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Molecular cloning, characterization and expression analysis of two catalase isozyme genes in barley

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Abstract

Clones representing two distinct barley catalase genes, *Cat1* and *Cat2*, were found in a cDNA library prepared from seedling polysomal mRNA. Both clones were sequenced, and their deduced amino acid sequences were found to have high homology with maize and rice catalase genes. *Cat1* had a 91% deduced amino acid sequence identity to CAT-1 of maize and 92% to CAT B of rice. *Cat2* had 72 and 79% amino acid sequence identities to maize CAT-2 and -3 and 89% to CAT A of rice. Barley, maize or rice isozymes could be divided into two distinct groups by amino acid homologies, with one group homologous to the mitochondria-associated CAT-3 of maize and the other homologous to the maize peroxisomal/glyoxysomal CAT-1. Both barley CATs contained possible peroxisomal targeting signals, but neither had favorable mitochondrial targeting sequences. *Cat1* mRNA occurred in whole endosperms (aleurones plus starchy endosperm), in isolated aleurones and in developing seeds, but *Cat2* mRNA was virtually absent. Both mRNAs displayed different developmental expression patterns in scutella of germinating seeds. *Cat2* mRNA predominated in etiolated seedling shoots and leaf blades. Barley genomic DNA contained two genes for *Cat1* and one gene for *Cat2*. The *Cat2* gene was mapped to the long arm of chromosome 4, 2.9 cM in telomeric orientation from the *mlo* locus conferring resistance to the powdery mildew fungus (*Erysiphe graminis* f.sp. *hordei*).

Introduction

The enzyme catalase (CAT, H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) protects aerobic organisms from oxygen free radicals by disproportionating H_2O_2 into O_2 and H_2O. As if to ensure that a functional catalase will always be available under different conditions, various species have evolved isozymal forms. Many of these have been cloned and characterized in bacteria [22] and

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers U20777 (*Cat1*) and U20778 (*Cat2*).

yeast [6, 36]. Catalase isozymal genes have also been cloned from a wide variety of dicot plants, including *Arabidopsis*, castor bean, cotton, mung bean, pea, soybean, sunflower, sweet potato, tobacco and tomato. In contrast, catalase isozymal genes have been cloned from only two monocots, maize [2, 29] and rice [23, 24]. The most highly characterized is the maize catalase gene/enzyme system, in which both developmental and temporal regulation occurs, with each isozyme being targeted to distinct tissues and subcellular compartments (reviewed in [31]).

Barley was previously thought to have one gene, or gene locus for catalase [17], but later genetic studies of isozymal variants provided evidence for two coding genes [1, 39]. Studies have also shown that barley contains catalases with differing enzymatic specificities, a peroxidatic form and a form with enhanced peroxidatic activity [14].

In addition to its importance in evolutionary and developmental studies, catalase may also be involved in plant disease and stress resistance. In tobacco, salicylic acid-mediated systemic acquired resistance to pathogens may involve binding of salicylic acid to catalase. This inactivates the enzyme, allowing H_2O_2 to increase to toxic levels and causing localized cell death [4]. Catalase may also be involved in the sensitivity of maize seedlings [27] and a variety of other plants [26] to chilling injury.

The following study reports the cDNA cloning, sequence analysis, developmental expression and mapping of the barley catalase genes. A system for grouping the catalase isozymes of monocots is proposed. The mapping of the barley *Cat2* gene completes the mapping of the catalase genes, which may prove useful in studies of disease and stress resistance.

Materials and methods

cDNA cloning and sequence analysis

Seedlings of barley (*Hordeum vulgare* L.) cv. Morex were imbibed and germinated at 16 °C, as described [34]; gibberellin treatment of isolated aleurones was also conducted as so described. RNA was extracted from kernels (starchy endosperm plus aleurone) and isolated aleurones by homogenization in Tris buffer containing aurintricarboxylic acid; after precipitation of β-glucans with Calcofluour white (Sigma) and phenol/chloroform extraction, RNA was precipitated with LiCl [34]. RNA was purified from scutella and etiolated seedling shoots (primary leaf plus coleoptile from the scutellar node and above) and from 8-day-old etiolated leaf blades by homogenization of tissue in guanidinium thiocyanate [5]. Genomic DNA was purified from etiolated leaves of cvs. Morex and Steptoe as described [34].

