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SURVEY AND SUMMARY

RNA helicases and abiotic stress

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ABSTRACT

RNA helicases function as molecular motors that rearrange RNA secondary structure, potentially performing roles in any cellular process involving RNA metabolism. Although RNA helicase association with a range of cellular functions is well documented, their importance in response to abiotic stress is only beginning to emerge. This review summarizes the available data on the expression, biochemistry and physiological function(s) of RNA helicases regulated by abiotic stress. Examples originate primarily from non-mammalian organisms while instances from mammalian sources are restricted to post-translational regulation of helicase biochemical activity. Common emerging themes include the requirement of a cold-induced helicase in non-homeothermic organisms, association and regulation of helicase activity by stress-induced phosphorylation cascades, altered nuclear–cytoplasmic shuttling in eukaryotes, association with the transcriptional apparatus and the diversity of biochemical activities catalyzed by a subgroup of stress-induced helicases. The data are placed in the context of a mechanism for RNA helicase involvement in cellular response to abiotic stress. It is proposed that stress-regulated helicases can catalyze a nonlinear, reversible sequence of RNA secondary structure rearrangements which function in RNA maturation or RNA proofreading, providing a mechanism by which helicase activity alters the activation state of target RNAs through regulation of the reaction equilibrium.

INTRODUCTION

The dependence of normal cellular function on the biochemical ability to alter RNA secondary structure is becoming increasingly evident. RNA in solution does not generally adopt the single-stranded linear conformation depicted in textbooks. Instead, RNA folds into extensive secondary structures via intra- and/or inter-molecular base pairings and diverse forms of long-range interactions. Functionally, therefore, RNA is similar to proteins in that the active form is based on the secondary and tertiary structure of the molecule and not necessarily the molecular sequence of the subunits which it is composed of. RNA secondary structure rearrangements are catalyzed predominantly by members of the two protein families either RNA helicases or RNA-binding proteins (RBPs). The majority of RNA helicases belong to the superfamily 2 (SF2) subclass of helicases characterized by sequence homology within a helicase domain consisting of eight or nine conserved amino acid motifs (1–3). SF2 consists of three subfamilies, termed DEAD, DEAH and DExH/D, based on variations within a common DEAD (Asp-Glu-Ala-Asp) motif (3). Amino acid sequences outside this 'core' helicase domain are not conserved and are believed to provide helicase specificity for target RNAs or protein–protein interactions. Biochemically, RNA helicases are molecular motors that unwind double-stranded RNA (dsRNA) thereby affecting the rearrangement of RNA secondary structure, traditionally associated with activation of RNA function. Recently, the range of enzymatic activities, and thus potential physiological activities, exhibited by RNA helicases has expanded to include rearrangement of ribonucleoprotein (RNP) complexes via the removal or 'clearing' of protein from RNA and the combination of both RNA unwinding and RNA annealing to promote RNA-strand exchange, potentially through a branch migration mechanism (3–7). These observations have dramatically increased the range of physiological activities catalyzed by RNA helicases, providing the potential for dynamic rearrangement of RNA or RNP structure.

RNA helicases are potentially required in any cellular process involving RNA maturation functioning in ribosome biogenesis, RNA splicing, transport, and turnover, transcription, translation initiation, RNAi, RNA editing, and development (1,3). With the exception of a few organisms, RNA helicase genes are encoded in viral, prokaryotic, archaea and eukaryotic genomes (1). Interestingly, helicases rarely exhibit functional complementarity despite exhibiting significant sequence and biochemical relatedness, implying that each helicase performs a unique function(s) in cellular physiology. Furthermore, it is becoming increasingly evident that an RNA helicase can perform more than a single physiological function. Although RNA helicases are associated with a diverse

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Table 1. RNA helicases involved in cellular response to abiotic stress

RNA helicase	Source	Stress	Biochemistry	Comments
CrhC (8,9)	*Anabaena PCC 7120*	Cold	5′ – 3′ unwinding (53)	Polar-biased localization (13)
CrhR (37)	*Synechocystis PCC 6803*	Light	Bidirectional RNA unwinding, RNA annealing, RNA-strand exchange (7)	Redox-regulated expression
CrhR (38)	*Synechocystis PCC 6803*	Salt/Cold		
CsdA (10)	*E.coli*	Cold	RNA destabilization (10); RNA unwinding (59)	Cold-adapted degradosome (24); 50S subunit biogenesis (58)
SrmB	*E.coli*	Cold?	RNA unwinding (59)	50S subunit biogenesis (57)
DeaD (11)	*M.burtonii*	Cold		
RhlE (62)	*P.syringae*	Cold		Cold-adapted degradosome
DED1	*S.pombe*	DED1 protein modification by heat and carbon source depletion (41)		Translation initiation (18,21); cell cycle progression (41,68)
DED1	*S.cerevisiae*	Cold (20)	RNA annealing, RNA-strand exchange54; RNA unwinding55; RNA-binding protein displacement (6)	Microarray analysis
Dbp2	*S.cerevisiae*	Cold (20)		Microarray analysis; mRNA decay and rRNA processing (23)
Dbp2	*S.pombe*			Cell cycle progression (69)
Tif2 (42)	*S.cerevisiae*	Lithium		
Dbp5p/Rat8p	*S.cerevisiae*	Ethanol and heat shock alter activity (65,66)		Nuclear mRNA export (65,66); transcription (67)
Los4 (30)/Cryophyte (31)	*Arabidopsis thaliana*	Cold		Nuclear mRNA export
Drh1 (32,33)	*A.thaliana*	Cold (+/−)		Microarray analysis
At5g08610/At1g59990 (34)	*A.thaliana*	Cold decreases transcript accumulation		Microarray analysis
HVD1 (36)	*Hordeum vulgare*	Salt		RGG and RSSS repeats
PDH45 (45)	*Pisum sativum*	Salt, cold, ABA, drought, wounding	3′–5′ DNA helicase (44)	DESD and SRT motifs
RHII/Gu (70)	Human	UV, anisomycin		c-Jun transcription cofactor
eIF-4A (47)	*Zea mays* roots	Hypoxia		Phosphorylation
eIF-4A (48)	Wheat germ	Heat		Phosphorylation
eIF-4A-8 (49)	*Nicotiana tabacum*	Pollen tube germination		Phosphorylation
PDH47 (43)	*P.sativum*	Cold, salt, heat, ABA	Bidirectional DNA unwinding; RNA unwinding	Phosphorylation by PKC *in vitro*
p68	HeLa	Expression altered in cancer cell lines (52)	Bidirectional RNA unwinding, RNA annealing, RNA-strand exchange (5)	Phosphorylation (51)

