DE-FG03-00ER15113 P. I. JOSEPH R. ECKER- THE SALK INSTITUTE

1. Award Amount \$382,600. (total costs). **Period of support:** 9/15/00- 2/14/04

2. Project Title: Molecular and Genetic Analysis of Hormone-Regulated Differential Cell Elongation in *Arabidopsis*.

3. Project Summary: We have utilized the response of *Arabidopsis* seedlings to the plant hormone ethylene to identify new genes involved in the regulation of ethylene biosynthesis, perception, signal transduction and differential cell growth. In building a genetic framework for the action of these genes, we have developed a molecular model that has facilitated our understanding of the molecular requirements of ethylene for cell elongation processes. The ethylene response pathway in *Arabidopsis* appears to be primarily linear and is defined by the genes: *ETR1*, *ETR2*, *ERS1*, *ERS2*, *EIN4*, *CTR1*, *EIN2*, *EIN3*, *EIN5*, *EIN6*, and *EIN*. Downstream branches identified by the *HLS1*, *EIR1*, and *AUX1* genes involve interactions with other hormonal (auxin) signals in the process of differential cell elongation in the hypocotyl hook. Cloning and characterization of *HLS1* (and three *HLL* genes) and *ETO1* (and *ETOL* genes) in my laboratory has been supported under this award. *HLS1* is required for differential elongation of cells in the hypocotyl and may act in the establishment of hormone gradients. Also during the previous period, we have identified and characterized a gene that genetically acts upstream of the ethylene receptors. *ETO1* encodes negative regulators of ethylene biosynthesis..

4. Human Resource Development: Participants supported under in this three year DOE-funded project have included: four postdoctoral fellows: Jose M. **Alonso**, (now Assistant Professor NCSU), Hitoshi **Yoshida** (now Research Scientist at), Kevin **Wang**, Hai Li and two graduate student Anna **Stepanova** (now postdoctoral fellow NCSU) and Ambika **Sundaresan** . Numerous undergraduates were also trained as part of this research program.

5. Publications:(2000-2004) (DOE supported personnel are listed in bold):

1. **Stepanova** A.N., and **Ecker**, J.R., (2000) Ethylene signaling: from mutants to molecules. *Curr. Opinion Plant Biol.* 3: 353-360
2. Curie, C*, **Alonso**, J.M*, Le Jean, M., **Ecker**, J.R., Briat, J.F. (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochem. J.* 347: 749-755
3. Jarillo, J. A., Capel, J., Gabrys, H., **Alonso**, J. M., **Ecker**, J. R. and Cashmore, A. R. (2001). Phototropin-related NPL1 controls chloroplast relocation induced by blue light. *Nature* 410: 952-954
4. **Alonso**, J.M. and **Ecker**, J.R.. 2001. The ethylene pathway: A paradigm for plant hormone signaling and interaction. *Science STKE*, <http://stke.sciencemag.org/cgi/content/full/oc-sigtrans;2001/70/rec1>.
5. Zheng, H.Y., Bednarek, S.Y., Sanderfoot, A. A, **Alonso**, J, **Ecker**, J.R., and Raikhel N.V. (2002) NPSN11 is a cell plate-associated SNARE protein that interacts with the syntaxin KNOLLE. *Plant Physiology* 129:530-539
6. Hu, J., Aguirre, M., Peto, C., **Alonso**, J., **Ecker**, J.R. and Chory, J.(2002) A Role for Peroxisomes in Photomorphogenesis and Development of *Arabidopsis*. *Science* 297: 405-409.
7. **Wang** KL, Li H, and **Ecker** JR (2002) .Ethylene biosynthesis and signaling networks. *Plant Cell*. 2002;14 Suppl:S131-51.
8. Friedrichsen DM, Nemhauser J, Muramitsu T, Maloof JN, **Alonso** J, **Ecker** JR, Furuya M, Chory J. (2002) Three Redundant Brassinosteroid Early Response Genes Encode Putative bHLH Transcription Factors Required for Normal Growth. *Genetics*. 162:1445-56.
9. Schroeder DF, Gahrtz M, Maxwell BB, Cook RK, Kan JM, **Alonso** JM, **Ecker** JR, and Chory J. (2002) De-etiolated 1 and damaged DNA binding protein 1 interact to regulate *Arabidopsis* photomorphogenesis. *Curr Biol*. 12:1462-72.
10. Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, Champion A, Kreis M, Zhang SQ, Hirt H, Wilson C,

