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TITLE: Cell Signalling and Phospholipid Metabolism

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RESULTS FROM PRIOR DOE SUPPORT

Our research for the past two years has involved the study of phosphoinositides and their potential role in regulating plant growth and development. Our initial goal was to document the sequence of events involved in inositol phospholipid metabolism in response to external stimuli. Our working hypothesis was that phosphatidylinositol bisphosphate (PIP_2) was in the plasma membrane of plants cells and would be hydrolyzed by phospholipase C to yield the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) and that IP_3 would mobilize intracellular calcium as has been shown for animal cells.

Our results with both carrot suspension culture cells and sunflower hypocotyl indicate that this paradigm is not the primary mechanism of signal transduction in these systems. We have observed very rapid, within 5 sec, stimulation of phosphatidylinositol monophosphate (PIP) kinase which resulted in an increase in PIP_2. However, there was no evidence for activation of phospholipase C. In addition, we have shown that PIP and PIP_2 can activate the plasma membrane ATPase. The results of these studies are described briefly in the paragraphs below.

Inositol phospholipids are localized in distinct membrane fractions. If PIP and PIP_2 play a role in the transduction of external signals, they should be present in the plasma membrane. We used the fusogenic carrot suspension culture cells as a model system to study the distribution of inositol phospholipids in various membrane fractions and organelles. Cells were labeled 12 to 18 h with *myo*[2-^{3}H] inositol and the membranes were isolated by aqueous two-phase partitioning. The plasma membrane was enriched in PIP and PIP_2 compared to the intracellular membranes (Wheeler and Boss, 1987).

Interestingly, unlike most animal cells, lysophosphoinositides also were very prevalent. These lipids were shown not to be generated during extraction and like the parent lipids, they partitioned into distinct membrane fractions. For example, over 96% of the recovered lysophosphatidylinositol (LPI) was found in the lower phase fraction (intracellular membranes) and about 4 % in the plasma membrane fraction and yet lysophosphatidylinositol monophosphate (LPIP) was more prevalent in the plasma membrane fraction.

The tonoplast (vacuolar membrane) and isolated vacuoles also were found to contain LPIP as the predominant inositol lipid other than PI. The tonoplast, however, contained little or no PIP or LPI (Cho, Memon, and Boss, Appendix I). Isolated nuclei, on the other hand, had detectable levels of PIP_2, PIP and LPI as well as LPIP and PI. In spite of the fact that [^{3}H]PIP_2 was 5.3% of the total inositol lipid recovered from the nuclei, the total inositol lipid per mg protein or per [^{14}C] myristate-labeled lipid was much less than that found in other membranes or organelles studied (Hendrix, Assefa, and Boss, Protoplasma, in press). However, the fact that the inositol phospholipids and lysolipids were found in the nuclear membrane is of interest because of their purported role in regulating cell proliferation (Berridge, Biochim Biophys Acta 907:33-45).

In addition to characterizing the [^{3}H] inositol-labeled lipids present in the fusogenic carrot cells, we assayed each fraction for lipid kinase activity using [γ ^{32}P]ATP. All membrane fractions had diacylglycerol (DAG) and PI kinase activity. The plasma membrane and nuclear membrane had

PIP kinase activity using endogenous substrate. These data are consistent with the fact that PIP_2 was only found associated with plasma membrane and nuclear membrane. The fact that PI kinase activity was found in the tonoplast even though PIP is not there suggested that PIP is either rapidly metabolized by the tonoplast or that in vivo conditions such as pH and substrate availability do not favor PIP biosynthesis.

The carrot inositol lipids have been identified by fast atom bombardment mass spectrometry. Lipids were separated by thin-layer and column chromatography. Individual samples were analyzed in a fast atom bombardment mass spectrometer using the negative ion mode. These studies were considered to be an important step for complete characterization of the inositol lipids since prior to this, identification of the inositol phospholipids in plants had only been by comigration of radiolabeled spots with standards and since lysolipids comigrate with the standards in some solvent systems. The data were present at the Conference on Mass Spectrometry and Allied Topics, May 1989 and a manuscript has been submitted for publication to Lipids (Van Breemen, Wheeler and Boss).

