Final Report 2000-2003 (one year no cost extension for 2004)

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The long term goal of this laboratory is to elucidate a detailed molecular description of the process of initiation of protein synthesis and its regulation. The specific goals of the project were:

1. Development of an *in vivo* [^{32}P]- and/or [^{35}S]-labeling system for proteins using *Arabidopsis* suspension cells.
2. Develop an *in vitro* protein synthesis assay from *Arabidopsis* suspension cells.
3. Develop an assay for locating *Arabidopsis* kinases that phosphorylate the initiation factors.
4. Begin to identify *Arabidopsis* kinases that are involved in phosphorylation of the initiation factors

We developed methods for using *Arabidopsis* cell culture as a source of protein synthesis machinery. We developed an extraction method of suspension cells that yields extracts in the 12-17 mg/ml range and we are able to purify active initiation factors from these extracts. These extracts are of sufficient concentration for further development of an *in vitro* system and for immunoprecipitation. We have obtained *Arabidopsis* specific rabbit antisera to recombinant eIF4G, eIF(iso)4G, eIF4B, eIF4E and eIF(iso)4E. The antisera to eIF4E and eIF(iso)4E do not cross-react with each other which will be most valuable in our further studies to understand the role of these two cap-binding proteins. We have tested these antisera in immunprecipitation reactions and they are specific for their respective proteins.

Analysis of expressed eIF4B suggests that interaction between eIF4F and eIF(iso)4F with eIF4B differ among various mRNAs tested. This suggests that a level of control may be exerted by eIF4B during initiation (Mayberry and Browning, manuscript in preparation).

Under continued development (current grant period) is a system that will express all 3 subunits of *Arabidopsis* eIF2 for development of highly specific antisera. It is also hoped that such an expression system will yield an active eIF2 complex that will be used as a substrate for kinases.

Continued development of the *Arabidopsis* protein synthesis system will allow us to go much further in understanding the regulation of protein synthesis factors by phosphoryiation, and how it relates to signaling pathways in plants. In addition, we will be able to use a number of genomic and proteomic approaches including DNA array analysis, transgenic plants and mass spectrometry to study the regulation of plant protein synthesis via phosphorylation.

The following papers were published during the grant period:

Le, H., Browning, K.S. and Gallie, D.R. (2000) The Phosphorylation State of PABP Specifies Its Binding to Poly(A) RNA and Its Interaction with eIF4F, eIFiso4F, and eIF4B. *J. Biol. Chem.* **275:** 17452-17462.

Burks, E.A., Bezerra, P., Gallie, D.R. and Browning, K.S. (2001) Plant initiation factor 3 subunit composition resembles mammalian initiation factor 3 and has a novel subunit. *J. Biol. Chem.* **276:** 2122-2131.

Browning, K.S., Gallie, D.R., Hershey, J.W.B., Hinnebusch, A.G., Maitra, U., Merrick, W.C. and Norbury, C. (2001) Unified nomenclature for the subunits of eukaryotic initiation factor 3. *TIBS* **26:** 284.

Miller, W.A., Waterhouse, P.M., Brown, J.W., Browning, K.S. (2001) The RNA World in Plants: Post-Transcriptional Control III. Plant Cell **13:** 1710-1717.
http://www.plantcell.org/cgi/content/full/13/8/1710?view=full&pmid=11487687

Gallie, D.R., Browning, K.S. (2001) eIF4G functionally differs from eIFiso4G in promoting internal initiation, cap-independent translation, and translation of structured mRNAs. *J. Biol. Chem.* **276:** 36951-36960.
http://www.jbc.org/cgi/reprint/276/40/36951.pdf

Hyun-Sook Park, Axel Himmelbach, Karen S. Browning, Thomas Hohn, and Lyubov A. Ryabova. (2001) A Plant Viral "Reinitiation" Factor Interacts with the Host Translational Machinery. *Cell* **106:** 723–733.
http://download.cell.com/cellpress/pdfs/cell/106/6/CELL.106_6_723.523.pdf

Duprat, A., Caranta, C., Revers, F., Menand, B., Browning, K.S., Robaglia, C. (2002) The Arabidopsis eukaryotic initiation factor (iso)4E is dispensable for plant growth but required for susceptibility to potyviruses. *Plant Journal* **32:** 927-34
http://www.blackwell-synergy.com/doi/full/10.1046/j.1365-313X.2002.01481.x

Park, H.S., Browning, K.S., Hohn, T., and Ryabova, L.A. (2004) Eucaryotic initiation factor 4B controls eIF3-mediated ribosomal entry of viral reinitiation factor. *EMBO J.* **23**: 1381-91.
http://www.nature.com/cgi-taf/dynapage.taf?file=/emboj/journal/v23/n6/full/7600140a.html