“REGULATION OF SENESCENCE BY SUGAR METABOLISM”

David Granot - Israel

Richard Amasino - USA

ABSTRACT

Research objectives

- a. Analyze transgenic plants that undergo rapid senescence due to increased expression of hexokinase.
- b. Determine if hexokinase-induced senescence accelerates natural senescence using senescence specific promoters that drive expression of a reporter gene (GUS) and a cytokinin producing gene (IPT - isopentyl transferase).
- c. Isolate and analyze plant genes that suppress sugar-induced cell death (SICD) in yeast, genes that potentially are involved in programmed cell death and senescence in plants.

Background to the topic

Leaf senescence is a regulated process of programmed cell death (PCD) in which metabolites are recycled to other active parts of the plant. Senescence associated genes (SAGs) are expressed throughout leaf senescence. Sugar flux and metabolism is thought to play a fundamental regulatory role in senescence. We found that transgenic tomato plants with high hexokinase activity, the initial enzymatic step of sugar (hexose) metabolism, undergo rapid leaf senescence, directly correlated with hexokinase activity. These plants provide a unique opportunity to analyze the regulatory role of sugar metabolism in senescence, and its relation to cytokinin, a senescence-inhibiting hormone. In addition, we found that sugar induces programmed cells death of yeast cells in direct correlation to hexokinase activity. We proposed to use the sugar induced cell death (SICD) to isolate Arabidopsis genes that suppress SICD. Such genes could potentially be involved in senescence induced PCD in plants.

Major conclusions

The promoters of Arabidopsis senescence-associated genes, SAG12 and SAG13, are expressed in senescing tomato leaves similar to their expression in Arabidopsis leaves, indicating that these promoters are good senescence markers for tomato plants. Increased hexokinase activity accelerated senescence and induced expression of pSAG12 and pSAG13 promoters in tomato plants, suggesting that sugar regulate natural senescence via hexokinase.

Expression of IPT, a cytokinin producing gene, under pSAG12 and pSAG13 promoters, delayed senescence of tomato leaves. Yet, senescence accelerated by hexokinase was epistatic over cytokinin, indicating that sugar regulation of senescence is dominant over the senescence-inhibiting hormone.

A gene designated *SFPI*, which is similar to the major superfamily monosaccharide transporters, is induced during leaf senescence in Arabidopsis and may be involved in sugar transport during senescence. Accordingly, adult leaves accumulate sugars that may accelerate hexokinase activity.

Light status of the entire plant affects the senescence of individual leaves. When individual leaves are darkened, senescence is induced in the covered leaves. However, whole adult plant placed in darkness show delayed senescence.

In a search for Arabidopsis genes that suppress SICD we isolated 8 cDNA clones which confer partial resistance to SICD. One of the clones encodes a vesicle associated membrane protein – VAMP. This is the first evidence that vesicle trafficking might be involved in cell death.

Implications

Increased hexokinase activity accelerates senescence. We hypothesized that, reduced hexokinase activity may delay senescence. Preliminary experiments using a hexokinase inhibitor support this possible implication. Currently we are analyzing various practical approaches to delay leaf senescence via hexokinase inhibition.

ACHIEVEMENTS

1. Involvement of hexokinase in the regulation of leaf senescence and its relation to cytokinin

It has previously been suggested that sugar regulate leaf senescence. However, the mechanism by which sugar regulates senescence is not known. Our initial observation that overexpression of Arabidopsis hexokinase (*AtHXK1*) accelerates tomato leaf senescence suggested that hexokinase, the first enzyme in hexose metabolism, is involved in the regulation of senescence. In order to assess this hypothesis we generated transgenic tomato plants that express the GUS (glucuronidase) reporter gene under Arabidopsis senescence specific promoters, pSAG12 and pSAG13. Both promoters are expressed in tomato senescing leaves similar to their expression in Arabidopsis leaves (Figure 1), suggesting conservation of the senescence processes in various plants. A cross between plants overexpressing *AtHXK1* and plants expressing SAG12::GUS and SAG13::GUS produced plants expressing both *AtHXK1* and either SAG12::GUS or SAG13::GUS. Expression of GUS in these plants was directly correlated with the accelerated senescence suggesting that hexokinase accelerates natural senescence.

