK
LAM2            SYVGCIKNLEI      SRSTFDLLRNSYGVRKGCGCALEPIQSVSFLRGGYVEMPP
LAM3            SFHGCIKNVVL     DAQLLDFTHATGSEQVELDTCLLAEEPMQS
LAM4            SIPACIGEIMV     NGQQLDKDRPLSA  SAVDRCYVVAQEGTFFEGSGYAALVKEG
LAM5            SFRGCVRNLRL SRGSQVQSLDLSRAFDLQGVFPHSCPGPEP
co              SFxGCIxNLxL gggggxxxxxxxxxxxxggxxxxxCxxxxx

CRB1         ILGNSFSGCLLDGPG LQFVNNSTVQNVVFGHC PLTPGPCSDHDLFTRL
CRB2         HQPSAFVGCMQDIMVNGKWIFPDEQDANISYTKLENVQSGCP
CRB3        *DSRDYFKGIIQDVKVSNGSLNLIVEMYSLNVTDVQVNAKPLGAVTIDRASVLPGEV
CRB4         QQGSQFKGCVGEARLGDLLLPYFSMAELYSRTNVSVQQKAQFRLNATRPEE
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Fig. 1. Homology of the five C-terminal domains of laminin A-chain (LAM1–LAM5) with the four cysteine-poor regions of Drosophila crb (CRB1–CRB4). The consensus sequence (co) comprising the motifs characteristic of the five laminin repeats are shown. Note that most of these motifs are present in the four repeats of crb.

fragment E3 corresponding to the fourth and fifth C-terminal repeats of laminin A-chain [7], mediates cell adhesion [9], and contains binding sites for collagen IV, heparin and basement membrane heparin sulfate proteoglycan [10,11]. Antisera against fragment E3 were found to block the neurite outgrowth promoting activity of laminin [12], to inhibit epithelial cell polarization [13], and to prevent interaction with type IV collagen [10].

The fact that the C-terminal repeats of laminin A are critical for epithelial cell polarity [13] is especially intriguing in view of the involvement of crb in epithelial organization. It is tempting to assume that the homologous domains of the crb protein and laminin A may be involved in similar interactions that are essential for the organization of epithelia.

The exact nature of the interaction critical for epithelial organization by fragment E3 of laminin A chain is only vaguely understood. Since E3 contains binding sites for heparin [11], basement membrane heparan sulfate proteoglycan, it is possible that integral membrane proteoglycans can function as receptors for the E3 domains. Nevertheless, in the case of at least some cell lines integrins are involved in E3-mediated adhesion: it has been shown that adhesion of certain cell lines to fragment E3 is inhibited by antibodies against the integrin $\beta_1$ subunit [9].

The role of integrins in determining epithelial polarization is clear from studies on their cellular distribution on keratinocytes. $\alpha_E$-$\beta_4$ integrins were found to be localized in contact with the basement membrane, whereas $\alpha_2\,\beta_1/\alpha_3\,\beta_1$ integrins were localized on lateral surfaces and are possibly involved in cell–cell adhesion [14]. In view of the involvement of $\alpha_2\,\beta_1$ and $\alpha_3\,\beta_1$ integrins in cell adhesion it has been suggested that (in addition to matrix components) a cell-surface ligand must exist for these receptors [14]. It is possible that this hypothetical cell surface ligand of $\beta_1$ integrins is related to the C-terminal domains of laminin A-chain and may thus be similar to crb.

This suggestion would harmonize with the known distribution of crb. This protein of Drosophila shows a

pronounced polarity in its cellular distribution: it is localized on the apical membranes of epithelial cells at the borders between cells [1]. The concentration of crb at borders where cells make contact with each other is compatible with its involvement in cell–cell interactions.

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