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The Effect of Temperature on Plant Growth and Development

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Introduction

Changes in temperature will have an effect on all thermosensitive metabolic processes in the plant cell, in addition they may lead to modification of structural components of the cell. Because plants are immobile, they must adapt their metabolism to changes in temperature to minimize that damage and growth perturbations. Two recent reviews have been published (Ougham and Howarth, 1988; Ho and Sacks, 1989) which discuss the effects of temperature, particularly high temperatures, on plants. Reviews of the heat-shock response in plants have also been published recently (Kimpel and Key, 1985; Schöffl, Baumann and Raschke, 1988) and also a survey of plant reactions to a range of environmental stresses including temperature (Sacks and Ho, 1986).

This review describes the effects of temperature changes on the physiology and biochemistry of plant tissues, with special consideration of gene expression, particularly in the rapidly growing field of low temperature studies. We will not discuss in detail the studies on chilling injury of plants, which have been reviewed by Lyons, Raison and Steponkus (1979), Minorsky (1985), Wang (1982) and Parkin *et al.* (1989), and will only present the studies of structural changes in membranes and proteins in the context of acclimation and metabolism, since this topic has been reviewed recently (Jaenicke, 1990; Steponkus, Lynch and Vemura, 1990; Williams, 1990).

Studies on the mechanism by which plants perceive temperature change, on the signal transduction leading to modification of gene expression and on the biochemical/physiological role of the genes, are such that rapid advances can

Abbreviations: ABA, abscisic acid; CAP, cold acclimation protein; cDNA, complementary DNA; ER, endoplasmic reticulum; HSP, heat-shock protein; LT_{50}, temperature inducing 50% electrolyte leakage; PAGE, polyacrylamide gel electrophoresis; RFLP, restriction fragment length polymorphism; Rubisco, ribulose bisphosphate carboxylase.

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be expected. This is a field in which the analytical techniques of molecular biology will have a major impact.

High-temperature responses

In almost all eukaryotic tissues investigated, a rapid elevation of the temperature above a required threshold results in the cessation of the synthesis of most proteins in the cell and the induced synthesis of a set of heat-shock proteins (HSPs), (Schöffl, Baumann and Raschke, 1988). The temperature at which this response occurs varies between species, in sorghum (*Sorghum* sp., a tropical grass) it is between 37°C and 40°C depending on the cultivar (Ougham and Stoddart, 1986); in temperate grasses it is between 30°C and 35°C (Ougham, 1987). HSPs in plants fall into three size groups:

1. large, 68 000–104 000 Da, which have features in common with those produced in other organisms;
2. intermediate, 20 000–23 000 Da; and
3. small, 15 000–18 000 Da, which are characteristic of the higher plant heat-shock response.

It has been suggested that these latter proteins would be more appropriately called heat-stress proteins since they are produced in response to more gradual temperature changes (2·5°C h^{-1}) which are equivalent to those found in the field (Ho and Sacks, 1989). Baszczynaki, Walden and Atkinson (1985) have shown that in maize (*Zea mays*), the synthesis of HSPs is dependent on the appearance of HSP mRNA in the cytoplasm. HSPs have been implicated in the development of thermotolerance (Schlesinger, Ashburner and Tissieres, 1982). Key *et al.* (1985) have shown that pretreatment of soybean (*Glycine max*) seedlings at 40°C for 2 h produces thermotolerance to a subsequent treatment at 45°C for 2 h, such that they will resume normal growth at 30°C. HSPs are produced at both 40°C and 45°C. Without the 40°C pretreatment, a treatment of 45°C for 2 h is lethal. A similar result has been seen in barley (*Hordeum vulgare*) (Marmiroli *et al.*, 1986) and *Sorghum* sp. (Ougham and Stoddart, 1986).

The response of all plant tissues to high temperature treatments is not identical. Germinating pollen has been reported not to synthesize HSP in *Tradescantia* (Xiao and Mascarenhas, 1985) and to produce unique polypeptides in maize (Cooper, Ho and Hauptmann, 1984). In a study of the heat-shock response of *Sorghum* seeds during imbibition and germination, Howarth (1990) has shown that the mRNA for the high molecular weight HSPs is present in *Sorghum* embryos prior to treatment. Most of the proteins encoded by this mRNA are not translated at normal temperatures, the exceptions are HSP72 and HSP83 which may have a role in the very early stages of imbibition. The low molecular weight HSPs, however, require *de novo* transcription. Thus, in *Sorghum*, an elevated temperature treatment evokes a change in both translational and transcriptional controls. The presence of heat-shock mRNA in seeds may be due to residual RNA resulting from heat stress during seed development. In most tissues HSP mRNAs are

short lived following the return to normal temperature (Schöffl and Key, 1982) but degradation may be prevented in dehydrated tissue. After 24 h of imbibition by *Sorghum* seeds no stored HSP RNA exists.

Several higher plant HSPs have been cloned. The small (15 000–18 000 Da) HSPs, which are characteristic of higher plants, are the most abundant proteins produced with high-temperature stress. A number of the genes for these proteins have been sequenced and shown to share homologous sequences (Schöffl, Baumann and Raschke, 1988). In soybean, the homology is about 90% for members of the same gene family and about 50% for other families. The shared characteristics are particularly pronounced in the C-terminal halves of the predicted proteins. Their synthesis is primarily regulated by a transcriptional control, in addition there is some evidence of elevated translation during heat shock. There is considerable homology among the high molecular weight HSPs, thus the maize *hsp70* has 75% sequence homology with the coding region of *Drosophila hsp70* (Goldberg, 1979). Similarly, a consensus heat-shock 5′ regulatory sequence has been found in plants at −48 to −62 base pairs (Schöffl, Raschke and Nago, 1984) and a secondary heat-shock consensus control sequence has been found by Nagao *et al.* (1985). Functional analysis suggests a bipartite promoter structure, such that maximal enhancement of transcription in transgenic tobacco (*Nicotiana tabacum*) requires both the proximal heat-shock regulatory consensus sequence and the more distally located 'simple' sequences (Schöffl, Baumann and Raschke, 1988). Unique to heat-shock promoters is the high copy number of some heat-shock-promoter elements (6–9 copies) in a short region, and their special overlapping configuration. The overlap of two heat-shock-promoter elements seems to be the basic functional unit of a plant heat-shock promoter (Schöffl, Rieping and Raschke, 1990). These results support the view that the molecular mechanism for the induction of HSPs is also highly conserved. The only plant HSP that has been identified is ubiquitin (Burke, Callis and Vierstra, 1988).

The reduced rate, or cessation, of synthesis of 'normal' proteins by heat shock has been studied in germinating barley aleurone cells. Treatment of barley aleurone tissue at 40°C arrests α-amylase (EC 3.2.1.1) synthesis and this is correlated with a reduction in the levels of the normally stable α-amylase mRNA (Belanger, Brodl and Ho, 1986). α-amylase mRNA is translated in the endoplasmic reticulum (ER), it has been suggested that during heat shock the dissociation of ER lamellae disrupts the association of α-amylase mRNA and ER-bound ribosomes. This, in turn, leads to the breakdown of mRNA (Brown and Brodl, 1988). Interestingly, actin mRNA levels do not fall in response to heat shock in this tissue and actin is translated on free polysomes (Brodl and Ho, 1986). In contrast to these studies, it has been shown that the ER-bound polysomal synthesis of storage proteins is not decreased in heat-stressed maturing soybean cotyledons (Bray and Beachy, 1985), neither is the ER membrane disrupted. Both α-amylase synthesis in barley and storage protein synthesis in soybean are controlled by plant hormones. In particular, abscisic acid (ABA) treatment stimulates storage protein synthesis but antagonizes gibberellic acid stimulation of α-amylase

synthesis. The contrasting effect of high-temperature stress on the synthesis of these proteins may indicate profound differences in the ER membrane on which synthesis occurs.