range of biotic cellular functions, there have been relatively few reports of RNA helicase involvement in cellular response to abiotic stress.

All cells experience a range of stress conditions, either abiotic or biotic, that tend to decrease cellular fitness. Since organisms frequently encounter abiotic stress in their natural habitats it follows that RNA helicase activity may be required for cellular adaptation to the altered environmental conditions. Reports have emerged recently indicating that RNA helicase expression or activity is regulated not only with respect to participation in housekeeping processes such as those mentioned above, but also in response to changes in specific environmental variables, including temperature, light, oxygen and osmolarity. Environmental regulation of RNA helicase gene expression in all probability is controlled through sensing and response pathways that are themselves activated by the corresponding environmental stress. RNA helicases, therefore, occupy a pivotal position in the regulation of gene expression. Their own expression can be environmentally regulated with the resulting induction of RNA helicase biochemical activity providing the ability to regulate either the expression or activity of downstream target mRNAs or functional RNAs, respectively. Furthermore, activity of the protein products resulting from translation of target mRNAs can then contribute to cellular adaptation to the inducing stress conditions.

The known RNA helicase genes whose expression is regulated in response to abiotic stress and their characteristics are summarized in Table 1. This review provides an overview of the regulation of RNA helicase gene expression or enzymatic activity in response to abiotic stress. It also examines the range of biochemical activities and the physiological functions ascribed to these environmentally regulated RNA helicases. Finally, the potential biochemical activities of environmentally regulated helicases are placed in context of cellular adaptation to abiotic stress.

RNA HELICASE EXPRESSION IN RESPONSE TO ABIOTIC STRESS

Cold-regulated expression

Archaea and prokaryotic RNA helicases. Abiotic stress induction of RNA helicase gene expression frequently involves regulation in response to growth at suboptimal temperatures in eubacteria and archaea. This is not surprising as temperature fluctuation is potentially the stress most frequently encountered by these organisms. Temperatures below those required for optimal growth elicit the so-called cold shock response, leading to the *de novo* synthesis of cold-induced proteins (CIPs). The mechanisms regulating CIP induction are poorly understood but the expression is generally, but not always transient and is accompanied by the concomitant, and seemingly contradictory, repression of global protein synthesis. CIP activity contributes to cellular adaptation to cold stress, eventually leading to resumption of normal growth. The RNA helicase genes, *crhC, csdA* and *deaD*, whose expression is regulated by a reduction in growth temperature, have been characterized in the photosynthetic cyanobacterium *Anabaena* sp. strain PCC 7120 (8,9), *Escherichia coli* (10), and the cold-adapted Antarctic methanogenic archaeon, *Methanococcoides burtonii* (11), respectively. Mechanisms involved in low temperature regulated expression have been investigated for *crhC* whose expression is low temperature specific as a range of additional abiotic stresses do not induce transcript accumulation (8). Induction is not transient and the mechanism involves low temperature enhancement of *crhC* transcript stability (9), a common theme in cold shock gene expression (12). Physiologically, CrhC is tightly associated with the plasma membrane, predominantly at the cell poles (13), implying a role in RNA metabolism that preferentially occurs at this location during cold stress. Expression of cold-regulated RNA helicase genes have also been identified by transcriptional profiling in the hyperthermophilic methanoarchaeon, *Methanococcus jannaschii* (14) and the Gram-positive bacterium, *Bacillus subtilis* (15). Genetic analysis of the *Bacillus* genes, *cshA* and *cshB*, indicated inactivation of both is lethal, emphasizing the requirement for RNA helicase activity during cold stress (16). Furthermore, FRET analysis revealed that CshB colocalizes with the RNA-binding cold shock protein (CSP), CspB, during active transcription. These observations position cold-induced RBPs and RNA helicases in close proximity with mRNA, implying an active role in translation initiation at low temperature (Figure 2). Related to the polar localization of CrhC, a portion of the cellular CshB localizes to the cell poles in *Bacillus*, although it is also distributed throughout the cell (16). It is interesting to note that all of the cold-regulated RNA helicase genes discussed above encode proteins belonging to the DEAD-box family.

