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Cloning, Characterization, and Expression of a cDNA Encoding a 50-Kilodalton Protein Specifically Induced by Cold Acclimation in Wheat[1]

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ABSTRACT

We have isolated, sequenced, and expressed a cold-specific cDNA clone, *Wcs120*, that specifically hybridizes to a major mRNA species of approximately 1650 nucleotides from cold-acclimated wheat (*Triticum aestivum* L.). The accumulation of this mRNA was induced in less than 24 hours of cold treatment, and remained at a high steady-state level during the entire period of cold acclimation in the two freezing-tolerant genotypes of wheat tested. The expression of *Wcs120* was transient in a less-tolerant genotype even though the genomic organization of the *Wcs120* and the relative copy number were the same in the three genotypes. The mRNA level decreased rapidly during deacclimation and was not induced by heat shock, drought, or abscisic acid. The *Wcs120* cDNA contains a long open reading frame encoding a protein of 390 amino acids. The encoded protein is boiling stable, highly hydrophilic, and has a compositional bias for glycine (26.7%), threonine (16.7%), and histidine (10.8%), although cysteine, phenylalanine, and tryptophan were absent. The WCS120 protein contains two repeated domains. Domain A has the consensus amino acid sequence GEKKGVMENIKEKLPGGHGDHQQ, which is repeated 6 times, whereas domain B has the sequence TGGTYGQQGHTGTT, which is repeated 11 times. The two domains were also found in barley dehydrins and rice abscisic acid-induced protein families. The expression of this cDNA in *Escherichia coli*, using the T_7 RNA polymerase promoter, produced a protein of 50 kilodaltons with an isoelectric point of 7.3, and this product comigrated with a major protein synthesized *in vivo* and *in vitro* during cold acclimation.

Cold-tolerant plants require a growth period at low temperature to trigger the appropriate genes needed for subsequent winter survival. During this period of cold acclimation, numerous biochemical, physiological, and metabolic functions are altered in plants. Previous biochemical and molecular analyses have demonstrated differential gene expression and the synthesis of a specific subset of proteins during cold acclimation in several species (2, 3, 5–7, 10, 14–16, 20). Using differential hybridization, several cold-regulated cDNAs have been recovered. Accumulation of the corresponding mRNAs for some of these cDNAs is also regulated by water stress and/or ABA (14, 15), whereas others are specifically regulated by low temperature (16).

Desiccation often accompanies cold acclimation and freezing stress (10). Therefore, it is not surprising that genes responsive to water deficit and ABA are also induced during cold acclimation. Members of such a gene family, *Rab*,[2] have been shown to be induced by drought and ABA in rice (17), and by cold in maize (3). Similarly, a set of genes encoding proteins called dehydrins are induced by drought in barley and maize (4). LEA proteins are also synthesized by many plants during embryogenesis while the embryo is undergoing desiccation (1). These desiccation-induced proteins, LEA and RAB, are also induced by ABA. These are extremely hydrophilic proteins and are thought to provide protection to cellular constituents during desiccation stress (1). However, there is no evidence that LEA and dehydrins are induced or regulated by cold.

Using genotypes of wheat differing in their capacity to develop freezing tolerance, we have demonstrated (5, 6, 20) a correlation between the level of synthesis of a specific set of proteins and the capacity of each genotype to develop freezing tolerance. To understand the function of these proteins and the molecular basis for freezing tolerance, we began the isolation and analysis of the genes involved in the cold acclimation of wheat. Here we report the analysis of a cDNA clone, *Wcs120*, representing an abundant cold-induced mRNA. This mRNA is specifically induced by low temperature. The level remains high during cold acclimation in two freezing-tolerant wheat (*Triticum aestivum* L.) varieties but declines in a less tolerant spring variety. The possible function of the gene product is also discussed.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Spring wheat (*Triticum aestivum* L. cv Glenlea) and winter wheat (*T. aestivum* L. cv Fredrick and cv Norstar) were grown

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[2] Abbreviations: *Rab*, rice abscisic acid-induced; LEA, late embryogenesis abundant; LT_{50}, 50% killing temperature; ORF, open reading frame; kb, kilobase; IPTG, isopropyl-β-D-thiogalactopyranoside.

under controlled environments. They were cold-acclimated as previously described (6). For ABA treatment, 7-d-old seedlings were watered daily with Hoagland solution containing 10^{-5} M ABA (Sigma) for 4 d under normal growth conditions (24/20°C, day/night with a 15-h light period, 450 μmol·m^{-2}·s^{-1}). As expected, ABA-treated plants showed a reduced growth rate compared with the control, indicating that ABA elicited the proper physiological response. Water stress was induced by withholding water until the plants became visibly wilted. Heat-shock was performed by incubating seedlings at 40°C for 3 h. This treatment was sufficient to induce typical heat-shock proteins as described in our previous work (5). Deacclimation was performed by returning the cold-acclimated (36 d at 4°C) plants to normal growth conditions for 1 or 5 d.

