Survival of plants under low oxygen conditions

Plants and animals are obligate aerobic organisms. Oxygen is essential as a terminal electron acceptor in respiration and is also required in many other biochemical reactions. Adverse weather conditions such as heavy rainfall or flooding and melting of snow in cold climate regions, can cause soil waterlogging and restrict oxygen supply to living organisms. It was essential to evolve survival mechanisms for these temporarily adverse conditions and prokaryotic and eukaryotic organisms have achieved this using a variety of different strategies (1, 2).

Plants are confronted with peculiar problems, being highly differentiated organisms, anchored with their roots in the soil, and having no central nervous system, nor an oxygen transporting blood stream like animals. Plants show an amazing variability in the capacity to tolerate low oxygen stress. Aquatic plants are morphologically and physiologically very different from land plants, but even land plants themselves show a lot of variation in low oxygen tolerance.

It is clear that the response to low oxygen stress in plants is complex and involves many physiological as well as morphological changes. A lot of research effort has been invested in understanding the physiological basis of the low oxygen response. This work was often carried out on plants with varying degrees of tolerance to the stress, which often complicated interpretation and extrapolation of the data.

The aim of our laboratory is to understand the molecular basis of the response to low oxygen conditions, with the long-term aim of designing strategies to improve flooding-tolerance of important crop plants. We have chosen Arabidopsis as a model because of the molecular biology resources available for this plant.

Molecular changes in plants upon low oxygen treatment

One way to understand the physiological response of plants to low oxygen conditions is to identify the genes that are activated under these conditions. Two-dimensional electrophoresis (2D-EF) showed that about 20 anaerobic proteins (ANPs) are expressed in maize roots under low oxygen conditions (3). In contrast to other stresses, the function of many of these genes is known. ADH (alcohol dehydrogenase) was the first anaerobic gene identified (4) and has served as a model ever since (5).

The function of many other anaerobic proteins (ANPs) were identified by proteome analysis, a combination of 2D-EF, pulse labelling, immunology and protein microsequencing techniques. Most of the ANPs are involved in sugar metabolism and fermentation (6). Recently, 2D-electrophoresis was combined with the power of mass spectrometry to identify another 46 proteins involved in the acclimation response of maize roots, showing that proteins from a wider range of cellular functions are also induced by low oxygen stress (7).

Obviously, metabolic adaptation is important during the acclimation period to low oxygen conditions. This may not be surprising, since oxygen is an important substrate of cell metabolism. A reduction in oxygen supply leads to an 18-fold reduction in ATP production, so plant roots have to compensate for this loss by accelerating sugar metabolism and glycolysis. Induction of fermentation pathway enzymes is required to regenerate NAD+ reducing power.

Can flooding tolerance be obtained by metabolic engineering?

Despite this extensive knowledge, it remains unclear what determines the difference between flooding-sensitive and flooding-tolerant plant species. This question is very difficult to answer, because of large morphological differences between different plant species. There are few examples of tolerant and intolerant varieties within one species, so a metabolic engineering approach using transgenic plants might be the only way to address this question.

There is an assumption that plant species with a more active ethanol fermentation compared to lactic acid fermentation metabolism are more flooding-tolerant (8, 9). Unlike lactic acid, ethanol does not cause cytosolic acidosis, which causes cell death under low oxygen stress. But recent data suggest that the origin of acidosis might not be purely metabolic, but could involve proton pumps (9, 10).

Most higher plants induce ethanol, lactic acid and alanine fermentation pathways de novo during low oxygen stress. These pathways consist of only one or two enzymatic reactions, making them a relatively easy target to carry out metabolic engineering. We have cloned the genes involved in these three pathways in Arabidopsis and produced sense and antisense overexpressing plants, in order to find out their contribution to flooding tolerance.

Using a survival assay for low oxygen conditions (11), we were able to show that overexpression of pyruvate decarboxylase, the first enzyme of the alcohol fermentation pathway, improves survival in Arabidopsis. We are currently investigating how overexpression of the entire alcohol fermentation pathway, and down-regulation of lactic acid fermentation, can further improve survival.