Effects of low temperature on growth and metabolism

EFFECTS OF LOW TEMPERATURE ON GROWTH

Plants adapted to survive or grow in the cold (temperate species) do not express this adaptation except following a period of acclimation, which is usually a period of growth in the cold. Acclimation of temperate cereals to low temperature results in an alteration of growth and in frost-hardening.

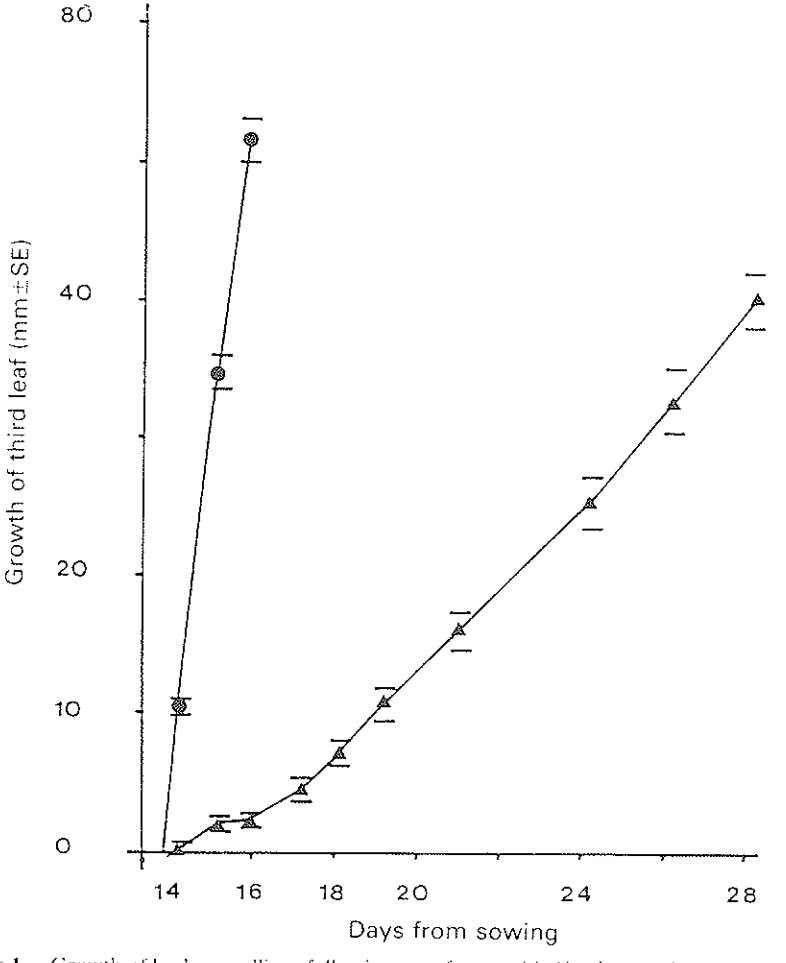

Figure 1. Growth of barley seedlings following transfer to cold. *Hordeum vulgare* cv. Igri seeds were grown for 14 days in 20°C day/15°C night, 10 h d^{-1}, 170 μmol m^{-2} s^{-1} photon flux density. Half were then transferred to a cold environment: 6°C day/2°C night, 10 h d^{-1}, 150 μmol m^{-2} s^{-1} (LT ▲). The remainder continued in the warm environment (HT ●). Growth of third leaf: average change in height after transfer to cold on day 14 (mm ± SE). (From Hughes and Pearce, 1988.)

Figure 1 shows the acclimation for growth of the third true leaf of barley seedlings transferred to a 6°C day/2°C night environment after 14 days' growth at 20°C day/15°C night and the growth of a parallel group maintained under the 20°C day/15°C night temperature regime. The growth rate of plants in the low temperature treatment was faster after 2 days' acclimation than immediately after transfer to the cold. Leaf emergence also acclimated (Hughes and Pearce, 1988).

Plant species have been classified into three groups, depending on the temperature below which injury occurs: chilling-sensitive, 10–15°C, freezing-sensitive, 0°C; and freezing-resistant plants, which are able to survive subzero temperatures (Pollock and Eagles, 1988). Acclimation for protection against low-temperature injury will depend on the group to which the plant species belongs. Mohapatra, Poole and Dhindsa (1987) have shown that alfalfa (*Medicago sativa*) will acclimate during a 2 day period at 4°C such that survival of seedlings at –10°C rises from 6% for plants grown continuously at 20°C to 40% for the acclimated group. Gilmour, Hajela and Thomashow (1988) used the electrolyte leakage technique to measure freezing damage to *Arabidopsis thaliana* leaf tissue following acclimation at 4°C. In this material the temperature for 50% electrolyte leakage (LT_{50}) was –3°C for plants grown continuously at 24°C and rose to –8 to –10°C after 9 days at 4°C.

In a number of studies, a strong negative correlation has been described between growth at low temperatures and the freezing tolerance of different ecotypes (Pollock and Eagles, 1988). It is not known whether or not this relationship is causal and there is some evidence that the relationship can be broken under appropriate selection.

PHYSICAL EFFECTS OF LOW TEMPERATURE ON CELL CONSTITUENTS

Lyons (1972) proposed that the low-temperature-induced changes in the molecular ordering of membrane lipids lead directly to injury in chill-sensitive plants. Since then the phase transitions of the lipid components of membranes have been widely studied (*see* Minorsky, 1985; Williams, 1990), primarily in the context of chilling injury. Using electron-spin resonance spectroscopy, Wade, Breidenbach and Lyons (1974) demonstrated phase changes at 10°C in mitochondria, glyoxysome and proplastid membranes of germinating castor bean (*Ricinus communis*) endosperm tissue. The physical phase-transition changes the membrane from a flexible liquid-crystalline to a solid-gel structure (Wang, 1982). This type of physical change has been proposed as the primary stimulus of low temperature in plant cells and, as such, would clearly be important not only in determining the extent of injury in a sensitive cell but also in transduction of the 'altered temperature' signal to bring about changes in cell metabolism. Minorsky (1985, 1989) has proposed a pathway of the main events leading to cooling stimulation in plant cells involving changes in the concentration of cytosolic calcium (*Figure 2*). The only direct measurements of free calcium (Ca^{2+}) in plant cells are in the alga, *Chara* spp. (Williamson and Ashley, 1982), thus this hypothesis remains to be tested experimentally.

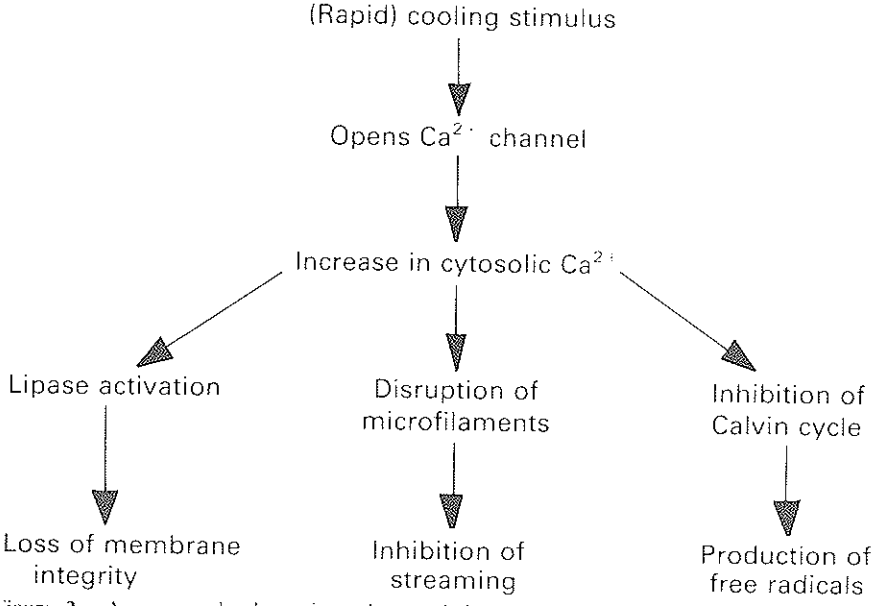

Figure 2. A suggested schematic pathway of the changes elicited by rapid cooling (after Minorsky 1985, 1989).