Construction and Screening of the cDNA Library

Poly(A)$^+$ RNA was isolated from cold-acclimated winter wheat Norstar (6). A cDNA library was constructed in lambda ZAPII (Stratagene) using *Eco*RI-*Not*I linkers from Pharmacia, and transformed into *Escherichia coli* strain XL1 blue. The cDNA library was screened with ^{32}P-labeled cDNA probes prepared from poly(A)$^+$ RNA isolated from cold-acclimated and nonacclimated wheat plants. The colonies showing an increased hybridization signal with cold-acclimated cDNA probes were selected and purified. The screening of the library and all the recombinant DNA techniques were performed as described (25). A clone, p2.1, like the majority of the clones, did not display differential hybridization during cold acclimation and was used to control the equal loading of RNA on the northern blots.

Northern and Southern Blot Analysis

Poly(A)$^+$ RNA (4 μg) samples were mixed with ethidium bromide before electrophoresis on formaldehyde agarose gels, as suggested (24). After electrophoresis, RNA or DNA was transferred to nitrocellulose membranes (BAS-85, Schleicher & Schuell) in 20 × SSC. The filters were air-dried then baked for 1 h at 80°C prior to hybridization with the ^{32}P-labeled p*Wcs120* insert (22). Filters were washed at 55°C with several buffer changes of decreasing SSC concentration (5 to 0.1×) and then autoradiographed on Kodak XRP films with intensifying screens (DuPont, Cronex Lightning plus) at −80°C.

Genomic DNA was prepared from shoots of 8-d-old seedlings as previously described (23), and DNA samples (10 μg) were digested with appropriate restriction endonucleases prior to electrophoresis. Slot blotting was performed as recommended by the manufacturer of the apparatus (Schleicher & Schuell).

DNA Sequence Analysis

Plasmid DNA was prepared, and deletion subclones were generated using Exonuclease III and VII, as described (29). Plasmids were sequenced by the dideoxynucleotide chain-termination method (26).

A computer-aided search of protein sequences was carried out with the Genetic Computer Group's Sequence Analysis Software package, version 6.0, with a Vax computer (University of Montreal). The database was searched with the TFASTA program.

Expression of *Wcs120* in *E. coli*

Plasmid p*Wcs120* was mutated using polymerase chain reaction to introduce a *Nde*I restriction site at the ATG start codon and a *Bam*HI site just after the stop codon. For this purpose, two oligonucleotides were synthesized using the Gene Assembler from Pharmacia. The first oligonucleotide, 5′-AGTGAGGAtCcCAGCGCcAtATGGAGAAC-3′, was homologous to the coding strand of *Wcs120* with the exception of four nucleotides (lowercase) to introduce *Bam*HI and *Nde*I sites. The second oligonucleotide, 5′-GTTGTCCG-GTGgAtcCTTAAAC-3′, was complementary to the coding strand with the exception of three nucleotides to produce a *Bam*HI site. Amplification using the *Taq*I DNA polymerase (Perking-Elmer Cetus corporation) and subcloning into pUC9 and pET vectors was performed (18). The inserted, amplified fragment was then digested with *Nde*I and *Bam*HI and ligated into *Nde*I-*Bam*HI-digested plasmid pET11a. This placed the entire coding frame, including the start methionine codon, directly downstream of the T_7 promoter to allow a high level of expression in *E. coli*. Expression was performed in BL21 (DE3). At an A_{600} of 0.6, 1 mM IPTG was added to the bacterial suspension, and 3 h later the bacteria were collected by centrifugation and resuspended in 0.1 volume of electrophoresis buffer for analysis.