Inositol trisphosphate was not detectable in cell extracts and was rapidly metabolized in a cell free system. If IP_3 is a second messenger, then according to the animal paradigm it should increase in response to external stimuli. Quantitating IP_3, however, in plants is thwarted by the fact that inositol is metabolized to glucuronic acid and other cell wall metabolites which will coelute with the inositol phosphates on anion exchange columns. Therefore, one must use HPLC or paper electrophoresis to obtain adequate separation. We chose the latter because of the availability of the equipment and expertise of Dr. Scott Chilton of the Botany Department. No IP_3 was detected in extracts of carrot cells labeled overnight (Rincón, Chen, and Boss, 1989). There were two possible explanations: (a) IP_3 was below the detection limits of our assay, or (b) IP_3 was metabolized as soon as it was produced.

To test the latter hypothesis, we isolated a microsomal membrane and soluble protein fraction (40,000*g* pellet and supernatant, respectively) from the carrot cells and monitored the metabolism of IP_3 (Memon, Rincón, and Boss, 1989, Appendix I). With the membrane fraction 90 % of the IP_3 was metabolized in 15 min at a rate of 55 pmoles mg^{-1}protein min^{-1} and IP_2 was the major product. With the soluble fraction, the rate of metabolism was slightly faster (70 pmoles mg^{-1}protein min^{-1}); 99 % of the IP_3 was metabolized and IP_2 and IP were the major products at 60 and 40 % of the recovered ^3H, respectively. The metabolism of IP_3 in both fractions was not sensitive to 10 mM lithium, a commonly used inhibitor of I-1,4-P_2 phosphatase in animal cells. However, we did observe inhibition of IP_3 metabolism when 1 mM molybdate was added to the membrane fraction.

The amount of PIP_2 present in these and other plant cells has been found to be very low. Since the vacuole has been shown by others (Schumaker and Sze, J Biol Chem 262:3944, 1987; Ranjeva et al., FEBS Lett 230:137, 1988) to be the IP_3-sensitive calcium store in plants, if IP_3 is rapidly metabolized by membrane and soluble phosphatases it is questionable whether or not sufficient IP_3 would reach the vacuole prior to being metabolized.

The phosphoinositides alone can alter plant membrane function. The polyphosphoinositides, PIP and PIP_2 increase the plasma membrane, vanadate-sensitive ATPase activity by about two fold.

The response was seen with both sunflower and carrot plasma membrane vesicles isolated by aqueous two-phase partitioning. The effect is not due to breakdown of the lipids since none of the metabolites (DAG, IP_3, or LPI) had the same effect (Memon, Chen, and Boss, Appendix I). This means that by altering the ratio of PI to PIP to PIP_2 (i.e. by regulating the phosphoinositide kinases or phosphatases) one could affect the plasma membrane ATPase.

PIP kinase activity is affected by external stimuli. Using two different external stimuli, light and a mixture of fungal cellulases, we have shown that PIP kinase activity is affected within seconds and there is a concomitant change in the plasma membrane ATPase activity. As little as 10 sec of light in vivo decreases the PIP kinase activity by 43 % when assayed in an in vitro phosphorylation system. There is a concomitant decrease in the vanadate-sensitive ATPase activity. These data are summarized in the attached abstract by Memon and Boss (Appendix I) and the manuscript is in preparation.

Using carrot culture cells we observed that a 5 sec exposure of cells to fungal cellulases increased the PIP kinase activity of isolated carrot plasma membranes and increased the plasma membrane ATPase activity. Studies of whole cell extracts after exposure to fungal enzymes indicated that there was no detectable IP_3 or evidence for changes in the distribution of other inositol lipids except for LPIP which decreased. The increase in kinase activity was detected with a 0.04 % mixture of enzymes. The ATPase activation was not detected at this concentration but was seen at 0.2 %. These data are summarized in the attached abstract by Chen and Boss and the manuscript is in preparation.

