Genetic engineering for high-level tolerance to abiotic stresses through over-expression of transcription factor genes: The next frontier

Abiotic stresses (such as high salt levels, low water availability, excess water and high and low temperatures) adversely affect growth and yield of crop plants. There is a great deal of urgency in improving the performance of crops against such stress factors. During the past five years (1993–98), a range of genes [such as those linked to osmoregulation and chaperoning activities, unsaturase, antifreeze protein (AFP) genes and late embryogenesis abundant (LEA) protein genes] have been employed for raising stress-tolerant plants and the success has been to varying extents[1]. However, high-level tolerance against abiotic stresses still remains a major challenge[1]. A general criticism against genetic engineering for tolerance to abiotic stress factors has been that since response of plants to these stresses is often multigenic, it is not possible to affect the whole cascade of cellular changes when single genes are employed. If so, expression of the entire battery of stress-responsive genes such as genes for different heat shock proteins (HSPs; induced as a response to high temperature stress) or genes for cold-regulated proteins (COR; induced as a response to low temperature stress) would have a greater beneficial effect on stress tolerance than the individual genes. This possibility has been recently tested and proven to be far more successful[2,3].

Basic molecular biology research has established that the expression of a given gene is governed by the promoter sequence present mostly at the 5′ end of the gene. The promoter sequences determine the strength of the expression (i.e. strong or weak expression) as well as provide specificity to the pattern of gene expression (temporal and/or spatial). For instance, regulation of heat shock (HS) genes (referred to as *hs* or *hsp* genes) is mediated by a core DNA sequence called heat shock element (HSE), located in the promoter region of the *hs* genes (Figure 1). Presence of at least three five base pair modules (nGAAn) arranged as contiguous repeats – nGAAnnTTCnnGAAn – is the key feature of HSEs. Likewise, regulation of *cor* genes is mediated by *cis*-acting CRT (C-repeat)/DRE (drought-responsive element) sequence that stimulates transcription in response to low temperature (and water stress)[4]. The *cis*-acting promoter sequences interact with specific proteins for their activation. Such proteins are generally termed as transcription factors. For the regulation of HS promoter, positively-acting transcription factors termed heat shock factors (HSFs) have also been identified that bind specifically to HSEs[5]. For the regulation of *cor* genes, CBF1 (CRT/DRE binding factor 1) is implicated to be the gene regulator[6].

While the precise interactions between the *cis*-acting DNA sequences and the *trans*-acting protein factors are still being looked into, a new level of hierarchy has emerged for controlling expression of stress-responsive genes. This hierarchy suggests that the stress-responsive genes may be over-expressed through over-expression of the transcription factor genes. The novelty as well as importance of this approach lies in the fact that the *cis*-acting promoter sequences of different stress-responsive genes , (which are induced as a response to the same stress factor) are similar, and thus can be governed at the same time through manipulation of the transcription factor genes. For instance, *cis*-acting CRT sequence is present in the promoters of multiple *cor* genes including those encoding COR15a, COR78 and COR6.6 proteins[2] and *cis*-acting HSE sequence is present in almost all *hs* genes sequenced so far[5].

Banking on this background, Jaglo-Ottosen *et al.*[2] have produced transgenic *Arabidopsis* plants that over-express CBF1. This was achieved by placing a cDNA encoding CBF1 under the control of strong cauliflower mosaic virus (CaMV) 35S promoter and transforming the chimeric gene into *Arabidopsis*. Specific transformed line exhibiting higher level of accumulation of CBF1 corresponding transcript, also showed greater than normal amounts of COR6.6, COR15a, COR47 and COR78 corresponding transcripts without a low temperature stimulus. Importantly, it was found that CBF1 over-expression increased the tolerance of plants to freezing stress. According to these authors[2], this scheme may well be a generalized way for improving freezing stress tolerance because CRT/DRE DNA regulatory elements have a widespread occurrence.