Protein Purification and Analysis

To purify the expressed protein, bacterial cells were suspended in 5 to 10% of the culture volume of 50 mM Tris (pH 8.0), 10 mM EDTA. After one freeze-thaw cycle, the cells were disrupted by sonication and the lysate was centrifuged at 15,000*g* for 15 min. The supernatant was boiled for 20 min and then centrifuged at 15,000*g* for 20 min to eliminate insoluble proteins. The boiling-stable proteins were precipitated from the supernatant with ice-cold acetone and collected by centrifugation. The proteins were solubilized in electrophoresis buffer and separated on a 10% preparative polyacrylamide gel (13). The expressed protein was excised and electroeluted for 3 h. The eluted protein was then precipitated with acetone and analyzed by two-dimensional gel electrophoresis as previously described.

To compare the *E. coli*-expressed protein with that synthesized *in vitro*, Poly(A)$^+$ RNA from cold-acclimated and nonacclimated wheat were translated in a wheat germ system and analyzed by two-dimensional gel electrophoresis as previously described (6).

RESULTS

Isolation of a Cold-Specific cDNA Encoding a 50-kD Protein

cDNAs corresponding to cold-regulated mRNAs were isolated by differential screening and confirmed by northern blot analysis. One clone, representing approximately 0.1% of

the library based on reprobing with the purified insert, was chosen for further characterization. This 1.5-kb clone was designated *Wcs120* for wheat cold-specific gene. Northern analysis (Fig. 1) showed that *Wcs120* hybridized preferentially to mRNA induced during cold acclimation of the three wheat genotypes tested. In nonacclimated plants, this mRNA was barely detectable during normal growth (NA_7 and NA_{12}). The size of the major mRNA species was estimated at 1.65 kb and that of the cDNA insert was 1522 base pairs. Other mRNA species, ranging in size from 0.8 to 5 kb, shared some homology with the cloned cDNA. After washing at higher stringency (68°C, 0.1 × SSC), only the 1.65-kb and a minor 1.85-kb mRNA band hybridized.

In two freezing-tolerant genotypes, Fredrick and Norstar (LT_{50} of −16 and −19°C, respectively), the *Wcs120* mRNA increased to its highest level after only 24 h of cold acclimation at 4°C and remained at a high level throughout the required cold-acclimation period (36 d) (Figs. 1B and C: A_1, A_6, and A_{36}). The abundance of mRNA during the acclimation period was genotype-dependent, because in the less freezing-tolerant genotype Glenlea (LT_{50} of −8°C), transcripts declined sharply during the later part of the acclimation period, *i.e.* after 36 d (Fig. 1A: A_1, A_6, A_{36}). After 1 d of cold treatment, the *Wcs120* levels were over 20 times that of the nonacclimated control plants for all three genotypes. When cold-acclimated plants were deacclimated at 24°C, message abundance declined to the level of control plants (Fig. 1: D_1, D_5). After 24 h at 24°C, before the plants showed any significant loss in their freezing tolerance, the level of mRNA was already markedly decreased. These results indicated that the accumulation of *Wcs120* mRNA was up-regulated by low temperature and that message levels correlated with the capacity of each genotype to develop and maintain freezing tolerance.

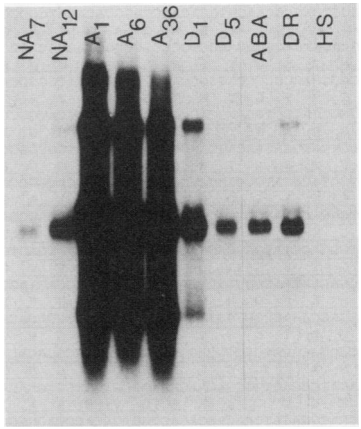

Figure 2. Northern blot analysis of mRNA hybridized with *Wcs120* after different treatments. Poly(A)$^+$ RNAs (4 μg) isolated from plants grown as described in "Materials and Methods" were separated and probed as described in Figure 1. ABA, Plants treated with ABA for 4 d; DR, water-stressed plants visibly wilted; HS, plants treated at 40°C for 3 h. Other symbols as in Figure 1. Exposure was for 20 h.

