Plant Molecular Biology **24**: 879–888, 1994.

Low-temperature-responsive barley genes have different control mechanisms

M.A. Dunn [1]*, N.J. Goddard [1], L. Zhang [3], R.S. Pearce [2] and M.A. Hughes [1]
[1] *Department of Biochemistry and Genetics, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK (* author for correspondence);* [2] *Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK;* [3] *Present address: Department of Cell Biology, IGER, Plas Gogerddan, Aberystwyth, SY23 3EB, UK*

Received 11 May 1993; accepted in revised form 16 December 1993

Key words: barley, cold acclimation, gene expression, low temperature genes, nuclear run-on transcription

Abstract

Several low-temperature-responsive (LTR) genes from barley have been shown to have high steady-state transcript levels. Run-on transcription was used to determine the control of expression of these LTR genes. Six of these are shown to be transcriptionally regulated (*blt* 4/9, *blt* 101, *blt* 1015, *blt* 63, *blt* 49, *blt* 410) whilst three are post-transcriptionally regulated (*blt* 14, *blt* 411, *blt* 801). Two transcriptionally regulated genes (*blt* 4/9 and *blt* 101) and one post-transcriptionally regulated gene (*blt* 14) have been used in expression studies. The time course for the appearance and decay of these transcripts is given. Initial appearance and steady-state levels of individual transcripts have different temperature characteristics but no single gene correlates with the cold acclimation response. We suggest that these different response profiles may represent a means of fine-tuning the low-temperature response. One gene, *blt* 4/9, also accumulated high steady-state levels of transcript in response to drought and a nutrient stress. However, only drought has an acclimating effect on barley plants.

Introduction

The response of plants to a low positive temperature treatment is a complex phenomenon which, in many temperate species, acclimates them to frost tolerance to varying degrees. This process has been shown to be accompanied by altered gene expression [14], indeed when *de novo* protein synthesis is inhibited by cyclohexamide plants that fail to acclimate [4, 29]. The ability to acclimate to frost tolerance is under genetic control with different species and cultivars showing different levels of frost hardiness. The most extensively studied species is wheat where frost hardiness has been demonstrated to be a multigenic additive trait with major contributions from a few identifiable genes [30, 32]. In barley, segregation of frost hardiness and spring growth habit has been studied in doubled haploid progeny of crosses between winter and spring cultivars [6]. Such studies demonstrate that, although the low-temperature response is complex, in cereals a relatively small number of genes are expected to have major effects.

Cellular and metabolic changes that occur during hardening have been studied and these include increased levels of sugars, soluble proteins, proline and organic acids as well as the

appearance of new isozymes and altered lipid membrane composition [14, 31]. Some of these changes may result from the accumulation of metabolites due to a reduction in growth rate but the fact that plants inhibited in protein synthesis are unable to acclimate indicates that new or increased levels of some gene products are essential for acclimation.

A small number of low-temperature-responsive (LTR) genes have been cloned from *Arabidopsis* [13, 17–19, 25], alfalfa [22], spinach [23], *Brassica napus* [33], wheat [19], barley [3, 7–9, 11] and rye [35]. These genes represent mRNAs which at steady-state mRNA levels, are induced or enhanced by low temperature. The number of LTR genes cloned is relatively small and as yet no *in vivo* function has been assigned to any of them. However, one LTR *Arabidopsis* gene product has been shown to have cryoprotective activity *in vitro* [20] and a barley LTR gene (*blt* 4) has significant homology to plant lipid transfer proteins [15] which have been demonstrated to transfer phospholipids between membranes *in vitro* [2]. A second barley LTR gene (*blt* 63) encodes a translation elongation factor 1α [9].

The complex nature of the low-temperature response in plants is illustrated by the fact that many of the low-temperature-responsive genes investigated are also inducible by the phytohormone abscisic acid and by water stress [13, 17]. However, since abscisic acid-insensitive mutants of *Arabidopsis* are capable of expressing LTR genes and acclimating in response to cold, independent control mechanisms have been proposed for low-temperature and abscisic acid [10, 24]. Further illustration of the complexity of the low-temperature response is provided by the fact that *Arabidopsis* LTR genes show both transcriptional and post-transcriptional control [13]. In this paper, we present evidence that different barley LTR genes are regulated in different ways, are detectable at different temperatures and are distinguished by their response to other stresses. These data demonstrate a complexity of the plant response to low temperature not revealed by physiological and biochemical studies.

Materials and methods

Plant material

Run-on transcription

Hordeum vulgare. L. cv. Igri were sown in John Innes No. 2 compost and grown for 16 days in 20 °C day/15 °C night, 10-h day, 170 μmol m^{-2} s^{-1} photon flux density. Half the plants were transferred to the cold: 6 °C day/2 °C night, 10-h day, 150 μmol m^{-2} s^{-1}, for a further 12 days. The remainder continued in the original environment for a further 3 days. Plants were harvested at the same developmental stage (3–4 leaves). Meristematic tissue was removed as described previously, [15] frozen in liquid N_2 and stored at -80 °C.