In summary, as cold-regulated RNA helicase gene expression is a common feature in a range of prokaryotic organisms, including psychrophilic, mesophilic and thermophilic species, it is likely that a cold-regulated helicase is expressed in the majority of eubacteria and archaea. The data are consistent with the hypothesis that the RNA secondary structure rearrangement activity provided by a cold-regulated helicase is an ancient and universal requirement for normal cellular function at reduced temperature, possibly originating in the 'RNA world'. The extensive range of growth habitats in which such an RNA helicase is induced, indicates that this requirement is not a function of absolute temperature but rather in response to a downshift from the organisms' optimal growth temperature.

mRNA stability. A significant aspect affecting RNA helicase expression in response to cold stress is the regulation of transcript stability. For example, *crhC* transcripts are significantly stabilized in response to a temperature downshift (9). Although the mechanism remains unknown, mRNA stabilization is an important factor regulating expression of other low temperature-induced genes including *cspA, pnp, hupB* and *infB* (12). Stabilization of the *E.coli cspA* gene requires the 5′-untranslated region (5′-UTR), which is unusually long with respect to the average bacterial mRNA (17). It is presumed that destabilization arises through the action of a nuclease, potentially RNaseE, followed by PNPase degradation (17). A similar scenario has been observed in cyanobacteria, in that the extended 5′-UTR of *crhC* is required for transcript stabilization and accumulation at low temperature (J. M. Brown, D. Chamot and G. W. Owttrim, unpublished data). Thus, low temperature-induced RNA helicase expression involves a combination of both *cis*- and *trans*-acting factors, with mRNA stabilization performing a crucial role in transcript accumulation. Enhancement of mRNA stability would restrict helicase activity to conditions where the cell experiences the specific abiotic stress, providing the ability to coordinate target RNA function downstream of helicase synthesis.

Eurkaryotic RNA helicases. The requirement for a cold-induced RNA helicase also extends to eukaryotic microorganisms. Physiological functions for the majority of the >30 yeast RNA helicases has been described (18,19). Genome-wide transcript profiling in *Saccharomyces cerevisiae* identified the RNA helicases DED1 and Dbp2 as early cold response genes (20). DED1 and Dbp2 are involved in translation initiation (21,22) and nonsense-mediated mRNA decay and rRNA processing (23), respectively, thereby linking helicase activity with the maintenance of these processes during low temperature stress. RNA helicases are well-known components of the degradosome in prokaryotic systems and rRNA processing machinery in yeast (24,25). As proposed for prokaryotes, DED1 could replace the optimal growth temperature helicase in mRNA turnover during cold stress in yeast.

Reports of cold stress-regulated RNA helicase genes in higher eukaryotes are less common. Higher plant genomes encode a number of RNA helicase genes (26) whose expression and polyadenylation patterns are tissue specific (27–29). Recently, a temperature-regulated RNA helicase, LOS4, has been linked with developmental processes including flowering and vernalization in *Arabidopsis* (30,31). Similar to bacteria and yeast, temperate plant cold acclimation involves induction of specific cold-responsive (COR) genes. COR gene expression is activated by the C-repeat-binding factor (CBF) family of transcriptional activators which are also rapidly and transiently induced at low temperature. The allelic *Arabidopsis* RNA helicase mutants, *los4-1* and *los4-2*/

cryophyte, reveal that the LOS4 RNA helicase is an early regulator of CBF transcription factor expression in response to plant chilling (30,31). LOS4 inactivation renders the plants sensitive to chilling although the two mutants differentially affect the CBF–COR cold response pathway; *los4-1* reduces whereas *los4-2* enhances the expression of CBFs and their downstream target genes. This divergent response is mediated through a differential effect on nuclear mRNA export that *los4-1* inhibits and *los4-2* enhances at low temperature (31). The conventional explanation is that the lack of CBF expression in *los4* mutants derives from a direct effect on CBF mRNA export and the corresponding lack of CBF protein. However, a variety of observations indicate that LOS4 may perform more specific roles in low temperature response, unrelated to its function in mRNA export. This pleiotrophic phenotype includes germination at temperatures that are generally inhibitory to wild-type seeds and early flowering. These processes also involve the plant stress hormone, absisic acid (ABA), to which the *los4* mutants are sensitive. Inactivation of the cold responsive LOS4 helicase therefore also affects cellular ability to respond to the plant stress hormone. Investigation of the effect on germination indicated that LOS4 appears to be required for the formation of a germination inhibitor, an intriguing observation, as it suggests that helicase activity is necessary for the formation of a protein that inhibits the environmental sensing-signal transduction pathway required for germination (31). The LOS4 helicase is therefore important in a range of physiological processes involving plant development, in addition to or in conjunction with response to low temperature. Genome-wide transcript analysis has identified additional *Arabidopsis* RNA helicase genes whose expression is cold stress regulated. *drh1* was initially observed to be moderately cold induced (32); however, more extensive profiling did not verify these results (33). Similar analysis indicated a unique situation in that the expression of two additional putative *Arabidopsis* RNA helicase genes, At5g08610 and At1g59990, decreases in response to cold stress (34). The expression pattern of the latter two genes was identical in response to cold, salt and osmotic stress, reflecting the integration of cellular response to these variables. It is expected that abiotic-stress induced reduction in RNA helicase expression will be observed more frequently in the future as this scenario has the potential to down regulate pathways whose functioning is detrimental during stress conditions.

Organelle localized RNA helicases. Organelles are a prime target for stress-induced physiological affects which, combined with the presence of RNA helicases in both mitochondria (35) and chloroplasts (28), suggest that stress-regulated helicases could function in these organelles. However, to date, only one stress-induced organelle-localized RNA helicase has been reported. Expression of this gene, HVD1, is induced by both cold and salt stress in barley chloroplasts (36). In addition to the conserved DEAD-box helicase domains, HVD1 also contains RGG and RSS RNA-binding motifs found in a range of RBPs involved in non-specific RNA binding. The C-terminal RNA-binding domain sequence does not exhibit homology with other proteins suggesting that HVD1 is a novel, stress-regulated RNA helicase. This is the first evidence that RNA helicase activity is required for response to environmental stress in organelles. The low temperature regulated expression of a variety of cytosolic and organellar RNA helicases in plant systems is reminiscent of the situation in bacteria and archaea, providing a common theme which reflects an underlying requirement for cold-regulated RNA helicase activity in non-homoeothermic organisms.