Specificity of the Induction of *Wcs120* mRNA

To determine if the *Wcs120* mRNA accumulation was specifically regulated by low temperature, we measured it after water stress, heat-shock, and the application of exogenous ABA, a hormone known to enhance cold tolerance in callus and cell suspension cultures (10, 19, 21) and to a limited degree in intact plants (15). *Wcs120* mRNA was not induced by these factors (Fig. 2; note that the exposure time is 10 times longer than for Fig. 1). To confirm this observation, it was of interest to determine whether ABA is in fact taken up by the plants in quantities sufficient to elicit the typical

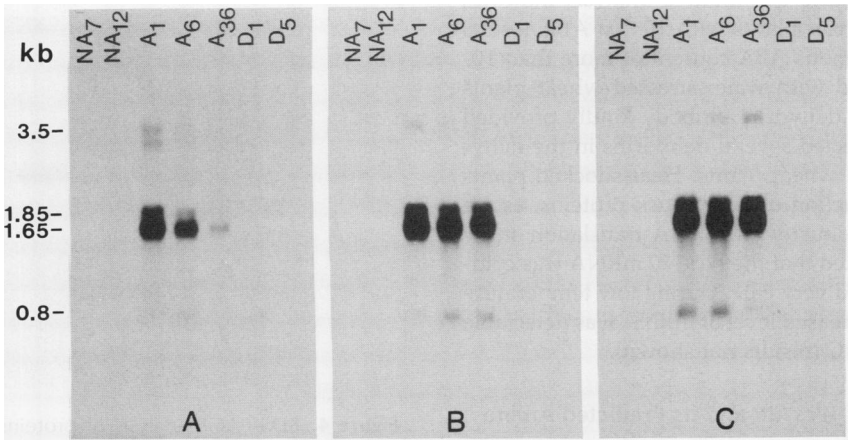

Figure 1. Northern blot analysis of mRNA hybridized with *Wcs120* during cold acclimation and deacclimation of wheat. Poly(A)$^+$ RNAs (4 μg) isolated from nonacclimated, cold-acclimated, and deacclimated wheat plants were separated by agarose gel electrophoresis in the presence of formaldehyde and then transfered to nitrocellulose membranes. The blots were probed with ^{32}P-labeled cDNA insert from plasmid p*Wcs120*. The final wash was at 55°C in 0.1 × SSC containing 0.1% SDS. Bands were visualized by autoradiography. A control probe (p2.1) was used to verify the equal RNA loading on the gel. NA_7 and NA_{12}, Control plants (nonacclimated) grown for 7 and 12 d; A_1, A_6, and A_{36}, plants cold acclimated for 1, 6, and 36 d; D_1 and D_5, plants deacclimated for 1 and 5 d. The plants used in the deacclimation experiment had been cold acclimated for 36 d. A, Glenlea; B, Fredrick; C, Norstar. Exposure was for 2 h.

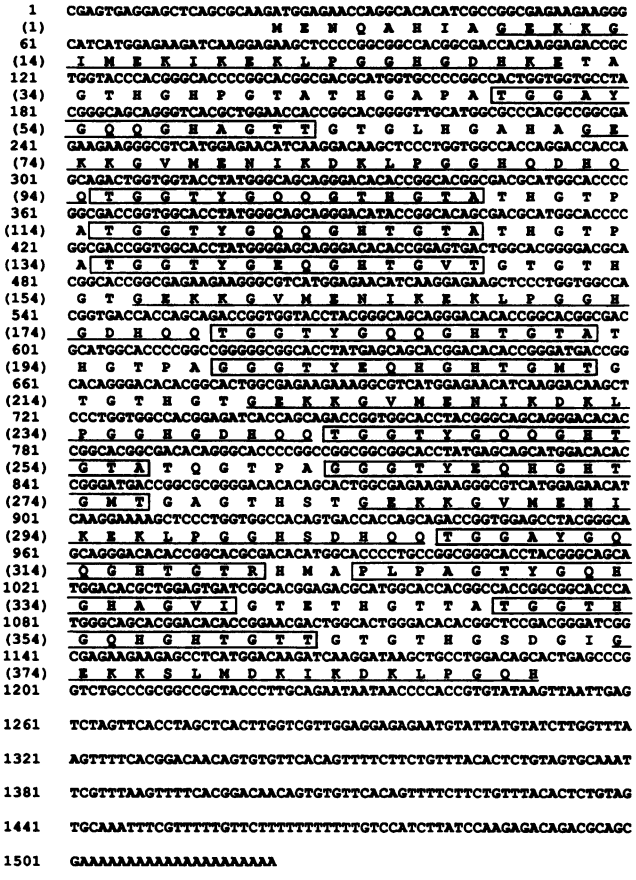

Figure 3. Nucleotide and deduced amino acid sequence of *Wcs120*. The DNA sequence was obtained on both strands by the chain termination method. Domain A (repeated 6 times) is underlined, and domain B (repeated 11 times) is boxed. GenBank Accession No.: M93342.