Time course

H. vulgare cv. Igri grown at 20 °C/15 °C as previously described [16] was transferred to LT 6 °C/2 °C, 10-h day, and samples were harvested for RNA extraction after 1, 3 and 7 days. Some plants which had been subjected to this low-temperature treatment for 7 days were transferred back to control 20 °C/15 °C conditions and samples harvested for RNA extraction after 6, 35 and 100 h.

Temperature transfer and frost regrowth tests

Plants were grown to the third leaf stage at 18 °C day/10 °C night (10-h day). One group of plants remained at 18 °C/10 °C whilst the others were transferred to one of the following regimes; constant 12 °C, constant 9 °C, constant 6 °C or 6 °C day/2 °C night for either 7 or 14 days. All plants had a 10-h day. Some plants acclimated at 6 °C/2 °C for 14 days were further transferred to 4 °C day/ − 4 °C night (10-h day) for 7 days.

Plants were harvested for RNA extraction or used to measure hardiness by regrowth after frost tests as described in Pearce [28]. Regrowth was measured after 7 and 14 days.

Drought and nutrient stress

Plants were subjected to drought stress as previously described [8]. Plants were subjected to

nutrient stress by using vermiculite as the growth medium and grown in the control temperature regime of 20 °C day/15 °C night without nutrient supply.

RNA extraction

Total RNA was extracted from 4–5 g (80–100 plants) leaf sheath base material as described [7].

Northern blot analysis

Approximately 5 μg denatured total cellular RNA per well was electrophoresed in 1% agarose gels in the presence of formaldehyde, transferred to Hybond-N membranes (Amersham International) and probed as described [7].

Filters were washed at high stringency, 2 × SSPE (1 × SSPE is 0.18 M sodium chloride, 0.01 M sodium phosphate, 0.001 M EDTA pH 7.7), 0.1% w/v SDS at room temperature for 10 min twice; 1 × SSPE, 0.1% (w/v) SDS at 65 °C for 15 min; 0.1 × SSPE, 0.1% w/v SDS at 65 for 10 min twice, and exposed to autoradiography using Fuji RX film.

Densitometric analysis

Autoradiograph peaks were analysed using a Chromoscan 3 densitometer (Joyce Loebl).

Nuclear run-on transcription

Nuclei were isolated from low temperature (6 °C/2 °C) and control temperature (20 °C/15 °C) barley shoot meristematic tissue using the following modifications of the method of Cox and Goldberg [5]: 10 g (200 plants) plant material was ground to a fine powder in liquid nitrogen using a mortar and pestle; the final nuclei pellet was resuspended in 250 μl of resuspension buffer (50 mM Tris-HCl pH 8.5, 5 mM $MgCl_2$, 50% v/v glycerol).

Dot blots carrying 5 μg linearised recombinant plasmid DNA per well were prepared. Plasmid DNA was denatured by boiling 15 min with 0.1 vol 1 M NaOH, neutralised with 0.1 vol 1 M HCl, and equal volume of 20 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) added. Samples were applied to pre-wetted nitrocellulose in a BioRad dot blot vacuum manifold, washed with 6 × SSC. The filter was baked at 80 °C for 2 h.

Approximately 2×10^7 nuclei were used for run-on transcription using the modified method of Cox and Goldberg [5]. 2×10^7 nuclei were incubated with 75 units RNase inhibitor (Pharmacia), 100 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 0.3 μM phosphocreatine, 0.5 mM each ATP, GTP, CTP, (BCL), 0.25 mg/ml creatine phosphokinase, 250 μCi [^{32}P]UTP (Amersham SP6/T7 grade, 800 Ci/mmol) 30 °C, 20 min, RNA was extracted from nuclei after digestion with 2×10^3 units DNase (RNase-free, Pharmacia) at 30 °C, 10 min, then made 10 μM Tris-HCl, pH 7.6, 5 mM EDTA, 1% SDS and incubated with Proteinase K (100 μg/ml, Sigma) at 42 °C, 30 min. 50 μg carrier tRNA (yeast, BRL) were added and the reaction mixture was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (IAA) (25:24:1) twice, chloroform/IAA (24:1) once and precipitated at −20 °C, 7 h, with 0.1 vol sodium acetate 2.5 vol ethanol. RNA was recovered by centrifugation and redissolved in 100 μl sterile water, made 5 × SSPE. Nitrocellulose filters were prehybridised in 5 ml prehybridisation solution (5 × SSPE; 5 × Denhardt's solution (0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone (PVP360)); 0.1% SDS, 250 μg/ml tRNA, 100 μg/ml salmon DNA) in a 10 ml universal for 16 h at 42 °C. [^{32}P]UTP-labelled RNA was denatured at 90 °C for 10 min and added to prehybridisation solution, made 10% w/v dextran sulphate and incubated at 42 °C for 42 h. Filters were washed in 2 × SSC, 0.1% SDS for 5 min at 20 °C twice and 0.2 × SSC for 30 min at 50 °C three times and exposed to autoradiography using pre-flashed Fuji RX100 film.