Other abiotic stresses

Prokaryotic RNA helicases. Regulation of RNA helicase expression in prokaryotes and archaea in the response to abiotic stresses other than low temperature is limited. Fluctuation in light quality and quantity is a stress frequently encountered by photosynthetic organisms, whose ability to sense and respond to dynamic light conditions is crucial for their survival. Expression of *crhR*, from the photosynthetic cyanobacterium *Synechocystis* sp. strain PCC 6803, is regulated by light-driven changes in the redox status of the electron transport chain between Q_A in photosystem II and Q_o in cyt b_6f (37). Alteration of electron carrier redox poise can be initiated by either photosynthetic light harvesting or glucose metabolism. Interestingly, the data indicate that cyanobacteria do not sense light directly but rather the light-driven changes in redox potential of electron carriers in the electron transport pathway (37). *crhR* has also been characterized as being induced by cold and salt stress (7,38). The cells used in these experiments were grown under constant illumination, and the enhanced reduction of the plant electron transport chain, observed in response to these stress factors, presumably contributes to the observed increase in *crhR* transcript accumulation (39). This regulation implies that CrhR functions in an aspect of RNA metabolism related to survival in the light, presumably involving maintenance of optimal photosynthetic capacity, either light harvesting or carbon acquisition and fixation. The only other example of abiotic RNA helicase gene expression regulation from prokaryotic sources originated from a genetic screen to identify oxidative stress genes in the anaerobic Gram-positive bacterium, *Clostridium perfringens* (40). Intriguingly, inactivation of this DEAD-box RNA helicase resulted in enhanced resistance to oxidative stress rather than acting to reduce the perceived stress, as is the case for the majority of abiotic regulated RNA helicases. This is a novel observation as it implies that helicase activity exacerbates the abiotic stress effect. Determination of the mechanism controlling this response will be insightful to understanding RNA helicase involvement in the oxidative stress response.

Yeast. In *Schizosaccharomyces pombe ded1* expression is induced in response to nitrogen or glucose depletion and heat shock, in addition to low temperature, but not in response to DNA damage or oxidative or osmotic stress (41). As with other helicases, the mechanisms resulting in differential DED1 expression are not known. Post-transcriptional events may also regulate DED1 activity, as induction results in the *de novo* production of two forms of the DED1 protein. The exact nature of the protein modification(s) remains to be determined; however, phosphorylation via the mitogen-activated signal transduction pathway that regulates *Saccharomyces* response to heat stress does not appear to

be involved (41). The diverse range of environmental variables that affect *ded1* expression suggests DED1 functions in a general stress response pathway. The range of abiotic stresses that alter RNA helicase expression was expanded recently by the initial report of induction in response to metal stress. Lithium-induced metal stress enhances expression of the *S.cerevisiae* RNA helicase, *tif2* (42). Importantly, overexpression of *tif2* conveys lithium tolerance *in vivo*, implying a direct link between TIF2 helicase activity and the ability to tolerate lithium exposure. This observation provides a potential link between cold and metal stress responses, as a major component of lithium toxicity involves the inhibition of protein synthesis at the level of translation initiation, identical to the major physiological effect of low temperature. Lithium induces removal of TIF2 from the translation initiation complex, a process reversed by overexpression of SIT4, a protein phosphatase component of the TOR kinase pathway (42). The results therefore present evidence-linking alteration of both RNA helicase expression and activity with a stress-induced signal transduction pathway. It therefore appears that cellular adaptation to the inhibition of translation initiation induced by stresses as divergent as metal toxicity and low temperature share a related mechanism requiring RNA helicase expression and activity.

Higher plants. In plants, two DEAD-box-related helicases, termed pea DNA helicase 47 (PDH47) and PDH45 are induced by a variety of abiotic stresses, suggesting that they are components of a general stress response mechanism. PDH47 expression is differentially induced in a tissue-specific manner with induction by cold and salinity stress in shoots and roots and heat and ABA treatment in roots (43). PDH47 was localized to both the cytosol and nucleus, suggesting shuttling between the compartments (43). The second characterized helicase, PDH45 is a unique member of the DEAD-box family of RNA helicases as it contains DESD and SRT instead of the characteristic DEAD and SAT motifs (44). PDH45 transcript is induced in pea seedlings in response to a range of abiotic stresses including salt (specifically Na^+), dehydration, wounding and low temperature, leading to the suggestion that *pdh45* transcript accumulates in response to general water stress caused by desiccation (45). The physiological importance and conservation of PDH45 function in the salt-stress response was demonstrated by the observation that constitutive expression of PDH45 conveys salt tolerance in tobacco (45). As described for TIF2, these results imply that PDH45 performs a crucial function, directly involved in cellular response to a specific abiotic stress. Although the exact mechanism of PDH45-mediated tolerance of salinity stress remains to be determined, PDH45 and PDH47 transcripts are also induced by ABA, the plant abiotic stress hormone (46). This observation, combined with the *los4* mutation described above, enticingly associates RNA helicase expression with ABA-induced stress sensing signal transduction pathways.

Mammals. Examples of abiotic regulation of RNA helicase expression originate primarily from non-mammalian organisms, while instances from mammalian systems are currently restricted to post-translational regulation of helicase biochemical activity. Whether this represents a fundamental difference with respect to RNA helicase regulation mechanisms or is related to the reduced level of abiotic stress to which mammalian cells are generally exposed remains to be determined.