Minorsky (1989) points out that the rate at which the temperature is lowered is important in determining the plant response. A number of authors have shown that when plants are gradually cooled to low, non-stressful, temperatures, the vacuolar-to-outside membrane potential differences of cells undergo slight monotonic depolarizations. However, rapid cooling causes strong transient depolarizations (Minorsky, 1989). Rapid cooling has also been shown to bring about an 'action-potential' response in plant cells (Opritov, Pyatygin and Retivin, 1982; Minorsky, 1989). This type of study has shown a loss of sensitivity of plant cells to temperature changes as the temperature approaches the lower limit for growth and activity of that species (Jones and Wilson, 1982). In addition to the purely physical effects, rapid cooling can evoke physiological effects (e.g. inhibition of growth, protoplasmic streaming, phloem translocation) which may be due to this type of stimulation. Davies (1987) has discussed the mechanism by which rapid ionic changes in plant cells could affect gene expression.

As the major components of the plant cell, proteins provide the basic elements of cellular organization and metabolism. The stability of proteins is limited at low temperature and comparative studies have been carried out on proteins from psychrophilic, mesophilic and thermophilic organisms (Jaenicke, 1990). So far it has not been possible to attribute specific increments in the free energy of stabilization to well-defined amino-acid changes and, in fact, the amino-acid sequences of specific proteins from these organisms are found to be highly homologous. There is no evidence in the literature for temperature-related changes in protein structure, stability or activity being involved in temperature sensing mechanisms.

It is not known to what extent previously or continuously expressed gene

products, which are altered by the change in temperature, may contribute to the process of acclimation. The role of lipid alterations in the cold acclimation process is controversial. Steponkus, Lynch and Vemura (1990) have studied the lipid composition of the plasma membrane of rye (*Secale cereale*) leaves. This is unique in comparison with other plant cell membranes, and changes during cold acclimation. For example, free sterols increase during acclimation, from 33 to 44 mol% of total sterols, whereas acylated sterylglucosides decrease from 4 to 1 mol%. Cold acclimation alters the proportion of nearly every lipid component, but there is no molecular species which is unique to the plasma membrane isolated from either treatment. Steponkus, Lynch and Vemura (1990) have shown that the lipid composition of plasma membranes can be modified by fusing leaf protoplasts and liposomes. The behaviour of the resulting protoplast plasma membrane is also changed such that non-acclimated protoplasts subsequently demonstrate characteristics of protoplasts isolated from acclimated tissue. Steponkus attributes the increased cryostability of these membranes to the alteration of lipid–lipid interactions due to the different proportions of lipids present.

LOW-TEMPERATURE EFFECTS ON METABOLISM

In view of the profound effect that significant changes in temperature might be expected to have on the kinetics of most biochemical reactions, it is not surprising that a very wide range of metabolic processes have been studied in relation to temperature. It is, however, clear that there is no consensus as to either the primary system, or systems, which sense temperature change or the primary system, or systems, which are changed during acclimation to allow normal growth at a lower temperature or frost-resistance during freezing. One feature of this field of work is that many groups work on very different experimental systems, and hence plant tissues, and comparisons between these are therefore often very difficult to interpret. Thus, conclusions about low-temperature injury to the storage organ of a chill-sensitive species cannot be translated to acclimation changes in meristematic tissue of a winter-hardy plant. A range of fields of study will be presented to provide a review of the most active areas. Many of these studies have the common feature that comparisons are made between the metabolism of cultivars or related species which differ in their response to low temperature. Metabolic processes which differ between these are potentially associated with the difference in low-temperature response. However, in many cases comparisons are made between materials which may differ in characters other than the low-temperature response, this is particularly true when inter-species comparisons are made. In addition, it is very difficult in these metabolic studies to distinguish between primary and secondary components in the low-temperature response of the plant cell.

Lipids

Studies on the physical properties of membrane lipids have been discussed previously. A number of comparative studies of lipid metabolism have been

carried out. Huner *et al.* (1989) compared the levels of *trans*-$\triangle^3$-hexadecanoic acid in phosphatidyl(D)glycerol of leaves in acclimating plants from eight cultivars of wheat (*Triticum aestivum*) and rye (*Secale cereale*) which differ in their freezing tolerance. The levels of this thylakoid lipid fall during low-temperature (5°C) growth and the decrease is correlated with the freezing tolerance of the cultivar. This strong relationship, seen in cereal cultivars, was not observed in the dicotyledonous species tested. The *trans*-$\triangle^3$-hexadecanoic acid changes were associated with a change in the *in vitro* organization of the light-harvesting complex in photosystem II. It is suggested by the authors that this change reflects a mechanism for regulating energy distribution within the photosynthetic apparatus to counteract the potentially deleterious effect of low-temperature-induced photo-inhibition. This mechanism cannot be considered a general one, however, since no low-temperature-induced changes of *trans*-$\triangle^3$-hexadecanoic acid occur in cold-hardened spinach (*Spinacea oleracea*), pea (*Pisum sativum sativum*), broadbean (*Vicia faba*) and periwinkle (*Vinca minor*). In addition, an *Arabidopsis thaliana* mutant which produces no *trans*-$\triangle^3$-hexadecanoic acid will acclimate to an LT_{50} of −14°C.

Galactolipase (EC 3.1.1.26) has also been suggested to be important in chill sensitivity in plants (Gemel and Kaniuga, 1987), although not all plants show a good correlation between galactolipase activity and free fatty acid accumulation during chilling stress.

Carbohydrates

Fructans are storage carbohydrates typically found in a range of temperate, perennial, monocotyledonous species (Pollock, 1986) and are characteristic of species which are active at low temperatures. They have been suggested as possible cryoprotectants; however, they are largely located in the vacuole and as such would not influence the cytosol or organelles. In addition, inter-species comparisons have shown that there is no correlation with the fructan chain length and frost-tolerance of temperate grasses (Pollock, 1986), which also argues against such a role, since the low molecular weight and osmotically active fructans would be expected to be more effective. However, Suzuki (1989) reports some change in the molecular size distribution to a lower degree of polymerization during acclimation in hardy grasses. Excess starch accumulation in chloroplasts at low temperature can lead to loss of photosynthetic activity and it has been suggested that fructan-storing species can retain a low-starch chloroplast content by converting photosynthetically produced sugars to soluble fructans, which have a vacuolar location (Nelson and Spollen, 1987). This would provide an explanation for the ability of fructan-accumulating species to continue active photosynthesis and growth at low temperatures.

The accumulation of sugars in hardy plants has also been studied extensively in relation to the mechanism of freezing resistance and the role of sugars as cryoprotectants (Sakai and Larcher, 1987). Acclimation of cotton (*Gossypium herbaceum*) plants (chill-sensitive) at 15°C day and 10°C night prevents injury at 5°C and was shown to increase the leaf sugar and starch

content (Guinn, 1971). Calderon and Pontis (1985) studied sucrose biosynthesis in wheat (*Triticum aestivum*) plants subjected to a chilling shock of 4°C. This temperature shift increased the level of sucrose and fructans in leaf tissue. The only enzyme, related to sucrose metabolism, affected by the temperature treatment was sucrose synthase (EC 2.4.1.13), whose activity rose within 1 h of the shift. It was suggested that this increased activity of sucrose synthase in the cold-treated leaves is associated with the transport of sucrose to the vacuole where fructans are synthesized and stored.

