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268 Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects

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Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects

Organized by
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I N D E X

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POSTERS:

P R O G R A M M E

SALT TOLERANCE IN MICROORGANISMS AND PLANTS: PHYSIOLOGICAL AND MOLECULAR ASPECTS

Monday, November 11th

Welcome: R. Serrano & J.A.Pintor-Toro

Introduction: A. Läuchli
The social and scientific relevance of salt tolerance studies.

1st Session - Chairman: A. Läuchli
Physiology of salt tolerance in plants.

André Läuchli - Ion transport in higher plants under salt stress.

R.Gareth Wyn Jones - Physiology and genetics of salt tolerance in wheat.

Ulrich Lüttge - Ecological adaptation of CAM plants to salinity. A view on different levels of scaling from the biogeographical level to levels of whole plants, cells, membranes and molecules.

Christopher J. Lamb - Integration of plant stress responses into developmental programs.

L. Reinhold - Comparative studies of the functioning of various cellular membranes in halophytic and glycophytic plants and cell lines.

Eduardo Blumwald - Sodium transport in higher plant vacuoles: Biochemical and physiological characterization of the Na^+/H^+ antiport and the cation channel.

Tuesday, November 12th

2nd Session - Chairman: P.M. Hasegawa
Genes regulated by salt stress in plants.

P.M. Hasegawa - Developmental, hormonal and environmental regulation of the osmotin gene.

José A. Pintor-Toro - Salt stress induces genes in tomato.

V. Conejero - Evidence that plants are endowed with systems for coordinated responses elicited by agents ranging from viroids to salt.

Ralph S. Quatrano - The interaction between salt and abscisic acid in the control of gene expression.

Montserrat Pagès - Abscisic acid and water stress responsive genes in maize.

3rd Session - Chairman: W. Epstein
Bacterial models of salt tolerance.

E. Padan - Molecular physiology of Na^+/H^+ antiporters, key enzymes in adaptation of bacteria to high salinity and alkaline pH.

W. Epstein - Accumulation of ionic osmolytes in adaptation of *Escherichia coli* to elevated osmolarity.

Christopher F. Higgins - Osmotic regulation of gene expression: a role for DNA supercoiling and chromatin structure.

Wednesday, November 13th

4th Session - Chairman: A. Rodríguez-Navarro
Eukaryotic microorganisms models of salt tolerance.

Uri Pick - Regulation of glycerol synthesis and of Na^+ in the halotolerant alga *Dunaliella*.

Lennart Adler - Salt tolerance in yeasts: Role of glycerol.

Ramón Serrano - Isolation and characterization of genes of the yeast *Saccharomyces cerevisiae* involved in salt tolerance mechanisms.

A. Rodríguez-Navarro The sodium efflux system of *Saccharomyces cerevisiae*.

R.F. Gaber - Ion transporter genes in yeast.

INTRODUCTION TO THE MEETING

THE SOCIAL AND SCIENTIFIC RELEVANCE OF SALT TOLERANCE STUDIES

André Läuchli
Department of Land, Air and Water Resources
University of California
Davis CA 59616, U.S.A.

Salting our agricultural lands is a 6000 year old problem! History teaches us that between 4000 and 2000 B.C. the Sumerians ruined their land and culture in the valleys of the Euphrates and Tigris in Mesopotamia by their irrigation practices. In this century history has repeated itself in many parts of the world. The vast river systems in semi-arid regions with extensive irrigated agriculture not only supply the water but also the salts dissolved in it, and thus are major contributors to the salinity problems in these regions. It is estimated that 30 to 50% of the 230 million ha land under irrigation is presently affected by salinity. Salinity is a worldwide threat to agriculture. The economic costs of salinity damage to agriculture are rising. For example, the Colorado River in the southwestern United States transports annually about 10 million tons of salt to the main agricultural areas in the southwest of the U.S. and northwestern Mexico causing considerable declines in crop productivity. Economic losses in the fertile Central Valley of California are expected to increase possibly tenfold between 1980 and the turn of the century. In addition, there is the acute danger of a rise in toxic trace elements such as selenium in waters and soils, threatening wildlife habitats, and animal and human food crops in some areas. Examples are China and the western United States.

What are the salinity management options? Irrigated lands must have adequate drainage to avoid rising saline water tables and crop damage. However, installation of drainage systems is very costly. Improved irrigation efficiency will assist in decreasing the need for artificial drainage. Genetic approaches for developing economic salt-tolerant crops has considerable potential of making important contributions to food production in many countries, particularly where lands and waters contain

naturally occurring salinity and in coastal regions where seawater intrusion can create severe salinity problems.

In screening genetic lines for salt tolerance, a useful approach is to identify physiological markers for tolerance. A recent example from the research by Professors Dvorak and Epstein at the University of California at Davis will be given: the ratio potassium to sodium in the flag leaf was found to correlate well with plant performance of crosses between bread wheat and one of its salt-tolerant relatives under saline conditions.

PHYSIOLOGY OF SALT TOLERANCE IN PLANTS

ION TRANSPORT IN HIGHER PLANTS UNDER SALT STRESS

André Läuchli
Department of Land, Air and Water Resources
University of California
Davis, CA 95616, U.S.A.

The processes and characteristics of ion transport in higher plants have been investigated traditionally at the macroscopic scale of whole organs. More recently, higher resolution approaches at the subcellular level have been used increasingly, either with isolated cell organelles or membrane vesicles. In my laboratory, quantitative approaches with spatial resolution at the cell or tissue scale are emphasized. The primary focus is to use electron probe X-ray microanalysis, as well as determine the spatial distribution of ion contents and ion deposition rates at approximately the millimeter scale. An additional method in use is NMR-spectroscopy for determining compartmentation of phosphate and pH distribution between cytoplasm and vacuole. These methods are applied to how salt stress affects ion transport in the intact plant and how these effects are related to inhibition of growth.

By means of electron probe X-ray microanalysis the role of the shoot apical meristem of lettuce in controlling ion transport to the developing leaves and regulating their growth was investigated under salt stress. Ion concentrations in tissues basal to the meristem were influenced considerably under stress. In particular, total calcium was reduced in basal regions below the meristem and also in very young leaves. Salinization disturbs the nutrition of the shoot apical meristem which is involved in regulation of emergence and growth of leaves.

In collaboration with Professor Silk the spatial distribution of growth, ion contents and ion deposition rates are investigated in roots and leaves of plants under salt stress. Ion deposition rates are derived from the combination of spatial distribution data for growth and ion contents using the continuity equation. In the cotton root the selectivity of K deposition over that of Na was lost at high NaCl concentration but fully restored by supplemental calcium in the apical 2 mm region. In the shoot of sorghum, salt stress dramatically affected the spatial distribution of ions in developing leaves. Potassium increased greatly in a leaf which developed during salt stress but only in the region apical to the growth zone. Sodium, however, increased within the growth zone while salt stress decreased Ca in the growth zone to low, deficient levels. These changes in ion distribution and allocation are related to the spatial and temporal aspects of leaf growth.

Physiology and Genetics of Salt Tolerance in Wheat

R. Gareth Wyn Jones

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University College of North Wales
Bangor, Gwynedd, Wales

The availability of many chromosomally-engineered lines of hexaploid and tetraploid wheats and of hybrids between Triticum species and many wild wheat grasses, which have natural salt and drought tolerance, makes this material particularly attractive for the study of salinity and alkalinity tolerance in cereals. Further there is a major problem of salt stress in the field in many semi-arid third world country where wheat is a major crop.

In this presentation I will describe studies on hexaploid and tetraploid wheats and on a Thinopyrum bessarabicum-wheat hybrid utilizing material generated in a number of collaborating laboratories. Previous work has shown that a major trait (gene) for leaf K^+/Na^+ discrimination is located on the long arm of the 4D chromosome of hexaploid wheat. This trait is constitutive, effective at all concentrations and probably operates at the symplasm-xylem boundary of roots, affecting xylem loading, with possibly some influence on Na^+ efflux from cortical cells but no detectable effect on K or Na influx. In generating these results Dr. Gorham and my other colleagues were dependent on genetic stocks from Professor E. Sears, Dr. Colin Law, Dr. Lionel Joppa.

There are several results from our laboratory and other sources suggesting that K^+/Na^+ discrimination in leaves is an indicator of tolerance. Dr. Rana and his colleagues at CSSRI Kanal, India, showed that hexaploid wheats were more resistent to sodic soils that tetraploid (durum) wheats and related this observation to the higher Na uptake into the leaves of the latter type. Dr. Jan Dvorak in Davis using the 4A/4D langdon substitution line of Joppa as one parent and the Capelli line containing the ph mutants to promote crossing-over, as the other has produced several dozen recombinant lines containing fragments of the 4D chromosome introducted into the genome of a Capelli tetraploid wheat. These lines have been screened in Bangor for the introduction and expression of the L4D discrimination trait with about 15-18 lines coming out positive. We have sought to further test these lines in artificial sodic soils but have experienced technical difficulties with the preparation and maintenance of the soil substrate. In a successful trail with 1 pair of lines and hexa-and-tetraploid controls, the K^+/Na^+ transformed tetraploid line showed markedly improved grain yield in soils of ESP's 17.5 and 32.5.

Within the hexaploid wheats the relationship between grain yield and ion uptake traits has been explored in families derived from selfed individual progeny of two contrasting wheat culturers Tobari and Pato. The families were selected for uniform leaf Na contents.

Using this material and the reciprocal crosses, a very strong correlation, significant at the 99.5% level, was observed between grain yield and Cl- exclusion and K+/Na+ ratio in leaves. It is not known if the variation in K+/Na+ ratio within the leaves of hexaploid leaves can be attributed to allelic difference in the same major gene on the L4D chromosome discussed above. These results also emphasize the importance of the regulation of Cl- fluxes (not regulated by that trait), in the general problem of salinity although possibly not sodicity tolerance.

The physiological responses of Thinopyrum bessarabicum to salt stress were discussed briefly including the transient uptake of Na+ and Cl- in response to a salt challenge. The wheat T.bessarabicum-hybrid in hydroponic and field trials expressed much of the tolerance of the wild wheat grass but had a low yield potential and other undesirable agronomic traits. The youngest leaves of the hybrid contained the high K+/Na+ ratio and low Cl- levels characteristic of the wheat grass. However the high betaine content was not expressed.

These results emphasize the importance of the regulation of the fluxes of the major genes from root to shoot and from leaf to leaf in tolerance. In the case of one trait, the K+/Na+ discrimination trait from the D chromosome of Aegilops squarrosa we have considerable physiological understanding and hopefully indications that the introgression of the trait into durum wheat will improve their tolerance to sodic soils. However other traits are observed in other species. No single trait will confer salinity tolerance and it is clear that there are several strategies involving various combinations of traits that confer tolerance even in closely related cereals.