Results

Nuclear run-on transcription

We investigated the regulation of nine barley LTR genes, all of which have been shown to have enhanced steady-state levels of mRNA in 3–4 leaf plants of the winter barley cultivar Igri, in response to a two-week low-temperature treatment of 6 °C/2 °C (*blt* 4 [8]; *blt* 14 [7]; *blt* 63 [9]; *blt* 101 [11]; *blt* 49, *blt* 410, *blt* 411, *blt* 801, *blt* 1015; M.A. Dunn and M.A. Hughes, unpublished data). To determine whether the increased steady-state mRNA levels of LTR genes, observed when plants are transferred to a low-temperature regime, represents transcriptional or post-transcriptional regulation of these genes, nuclei were isolated from low temperature (LT) (6 °C/2 °C) and control (HT) (20 °C/15 °C) barley shoot meristematic material and used in an *in vitro* run-on transcription assay. The results shown in Figs. 1A and 1B indicate that four genes (*blt* 4/9, *blt* 101, *blt* 1015 and *blt* 410) have high levels of transcript present at low temperature but very low levels at the control, indicating that these genes are transcriptionally regulated in response to low temperature. Two members of the *blt* 4 gene family, *blt* 4/1 (previously referred to as *blt* 4 [8]) and *blt* 4/9, cross-hybridize and are indistinguishable in expression studies using complete cDNA sequences although the 3′ non-coding sequence of *blt* 4/9 (*blt* 4/9-3′) has no homology with *blt* 4/1 (unpublished data). Two genes (*blt* 63 and *blt* 49) have high levels of their respective transcripts at the control temperature, both of which increase markedly with the low-temperature treatment (Fig. 1b), indicating that these genes are also transcriptionally regulated at LT.

In contrast to the LT transcriptional response, two genes (*blt* 14 and *blt* 411) have very low levels of detectable transcript in both temperature treatments, indicating low transcription rates, in both LT and control temperature. One gene (*blt* 801) which also accumulates to high steady-state levels at LT appears to be transcriptionally down-regulated at low temperature (Fig. 1b), although transcription rates at both temperatures

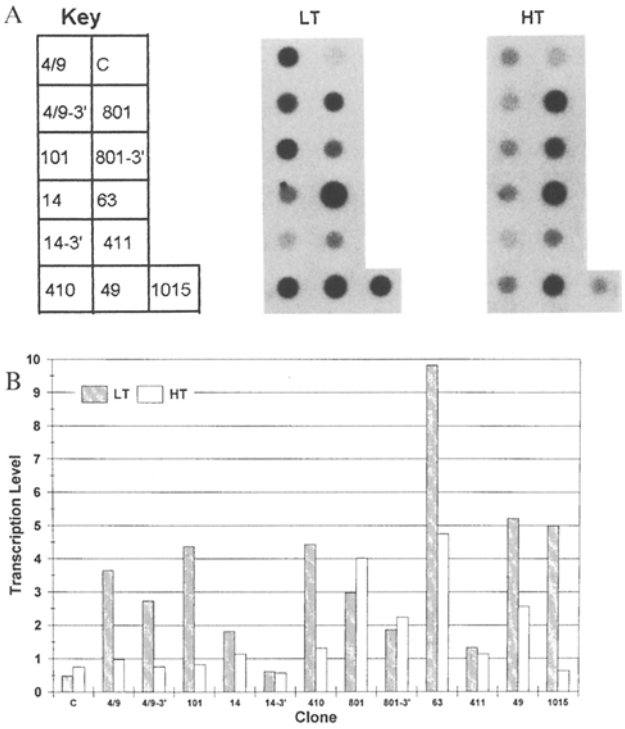

Fig. 1. Nuclear run-on transcription analysis. A. Duplicate nitrocellulose filters contain 5 μg linearised recombinant DNA per well. Filters hybridised with [^{32}P]UTP-labelled nuclear run-on transcripts. B. Densitometric analysis of run-on transcription assay. Transcription level: integral of autoradiographic peak in arbitrary units. C, non-recombinant plasmid; 4/9 (*blt* 4/9), 4/9-3′ (*blt* 4/9 non-coding 3′ end), 101 (*blt* 101), 14 (*blt* 14), 14-3′ (*blt* 14 non-coding 3′ end), 410 (*blt* 410), 801 (*blt* 801), 801-3′ (*blt* 801 non-coding 3′ end), 63 (*blt* 63), 411 (*blt* 411), 49 (*blt* 49), 1015 (*blt* 1015) cDNA recombinant plasmids. HT, plants grown at 20 °C/15 °C; LT, plants grown at 6 °C/2 °C.

are higher than those of *blt* 14 and *blt* 411. Since all three transcripts (*blt* 14, *blt* 411, *blt* 801) accumulate at LT but non HT, these results suggest that low temperature stabilises the transcripts of these three genes and that their regulation is therefore post-transcriptional. *Blt* 14-3′ represents the non-coding region of this gene which distinguishes between members of the *blt* 14 multigene family.