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- Heberle-Bors E, Ellis BE, Morris PC, Innes RW, **Ecker JR**, Scheel D, Klessig DF, Machida Y, Mundy J, Ohashi Y, Walker JC (2002) Mitogen-activated protein kinase cascades in plants: a new nomenclature. *Trends Plant Sci.* 7: 301-308
11. Zhao Y, Hull AK, Gupta NR, Goss KA, **Alonso J**, **Ecker JR**, Normanly J, Chory J, and Celenza JL. (2002) Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes Dev.* 16:3100-12.
 12. Strand A, Asami T, **Alonso J**, **Ecker JR**, and Chory J. (2003) Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrin IX. *Nature.* 421:79-83.
 13. Ullah, H., Chen, J-G., Temple, B., Boyes, D., **Alonso, J.**, Davis, K., **Ecker, J.R.**, Jones, A.M. (2003) The beta subunit of the Arabidopsis G protein negatively regulates auxin-induced cell division. *Plant Cell* 15: 1–17
 14. Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, **Alonso J**, **Ecker J.R.**, and Shinozaki K (2002) ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis. *Plant and Cell Physiology* 43: 1473-1483
 15. Larkin, R.M., **Alonso, J.M.**, **Ecker, J.R.**, and Chory, J. (2003) GUN4, a regulator of chlorophyll synthesis and intracellular signaling, *Science* 299:902-906
 16. Mackey, D., Belkadir, Y., **Alonso, J.M.**, **Ecker, J.R.**, and Dangl, J.L. (2003) Arabidopsis RIN4 is a target of the Type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* 112:379-389
 17. **Alonso, J.M.**, **Stepanova, A.N.**, Solano, R., Wisman, E., Ferrari, S., Ausubel, F.M., and **Ecker, J.R.** (2003) Five Components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in Arabidopsis *Proc. Natl. Acad. Sci. USA* 100: 2992-2997
 18. Jones, A.M, **Ecker, J.R.**, and Chen, J-G. (2003) A Reevaluation of the Role of the Heterotrimeric G Protein in Coupling Light Responses in Arabidopsis *Plant Physiol.* 131: 1623-1627.
 19. **Alonso, J.M.**, **Stepanova, A.N.**, Lisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C.C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D.E., Marchand, T., Risseuw, E., Brogden, D., Zeko, A., Crosby, W.L., Berry, C.C. and **Ecker, J.R.**, (2003) Genome-wide Insertional mutagenesis of Arabidopsis thaliana. *Science*, **301**:653-657
 - Michael, T.P., Salomé, P.A., Yu, H.J., Spencer, T.R., Sharp, E.L. McPeck, M.A., **Alonso, J.M.**, **Ecker J.R.**, and McClung, C.R. (2003) Enhanced fitness conferred by naturally occurring variation in the circadian clock, *Science*, 302:1049-1053.
 20. **Wang, K. L.**, **Yoshida, H.**, **Lurin, C.**, and **Ecker, J. R.** (2004). Regulation of ethylene gas biosynthesis by the Arabidopsis ETO1 protein. *Nature* 428, 945-950.

6. DESCRIPTION OF RESEARCH

A) SUMMARIES OF FINDINGS.