ECOLOGICAL ADAPTATION OF CAM PLANTS TO SALINITY. A VIEW ON DIFFERENT LEVELS OF SCALING FROM THE BIOGEOGRAPHICAL LEVEL TO LEVELS OF WHOLE PLANTS, CELLS, MEMBRANES AND MOLECULES

Ulrich Lüttge, Institut für Botanik, Technische Hochschule Darmstadt, D W-6100 Darmstadt, FRG

The use of crassulacean acid metabolism (CAM) in adaptation to salinity is illustrated for two different life forms of CAM-plants:

CACTACEAE	AIZOACEAE
Perennials	**Annuals**
Subpilosocereus ottonis	*Mesembryanthemum crystallinum*
Cereus validus	*Mesembryanthemum nodiflorum*
Constitutive CAM	**Inducible CAM**
Stress avoidance:	**Stress tolerance:**
Salt excluders	Salt includers

The perennial cacti operate with seasonal cycles of water relations and modes of gas exchange on the whole plant level. The Aizoaceae reveal involvement of adaptive mechanisms with an annual time behaviour at an array of levels with different scaling:

- On *the whole plant level* the form of growth, salt and water relations, and gas exchange;
- on *the cell level* metabolic regulation and enzyme activities;
- on *the membrane level* activities of the H^+-ATPase at the tonoplast;
- on *the protein level* the peptide subunit pattern of the H^+-ATPase holoenzyme;
- on *the genetic level* inductive regulation of enzymes.

Like with fractals on each of the subsequent levels with increasingly finer scaling one encounters similarly complex systems with feedback coupling and non-linear behaviour.

INTEGRATION OF PLANT STRESS RESPONSES INTO DEVELOPMENTAL PROGRAMS. Christopher J. Lamb, Plant Biology Laboratory, Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Induction of phytoalexins, lytic enzymes and wall reinforcement involves transcriptional activation of defense genes, in some cases within 2-3 minutes of an elicitation signal. Rapid response genes include those encoding chitinase and phenylpropanoid biosynthetic enzymes for production of phytoalexins and lignin monomers. Genes encoding cell wall hydroxyproline-rich glycoproteins are induced more slowly and at a distance from the initial perturbation, in response to several distinct endogenous intercellular stress signals. Recent studies to characterize *cis*-acting nucleotide sequences and *trans*-acting factors involved in these complex patterns of defense gene activation will be described. Many defense genes undergo developmental as well as environmental regulation and recent findings on the tissue- and cell-type-specific expression of defense genes and the molecular mechanisms underlying the interplay between developmental programs and environmental stimuli will be considered. Moreover, expression of a bean phenylalanine ammonia-lyase (PAL) gene in transgenic tobacco under the control of a chimeric CaMV 35S - PAL promoter causes abnormal development. The phenotypes involve both inhibition of the synthesis of bulk phenylpropanoid products such as lignin and flavonoid pigments and also hormonal-like effects suggesting dysfunction of novel signal systems based on phenylpropanoid compounds. We have recently observed the apparent disappearance of two specific cell wall structural proteins in response to fungal elicitor or glutathione. This response, which reflects the insolubilization of these major wall proteins by H2O2-mediated oxidative cross-linking, is initiated within 1 to 2 minutes of elicitor addition, and complete within 5 minutes. We propose that this protein cross-linking, which presumably toughens the cell wall, is a novel, ultra-rapid defense mechanism.

Comparative studies of the functioning of various cellular membranes in halophytic and glycophytic plants and cell lines. L. Reinhold, Y. Braun, S. Cooper, M. Hassidim, M. Schwarz and H.R. Lerner. Department of Botany, The Hebrew University of Jerusalem, 91904 Israel.

Na^+/H^+ antiporters have been reported in tonoplast fractions isolated from a number of plants, all of them salt-tolerant. We present evidence that monovalent cation/proton antiporters are not confined to salt-tolerant plants; nor are they confined to the tonoplast. The antiporters of salt-tolerant and glycophytic cells will be contrasted, principally with regard to cation transported. The rigorous criteria which must be fulfilled before it can be concluded that observed cation exchange is mediated by a membrane component will also be discussed.

Mitochondrial performance in the face of rising salinity will be briefly compared in wild type and NaCl-adapted tobacco cell lines. Though O_2 uptake is progressively inhibited with rising NaCl concentration in the case of both cell lines, phosphorylation is maintained at a higher level in that of the NaCl-adapted. The evidence suggests that increased proton permeability of the inner membrane is a probable cause for the loss of phosphorylating ability in the wild type.

SODIUM TRANSPORT IN HIGHER PLANT VACUOLES: Biochemical and Physiological Characterization of the Na^+/H^+ Antiport and the Cation Channel

Eduardo Blumwald

Centre for Plant Biotechnology, Department of Botany, University of Toronto, 25 Willcocks St. Toronto, Ontario M5S 3B2, CANADA

The vacuolar Na^+/H^+ antiport has been characterized in sugar beet (Betavulgaris L.) cell suspension cultures. Increasing the NaCl concentration in the growth media resulted in an increased transport activity of the antiport. Moreover, when cell suspensions were grown in media supplemented with 1 mM amiloride (an inhibitor of Na^+/H^+ antiport activity) there was an additional increase in the activity of the antiport. Cell suspension cultures, grown in the presence and absence of NaCl and amiloride, were in vivo labeled with [^{35}S]-methionine, and tonoplast protein profiles compared by SDS-PAGE and autoradiography. Our results showed the increase of a 170 kD vacuolar polypeptide. A polypeptide with the same molecular mass was also found to incorporate [^{3}H]-MIA, an amiloride analogue. Polyclonal antiserum was produced in mouse by immunization with the 170 kDa polypeptide. Western blot analysis of tonoplast proteins revealed that this antibody specifically precipitated the 170 kDa polypeptide. Thus suggesting that this polypeptide is a component of the Na^+/H^+ antiport. Pretreatment of tonoplast vesicles with ammonium sulfate-purified and dialyzed immunoglobulins inhibited the Na^+/H^+ antiport activity. These antibodies are presently being used to test cross-reactivity in tonoplast from halophytes and glycophytes.

The voltage patch technique was used in the whole cell and isolated outside-out patch configurations to record ionic current in the tonoplat of glycophytes (tomato) and halophytes (sugarbeet). Halophyte tonoplast channels displayed inward rectification (low conductance at positive membrane potentials), while glycophyte channels displayed a constant conductance at positive and negative membrane potentials. The observed low channel conductance at physiological membrane potentials in halophytes would prevent a significant loss of the Na^+ accumulated in the vacuole through the operation of the Na^+/H^+ antiport, while channel rectification in glycophytes would have have no physiological significance.

GENES REGULATED BY SALT STRESS IN PLANTS

DEVELOPMENTAL, HORMONAL AND ENVIRONMENTAL REGULATION OF THE OSMOTIN GENE.

P.M. Hasegawa, D.E. Nelson, K.G. Raghothama, P.C. LaRosa, A. Kononowicz, Z. Chen, A. Casas, M. Paino D'Urzo, I. Ilahi, D. Liu, N.K. Singh, M. Binzel, M.A. Botella, M. Narasimhan, X. Niu, E. Perez-Prat, J.-K. Zhu, V. Valpuesta, and R.A. Bressan.

Dept. of Horticulture, Purdue University, West Lafayette, Indiana 47907.

Osmotin is a 26 kDa protein, classified as a family-5 pathogenesis related protein, that accumulates as a function of osmotic adaptation. The osmotin gene is regulated by osmotic stress and several other factors including abscisic acid (ABA), ethylene, wounding, virus infection and spatial and temporal development of the plant. Nuclear run on experiments with ABA induction indicate transcriptional regulation. A number of regulatory conditions result in increased accumulation of osmotin mRNA but not protein suggesting that post-transcriptional regulation also occurs. Osmotin/GUS promoter fusion studies indicate a very tissue-specific pattern of promoter control including an interestingly high level of expression in mature pollen. Promoter-deletion/gel retardation studies indicate the presence of more than one cis-acting element that binds nuclear factors. Transgenic plants with chimaeric osmotin genes that over- and under-produce osmotin were made and are being used to test the possible function of this gene in osmotic stress tolerance. Two osmotin cDNA clones were isolated from an *Atriplex nummularia* cDNA library. One of these halophytic genes is highly induced by NaCl, the other is mostly constitutive. They also have distinctly different patterns of tissue expression.

We have been also examining the roles of ion motive ATPases in osmotic stress tolerance. cDNA probes encoding the 70 kDa subunit of the tonoplast H^+-ATPase, the plasma membrane H^+-ATPase and an E_1E_2-type Ca^{2+}-ATPase have been isolated and used in Northern blot analysis. The levels of the mRNAs of the ATPases are regulated by NaCl.

SALT STRESS INDUCES GENES IN TOMATO

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Salt stress induces important alterations in plant metabolism affecting plant growth and development in a complex way. Deleterious effects of salt stress can be due to either internal accumulation of ions to toxic levels, diminished water availability by osmotic effects or altered mineral nutrition. Some physiological changes, generally interpreted as adaptive, have been shown to occur in salt-stressed plants. These include alterations in ionic transport, lipid composition and accumulation of organic osmolites. Salt stress-induced changes in gene expression have been reported in different biological systems. In a first step of our work, we analyzed the protein patterns of tomato plants treated with different NaCl concetrations and abscisic acid (ABA). we concluded that 1) Salt induced specific proteins in different development stages, 2) Some of these proteins were induced in every stage analyzed and 3) Most of salt-induced proteins were also induced by ABA.

In a second step, cDNAs encoding salt- and ABA-inducible proteins were isolated by differential hybridization. One of thee clones, TAS14, has been characterized. TAS14 mRNA is induced by salt and ABA in every organ analyzed, independently of the plant development stage. However, TAS14 protein has been only detected in aerial parts and it is accumulated preferentially in stems of salt-treated plants. Immunocytological methods were used to determine the distribution of the TAS14 protein in tomato plants. Alkaline phosphatase-labeled secondary antibody showed a high concetration of the TAS14 protein in the adventitious root primordia, in the procambium of the apical meristem, leaf primordia and associated with the vascular system in leaves. TAS14, as revealed by immunoelectron microscopy, is basically localized in the nucleus and also detected in the cytoplasm, although not associated with any specific organelle.

Transgenic tobacco plants expressing constitutively TAS14 gene show anomalous floral fenotypes.