Catalase expression was analyzed on RNA blots, and gene organization was analyzed on Southern blots, both at 62.5 °C, as described [34]. Blots were hybridized with either the full-length catalase cDNA inserts or with restriction fragments representing about 420 bp of their 3′ ends. To monitor the specificity of hybridization, samples of full-length barley *Cat1* and *Cat2* clones were linearized with *Sac* I and electrophoresed on the same gels. Autoradiograms were prepared by exposing Kodak XAR-5 film with blot filters between intensifying screens.

Catalase clones were isolated from a cDNA library prepared from 3- and 4-day-old Morex seedling polysomal poly(A)$^+$ mRNA [33]. Clones were detected by probing plaque lift filters with α-^{32}P-dCTP-labeled insert DNA from the 290 bp 5′ *Eco* RI/*Sma* I fragment of the *Cat2* catalase cDNA clone from maize [2]. Probing conditions were the same as those used for Southern blots, except that hybridization and rinsing were at 42 °C. Positive plaques were purified, and phagemids were converted to plasmids by *in vivo* excision with R408 helper phage. The longest clones were sequenced by dideoxy chain termination [30]. Data was analyzed using the Genetics Computer Group software [8].

RFLP and linkage analysis

RFLP hybridization filters were prepared after separation of barley genomic DNA in high-

resolution polyacrylamide gels [9]. The restriction enzyme used to display the RFLP with the *Cat2* cDNA was *Rsa* I. Calculation of linkage to the *mlo* locus has been described [15].

Results

Sequence analysis of catalase cDNA clones

Restriction digests of cDNA clones revealed that all potential clones fell into two classes. The two longest clones in each class were sequenced and found to be homologous to catalases from other plants. Homologies were highest to those from maize and rice, especially in the 5′ regions. The deduced amino acid sequences were more highly related to catalases of other species, rather than to each other (Fig. 1). The deduced amino acid sequence of one clone was 91% identical to CAT-1 of maize [29] and 92% identical to CAT B of rice [24]; this gene was labeled as '*Cat1*'. The other barley clone was 72%, 73% and 79% identical to CAT-1,-2 and -3 of maize, respectively, and 89% identical to CAT A of rice [23]; this gene was labeled as '*Cat2*'. Even though the deduced amino acid sequences for barley *Cat1* and *Cat2* were similar to each other (72%), their nucleic acid sequences were more loosely related. The homology was only 67% between their coding regions, and a 49% homology in their 3′ UTRs could only be found after liberal gap substitutions. Based upon amino acid homologies, we propose that barley CAT-1, maize CAT-1 and rice CAT B form a distinct group (I) of related monocot isozymes (Fig. 1). Barley CAT-2, maize CAT-3 and rice CAT A form a second group (II) of closely related isozymes. Maize CAT-2 is slightly more homologous to barley CAT-1 (78%) than barley CAT-2 (73%) and so is placed with group I. A BLAST [8] sequence alignment also revealed that barley CAT-1 is much more closely related to dicot CATs than is barley CAT-2 (data not shown).

Both barley *Cat1* and *Cat2* sequences contain uninterrupted open reading frames upstream from the ATG codon specifying the N-terminal methionine proposed for other cereal catalases (Fig. 1), but it is not known whether this upstream region is indeed translated. *Cat1* contains a sequence surrounding the ATG codon, CGGCCATGG, which is similar to the CCGCCATGG sequence identified as optimal for initiation by eukaryotic ribosomes [20]. However, the ATG of *Cat2* is contained within an unrelated GAAGGATGG sequence. The 76 bp and 58 bp 5′ UTRs of *Cat1* and *Cat2* are very C-rich (55 and 41%, respectively), and there are no regions of homology between the two. In the N-terminal sequence up to the first active site residue (H65), barley CAT-1 is quite similar to maize CAT-1 and rice CAT B. The comparable region of barley CAT-2 is more similar to those of maize CAT-3 and rice CAT A. While this region of maize CAT-2 contains homologies to both groups, it is divergent enough to possibly be considered distinct from both. However, its overall coding sequence is more related to barley CAT-1 than CAT-2. The deduced amino acid sequences for *Cat1* and *Cat2* specify proteins of 492 and 494 amino acids in length, respectively. Possible peroxisomal targeting sequences occurs six amino acids from the carboxy terminus of all group II CATs (SRL) and near the N-terminus of group I CATs (SSS). The region from P39 to V64 is predicted to form an amphiphilic α-helix with 7 (CAT-1) to 8 (CAT-2) amino acids presenting an uninterrupted hydrophobic face (Helicalwheel projection [8]; data not shown).