The enzyme phosphofructokinase (EC 2.7.1.11) is important in the control of hexose phosphate oxidation in potato (*Solanum tuberosum*) tubers. The enzyme is cold-labile and has been shown to be involved in the phenomenon of low-temperature sweetening of potato tubers (Dixon, Franks and Ap Rees, 1981). It is argued that the reduction in phosphofructokinase activity at low temperatures could reduce carbohydrate oxidation to a greater extent than other reactions that metabolize hexose phosphates, and this would lead to a diversion of these compounds to sucrose. Recently, Hammond, Burrell and Kruger (1990) have compared the temperature coefficients of the four forms of tuber phosphofructokinase from potato cultivars which differ in their ability to accumulate sugars at low temperature. These results were consistent with the proposal that inactivation of this enzyme leads to increased sucrose synthesis in tubers kept at low temperatures. One other enzyme (pyruvate orthodikinase, EC 2.7.9.1), which functions in the carboxylation phase of the C4 pathway, has been shown to be cold-labile (Sugiyama *et al.*, 1979). A survey of C4 plant species showed that the most cold-labile enzyme was found in those species which were the most cold sensitive.

Proline

Lalk and Dörffling (1985), in a comparative study of two winter wheat cultivars which differed in their freezing resistance, showed that levels of the amino acid, proline, increased during acclimation and that the more frost-resistant variety, Holme, had significantly more proline that the variety, Amandus. In a related study, Machackova, Hanisova and Krekule (1989) showed that proline accumulation occurred during acclimation of all the winter wheat cultivars tested but that in the frost-resistant cultivar, Mironovska, it accumulated to twice the level found in the non-resistant cultivar, Slavia.

Potato plants have been regenerated from callus selected for resistance to the presence of the amino-acid analogue, hydroxyproline, in the culture medium (van Swaaij *et al.*, 1987). Tuber-propagated regenerants generally showed increased frost tolerance, both at the plant and the cell level, and this was associated with increased levels of both proline and total amino acids. However, although proline levels were also higher in the tubers of hydroxyproline-resistant regenerants, these organs showed no differences in frost sensitivity. The role of this accumulation of proline during acclimation leading to frost resistance has been questioned. It has been proposed as a cryoprotectant and as a osmoregulator, since water stress is associated with

cellular freezing injury. It has also been suggested that this accumulation is merely a general symptom of stress (Hanson and Hitz, 1982).

Abscisic acid

There are a large number of reports which implicate the plant hormone, abscisic acid (ABA), in higher plant stress responses. Both Lalk and Dörffling (1985) and Machackova, Hanisova and Krekule (1989) studying frost resistance in winter wheat cultivars, showed that ABA accumulates during acclimation and that there was a relationship between ABA levels and levels of frost resistance. Chen, Li and Brenner (1983) have also shown that ABA levels rise during acclimation of *Solanum commersonii*, whereas no increase is seen in cold treatment of the frost-sensitive cultivated potato *Solanum tuberosum*. Exogenously applied ABA has also been reported to induce frost tolerance at non-acclimating temperatures in a number of systems, including *S. commersonii* stem cultures (Chen, Li and Brenner, 1983), suspension cultures of bromegrass (*Bromus inermis*) (Robertson *et al.*, 1987) and *Brassica napus* (Orr, Keller and Singh, 1986) and plantlets of *Arabidopsis thaliana* (Lang, Heino and Palva, 1989). The role of ABA will be discussed later, but the evidence suggests that it is involved in the generalized stress response of plant cells and not confined to low-temperature responses. Ward and Lawler (1990) suggest that ABA functions as a regulatory link between the photosynthetic apparatus and environmental perturbations, including temperature.

IDENTIFICATION OF GENES IMPLICATED IN THE LOW-TEMPERATURE RESPONSE

Temperate plants, possessing the appropriate genes, are able to acclimate to low-temperature growth and survive frosts (acquisition of frost hardiness) following a period of low positive temperature treatment. In addition, low-temperature treatment is required to trigger flower development in some species or cultivars (vernalization). Thus, the response of a plant to low-temperature treatment is complex and is known to be determined by its genotype (Doll, Haahr and Sogaard, 1989). The exact gene products and their physiological roles in cold acclimation are not known. Genetic dissection of the genes involved in two characters associated with low temperature growth, vernalization requirement and frost resistance, has been attempted for a number of cereal species. These have been studied by conventional breeding and genetic analysis, and using doubled haploid and monosomic substitution lines of barley (Doll, Haahr and Sogaard, 1989), wheat (Brule-Babel and Fowler, 1988; Fletcher, 1988; Fletcher and Cullis, 1988; Sutka, 1989) and rye (Brule-Babel and Fowler, 1989).

Two of the above studies analysed the progeny of crosses between spring (no vernalization requirement) and winter (vernalization requirement) cultivars for inheritance of spring or winter growth habit and of frost hardiness. Doll, Haahr and Sogaard (1989) studied the doubled haploid progeny lines

from four crosses between one winter and four spring barley cultivars. Their results indicated that two pairs of alleles govern growth habit, with the spring habit being dominant over the winter habit. The length of time required for vernalization varied from 2 to 9 weeks, indicating additional genetic variation or modifying genes. Brule-Babel and Fowler (1988, 1989) studied the progeny of crosses between one spring and two winter rye cultivars and crosses between one spring and four winter wheat cultivars of varying frost hardiness, in all possible combinations. Three generations of progeny were analysed. The authors concluded that a single dominant nuclear gene was responsible for spring versus winter growth habit in rye with some modifying or recessive alleles possibly implicated. A similar experiment in wheat, using a spring cultivar possessing a single dominant allele and four winter cultivars showed the expected segregation of subsequent generations into spring and winter types. Fletcher (1988), using wheat chromosomal substitution lines Chinese Spring (Cheyenne), located a major vernalization response on chromosome 5D which segregated as a single chromosomal gene in subsequent crosses. However, he found evidence of further modifying genes. All of these studies identified one or two dominant alleles responsible for spring growth habit (no vernalization requirement) in cereals with some modifying genes. Earlier work by Pugsley (1971, 1972, 1973) in which the segregating progeny of crosses between a number of spring and winter wheat cultivars were analysed, identified four *Vrn* genes governing growth habit. The inheritance of dominant alleles of any of these eliminated vernalization requirement.

Cold hardiness is a more complex character, apparently controlled by multiple alleles and showing continuous variation. However, analysis of doubled haploid progeny lines in barley (Doll, Haahr and Sogaard, 1989), crosses between non-hardy and hardy cultivars of rye and wheat (Brule-Babel and Fowler, 1988, 1989), and analysis of substitution lines and their progeny (Fletcher and Cullis, 1988; Sutka, 1989; Sutka and Snape, 1989) showed that a major component of cold hardiness is either tightly linked to, or a pleiotropic effect of, the recessive alleles for vernalization requirement (winter growth habit) since all plants requiring vernalization also showed good cold hardiness. However, these studies also showed that good winter hardiness could be inherited with spring growth habit (Doll, Haahr and Sogaard, 1989; Brule-Babel and Fowler, 1988). Studies of wheat substitution lines have shown that 10 of the 21 pairs of chromosomes are involved in winter hardiness, with two of these most frequently implicated (Sutka, 1989). Such studies are important because they demonstrate that it is possible to identify and locate genes which have a major effect on these characters. The combination of molecular analysis and the genetical material described above will allow the identification of gene products involved in these responses and will be discussed further.

The genetic basis of frost tolerance and vernalization requirement has, to some extent, been addressed for cereals, as outlined above. However, there is a dearth of information available for other species, although both the ability to acclimate and the requirement for vernalization are clearly inherited characters. Genetic variation between white clover (*Trifolium repens*) culti-

vars, with respect to flowering, was reported by Beatty and Gardner (1961). The induction of flowering was triggered by daylength in some cultivars and by low-temperature treatment in others. The optimal daylength for induction also varied between cultivars. Norris (1990) performed crosses between vernalization-requiring and non-vernalization-requiring plants from a white clover variety, Menna, and found only 40% required vernalization (50% of Menna seed requires vernalization). This is consistent with the presence of dominant and recessive alleles as described for cereals, but the possible number of vernalization genes was not discussed.