POST-TRANSLATIONAL REGULATION OF STRESS-INDUCED RNA HELICASE EXPRESSION AND ACTIVITY

Phosphorylation

Although environmental signal transduction frequently involves phosphorylation cascades, reports of RNA helicase phosphorylation are relatively rare and occur primarily in plant systems (Table 1). Inhibition of translation initiation is a common physiological response to both hypoxia and cold stress. In maize roots, hypoxia inhibits translation initiation through a process involving rapid phosphorylation of a member of the eIF-4A RNA helicase gene family (47). The hypoxia-induced decrease in cytoplasmic pH acts as a secondary messenger, regulating protein kinase and/or phosphatase activity that determines the phosphorylation state of eIF-4A. Similar to hypoxic stress, a wheat eIF-4A isoform is phosphorylated in response to heat stress (48). Thermal induction of eIF-4A phosphorylation is only observed in response to prolonged stress, suggesting a function in the adaptive process and not the immediate reduction in translation initiation (48). eIF-4A is encoded by a large gene family in tobacco (27,28) and is generally associated with translation initiation (1). Tobacco eIF-4A phosphorylation is also associated with plant reproduction as one member of the family, eIF-4A8, is expressed exclusively in pollen and is phosphorylated during a specific stage of pollen tube germination (49). Alteration of helicase activity by phosphorylation has also been reported in pea, in which protein kinase C (PKC)-mediated phosphorylation enhances the DNA unwinding and ATPase activities of PDH47 *in vitro* (43). Interestingly, although the PDH47 amino acid sequence identifies it as a DEAD-box RNA helicase, exhibiting 93% identity with tobacco eIF-4A (27,28), the recombinant protein unwinds dsDNA, DNA–RNA hybrids and dsRNA (43). PDH47 is also reported to stimulate mRNA translation in a wheat germ derived *in vitro* translation assay (43). These observations suggest PDH47 performs a role in translation initiation or another aspect of nucleic acid metabolism, based on its nuclear localization and DNA-unwinding activity. RNA helicase phosphorylation is therefore a common physiological response to abiotic stress in plant systems. In mammalian systems, intriguing reports have linked phosphorylation of p68 with cancer development and tumor cell proliferation. p68 is a nuclear-localized helicase associated with a number of processes in eukaryotic cells, including functioning as a transcriptional coactivator of the p53 tumor suppressor (50). Importantly, tyrosine phosphorylation has been shown to directly affect p68 biochemical activity, inhibiting ATPase and unwinding activity and thus p68 function in pre-mRNA splicing (51). Phenotypic consequences are also evident as p68 phosphorylation on multiple sites is specifically observed in a variety of cancer cells, implying a role in cell transformation (52). Physiologically, differential phosphorylation

of p68 at a number of tyrosine, serine or threonine residues provides the potential for regulation of p68 activity through diverse signal transduction pathways in response to a variety of external stimuli (51).

Thus, while examples of RNA helicase activity regulated at the post-translational level by protein modification are accumulating, they are currently restricted to eukaryotic systems, suggesting that regulation of RNA helicase activity differs between prokaryotes and eukaryotes. Since environmental sensing and signal transduction frequently involve phosphorylation cascades, the regulation of RNA helicase activity by these archetypal regulatory mechanisms warrants further investigation which will undoubtedly enhance our understanding of RNA helicase regulation in response to abiotic stress.

BIOCHEMISTRY OF STRESS-INDUCED RNA HELICASES

Biochemical activities characteristic of RNA helicases, namely RNA-dependent ATPase and RNA unwinding, have been demonstrated for a relatively small proportion of the RNA helicase-related sequences in the public databases. This also applies to stress-regulated helicases. Biochemical activity of stress-induced RNA helicases is summarized in Table 1. Extensive biochemical analysis has been performed on two RNA helicases from photosynthetic Gram-negative species of cyanobacteria. Expression of CrhC, from *Anabaena*, is specifically regulated by temperature with transcript accumulation only occurring at temperatures below 30°C (8,9). CrhC exhibits both RNA-dependent ATPase and RNA-unwinding activities characteristic of RNA helicases (53). RNA unwinding proceeds unidirectionally, 5′–3′, over short duplexes suggesting CrhC possesses low processivity. Low processivity is a characteristic of the prototypical RNA helicase, eIF-4A, required for translation of the majority of mRNAs in eukaryotic cells (19). Whether CrhC performs a similar function in *Anabaena* during cold stress remains to be determined. The redox-regulated cyanobacterial helicase, CrhR, exhibits radically different biochemical properties, catalyzing a range of RNA structural rearrangements in addition to those characteristic of RNA helicase activity (7). CrhR catalyzes annealing of complementary single-stranded RNA (ssRNA) into dsRNA and combines the helicase and annealing activities to promote RNA-strand exchange, a reaction that potentially proceeds through an RNA branch migration mechanism. Similar RNA secondary structure rearrangements are catalyzed by three other RNA helicases, the highly related mammalian p68 and p72 enzymes (5) and DED1 (54). The mechanism by which cells balance the annealing and unwinding reactions catalyzed by these enzymes is a major question. Analysis of DED1 activity has addressed this question, indicating the balance between the two reactions is modulated by the ATP:ADP ratio in conjunction with the structure of the RNA substrate (54). The ability of CrhR, p68/p72 and DED1 to catalyze both RNA annealing and unwinding concurrently provides the potential for rapid and efficient conversion between alternative RNA structures. In addition, DED1 also promotes protein displacement from RNA, indicating the ability to remodel both RNA secondary structure (55) and RNP complexes (6). Insightfully, DED1 catalyzed RNP remodeling occurs on ssRNA, in the absence of duplex unwinding. The combined results suggest that the 'clearance' of RBPs is the physiological function performed by DED1, and possibly other DExH/D proteins, *in vivo* (6). It will be informative to determine which, if any, of these biochemical activities are associated with a specific physiological function. Structurally, the identified RNA helicases catalyzing RNA annealing appear to comprise a unique subclass of SF2 RNA helicases which contain RG-rich C-terminal regions (54). It is possible that this RG-rich domain is required to promote RNA-strand annealing. In the broader context of general nucleic acid metabolism, strand exchange and branch migration are also characteristics of RecA and RuvB, required for rearrangement of DNA secondary structure. Indeed, it has become clear that these DNA and RNA secondary structure rearranging proteins are related not only mechanistically but also structurally (56). A number of cellular functions also require nucleic acid secondary structure rearrangement whose mechanism involves a combination of helicase and annealing activities catalyzed by independent proteins (56). These RNA-binding proteins function as nucleic acid chaperones, catalyzing secondary structure rearrangement in the absence ATP hydrolysis. CrhR and DED1 utilization of ATP hydrolysis to drive these rearrangements distinguishes them from the chaperone-catalyzed reactions.