Time course for temperature response

Three genes (*blt* 4/9, *blt* 14 and *blt* 101) whose transcripts reach high steady-state levels in LT-

treated shoot meristems but whose control differs, were used for further expression studies. *Blt* 4/9 and *blt* 101 are both transcriptionally regulated at LT, whereas *blt* 14 is post-transcriptionally controlled (Fig. 1).

The rates of appearance and decay of transcripts of these three LTR genes after transfer to low temperature (6 °C/2 °C) (acclimation) and return to the control temperature (20 °C/15 °C) (deacclimation, unpublished data) are shown in Fig. 2. Figure 2a shows northern blot analysis of steady-state homologous mRNA levels of the genes *blt* 4/9, *blt* 14 and *blt* 101 in plant material grown under standard low temperature (6 °C day/2 °C night) and control (20 °C day/15 °C night) conditions as described by Hughes and Pearce [16]. All three genes shown a strong differential response, *blt* 14 and *blt* 101 have no detectable signal at the control temperature in contrast to *blt* 4/9 which has low basal levels. After transfer from control to low temperature, steady-state transcripts increase to almost maximal levels after seven days (Fig. 2b) with the transcripts of *blt* 101 and *blt* 4/9 appearing more rapidly than that of *blt* 14.

Transfer back to the control temperature (20 °C/15 °C) resulted in a decrease in transcript levels over a four-day period (Fig. 2c). *Blt* 101 decreased most rapidly, having almost disappeared after four days. Steady-state levels of *blt* 4/9 and *blt* 14, although decreasing were still relatively high after four days.

Expression of LTR genes and freezing tolerance

The expression of the LTR genes *blt* 4/9, *blt* 14 and *blt* 101 (as steady-state mRNA levels) is shown at a range of temperatures in Fig. 3. Plants were grown at 18 °C day/10 °C night temperatures before transfer. After seven days treatment both *blt* 101 and *blt* 14 mRNAs are detectable at constant 9 °C whereas maximum levels in this experiment are seen at 6 °C/2 °C. After fourteen days, low levels of both *blt* 101 and *blt* 14 mRNAs are detectable at 18 °C/10 °C and 12 °C with further increases at constant 9 °C, constant 6 °C and 6 °C/2 °C. In contrast to these two genes, after both 7 and 14 days, the *blt* 4/9 transcript decreases on transfer from 18 °C/10 °C to constant 12 °C but shows an increase similar to *blt* 101 and *blt* 14 between 9 °C and 6 °C/2 °C. The presence of *blt* 4/9 transcript at 18 °C/10 °C

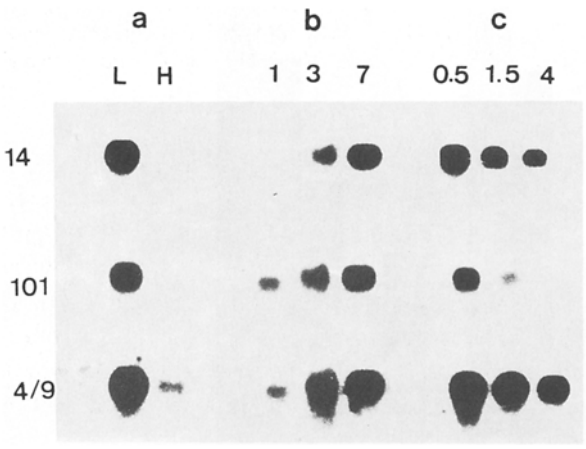

Fig. 2. Northern blot analysis of accumulation and decay of transcripts of *blt* 14, *blt* 101 and *blt* 4/9 during acclimation and deacclimation. a, L, 6 °C/2 °C; H, 20 °C/15 °C (standard conditions); b, transferred from 20 °C/15 °C to 6 °C/2 °C for 1, 3, 7 days; c, transferred from 6 °C/2 °C to 20 °C/15 °C for 0.5, 1.5, 4 days.

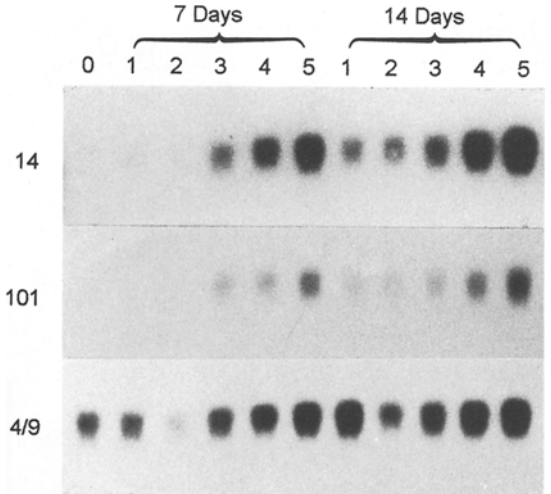

Fig. 3. Northern blot analysis of *blt* 14, *blt* 101 and *blt* 4 steady-state transcript levels after 7 days or 14 days at 18 °C/10 °C (1), 12 °C (2), 9 °C (3), 6 °C (4), 6 °C/2 °C (5); 18 °C/10 °C grown plants immediately before transfer (0). 14, *blt* 14; 101, *blt* 101; 4/9, *blt* 4/9.