EVIDENCE THAT PLANTS ARE ENDOWED WITH SYSTEMS FOR COORDINATED RESPONSES ELICITED BY AGENTS RANGING FROM VIROIDS TO SALT

Conejero, V., Bellés, J. M., Domingo, C., Fayos, J., Frank, R., García-Breijo, F., Garro, R., Hernández-Yago, J., Rodrigo, I., Saurí, A., Tornero, P. and Vera, P.
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Studying comparatively the plant reaction induced by pathogens with very different molecular complexity, including biotic (citrus exocortis viroid (**CEVd**)) and abiotic (Ag^+ ions) agents we arrived to the conclusion that plants have an ethylene-mediated general mechanism of response susceptible of being triggered by very different kinds of elicitors. The major components of this response would be a pathological developmental syndrome, the physiological condition of enhanced resistance and the synthesis and accumulation of a set of *pathogenesis-related* (PR) proteins. (1, 2, 3).

Our finding is in accordance with the general idea that evolution appears to have been guided by a principle of mechanistic economy when diversification has not been essential. But economy is not the only advantage of having a number of interlinked reactions to form a general system of response. Such a system would have at least two additional advantages compared with independent transduction chains: control would be easier, and attack by certain agents would elicit defenses against subsequent aggressions by other afflicting agents (3).

Data illustrating the above outlined model will be presented. More specifically, we present data relevant to the viroid induction of an osmotin-like protein (PR P23) (4). The subcellular localization and differential tissue accumulation of this pathogenesis-related protein depending on the nature of the elicitor (viroid or salt) will be also presented.

References

1.- Conejero, V. (1982) *IV International Conference on Comparative Virology. Control of Viral Diseases*. Banff, Alberta, Canada (Abstr. W-12-1).
2.- Granell, A., Bellés, J.M. and Conejero, V. (1987) *Physiol. Mol. Plant Pathol.* 31, 83-90.
3.- Conejero, V, Bellés, JM, García-Breijo, F, Garro, R, Hernández-Yago, J, Rodrigo, I, Vera, P (1990) Signalling in viroid pathogenesis. *In* Fraser RSS (ed) *Recognition and Response in Plant-Virus Interactions*. Springer-Verlag, Heidelberg pp 233-263.
4.- Rodrigo, I., Vera, P., Frank, R. & Conejero, V. (1991) *Plant Mol. Biol.* **16**, 931-934.

THE INTERACTION BETWEEN SALT AND ABSCISIC ACID IN THE CONTROL OF GENE EXPRESSION.

Ralph S. Quatrano

Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280 U.S. A.

Over a 3-5 day culture period in the presence of 50 uM ABA, immature wheat embryos undergo an accelerated but normal morphogenesis and accumulation of mature embryo proteins. One such protein (Em) is an abundant, hydrophilic protein found in the mature embryo. Characterization of the expression of this gene and the pattern of its accumulation in response to ABA indicates that its regulation occurs at the level of transcription and mRNA stability. Increasing the levels of ABA or sensitivity to ABA in seedling tissue or in suspension cultures by desiccation or high salinity results in increased levels of Em mRNA. The effect of salt stress on Em gene expression in rice suspension cells appears to operate through two pathways; one is mediated through increases in the level of ABA, the other is via a unique salt response pathway that includes an intermediate that is common to both the salt and ABA response chains, and results in an increased tissue sensitivity to endogenous ABA .

To determine the cis elements and trans-acting factors involved in the salt and ABA response, fusions of the intact and deleted 5' promoter regions of the Em gene with the glucuronidase (GUS) reporter gene have been tested for expression in transient assays using rice protoplasts and in transgenic tobacco plants. Results from both assays indicate that a 646bp region (-554 to +92) from the 5' untranslated region of the Em gene contains all the information needed for ABA regulation and embryo-specific expression. A detailed analysis of the sequences in the ABA response element [ABRE] involved in ABA-modulated regulation of chimeric genes in a transient assay reveals a 20bp sequence that can confer ABA-responsiveness on a non-responsive CaMV viral promoter. Nuclear extracts from embryonic cells contain protein factors which specifically bind to the ABRE, as evidenced by gel-shift and footprinting experiments. A cDNA clone (GC19) was isolated by screening a lambda gt11 expression library. The protein product of GC19 is a DNA binding protein (EmBP-1) of the bZIP class that interacts specifically with an 8bp sequence (CACGTGGC) in the ABRE. A 2bp mutation in this sequence prevents binding of EmBP-1 and reduces the ability of the ABRE to respond to ABA in the transient assay. The 8bp EmBP-1 target sequence (i.e. G-box) is conserved in several other ABA-responsive promoters and in promoters from plant genes that respond to signals other than ABA, e.g. light. Results from competitive gel-shift and transient expression assays using G-box containing 20bp sequences of these genes will be presented as an approach to define the specificity of interactions. The deduced amino acid sequence of EmBP-1 contains conserved basic and leucine zipper domains found in other plant transcription factors of the bZIP class. Preliminary evidence for the interaction of EmBP-1 with the maize regulatory gene product Vp1 during ABA-regulated gene expression will be presented.

Supported by the U.S.D.A. Competitive Grants Program (89-37262-4456) and NIH (GM 44288).

ABSCISIC ACID AND WATER STRESS RESPONSIVE GENES IN MAIZE

Montserrat Pagès, Maria Pla, Eva Poca, Miguel Angel Freire, Anders B. Jensen, Cesar Arenas and Adela Goday.

Earlier studies on the regulation of gene expression during embryogenesis in *Zea mays* L. have shown that a group of stage-specific mRNAs and proteins which accumulate in late embryogenesis are induced precociously in immature embryos when abscisic acid (ABA) is supplied in the culture medium (Sanchez-Martinez et al. 1986). cDNA and genomic clones for some of these mRNAs have been previously obtained and extensively characterized (Goday et al 1988, Pla et al 1989).

Expression of maize *rab* genes can be induced in tissues other than those of the embryo, and at other stages besides embryo development by ABA treatment or water-stress (Gomez et al 1988, Pla et al 1989). Several genes showing similar behavior have been identified in different plant systems (Mundy and Chua 1988, Bartels et al. 1990, Claes et al. 1990).

In the maize *viviparous* mutants, the embryo fails to become dormant, but precociously germinate in the mother plant without having first undergone the normal period of desiccation. The ABA concentration of *vp1* seedlings increases in response to water deficit. However *vp1* mutant is insensitive to exogenously supplied ABA and is considered to be defective in its responsiveness to this hormone (Mc Daniel et al.1977, Robichaud et al. 1980). The ABA-deficient *vp2* is carotenoid deficient. Seedlings rescued from *vp2* kernels contain less ABA than do wild seedlings and the ABA concentration does not increase in response to water deficit (Neill et al 1986). *vp2* embryos respond to exogenously applied ABA by undergoing maturation processes rather than precocious germination (Robichaud et al 1980).

Two different *rab* genes have been identified as ABA-inducible in wild type, *vp2* and *vp1* embryos and young leaves, and *rab* mRNAs are also induced by water-stress in leaves of wild type and *vp1* plants but not in *vp2* plants. This study has highlighted interesting features at the level of gene regulation in these mutants. First, the low level of *rab* message detected in mature *vp1* embryos may reflect a selective regulation of *rab* by *vp1* at the level of gene expression during embryo development. Second, we have observed a differential regulation of *rab* mRNA in embryos and vegetative organs of the ABA-deficient *vp2* mutants.

In transgenic Arabidopsis and tobacco plants 5' upstream sequences of the *rab17* gene confer the appropiate patterns of expression on the reporter gene. This construct results in high levels of CAT or GUS activity in mature embryos and in vegatative tissues subjected to water stress. However the relative percentage of enzyme activity is lower in leaves than in embryos. This indicate again a differential regulation of the *rab*promoter in embryo and vegetative organs.

Transient expression studies have shown that the promoter region of *rab* genes contain ABA-responsive DNA motifs which confer ABA responsiveness on the reporter gene in cereal protoplasts (Marcotte et al.1989, Mundy et al 1990, Vilardell et al 1991). Moreover a leucine zipper protein binding to one of the DNA motifs has been recently characterized (Guiltinan et al. 1990). Gel retardation assays indicate that nuclear proteins from embryo and water stressed leaves interact specifically with a short DNA fragment (containing the ABRE) from *rab* genes.However they generate specific complexes of different electrophoretoic mobility which are stable in the presence of detergent and high salt. These results suggest that distinct regulatory factors may be involved in the hormone action during embryo development and in vegetative tissues subjected to osmotic stress.

REFERENCES

-Bartels D, Schneider K, Terstappen G, Piatkowski D, Salamini F. (1990) Planta 181 27-34.
-Claes B, Dekeyser R, Villaroel R, Van den Bulcke M, Bauw G, Van Montagu M, Caplan A. (1990). The Plant Cell 2, 19-27.
-Goday A, Sanchez-Martinez D, Gomez J, Puigdomenech P,Pages M (1988). Plant Physiol. 88, 564-569.
-Gomez J Sanchez-Martinez D, Stiefel V Rigau J Puigdomenech P and Pages M. Nature 334, 262-264.
-Guiltinan MJ, Marcotte WR, Quatrano R.(1990) Science.
-Marcotte WR, Bayley ChC, Quatrano RS (1988). Nature 335', 454-457.
-Marcotte WR, Russell SH, Quatrano RS (1989). Plant Cell 1, 969-976.
-Mundy J, Chua NH (1988). EMBO J. 7, 2279-2286.
-Mundy J, Yamaguchi-Shinozaki K, Chua NH (1990). Proc. Natl. Acad. Sci. USA 87,1406-1410.
-Neill SJ, Horgan R, Parry AD (1986). Planta 169, 87-96.
-Pla M, Goday A, Vilardell J, Gomez J, Pages M (1989). Plant Mol. Biol.13, 385-394.
-Sanchez-Martinez D, Puigdomenech P, Pages M (1986). Plant Physiol. 82, 543-549.
-Skriver K, Mundy J (1990). Plant Cell 2, 503-512.
-Vilardell J, Goday A, Freire MA, Torrent M, Martinez MC,Torne JM, Pages M (1990). Plant Mol.Biol.14, 423-432.

BACTERIAL MODELS OF SALT TOLERANCE

Molecular physiology of Na^+/H^+ antiporters, key enzymes in adaptation of bacteria to high salinity and alkaline pH.

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Sodium proton antiporters have been suggested to play a major role in regulation of Na^+ and H^+ metabolism both in eukaryotes and prokaryotes (1). Cloning of two genes, *nhaA* and *nhaB* encoding Na^+/H^+ antiporters in *Escherichia coli* (2,3) allowed us to develop molecular tools to study the antiporter proteins as well as their role in adaptation of bacteria to high salinity and pH.

The NhaA protein has been purified and reconstituted into proteoliposomes in a functional form (4). Kinetic studies showed that the antiporter is electrogenic both at acid and alkaline pH and is activated by alkaline pH. This activation (2000 fold increase) is consistent with the proposed role of the antiporter in regulation of internal pH at the alkaline pH range.