CHANGES IN GENE EXPRESSION IN RESPONSE TO LOW TEMPERATURE

Proteins

There is evidence from a number of studies that alterations in the profile of proteins is associated with low-temperature treatments of both acclimating and non-acclimating plant species. In all of the reported investigations a number of new or enhanced proteins appear while a few are reported to be absent or reduced. The approaches to identifying and studying these changes include direct comparison of stained protein extracts, autoradiography of *in vivo* radiolabelled proteins and autoradiography of *in vitro* translation products of extracted messenger RNA. In each case the proteins are separated according to their electrophoretic mobility by either one- or two-dimensional polyacrylamide gel electrophoresis (PAGE). Comparison of extracted proteins separated by one- or two-dimensional electrophoresis is a fairly crude technique since the large amounts of long-lived proteins present in the extract may obscure small differences. The identification of certain proteins also depends on their stability during extraction and sample preparation. In our experience, low-temperature-induced proteins from barley were extremely labile and could only be identified if extracts were prepared rapidly in the cold. In contrast, such differences were a prominent feature of the *in vitro* translation profile of the same barley material (*Figure 3*). Despite this limitation, a number of studies of the proteins present following low-temperature treatments have identified differences between plants subjected to such treatment and warm-grown controls. The main advantage of this approach is that it is relatively simple to carry out and is often used in preliminary studies or in conjunction with other methods of identifying *de novo* protein synthesis. Novel proteins were identified in wheat seedling extracts by Sarhan and Perras (1987) who extended their study to a comparison of *in vivo* labelled proteins from leaves, crown and roots (Perras and Sarhan, 1989). A similar preliminary study of alfalfa proteins was also followed up by *in vivo* labelling (Mohapatra, Poole and Dhindsa, 1987, 1988; Mohapatra *et al.*, 1989). Changes in the soluble proteins of shoots following cold acclimation of winter wheat and rye were observed by Cloutier (1983) who also analysed plants subjected to desiccation and identified one protein with the same electrophoretic mobility common to both stresses. Guy and Haskell (1989) separated and stained subcellular fractions of spinach seed-

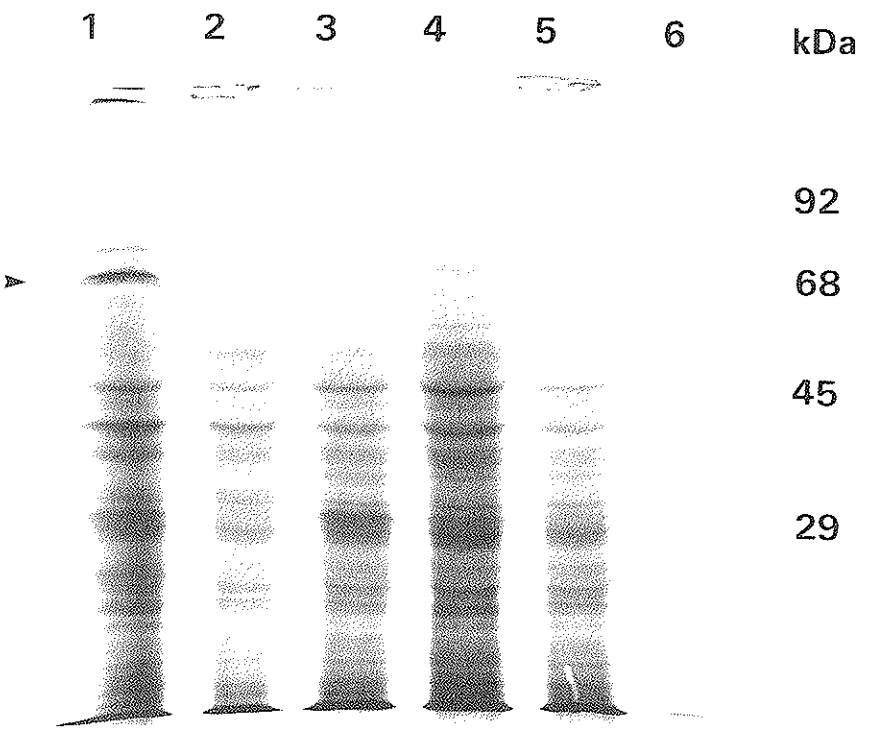

Figure 3. Analysis of altered gene expression following temperature treatment of barley plants. Autoradiograph of SDS-polyacrylamide gel of *in vitro* translation products from total RNA isolated from leaf sheath bases taken from leaves which were still expanding. Temperature treatment: lane 1, 5°C 10 h d^{-1}, 235 μmol m^{-2} s^{-1} and 1°C night for 50 d (germinated in treatment 2 for 10 d); lane 2, 16°C 16 h d^{-1}, 210 μmol m^{-2} s^{-1} and 16°C night for 19 d (HT); lane 3, 20°C 10 h d^{-1}, 170 μmol m^{-2} s^{-1} and 15°C night for 14 d (HT); lane 4, plants grown under the same conditions as lane 3 for 14 d, then transferred to a regime of 6°C 10 h d^{-1}, 150 μmol m^{-2} s^{-1} and 2°C night for 2 d (LT); lane 5, plants grown under the same conditions as lane 3 for 16 d (HT); lane 6, control translation, no added RNA. Growth of plants in treatments 3–5 are recorded in *Figure 1*. The major polypeptide present differentially in the cold-treated plants (lanes 1 and 4) is marked with an arrow. Markers are shown on the right. (From Hughes and Pearce, 1988).

lings and identified a low molecular mass protein associated with chloroplasts and three nuclear proteins, all of which increased in response to low-temperature treatment.

A central feature of the work described above is an altered protein profile associated with low-temperature treatments and the acquisition of freezing tolerance. These changes could be due to modification of existing proteins. However, it has been demonstrated by Robertson *et al.* (1987) and Chen and Li (1982) that cold acclimation does not occur in the presence of cycloheximide, an inhibitor of protein synthesis, indicating that the synthesis of novel proteins is a necessary prerequisite for acclimation. The role of proteins as cryoprotectants was demonstrated by Hincha, Heber and Schmitt (1990) who showed that protein fractions from frost-hardy leaves of cabbage (*Brassica oleracea*) or spinach (*Spinacea oleracea*) will protect isolated thylakoids from

mechanical rupture *in vitro*, whereas extracts from non-hardy leaves will not. Individual proteins were not identified. It is to be expected that novel proteins represent at least *de novo* translation of pre-existing mRNAs if not *de novo* transcription. Where it is possible to identify protein differences from crude extracts, or from *in vivo* labelled tissue, and make comparisons with *in vitro* translation products, inferences about the possible processing and turnover of proteins may be made. Several of the studies referred to here compare crude extracts, *in vivo* labelled proteins and *in vitro* translation products, or some combination of these, and will be discussed further.

In vivo *labelling*

In vivo labelling has been successfully used to identify novel proteins produced in response to cold in a number of different species, including *Arabidopsis thaliana* (Kurkela *et al.*, 1988; Lang, Heino and Palva, 1989), wheat (Perras and Sarhan, 1989), barley (Marmiroli *et al.*, 1989), alfalfa (Mohapatra, Poole and Dhindsa, 1987), *Brassica napus* (Meza-Basso *et al.*, 1986; Johnson-Flanagan and Singh, 1987), spinach (Guy, Niemi and Brambl, 1985) and bromegrass (Robertson *et al.*, 1987). Where whole plant *in vivo* labelling was performed, it was possible to analyse proteins from different parts of the plant, i.e. roots, leaves and crown, to identify different sets of tissue-specific, cold-induced proteins (Marmiroli *et al.*, 1989; Perras and Sarhan, 1989). In addition to low-temperature treatments, some studies included either heat-shock treatment of similar plant material (Marmiroli *et al.*, 1989) or treatment with ABA (Robertson *et al.*, 1987; Lang, Heino and Palva, 1989). The ABA treatment of *A. thaliana* by Lang, Heino and Palva (1989) resulted in the acquisition of frost tolerance within 1°C of the maximum induced by low-temperature treatment. A subset of eight novel polypeptides common to both treatments was identified. A further seven polypeptides were ABA specific and three were low-temperature specific.

