The Plant Cell, Vol. 12, 457, April 2000, www.plantcell.org

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HSP101: A Key Component for the Acquisition of Thermotolerance in Plants

HEAT SHOCK RESPONSE

A sudden elevation in temperature triggers a stress response found in all organisms that brings about a global transition in gene expression. Typically, the expression of most genes is either shut down or greatly attenuated, and a specific group of genes, called heat shock (HS) genes, is rapidly induced to high levels (Schlesinger et al., 1982). Proteins encoded by HS genes enable cells to survive the harmful effects of heat by two general strategies: one group of heat shock proteins (HSPs) acts as molecular chaperones that counteract protein denaturation and aggregation, and other HSPs, including ubiquitin and certain proteases, target nonnative proteins for degradation. The HS response is transient in nature, usually peaking 1 to 2 hr after onset, providing protection from acute episodes of thermal stress.

Most major classes of HSPs are present in plants and include the small HSPs (ranging in molecular weight from 15 to 28 kD), HSP60, HSP70 (and a constitutively expressed HS cognate protein, HSC70), HSP90, and HSP100 (Vierling, 1991). As is the case in other organisms, refolding of nonnative proteins in plants is thought to occur in complexes ("refolding machines") containing HSC70/HSP70 and DnaJ homologs (Lee and Vierling, 2000). The robust synthesis of the numerous small HSPs in the HS response differentiates plants from organisms such as Drosophila, in which expression of HSP70 dominates the response.

HSPs facilitate growth and survival of plants not only over the course of transient extremes of temperature but also under conditions of severe heat stress whereby lethal temperatures can be tolerated for short periods. Protection from severe heat stress usually requires a preconditioning by prior exposure to moderate HS conditions. This phenomenon, known as induced or acquired thermotolerance, has long tantalized researchers with the prospect that organisms, including plants, may be engineered to better tolerate heat stress. One HSP class that might conceivably be manipulated so as to optimize thermotolerance is HSP100 (Sanchez et al., 1992).

HSP101 IS LINKED TO THERMOTOLERANCE IN ARABIDOPSIS

On pages 479–492 of this issue of THE PLANT CELL, **Queitsch et al.** provide compelling evidence that HSP101 from Arabidopsis is essential for survival under severe heat stress and for the acquisition of thermotolerance. Their approach is to utilize the constitutive cauliflower mosaic virus 35S promoter to overexpress HSP101 sense and antisense mRNAs in transformed plants. Their findings were anticipated by two earlier reports in which soybean and Arabidopsis members of the HSP100 class were able to partially compensate for deletion of HSP104 in yeast cells (Lee et al., 1994; Schirmer et al., 1994). Underexpression of HSP101 was obtained by Queitsch et al. in two ways: inadvertently by cosupression and by synthesizing antisense transcripts. The authors show that alterations in thermotolerance are tightly correlated with the levels of a single protein, HSP101.

In previous studies, expression of constitutively active plant heat shock transcription factors (HSFs) had only increased basal thermotolerance, but acquired (induced) thermotolerance was unaffected (Lee et al., 1995; Prändl et al., 1998). Here, Queitsch et al. show that alterations in HSP101 expression influence both basal and acquired thermotolerance. The requirement of HSP101 for acquired thermotolerance is demonstrated by exposure of seedlings to the lethal temperature of 45°C for 2 hr with and without preconditioning at 38°C. All plants are killed by direct exposure to the severe heat stress. Pretreatment at 38°C, however, enables wild-type and vector-only controls to survive the subsequent severe heat, whereas plants with reduced HSP101 levels are drastically impaired or killed despite pretreatment. This result provides clear evidence that HSP101 expression is essential for induced thermotolerance.

In a more quantitative assay, the authors monitor hypocotyl elongation during induced thermotolerance. Hypocotyls from seedlings grown for 2.5 days at 22°C and then exposed, either with or without a heat pretreatment, to severe heat stress fail to elongate if HSP101 expression is severely compromised. Preconditioned HSP101 antisense plants thus act like wild-type plants that had received no prestress treatment, clearly demonstrating the requirement for HSP101 in induced thermotolerance.