which is reduced at 12 °C indicates that upregulation of *blt* 4/9 begins between 12 °C and 10 °C, whereas *blt* 14 and *blt* 101 are not upregulated until below 10 °C. Control plants grown at 20 °C/15 °C show only basal levels similar to those grown at 12 °C (Fig. 1a).

The freezing tolerance of plants given the temperature treatments shown in Fig. 3 was measured by regrowth of the crown and expressed as an LT_{50}, that is the temperature at which 50% of the plants survived. Figure 4 shows that freezing tolerance is related to the pattern of accumulation of *blt* 4/9 mRNA between 18 °C/10 °C and 6 °C/2 °C. Thus the LT_{50} of plants grown at 18 °C/10 °C is significantly lower than those transferred to constant 12 °C for one week, indicating deacclimation between 10 °C and 12 °C. A relationship between *blt* 4/9 mRNA levels and frost tolerance is also present after 14 days LT treatment where there is a general increase in frost tolerance at all temperatures except constant 6 °C.

Figure 5 shows that transfer of plants from 6 °C/2 °C to 4 °C/−4 °C results in a further increase in the steady-state levels of all three transcripts; however, the increase in levels of *blt* 101 and *blt* 14 is proportionately much greater than that of *blt* 4/9. The LT_{50} of crown regrowth is also reduced by this sequential lower-temperature treatment.

Effects of drought and nutrient stress

It has previously been shown that a number of LTR genes are also induced by drought and that drought treatment can acclimate plants for frost tolerance within a few degrees of that induced by a low positive temperature treatment [32]. The responses of the LTR barley genes, *blt* 4/9, *blt* 14

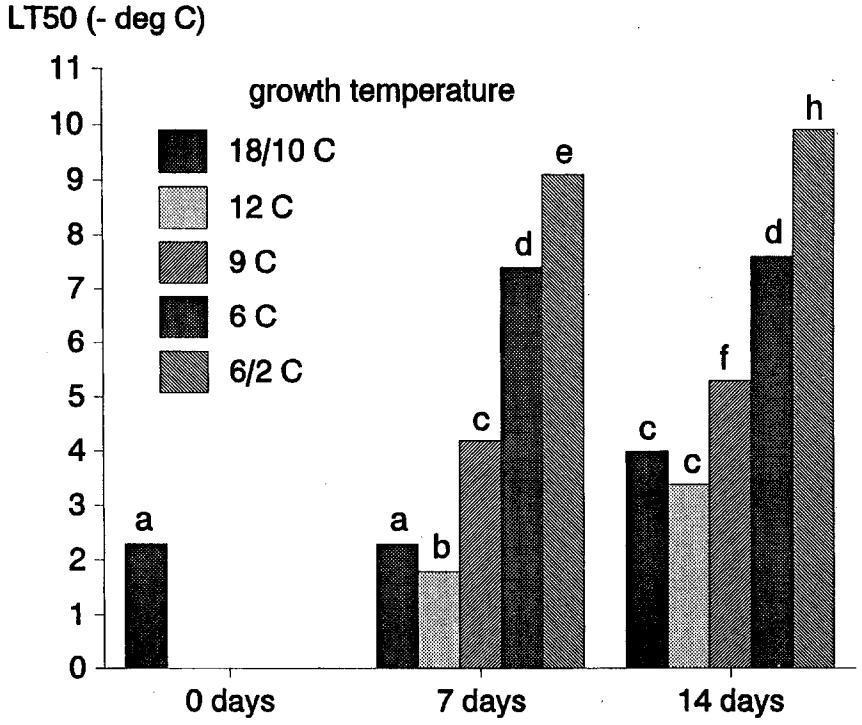

Fig. 4. Frost hardiness of plants grown at different temperatures for 7 or 14 days after growth at 18 °C/10 °C (0 days). 18 °C day/10 °C night; constant 12 °C; constant 9 °C; constant 6 °C; 6 °C day/2 °C night. LT_{50}, temperature at which 50% of plants regrow. Treatments with different letters are significantly different at $P<0.05$, based on a χ^2 test of survival data ($n=16$).