1. Five Components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in Arabidopsis. Alonso, J.M., Stepanova, A.N., Solano, R., Wisman, E., Ferrari, S., Ausubel, F.M., and Ecker, J.R. (2003) Proc. Natl. Acad. Sci. USA 100: 2992-2997

Five ethylene-insensitive loci (*wei1–wei5*) were identified by using a low-dose screen for “weak” ethylene-insensitive mutants. *wei1*, *wei2*, and *wei3* seedlings showed hormone insensitivity only in roots, whereas *wei4* and *wei5* displayed insensitivity in both roots and hypocotyls. The genes corresponding to *wei1*, *wei4*, and *wei5* were isolated using a positional cloning approach. The *wei1* mutant harbored a recessive mutation in *TIR1*, which encodes a component of the SCF protein ubiquitin ligase involved in the auxin response. *Wei4*, a dominant mutant, resulted from a mutation in the ethylene receptor *ERS*, whereas *wei5*, a semidominant mutant, was caused by a mutation in the *EIN3*-related transcription factor gene *EIL1*. The simultaneous loss of functional *WEI5_EIL1* and *EIN3* nearly completely abolished the ethylene response in etiolated seedlings, and adult plants were highly susceptible to infection by the necrotrophic fungal pathogen *Botrytis cinerea*. Moreover, *wei5_eil1_ein3* double mutants were able to fully suppress constitutive signaling caused by *ctr1*, suggesting a synergistic interaction among these gene products. Unlike previously known root ethylene-insensitive mutants, *wei2* and *wei3* were not affected in their response to auxin and showed a normal response to gravity. Genetic mapping studies indicate that

wei2 and *wei3* correspond to previously unidentified ethylene pathway genes that may control cell-elongation processes functioning at the intersection of the ethylene and auxin response pathways.

2. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Alonso, J.M., Stepanova, A.N., 22 co-authors and Ecker, J.R. *Science*, **301:653-657.**

Over 225,000 independent *Agrobacterium* T-DNA insertion events in the genome of the reference plant *Arabidopsis thaliana* have been created that represent near saturation of the gene space. The precise locations were determined for more than 88,000 T-DNA insertions, resulting in the identification of mutations in more than 21,700 of the ~ 29,454 predicted *Arabidopsis* genes. Genome-wide analysis of the distribution of integration events revealed the existence of a large integration site bias at both the chromosome and gene levels. Insertion mutations were identified in genes that are regulated in response to the plant hormone ethylene.

3. ETO1 negatively regulates ethylene biosynthesis by direct interaction with ACS5 in *Arabidopsis*. Wang, L-C., Yoshida, H., Lurin, C., Harper, J.F. and Ecker, J.R. *Nature*

Mutations in *ETHYLENE-OVERPRODUCER1 (ETO1)* cause overproduction of the plant hormone ethylene in *Arabidopsis thaliana*. *ETO1* belongs to a novel plant gene family encoding proteins with BTB/POZ (Broad-Complex, Tramtrack, Bric-à-brac /Poxvirus, Zinc finger) and TPR (Tetratricopeptide Repeat) domains. A functional *AMINOCYCLOPROPANE CARBOXYLIC ACID SYNTHASE 5 (ACS5)*, which encodes a key enzyme controlling the rate-limiting step of ethylene biosynthesis, is required for the *eto1* phenotype. ETO1 directly interacts with the carboxyl terminus of ACS5. Interaction with ETO1 not only inhibits ACS5 activity, but also promotes ACS5 protein degradation via the proteasome-dependent pathway *in planta*. Phosphorylation of ACS5 *in vitro* by CALCIUM-DEPENDENT PROTEIN KINASE1 (AtCPK1) from *Arabidopsis thaliana* reduces its affinity for ETO1, suggesting phosphorylation by kinases may play a molecular switch for ACS activity. Our results reveal the molecular mechanism that ACS5 activity is negatively regulated by direct interaction with ETO1 and relief of ETO1 inhibition by phosphorylation of ACS5. 428, 945-950