Inactivation of *nhaA* in the chromosome showed that *nhaA* is indispensable for adaptation to high salinity and alkaline pH in *Escherichia coli* (5). Furthermore study of the Δ*nhaA* mutant revealed the existence of *nhaB*, an additional antiporter system. It differs from *nhaA* both in having a 15 fold lower affinity to Li^+ and in being insensitive to pH.

The existence of at least two antiporters imply that understanding their regulation is essential for delineation of their role in homeostasis of the cell. Monitoring the β-galactosidase activity of a chromosomal translation fusion of *nhaA'-'lacZ* showed that at pH 7.5 the gene is induced, within 1h, by 100 mM of both Li^+ and Na^+. Alkaline pH potentiates the effect of external Na^+.

A new gene located downstream of *nhaA*, *nhaR*, has been identified. A deletion of this gene suggested that *nhaR* plays a role in regulation of expression of *nhaA*. Based on sequence comparisons *nhaR* belongs to a family of transcription activators represented by the *oxyR/lysR* family. The pattern of regulation of *nhaA* reflects its importance in adaptation to high salinity and alkaline pH in *E. coli* (2-5).

References

1. Padan, E., Zilberstein, D. and Schuldiner, S. (1989) Biochim. Biophys. Acta *650*: 151-166.
2. Goldberg, E.B., Arbel, T., Chen, J., Karpel, R., Mackie, G.A., Schuldiner, S. and Padan, E. (1987) Proc. Natl. Acad. Sci. U.S.A. *84*: 2615-2619.
3. Karpel, R., Olami, Y., Taglicht, D., Schuldiner, S. and Padan, E. (1988) J. Biol. Chem. *263*: 10408-10414.
4. Taglicht, D., Padan, E. and Schuldiner, S. (1991) J. Biol. Chem. (in Press).
5. Padan, E., Maisler, N., Taglicht, D., Karpel, R. and Schuldiner, S. (1989) J. Biol. Chem. *264*: 20297-20302.

Accumulation of ionic osmolytes in adaptation of Escherichia coli to elevated osmolarity. W. Epstein & D. McLaggan. Dept. of Molecular Genetics & Cell Biology, Univ. of Chicago, Chicago, IL 60637 USA

Bacteria adapt to media of different osmolarities by adjusting cytoplasmic osmolytes to maintain turgor pressure within narrow limits. The increase in turgor produced by reducing external osmolarity (downshock) leads to a loss of osmolytes that is selective if the change in turgor is moderate in rate but non-selective if the change is too rapid. An increase in medium osmolarity (upshock) reduces turgor and triggers a wide range of changes designed to increase cytoplasmic osmolytes. Our studies have dealt primarily with the early response to upshock that occur in the absence of protein synthesis, including: i) the uptake of K^+, ii) the accumulation (by transport or synthesis) of glutamate, iii) the uptake of phosphate, and iv) major changes in pools of phosphorylated intermediates.

Stimulation of the uptake of K^+ is the most rapid response, occuring without lag. This response is shown by all three saturable systems, Kdp, TrkA and TrkD that mediate uptake of this ion. Uptake of phosphate begins about 2 min. (at 37° C) after upshock, while accumulation of glutamate begins after 5 min. The kinetics of glutamate accumulation are essentially identical whether glutamate is synthesized by glutamate dehydrogenase or by glutamate synthase, indicating that the activity of each enzyme is regulated.

When synthesis of glutamate or uptake of phosphate is blocked, adaptation to upshock is incomplete in that K^+ uptake is reduced by 50%. The limitation of K^+ uptake appears to be due to a reduction in membrane potential. In the presence of phosphate but the absence of glutamate synthesis, cells appear to have difficulty regulating the internal pH, implicating glutamate ion as important for pH regulation during upshock.

There are major transient changes in pools of many phosphorylated compounds in upshock. A few persist indefinitely while most are transient when adaptation is complete but persist when cells cannot synthesize glutamate.

The uptake of K^+ and the accumulation of glutamate serve not only to increase osmolarity but also as second messengers that trigger the activation of enzymes and/or changes in gene expression that are important in the accumulation of the compatible solutes accumulated to high concentrations in E. coli and many other bacteria: trehalose, proline and betaine. K^+, glutamate and compatible solutes appear to be regulated by a series of key steps, in which low turgor probably affects primarily movement of K^+ while most of the other changes are in response to the changes in cell K^+ pools and/or pools of glutamate.

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Osmotic Regulation of gene expression: a role for DNA supercoiling and chromatin structure

Many physiological changes are observed in bacteria in response to osmotic upshock. At least some of these are a result in alterations in transcription patterns. For some genes, such as the *kdp* potassium transporter or the *ompC/ompF* porins, specific membrane receptors sense changes in osmolarity which are transmitted to specific DNA-binding regulatory proteinsa. However, for other genes (such as the *proU* glycine betaine uptake system) no specific regulator has been identified (despite much effort). Instead, changes in DNA supercoiling appear to play a role in regulation (Higgins *et al.*, 1988; Dorman *et al.*, 1990). Several lines of evidence show that opsmotic upshock causes an increase in the superhelicity of plasmid and chromosomal DNA. Additionally, promoters such as *proU* respond to factors such as DNA gyrase inhibitors, which perturb supercoiling.

Current studies will be described, aimed at understanding why promoters such as *proU* are so sensitive to changes in DNA topology, and the mechanisms by which osmolarity leads to a change in DNA supercoiling. The histone-like protein H-NS (also called H1) appears to provide at least part of the answer to both questions. H-NS is one of the two major, chromatin associated proteins in the cell and appears to play an important role in determining nucleoid structure. The H-NS protein is encoded by the *osmZ* locus, originally identified because of its effects on *proU* expression and supercoiling (Hulton *et al.*, 1990; Higgins *et al.*, 1991). This protein binds at bends in the *proU* promoter region and, probably via topological effects, influences RNA polymerase function. Additionally, we have found that H-NS exists as multiple isoforms in the cell. Our working hypothesis is that the ratio of these isoforms varies in response to osmotic upshock and that the isoforms have different DNA binding properties. This could, potentially, provide a mechanism by which osmolarity could induce changes in DNA supercoiling.

References.

Dorman, C.J. *et al.* (1990) Nature 344: 789-792
Higgins, C.F. *et al.* (1988). Cell 52: 569-584
Higgins, C.F. *et al.* (1990). Mol. Microbiol. 4: 2007-2012.
Hulton, C.S.J. *et al.* (1990). Cell 63: 631-642

EUKARYOTIC MICROORGANISMS MODELS OF SALT TOLERANCE

Regulation of glycerol synthesis and of Na^+ in the halotolerant alga *Dunaliella*

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The unicellular green alga *Dunaliella* can adapt to an exceptionally wide range of salt concentrations, ranging from 0.1M to 5M NaCl. The capacity to adapt to high salt concentrations is achieved by massive production of glycerol, which serves as an intracellular osmotic element, and by efficient elimination of Na^+ from the cells.

The mechanism which regulates glycerol production is not well understood. The following approaches have been utilized to clarify this issue: a) A selection procedure for obtaining osmotically-defective mutants has been developed, which clarified the metabolic pathway of glycerol synthesis; b) Changes in phosphometabolite precursors of glycerol, following osmotic shocks, enabled to identify the checkpoint regulatory enzyme in the process; c) Rapid changes in the structure, permeability and phospholipid metabolism within the plasma membrane suggest that the sensor mechanism which triggers glycerol production resides in the cell membrane and is activated by volume changes.

Maintenance of low cytoplasmic Na^+ concentrations in *Dunaliella* is perturbed by a salt stress or by intracellular acidification, which induce a transient influx of Na^+ into the cells. Na^+ influx is mediated by a Li^+ and amiloride-sensitive Na^+/H^+ antiporter, which has been characterized in plasma membrane preparations. Over-expression of the Na^+/H^+ antiporter and labeling studies with a photoafinity amiloride derivative suggest that at least two polypeptides compose the Na^+/H^+ antiporter. Na^+ elimination is catalyzed by an energy-dependent, vanadate-sensitive mechanism presumably a Na^+-ATPase. The results suggest that Na^+ homeostasis in *Dunaliella* is achieved by the Na^+/H^+ antiporter, whose function is primarily pH regulation and by the putative Na^+-ATPase which eliminates Na^+ from the cells.

SALT TOLERANCE IN YEASTS: ROLE OF GLYCEROL.

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The response of *Saccharomyces cerevisiae* and the marine yeast *Debaryomyces hansenii* to increased environmental salinity involves an increased production and intracellular accumulation of glycerol. ^{13}C NMR spectroscopy and studies using continuous culture show that glycerol has an exclusive role as an osmolyte in these organisms. A direct support for this came from a mutant of *D. hansenii* with impaired glycerol production which could not grow at high salinity unless low concentrations of glycerol was exogenously added. The supplied glycerol was rapidly taken up by the mutant and used for intracellular adjustments.

The pathways for the glycerol metabolism are similar in the two yeasts although *D. hansenii* has an active transport system for glycerol which *S. cerevisiae* has not. Membrane permeability to glycerol is, however, not uncontrolled in *S. cerevisiae*.

The cytoplasmic glycerol 3-phosphate dehydrogenase (GPD) which is located at the branch point between the glycolytic flux and the glycerol producing pathway is activated at the protein level by K^+ or Na^+ in *D. hansenii*. In *S. cerevisiae* a putative GPD gene has been cloned by reverse genetics and several lines of evidence indicate that expression of this gene is controlled by external osmolarity. Two dimensional gel electrophoresis coupled to computerized quantification revealed that the pattern of control shown by GPD represents one of several different temporal patterns of protein synthesis following a salt shock. To identify other genes critical for glycerol accumulation, mutants defective in this response were isolated. The mutants which are osmosensitive define four complementation groups. Two (OSG1 and OSG2) are affected in the GPD activity while OSG4 is unable to retain produced glycerol intracellularly. Complementing DNA has been isolated but the genes are not yet sequenced. This and other non-related strategies to disclose important components in the osmoregulatory stimulus response pathway will be discussed.

