Research at the Division of Plant Biochemistry involves membrane structure and function, signal transduction and gene regulation. This includes basic research on the plant plasma membrane, its transport capacities and its role in signal transduction. It also includes research on membrane protein complexes involved in the bioenergetic reactions of photosynthesis and respiration.

The plasma membrane proton pump

Plants actively extract nutrients (NPK etc) from the soil, and actively transport products of photosynthesis (such as sucrose) to those parts of the plant that do not carry out photosynthesis (such as the roots). The key enzyme in these processes is the plasma membrane H⁺-ATPase. This enzyme pumps protons across the plasma membrane and thereby generates the proton and electrical gradient that is the driving force for secondary active transport executed by carriers and chan-
nels. The proton pump also has a role in signal transduction by generating signals in the form of transient changes in cytosolic pH and membrane potential. Since the plasma membrane H\textsuperscript{+}-ATPase is a key player in many processes we are investigating its regulation. We have shown that proton pumping is activated by phosphorylation of a threonine residue in the C terminus of the enzyme and concomitant binding of a regulatory protein (a 14-3-3 protein) to the phosphorylated threonine residue. Future studies will focus on the 14-3-3/H\textsuperscript{+}-ATPase interaction, the protein kinase phosphorylating the H\textsuperscript{+}-ATPase, and on other proteins involved in signal transduction pathways that regulate the activity of the H\textsuperscript{+}-ATPase.

(Prof. Christer Larsson, Prof. Marianne Sommarin)

Aquaporins channel water through membranes

Plant roots constantly absorb water from the soil. To enter the water-conducting vessels and each individual cell of a plant, the water molecules must cross membranes. Movement of water across biological membranes was until recently thought to be accomplished only by diffusion of water molecules across the lipid bilayer. This has turned out to be wrong, and a group of channel-forming, integral membrane proteins that specifically allow water molecules to pass through membranes has been identified. These water channel proteins are termed aquaporins. In Arabidopsis, more than 30 genes code for aquaporin homologues. Some of these genes code for highly abundant, constitutively expressed aquaporins and some genes are known to be temporally and spatially regulated during development and in response to drought stress. We have shown that aquaporins may constitute up to 30% of the total protein of both the plasma membrane and the vacuolar membrane, and that water flow through an aquaporin may be regulated by phosphorylation of the protein. Future projects will deal with regulation of expression of all the genes that code for aquaporins, and with regulation of water transport activity at the protein level.

(Dr Per Kjellbom, Dr Urban Johanson, Prof. Christer Larsson)

Aquaporin structure. Aquaporins have an internal homology, the N-terminal half being homologous to the C-terminal half, although the two halves are inserted inversely in the membrane (helices 1, 2 and 3 correspond to helices 4, 5 and 6, respectively). Water flow through aquaporins is regulated by phosphorylation of conserved serine residues located in the first cytosolic loop and in the C terminus.
Lipid-based signal transduction

Plants respond to a variety of environmental signals. Yet, for plants, the molecular mechanisms by which the information is received, transduced, and converted into specific intracellular responses still remain elusive. Thus, an entire signalling pathway from environmental signal to intracellular physiological response has to date not been demonstrated for any plant cell. We are focussing on the role of the phosphoinositide system in signal transduction. We have identified, characterised, and cloned several of the enzymes involved in phosphoinositide metabolism, and demonstrated that salt stress rapidly and dramatically increases the level of a key lipid, phosphatidylinositol 4,5-bisphosphate, in this signal transduction pathway. Future studies will focus on the regulation of the enzymes involved and on identifying interacting components, e.g. in the cytoskeleton, in order to understand the complex signalling involving phosphoinositides. This knowledge is required for understanding how plants react and adapt to the environment and for the development of stress-tolerant plants and of plants as an environmentally-safe production system for new or modified products.

(Prof. Christophe Pical, Prof. Marianne Sommarin)

Photosynthesis under stress

Light is of course essential for photosynthesis. Yet, high light intensities are a severe stress for plants causing oxidative damages - what we call photoinhibition. We are studying systems that have evolved to protect plants against photoinhibition. Carotenoids and particularly the xanthophylls are important components in some of the protective mechanisms. The work is focused on the regulation of a protective process called the xanthophyll cycle, on the enzymes involved in the interconversion of xanthophylls, and on the role of individual xanthophylls in protection. One enzyme, violaxanthin de-epoxidase, has been isolated and cloned. Attempts are now made to overproduce this enzyme for structural studies.

(Dr Hans-Erik Åkerlund)
Redox signalling by a two-component pathway. A redox sensor is a membrane phosphoprotein that becomes phosphorylated on a histidine side chain (His) when oxidised or reduced by components of an electron transport chain. Its substrate, the redox response regulator, is a sequence-specific DNA-binding protein that becomes phosphorylated on aspartate (Asp), regulating transcription. Two-component redox signalling links photosynthesis and respiration with transcription in bacteria: we predict that this mechanism has been conserved in the evolution of eukaryotic cells, providing a function for the genetic systems of chloroplasts and mitochondria.

The xanthophyll cycle of higher plants. DHA, dehydroascorbate; Asc−, ascorbate; AscH, ascorbic acid; sVDE and bVDE, soluble and bound violaxanthin de-epoxidase; ZE, zeaxanthin epoxidase.

Structural, regulatory and evolutionary aspects of phosphoproteins and redox signalling in chloroplasts, mitochondria and photosynthetic prokaryotes

"Redox signalling" may be defined as the regulatory coupling between biological electron transfer and gene expression. Electron transfer is essential for transduction of energy in living cells, including transduction of light energy in photosynthesis. Redox signalling controls gene expression at every level, from DNA replication and transcription to post-translational modification of protein structure and function. Redox control of protein phosphorylation in chloroplast thylakoid membranes exerts its regulatory effects by means of specific effects on protein structure and on molecular recognition. We find that rapid redox control of chloroplast transcription determines the stoichiometry of the two photosystems in the thylakoid membrane. This discovery is consistent with the proposal that redox control of transcription during photosynthesis and respiration has determined the composition of chloroplast and mitochondrial genomes. Future work will be directed at finding and describing the regulatory components that have been conserved in the transition from prokaryote to eukaryotic organelle, in elucidating their regulatory roles, and in providing a structural description of their functional effects.

(Prof. John F. Allen)