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PROGRESS REPORT 8/16/89 through 8/15/92
DOE Grant Number DE-FG09-86ER13602 PI - Joe L. Key

I. Publications/Manuscripts in Preparation

Nagao, R. T. and Joe L. Key. 1989. Heat Shock Protein Genes of Plants. In: Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, (Eds. I. Vasil and J. Schell), Academic Press, Inc., New York, pp. 297-328.

Nagao, Ronald T. 1989. The Heat Shock Response in Plants: Short-Term Treatment Regimes in Thermotolerance. NATO ASI Series, Vol G19. Environmental Stress in Plants (Ed. J. H. Cherry) Springer-Verlag, Berlin, Heidelberg, pp 331-342.

Nagao, R. T., J. A. Kimpel, and Joe L. Key. 1990. Molecular and Cellular Biology of the Heat Shock Response. In: Genomic Responses to Environmental Stresses, (Ed. J. Scandalious), Academic Press, Inc., New York, pp. 235-274.

Conner, T. W., P. R. Lafayette, R. T. Nagao and Joe L. Key. 1990. Sequence and Expression of a HSP83 from *Arabidopsis thaliana*. Plant Physiol. 94:1689-1695.

Kimpel, J. A., R. T. Nagao, V. Goekjian and Joe L. Key. 1990. Regulation of the Heat Shock Response in Soybean Seedlings. Plant Physiol. 94:988-995.

Gurley, William B. and Joe L. Key. 1991. Transcriptional Regulation of the Heat Shock Response: A Plant Perspective. Biochemistry 30:1-12.

Roberts, J. K. and Joe L. Key. 1991. Isolation and Characterization of a Gene which Encodes a 70 kD Heat Shock Protein of Soybean. Plant Mol. Biol. 16:671-683.

Helm, K. W., P. R. LaFayette, R. T. Nagao, Joe L. Key and E. Vierling. 1993. Members of the Superfamily of Eukaryotic Small Heat Shock Proteins in the Plant Endomembrane System. Mol. Cell Biol. 13:238-247.

Research Progress

The major research activities proposed for the renewal period were focused on (1) defining regulatory mechanisms operative in the heat shock (HS) response and (2) assessing the mechanism of HS-induced thermotolerance. The approaches which were proposed to assess the mechanisms operative in these highly related and

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interdependent processes included (1a) regulation of HS gene expression including nuclear run-off transcription studies in conjunction with analysis of the mRNA levels under various HS regimes, (1b) the use of amino acid analogs and protein synthesis inhibitors to study self-regulation in the context of levels of heat shock protein (hsp) synthesis, (1c) development of mutants of transgenic *Arabidopsis* plants and/or transformed soybean cells in culture altered in the HS response as approaches to analyze self-regulation (or autoregulation) of the HS-response; (2a) use of transformed soybean cell cultures to study thermotolerance, (2b) subcellular localization of hsp during HS and their redistribution during recovery from HS, (2c) the characterization of membrane-associated hsp and HS effects on membrane transport, and (2d) the influence of over- and underexpression of selected HS mRNAs/hsp on phenotype (especially thermotolerance and growth habit).

Regulation of the HS Response.

Regulation of HS Gene Expression - At an "optimum" HS temperature (e.g. 40°C for soybean seedlings), HS mRNAs accumulate for 1 to 2 hr and then gradually decline during continuous HS; return of seedlings to 30°C after a 1- to 3-hr HS at 40°C results in the rapid depletion of HS mRNAs. Depletion is much slower when seedlings are grown at 40°C over the same period. A brief (e.g. 5 to 10 min) HS at 45°C followed by growth at 30°C results in accumulation and depletion of HS mRNAs similarly to accumulation and depletion during a continuous 40°C HS. Further, a second HS at 40°C or 45°C, following an initial 2-hr 40°C HS and a 3-hr recovery at 30°C, results in a new round of HS mRNA accumulation and hsp synthesis. During the initial 40°C continuous HS, transcription of HS genes is initiated very rapidly and continues at a relatively high level for about 2 to 3 hr based on nuclear run-off transcription studies; transcription is not detected by about 4 hr. However, HS mRNAs persist for several hours (about 15% to 20% of peak level at 12 hr, varying somewhat with different families of HS mRNAs). These results on transcription combined with steady-state mRNA levels imply that HS mRNAs generally have a 3- to 4-fold greater stability at HS temperatures than at normal growing temperatures. A brief 45°C HS followed by return of seedlings to 30°C treatment results in rates of transcription based on nuclear run-off analyses similar to a continuous 40°C HS. Thus, active transcription of HS genes under these very different regimes indicates that transcription of the HS genes must involve more than a simple sensing of the ambient temperature and "activation" of the HS transcription factor (HSF). During a second HS, following initial HS and recovery, HS mRNAs accumulate as noted above; nuclear run-off transcription analyses show that activation of HS gene transcription occurs, but the apparent rate of transcription is significantly lower than during the initial HS and slows (ceases) much sooner. The relative transcription rates and steady-state mRNA levels which are achieved during the initial and second HS treatments are suggestive that HS mRNAs may be more stable during the second HS. Autoregulation or self-regulation of the HS response is clearly observed at the level of nuclear run-off transcription of HS genes.