A test of the role of HSP101 in basal thermotolerance is presented with germinating seeds. Arabidopsis seeds are remarkably resistant to the effects of severe heat stress during early germination, being able to survive for up to 2 hr at 47°C without pretreatment. After 48 hr of imbibition, however, seedlings are killed under such conditions, which correlates with a rapid depletion of HSP101 (S.-W. Hong, N. Wehmeyer,

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and E. Vierling, unpublished results). Indeed, Queitsch et al. show that seeds of transformed plants expressing reduced levels of HSP101 are no longer able to survive severe heat stress.

CONSTITUTIVE EXPRESSION OF HSP101 MAY IMPROVE PLANT SURVIVAL

Constitutive overexpression of HSP101 results in plants containing from 40 to 85% of the levels of HSP101 normally induced by heat pretreatment. Overexpressing HSP101 in 14-day-old seedlings does not result in full thermotolerance inasmuch as they are not able to survive 47°C without pretreatment. However, overexpression of HSP101 enables seedlings to survive shorter exposures to slightly less severe temperatures (without preconditioning) that otherwise would impair wild-type plants drastically. A similar result is obtained when newly germinated seedlings are heat shocked after 3 days of germination, when stores of HSP101 are depleted. Again, seedlings constitutively overexpressing HSP101 show a dramatic improvement over wild-type and antisense plants in survival at 45°C for limited time periods. Thus, the constitutive overexpression of a single protein, HSP101, can extend the period of high thermotolerance inherent to seeds through to the early stages of germination.

The essential requirement for HSP101 in survival of severe heat stress has recently been confirmed in a related report in which a mutated gene for HSP101, designated *hot1*, was identified after screening more than 17,000 mutagenized Arabidopsis seeds (Hong and Vierling, 2000). The mutant HSP101 was produced at normal levels, but mutant plants lacked the ability to induce thermotolerance, and seeds were no longer able to survive severe heat stress. The *hot1* mutant plants appear to grow and metabolize normally and are able to develop a normal HS response after moderate heat stress (38°C for 2 hr). In accordance with the present study of Queitsch et al., the *hot1* mutation can be rescued by transformation with wild-type HSP101. Interestingly, four other mutants at unlinked loci were also obtained, indicating that multiple proteins (possibly HSPs) are required for induced thermotolerance in plants. These mutant lines provide a significant toehold for the genetic dissection of pathways involved in thermotolerance.

THE GENERAL ROLE OF HSP101

Under moderate heat stress, the small HSPs form granules in the cytoplasm and provide surfaces that stabilize nonnative proteins and retard irreversible aggregation (Neumann et al., 1987, 1989; Nover et al., 1989). Recent studies in pea have further demonstrated that a member of the small HSPs, HSP18.1, prevents thermal aggregation of proteins by binding to nonnative forms and maintaining them in a state that can be refolded (Lee et al., 1997). These stabilized proteins are then restored to the native state by "refolding machines" that involve HSP70 and DnaJ homologs (Lee and Vierling, 2000). During severe temperature stress, when this system is not able to keep up with denaturation, insoluble complexes accumulate. HSP100 functions to resolubilize these aggregates by acting as an ATP-dependent "molecular crowbar," transferring proteins liberated from the insoluble complexes to the HSP70/DnaJ refolding machine (Schirmer et al., 1996; Glover and Lindquist, 1998).

HSP100 is not employed in all organisms to solubilize aggregated proteins. For example, in Drosophila and vertebrates, where elevated levels of HSP70 and other HSPs are apparently sufficient to keep aggregation in check and accommodate refolding even under severe heat stress, homologs of yeast HSP104 are absent. Perhaps the sessile lifestyle of plants necessitates a more vigorous stress response system, incorporating both small HSPs that are abundantly expressed so as to prevent aggregation during moderate heat stresses and the HSP101 system that facilitates recovery from severe stress episodes.

