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CHARACTERIZATION OF THE WHEAT cDNA ENCODING THE β SUBUNIT OF THE MITOCHONDRIAL ATP SYNTHASE

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ABSTRACT

A wheat cDNA encoding an open reading frame of 553 amino acids with a deduced amino acid sequence corresponding to the mitochondrial β subunit of the synthase was isolated. The expression of the β ATPase was investigated in leaves of 7-day-old wheat plants, and a decrease in the abundance of transcripts along the leaf was observed. The cDNA of the β ATPase was mapped on the group 1 chromosomes of wheat. Phylogenetic analysis of the mitochondrial β subunit of the ATPase complex is described.

INTRODUCTION

The proton-translocating F1-F0 ATPase (ATP synthase) complex plays an essential role in the terminal step of the energy production of living organisms. The structure, function, and biogenesis of F1-F0 ATPase complex have been studied in many organisms (Futai et al., 1989; Hamasur and Glaser, 1992). The structure of the enzyme is highly conserved in prokaryotic and eukaryotic organisms. The enzyme consists of two distinct parts, a hydrophilic F1 part containing the nucleotide binding site that catalyzes the ATP hydrolysis, and a hydrophobic F0 part that conducts protons through the membrana (Senior, 1988). The hydrophilic F1 portion has similar structures in both prokaryotes and eukaryotes, and consists of five subunits α, β, χ, δ, and ε, with a stoichiometry of 3:3:1:1:1. In contrast, the subunit composition of the hydrophobic membrane-bound F0 varies between species (Walker et al., 1985). The mitochondrial enzyme complex has been purified from mammals, yeast, and plants (Tzagoloff et al., 1979; Hatefi, 1985; Hamasur and Glaser, 1992).

In plant cells, the ATP-synthesizing complex is present in both chloroplasts and mitochondria. The β subunit of the mitochondrial F1-F0 ATP synthase is a nuclear-encoded protein that contains at least one nucleotide-binding domain, and its maximal catalytic activity is achieved by interaction between the α and β subunits (Senior and Wise, 1983; Pedersen and Amzel, 1993). A high degree of sequence conservation among the β subunits of bacterial, chloroplast, and mitochondrial complexes has been

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found (Runswick and Walker, 1983).

The β subunit of the mitochondrial ATP synthase was shown to be encoded by two nuclear genes in *Nicotiana plumbaginifolia* (Boutry and Chua, 1985). In maize two cDNAs encoding β ATP synthase were isolated (Ehrenshaft and Bramble, 1990; Winning et al., 1990), and the nucleotide sequences of rice (Sakamoto et al., 1992), carrot (accession number X60303), and rubber tree (Chye and Tan, 1992) have been published. Comparison of the N-terminals among plant mitochondrial β ATPase subunits reveals high levels of similarity (Hamasur and Glaser, 1992).

In this study the cDNA coding for the wheat mitochondrial subunit β of the F1 ATPase complex was isolated. The sequence was chromosomally mapped and the phylogenetic analysis is described.

MATERIALS AND METHODS

cDNA LIBRARY SCREENING, DNA LABELING, CLONE PURIFICATION, AND DNA SEQUENCING

A wheat root cDNA λGT11 library (Clonetech, Palo Alto, CA) was screened with a 1536 bp NcoI-SalI fragment of the *Nicotiana plumbaginifolia* ATPase subunit β DNA (Bountry and Chua, 1985) at 60 °C (hybridization in 5 × SSPE, 2.5 × Denhart, 0.25 w/v SDS), and final washes were done in 0.2 × SSPE, 0.1% SDS at 60 °C. (1 × SSPE = 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA pH 7.4). The DNA fragments were labeled by the multiprime DNA labeling system (Amersham). Five positive clones of 1.1–1.2 Kbp were isolated and cloned at the EcoRI site of Bluescript plasmid vector for sequence analysis. The sequence was determined using the sequence version 2 enzyme kit (USB). Four clones contained the 3' COOH terminal of the cDNA from nucleotide +768 to ± 1801 and one clone contained the 5' NH_2 terminus of the cDNA from nucleotide –40 to ± 1132. Therefore the clones covered by overlapping the full coding sequence.