physiological response. The quantitative analysis indicates that the treatment of wheat plants with 10^{-5} M ABA results in an increase in endogenous ABA content of more than 10-fold, to a level observed with water-stressed wheat plants (8). In addition, using a dehydrin antibody kindly provided by Dr. T. Close, we detected several dehydrins in the dehydrated and ABA-treated wheat plants. Heat-shocked plants showed the typical induction of heat shock proteins, as we have shown in our previous *in vitro* RNA translation study (5). These results suggested that the *Wcs120* mRNA was cold-specific. It is also induced very early during low temperature exposure, because an increased level of mRNA was detectable after 3 h of growth at 4°C (results not shown).

Nucleotide Sequence of *Wcs120* and Its Predicted Amino Acid Sequence

The nucleotide sequence of the *Wcs120* cDNA clone contains a long ORF of 390 amino acids with an ATG codon at nucleotide 23 and the poly(A) tail starting 306 nucleotides downstream from the stop codon (Fig. 3). The calculated molecular mass of the polypeptide is 39,022 D with an isoelectric point of 7.77. The predicted protein has a compositional bias for Gly (26.7%), Thr (16.7%), and His (10.8%). These three amino acids account for 54% of the polypeptide, whereas Cys, Phe, and Trp are absent. The predicted protein contains two repeated domains. The A repeat is basic and has the consensus sequence GEKKGVMENIKEKLPGGHGDHQQ, which is repeated six times in the ORF (underlined, Fig. 3). The B repeat (boxed) contains 14 amino acids (consensus sequence TGGTYGQQGHTGTT) and is repeated 11 times.

A computer search revealed that repeats A and B are found in the dehydrin and RAB protein families, with the exception that the B repeat was not found in dehydrins 8 and 9 (4). Outside these repeats, very little homology was found between WCS120 and these two protein families with the exception that all the predicted polypeptides start with ME and, at the carboxy tail, share a stretch of 18 highly conserved amino acids ending with QH. These similarities suggest that WCS120 could share a function with the dehydrins and RAB families. On the other hand, the conserved sequence SGSSSSSSS, found in all RAB and dehydrin proteins, was conspicuously absent in WCS120.

Identification of the Polypeptide Encoded by *Wcs120*

To identify the encoded protein, the *Wcs120* ORF was expressed in *E. coli*. The protein was purified from *E. coli* (Fig. 4) and compared with the *in vitro* translation products of RNA isolated from nonacclimated and cold-acclimated plants (Fig. 5). The protein synthesized by bacteria was boiling stable and co-migrated with a 50-kD protein produced by mRNAs isolated from the cold-acclimated plants. The protein produced in the bacteria also co-migrated with a protein that accumulated *in vivo* during cold acclimation, suggesting that little or no posttranslational modifications were occurring in the intact plants and that, in both species, the first initiation codon, at position 23, was probably used.

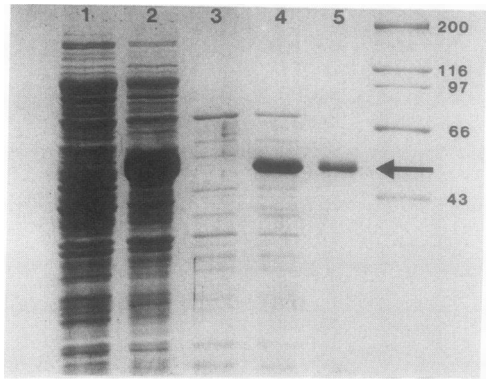

Figure 4. SDS-PAGE analysis of proteins present in *E. coli* transformed with plasmid pE*Wcs120*. Lane 1, non-transformed; lane 2, transformed and induced with 1 mM IPTG; lane 3, nontransformed boiled extract; lane 4, transformed and induced boiled extract; lane 5, induced purified protein. The proteins were analyzed on a 10% polyacrylamide gel and visualized by staining with Coomassie blue R-250. The arrow indicates the presence of the 50-kD protein in pE*Wcs120*-transformed cells induced with IPTG. The molecular mass markers are shown on the right side (kD).