EXPERIMENTAL OPTIMIZATION OF THERMOTOLERANCE IN PLANTS

Inducible thermotolerance provides organisms with a well-adapted and highly effective system for minimizing the deleterious effects of protein denaturation that accompany short-term increases in temperature. Thermotolerance must generally be triggered prior to severe stress by exposure to moderate heat stress. In some situations, on the other hand, the requirement for preconditioning is met by developmental induction of HSPs, as is the case in Arabidopsis seeds. Most experimental attempts to extend the upper temperature range survivable by induced thermotolerance have not been successful; however, various strategies to eliminate the induction or pretreatment aspect of thermotolerance have shown some degree of success.

In plants, two strategies have been utilized to optimize thermotolerance experimentally. The first strategy involves the expression of various HSFs, and the second alters levels of individual HSPs. Expression of an antisense HSP70 gene in Arabidopsis resulted in a reduction in acquired thermotolerance, demonstrating the involvement of HSP70/HSC70 (Lee and Schöffl, 1996); however, overexpression of HSP70 was not implemented. Increased expression of the small HSPs has been linked to increased thermotolerance in a grass (Park et al., 1996), and Kim et al. (1997) reported an increase in ther-

motolerance when a small HSP gene from Chinese cabbage was overexpressed in tobacco. In carrot cells, over- and underexpression (Malik et al., 1999) of HSP17.7 clearly correlated to improved and compromised degrees of thermotolerance, respectively, thereby confirming the feasibility of enhancing thermotolerance in plants by manipulating cellular levels of a specific HSP. Unfortunately, other attempts to increase thermotolerance, by overexpression of small HSPs, have proved less successful (e.g., Osteryoung et al., 1994).

In most cases, increasing the expression of constitutively active HSFs in plants leads to an increase in HSP synthesis and thermotolerance; however, this strategy has encountered mixed results. Overexpression of AtHsf1 in Arabidopsis did not lead to an increase in expression of HS genes (Lee and Schöffl, 1996). Surprisingly, when AtHsf1 was translationally fused to GUS, however, the chimeric factor was constitutively active and caused an elevation in the levels of at least one HSP, namely, AtHSP18, and conferred a modest increase in basal tolerance (Lee et al., 1995). Interestingly, no corresponding increase was obtained in acquired thermotolerance. A similar result was seen when AtHsf3 was overexpressed, except that this Hsf was constitutively active, derepressing the expression of at least the small HSPs and resulting in an increase in thermotolerance (Prändl et al., 1998). Although thermotolerance can thus be improved by manipulating HSFs, the approach may potentially lead to unwanted consequences because many of the HSPs induced may have roles in normal growth and development.

MANIPULATION OF HSP101 TOWARD ENGINEERING THERMOTOLERANT PLANTS

The finding that constitutive expression of HSP101 can nearly eliminate the need to precondition plants to survive severe heat stress has important implications for efforts to improve stress tolerance in plants. It may be possible to greatly reduce the vulnerability of certain tissues or developmental stages to heat stress by boosting the levels of HSP101. For example, pollen released by many crops, including maize (Herrero and Johnson, 1980), is often deactivated by the midday heat. Perhaps engineering the expression of HSP101 in pollen might improve fertilization during periods of thermal stress, although additional HSPs may also need boosting because pollen does not show the normal HS response (Cooper et al., 1984; Frova et al., 1989; Dupuis and Dumas, 1990; Hopf et al., 1992). Other applications may include the increase in seed thermotolerance and the prolonging of thermotolerance from the seed into germination, as presented in this issue. The fact that over- and underexpression of HSP101, as shown in the present study by Queitsch et al., does not affect normal growth and development makes this protein an especially attractive target for engineered expression. Ultimately, a combination of approaches may yield the best protection, but the manipulation of the genes that encode proteins of the HSP100 class is likely to be a key component of any future strategy.

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