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Providencia may help find a function for a novel, widespread protein family

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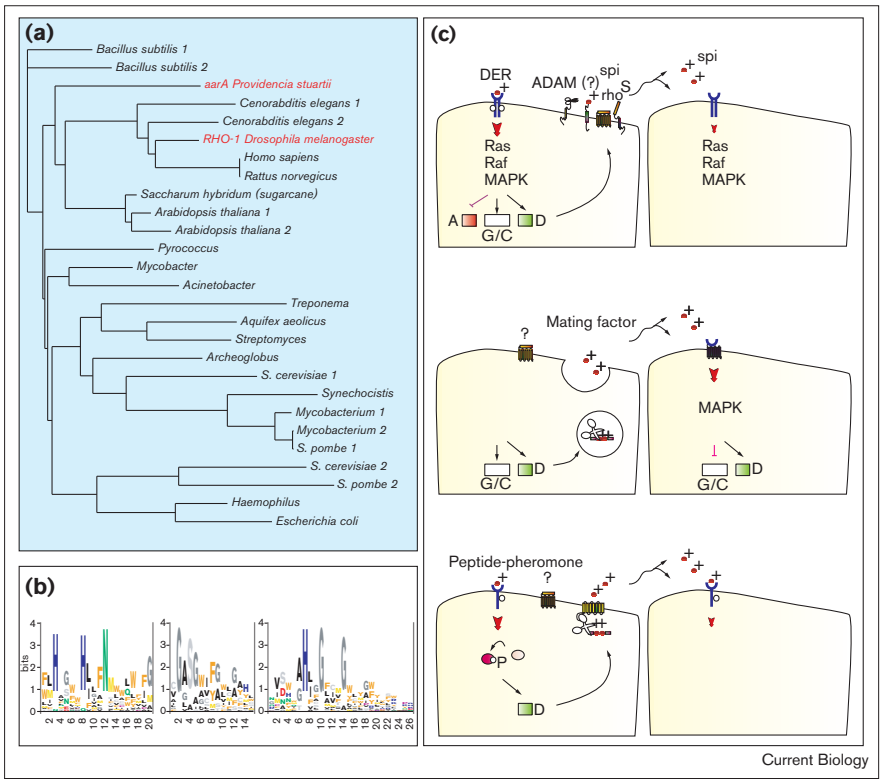
The seven transmembrane domain protein Rhomboid (RHO) is a key component of EGF-receptor signalling in *Drosophila*. Genetic evidence indicates it is needed in the activation of the TGFα-like ligand of the *Drosophila* EGFR (DER), Spitz (SPI). Most of the pathway components, including DER and a transmembrane, inactive form of Spitz, have a broad distribution. The limiting step for activation of the pathway is thought to be Spitz proteolytic release (reviewed in [1]). Like mammalian TGFα, SPI is likely to be cleaved by an ADAM-family metalloprotease, but no evidence has been obtained for this in flies. Instead, Star (S), a single-pass transmembrane protein, and RHO have been implicated in SPI activation. The mechanism underlying SPI activation by RHO and S is not known, but neither is directly required for SPI proteolytic cleavage [2].

The puzzling observation that removal of *rho* (now *rho-1*) has little or no effect on eye development [3], despite a strong requirement for active SPI, was recently resolved when a new member of the *rho* family was found which is expressed specifically in the eye. One of the first fly mutations affecting eye development to be isolated in the laboratory, *rhoughoid*, was found to uncover a new *rhomboid*, named *rhomboid-3*, that accounts for RHO function during eye development [4]. Additional *rho*-like sequences were also reported in the *Drosophila* genome [4], in *C. elegans*, rat and human [5]. Moreover an entire protein family comprising 47 entries has been assembled at the InterPro database (Integrated resource of protein domains and functional sites, accession number IPR002610). Entries include archaea, Gram-negative and Gram-positive bacteria, the two major yeasts (2 entries each), *Arabidopsis* (5), sugarcane (1), *Caenorhabditis elegans* (4), *Drosophila* (8), rat (1) and human (1). Most of the identified sequences predict multi-transmembrane domain proteins. The conserved region spans the transmembrane domains, where a stretch corresponding to domains 2–3 in Rho-1,2 and 3, respectively, contains three strongly conserved histidyl and one asparagyl residue in combination with a GASG motif. Figure 1a,b shows the most conserved residues within the RHO domain and a phylogenetic tree based on the alignment of this region for representative entries. In contrast, the amino terminus is highly divergent already within the *Drosophila* Rhomboid group. The fact that RHO-like proteins are represented in such diverse phyla implies a more general cellular role than EGFR activation. The conservation of key amino acids within the transmembrane domains could correspond to a catalytic site or channel [4] with similar function in proteins with otherwise divergent roles.