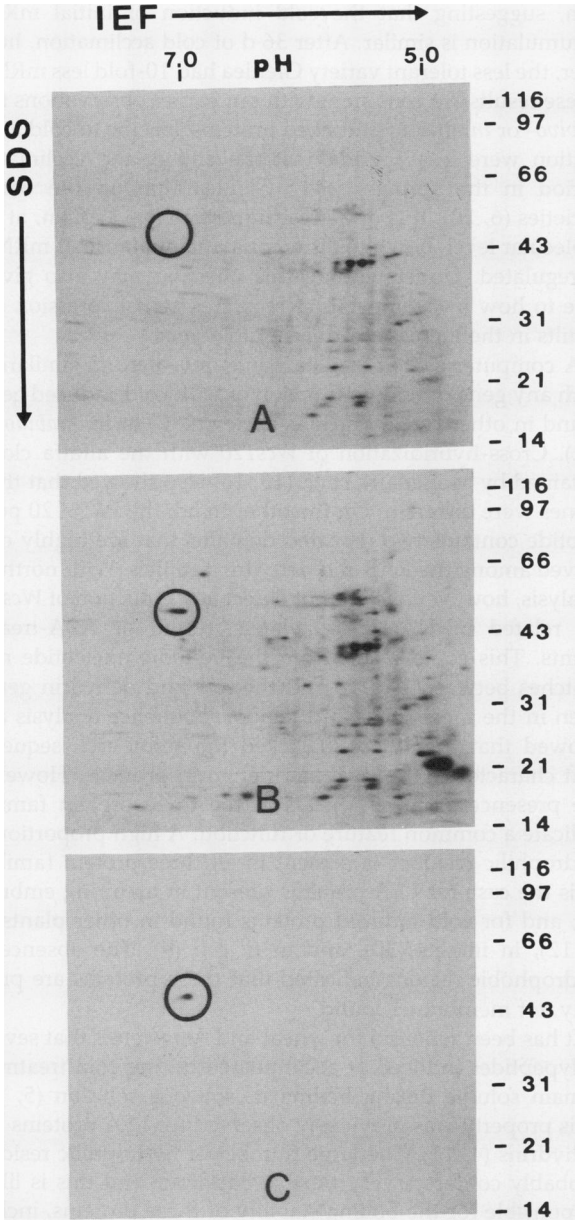

Figure 5. Two-dimensional gel electrophoretic analysis. A, *In vitro* translation products of mRNAs isolated from control (nonacclimated) winter wheat Fredrick. B, *In vitro* translation products of mRNAs isolated from cold-acclimated winter wheat Fredrick. Circle indicates the 50 kD-protein induced during cold acclimation. C, Purified protein expressed in *E. coli* transformed with pE*Wcs120*. The protein had identical molecular weight and isoelectric point as that synthesized *in vitro* and *in vivo*.

A discrepancy between the calculated and apparent molecular masses on SDS-PAGE was observed (39 *versus* 50 kD). This discrepancy likely results from the avid binding of SDS, as suggested for other plant stress proteins with skewed amino acid composition (28).

Genomic Organization and Relative Gene Copy Number in the Three Genotypes

Genomic DNA from the three genotypes was purified and digested with several restriction enzymes to determine if the