The nucleotide sequence data appear in the EMBL Gene Bank under the accession number X7454 *T. aestivum* atp2

RNA ISOLATION AND NORTHERN BLOT ANALYSIS

Leaves were detached from 7-day-old wheat plantlets and cut into 1-cm pieces representing each 0.5 cm of the leaf (numbered lanes 1–7 in Fig. 2) starting from the leaf base. Centimeters 6–7 from the leaf base are represented in lane 8 (Fig. 2). The plant material was extracted by the guanidium-HCl method (Logeman et al., 1987). Total RNA was separated on 1.2% agarose gel under denaturative conditions and transferred to a nylon membrane (Hybond N, Amersham, UK). The membrane was hybridized with the wheat 1.2 kbp fragment representing the N terminus of the clone, and after the probe was washed it was rehybridized with the wheat 26S rRNA EcoRI BamHI 900 bp' fragment from pTA71 for RNA quantitation (Gerlach and Bedbrook, 1979). The quantitation was measured with a phospho imager and scanned. Parallel filters were hybridized with a 900 bp SacI-EcoRI fragment of the maize mitochondrial ATPase subunit α (Braun and Levings, 1985).

The mapping of the ATPase on the wheat genone was performed as described in Devos et al. (1992).

RESULTS AND DISCUSSION

ISOLATION OF THE cDNA ENCODING THE β SUBUNIT OF THE WHEAT MITOCHONDRIAL F1 ATPASE

Wheat *Triticum aestivum* cDNA clones were isolated from a library constructed using root tip mRNA in a λGT11 vector. The library was screened using the 1536 bp NcoI-SalI fragment of *N. plumbaginifolia atp* b radiolabeled probe (Boutry and Chua, 1985). Five positive clones were obtained from screening 150,000 plaques. The cDNA sequences of four clones were identical representing, 1100 bp of the cDNA at the 3' end and including the poly A stretch. One clone of 1,200 bp had the N'-terminal part of the gene and a 364 bp sequence overlapping the other clones. The combined cDNA clones represent the full length *atp* β cDNA consisting of 1,891 bp, of which 1,659 bp comprise the open reading frame starting at position 14 and stopping at position 1673 with a TGA stop codon. The deduced 553 amino acid sequence represents a 59 kDa protein homologous to other mitochondrial F1-ATPase β subunits (Fig. 1). Similar to other proteins which are imported into organelles, the wheat β ATPase possesses a presequence at its N-terminal which has been demonstrated in other species to function as a targeting signal that is cleaved upon transport (Hamasur and Glaser, 1992).

The deduced amino acid sequence of the wheat F1β ATPase was compared to the mature protein isolated from spinach and *N. plumbaginiflia* (Boutry and Chua, 1985; Hamasur and Glaser, 1992; Chaumont et al., 1994). Since the sequences are well conserved we speculate that the wheat mature protein starts with alanine at position 48, corresponding to alanine at position 55 in *N. plumbaginifolia* (Chaumont et al., 1994).

The wheat mitochondrial presequence is rich in positively charged and hydroxylated amino acid residues. Twelve out of 18 amino acids at the amino terminus are serines, leucines, and arginines, which resembles other mitochondrial targeting sequences (von Heijne et al., 1989). Several conserved domains which have been described as important for catalytic activities of the β subunit of the ATPase complex in other organisms have been identified in the deduced amino acid sequence of the wheat F1β ATPase. A catalytic adenine nucleotide binding site with a conserved tyrosine at position 427, was present within a highly conserved dodecapeptide starting at position 418; a noncatalytic binding site with a conserved tyrosine at position 443 (Wise et al., 1987); and a glutamic acid residue at position 270 within another highly conserved segment between residues 253 to 273, proposed to be involved in magnesium binding to the active site of the subunits (Yoshida et al., 1981). These motifs are all present in the wheat-deduced sequence when compared to bacteria, yeast, chloroplast, and plant mitochondria F1β ATPase (Howe et al., 1985; Takeda et al., 1985; Wise et al., 1987; Ehrenshaft and Bramble, 1990). In addition, a tripartite domain defined in adenylate kinase to be essential for Mg ATP binding (Fry et al., 1986) was detected in the wheat F1β ATPase. (The characteristic fragments start at positions 227 (GGAGVGKT), 239 (LI), and 321 (GQDVLLFID).