The 3′ UTR of *Cat1* contains three possible polyadenylation signals (data not shown; [16]). Each signal sequence (AATAAT) is clustered in a 19 bp region which begins 224 bases from the TAA termination codon and ends 10 bases before the first, and most frequently used poly(A) addition site. A second site, which extends the 3′ UTR by 68 bases is also used. This extended sequence is contained in the full-length 1879 bp *Cat1* clone and also in an earlier *Cat1* clone (86-4, GenBank accession number U16132). The 207 bp 3′ UTR of *Cat2* contains no recognizable polyadenylation signal in an appropriate position. Two AATAAG sequences and one AATAGA could serve the signal function, but the closest is 65 bp from the actual polyadenylation site.

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                1                   20                  40                  60
HVCAT1          MDPYKHRPTS GANSAYWTTN SGAPVWNNNN ALTVGHRGPI LLEDYHLIEK LAQFDRERIP
ZMCAT1          M.......S. .SN.SF.... .........S .....Q.... .......... ..........
OSCATB          M......AS. ....TF.... .........S .....E.... .......... ..........
ZMCAT2          M.....ARRA PSTPVL-DH. .-..RVEQRQ L....A.... ....L-VV.. ..N.......
                     ** *
HVCAT2          M..C.F..S. SFDTKTT... A.Q....D.E .....P.... .......L.. I.H.A.....
OSCATA          M..C.F..S. SFDTKTT... A......D.E .....P.... .......... V.H.A.....
ZMCAT3          M..T.F..S. SHDTTVT... A......D.E .....P.... .......... V.H.......

                  70                            100                           125
HVCAT1  ERVVHARGAS AKGFFEVTHD VSQLTCADFL RAPGVQTPVI VRFSTVVHER GSPE.LRDPR GFAVK
ZMCAT1  .......... .......... ..H....... .......... .......... .......... .....
OSCATB  .......... .......... I.H....... .......... .......... .......... .....
ZMCAT2  .......... .......... ITH....... .......... ......I... .......... .....

HVCAT2  .......... ..... C... .TG....... ....AR.... ......I... .....I.... .....
OSCATA  .......... ......C... .TDI...... .S..A..... ......I... ..Q..I.... .....
ZMCAT3  .......... ......S... .TS....... .....R.... ....Q.IP.P ..GR.I..A. .....

                135                           165                           190
HVCAT1  FYTREGNFDL VGNNMPVFFI RDGMKFPDMV HAFKPSPKTN MQENWRVVDF FSHHPESLHM FTFLF
ZMCAT1  .......... .......... .......... .....N.... L.....I... .......... .....
OSCATB  .......... ......G... .......... .......... ......I... .......... .S...
ZMCAT2  .......W.. ....F..... ...I...... ..L..N.R.H I.D...IL.. .......... .S...

HVCAT2  .......W.. L...F..... ...I....VI .....N.KSH V..Y...F.. L........T .F...
OSCATA  .......W.. L...F..... ...I....VI .....N.RSH V..Y...F.. L........T .F...
ZMCAT3  .......W.. L...F..... ...I....VI .....N.RSH V..Y...F.. L..L.....T .F...

                200                           230                           255
HVCAT1  DDVGIPLNYR HMDGFGVNTY TLISRDGKAH LVKFHWKPTC GVKCLLDDEA VTVGGTCHTH ATKDL
ZMCAT1  .......... ..E....... S..N....P. .......... .......N.. ........S. .....
OSCATB  .......... ..E....... ...NK...P. .......... .......... ........S. .....
ZMCAT2  ......AD.. ....S..H.. ..V..A.TVT Y.....R... ..RS.M.... .RC.AN-.S. .....

HVCAT2  ......TD.. .......... .FV..A..S. Y.....R... ..S..M.... TL...KN.S. ..Q..
OSCATA  ......TD.. .......... .FVT..A..R Y.......... ..S..M.... TL...KN.S. ..Q..
ZMCAT3  .H..V.SD.. ..E....... .FV.AA...Q Y.......... .ERSI.T..E AR...RN.S. -.Q..