Gene expression under low oxygen conditions

We have mapped the promoter elements of the ADH1 gene with the aim of identifying the factors regulating the anaerobic response genes. The anaerobic response element (ARE) was identified in the maize and Arabidopsis ADH1 promoters (12, 13) (Fig. 1) and is a bipartite element consisting of GT- and GC-motifs which are both crucial for gene expres-
Molecular Basis of the Anaerobic Response in Plants (contin.)

**Different stages in the low oxygen response?**

Treatment of plants with the protein synthesis inhibitor cycloheximide during anaerobic treatment prevents induction of ADH1 mRNA. This indicates that protein synthesis is required prior to induction of anaerobic genes such as ADH1. AtMYB2 induction does not require protein synthesis (15). Cycloheximide treatment caused up-regulation of AtMYB2 mRNA levels under both control and low oxygen conditions.

This has been reported for several other signal transduction components and could mean that cycloheximide acts as a stabiliser of mRNAs which normally turn over quickly (16). This could mean that AtMYB2 mRNA levels, and possibly other regulatory factors of the anaerobic response, are regulated post-transcriptionally. Studying the regulation of AtMYB2 could therefore provide us with an understanding of how the anaerobic response is activated.

The first stage in the anaerobic response obviously consists of putting in place the regulatory machinery required for induction of the second stage genes, such as ADH1. This provides a metabolic adaptation to supply energy and metabolic components for survival of short-term low oxygen stress. The second stage could also help to establish a third stage in the response: the provision of longer term survival strategies.

One example in some plants is the development of aerenchyma, root cortical air spaces promoting air transport from shoot to root (17, 18). Aerenchyma formation requires the hormone ethylene. Amongst the genes induced by low oxygen conditions are ethylene biosynthetic enzymes (19).
would be detectable on 2D-gels. Other restrictions are the limited pH range of the first dimension isoelectric focusing gels, limiting detection of certain transcription factors with high pI values, and the inability to identify insoluble and down-regulated proteins.

Molecular biologists have been dreaming of a method to look at gene expression at the genome level, and the microarray technology allows this to be achieved (20, 21). Up to 10,000 DNA samples (genomic DNA, cDNAs, oligonucleotides) can be spotted on one glass microscope slide using a robot and analysed with fluorescent RNA probes. We have recently started this approach with the intention of identifying regulatory factors involved in low-oxygen-induced gene expression.

A cDNA library was made from anaerobically-induced Arabidopsis roots, and 2 sets of microarray slides were prepared: a 3.5K array, containing 1,000 anaerobic cDNA clones plus 2,500 Arabidopsis EST clones, and a 10K array containing 10,000 random anaerobic cDNA clones. We screened the 3.5K array using mRNA from different time points during anaerobic treatment, and established a list of more than 200 genes that are up-regulated or down-regulated.

The list contains genes from a wide variety of cellular processes, including signal transduction components and transcription factors.

Microarrays will allow us to answer many questions about the anaerobic response in plants. We will be able to look at different time points of anaerobic treatment and compare the genes that are induced or repressed. We will also be able to identify the genes that are shared by other environmental stresses, by using mRNA probes from different stress treatments. By using RNA of cycloheximide-treated plants, we will be able to identify the earlier regulatory components that are stabilised by cycloheximide.

We will look at plants overproducing AtMYB2 and identify the genes that are affected by this factor. It might also be possible to identify quantitative and qualitative differences in the set of expressed genes in flooding-tolerant and flooding-sensitive plant species (provided there is enough cross-hybridisation) and identify key genes in determining tolerance.

The opportunities offered by microarrays are seemingly limitless. However, the real challenges have become the bottlenecks of computing and database management, and the question of how to exploit this sudden wealth of information. This will involve testing the relevance of several genes by producing lots of transgenic plants with sense and antisense over-expression constructs, or large scale screening of insertion mutant lines to find any mutant phenotype associated with a particular gene. The speed and reliability of the microarray approach can also be exploited to analyse transgenic or mutant lines. This should enhance our efforts to identify those genes relevant for the anaerobic response.

References

DEHYDRATION, SALINITY, WOUNDING
COLD
LOW OXYGEN
ARE

ABA

G-Box
(-214 to -208)
GT-Motif
(-159 to -149)
GC-Motif
(-147 to -143)
TATA-box
(-31 to -23)

Fig. 1