An observation that could have profound implications for understanding the function and evolution of this novel protein family has so far escaped attention. The only other member of the *rho* family that has been genetically characterised is the *aarA* locus of *Providencia stuartii*, a gram-negative bacterium responsible for urinary tract infections in humans [6,7]. Most bacteria possess means of cell-to-cell signalling to sense and monitor population density. The best studied phenomenon is known as quorum sensing and was first identified in bioluminescent symbiotic bacteria. The costly production of luminescence is suppressed in free-living bacteria and triggered only when a certain population size (quorum) is reached within the host. Since this discovery a great number of molecules and signalling systems have been elucidated that are deployed to regulate a variety of cellular events [8]. Most relevant to this work, oligopeptides were among the first class of prokaryotic communication molecules to be discovered. By analogy with many eukaryotic signalling molecules, oligopeptide pheromones are synthesized as large precursor proteins which are post-translationally processed into smaller active units, transported outside the cell and recognised by surface receptors belonging to two-component protein kinase regulatory systems (Figure 1c). This signalling paradigm has so far been reported only for Gram-positive bacteria, but is likely to exist for Gram-negatives as well, as oligopeptide-receptor-like molecules have been found in some Gram-negative bacteria [8].

In *Providencia stuartii* the accumulation of an extracellular factor of unknown nature (acetyltransferase repressing factor or AR-factor) regulates a chromosomal acetyltransferase (*aac(2′)-Ia*) in a density-dependent manner. The *aarA* locus was identified in a search for regulatory loci controlling *aac(2′)-Ia* expression [7]. Its sequence predicts a seven transmembrane domain protein with a significant Rhomboid-family signature. Strikingly, the genetic evidence obtained in *Providencia* suggests *aarA* involvement in the biosynthesis or export of the activating signal [6], just like the *Drosophila* RHOs are required for the activation of SPI. If multi-transmembrane domain proteins of the Rhomboid family were proved to be involved in cell-to-cell communication in such different organisms as *Drosophila* and

Figure 1



(a) Relationships among Rhomboid domains of representative protein family members represented as a phylogenetic tree. The RHO domain of proteins included in the protein family database (PF01694) was aligned with ClustalW and analysed with Protdist (from the Phylip package, see Supplementary material). Branch length represents the distance between the various groups as calculated by Protdist. The entries discussed in the text are in red. **(b)** Conserved amino acids within the RHO domain. The ClustalW alignment mentioned in (a) was submitted to Blockmaker. The Block maker server (blockmaker@blocks.fhcrc.org) finds blocks in a group of related protein sequences. Blocks are short multiply aligned ungapped segments corresponding to the most highly conserved regions of proteins. The three blocks shown represent the most conserved regions within the RHO domain, the size of each amino-acid residue is directly proportional to its conservation. **(c)** Comparison of cell-to-cell communication strategies in (top) *Drosophila* eye development, (centre) yeast reproductive cycle and (bottom) peptide-mediated quorum sensing in bacteria. Signal transduction mechanisms are extremely simplified. A schematic output of the signal is represented in the boxes, where A stands for apoptosis, G/C for cell growth/division and D for differentiation. The ABC transporter complex is believed to be responsible for processing and export of peptide signals in bacteria and is represented as a multidomain transmembrane complex. In all panels RHO-family proteins are shown as seven transmembrane domain proteins, in orange.

Providencia, it would be tempting to speculate about their role in the evolution of multicellularity, as cell communication had to be one of the central problems to solve for early multicellular organisms. To fully understand the biological function of the RHO protein family a number of key questions remain to be answered. First, what is the biochemical function of RHO proteins? The paucity of functional information available on RHO family members means that we do not yet know if the correlation of structure and function between *Drosophila* and *Providencia* RHOs is just a coincidence or part of a trend. In addition to bacteria, yeast is known to deploy peptide pheromones for cell signalling during sexual reproduction. Again, the peptide is synthesized as a larger protein that is then post-translationally cleaved and exported to be recognised by a specific receptor. Interestingly, the signal transduction pathway activated by this receptor is a variant of the MAPK pathway, that used by the *Drosophila* EGFR. Elucidating the role of the newly identified RHO-like protein in the two major yeasts and in other model systems, will be crucial for unravelling the function of this widespread protein family.

Supplementary material

Supplementary material including the alignment of the RHO domain proteins is available at http://current-biology.com/supmat/supmatin.htm.

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