ISOLATION AND CHARACTERIZATION OF GENES OF THE YEAST SACCHAROMYCES CEREVISIAE INVOLVED IN SALT TOLERANCE MECHANISMS

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The progressive salinization of irrigated land poses a threat to the future of agriculture in arid regions[1,2]. Genetic engineering has the potential to improve the salinity tolerance of crops[3]. However genes involved in regulating salt tolerance have first to be identified. Although many plant genes induced by salinity have been cloned[4,5], most of them probably correspond to non-specific stress responses[6,7]. The yeast *Saccharomyces cerevisiae* could serve as a convenient model system to uncover rate-limiting steps[8] for growth under salt stress. This yeast shares basic ion transport mechanisms with plants[9] and genes can easily be isolated by complementation of the desired phenotype[10]. We have isolated a novel yeast gene, *HAL1*, which upon overexpression improves growth under salt stress. In addition, disruption of this gene decreases salt tolerance. *HAL1* encodes a basic protein of 32 kDa induced by osmotic stress and unrelated to sequences in data banks. As overexpression of *HAL1* increases intracellular potassium, this novel protein could be involved in the mechanisms which determine intracellular ion homeostasis in fungi and plants.

The sodium efflux system of Saccharomyces cerevisiae

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In 1954 Conway, Ryan and Carton [1] demonstrated that yeast cells were able to pump Na^+ out of the cell, uphill the Na^+ concentration gradient across the membrane. This Na^+ pump of yeast cells was of a different type than that in animal cells, and Conway and Kerman proposed that it was a redox pump [2]. The research on this pump was not very active, and until twenty seven years later it was not found that Li^+ efflux was dependent on the ATP concentration, sensitive to DCCD and DES, and that it functioned as a Li^+/H^+ antiport [3]. This suggested that instead of a redox pump, Li^+ efflux could be coupled to pH or to ATP hydrolysis. However, using whole cells the prediction of the mechanism involved was difficult because many fluxes occur simultaneously. At that moment, progress in the field required a molecular approach.

After failing to obtain mutants with reduced Li^+ or Na^+ effluxes, we cloned a gene involved in Na^+ and Li^+ effluxes by complementing the Li^+ sensitivity of a low Li^+-efflux strain with a gene library. Disruption of this gene (ENA1) changed a Li^+ tolerant strain into an extremely Li^+-sensitive strain, which lacked Na^+ and Li^+ effluxes at alkaline pH values. This mutant was also sensitive to alkaline pH values.

The sequence of ENA1 revealed that it encoded a P-ATPase, and we also found that two additional copies of ENA1 were in tandem with it. The first copy (ENA2) presented a promoter region different from that of ENA1, and also 13 different amino acids in the putative encoded protein. The second copy (ENA3) is apparently identical to ENA2.

The ENA1 gene has also been cloned by its homology with PMA1, the gene encoding the H^+-pump ATPase of the plasma membrane of yeast. This gene was named PMR2, and it was proposed to encode a Ca^{++}-ATPase on the basis of homology considerations [4].

So far, purification of the product of ENA1 has not been possible, and a physical demonstration of the function of the product of ENA1 is lacking. However, it seems unlikely that this product is a Ca^{++} pump. We have found that Ca^{++} concentration does not affect Li^+ efflux, and that Na^+, but not Ca^{++}, is involved in the regulation of the expression of ENA1 and ENA2.

[1] Conway, E.J., Ryan, H., Carton, E. (1954) Biochem. J. 58:158-167
[2] Conway, E.J., Kernan, R.P. (1955) Biochem. J. 61:32-36
[3] Rodríguez-Navarro, A., Pérez-Lloveres, C. (1981) Biochim. Biophys. Acta 640:352-358
[4] Rudolph, H.K., Antebi, A., Fink, G., Buckley, C.M., et. al. (1989) Cell 58:133-145

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Ion Transporter Genes in Yeast

The uptake of potassium in *S. cerevisiae* is primarily mediated by two transporters encoded by the genes *TRK1* and *TRK2*. *TRK1* encodes a high-affinity K^+ transporter, the structure of which is unrelated to ion transporters found in animal cells. TRK1 contains 12 putative membrane-spanning domains (M1 - M12) and a large, 650-amino-acid domain that our evidence suggests is located within the cytoplasm. We are currently utilizing molecular genetic approaches to gain a more detailed understanding of the structure and function of TRK1.

Deletion of *TRK1* is not lethal but results in a 50-fold increase in the amount of K^+ required to support normal growth. This negative phenotype allowed the isolation of Trk^+ pseudorevertants from *trk1Δ* cells. Two of the genes identified among these pseudorevertants, *RPD1* and *RPD3*, are required for full repression of *TRK2*, the gene encoding the low-affinity K^+ transporter. In addition, *RPD1* and *RPD3* are required for the full repression and the full activation of many (diverse) yeast genes.

Molecular analysis of *TRK2* indicated that it encodes a 100 kD protein, the overall structure of which is very similar to *TRK1*. Like TRK1, TRK2 contains 12 putative transmembrane domains and a large hydrophilic region between M3 and M4. The most highly conserved regions of the two proteins are the putative transmembrane domains; other regions of the two transporters exhibit significantly greater divergence. Overexpression of *TRK2* can completely suppress the increased K^+ requirement of *trk1Δ* cells. This can occur through loss of repression of *TRK2* because of *trans*-acting mutations in *RPD1* or *RPD3*, or, by *cis*-acting mutations in the promoter of *TRK2*.

TRK1 and *TRK2* are nonessential genes. Deletion of *TRK2* in a *trk1Δ* cell results in a 10-fold increase in the concentration of K^+ required to support growth. Expression of *TRK1* in *trk2Δ* cells results in cells that exhibit fully wild-type K^+ uptake. Thus, TRK1 and TRK2 are functionally independent. In addition to a high K^+ requirement (30-50 mM), *trk1Δ trk2Δ* cells exhibit hypersensitivity of low pH. Since this phenotype can be suppressed by very high concentrations of K^+ (> 300 mM), we believe that the low pH hypersensitivy reflects the inability of *trk1Δ trk2Δ* cells to take up K^+ in low pH medium.

Suppressor mutations that suppress the high K^+ requirement and/or the low pH hypersensitivity of *trk1Δ trk2Δ* cells have been obtained and analyzed. The results of these studies will be presented in further detail.

POSTERS

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EFFECT OF DIFFERENT SALTS AND SUPPLY - Ca^{2+} ON WATER RELATIONS AND GAS EXCHANGE PARAMETERS IN CITRUS LEAVES.

Leaf gas exchange, water relations and ion content were measured on one-year- old sweet orange seedlings C. sinensis (L.) Osbeck cv. Hamlin. Different salts were used (NaCl, KCl and NaNO3) to determine the specific effects of chloride and sodium on these parameters.

Reduction in dry weight and defoliation was higher in plants treated with chloride salts . Therefore, plants showed a progressive accumulation of chloride which caused a sharp reduction in photosynthesis and stomatal conductance. By contrast, these parameters were not affected by Na+ accumulation in leaves. Salt treatments also caused a reduction in K+ content. Water potential in leaves reached values near -1.8 MPa, with 60 mM NaCl or 60 mM KCl. This reduction was offset by a reduction in osmotic potential so that turgor was maintained at or above control values. This reduction was closely correlated with proline concentration in leaves,, which was increased by chloride salts.

Addition of Ca2+ (10 mM and 30 mM) increased the dry weight in plants treated with 45 mM NaCl or 45 mM KCl and decreased the defoliation by about 50 %. Therefore, Ca2+ decreased Cl- and Na+ concentration in leaves and increased K+ concentration.

Photosynthesis and stomatal conductance increased their

values in plants treated with 45 mM NaCl or 45 KCl with increasing Ca2+ treatments.Hoewer, both parameters were not appreciably reduced in plants treated with NaNO3.

Addition of Ca2+ counteracted the effects on reductions in leaf water and osmotic potentials. Therefore, Ca2+ inhibited proline accumulation in leaves and that affected the reduction in osmotic potential.

Changes on the parameters studied was attributed to a higher chloride concentration in Citrus leaves.

SODIUM TOLERANCE IN SUNFLOWER AND BEAN PLANTS. ROLE OF POTASSIUM.

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In most crop species, Na^+ content in plants is low [1], and Na^+ tolerance has been related, in some cases, to the plant capability of keeping low Na^+/K^+ ratios in the shoot [2]. Since this ratio depends on the ratio between K^+ and Na^+ fluxes, Na^+ tolerance should be explained in terms of these fluxes. To test this hypothesis, we have studied the relationship between K^+ and Na^+ fluxes and Na^+ tolerance in the moderately salt tolerant sunflower plants and in the salt sensitive bean plants.

When Na^+-loaded seedlings of sunflower and bean plants, with approximately 75 μmol $(g.fw)^{-1}$ in the root, were transferred to a Na^+ nutrient solution (50 mM Na^+, 5 mM K^+) both species took up Na^+ from the nutrient solution, but at a much lower rate in sunflower plants. In these plants, the rate of Na^+ gain was lower than the rate of weight gain, Na^+ was diluted and the plants thrived. In contrast, bean plants taken up Na^+ more rapidly than the gain in weight, Na^+ concentrated and the plants eventually stopped growing. Na^+-loaded plants were also low in K^+, and in the 50 mM Na^+, 5 mM K^+ nutrient solution, both sunflower and bean plants took up K^+ very actively. No significant differences were found between species. In both cases, two days were enough to raise the K^+ content to a normal value.

Unidirectional Na^+ efflux from the roots of sunflower and bean plants to the Na^+ nutrient solution (50 mM Na^+, 5 mM K^+) and to a Na^+-free nutrient solution did not show significant differences between the two kind of plants. In the absence of external Na^+, the half life of Na^+ in the root was approximately two days. Addition of external Na^+ did not inhibit the Na^+ efflux observed in the absence of external Na^+. Na^+ fluxes between roots and shoots were higher in sunflower plants than in bean plants. As a consequence, sunflower plants presented a higher Na^+ content than bean plants in all conditions, but also a higher capability to dilute the cation.

The results suggest that the most significant difference between bean and sunflower plants is the K^+-Na^+ uptake system, much more efficient to discriminate between Na^+ and K^+ in sunflower plants. This difference can account for the different Na^+ tolerance between both species.

REFERENCES.

1.- Greenway, H., and Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31:149-190.

2.- Winter, E. (1982). Salt tolerance of Trifolium alexandrinum L. II. Ion balance in relation to its salt tolerance. Aust. J. Plant Physiol. 9:227-237.