Influence of Amino Acid Analogs and Cycloheximide on Induction and Duration of the HS Response - The influence of azetidine-2-carboxylic acid on induction of a "HS response" was studied in detail, with lesser studies using canavanine. Azetidine (0.5mM) induced the accumulation of most classes (families) of soybean HS mRNAs; the accumulation was slower than at an optimum HS, but the levels continued to increase for 6 or more hr, in contrast to 1 to 2 hr for HS. The families represented by cDNA clones pCE53, pCE54, pCE75, pEV1, pEV2, and pSB70 responded similarly relative to HS mRNA accumulation (see Fig. 1). However, the cDNA clone pEV3 which is representative of a family of nuclear-encoded, chloroplast-localized hsp was not significantly induced by the analog. HS mRNAs corresponding to the pFS2033 family likewise were not significantly induced by analogs. However, mRNA detected by cDNA clone pEV6 (highly related at the DNA sequence level to pFS2033) did accumulate substantially in response to analog. This family(s) of mRNAs and some of the unique characteristics which have been identified will be covered in 2c below. A 40°C HS in the presence of azetidine results in a rather normal pattern of accumulation of HS mRNAs corresponding to all of the cDNA clone families noted above. Analog treatment did result in impairment of the self-regulation response in that HS mRNAs accumulated for a longer time period than HS in absence of analog (1 to 2 hr), and the mRNAs persisted at relatively higher levels for longer times (see Fig. 1). However, the maximum level of HS mRNAs that accumulated in analog- or HS-analog-treated tissue generally was somewhat lower than for HS alone; a marked exception to this pattern is exemplified by the pCE54 family (general stress proteins which accumulated to levels well above that induced by HS). As might be predicted if some protein(s) which accumulates during HS relates to the self-regulation response, cycloheximide (CH) treatment extended the period of HS mRNA accumulation, and depletion of the mRNAs during continuous HS was significantly impaired (CH concentration was adjusted to give about 60% inhibition of protein synthesis).

Several interesting observations emerged from the analysis of hsp accumulation (i.e. radioactive amino acid incorporation) during analog or analog plus HS treatment. On a relative basis, substantial amino acid was incorporated into high molecular weight hsps (e.g. hsp70, hsp83, and hsp92 groups) while little was apparently incorporated into the lower molecular weight hsps (e.g. 15 to 18kD families). Antibodies to one family (pCE53) of the 15 to 18kD hsps were used in Western blot analyses; these hsps accumulated to disproportionately high levels following analog treatment (yet much lower than with HS) based on relative labeled amino acid incorporation. These analyses are still underway, with the intent to compare hsp70, hsp83, and the pCE53 family on Western blots and 1D/2D gel analysis of labeled amino acid incorporation into the different proteins. Potentially of more interest are the observations on hsps in the 92 to 110kD range. With HS treatment, synthesis of hsp92/110 is not detected after 2 to 3 hr of 40°C HS while synthesis of most or all other hsps continues at decreasing levels out to 8 hr or more. Hsp92/110 continues to be synthesized in analog-treated tissue and analog/HS-treated tissue at least out to 12 hr of treatment. This raises the intriguing possibility that this family of proteins may function in the self-regulation system based on these

correlative data. Also of interest was the observation that analog treatment induced two or more proteins related to the pCE53 family which reacted on immunoblots with this antibody that were not induced by HS. We had observed earlier that cadmium (Cd) induced 5 or 6 mRNAs homologous to pCE53 in addition to the 13 induced by HS. In the case of cadmium treatment, the same genes or genes identical in the coding region were induced by HS and Cd. There is no reason to believe that analog and HS do not induce the same 13 genes of the pCE53 family, with analog inducing additional highly related genes. Some additional hsp (in addition to the pCE53 proteins) react with the antibody used in these studies.