The helicase catalyzed RNA annealing, unwinding and protein displacement activities provide the opportunity for a variety of structural rearrangements involving both RNA secondary and RNP structure. As proposed in Figure 1, helicase alteration of an initial RNA secondary structure and concomitant removal of RBPs potentially activates the RNA, allowing binding and function of a second set of RBPs on the new RNA structure. Repetition of this scenario will generate a linear series of sequential RNA modification steps, the prime example being RNA splicing in eukaryotic systems. The discovery of RNA helicase catalyzed annealing expands this scenario by allowing a nonlinear, reversible sequence of RNA secondary structure rearrangements. The reversible interconversion of RNA or RNP structure provides the ability to control the equilibrium of the maturation/functional pathway, providing a mechanism to regulate downstream events. A reversible, cycling of RNA secondary structure is also applicable to the regulation of pathways requiring single RNA unwinding events, e.g. translation initiation or degradation, or potentially reactions in which a functionally active RNA changes structure as a consequence of each catalyzed reaction. Indeed, the annealing activity could perform an RNA secondary structure 'proofreading' function, whereby an improperly folded RNA could be returned to its original conformation, facilitating accurate RNA maturation or function. In conclusion, identification of biochemical activities associated with specific physiological functions will be a primary goal of future research. Determination of the mechanisms by which the physiological and biochemical functions are regulated will be an even more revealing result.

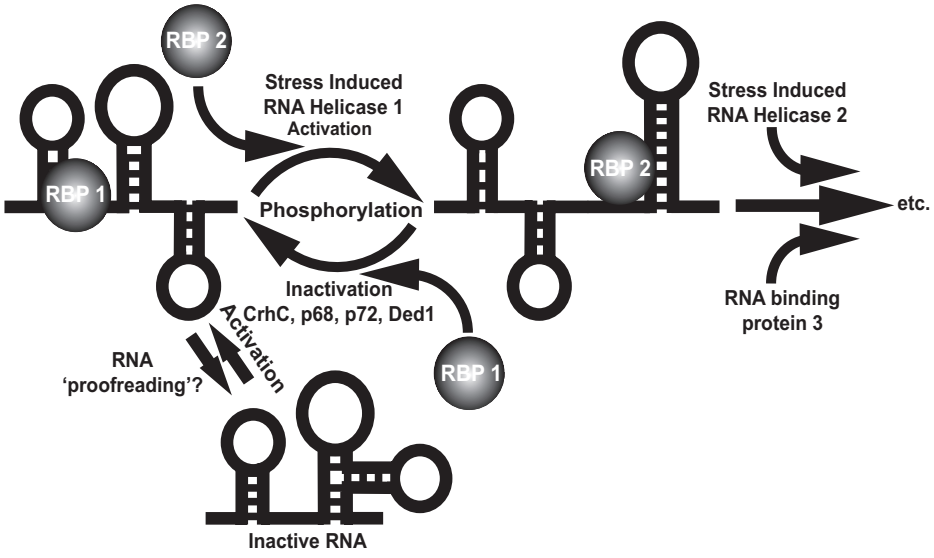

Figure 1. Helicase activation of RNA substrates. The traditional proposed function of RNA helicases involves unwinding of dsRNA in a linear unidirectional reaction. The discovery of helicases with additional biochemical activities, including the 'clearing' of proteins and annealing of complementary RNAs indicates the potential for expanded roles in RNA metabolic pathways. Three different roles are shown here. A linear sequence of RNA maturation steps, each of which is catalyzed by a unique helicase which can concomitantly rearrange RNA secondary structure and clear proteins from the RNA. Successive rounds of helicase RNP remodeling combined with RBP action will generate a linear series of steps catalyzing RNA maturation, thereby activating the RNA substrate. The discovery of RNA helicases that can anneal complementary RNAs (DED1, p68, p72 and CrhR) provides the ability to reverse a helicase driven activation-unwinding reaction, thereby maintaining the substrate RNA in an inactive or immature state. Finally, the ability to both unwind and anneal RNA substrates provides the potential for RNA proofreading, enabling improperly folded RNAs to be reactivated. The reversibility of each of these reactions also provides the potential to regulate the equilibrium of the reaction. RNA helicase phosphorylation in response to stress activated signal transduction pathways is another mechanism by which RNA helicase activity can be regulated. RBP, RNA-binding protein.

PHYSIOLOGICAL FUNCTIONS OF ABIOTIC STRESS-REGULATED RNA HELICASES

Ribosome biogenesis

Potential physiological functions for stress-regulated RNA helicases have been identified in a few cases. In *E.coli*, three of the five DEAD-box RNA helicases are required for ribosome biogenesis, with two proposed to function in response to low temperature. *E.coli* SrmB is required early in 50S ribosomal subunit assembly and, although not reported to be a CIP, has been proposed to rearrange an RNA secondary structure that is thermodynamically stabilized at low temperature (57). Charollais *et al*. (58) reported that the cold-regulated helicase, CsdA, also performs a role in 50S subunit biogenesis in *E.coli*, although at a later stage than SrmB. Thus, CsdA potentially performs multiple roles in translation initiation (10) and ribosome maturation in response to cold stress, presumably involving rearrangement of RNA secondary structure. In support of this proposal, CsdA has been shown recently to unwind dsRNA (59).