A summary of proteins which appeared or increased following low temperature and other treatments is shown in *Table 1*. Direct comparisons between species are difficult to make because the low-temperature conditions used varied both in temperature and length of application and material of various ages was used. It is quite possible that similar polypeptides could be induced at different temperatures depending on the cold tolerance of the species or cultivar. A study of non-hardy rice (*Oryza sativa*) demonstrated that a set of proteins, induced or repressed in a cold-sensitive variety by a 15°C/10°C treatment, requires a treatment of 11°C/6°C in a cold tolerant variety (Hahn and Walbot, 1989). In Table 1 proteins are grouped according to size (large >60 000 Da, intermediate 20 000–60 000 Da and small <20 000 Da). In this way, we can compare the range of proteins for each treatment, bearing in mind the possibility that different experimenters will assign different sizes to identical polypeptides due to the errors inherent in estimating molecular mass by relative mobilities in gels. The number of different polypeptides induced by low temperature in plants may therefore be overestimated.

Guy and Haskell (1989) used *in vivo* labelling in an attempt to identify the location of three high molecular mass cold acclimation proteins (CAPs)

Table 1. Proteins induced (or repressed) by low temperature and/or ABA

Authors	Source	Treatment		Protein molecular mass (kDa)					
		T°C	Time	<40	40–59	60–79	80–100	>100	Notes
Robertson *et al.* (1987)	Bromegrass (*Bromus inermis*) (cell cultures)	3°C	4 d		47 48				
		ABA		9 16 24 26			87		enhanced
				21	47 48				new
Gilmour, Hajela and Thomashow (1988)	*Arabidopsis thaliana* (leaf, tissue culture)	4°C repressed	1 d	23	4×47			4×160	
Lang, Heino and Palva (1989)	*A. thaliana* (whole plant)	4°C/2°C	5 d	20 30 32 33	45 57 58	69	2×80		
		ABA		20 30 32	45 58				+7 not common to LT
Kurkela *et al.* (1988)	*A. thaliana* (whole plant)	4°C	7 d	24 30	45	60 69	85	150	
Mohapatra, Poole and Dhindsa (1988)	Alfalfa (*Medicago falcata*) (seedlings)	4°C soluble	17 d	20		70		170	9 new proteins
		membrane		20	40	75			10 new proteins
		repressed		25		66			6 in range 25–66
Marmiroli *et al.* (1986, 1989)	Barley (*Hordeum vulgare*) (seedlings, shoots and roots	6°C	4 h						
		roots			52*	69	87 100		
		both				61 67 77		107	
		repressed		32*	56				
Sarhan and Perras (1987) Perras and Sarhan (1989)	Wheat (*Triticum aestivum*) (seedlings)	6°C/2°C	30 d	31 38	43 52	68 74 77		200 180	
		repressed		34	42			157	
Guy and Haskell (1989)	Spinach (*Spinacea oleracea*) (whole plant)	5°C	7 d			70 79	85	117 160	
Hahn and Walbot (1989)	Rice (*Oryza sativa*) (seedling leaves)	11°C/6°C repressed	14 d	21 25 26		75	95		

* Winter only. † Spring only. LT, low temperature.

within cells of spinach and determine their amino-acid composition. They were able to eliminate accumulation in mitochondria or chloroplasts but further localization was not possible. Following radiolabelling with [^{32}P]orthophosphate, one high molecular weight protein co-migrated with the largest of the CAPs, indicating phosphorylation of this protein. This protein was also detectable at a low level by ^{32}P labelling in non-acclimated tissue, although previous [^{32}S]methionine *in vivo* labelling and protein studies failed to detect it. Heat shock dramatically reduced ^{32}P incorporation and, although it is not clear whether this was due to dephosphorylation or reduced protein synthesis, the authors suggest that its possible role in a cascade system of regulation, control of enzyme activity or stimulus/response coupling is indicated.

A general feature of low-temperature-induced proteins is that they continue to be produced throughout cold treatment but decline if a shift to non-acclimating temperatures is made (Mohapatra, Poole and Dhindsa, 1987); with a few exceptions, the profile of other proteins in the plant remains the same. This is in contrast to heat-shock protein production, which declines with time, and where the synthesis of other cellular proteins is repressed (Kurkela *et al.*, 1988; Guy, Niemi and Brambl, 1985).

In vitro *studies*

In vitro translation of mRNA extracted from plant tissue represents the most effective method of evaluating *de novo* transcription and thus the potential for novel protein synthesis. It must be noted, however, that new mRNAs will not necessarily be translated, neither does the quantity of message present necessarily reflect the abundance of its putative protein product, since the half-lives of individual mRNAs and polypeptides are variable. A number of studies have identified *in vitro* translation products which represent new or increased abundance of mRNAs following low-temperature treatments. These are summarized in *Table 2*. There is little doubt that *de novo* protein synthesis is necessary for cold acclimation, since its prevention results in failure to acclimate (Chen and Li, 1982; Robertson *et al.*, 1987) and there is every reason to suppose that novel gene products resulting from low-temperature treatment have physiological roles in acclimation to low-temperature growth, frost tolerance and vernalization. It might be argued that transcription products which become more abundant following low-temperature treatment simply reflect changes in the stability of their mRNAs or the reduced levels of activity of RNases. We have shown, however, that this is not a general feature of low-temperature treated material. The abundance of most mRNA species is the same in low-temperature-treated and warm-grown barley seedlings of equivalent development (Dunn *et al.*, 1990). It has also been noted by others that the general profile of translatable mRNA is little changed by low-temperature treatments (Mohapatra, Poole and Dhindsa, 1987; Kurkela *et al.*, 1988). It therefore seems likely that specific mRNAs, which increase in abundance in response to low temperature, encode gene products involved in acclimation or vernalization. Genes

that control vernalization requirement and frost resistance have been identified in some species, as discussed earlier (Brule-Babel and Fowler, 1988; Fletcher, 1988; Fletcher and Cullis, 1988; Sutka, 1989; Sutka and Snape, 1989) and it is therefore possible that the transcripts of these genes might be identified from acclimating plant material.

Table 2. Protein products of *in vitro* translation of mRNA induced (or repressed) by low temperature

Authors	Source	Treatment		Protein molecular mass (kDa)				
		T°C	Time	<40	40–59	60–79	80–100	>100
Cattivelli and Bartels (1989)	Barley (*Hordeum vulgare*) (coleoptiles)	5°C	6 h			75		
		1°C	24 h		45	75		
		1°C	72 h	2×20	45	75		
Gilmour, Hajela and Thomashow (1988)	*Arabidopsis thaliana* (19-day-old leaf tissue culture)	4°C repressed	1–3 d	23 29	4×47			160
Hughes and Pearce (1988)	Barley (*Hordeum vulgare*) (shoot meristems)	6°C/2°C	48 h			77		
Kurkela *et al.* (1988)	*Arabidopsis thaliana* (3-week whole plants)	4°C	24 h	24	45			150
Schaffer and Fischer (1988)	Tomato (*Lycopersicum esculentum*) (fruit)	4°C	21 d	17	41			
Johnson-Flanagan and Singh (1987)	*Brassica napus* (tissue culture)	4°C	2–8 d	17 20				
Mohapatra, Poole and Dhindsa (1987)	Alfalfa (*Medicago falcata*) (7-day-old seedlings)	4°C	17 d	16 27 33		60	90	
Meza-Basso *et al.* (1986)	*Brassica napus* (2-day-old seedlings)	0°C repressed	48 h	2×25 2×20 2×15 2×23	4×40 45 4×42	75		
Guy, Niemi and Brambl (1985)	Spinach (*Spinacea oleracea*)(leaf)	5°C repressed	2–16d	19 31 13 23	43	72 68	82	180

Most of the *in vitro* translation studies that have been reported compared *in vitro* translation products from low-temperature-treated material with controls grown at a non-acclimating temperature, usually above 10°C. Species such as barley, which have cultivars varying in their genetic competence to acquire frost tolerance and in their growth habit (winter v. spring), provide an additional source of material for these studies. *Table 2* divides the *in vitro* translation products identified by various authors, into groups according to their molecular mass in order to evaluate the range of polypeptides observed in different species. The plant material and the cold treatment given varied between studies, again making direct comparisons difficult. We will discuss these data by dividing the experiments into broadly similar groups, according

to developmental stage or tissue used in the study, and whether the species was dicotyledonous or monocotyledonous.