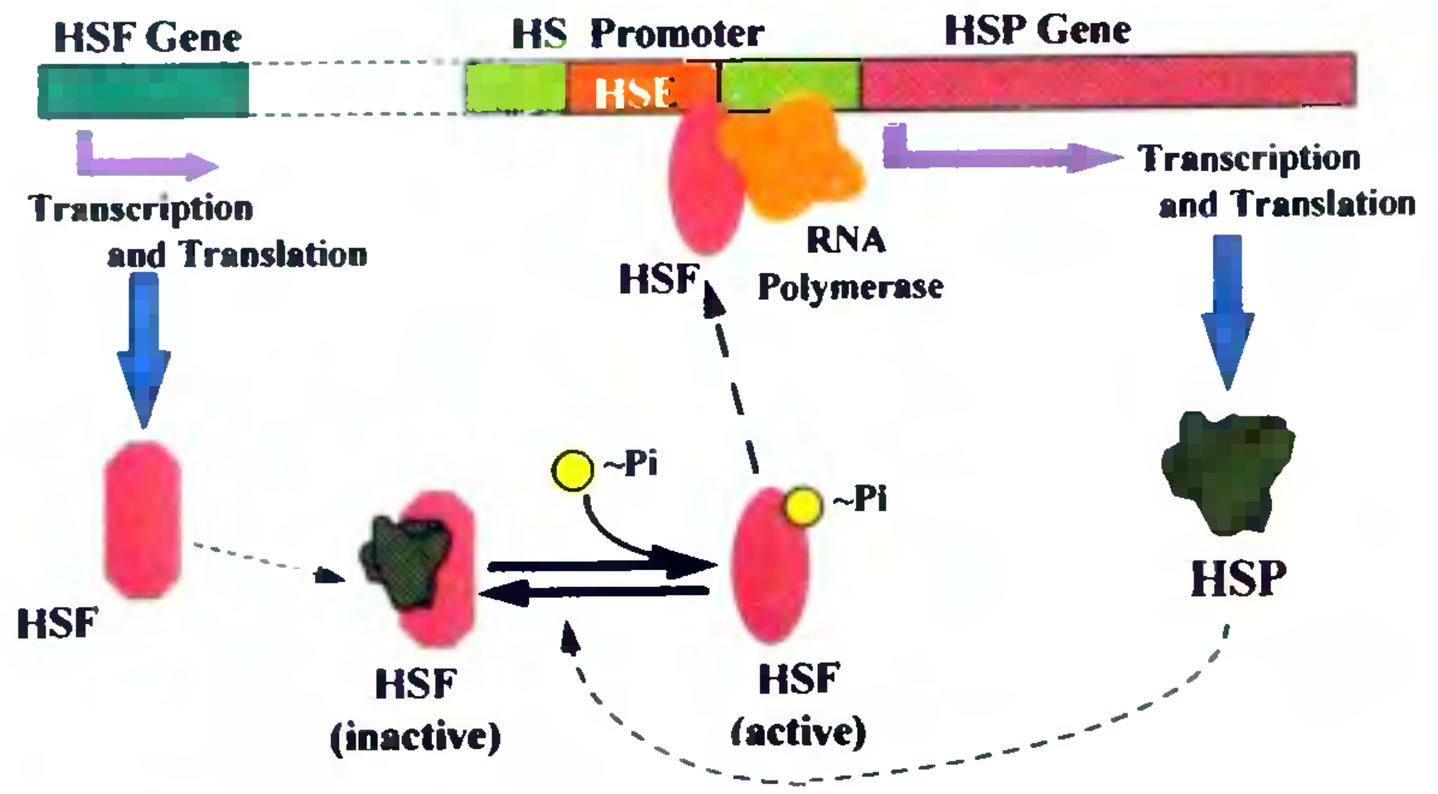

Figure 1. A model for the regulation mechanism for transcription of HSPs. Induction of *hs* gene transcription occurs when HSF binds to HSE upstream from the *hs* gene. Activation of HSF is related to phosphorylation of the HSFs and releasing them from binding to a HSP during HS. The response of HSP gene transcription to HS is a fine-tuned process that opens up many possibilities for genetic engineering. HS: Heat shock; HSP: Heat shock protein; HSE: Heat shock element; HSF: Heat shock factor.