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The mitochondrial monothiol glutaredoxin S15 is essential for iron-sulfur protein maturation in Arabidopsis thaliana.

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The iron-sulfur cluster (ISC) is an ancient and essential cofactor of many

proteins involved in electron transfer and metabolic reactions. In Arabidopsis, three pathways exist for the maturation of iron-sulfur proteins in the cytosol, plastids, and mitochondria. We functionally characterized the role of mitochondrial glutaredoxin S15 (GRXS15) in biogenesis of ISC containing aconitase through a combination of genetic, physiological, and biochemical approaches. Two Arabidopsis T-DNA insertion mutants were identified as null mutants with early embryonic lethal phenotypes that could be rescued by GRXS15. Furthermore, we showed that recombinant GRXS15 is able to coordinate and transfer an ISC and that this coordination depends on reduced glutathione (GSH). We found the Arabidopsis GRXS15 able to complement growth defects based on disturbed ISC protein assembly of a yeast Δgrx5 mutant. Modeling of GRXS15 onto the crystal structures of related nonplant proteins highlighted amino acid residues that after mutation diminished GSH and subsequently ISC coordination, as well as the ability to rescue the yeast mutant. When used for plant complementation, one of these mutant variants, GRXS15K83/A, led to severe developmental delay and a pronounced decrease in aconitase activity by approximately 65%. These results indicate that mitochondrial GRXS15 is an essential protein in Arabidopsis, required for full activity of iron-sulfur proteins.

Keith

Species-Specific Structural and Functional Divergence of α-Crystallins: Zebrafish αBa- and Rodent αA^{ins}-Crystallin Encode Activated Chaperones

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In addition to contributing to lens optical properties, the α-crystallins are small heat shock proteins that possess chaperone activity and are predicted to bind and sequester destabilized proteins to delay cataract formation. The current model of α-crystallin chaperone mechanism envisions a transition from the native oligomer to an activated form that has higher affinity to non-native states of the substrate. Previous studies have suggested that this oligomeric plasticity is encoded in the primary sequence and controls access to high affinity binding sites within the N-terminal domain. Here, we further examined the role of sequence variation in the context of species-specific α-crystallins from rat and zebrafish. Alternative splicing of the αA gene in rodents produces αA^{ins}, which is distinguished by a longer N-terminal domain. The zebrafish genome includes duplicate αB-crystallin genes, αBa and αBb, which display divergent primary sequence and tissue expression patterns. Equilibrium binding experiments were employed to quantitatively define chaperone interactions with a destabilized model substrate, T4 lysozyme. In combination with multiangle light scattering, we show that rat αA^{ins} and zebrafish α-crystallins display distinct global structural properties and chaperone activities. Notably, we find that αA^{ins} and αBa demonstrate substantially enhanced chaperone function relative to other α-crystallins, binding the same substrate more than 2 orders of magnitude higher affinity and mimicking the activity of fully activated mammalian small heat shock proteins. These results emphasize the role of sequence divergence as an evolutionary strategy to tune chaperone function to the requirements of the tissues and organisms in which they are expressed.

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