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Analysis of family 24 of the *Arabidopsis thaliana* J-domain chaperone proteins

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The *Arabidopsis thaliana* genome includes an unexpectedly large and diverse group of J-domain chaperone proteins. The 93 *A. thaliana* J-domain protein sequences have been grouped into 51 families, many of which do not have any well-studied counterpart in microbes or mammalian cells. Based upon the results of silico analyses, three proteins, atDjC43, atDjC48, and atDjC49, were assigned to Family 24. Homologous proteins are present in maize, rice, and soybean, and in a variety of animals, but none has been characterized. Members of Family 24 have approximately 300 amino acid residues; besides the J-domain the only prominent structural feature is a predicted transmembrane helix near the C-terminus. Preliminary experiments were conducted to try to understand the multiplicity of plant J-domain proteins, and to address the question of redundancy versus specialization. In order to determine where and when these three J-domain proteins are expressed, primers were designed for semi-quantitative RT-PCR. Total RNA was isolated from the rosette leaves and roots of 4-week old *A. thaliana* ecotype Columbia plants, and from flowers and green siliques. The results establish a pattern of organ-specific expression. We are also using T-DNA insertion knockout plants for our analyses. We currently have homozygous KO plants for atDjC48, and are in the final stages of screening for atDjC43 knockouts. It is difficult to express eukaryotic integral membrane proteins in bacteria. The sequence encoding the C-terminal transmembrane helix was deleted from the atDjC48 reading frame. C sequence was then cloned into the pCal-n vector for expression in Escherichia coli as a chimera with the CaM-Binding-Peptide.The atDjC48 The recombinant protein will be assayed for activity in vitro. Using these diverse strategies, we hope to gain insight into the roles of this family of molecular chaperone proteins.