DEVELOPMENTAL REGULATION OF A VERTEBRATE HOMOLOGUE OF THE DROSOPHILA DLG TUMOR SUPPRESSOR GENE

Philippe GORRY, François VAUTIER, Valérie LE MORVAN, Pierre COSTET and Jean-Yves. DANIEL
Laboratoire de Biologie Cellulaire, Faculté de Médecine, University V. Ségalen, 33076 Bordeaux, France

Introduction

A fundamental goal in cell biology is to understand the structural basis of cytoarchitecture and cell shape in highly-specialised cells such as epithelial cells and neurones. Three main types of cell junctions occur in most vertebrate tissues: desmosomes, tight junctions, and gap junctions. In the epithelia of vertebrates, tight junctions form a belt around the cell, maintaining apical-basal cell polarity. Some components of tight junctions have been identified (Figure n°1). However critical tests of the developmental functions of tight junctions have not yet been reported.

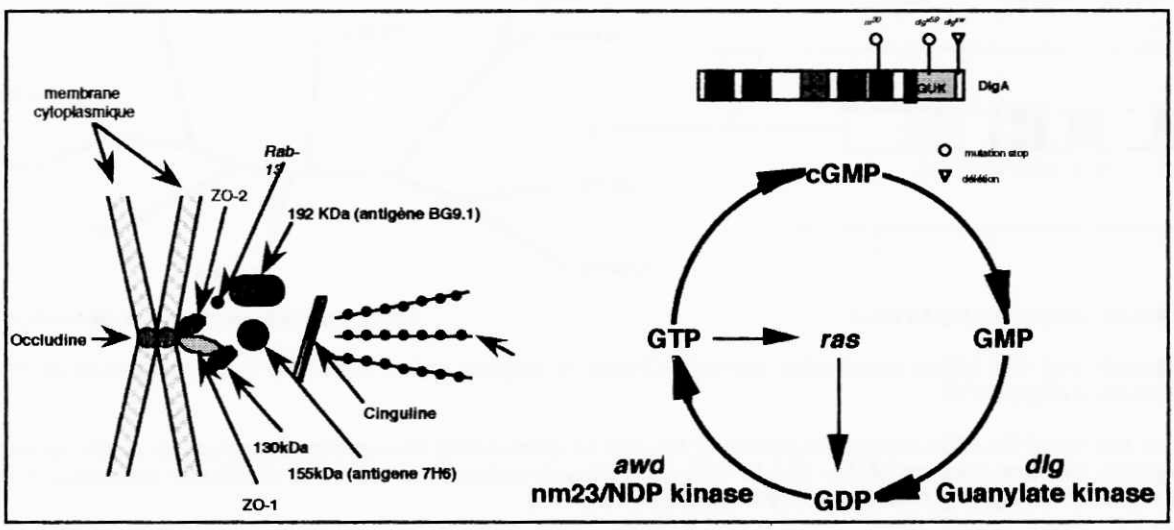

Figure n°1: Zona Occludens

Figure n°2: dlg mutations and cGMP metabolism

In Drosophila, mutations in some genes cause either hyperplastic or neoplastic overgrowth of various proliferating cell populations in the embryonic, larval and adult stages: these genes have been classified as tumor suppressor genes.

Mutation in the lethal(1)discs large (dlg) gene cause neoplastic imaginal overgrowth. It encodes a 960 aa protein DlgA that is expressed in most epithelia. The C-terminal of DlgA show strong homology to yeast guanylate kinase (GUK), an enzyme that transfers a phosphate group from ATP to GMP. DlgA also contains a SH3 domain, which is found in many membrane-associated signal transduction proteins (Figure n°2). The N-terminal half of the molecule contains 3 copies of a 91 aa motif called PDZ/DHR (GLGF). The PDZ repeat might be a protein-binding domain since interaction between NMDA receptor subunits and one of the PDZ domain of the postsynaptic density protein PSD95 have been demonstrated to interact.

dlg belongs to a growing family of gene products referred to MAGUKs (membrane-associated guanylate kinase homologs); some of them have been associated with cell junctions in vertebrates. ZO-1 is a major component of epithelial and endothelial tight junctions. It shows clear homology to DlgA but differs from it in the presence of a large proline-rich C-terminal extension. The second mammalian MAGUK is ZO-2, another protein component of epithelial tight junctions. Finally it has been demonstrated that *lin-2*, which is a component of the let-23/EGF signal transduction pathway in C.elegans, encodes also for a MAGUK protein (Figure n°3).