                265                           295                           325
HVCAT1  TDSIAA-GNY PEWKLFIQTI DADHEDRF-D FDPLDVTKTW PEDIIPLQPV GRMVLNKNID NFFAE
ZMCAT1  Y.....-... .....Y.... .L....K... .......... .......... ........V. .....
OSCATB  ......-... .....Y.... .P........ .......... .......... .......... .....
ZMCAT2  ..A...-..F ...T.Y...M .PEM...LD. L.......... ...TF..... ..L...R... .....

HVCAT2  Y...D.-..F ......V.V. .P.E...... .....D.... ...LV..... ..L..DR.V. ...N.
OSCATA  Y.....-..F ......V.V. .PEE.E.... .....D.... ...EV..R.. ..L...R.V. ...N.
ZMCAT3  Y.....E.SF ...T.YV.VM .PAQQEQY.. .....D.... ...LL..R.. ..L..DR.V. ..LN.

                335                           365                           390
HVCAT1  NEQLAFCPAV TVPGIHYSDD KLLQTRIFSY ADTQRHRLGP NYLMLPVNAP KCAHHNNHHD GLMNF
ZMCAT1  ...I.....I S..A...... .......... .......... .......... .......... .F...
OSCATB  .........I I......... .......... .......... .......... ...Y...... .S...
ZMCAT2  ........GL I....Y.... .......... S......... ...L..A... ........Y. .S...

HVCAT2  ......G.GL V....Y.... .M..C.V.A. .....Y.... .......... ..GFK...Y. .A...
OSCATA  ......G.GL V....Y.... .M..C.V.A. .....Y.... .......... ........Y. .A...
ZMCAT3  ......G.GL V....Y.... .M..C.V.A. .....Y.... .......... R.GT....Y. .A..L

                400                           430                           455
HVCAT1  IHRDEEVNYF PSRFDPTRHA E--KYPMPPR VLSGCREKCI IDKENNFKQA GERYRSFDPA RQDRF
ZMCAT1  M......... ......A... ....V.I... ..TR...... .Q........ .......... .....
OSCATB  M......... .....AA... ....V.I... ..T......V .......Q.. .......... .....
ZMCAT2  M..H...D.. ...Y.AV.N. P..R..I.TA HIA.R...TV .S........P .....AM... ..E..

HVCAT2  M......D.Y ...HA.L... .PASF.V.T. PVV.K...TR .K...D.V.P ......WA.D .....
OSCATA  M......D.Y ...HA.L... PPT--.IT.. PVV.R.Q.AT .H.Q.D...P ......WA.D ..E..
ZMCAT3  M......D.Y ...HAR-.CG RAAPT.L... PVA.R...AT .R.P.D...P ......W.AD .....

                465                           ***   501
HVCAT1  LQRWVDALTD ARVTHEIQSI WVSYWSQCDA SLGQKLASRL KIKPNM
ZMCAT1  I........H P.....HRT. .I........ A.....P... NL..S.
OSCATB  ........S. P.I...LRG. .I........ .......... NL....
ZMCAT2  IT......S. P.L....RT. .L.N...A.R .......... SA..S.

HVCAT2  VR.FS...AH PK.S..LRV. .IDFL.K..K .C.M.V.N.. NV..S.
OSCATA  I-AVRRRVAH PK.SP.LRA. ..N.L....E ...V.I.N.. NV..S.
ZMCAT3  VKAIRRLART PKRQP.LR.. .IDLLAKV.. ...M.I.T.. NM.A..
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Tissue-specific and temporal Cat gene expression

A distinct pattern for differential expression of the *Cat* genes was found when RNAs isolated from various tissues were analyzed on northern blots. In whole endosperms (aleurones plus starchy endosperms) of seedlings which had developed for 1 to 7 days from the onset of imbibition, *Cat1* mRNA was already present at moderate levels by day 1. Its levels increased to a peak at day 4 and then declined modestly thereafter (Fig. 2A). *Cat2* mRNA was virtually absent from whole endosperms (Fig. 2B). *Cat1* was also strongly expressed in scutella of seedlings (Fig. 2C). It reached peak levels by day 2 and declined only slightly thereafter. Much less *Cat2* mRNA was found in scutella. While it also rapidly reached peak levels at day 2, it then rapidly declined and was almost undetectable by day 6 (Fig. 2D). While *Cat1* was strongly expressed in whole endosperms and scutella, it was almost undetectable in etiolated shoots (Fig. 2E). Instead, *Cat2* was expressed, but its mRNA levels in shoots remained low until day 4. At this time, rapid shoot expansion commenced, and *Cat2* increased strongly (Fig. 2F).

