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Studies on harpin- mediated cell death events in Saccharomyces cerevisiae

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Background: 'Harpin' a novel protein isolated from Pseudomonas syringae pv. syringae, a phytopathogenic bacteria elicits hypersensitive response (HR) during host-pathogen interactions resulting in necrotic cell death in leaf tissues particularly in Nicotiana tobaccum cv. xanthi. Harpin is hydrophilic, heat-stable, glycine-rich, amyloid forming protein which elicits HR when infiltrated into the apoplast of certain plants and absence of homology in amino acid sequence with other known proteins makes it highly difficult to predict the three-dimensional structure and establish the structure-function relationship. The present study is oriented in understanding the physiological mechanism of action of harpin using 'Saccharomyces cerevisiae' as a model system with relevance to its biophysical features. Apart from inducing cell death in plants and yeast, harpin also induces apoptosis in 'cancer cell lines'.

Methods: In the yeast cell harpin is endogenously expressed by cloning in a yeast expression vector under a galactose (GAL1) promoter and selectively expressed by induction in galactose medium. Flow cytometric and Fluorescent microscopy techniques have been used to study the ROS release, mitochondrial potential, nuclear fragmentation and other cell death studies in the yeast system. Biochemical assays were done to evaluate various mitochondrial enzymes. CD spectroscopy, Differential scanning calorimetry, Dynamic light scattering and Atomic force microscopy were used to study the various bio-physical features of the protein.

Results: Harpin expression in Saccharomyces cerevisiae under a galactose inducible promoter resulted in yeast cell death. Flow cytometric and biochemical studies on the yeast system revealed ROS release, alteration in mitochondrial potential, changes in cell cycle events and reduction in the specific activity of mitochondrial ETC enzymes, indicating the possible role of mitochondria and metacaspases in harpin-induced yeast cell death. Harpin treatment in cancer cell lines lead to apoptotic cell death as indicated by the cellular studies. Bio-physical studies paved the way to understand the thermal stability, aggregation and structure-functional activity of harpin.

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