IMPACT OF OSMOPROTECTANTS ON SALT - INDUCED GROWTH INHIBITION

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A number of genes have been cloned from plants that are induced by osmotic stress. None have been clearly implicated in the synthesis of osmoprotectants. Nor is it at all clear which of the most investigated osmoprotectants, proline and glycine betaine, contribute significantly to the intracellular osmolarity. They may play important roles in only a few, as yet unidentified compartments, or to protect only a subset of the sum of cellular compartments, or they may simply contribute to, but not determine, osmotic adaptation. We have initiated a program to investigate the effects of treatments with these chemicals on gene expression during stress. Sterile rice seedlings were grown in increasing concentrations of proline or proline plus 1% (171 mM) NaCl. Proline alone is a growth inhibitor. Fifty millimolar proline inhibits rice growth as much as 171 mM NaCl. The combination inhibits growth synergistically. When NaCl-treated plants were subsequently assayed for expression of the salt-induced gene, *salT*, we found a near-linear increase in expression as external proline levels increased from 0 to 200 mM. The combined treatment of 171 mM NaCl and 200 mM proline gave approximately 20-fold more *salT* expression than NaCl alone. We next compared the effects of low concentrations of proline, betaine, and trehalose on *salT* expression. We first saw that 1 to 10 mM proline, and 5 to 10 mM betaine were growth inhibitory. By contrast, 10 mM trehalose enhanced growth by 10%. Second, at the concentrations tested, proline did not reduce the inhibition of sheath growth, although 1 to 10 mM did permit the leaf lamina to grow longer. By contrast, betaine afforded protection in the sheath, and trehalose in both sheath and leaves. When these plants were then hybridized with *salT*, strong induction of the genes is seen in leaves of proline- or betaine-grown plants, but not in trehalose-grown ones. From these studies, we propose that trehalose may be a more useful osmoprotectant than the others tested here. Moreover, since low concentrations of proline induce *salT* (and possibly other osmoprotectant genes from studies currently being conducted), the primary value of changes in free proline in the cell may be to create a highly diffusible, easily catabolized inducer of other stress-induced genes.

EVIDENCE FOR CALCIUM-CALMODULIN REGULATOR OF THE SPECIFIC K/H ANTIPORTER OF SOIL SEED RAPE

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The mode of regulation of a specific K^+/H^+ antiporter for which we have recently brought evidence (1), in *Brassica napus* plasmalemma and tonoplast membrane vesicles has been investigated. The antiporter must presumably be tightly regulated in vivo since it mediates extrusion of K^+ from the cell coupled with H^+ influx.

We present the following evidence, that this exchange activity is Ca^{2+}-calmodulin regulated: Dissipation by K^+ of a previously established transmembrane pH gradient was inhibited when Ca^{2+} was depleted by addition of imM EGTA. It was also inhibited by addition of the calmodulin antagonist, compound 48/80 (10 µgr/ml). Each treatment brought about approximately 70% inhibition of the antiport activity. This inhibition, investigated here in plasma membrane vesicles, prepared by separation on a sucrose gradient, showed no dependence on ATP.

1. Cooper S, Lerner HR, Reinhold L (1991) Evidence for a highly specific K^+/H^+ antiporter in membrane vesicles from oil-seed rape hypocotyls. Plant Physiol. 97. in press.

Spatial distribution of water and cations in salt-treated plants of Lycopersicon spp.

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Ion contents and succulence seem to be valuable physiological traits on selection of salt tolerant genotypes in tomato, but they would change according to leaf age; that's why we decided to determine their spatial distribution (leaf-to-leaf profiles) in the shoot of plants of Lycopersicon species with differences on salt tolerance.

Plants of L. esculentum cv. "MONEYMAKER", L. pimpinellifolium (accession PE-2) and L. cheesmanii (accession LA-1401) were grown on gravel with Hoagland's solution, plus 0% (control) and 40% (salt treatment) of Artificial Sea Water, for 30 days. Fresh and dry weights were recorded and cations determined in dried leaves.

According to dry weight, PE-2 and LA-1401 seem to be more salt tolerant than the cultivated accession, there being few differences between both of them.

Sodium concentration in salt-treated plants increased uniformly from the youngest analized leaf to the oldest one, in MONEYMAKER; in PE-2 and LA-1401, it was almost constant along the three youngest analized leaves, then increased (more in the former) and became constant again along the oldest leaves. In young leaves, it kept close to the control, in PE-2, and was clearly higher than the control, in LA-1401 and MONEYMAKER.

According to our results, differences of Na concentration between young and old leaves always occur in tomato, irrespectively of salt tolerance. Which could be related to salt tolerance would be the Na concentration pattern; a genotype would be tolerant if Na concentration, in old leaves, was constant and, at least, as high as that of the substrate, and, in young leaves, constant and lower. With the pattern described, young leaves would be kept out of high Na concentration while old ones would be used for storing excess of Na.

Total sodium plus potassium profile in salt-treated plants was vertical and nearly parallel to that of the control all along the plant, in LA-1401; in PE-2 and MONEYMAKER, both values and differences between treatments increased from young to old leaves.

Calcium concentration in salt-treated plants kept very close to the control all along the plant, in LA-1401 and MONEYMAKER; in PE-2, it was always smaller than the control, differences between treatments increasing from young to old leaves.

According to our results, the hypothesis of Cramer at al (1.991), who suggested that Ca might be a limiting factor in the growing zone, could be discarded because Ca concentration was similar to each other in this zone of salt-treated and control plants, while the latter grew far better.

Succulence in salt-treated plants increased from young to old leaves, values of youngest analized leaves being close to the control, within all the accessions. The differences between treatments increased from top to bottom, being higher, at old leaves level, in LA-1401 and PE-2.

Increase in succulence could be related to increase in ion concentration in MONEYMAKER and PE-2; LA-1401 increased succulence while ion concentration (Na+K) remained steady. So, succulence could be seen as an adaptative response to salinity in PE-2 and MONEYMAKER, but not in LA-1401.

SALINITY AFFECTS THE EXPRESSION OF DNA SEQUENCES SUBMITTED TO SALT-DEPENDENT MODIFICATIONS IN THE HALOPHILIC ARCHAEBACTERIUM HALOFERAX MEDITERRANEI.

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Some halobacteria (extremely halophilic archaebacteria) are able to grow over a wide range of salt concentrations. Specifically, representatives of the genus Haloferax can grow from 10% salts to concentrations of saturation for NaCl. To be able to grow at such a different salinities the organism has to suffer considerable physiological changes, many of which could be regulated by expression of different genes. It has been shown recently that the mc-vac gene encoding the major gas vesicle protein of Haloferax mediterranei has markedly different levels of expression at different salt concentrations (Englert et al., 1990).

We have described in Haloferax mediterranei the existence of certain PstI sites (at least 16 in the genome) that appear to be more or less susceptible to cleavage by the enzyme depending on the salt concentration at which the cells have grown (Juez et al., 1990). To determine whether such salt-dependent DNA modifications are related to the genes nearby we have studied the expression of the DNA stretches located around two of the differently modified PstI sites, by Northern blot. Our results show that, in fact, at least one of the species of mRNA codified by each fragment presented markedly different levels of expression depending on the salt concentration of the growth medium. In the case of fragment m61 the transcript level enhancement by high salinity was even more marked than that of the mc-vac gene. These DNA fragments are also being sequenced to determine the corresponding ORFs and their function. The sequences surrounding the PstI sites studied here show peculiarities such as purine-pyrimidine alternancy and inverted and direct repeats similar to those described in promotor regions of certain halobacterial genes. Our results could derive from a putative mechanism of regulation of gene expression by salt through modification of the DNA sequence at or around promoters. Further studies are in progress to clarify the nature of these DNA salt-dependent modifications and their possible role in haloadaptation of halophilic archaebacteria.

LITERATURE CITED

Englert, C., Horne, M. and Pfeifer, F. (1990) Expression of the major gas vesicle protein gene in the halophilic archaebacterium Haloferax mediterranei is modulated by salt. Mol. Gen. Genet. **222:** 225-232.

Juez, G., Rodríguez-Valera, F., Herrero, N. and Mojica, F.J.M. (1990) Evidence for salt-associated restriction pattern mofifications in the archaebacterium Haloferax mediterranei. J. Bacteriol. **172:** 7278-7281.

STRUCTURE AND REGULATION OF COTTON *LEA* GENES

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Late embryogenesis-abundant (*Lea*) genes are highly expressed during the terminal postabscission stage of plant embryogenesis. They have been postulated to be involved in desiccation protection or repair, and some of them are expressed in embryos and other tissues in response to water, osmotic, and salt stress and by application of abscisic acid, a hormone often implicated as a transduction signal for these stresses. Those so far sequenced are hydrophilic and some have amino acid repeats suggestive of structural proteins.

We have sequenced genes or mRNAs of an additional 5 cotton *Lea* and *LeaA* genes, bringing to 11 the number sequenced in cotton. Significantly, 3 of the new ones (*Lea5*-D73, *Lea14*-D95, and *Lea11*-D102) encode proteins that are not hydrophilic. *LeaA2*-D132 encodes a protein very similar to the cotton *Lea4*-D19 and wheat Em proteins, but contains two copies of what, from cotton and barley, is now recognized a tandom 20 amino acid repeat. The fifth, *Lea10*-D92, is the true cotton homolog of the so-called "Group 3" LEA proteins containing tandom copies of the 11 amino acid repeat.

When compared to several other cotton genes that are expressed in embryos at other stages of development, most cotton *Lea* and *LeaA* genes specifically contain in their upstream region several copies of elements similar to those described in wheat and rice that have been implicated as abscisic acid-responsive elements. The significance of these and other potential elements is being studied in transgenic tobacco and in transient expression in cotton embryo protoplasts, using sequences from 4 of the cotton *Lea* and *LeaA* genes. The main focus has been in defining the elements required for normal developmental expression during embryogenesis. These appear in most cases to reside within 250 NT of the transcription start. The results of these and additional studies of developmental and stress responsiveness will be discussed.

SALT TOLERANCE IN THE HALOPHYTIC WILD RICE, PORTERESIA COARCTATA

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Porteresia coarctata, a halophytic species of the tribe Oryzeae that grows in brackish water around the Bay of Bengal, is a potential source for salt tolerance genes for rice improvement. Tolerance to salinity has been found to depend largely on the presence of unicellular salt glands on the leaf surface. The effect of salinity on growth, ion distribution and secretion is discussed, with emphasis on salt gland ultrastructure and cytochemical localization of ATPase activity in salt-treated and salt-free grown leaves.