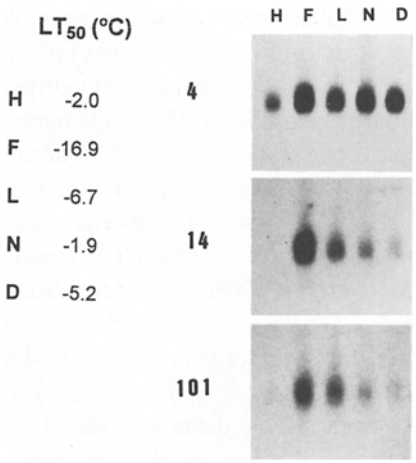

Fig. 5. Northern blot analysis of the response of *blt* 4, *blt* 14 and *blt* 101 to freezing, nutrient and drought stress. H, 20 °C/15 °C; F, 4 °C/−4 °C; L, 6 °C/2 °C; N, nutrient stress; D, drought stress. 4, *blt* 4; 14, *blt* 14; 101, *blt* 101. Frost hardiness is shown as LT_{50}, temperature at which 50% of plants regrow ($n = 16$).

and *blt* 101, to both drought and a nutrient stress are shown in Fig. 5. Northern blot analysis of steady-state mRNA levels shows that *blt* 4/9 is responsive to both drought and the nutrient stress whereas *blt* 14 and *blt* 101 are only slightly induced by these treatments. Drought stress acclimates plants for frost tolerance to within 1.5 °C (LT_{50}) of the low-temperature (6 °C/2 °C) treatment, however the nutrient stress used in this experiment did not result in any increase in frost tolerance.

Discussion

In barley, changes in gene expression in response to low temperature result in a set of elevated mRNAs which may encode proteins having a role in acclimation for frost tolerance. Whether all newly expressed or enhanced proteins are part of this process is not clear. In this study we have analysed the expression and control mechanisms of several LTR genes in relation to acclimation for frost tolerance. The data presented here indicate that, although three genes (*blt* 4/9, *blt* 14, *blt* 101) show similarly increased steady-state mRNA levels in response to a temperature shift from 20 °C day/15 °C night (or 18 °C day/10 °C night) to 6 °C day/2 °C night, each gene has unique response characteristics.

Some caution is necessary in interpreting the expression of *blt* 4/9 since this represents one of two cross-hybridising members of the *blt* 4 multigene family. There is likely, therefore, to be some cross-hybridisation between the transcripts of these two genes or those of other members of this gene family. The use of a 3′-specific probe for *blt* 4/9 in the run-on transcription assay confirms that *blt* 4/9 is low-temperature-responsive. Further work is underway to assign specific responses to each member of the gene family.

Run-on transcription data show that *blt* 4/9 and *blt* 101 are transcriptionally regulated in response to LT whereas *blt* 14 is not (Fig. 1). The high steady-state mRNA level of *blt* 14 at 6 °C/2 °C is thus consistent with post-transcriptional stabilisation of the *blt* 14 message at low temperature since no changes in *blt* 14 transcript size have been detected after temperature transfer (data not shown). Further, the time course experiment (Fig. 2) shows that there is a more rapid increase in *blt* 101 and *blt* 4/9 transcript levels compared to *blt* 14, which appears to be transcribed at low levels in both temperature regimes. The relatively slow accumulation of *blt* 14 compared with *blt* 4 genes and *blt* 101, confirms the relatively slow rate of transcription of this gene indicated by run-on transcription. These three genes also differ in their response to a deacclimation treatment. Thus, on return to the control temperature, the transcript of *blt* 101 rapidly disappears whereas those of *blt* 14 and *blt* 4/9 decay more slowly. This may indicate that a stabilising factor is involved in post-transcriptional regulation of both *blt* 14 and *blt* 4/9.

These data are similar to the findings of Hajela *et al.* [13] who reported that low-temperature-regulated accumulation of the transcript of one *Arabidopsis* gene was primarily transcriptionally regulated whilst three other genes were post-transcriptionally regulated. The responses and decay of all four *Arabidopsis* transcripts were however more rapid than those of the barley LTR

genes. Two of the *Arabidopsis* genes showed almost identical patterns of temperature induction of transcript even though different control mechanisms were involved. Recently a LTR gene alfalfa [34] has been shown to be both transcriptionally and post-transcriptionally regulated with a transcription-dependent factor implicated in rapid decay of specific mRNA on deacclimation. It may be that a similar factor in barley affects some LTR genes and is responsible for different rates of decay. The precise nature of the control of stability of these LTR gene transcripts is currently under investigation.

Selective enrichment of *de novo* synthesised thiouridine labelled mRNA was used by Weretilnyk *et al.* [33] to study the synthesis of a low-temperature-responsive gene from *Brassica napus*. The results indicated that the transcript is primarily transcriptionally regulated although they could not exclude the possibility of post-transcriptional stabilisation since the labelling was carried out over an 18-hour period. In contrast to the technique used by Weretilnyk *et al.* [33], the run-on transcription assay labels nascent transcripts already initiated at the time of harvesting the plant material [1] and thus demonstrates more clearly differences in transcription rates *in vivo*.