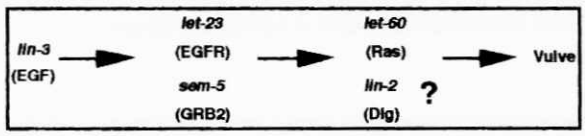

Figure n°3: ras signalling pathway during vulval induction in C. Elegans

Results

In the course of a chromosomal walking in the Friedreich ataxia chromosomic region 9q13-q21, the X104 sequence was isolated by exon trapping and characterized. Using single-strand conformation polymorphism SSCP procedure, mutations in affected patients were search in the coding sequence of the gene, but no causative mutations were found, suggesting that the gene was not candidate for Friedreich ataxia.

The X104 gene was further identified as the human homolog of dog ZO-2 tight junction protein during a genome data bank survey.

Using the human ZO-2 full length cDNA clone pJT5 as a probe, mouse ZO-2 cDNA was cloned from a day 10.5 mouse random library under high stringency hybridization condition. Two overlapping clones, pGB81 and pGB83 encoding the mouse ZO-2 were isolated (Figure n°4). DNA sequence analysis indicates that mZO-2 is a member of the Drosophila *dlg* tumor suppressor gene MAGUK family with 3 PDZ domains, SH3 and GUK domains, with strong overall homology, and 63 to 74% identity, to human ZO-2.

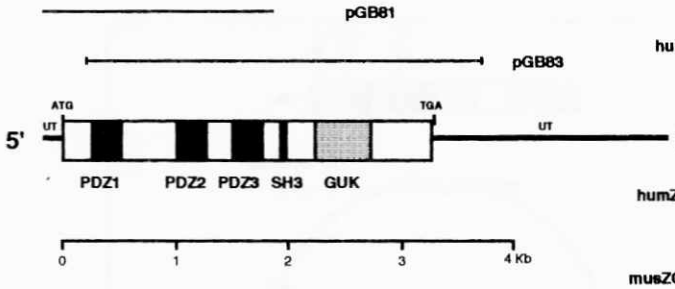

Figure n°4: Mouse cDNAs encoding for ZO-2

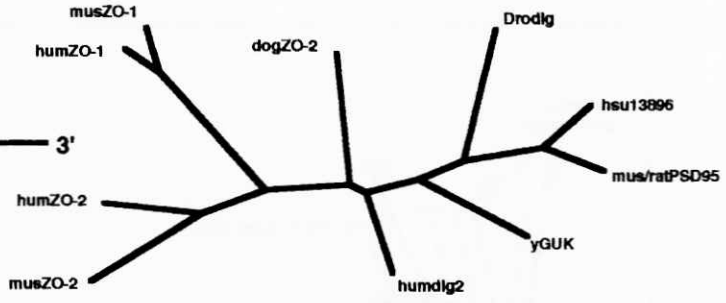

Figure n°5: Phylogenetic tree of the MAGUK family

A phylogenetic tree was further constructed using the Clustal IV program on the basis of a matrix calculated by Prettyplot with GCG package (Figure n°5).

We have examined the RNA expression pattern of the mZO-2 gene during mouse embryogenesis by *in situ* hybridization. Preliminary data on tissue sections of day 12.5 to 18.5 dpc embryos suggest that mZO-2 is specifically expressed in the gut and tightly regulated during lung and kidney organogenesis (Table n°1).

	14.5 d.p.c.	16.5 d.p.c.
Heart	-	-
Liver	-	-
Lung	+	+++
Kidney	+	++
Colon	+++	+++

Table n°1:

Discussion

The *in situ* hybridization data suggest a specific role for mZO-2 during mouse embryogenesis. However analysis of the expression pattern of other members of the MAGUK family, especially ZO-1, is required to appreciate the extend of the redundancy between these two tight junction proteins.

We would like to ask if tight junction proteins play a key role in cell shape and cell proliferation during vertebrate development. New technologies of mouse genetics are now being used to assess the functions of mammalian genes in vivo.

The creation of a null mutation in the mouse ZO-2 gene by homologous recombination *in vivo* will be of importance since no protein component of the tight junction has been knocked out in a vertebrate as yet.

References

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