Multiple mechanisms contribute to osmotic inducibility of *proU* operon expression in *Escherichia coli*: two osmoresponsive promoters, and a negative regulatory element within the first structural gene. J. Gowrishankar, C.S. Dattananda, and K. Rajkumari.
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The osmoprotective action of glycine betaine and L-proline during growth of *E. coli* in media of elevated osmolarity is dependent in part on the presence of a functional ProU transporter in these cells, encoded by three genes of the *proU* operon. Transcription of the *proU* operon is induced several hundred-fold in high-osmolarity growth media; such a magnitude of induction is the highest known for an osmoresponsive locus in any biological system, but the mechanisms involved are still not completely understood. A low copy promoter-cloning plasmid vector, with *lacZ* as the reporter gene, was used for assaying the osmoresponsive promoter activity of each of various lengths of *proU* DNA, generated both by the cloning of discrete restriction fragments and with an exonuclease III-mediated deletion approach. The results indicate that expression of *proU* in *E. coli* is directed from two promoters, one (P2) characterized earlier by other workers with start-site of transcription 60 nucleotides upstream of the initiation codon of the first structural gene (*proV*), and the other (P1) situated approximately 250 nucleotides upstream of *proV*. Furthermore, a long region of DNA contained within *proV* was shown to be involved in negative regulation of *proU* transcription; phage Mu dII1681-generated *lac* fusions in the early region of *proV* also exhibited partial derepression of *proU* regulation, in comparison with fusions further downstream in the operon. The sequences around promoters P1 and those around P2, and the promoter-downstream negative regulatory element, respectively conferred approximately 5-fold, 10-fold and 25-fold osmoresponsivity on *proU* expression. Within the region genetically defined to encode the negative regulatory element, there is a 116-nucleotide stretch which is absolutely conserved between the *proU* operons of *E. coli* and *Salmonella typhimurium*, and which has a capability of exhibiting alternative secondary structure. Insertion of this region of DNA into a pUC18 plasmid vector was associated with specific changes in topological profile in plasmid molecules isolated from cultures grown in high-osmolarity medium; a model to explain its possible role in *proU* regulation will be discussed.

TOBACCO OSMOTIN GENE DOES NOT HYBRIDIZE TO DIFFERENT LEGUMINOUS SPECIES.

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Plants have required the interplay of many gene products to evolve different types of adaptations to drought and salinity. However, with the current levels of knowledge of plant genetics and metabolism, genetic engineering can only be applied to biochemically definable traits involving only a single or very few gene products.

Osmotin is a mayor protein which accumulates in vacuolar inclusion bodies of tobacco cells adapted to low water potencials. Abscisic acid (ABA) is involved in the induction of osmotin synthesis and the osmotic agent is necessary for the protein to accumulate. Enhanced accumulation of osmotin has been associated with NaCl tolerance in tobacco cells. Proteins immunologically related to the tobacco osmotin have been found in other members of the Solanaceae, as well as in Alfalfa and Green Beans.

Our aim was to detect a possible osmotin homologous gene in Legumes by genomic DNA hybridization. Genomic DNA from Leguminous species: Medicago sativa, Medicago truncatula, Glycine max, Pisum sativum, Vicia sativa and Phaseolus vulgaris (Black and Green Breans) was cut with EcoRI, HindIII and XbaI, separated on an agarose gel, blotted and probed with a 561bps fragment corresponding to the coding region of the tobacco osmotin gene which was amplified by PCR from Nicotiana tabacum var. Bouton special. Genomic DNA from tobacco was used as control in the Southern blot.

Hybridization was not found in neither leguminous species when washed at low stringency conditions.

EXPRESSION OF DROUGHT - RELATED PROTEINS FROM THE RESURRECTION PLANT CRATEROSTIGMA PLANTAGINEUM IN TRANSGENIC TOBACCO

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Several proteins are induced during water stress in the resurrection plant Craterostigma and their corresponding cDNA clones have been isolated and sequenced (Bartels et al., 1990 ; Piatkowski et al., 1990). Three selected cDNAs, namely pcC6-19, pcC3-06 and pcC27-45 encode some of the most abundant proteins expressed during dehydration. The encoded product of pcC6-19 displays homology to desiccation-related genes expressed in embryos or dehydrated seedlings of several plants (Mundy and Chua, 1988 ; Close et al., 1989). The pcC3-06 cDNA clone contains tracts of sequences which are related to a cotton embryo gene (Baker et al., 1988). The third cDNA clone, pcC27-45, appears to encode a Craterostigma-specific protein (Piatkowski et al., 1990).

Each of the mentioned cDNAs was subcloned in the Agrobacterium Ti-derived plasmid pBin19 (Bevan,1984) under the control of a triplicated 35S promoter, to ensure high levels of expression in tobacco plants, and subsequently used to transform tobacco. Fully regenerated plants were screened for protein expression with specific antibodies raised from protein expressed in E. coli. Results concerning the physiological performance of these plants will be presented.

References

- Barker, J.C., Steele, C., Dure III, L (1988) Plant Mol. Biol. 11 : 277-291.

- Bartels, D., Schneider, K., Terstappen, G., Piatkowski, D., Salamini, F. (1990) Planta 181 : 27-34.

- Bevan, M. (1984) Nucl. Acids Res. 12 : 8711-8721.

- Close, T.J., Kortt, A.A., Chandler, P.M. (1989) Plant Mol. Biol. 13 : 95-108.

- Mundy, J., Chua, N.-H. (1988) EMBO J. 7 : 2279-2286.

- Piatkowski, D., Schneider, K., Salamini, F., Bartels, D. (1990) Plant Physiol. 94 : 1682-1688.

Expression of closely related Lea genes in cultured barley embryos indicates different transduction pathways for ABA, salt and mannitol

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The late embryogenesis abundant (Lea) genes, which encode putative "desiccation protectants", can be induced in immature and germinating embryos and in vegetative tissues by ABA and various applied osmotic stress factors. By screening a barley cDNA library with cotton Lea D19 cDNA we cloned three closely interrelated genes (B19.1, B19.3 og B19.4) with similarites to wheat Em. The only major difference between the coding sequences of the barley B19 genes is in copy number of a 20 amino acid sequence (1,3 and 4 copies in B19.1, B19.3 and B19.4, respectively). This amino acid motif is also found as a repeat in related Lea genes in dicots, indicating an important function of the repeat. The B19 genes are tightly clustered and putative ABA responsive elements are found in the upstream regions.
The B19 mRNAs show differential accumulation during development and in cultured embryos. B19.1 is the most abundant transcript of the three. In immature embryos all three genes are responsive to ABA and mannitol, however only B19.1 is strongly responsive to salt. Application of the ABA biosynthesis inhibitor norflurazon together with salt or mannitol reveals that the salt response is completely supressed by norflurazon, whereas the mannitol response is unaffected. In sum, these results insicate that; 1) the salt response and ABA response are mediated through different pathways that have common steps in the transduction chain. 2) endogenous and exogenous ABA have differential effects on gene expression. 3) the mannitol transduction pathway acts independently of the ABA pathway.

OSMOREGULATION IN ESCHERICHIA COLI: A CONTINUOUS EXCRETION OF ORGANIC OSMOPROTECTANTS

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It is known that *Escherichia coli* can osmoregulate by uptake of glycine betaine through the ProU and ProP systems and by synthesis of glycine betaine from choline by the Bet system. We report that the ProU system of *E. coli*, but not that of *Salmonella typhimurium*, can mediate low-affinity choline uptake. The high-affinity choline uptake system BetT of *E. coli* was shown to be activated at the level of transport by osmotic stress. Furthermore, choline which had been accumulated in stressed cells was excreted during uptake of glycine betaine. An *E. coli* strain which was defective in glycine betaine uptake was unable to retain glycine betaine synthesized from choline within the cytoplasm. We conclude that *E. coli* has efflux systems for both choline and glycine betaine, and that the cytoplasmic pool of glycine betaine is maintained through an equilibrium between synthesis, uptake, and efflux.

Na+-GLYCEROL CO-TRANSPORT SYSTEM IN DEBARYOMYCES HANSENII AS A PHENOMENUM WITH OSMOREGULATORY PURPOSES

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Debaryomyces hansenii is known to maintain osmotic balance when growing exponentially in glucose medium in the presence of NaCl by accumulating glycerol as compatible solute, establishing, for these two molecules, gradients across plasma membrane of opposite directions.

Evidence is presented here that the two gradients are linked trough a constitutive Na+/glycerol symport that uses sodium gradient as the driving force to maintain glycerol gradient. The symporter also accepts potassium ions as a co-substrate. It was also observed that glycerol uptake was accompanied by proton uptake when extracellular NaCl was present and that the protonophore CCCP induced collapse of glycerol gradient. This supports earlier proposals, by other authors, that the sodium gradient is maintained by an active sodium-proton exchange mechanism.

EFFECTS OF Ca^{2+} ON SALT-STRESS RESPONSE OF BARLEY ROOTS AS OBSERVED BY IN VIVO ^{31}P NMR AND IN VITRO ANALYSIS

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Time course experiments were carried out to study the effect of salinity (100 mM NaCl) and high Ca^{2+} concentration (6 mM) on excised barley roots. In vivo ^{31}P NMR data showed a pH alkalization of the vacuole after salt treatment. The salt-induced alcalization of vacuolar pH was reduced by addition of the high Ca^{2+} treatment. Slight acidification was observed in the cytoplasmic pH, follow by moderate alkalinization in all treatments. However pH alkalinization begun first at the high salt treatments. On the other hand, salinity increased Na^+ and decreased K^+ content in the tissue. At the low salt level (1 mM), high Ca^{2+} treatment increased the total Na^+. However, high Ca^{2+} treatment reduced Na^+ uptake rate at high salt concentration. No clear differences was observed in the cytoplasmic Pi content. However, Pi in vacuole was highly influenced by the treatments. In all treatment vacuolar Pi increased except in the high salt and low Ca treatment that decreased. High Ca increased vacuolar Pi at the control treatments.

EFFECT OF NaCl ON GROWTH METABOLISM AND ENERGETICS OF *SACCHAROMYCES CEREVISAE*

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A respiratory (petite) mutant of *Saccharomyces cerevisiae* IGC3507, was able to growth exponentially on a synthetic medium with NaCl concentrations up to 2 M. The specific growth rate (K) decreased linearly with NaCl concentration and this relation could be described by the equation:

$$K_{NaCl} = K_0 - K_0 \ (NaCl)/(NaCl)_{max}$$

in which K_{NaCl} and K_0 were the growth rates with and without salt respectively and $(NaCl)_{max}$ was upper limit of salt tolerance (at which growth can occur). In our case this value was 2.2 M.

We had show previously that the specific growth rate of this strain, cultivated under identical environmental conditions on the same medim, was a variable with normal distribution (1). Curiously enough, the variability decreased with the concentration of salt in the medium. Although the source of this variation remains obscure, it seems that these cells are capable to develop, under the same conditions, several steady-states,each one defined by a growth rate. The number of this possible steady-states would be limited by a salt stress.

Salt concentration also affected growth metabolism. In contrast with our hypothesis that salt should estimulate alcoholic fermentation to produce the extra ATP needed for Na pumping, alcohol production decreased about 308 at 0.5 and 1 M NaCl, and was similar to the control at 1.5 M. Glicerol production also decreased at 0.5 M NaCl but increased at 1 M and almost doubled the amount produced by the control at 1.5 M NaCl. Non-fermentative carbon dioxide, acetoin and diacetly were the excreted metabolites that increased their production monotonously with NaCl concentration.