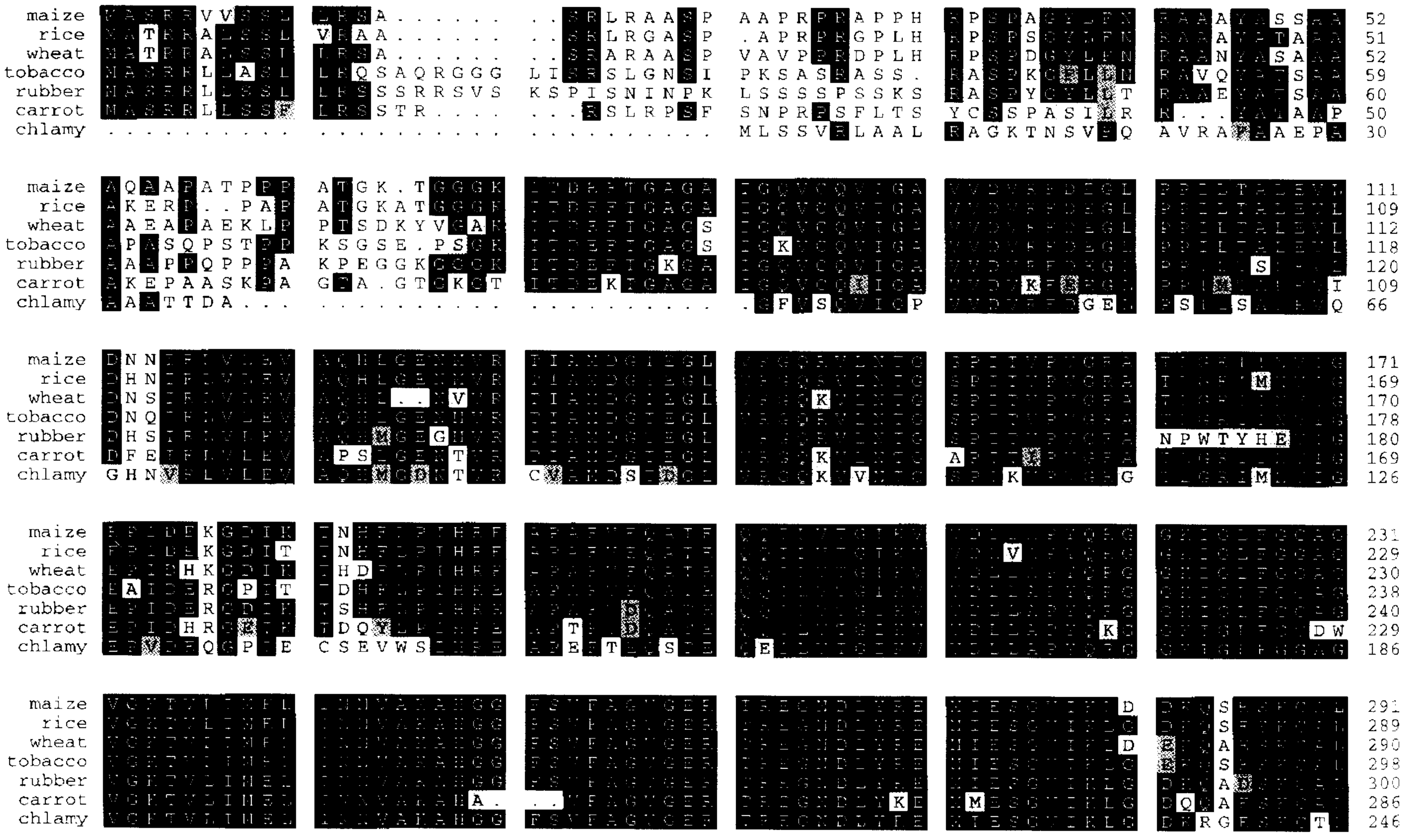

Fig. 1. Comparison of the deduced amino acid sequence of mitochondrial β APTase from wheat with sequences from maize (Winning et al., 1990), rice (Sakamoto et al., 1992), tobacco (Boutry and Chua, 1985), rubber tree (Chye and Tan, 1992), carrot (X60303), and *Chlamydomonas* (Franzen and Falk, 1992). The alignment was done using the program prettybox of the GCG package (Version 7). Identical residues are shown on black; conserved substitutions have grey background.