In summary, our studies suggest that in the two systems studied, carrot suspension culture cells and sunflower hypocotyls, the PIP kinase and PIP_2 phosphatase are key regulators of plant responses to external stimuli and that the negatively charged inositol polyphospholipids can, in and of themselves, regulate the plasma membrane ATPase and thereby affect plant growth and development. We found no evidence for the production of IP_3 in vivo nor for a loss in PIP or PIP_2 which would indicate phospholipase C activation. The paradigm originally proposed which involves the hydrolysis of PIP_2 to IP_3 and DAG does not appear to be of primary importance in these systems.

As a result of this research, we have developed a new hypothesis concerning the role of inositol phospholipids in signal transduction in these cells, one in which the inositol lipids directly interact with membrane proteins thereby altering enzyme activity and cytoskeletal interaction. This hypothesis is the basis for the attached proposal.

Other Research Activities: Dr. D. James Morré and I co-organized the First International Workshop on Second Messengers and Inositol Metabolism in Plants held at Purdue University, April, 1988. In addition, Dr. Morré and I co-edited the book entitled "Second Messengers in Plant Growth and Development" Alan R. Liss, N.Y. 1989 and are co-editing with Dr. Frank Loewus a book entitled "Inositol Metabolism in Plants" which recently went to press.

Other Research Currently Funded: The principle investigator has one project funded by the National Science Foundation. The NSF project is separate and has a different focus and goals

from this project on signal transduction which we are submitting to the Department of Energy. The goals of the NSF project are to characterize the biogenesis and metabolism of lysophosphatidylinositol monophosphate (LPIP) which is found in the plasma membrane of the fusogenic carrot cells and which correlates positively with their fusion potential and to study the effect of LPIP on membrane structure and function. The fact that both projects involve the inositol phospholipids has led to productive discussions in the laboratory and has been synergistic for both projects.

Publications since 1987 relating to this project:

Memon, A., Q. Chen and W. F. Boss. Inositol Phospholipids Activate Plasma Membrane ATPase in Plants. Biochem Biophys Res Comm, submitted.

Memon, A., M. Rincón and W. F. Boss. 1989. Inositol Trisphosphate Metabolism in Carrot (Daucus carota L.) Cells. Plant Physiol, submitted.

Hendrix, K. W., H. A. Assefa and W. F. Boss. 1989. Polyphosphoinositides are Present in Nuclei Isolated from Carrot Protoplasts. Protoplasma, in press.

Rincón, M. and W. F. Boss. 1989. Phosphoinositides as Second Messengers in Plants. In: Plant Inositide Metabolism. D. J. Morré, W. F. Boss and F. Loewus, eds. Alan R. Liss, in press.

Wheeler, J. J. and W. F. Boss. 1989. Inositol Lysolipids. In: Plant Inositides. D. J. Morré, W. F. Boss, and F. Loewus, eds. Alan R. Liss, in press.

Rincón, M. Q. Chen, and W. F. Boss. 1989. Characterization of Inositol Phosphates in Carrot (Daucus carota.) Cells. Plant Physiol 89:126-132.

Boss, W. F. 1989. Plant Phosphoinositide Metabolism: A Potential Mechanism for Signal Transduction. In: Second Messengers in Plant Growth and Development. W. F. Boss and D. J. Morré, eds. Alan R. Liss, New York. pp 29-56.

Boss, W. F. and D. J. Morré, eds. 1989. Second Messengers in Plant Growth and Development. One volume of a series entitled "Plant Biology." Series ed. N. S. Allen. Alan R. Liss, New York.

Blowers, D., and W. F. Boss, A. J. Trewavas. 1987. Rapid Changes in Plasma Membrane Protein Phosphorylation During Cell Wall Digestion. Plant Physiol 86:505-509.

Wheeler, J. J. and W. F. Boss. 1987. Polyphosphoinositides are Present in the Plasma Membranes Isolated from Fusogenic Carrot Cells. Plant Physiol. 85:389-392.

Rincón, M. and W. F. Boss. 1987. *Myo*-Inositol Trisphosphate Mobilizes Calcium from Fusogenic Carrot (Daucus carota L.) Protoplasts. Plant Physiol. 83:395-398.