Cat1 mRNA was strongly expressed in gibberellin-treated isolated aleurones from five-day-old deembryonated half-seeds, while *Cat2* was not expressed (Fig. 3). (Studies were not conducted to determine whether GA specifically affect *Cat* expression.) *Cat1* was also expressed in the developing seed, 25 days after pollination, although at much lower levels than found in the aleurone, while *Cat2* was very weakly expressed. Thus, most of the *Cat1* mRNA found in whole endosperms probably occurred in the aleurone, while a portion of that found in one-day-old germinating kernels could have represented mRNA deposited prior to seed maturation. While *Cat1* expression eclipsed

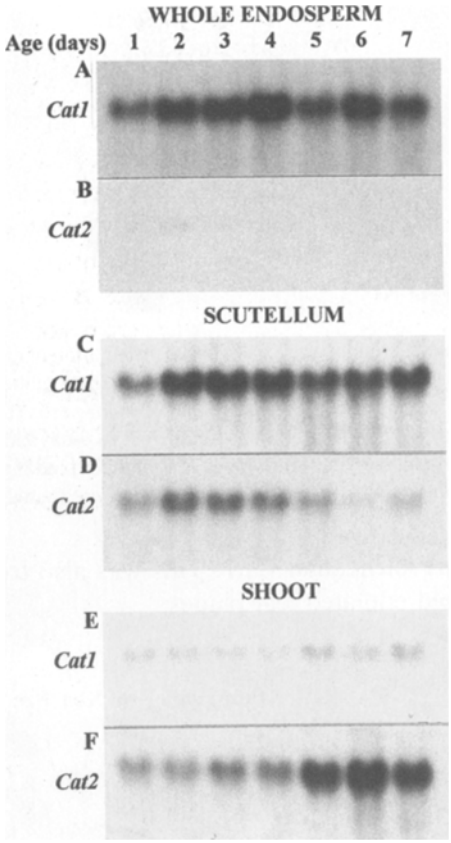

Fig. 2. Northern blot analysis of temporal and tissue-specific distribution of catalase mRNAs in barley seedlings. Duplicate blots were probed with a 3′ 400 bp fragment of the *Cat1* clone (panels A, C and E) or a 3′ 440 bp fragment of the *Cat2* clone (panels B, D and F). All lanes contained 15 μg of total RNA from whole endosperm (aleurones plus starchy endosperm, panels A and B), scutella (panels C and D) or etiolated shoots (leaf plus coleoptile, panels E and F). Age is given in terms of days from the onset of imbibition. Autoradiograms were produced by a 48 h exposure.

that of *Cat2* in the non-dividing tissues of the germinating seed (aleurone and scutellum), the opposite occurred in developing shoots (leaves plus coleoptiles), where *Cat2* mRNA was much

Fig. 1. Alignment of deduced amino acid sequence from barley *Cat1* and *Cat2* catalase cDNA clones and other cloned monocot catalases. The upper four sequences (group I) are separated by a space from the lower three sequences (group II) to distinguish them as possibly distinct isozymal groups. Sequences which are identical to barley CAT-1 are represented by a period, and numbering is with respect to barley CAT-1. Gaps inserted to create an alignment are represented by a dash. HVCAT1 and 2, barley CAT-1 and -2; ZMCAT1, 2 and 3, maize CAT-1, -2 and -3, OSCATA and B, rice CAT A and B. Asterisks denote possible microbody targeting signals (position 493–495 in group I and 9–11 in group II).

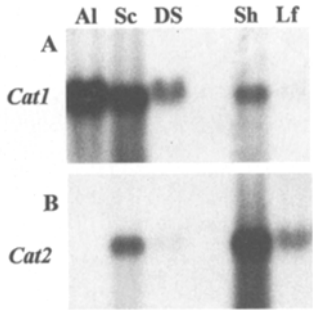

Fig. 3. Northern blot analysis of tissue distribution of catalase mRNAs. Duplicate blots containing RNA from gibberellin-treated aleurones (Al), scutella (Sc), developing seeds (DS) 25 days after pollination, etiolated shoots of 3- to 6-day old seedlings (Sh) and etiolated leaf blades of 8-day-old seedlings (Lf) were probed with either *Cat1* (panel A) or *Cat2* (panel B).

more prevalent than *Cat1*. This was also true of 8-day-old etiolated leaf blades.