Figure 1: Expression of SAG12 and SAG13 promoters in mature leaves of transgenic tomato plants.

We found that expression of *SFPI*, an Arabidopsis gene homologous to a monosaccharide transporter, is senescence associated, and is paralleled by an accumulation of monosaccharides (publication no. 5). This observation supports the possibility that accelerated hexose phosphorylation plays a role in senescence.

While sugar accumulation and increased hexokinase activity accelerate senescence, cytokinin, a plant hormone, delays senescence. Which of the two, hexokinase or cytokinin is dominant with regard to senescence? To answer this

question we produced transgenic tomato plants expressing IPT, a cytokinin-producing gene, under SAG12 and SAG13 promoters. Expression of SAG12::IPT and SAG13::IPT delayed senescence (Figure 2). These plants were crossed with plants overexpressing *AtHKK1* to obtain plants that express *AtHKK1* and either SAG12::IPT or SAG13::IPT. These “double” transgenic plants exhibited accelerated

senescence indicating that senescence induced by hexokinase is dominant over cytokinin.

1.2 Isolation and initial characterization of plant genes suppressing sugar induced cell death (SICD)

We used the SICD phenomenon to isolate *A. thaliana* cDNA clones that confer resistance to, or reduce the rate of SICD. Such clones may uncover genes involved in plant programmed cell death and senescence that are stimulated or induced by sugars. We isolated eight cDNA clones, which confer partial resistance to SICD. Data base comparison uncovered homology of four clones with four Arabidopsis genes that appear in the database. One (3.54) is an Arabidopsis gene (LHCP AB 165) for chlorophyll a/b binding protein, *CAB1*, the second (1.33) is Arabidopsis gibberellin-regulated (GASA4) mRNA, the third (1.29) is partially homologous to methionyl aminopeptidase and the forth clone (3.61) encodes for a synaptobrevin-like protein which is a vesicle associated membrane protein (VAMP). The other four clones are new, unidentified cDNAs that exist in Arabidopsis genomic DNA database. Most interesting (at this point) is clone 3.61, which encodes for a vesicle associated membrane protein (VAMP). It blocks apoptosis of yeast cells downstream of the oxidative burst (publication no. 4). This is the first functional evidence that vesicle trafficking is involved in cell death.

Agricultural and/or economic impacts

One major outcome of this research is the discovery that hexokinase regulates senescence. Since overexpression of hexokinase accelerates senescence, reduced activity of hexokinase may delay senescence. In a preliminary experiment we have analyzed the effect of a hexokinase inhibitor on leaf senescence of cut Lily flowers. The hexokinase inhibitor delayed leaf senescence (Figure 3). Regulating senescence by modulation of hexokinase activity could have an important agricultural implication.

Figure 3: A - Control B - Delayed leaf senescence in presence of hexokinase inhibitor.

We have initiated an antisense approach to reduce hexokinase activity in tomato plants and to

analyze its effect on leaf senescence. For that purpose we have recently isolated and characterized two tomato hexokinase genes, *LeHXX1* and *LeHXX2* (publications no. 2& 8). We also plan to generate tomato plants that over express Arabidopsis VAMP. That might prevent PCD and delay senescence.

Details of cooperation

The two research groups cooperated in a number of ways. The constructs of SAG::GUS and SAG::IPT were prepared in the US laboratory and were used by the Israeli PI to generate transgenic tomato plants. The Israeli group isolated and sequenced the SICD suppressing genes that were used by the US laboratory to screen for *Arabidopsis* knockouts. Binary constructs of hexokinase and fructokinase prepared in the Israeli laboratory were sent to the US group for Arabidopsis transformation.

The two PIs met twice and had a continuous e. mail contact. As a result of this collaboration, a second BARD grant has been recently approved.