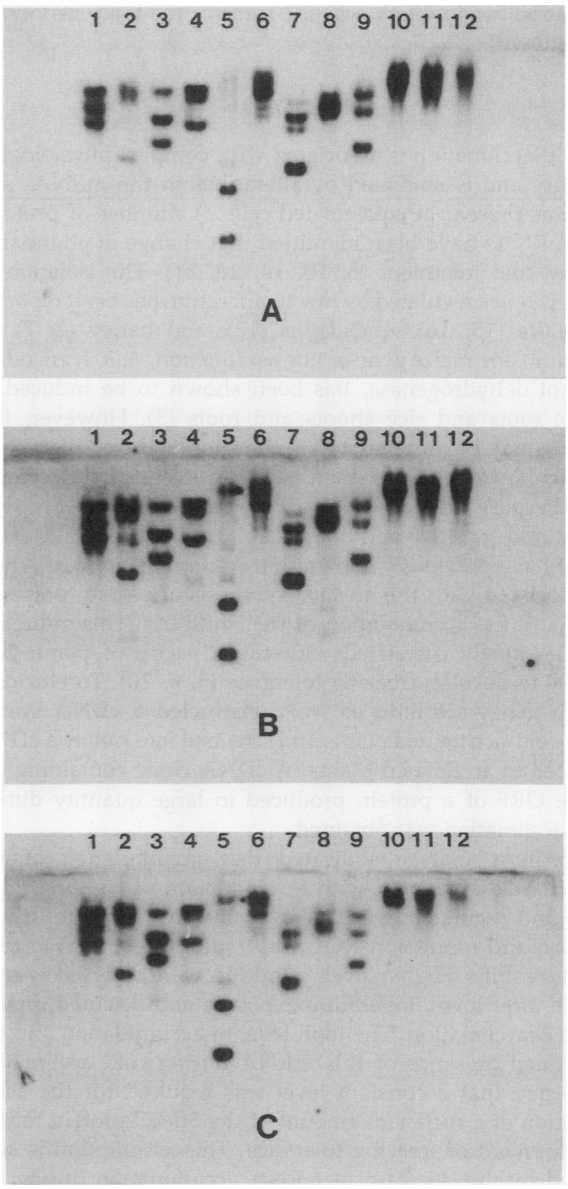

Figure 6. Southern blot analysis of wheat genomic DNA. Wheat DNA (10 μg) from three genotypes was digested with 12 different restriction enzymes, separated on agarose gel electrophoresis, transfered to nitrocellulose, and then probed with *Wcs120*. Lane 1, *Apa*I; lane 2, *Kpn*I; lane 3, *Sac*I; lane 4, *Hin*dIII; lane 5, *Pst*I; lane 6, *Pvu*II; lane 7, *Bam*HI; lane 8, *Eco*RI; lane 9, *Xba*I; lane 10, *Sal*I; lane 11, *Xho*I; and lane 12, *Sma*I. A, Glenlea; B, Fredrick; C, Norstar.

general organization was the same in the winter and spring varieties. Because all DNA fragments detected were present in the three genotypes, it appears that there were no major differences in this organization (Fig. 6). Because cultivated wheat is hexaploid, multiple bands are expected for enzyme sites outside the coding region (lanes 1, 4, 5, 6, 7, 8, 9, 10, 11). Three strong hybridizing fragments were detected after digestion with 12 enzymes, indicating that wheat contains only 3 loci corresponding to *Wcs120*. The other weakly hybridizing bands may correspond to other members of the putative gene family that were detected on northern blots (see Figs. 1 and 2). The relative gene copy number, as measured by slot blots, was the same in the three genotypes (not shown).

DISCUSSION

Cold acclimation is associated with complex physiological changes and is mediated by alterations in the mRNAs and proteins present in cold-treated cells. A number of proteins and mRNAs have been identified that change in abundance during cold treatment (6, 10, 14, 20, 21). The isolation of cDNA clones regulated by low temperature has been reported in alfalfa (15, 16), *Arabidopsis* (12), and barley (2, 7). In addition, one maize gene of known function, *Adh-1*, encoding alcohol dehydrogenase, has been shown to be induced in maize roots and rice shoots and roots (3). However, this change may be more related to the sudden drop in temperature (cold shock) rather than to the process of cold acclimation, because it is known that both maize and rice are relatively cold sensitive and do not acclimate.

We have previously shown that cold acclimation of wheat is associated with the *de novo* synthesis of several polypeptides and the accumulation of their mRNAs. This induction was positively correlated with the capacity of plants and tissues to develop freezing tolerance (5, 6, 20). To elucidate which genes are induced, we constructed a cDNA library from cold-acclimated plants and screened it to isolate a cDNA induced in acclimated plants. A cDNA clone containing the entire ORF of a protein produced in large quantity during cold acclimation was obtained.