Gene organization

Gene copy number and organization were assessed on duplicate Southern blots of genomic DNA cleaved with restriction enzymes which did not cut either of the full-length cDNA clones: *Apa* I, *Eco* RI, *Xba* I and *Sac* I. Blots were probed first with full-length insert DNA. They were then stripped and reprobed with either a 3′ 400 bp *Eco* RV fragment of *Cat1* or a 3′ 440 bp *Eco* RV/*Sma* I fragment of *Cat2*. When probed with full-length *Cat1*, several bands appeared, especially in the *Eco* RI lane (Fig. 4A). When reprobed with a 3′ fragment, only two bands appeared, indicating that either two *Cat1* genes exist or that the authentic gene is accompanied by a pseudogene (Fig. 4B).

The full-length *Cat2* clone detected one band of

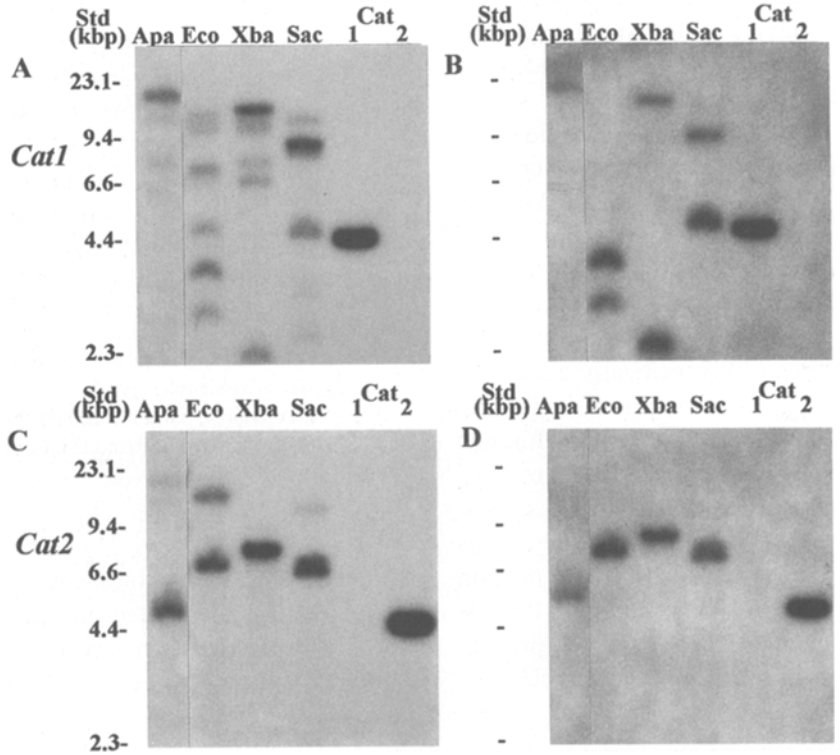

Fig. 4. Southern blot analysis of barley catalase nuclear gene organization. Genomic DNA (10 μg per lane) from etiolated leaves of cv. Morex was restriction-cut with *Apa* I, *Eco* RI, *Xba* I or *Sac* I. Duplicate blots were first probed with full-length insert DNA from the *Cat1* (panel A) and *Cat2* (panel C) clones. After autoradiography, the probe was stripped from the filters, and the filters were reprobed with a 3′ 400 bp *Cat1* fragment (panel B) or a 3′ 440 bp *Cat2* fragment (panel D). Lanes 5 and 6 in each blot contained 50 pg of plasmid DNA from either the full-length *Cat1* or *Cat2* cDNA clones. The clones were linearized with *Sac* I prior to electrophoresis. The positions of the molecular size marker DNAs (λ phage DNA cut with *Hind* III) are given to the left.

major intensity and one minor band in *Apa* I, *Eco* RI and *Sac* I lanes (Fig. 4C). After reprobing with the 3′ fragment, only the initial major band was detected, indicating that there is only one copy of the *Cat2* gene (Fig. 4D). A possible doublet, each close to 7 kb, occurred in the *Sac* I lane. These products could have resulted if a *Sac* I site is present in an intron. The complete lack of cross-hybridization between the full-length *Cat1* and *Cat2* clone controls (lanes 5 and 6) confirmed the specificity of the hybridization.

