Diversity in transport systems

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Summary

Each living cell is surrounded by a membrane which constitutes a barrier to water soluble compounds. In this way the information for the synthesis of metabolites and catalysts, which lies on the DNA, and the metabolites and catalysts themselves used by the cell for its replication, are held together in a restricted area, bordered by the membrane. In Chapter I it is argued that this property of biomembranes has been very important in the evolution of life. Evolution in the sense of Darwin, driven by random mutations of the information carrying molecules and subsequent selection of the fittest entities, can not proceed unless the effect which such a mutation has on metabolism is of benefit or disadvantage preferentially to the information carriers encoding it. Darwinian evolution in a pool of freely diffusing metabolites and information carriers is impossible. Consequently, diffusion of information carriers and metabolites has to be restricted, for example by binding to surfaces or by containment in a restricted three-dimensional area. The invention of the membrane by life has been of prime importance in its conquering of the three-dimensional world.

For growth and replication the cell needs to scavenge nutrients from its environment. The same impermeable membrane which was so beneficial for the evolution of life hinders the use of large water soluble molecules from the environment. It is therefore likely that life's origins were of autotrophic nature, meaning that it originally fixated simple membrane permeable molecules like CO or CO_2. With the development of protein chemistry proteinaceous transport systems became available which left the impermeability of the membrane intact, except for certain molecules to be taken up or excreted by the cell. The remaining chapters of this thesis deal with such transport systems.

In chapter II a method is described to measure the intracellular pH in bacteria by the use of a fluorescent pH indicator. The pH is an important factor in determining the activity of enzymes in the cell. The cell has developed several transport mechanisms to maintain the pH at a constant, for many bacteria usually neutral value. The method described here offers, in addition to the steady state methods developed by others, a possiblity to study such pH-stating mechanisms in the fast kinetic time-domain.

This method for measuring the cytoplasmic pH revealed the existence of a novel transport mechanism in *Lactococcus lactis*, responsible for the active excretion of the fluorescent probe from the cytosol. Its characterization is described in chapter III. Efflux via this transport system is probably driven by ATP or another high-energy phosphate intermediate. The physiological role of this transport system remains obscure. The pH indicator does not resemble any known physiological compound, while a mutant with decreased efflux rate

displayed no other obvious phenotype. One of our hypotheses is that it plays a role in general antibiotic resistance, although we found no antibiotic substrate. Heterotrophs like *Lactococcus lactis* live in competitive ecosystems, in which toxins are excreted by other bacteria, fungi, and host plants or animals as a part of defensive or offensive strategies. Bacteria would acquire resistance to toxins with cytoplasmic target sites by excreting them.

In chapter IV we describe another excretion system which clearly confers resistance to multiple drugs in *Lactococcus lactis*. The substrate range of this system suggests that there is a relation, at least at the level of the substrate recognition mechanism, with the multidrug resistance excretion pumps from eukaryotes. These eukaryotic pumps play ,among others, a role in the resistance of cancer cells to several unrelated chemotherapeutical drugs. It is our expectation that a more detailed characterization of this system in *Lactococcus lactis* will aid in understanding the molecular mechanism of multidrug resistance.

In chapter V a study of osmoregulatory mechanisms in *Lactococcus lactis* is described. Osmoregulation is the adaptation of organisms to changes in water activity, caused by e.g. changes in concentration of dissolved compounds, or by drying. Water plays an important role in the conformation and structural stability of proteins, and its activity in the cytoplasm therefore determines the catalytic activity of enzymes. An organism may adapt the water activity in the cytoplasm by changing intracellular concentrations of so-called osmolites; compounds with high solubility which have no deleterious effects on metabolism. Osmolites are synthesized by the organism, or accumulated from the environment. In the chapter two transport systems for the osmolites proline and betaine are described.

Finally, chapter VI describes a pH-regulatory and energy transducing system in the lactic acid bacterium *Lactobacillus buchneri*. This system is based on the rapid conversion of histidine to histamine by a cytoplasmic histidine decarboxylase with concomitant electrogenic exchange of extracellular histidine and intracellular histamine by a transport system. In this way both a pH gradient and an electrical potential difference across the membrane are created. A survey of the literature revealed that many bacteria display vigourous amino acid decarboxylation and amine excretion activity at low external pH values. This activity appears to protect the cell against cytoplasmic acidification, and may also offer an alternative energy source to glycolysis. Electrogenic exchange of the amino acid and the amine as demonstrated in this chapter may play an important role in pH regulation and energy transduction by amino acid decarboxylation.