RNA turnover

CsdA also has the potential to function in RNA turnover through its ability to interact with the degradosome, a multi-subunit complex required for RNA turnover in organelles and prokaryotes. At 37°C, the degradosome is composed of enolase, the RNases RNase E and PNPase, and the RNA helicase, RhlB which unwinds RNA secondary structure 3′–5′ in conjunction with PNPase-catalyzed RNA degradation (60). At 15°C, CsdA can structurally and functionally replace RhlB in the degradosome complex *in vitro*, implying that cold stress results in the formation of a cold-adapted degradosome (25). CsdA may be required to promote degradation of RNA whose secondary structure is stabilized by low temperature to a degree where RhlB is unable to unwind the structure. Although this is an interesting proposal, the *in vivo* physiological relevance of this interaction requires further investigation in light of the observation that RNase E appears to be promiscuous with respect to the RNA helicases with which it interacts. Binding studies indicate that RNase E has the potential to associate with SrmB, RhlE and CsdA *in vitro*, with RhlB, RhlE and CsdA being interchangeable with respect to the facilitation of structured RNA degradation (25,61). In support of the cold-adapted degradosome proposal, biochemical purification has also indicated that RhlE is a component of the degradosome in the cold-adapted, Antarctic bacterium, *Pseudomonas syringae* Lz4W (62). The accumulated data therefore indicate the potential for degradosome cold adaptation by association with a specific cold-induced RNA helicase in a range of bacteria, highlighting the requirement for RNA helicase activity to maintain RNA turnover during cold stress.

Nuclear mRNA export

mRNA transport through the nuclear pore complex is a frequent target of stress response mechanisms. In addition to the *Arabidopsis* LOS4 protein described above, the essential yeast DEAD-box RNA helicase, Dbp5p/Rat8p, is also

required for mRNA export from the nucleus (63). Dbp5p/Rat8p shuttles between the nucleus and cytoplasm and is required to dissociate the nuclear mRNA export receptor, Mex67p, during transfer through the nuclear pore complex (64). Interestingly, genetic analysis of the cold-sensitive defect observed in *rat8-7* mutants suggests that the requirement for the Mex67p-clearing activity of Dbp5p/Rat8p is enhanced at low temperature (64). Ethanol and heat stress also differentially affect Dbp5p/Rat8p function. Ethanol stress blocks bulk mRNA export, correlating with the nuclear accumulation of Dbp5p/Rat8p (65). In contrast, although bulk mRNA export is also blocked by heat shock, Dbp5p/Rat8p localization to the nuclear rim is not affected by this stress (65) where it specifically enhances nuclear export of heat shock mRNAs (66). Heat shock mRNA export requires the amino acid motif, NGQADP, found only in Dbp5p/Rat8p and orthologous proteins present in a range of organisms (66). This motif may be required to maintain mRNA export at elevated temperature and it would be informative to know if orthologs also promote differential mRNA export in response to heat shock in other systems. The results suggest that Dbp5p/Rat8p function in nuclear mRNA transport differs with respect to ethanol, heat and cold stress, indicating the potential for Dbp5/Rat8p interaction with different subgroups of mRNA transcripts in response to each stress. Dbp5/Rat8p function is more complex as genetic and physical association with components of the transcription factor IIH (TFIIH) complex have been observed, linking this helicase with early steps in transcription (67). Thus, it appears that the subfamily of RNA helicases related to Dbp5p/Rat8p has pleiotrophic functions in the nucleus and cytoplasm involving both transcription and mRNA export in response to a range of abiotic stresses. Determination of the ability of Dbp5p/Rat8p to rearrange RNA or RNP structure through removal of RBPs *in vitro* is clearly an objective that would significantly enhance understanding of Dbp5p/Rat8p physiology.

Cell cycle progression

Evidence that the yeast RNA helicase DED1 also performs roles in addition to its function in translation initiation was obtained by the observation that *ded1* inactivation leads to cell cycle arrest (41). A substantial body of evidence is now accumulating which links DED1 with cell cycle progression (41,68). DED1 physically interacts with the protein kinases, Chk1 and Cdc2, required for cell cycle response to DNA damage and the primary cyclin-dependent kinase regulating cell cycle progression, respectively (41). Unfortunately, the physiological function of the DED1–Chk1/Cdc2 interaction *in vivo* is unknown. DED1 is not the only stress-regulated yeast RNA helicase associated with cell cycle progression as Tallada *et al*. (69) have reported that overexpression of Dbp2 also resulted in cell cycle defects. It is interesting that Dbp2 is related to p68, a helicase which is also potentially associated with cell cycle progression as p68 is tyrosine phosphorylated only in cancer cells, functioning as a transcriptional coactivator of the p53 tumor suppressor (50,52). Thus the yeast RNA helicases, DED1 and Dbp2, which function in general RNA metabolism, also perform more specific roles that affect a diverse range of cellular functions including response to abiotic stress and cell cycle progression. Whether these pleiotrophic functions are independent of the roles performed by these helicases in general RNA metabolism remains to be determined.

Transcriptional regulation

Reports of stress-regulated RNA helicase involvement in mammalian systems are exceptionally rare. The one instance also links RNA helicase activity with the regulation of transcription. c-Jun is a transcriptional regulator activated by phosphorylation in response to a range of external stimuli including abiotic stress and differentiation signals. Tandem affinity chromatography identified that the human DEAD-box RNA helicase RHII/Gu interacts with c-Jun independently of c-Jun-mediated phosphorylation (70). This interaction is stimulated by UV or anisomycin treatment and is also associated with RHII/Gu translocation from the nucleolus to the nucleoplasm. In the absence of RHII/Gu, activation of both reporter and c-Jun target gene transcription is inhibited during neuronal cell differentiation. The results indicate that the RHII/Gu helicase is a transcriptional coactivator of c-Jun, with functions involving both RNA polymerase II-catalyzed transcription and stress responses in mammalian cells. Thus, the stress-regulated RNA helicases, Dbp5/Rat8p and RHII/Gu, have been directly associated with transcription.