Mapping of Cat2 *on the barley genome*

Mapping attempts were motivated by previous reports which, based on isozyme data, indicated that catalase genes are located on barley chromosomes 4 and 6 [1, 39] and by the desire to locate the *Mlo* powdery mildew resistance locus. The *Cat2* cDNA clone was used as a probe in Southern analysis of a series of *mlo* backcross lines [15]. This detected a RFLP in five out of eight *mlo* backcross lines (data not shown). This finding indicated a close genetic linkage to the *mlo* resistance locus which has been previously mapped with RFLP markers to barley chromosome 4 [15]. A detailed mapping was performed with the *Cat2* cDNA probe and DNA from 256 individuals of an F2 population from the cross *Hordeum vulgare* subsp. *vulgare* cv. Carlsberg II Mlo × cv. G. Zwciz. mlo11. A *Rsa* I polymorphism allowed us to map the *Cat2* gene within a 0.9 cM interval bordered by RFLP markers bAL88/2 and bAP91 and at a distance of ca. 2.8 cM in centromeric orientation from the *mlo* locus (Fig. 5).

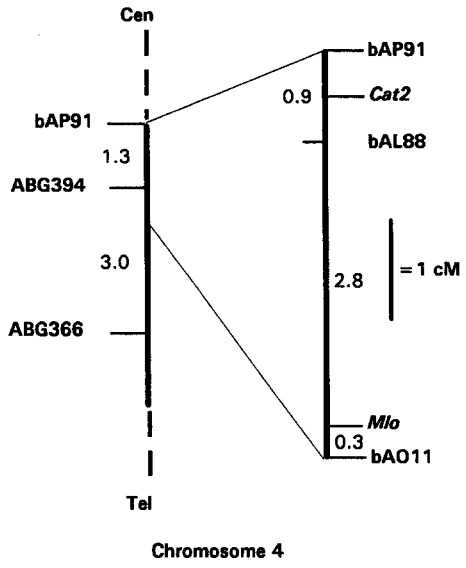

Fig. 5. Marker assisted mapping of the *Cat2* locus on chromosome 4 of the barley genome. The left map represents loci determined on the cv. Steptoe × cv. Morex genome map [18], and the right map represents loci determined by fine mapping conducted with a Carlsberg II Mlo × G. Zwciz. mlo 11 cross. *Cat2* is shown with respect to the powdery mildew resistance locus (*Mlo*). Positions are given with respect to the centromere (Cen) and telomere (Tel).

Discussion

The finding that the amino acid sequences of barley, rice and maize catalases fall into two distinct groups corroborates the distinctions made from enzymological studies of barley and maize [14]. Barley CAT-1, maize CAT-1 and rice CAT B clearly fall into one group (group I, Fig. 1), based upon amino acid homologies, while maize CAT-2 can more loosely be included in this group. Barley CAT-2, maize CAT–3 and rice CAT A form a second distinct group (II). Although these are the only monocot catalase genes sequenced to date, this pattern may hold for other monocot cereals. Enzymological studies [14] have shown that barley catalases can be distinguished as to having a peroxidatic (T-CAT) or enhanced peroxidatic (EP-CAT) function. In maize leaves, the EP-CAT form corresponds to CAT-3 isozyme, found only in mesophyll cells [38]. CAT-3 is also expressed preferentially in leaves of dark-grown maize [28]. Similarly, EP-CAT activity is found in dark-grown barley. While T-CAT activity in barley is greatly enhanced by light, EP-CAT activity is unaffected by light and contributes 10% of the total catalase activity in mature greenhouse-grown leaves [14]. By functional analogy and amino acid sequence homology, CAT-2 of barley would correspond to CAT-3 of maize. A mutation which greatly reduces levels of the T-CAT

(CAT-1) of barley weakens the plant so that it has difficulty surviving under photorespiratory conditions [17]. T-CAT is thought to correspond to a light-induced isozyme of barley whose mRNA has homology to *Cat2* of maize (Acevedo *et al.*, unpublished).