Mohapatra, Poole and Dhindsa (1987) and Meza-Basso *et al.* (1986) observed novel *in vitro* translation products from whole-seedling mRNA of alfalfa (*Medicago falcata*) and rapeseed (*Brassica napus*), respectively. However, the former were treated at 0°C and the latter at 4°C. Three new polypeptides (150 000, 45 000 and 24 000 Da) were detected in the *in vitro* translation products of the shoots of 21-day-old *A. thaliana* plants acclimated for 24 h at 4°C (Kurkela *et al.*, 1988). The authors also reported novel proteins of similar molecular mass to the *in vitro* translation products which appeared in the profile of *in vivo* labelled proteins during cold treatment, persisted up to 7 days and correlated with increased frost resistance. Additional proteins shown in the *in vivo* study are not explained, but it is possible that they are breakdown products of those shown by *in vitro* translation or accumulated products of long-lived low-level mRNA species. Cattivelli and Bartels (1989) compared the *in vitro* translation products of coleoptile material mRNA induced by low temperature in seedlings of a winter and a spring cultivar of barley. They studied the induction kinetics of novel, low-temperature-specific gene products induced by treatments of 5°C and 1°C. One high molecular mass (75 000 Da) polypeptide mRNA was induced rapidly, reaching its highest level of expression after 6 h at 5°C. mRNA for other novel polypeptides of lower molecular mass (45 000 and 20 000 Da) were only present after 1 and 4 days, respectively, at 1°C, although a number of other polypeptides increased several-fold following low-temperature treatment. Interestingly, Cattivelli and Bartels (1989) report only slight differences between the *in vitro* translation products of the spring and winter cultivars, with essentially the same set of stress-related proteins present in both.

The use of seedling coleoptile material for such a study must have limitations since the coleoptile contains relatively fewer actively dividing cells than leaf tissue, and fulfils its role and senesces at a relatively early stage of development. Hughes and Pearce (1988) used 14-day-old barley seedlings (winter cultivar Igri) in an *in vitro* translation study of mRNA extracted from leaf and stem meristems (coleoptiles removed) treated at 6°C/2°C for 50 days. It is these parts of the cereal plant that are crucial for survival (Steponkus, 1978; Pearce, 1980; Gusta, Fowler and Tyler, 1982) and they represent the part of the plant which perceives low temperature (Peacock, 1975; Thomas and Stoddart, 1984). The most prominent novel polypeptide, of approximately 77 000 Da, was present after 2 days but persisted and was increased after 50 days. This was absent from control plants of equivalent development. It seems likely that this gene product and the prominent 75 000 Da polypeptide described by Cattivelli and Bartels are in fact the same gene product. This gene is apparently continuously expressed and its product rapidly turned over throughout low-temperature treatment since, although there is no prominent protein difference detectable by electrophoresis of protein extracts (own unpublished results), the mRNA is a major species in the translatable RNA extracted from these plants.

The altered *in vitro* translation profiles of mature leaves during cold acclimation at 4°C or 5°C was studied first in spinach (Guy, Niemi and Brambl, 1985) and also in *Brassica napus* (Johnson-Flanagan and Singh, 1987) and *A. thaliana* (Gilmour, Hajela and Thomashow, 1988). The two groups last mentioned also reported novel gene products from *in vitro* translation of mRNA of low-temperature-treated cultured cells. With the exception of one tissue-culture polypeptide, the novel or increased polypeptides were common to both leaves and tissue culture. Guy, Niemi and Brambl (1985) identified two prominent high molecular weight polypeptides (82 000 Da and 180 000 Da) after 2 days, persisting, but not increasing in concentration, up to 16 days of cold acclimation and correlating with increasing freezing tolerance. Four more new polypeptides appeared after 8 days acclimation (72 000, 43 000, 31 000 and 19 000 Da) and, at the same time, three others disappeared. The polypeptides of 160 000 and 47 000 Da identified by Gilmour, Hajela and Thomashow (1988) in both tissue culture and leaf material may be the same as those identified as 150 000 and 45 000 Da by Kurkela *et al.* (1988). The significance of these leaf and tissue-culture studies for plant growth and development is not clear. Novel gene expression could be a general stress response in mature leaf tissue since these leaves are not growing, although their survival may be crucial for the further growth and development of the plant. Guy, Niemi and Brambl (1985) and Johnson-Flanagan and Singh (1987) also included an investigation of HSPs in leaf and suspension-cultured cells, respectively; the latter authors applied heat shock to both hardened (i.e. acclimated by exposure to low temperature) and non-hardened cells. In one-dimensional gel electrophoresis, two HSPs from spinach had the same electrophoretic mobility as two polypeptides induced at low temperature, but six other different HSPs were produced, whereas synthesis of most other cellular protein stopped, as noted earlier. There was no attempt to determine whether freezing tolerance was induced by heat shock. In contrast, heat shock of the *B. napus* cell suspension induced a number of distinct HSPs (shown by *in vivo* labelling with [^{35}S]methionine) in both hardened and non-hardened cells, but it is not possible to determine from the data whether any of the proteins characteristic of hardened tissue are identical with any induced by low-temperature treatment. However, heat shock neither increased nor decreased the hardening of the respective cells used.

A number of studies have considered the effect of the plant hormone abscisic acid (ABA) on acclimation to freezing and have correlated ABA application at non-acclimating temperatures with increasing frost tolerance, which paralleled that induced by low temperature to within 1° or 2°C of maximal tolerance. This effect has been demonstrated in whole plants of *A. thaliana* (Lang, Heino and Palva, 1989), stem-cultured *Solanum commersonii* (Chen, Li and Brenner, 1983), suspension cultures of *B. napus* (Orr, Keller and Singh, 1986), winter wheat, winter rye and bromegrass (Chen and Gusta, 1983; Reaney and Gusta, 1987; Robertson *et al.*, 1987). The *A. thaliana* studies further demonstrated that *de novo* protein synthesis, stimulated by ABA treatment, resulted in a subset of eight polypeptide species in common

with low-temperature treated material (Lang, Heino and Palva, 1989). Two common polypeptides were found for both treatments of bromegrass cell cultures (Robertson *et al.*, 1987) as determined by electrophoretic mobilities. However, not all the low-temperature-induced polypeptides were also induced by ABA. The acquisition of frost tolerance induced by ABA was prevented by cycloheximide, indicating the importance of *de novo* protein synthesis.

The evidence that ABA accumulates during acclimation in several plant species (*see* p. 170) and that its level is related to the degree of frost tolerance achieved, together with the demonstration that the acquisition of frost tolerance is related to induced gene expression, as shown by exogenous application of ABA outlined above, indicates a central role for ABA in acclimation as well as in other stress responses. The exact nature of its role has yet to be elucidated.