Northern analysis indicated that accumulation of the mRNA was associated with the induction of freezing tolerance and regulated specifically by low temperature. Its induction and repression were very rapid in response to temperature shifts. Higher levels of mRNA were detected as early as 3 h after low temperature exposure and declined rapidly upon deacclimation. The high level of accumulation and the continued presence of this mRNA during cold acclimation suggested that a constant level was required for the accumulation of a sufficient amount of the 50-kD protein for the development of freezing tolerance. This assumption is supported by the decrease in mRNA accumulation during the later part of the acclimation period in the spring genotype Glenlea, a variety with less capacity to develop freezing tolerance. This lower capacity could also be explained by a different genomic organization or gene copy number that could lead to a lower expression of the appropriate transcripts. Based on both Southern and slot blot analyses (data not shown), however, the relative gene copy number and gene organization proved to be the same for all three genotypes. Thus, the difference in tolerance is more likely the result of differences in gene regulation involving either transcription or mRNA stability. A comparable level of mRNA was present in all three genotypes after 1 d of cold acclimation, suggesting that the cold induction of initial mRNA accumulation is similar. After 36 d of cold acclimation, however, the less tolerant variety Glenlea had 10-fold less mRNA. These results are consistent with our earlier observations that *in vivo*- or *in vitro*-synthesized proteins specific to cold acclimation were less abundant at the end of the acclimation period in the spring variety Glenlea than in the winter varieties (6, 20). It is of a great importance to explain, at the molecular level, how the differential maintenance of mRNAs is regulated. Understanding this question may also give a clue to how low temperature regulates gene expression and results in the increase in freezing tolerance.

A computer search of data banks revealed no similarities with any gene of known function or with cold-induced genes found in other cereals such as barley (2, 7) or in *Arabidopsis* (12). Cross-hybridization of *Wcs120* with the alfalfa clones obtained by Mohapatra *et al.* (15, 16) also showed that these clones were different. On the other hand, the WCS120 polypeptide contains two repeated domains that are highly conserved among the RAB and dehydrin families. With northern analysis, however, we did not detect any induction of *Wcs120* (or related mRNAs) from water-stressed or ABA-treated plants. This could result from the periodic nucleotide mismatches between *Wcs120* and the *Rab* and dehydrin genes, even in the most conserved regions. Sequence analysis also showed that the WCS120 lacked the serine-rich sequence that characterize the RAB and dehydrin proteins. However, the presence of these repeats in the three protein families indicate a common feature or function. A high proportion of hydrophilic residues is present in all three protein families, as is the case for LEA proteins present in maturing embryos (1), and for cold-induced proteins found in other plants (2, 7, 12), in insects (30), and in *E. coli* (9). The absence of hydrophobic regions indicated that these proteins are probably not membrane-bound.

It has been reported for wheat and *Arabidopsis* that several polypeptides induced or accumulated during cold treatment remain soluble during boiling in aqueous solution (5, 14). This property was previously observed for LEA proteins and dehydrins (4, 11). The large number of hydrophilic residues probably confers a very flexible backbone and this is likely responsible for the boiling stability of these proteins, including the 50-kD protein identified in our work, because they would not have to renature after boiling. The high Gly content (26%) of the 50-kD protein may confer a high flexibility and mobility to the protein as found in several Gly-rich proteins such as elastin. The small size of the Gly molecule and its short side chain gives it a unique function in the structure of several proteins. It facilitates the formation of intramolecular hydrogen bonding and thus gives the protein a random coil conformation. This property allows the protein to stretch, bend, and expand in all directions, a property that could be useful to protect cellular structures against freezing or severe dehydration. Physicochemical characterization of this protein should help to verify this assumption. The sig-

nificance of these properties is still unclear but the high hydrophilicity may also be important in hydrogen bonding to the lattice of nascent ice crystals, thus modifying the structure or propagation of ice crystals, which may reduce intracellular freezing damage during winter. The high hydrophilicity of these proteins may also be important in trapping enough water inside the cell to prevent local dehydration that may occur during freezing or water stress (10, 27).

Drought stress was shown to increase the freezing tolerance (10). This suggests that some features must be common between the proteins induced during these different stresses.

The absence of the serine-rich repeat and the specific induction of *Wcs120* mRNA early during cold acclimation, before any increase in osmotic pressure, cell dehydration, or ABA content occurs (data not shown), suggested that the *Wcs120* gene was regulated differently from the *Rab* and dehydrin families. The molecular mass of the WCS120 protein is much higher than the known RAB and dehydrin protein masses. The striking recurrence of the common repeats in WCS120, RAB, and dehydrin proteins suggests that survival at low temperature and during water stress requires large amounts of these unusual proteins. The production of antibodies to WCS120 for immunohistochemical analysis and physiological studies is in progress. We also plan a more detailed physicochemical characterization of the purified protein to better understand its possible function. Preparation of transgenic plants with sense and antisense expression vectors should help in better defining the role of this protein during cold acclimation.

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