List of publications

1. Dai N., Schaffer A., Petreikov M., Shahak Y., Giller Y., Ratner K., Levine A. and Granot D. (1999). Overexpression of *Arabidopsis* hexokinase in tomato plants inhibits growth, reduces photosynthesis and induces rapid senescence. *Plant Cell* **11**: 1253-1266
2. Menu T., Rothan C., Dai N., Petreikov M., Etienne C., Destrac-Irvine A., Schaffer A., Granot D. and Brnice Ricard (2000). Cloning and characterization of a cDNA encoding hexokinase from tomato. *Plant Science* **160**: 209-218.
3. Himelblau, E. and Amasino R. M. (2001). Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. *Journal of Plant Physiology* **158**: 1317-1323
4. Levine A., Belengh B., Damari-Weisler H. and Granot D. (2001). Vesicle associated membrane protein of Arabidopsis blocks Bax-induced apoptosis in yeast downstream of oxidative burst. *The Journal of Biological Chemistry* **276**: 46284-46289
5. Quirino, B. F. Reiter, W. D. and Amasino, R. D. (2001). One of two tandem Arabidopsis genes homologous to monosaccharide transporters is senescence-associated. *Plant Molecular Biology* **46**: 447-457
6. Weaver L. M. and Amasino R. M. (2001). Senescence is induced in individually darkened Arabidopsis leaves, but inhibited in whole darkened plants. *Plant Physiology* **127**: 876-886

7. Dai N., German G., Matsevitze T., Hanael R., Swartzberg D., Yeselson Y., Petreikov M., Schaffer A.A. and Granot D. (2002). *LeFRK2*, a gene encoding the major fructokinase in tomato fruits, is not required for starch accumulation in developing fruits. ***Plant Science*** 162:423-430
8. Dai N., Kandel-Kfir M. Petreikov M., Hanael R., Levine I., Ricard B., Rothan C., Schaffer A.A. and Granot D. (2002). The tomato hexokinase *LeHXK1*: Cloning, mapping, expression pattern and phylogenetic relationships ***Plant Science*** (in press)
9. German M.A., Dai N., Chmelnitsky I., Sobolev I., Salts Y., Barg R., Schaffer A.A. and Granot D. (2002) *LEFRK4*, a novel tomato (*Lycopersicon esculentum* Mill) fructokinase specifically expressed in stamens. ***Plant Science*** (in press)
10. Silber A., Ben-Jaacov J., Ackerman A., Bar-Tal A., Matsevitze-Yosef T., Swartzberg D., Riov J. and Granot D. (2002). Phosphorus toxicity: the role of hexokinase. ***Plant and Soil*** (in press)

APPENDIX

- Dai N., Schaffer A., Petreikov M., Shahak Y., Giller Y., Ratner K., Levine A. and Granot D. (1999). Overexpression of *Arabidopsis* hexokinase in tomato plants inhibits growth, reduces photosynthesis and induces rapid senescence. ***Plant Cell*** 11: 1253-1266
- Menu T., Rothan C., Dai N., Petreikov M., Etienne C., Destrac-Irvine A., Schaffer A., Granot D. and Brnice Ricard (2000). Cloning and characterization of a cDNA encoding hexokinase from tomato. ***Plant Science*** 160: 209-218.
- Himelblau, E. and Amasino R. M. (2001). Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. ***Journal of Plant Physiology*** 158: 1317-1323
- Levine A., Belengh B., Damari-Weisler H. and Granot D. (2001). Vesicle associated membrane protein of *Arabidopsis* blocks Bax-induced apoptosis in yeast downstream of oxidative burst. ***The Journal of Biological Chemistry*** 276: 46284-46289
- Quirino, B. F. Reiter, W. D. and Amasino, R. D. (2001). One of two tandem *Arabidopsis* genes homologous to monosaccharide transporters is senescence-associated. ***Plant Molecular Biology*** 46: 447-457
- Weaver L. M. and Amasino R. M. (2001). Senescence is induced in individually darkened *Arabidopsis* leaves, but inhibited in whole darkened plants. ***Plant Physiology*** 127: 876-886