It was noted by several authors that a number of proteins are repressed by low-temperature treatment. Meza-Basso *et al* (1986) positively identified one of these as the 23 000 Da small subunit of ribulose bisphosphate carboxylase (EC 4.1.1.39) (Rubisco), which was absent from the *in vitro* translation products of low-temperature-treated seedings of *B. napus*. A 23 000 Da *in vitro* translation product is also absent from cold-treated *A. thaliana* leaf and callus (Gilmour, Hajela and Thomashow, 1988; Kurkela *et al.*, 1988) and spinach leaves (Guy, Niemi and Brambl, 1985), although this was not positively identified as Rubisco. In addition, Hahn and Walbot (1989) reported the suppression of synthesis of mRNA encoding Rubisco small subunit in cold-treated rice leaves.

CLONED GENES

The identification of differences in the profile of polypeptides induced by low temperature has led to the cloning and characterization of some cold-specific genes. At present, there are few such reports and only one gene product has been identified. However, the characterization of such genes and their protein products, using genetical material to analyse their pattern of expression and available data banks to identify related gene products, is a potentially powerful tool in the dissection of the response of plants to low temperature. The only low-temperature-specific gene product so far identified is a thiol protease from ripening tomatoes (Schaffer and Fischer, 1988). This represents part of a cold-stress response, not acclimation to growth, and as such may be unrelated to acclimation and be confined to storage tissue. Mohapatra *et al.* (1989) have isolated three cold-acclimation-specific genes in alfalfa seedlings which are unrelated to genes induced by heat shock, wounding, water stress or abscisic acid. Their expression, measured by mRNA levels, correlated with acclimation for freezing tolerance of different *Medicago* species.

A number of low-temperature-induced genes have been isolated from a cDNA library prepared from leaf meristematic tissue of winter barley, and the sequence and further analysis of one of these (BLT14) encoding a low

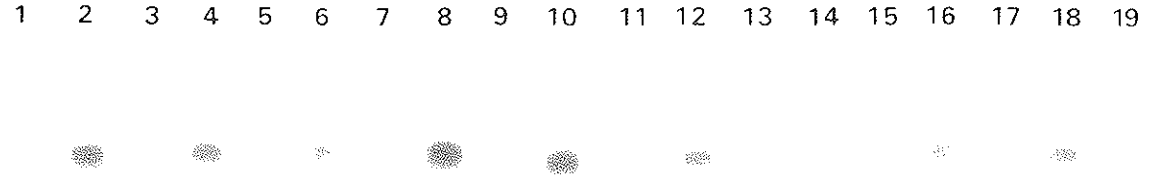

Figure 4. Analysis of altered expression of gene BLT14 following temperature treatment of barley plants. Northern blot analysis of total RNA extracted from meristematic shoot tissue of plants at the four-leaf stage of development. Membranes probed with complete cDNA of BLT14 (Dunn *et al.*, 1990), transcript size 519 bases. Temperature treatment: lanes 1, 3, 5, 7, 9, 11, 13, 15 and 17 plants grown at 20°C day (10 h d^{-1}) and 15°C night (21 days old); lanes 2, 4, 6, 8, 10, 12, 14, 16 and 18 plants grown at 20°C day and 15°C night for 14 days then transferred to 6°C day (10 h d^{-1}) and 2°C night (53 days old); lane 19, plants grown at 20°C day (10 h d^{-1}) and 15°C night but drought stressed. Plant material: lanes 1 and 2, cv. Vogelsanger Gold parent (winter habit, frost hardy); lanes 3 and 4, cv. Tystoffte Prentice parent (spring habit, frost sensitive); lanes 5, 6, 7 and 8, doubled haploid lines (winter habit, frost hardy); lanes 9, 10, 11 and 12, doubled haploid lines (spring habit, frost hardy); lanes 13, 14, 15 and 16 doubled haploid lines (spring habit, frost sensitive); lanes 17, 18 and 19, cv. Igri (winter habit, frost hardy). (Data produced by Zhang Lin.)

molecular weight polypeptide of 10 000 Da is reported by Dunn *et al.*, (1990). No homology with the sequence of any other cloned gene has been found in the EMBL and Gen Bank DNA databanks nor with protein sequences in the Protein Identification Resource of the US National Biomedical Research Foundation. Although the mRNA represents 0·2% of the total, this gene is present as a single copy on chromosome 2 of the barley genome. A number of less homologous related sequences are also present. Homologous sequences are present in wheat and rye but not oats (*Avena sativa*). In barley the gene shows restriction fragment length polymorphism (RFLP) in Southern blot analysis of genomic DNA, and cultivars fall into one of three distinct groups: A, B^1 or B^2. Winter cultivars examined are predominantly B^2 pattern and spring cultivars either A or B^1 pattern (*Table 3*).

Table 3. Allelic variation amongst spring and winter barley cultivars for the pBLT14 RFLP locus (from Dunn *et al.*, 1990)

Cultivar	RFLP alleles				
	*Bam*HI	*Eco*RI	*Hin*dIII	*Sst*I	*Dra*I
Spring					
Atem	A	A	A	A	A
Blenheim	A	A	A	A	A
Goldmarker	B	B	B^1	B^1	B
Golden Promise	A	A	A	A	A
Golf	B	B	B^1	B^1	B
Klaxon	B	B	B^1	B^1	B
Kym	B	B	B^1	B^1	B
Natasha	A	A	A	A	A
Triumph	A	A	A	A	A
Vista	...	B	B^1	B^1	...
Winter					
Frolic	B	B	B^1	B^1	A*
Gaulois	B	B	B^1	B^1	B
Halycon	B	B	B^1	B^1	A*
Igri	B	B	B^1	B^1	A*
Kaskade	B	B	B^1	B^1	A*
Koale	B	B	B^1	B^1	B
Magie	B	B	B^1	B^1	A*
Maris Otter	A	A	A	A	A
Panda	B	B	B^1	B^1	B
Fragment size					
A allele	5·5	6·0	11·1	14·0	1·6
B allele	1·5	2·0	14·0	17·0	2·5

* Allelic forms generated by *Dra*I but not the other four enzymes, for a particular variety.
B^1 and B^2 indicate allelic variants at minor loci only detectable at low stringency.

Analysis of RFLP in genomic DNA is currently being used in conjunction with an analysis of the pattern of gene expression in the parents and segregating doubled haploid progeny produced by Doll, Haahr and Sogaard (1989). *Figure 4* shows a Northern blot of total RNA extracted from the parent cultivars Vogelsanger Gold (winter, frost tolerant) and Tystoffte Prentice (spring, frost sensitive) and a selection of their doubled haploid progeny lines, which had either winter or spring growth habit and were either frost tolerant or frost sensitive. Also included in the figure is the winter cultivar Igri from which the cDNA library was prepared. Paired wells for each

plant contain shoot meristem mRNA extracts from controls (grown at 20°C day/16°C night) and low-temperature treatments (grown at 6°C day/2°C night) where plants from each treatment were grown to the same developmental stage (4 leaves). Clearly, it can be seen that this gene is expressed in all the low-temperature-treated plants and not in the high-temperature controls. Although there are differences between individual lines, there is none between the parent cultivars, and the differences between the doubled haploid lines do not correlate with either frost tolerance or vernalization requirement in this family. Lane 19 contains RNA from drought-stressed Igri of equivalent development and the hybridization signal shows that BLT14 is also weakly induced by drought. The analysis described here shows that the cold-induced barley gene, BLT14, is not involved in the control of the phenotypic differences between Vogelsanger Gold (winter habit, frost hardy) and Tystoffte Prentice (spring habit, frost sensitive). The effect of drought on the expression of this gene may indicate that it is a gene involved in a generalized stress response of the plant meristem. This is currently being investigated further. The combination of molecular techniques and well-characterized genetical material is a powerful tool which will allow rapid analysis of differentially expressed genes. We should expect to see rapid progress in the identification of genes, their products, control and function in the plants' response to growth and survival at low temperature.

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