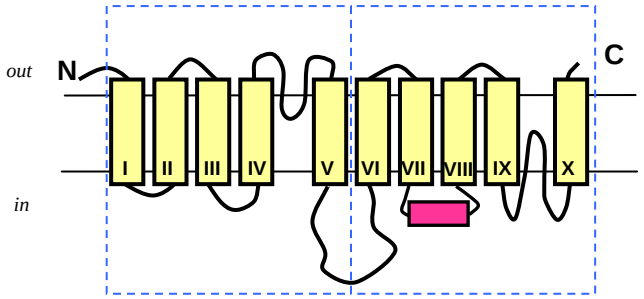
Molecular Microbiology: *Structural classification of membrane proteins*

Membrane proteins

The membrane surrounding biological cells functions like a 'fire wall' that protects the cell interior against the hostile environment. Proteins residing in the membrane open up 'ports' to allow necessary communication of the cell with the environment. Membrane proteins are present in all living organisms in large numbers. Many of these proteins are involved in transport of substances across the membrane, which is essential for proper functioning of a living cell. Transporter proteins are found in cells ranging from bacteria to mammalian cells and constitute about half of all transport proteins. They are involved in important cellular functions; besides the uptake of nutrients, secondary transporters are involved in processes like the excretion of metabolic end-products, metabolic energy generation, pH homeostasis, defence mechanisms, neurotransmission and brain function, and many more. Knowledge of these proteins is essential to understand the functioning of the cell as a whole.

Membrane proteins of the plasma membrane share a similar architecture; they fold as bundles of α-helices that are oriented perpendicular to membrane. The transmembrane α-helices are embedded in the hydrophobic interior of the membrane, while the connecting loop regions are sitting in the hydrophilic water phase. Occasionally, a loop region may fold back in between the transmembrane helices, so-called re-entrant loops.



Structural model of a membrane protein. Boxes represent transmembrane α-helices. Loop regions between TMSs IV and V and between TMSs IX and X represent pore loops that fold back in between the transmembrane helices. The loop in between TMSs VII and VIII folds into an amphipatic α-helix. Dashed boxes represent homologous N- and C-terminal domains. The protein shown is a citrate transporter of the pathogen *Klebsiella pneumoniae.*

Research question

In spite of their similar architecture, membrane proteins come in a great diversity. For instance, a classification scheme based on amino acid sequence identity shows over 100 families of secondary transporters, a type of transporters that translocate substrates across the membrane driven by ion gradients. It is unlikely that these families represent the same number of structures and mechanisms, but rather many families may be evolutionary related but have diverged so far that this is not recognized in the amino acid sequences anymore. On the other hand, recently reported crystal structures of 8 secondary transporter proteins revealed 7 different structures.

Solution

To bring some order in the great diversity of membrane proteins, it would be much better to have a classification based on structure rather than amino acid sequence. We have developed the MemGen classification system that clusters families of membrane proteins into structural classes based on hydropathy profile analysis. The rationale is that during evolution the structure of a protein, here represented by the hydropathy profile, is much better conserved than the amino acid sequence. Hydropathy profiles are proposed to report a specific fold and, therefore, are able to detect distant relationships between protein families. The analysis compares averaged hydropathy

profiles of families of membrane proteins and classifies the proteins in the families as having similar or distinct folds. The classification groups unrelated families of membrane proteins into structural classes.

Projects

We have many projects that focus on the validation of the MemGen classification scheme. An exhaustive search of the NCBI protein database resulted in slightly over 2000 unique sequences in structural class ST[3] of the classification that are distributed over 59 subfamilies and 32 families. All sequences in the class are postulated to share a common evolutionary origin and folding, even though sequence identity between many members cannot be detected anymore. The projects focus on determining the (common) fold of the proteins and other structural properties by biochemical techniques.

Techniques

Transporter genes are cloned by PCR and expressed in suitable host cells (usually strains of *Escherichia coli*). A variety of techniques is available to assess the folding of the protein in the membrane, all starting with genetic manipulation of the coding gene like site directed mutagenesis and gene fusions. Locations of specific sites in the protein are determined using reporter enzymes and/or labelling techniques. Detection of labelling is based on fluorescence or mass spectrometry. Functional analysis of the gene product is done by transport assays which involve the isolation of cytoplasmic membranes from cells expressing the gene and the use of radioactive tracers.

Information

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