Despite enzymatic distinctions, active site amino acids H65, S104 and N138, proximal heme-binding ligands P333, R351 and Y355 and distal heme-binding ligands V63, T105, F143 and F351 [7] are all conserved among monocots (Fig. 1) and other plants, animals and prokaryotes [25]. However, differences in neighboring amino acids occur between the two monocot catalase groups at active site N138 and at all three proximal heme ligands. These differences are conserved among all members of group II. The existence of catalase isozymes presents a dilemma in analyzing evolutionary relatedness. It may not be useful to focus upon a single catalase isozyme when utilizing catalase sequences to construct evolutionary trees [25]. A determination must first be made as to whether the isozymes within a species differ due to gene duplication and divergence or by ancient acquisition of an endosymbiont. An evolutionary tree of the Graminae, based upon prolamin sequences, places barley closer to rice, with maize more distantly related [3]. This is supported by our finding that barley CAT-1 and -2 are more closely related to rice A and B than to the maize CATs.

The SRL sequence located near the carboxyl terminus of barley CAT-1 (Fig. 1) suggests that it is targeted to peroxisomes and/or glyoxysomes [11, 12]. This tripeptide is shared by the other members of group I, including maize CAT-2, but not by those of group II. However, all group II members contain an SSS sequence near the N-terminus (position 9–11), which has been shown to be a peroxisomal targeting signal in cotton catalase [19]. In addition, all of the monocot CATs, with the exception of maize CAT-2, have a PILLEDYHL sequence near the N-terminus (position 39–47). This sequence is quite similar to a concensus peroxisomal targeting sequence occurring near the N-terminus of watermelon glyoxysomal malate dehydrogenase and some mammalian peroxisomal proteins (RL/I-X5-HL) [10]. All members of group II have almost exactly the same amino acid sequence up to the active site H65. Since the maize CAT-3 of this group is associated with mitochondria, the barley CAT sequences were analyzed for features found in mitochondrial presequences [13]. The first 64 amino acids of both are not particularly rich in the highly charged amino acids R, K and H, but the region from P39 to V64 is predicted to form an amphiphilic α-helix with an uninterrupted hydrophobic face. Thus, there is sufficient basis to predict that both barley CATs could be imported into subcellular organelles.

In maize, the CAT-1 isozyme is expressed at low levels in all tissues. Although this does not occur in barley, the distribution pattern of barley catalase mRNAs has similarities with the patterns found in maize [32]. Barley *Cat1* and *Cat2* mRNAs occur together in the scutellum (Fig. 2). Thus, there is an opportunity for enzyme subunit heterotetramers to form, as they do in maize scutella [32]. In scutella of maize seedlings, the CAT-1 isozyme appears first and then declines as the CAT–2 isozyme increases to much higher relative levels and, in some cases, persists for at least nine days. In the barley scutellum, *Cat2* mRNA declines early, while *Cat1* mRNA reaches higher levels and persists for at least 7 days. In etiolated leaves of barley, *Cat2* mRNA is abundant, while *Cat1* is almost absent. This is similar to maize, where *Cat3* is abundant, but *Cat2* is not expressed without exposure to light [35]. (We have not conducted studies to determine whether similar light responses occur in barley.) In the aleurone of maize, *Cat2* mRNA is abundant, while *Cat3* is absent. This is again similar to barley, where *Cat1* is abundant, but *Cat2* is absent. Thus, barley *Cat1* shares expression features with maize *Cat2*, and barley *Cat2* shares expression features with maize *Cat3* and the maize scutellar CAT-1 isozyme.

Preliminary data indicated that *Cat2* was closely associated with powdery mildew resistance gene (*mlo*) in barley (Schulze-Lefert and Hinze, unpublished). This was intriguing because of the association of catalase with systemic ac-

quired resistance (SAR, [4]) and the involvement of H_2O_2 in the hypersensitive resistance response [21]. However, fine mapping has shown that *Cat2* is in fact separated from *mlo* by 2.8 cM (Fig. 5). This mapping study has placed *Cat2* on the long arm of chromosome 4. A previous mapping study placed *Cat1* near the telomere of the short arm of chromosome 1 [18]. (This catalase locus was incorrectly named *Cat3* and is being changed to *Cat1*.) We do not know whether the two genes for *Cat1* are located nearby on the same chromosome or at a distant locus which may have been undetected by mapping efforts. In castor bean, the *Cat1* and *Cat2* catalase gene occur in a cis configuration, separated by only 2.4 kb [37].

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