All of the LTR genes which have been published have been selected by differential screening using mRNA derived probes from plant material treated between 0 °C and 5 °C and a control temperature treatment (between 15 °C and 25 °C). Subsequent expression studies on these genes have used the same temperature treatments [3, 10, 13, 17, 22–24, 33]. Although expression of a number of LTR genes has been shown to broadly correlate with cold acclimation [10, 17, 22, 23, 25, 36], the precise temperature at which transcripts appear and reach maximum steady-state levels is unreported. This information may be important in confirming which genes have a role in cold acclimation since temperatures lower than 12 °C are required to acclimate most frost-tolerant plants [12]. Genes which begin to be transcribed above 12 °C many however have a role in temperature sensing or conditioning the plant for subsequent acclimation response. Our investigation of transcript levels and acclimation at a range of temperature indicates fine-tuning of the response of barley genes to low temperatures. Thus the transcript level of *blt* 4/9 begins to increase between 12 °C and 10 °C whereas those of *blt* 101 and *blt* 14 only begin to increase at 9 °C (Fig. 3). *Blt* 101 and *blt* 14 show almost identical patterns of increase between 9 °C and −4 °C, which correlate well with the acquisition of increasing frost tolerance over this temperature range (Fig. 5). Transcript levels of *blt* 4/9, however, are not significantly increased by the sub-zero treatment. These data indicate that sequential events may occur in the low-temperature response, but it remains to be determined whether they are interdependent. It is also interesting that *blt* 101 and *blt* 14, which have almost identical patterns of transcript accumulation in response to different temperatures and other stresses (Fig. 5), have been shown to be regulated by different mechanisms (Fig. 1).

In common with several LTR genes from other species we have previously shown that *blt* 4 (*blt* 4/1) is ABA and drought responsive [15]. In contrast *blt* 4 and *blt* 101 are not [15, 11]. Here we show that a nutrient stress also induces *blt* 4/9 to levels comparable with drought, but although the drought treatment significantly increases freezing tolerance of the plants, the nutrient stress does not (Fig. 5). This indicates that the role of the *blt* 4 gene family in the plants' low temperature and drought responses is likely to be interactive with other more specific gene products.

It is interesting to note that the LTR genes which we have isolated from barley are different from those isolated in other studies, many of which were from *Arabidopsis* [13, 17, 24] or related dicots [23, 33]. The dicotyledonary LTR genes have homology to each other and other dehydrative stress-related genes. This different range of barley LTR genes may reflect the fact that leaf meristematic and crown (apical meristem) material, which is essential for survival of cereals at low temperature [26, 27], was the source of the cDNA library used in this study, which is in contrast to most other studies, where whole plants or mature leaf material was used.

Acknowledgement

This work was supported by AFRC Grant PG13/514.