As the observed changes could be due to the decrease in growth rate and not specifically to the effect of NaCl, the cells were cultivated in a continuous culture at the same dilution (growth) rate with (0.5 M) and without salt. The results were confirmed and apparently cells in 0.5 M NaCl needed less energy to growth, at the same rate, that those without salt. This result was also confirmed by microcalorimetric measurements: Control cells produced 37.8 Kcal per mol of glucose consumed, whereas those growtn at 0.5 M NaCl produced only 27.8 Kcal/mol. The reasons for this increase in energetic efficiency induced by moderate concentrations of NaCl are currently under study.

1.- J.M. Peinado & N. van Uden. Arch. Microbiol. 113:-303-307 (1977)

Acute changes in nucleotide pools during adaptation to salt stress by *Escherichia coli*

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Adaptation of *Escherichia coli* to salt stress (osmotic upshock) produces major changes in the pools of phosphorylated nucleotides. Cells with a mutation preventing trehalose synthesis growing in glucose minimal medium were subjected to an increase of 0.45 M NaCl, which permitted resumption of cell growth (doubling time 2.7 h) within 30 minutes; 0.65 M NaCl, after which the cells had difficulty resuming growth; and an intermediate upshock of 0.57 M NaCl. Cell samples labeled with ^{32}P were taken at intervals after upshock and separated by 2D TLC. Purine triphosphates decreased transiently prior to reaching levels approximately 2 fold higher than pre-stress conditions. The levels gradually decreased to pre-stress levels 30 - 40 minutes after upshock. Under severe salt stress (0.65 M NaCl), the decrease of the ATP and GTP pools was greater and prolonged; 2 h after upshock levels were still below pre-stress. Pools of purine diphosphates increased transiently, reaching peak levels within 1 - 2 minutes of upshock. The extent of increase was dependent on the extent of the salt stress and recovery of ADP pools to pre-stress levels was slower following a severe salt stress. Our studies indicate that changes in adenylate energy charge are responsible, at least in part, for the growth lag of *E.coli* in adapting to higher osmolarity. The alarmone ppGpp, found to accumulate in *E.coli* during carbohydrate starvation, nitrogen starvation or during inhibition of protein synthesis, was found to increase more than 10 fold during salt stress. The changes in phosphorylated nucleotides were largely dependent on the ability of the cell to take up potassium in response to salt stress rather than salt stress per se; most of the responses were reduced or absent during salt stress under potassium limiting conditions. An exception was dATP, the pools of which increased 2-3 fold in the absence of K^+ and remained elevated. In the presence of K^+ the levels of dATP recover to pre-stress levels within 35 min of upshock. (Supported by NSF grant DCB 8704059)

CLONING SEQUENCING AND EXPRESSION OF THE nhaB GENE FROM ESCHERICHIA COLI

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Na^+/H^+ antiporter activity is found in most living cells. In *E. coli* and other bacteria, the Na^+/H^+ antiporter is the main system responsible for the generation and maintenance of a sodium gradient (directed inward). A gene coding for this activity in *E. coli*, *nha*A, was cloned and sequenced. NhaA was overproduced and purified and it was found to be electrogenic and extremly sensitive to pH. Δ*nha*A mutants are sensitive to high concentrations of sodium and lithium in a pH dependent manner but also possess residual Na^+/H^+ antiport activity indicating the existence of alternative gene(s) which we tentatively named *nha*B.

In this work we have constructed a genomic library from the Δ*nhaA* mutant and screened it for its ability to correct the phenotype of this strain i.e, growth on LB medium supplemented with 0.5 M NaCl at pH 7.5. Using this approach pEL24, a pUC18 plasmid containing a 2.3Kb insert, was isolated. The antiporter activity of the Δ*nha*A strain containing pEL24, as measured in inverted membrane vesicles using acridine orange as a monitor of ΔpH, was found to be very high with Na^+ as with Li^+ ions and pH independent. The insert was sequenced and it shows an ORF with 1512 bp that encodes a hydrophobic 55.5 kDal protein with 12 putative trans-membrane segments. Metabolic labeling of the cloned gene product with the T7 polymerase system yielded a single, membrane associated band (apparent Mr 45-47 kDal).

In order to understand the role of *nha*B in cell physiology, Δ*nha*B and Δ*nha*AΔ*nha*B strains were constructed. This was done by replacing 1.0 Kb fragment from the ORF of *nha*B with a 1.3 Kb fragment containing chloramphenicol resistance gene and allowing for homologous recombination.

PROTEINS RELATED WITH SALT ADAPTATION IN C. Limonum CELLS.

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The response of plants to environmental stresses has atracted great interest in plant science studies in recent years, with particular attention to the analysis of gene expresion. The basic sumption in these studies is that plants able to survive under stress have an altered gene expression which is detected as changes in mRNA and proteins.

Citrus limonum cells adapted to salinity showed several changes in the pattern of protein synthesis when compared with unselected cells. Some spots were increased and several new spots could be recognized in adapted cells. The first group of 3 spots afected by cuantitative changes had a mol.wt. of 12kd and pI from neutral to basic. Qualitative changes (appearance of new polypeptides) were limited to 3 groups of polypeptides, one of acidic pI and 10kD and two of basic pI with 29 and 68 kD .

Antibody raised against the salt induced 26kD protein of tobacco (kindly provided by Dr. R.A. Bressan, Purdue University , USA) did not show any cross reaction with proteins from salt tolerant C. limonum cells.

Polypeptide changes associated with salt adaptation in C. Limonum cells are related with previous studies in tobacco , tomato and orange salt tolerant cell cultures. However, clear differences can be observed between species with the unique common presence of 25-29kD polypeptides. Those facts show that many salt-induced changes in proteins are species-specific and reflect an altered gene expresion caused by the adaptation process.

A probable lipid transfer gene is induced by NaCl in tomato plants and shows stem-specific accumulation

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A full-lenght tomato cDNA clone, TSW12, has been isolated and characterized. The nucleotide sequence of TSW12 includes an ORF coding for a basic protein of 114 amino acids which shows high similarity with reported plant lipid transfer proteins.
TSW12 gene expression is subjected to enviromental and developmental regulation: mRNA is accumulated during germination and its level is increased after NaCl or heat shock. In mature plants, this mRNA can only be detected upon NaCl, ABA or mannitol treatment, and its expression mainly occurs in stems.

ISOLATION AND CHARACTERIZATION OF PYRROLINE-5-CARBOXYLATE REDUCTASE GENE FROM *Arabidopsis*

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In higher plants, proline is believed to play a role in resistance to stresses such as salt, drought, and frost. In fact, proline accumulation is frequently observed in plants grown under such conditions. The precise role of this accumulated proline is not understood yet, but can be an osmoticum, a compatible solute-protecting enzyme. The source of this accumulation is mainly *de novo* synthesis. The proline biosynthetic pathway is not well understood in plants and the mechanisms of its enhancement after stress is not known.

We cloned and characterized a pyrroline-5-carboxylate acid reductase (P5CR) gene in *Arabidopsis thaliana*. P5CR (L-proline; $NADP^+$ 5-oxidoreductase; E.C. 1.5.1.2) catalyzes the last step of the pathway, the reduction of P5C to proline. The upstream region of the gene shows several interesting features: a palindromic sequence (-253) and 20-bp repeated elements (-61 to +118); a putative regulatory role of these repeats is being investigated. Genomic analysis revealed at least two copies of the P5CR gene. Homology of the coding region with known P5CR protein sequences will be reported. Expression of the P5CR gene has been studied; the transcript is 1.35 kb long and the expression is organ specific, not repressed by proline, and induced by salt.

THE SOD2 GENE: A NOVEL SODIUM/PROTON ANTIPORTER IN FISSION YEAST

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The sod2 (sodium 2) locus has been identified and shown to represent an electrogenic Na^+/H^+ antiporter functioning in sodium export. The sod2 gene has been cloned and sequenced. Sequence analysis shows that the predicted sod2 protein is highly hydrophobic and has at least 8 transmembrane segments. No significant sequence homology has been found between the sod2 protein, the bacterial ant and mammalian Na^+/H^+ antiporter. Overexpression of sod2 increases sodium export capacity and confers NaCl tolerance on cells. Disruption of sod2 blocks Na^+ export and causes hypersensitivity to NaCl. With a sod2 disruption, cells fails to grow at alkaline pH. The sod2 gene has been amplified in sod2 mutants. This kind of amplification could be stepwise selected. The degree of sod2 gene amplification correlates with the level of LiCl/NaCl tolerance.

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LIST OF INVITED SPEAKERS

Workshop on
SALT TOLERANCE IN MICROORGANISMS AND PLANTS: PHYSIOLOGICAL AND MOLECULAR ASPECTS

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253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
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J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

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Organized by M. Perucho and E. Martínez-Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. Mc Clelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 **Workshop on Yeast Transport and Energetics.**
Organized by A. Rodríguez-Navarro and R. Lagunas. Lectures by M. R. Chevallier, A. A. Eddy, Y. Eilam, G. F. Fuhrmann, A. Goffeau, M. Höfer, A. Kotyk, D. Kuschmitz, R. Lagunas, C. Leão, L. A. Okorokov, A. Peña, J. Ramos, A. Rodríguez-Navarro, W. A. Scheffers and J. M. Thevelein

265 **Workshop on Adhesion Receptors in the Immune System.**
Organized by T. A. Springer and F. Sánchez-Madrid. Lectures by S. J. Burakoff, A. L. Corbi-López, C. Figdor, B. Furie, J. C. Gutiérrez-Ramos, A. Hamann, N. Hogg,

L. Lasky, R. R. Lobb, J. A. López de Castro, B. Malissen, P. Moingeon, K. Okumura, J. C. Paulson, F. Sánchez-Madrid, S. Shaw, T. A. Springer, T. F. Tedder and A. F. Williams.

266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
Organized by F. X. Avilés. Lectures by T. L. Blundell, W. Bode, P. Carbonero, R. W.Carrell, C. S. Craik, T. E. Creighton, E. W. Davie, L. D. Fricker, H. Fritz, R. Huber, J. Kenny, H. Neurath, A. Puigserver, C. A. Ryan, J. J. Sánchez-Serrano, S. Shaltiel, R. L. Stevens, K. Suzuki, V. Turk, J. Vendrell and K. Wüthrich.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organized by J. M. Mato and J. Larner. Lectures by M. V. Chao, R. V. Farese, J. E. Felíu, G. N. Gaulton, H. U. Häring, C. Jacquemin, J. Larner, M. G. Low, M. Martín Lomas, J. M. Mato, E. Rodríguez-Boulan, G. Romero, G. Rougon, A. R. Saltiel, P. Strålfors and I. Varela-Nieto.

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