CONCLUSIONS AND FUTURE DIRECTIONS

This review examines the current literature identifying RNA helicases whose expression or activity is altered in response to abiotic stress. RNA helicases are encoded in essentially every organism, ranging from viruses to humans, performing essential roles in potentially any cellular function involving RNA metabolism. Although a number of these functions are documented with respect to housekeeping activities, the importance of stress-induced alteration of RNA helicase expression and activity is only just becoming evident. RNA helicases function in the cellular response to these abiotic stimuli through the alteration of nuclear mRNA export, translation initiation, mRNA decay, rRNA processing, cell cycle progression, transcription and helicase subcellular localization. The precise roles performed by the induced RNA helicases in cellular response to abiotic stresses remain elusive; however, they almost assuredly involve RNA helicase rearrangement of RNA or RNP structure. A general model for RNA helicase function in abiotic stress is presented in Figure 2. Although translation initiation at low temperature is diagramed, non-cold-regulated helicases many function similarly in response to other stresses. Cold-induced thermodynamic stabilization of RNA secondary structure inhibits normal RNA helix destabilizing mechanisms preventing proper RNA–protein interaction and subsequent RNA maturation or functioning. Under these conditions, interaction of a stress-induced helicase with the target RNA will actively unwind the RNA secondary structure providing ssRNA which functions in translation initiation. ssRBPs bind and coat the RNA, preventing spontaneous refolding of the newly liberated ssRNA, analogous to DNA helicase and ssDNA-binding protein function during DNA replication. The RBPs shown

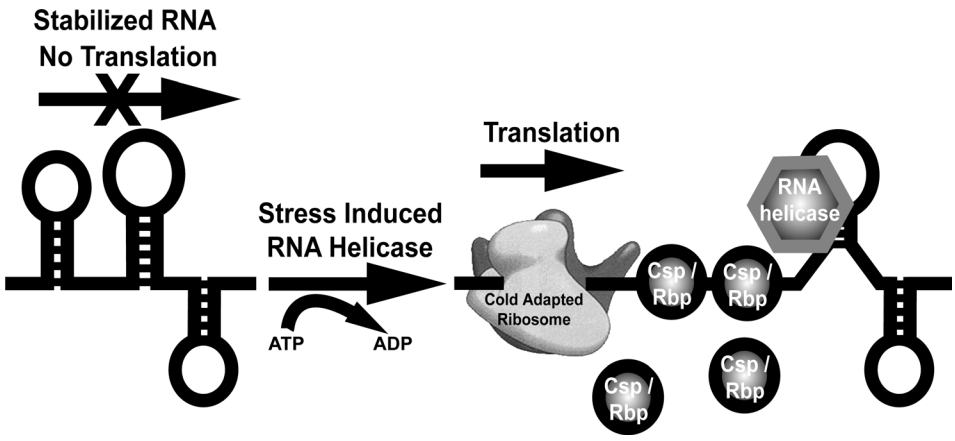

Figure 2. Roles for abiotic stress-induced RNA helicases. Stabilized, non-functional RNAs are recognized and unwound by the abiotic stress (low temperature in this example)-induced RNA helicase. Cold-induced RBPs belonging to the Rbp or Csp families in cyanobacteria (71) and *E.coli* or *Bacillus* (72), respectively, potentially bind to the RNA helicase-generated ssRNA, thereby inhibiting spontaneous reversion to dsRNA, and permit translation initiation to proceed. Similar scenarios can be envisioned for helicases involved in prokaryotic and eukaryotic RNA degradation pathways. It is also possible for this model to function on constitutively expressed RNAs that are not translated in the absence of a stress-induced helicase. Stress-induced production of an RNA helicase which interacts with a specific secondary structure in these stored RNAs can then initiate their translation, thereby allowing post-transcriptional regulation of an entire response system through regulated expression of a single RNA helicase gene.

belong to either the RBP or CSP families. Members of both gene families are cold induced in cyanobacteria or *E.coli* and other prokaryotes, respectively (71,72). A similar scenario can be proposed for helicase function in RNA turnover catalyzed by cold-adapted RNA degradation complexes in the prokaryotic degradosome and the eukaryotic exosome.

Although a relatively small percentage of the RNA helicase-related sequences in public databases have been identified as being stress-regulated, there are common themes emerging. It is quite evident that RNA helicases are capable of performing more than one, non-overlapping function in a cell, e.g. translation initiation and RNA turnover under normal and stress conditions, respectively. There also appear to be a variety of mechanisms by which stress-regulated helicases exert their influence. For example, stress-induced alteration of helicase localization within the cell provides the ability to perform diverse roles in different subcellular compartments while phosphorylation provides the opportunity to directly link helicase activity with environmental sensing-signal transduction phosphorylation cascades. Undoubtedly, examples of RNA helicases involved in abiotic stress responses will continue to increase. Although identification of RNA helicases intimately associated with cellular response to abiotic stress has begun, numerous fundamental questions remain unanswered. Future work will involve identification of (i) the pathways by which environmental stress regulates RNA helicase gene expression or activity; (ii) the specific RNA targets of stress-induced RNA helicases; (iii) RNA or RNP structure rearrangement activities associated with specific physiological functions; and (iv) the mechanisms by which the physiological and biochemical functions are integrated and regulated. Unfortunately, all too commonly, the physiology and biochemistry of RNA helicase function have not been linked. This is clearly an objective which will significantly enhance understanding of RNA helicase physiology in cellular response to abiotic stress.

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