References

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Smith JA, Seidman JG, Struhl K: Current Protocols in Molecular Biology, Supplement 9. Wiley-Interscience, New York (1989).
2. Breu V, Guerbette F, Kader J-C, Kannangara GC, Svenson B, von Wettstein-Knowles P: A 10 kD barley basic protein transfers phosphatidylcholine from liposomes to mitochondria. Carlsberg Res Commun 4: 81–84 (1989).
3. Cattivelli L, Bartels D: Molecular cloning and characterisation of cold-regulated genes in barley. Plant Physiol 93: 1504–1510 (1990).
4. Chen HH, Li PH: Potato cold acclimation. In: Li PH, Sakai A (eds) Plant Cold Hardinesss and Freezing Stress, vol 2, pp. 5–22. Academic Press, New York. (1982).
5. Cox KH, Goldberg RB: Analysis of gene expression. In: Shaw CH (ed) Plant Molecular Biology: A Practical Approach, pp. 1–35. IRL Press, Oxford (1988).
6. Doll H, Haahr V, Sogaard B: Relationship between vernalisation requirement and winter hardiness in doubled haploid lines of barley. Euphytica 42: 209–213 (1989).
7. Dunn MA, Hughes MA, Pearce RS, Jack PL: Molecular characterisation of a barley gene induced by cold treatment. J Exp Bot 41: 1405–1413 (1990).
8. Dunn MA, Hughes MA, Zhang L, Pearce RS, Quigley AS, Jack PL: Nucleotide sequence and molecular analysis of the low temperature induced cereal gene, *blt* 4. Mol Gen Genet 229: 389–394 (1991).
9. Dunn MA, Morris A, Jack PL, Hughes MA: A low temperature-responsive translation elongation factor 1α from barley (*Hordeum vulgare* L.). Plant Mol Biol 23: 221–225 (1993).
10. Gilmour SJ, Thomashow MF: Cold acclimation and cold-regulated gene expression in abscisic acid mutants of *Arabidopsis thaliana*. Plant Mol Biol 17: 1233–1240 (1991).
11. Goddard NJ, Dunn MA, Zhang L, White AJ, Jack PL, Hughes MA: Molecular analysis and spatial expression pattern of a low temperature-specific barley gene, *blt* 101. Plant Mol Biol 23: 871–879 (1993).
12. Graham D, Patterson BD: Responses of plants to low non-freezing temperatures: proteins, metabolism and acclimation. Annu Rev Plant Physiol 33: 347–372 (1982).
13. Hajela RK, Horvath DP, Gilmour SJ, Thomashow MF: Molecular cloning and expression of cor (cold-regulated) genes in *Arabidopsis thaliana*. Plant Physiol 93: 1246–1252 (1990).
14. Hughes MA, Dunn MA: The effect of temperature on plant growth and development. Biotechnol Genet Eng Rev 8: 161–188 (1990).
15. Hughes MA, Dunn MA, Pearce RS, White AJ, Zhang L: An abscisic-acid-responsive, low temperature barley gene has homology with a maize phospholipid transfer protein. Plant Cell Envir 15: 861–865 (1992).
16. Hughes MA, Pearce RS: Low temperature treatment of barley plants causes altered gene expression in shoot meristems. J Exp Bot 39: 1461–1467 (1988).
17. Kurkela S, Franck M: Cloning and characterisation of a cold- and ABA-inducible *Arabidopsis* gene. Plant Mol Biol 15: 137–144 (1990).
18. Kurkela S, Borg-Franck M: Structure and expression of *kin* 2, one of two cold- and ABA-induced genes of *Arabidopsis thaliana*. Plant Mol Biol 19: 689–692 (1992).
19. Lin C, Guo WW, Everson E, Thomashow MF: Cold acclimation in *Arabidopsis* and wheat: a response associated with expression of related genes encoding 'boiling-stable' polypeptides. Plant Physiol 94: 1078–1083 (1990).
20. Lin C, Thomashow MF: A cold-regulated *Arabidopsis* gene encodes a polypeptide having potent cryoprotective activity. Biochem Biophys Res Comm 183: 1103–1108 (1992).
21. Lynch DV, Steponkus PL: Plasma membrane lipid alterations associated with cold acclimation of winter rye seedlings (*Secale cereale* L. cv. Puma). Plant Physiol 83: 761–767 (1987).
22. Mohapatra SS, Wolfram L, Poole RJ, Dhindsa RS: Molecular cloning and relationship to freezing tolerance of cold-acclimation-specific genes of alfalfa. Plant Physiol 89: 375–380 (1989).
23. Neven LG, Haskell DW, Hofig A, Li Q-B, Guy CL: Characterisation of a spinach gene responsive to low temperature and water stress. Plant Mol Biol 21: 291–305 (1993).
24. Nordin K, Heino P, Palva ET: Separate signal pathways regulate the expression of a low-temperature-induced gene in *Arabidopsis thaliana* (L) Heynh. Plant Mol Biol 16: 1061–1071 (1991).
25. Nordin K, Vahala T, Palva ET: Differential expression of two related, low-temperature-induced genes in *Arabidopsis thaliana* (L.). Heynh. Plant Mol Biol 21: 641–653 (1993).
26. Olien CR: Freezing processes in the Crown of 'Hudson' barley (*Hordeum vulgare* L.). Crop Sci 4: 91–95 (1964).
27. Peacock JM: Temperature and leaf growth of *Lolium perenne*. II. The site of temperature perception. Ann Appl Ecol 12: 115–123 (1975).
28. Pearce RS: Relative hardiness to freezing of laminae, roots and tillers of tall fescue. New Phytol 84: 449–463 (1980).
29. Robertson AJ, Gusta LV, Reaney MJT, Ishikawe M: Protein synthesis in bromegrass (*Bromus inermis* Leyss) cultured cells during the induction of frost tolerance by abscisic acid or low temperature. Plant Physiol 84: 1331–1336 (1987).

30. Sutka J, Snape JW: Location of a gene for frost resistance on chromosome 5A of wheat. Euphytica 42: 41–44 (1989).
31. Steponkus PL, Uemura M, Balsamo RA, Arvinte T, Lynch DV: Transformation of the cryobehaviour of rye protoplasts by modification of the plasma membrane lipid composition. Proc Natl Acad Sci USA 85: 9026–9030 (1988).
32. Thomashow MF: Molecular genetics of cold acclimation in higher plants. Adv Genet 28: 99–129 (1990).
33. Weretilnyk E, Orr W, White TC, Lu B, Singh J: Characterisation of three related low-temperature-regulated cDNAs from winter *Brassica napus*. Plant Physiol 101: 171–177 (1993).
34. Wolfraim LA, Langis R, Tyson H, Dhindsa RS: cDNA sequence, expression and transcript stability of a cold acclimation-specific gene, *cas* 18, alfalfa (*Medicago falcata*) cells. Plant Physiol 101: 1275–1282 (1993).
35. Zhang L, Dunn MA, Pearce RS, Hughes MA: Analysis of organ specificity of a low temperature responsive gene family in rye (*Secale cereale* L.). J Exp Bot 44: 1787–1793 (1993).
36. Zhu B, Chen THH, Li PH: Expression of an ABA responsive osmotin-like gene during the induction of freezing tolerance in *Solanum commersonii*. Plant Mol Biol 21: 729–735 (1993).