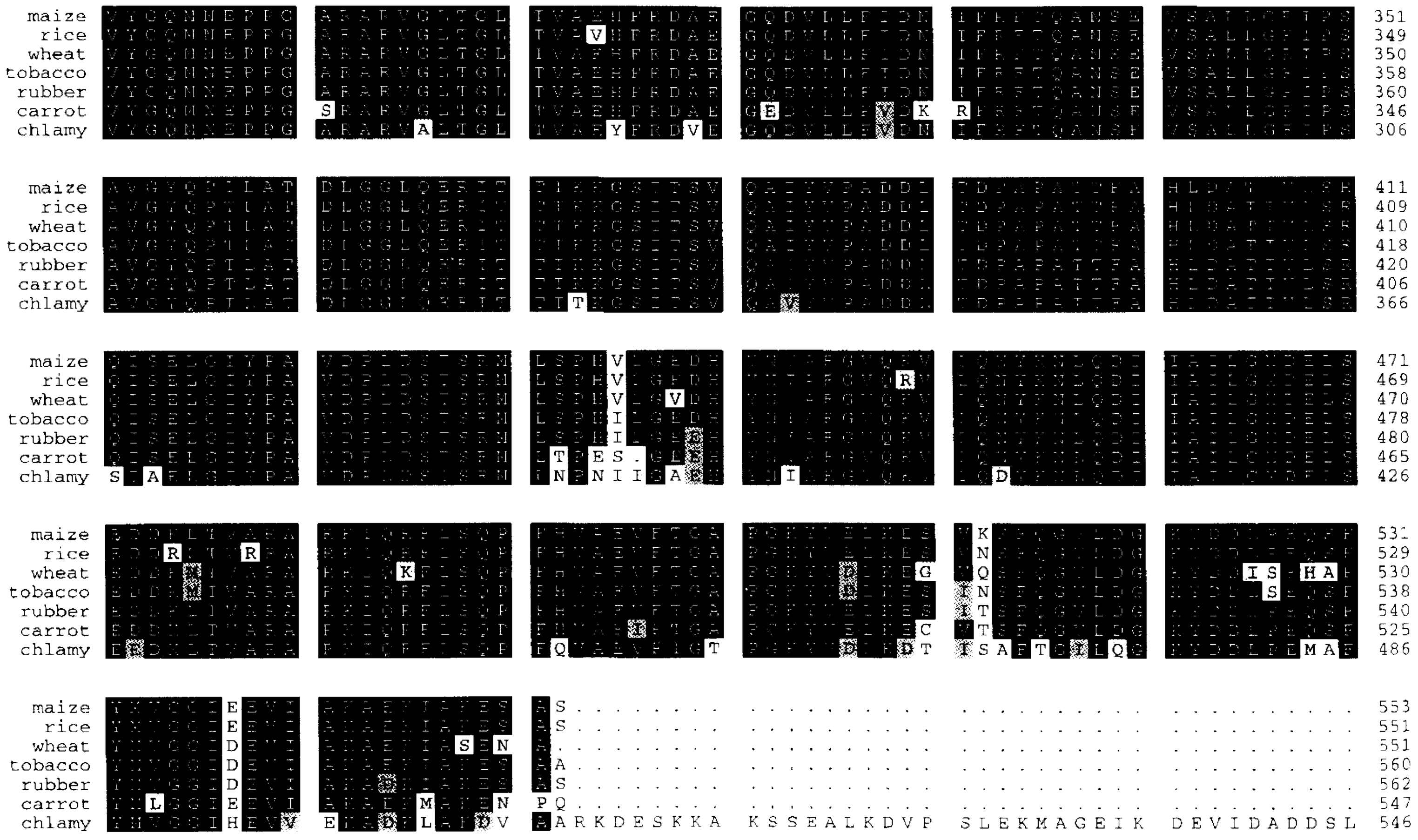

Fig. 1. Continued.

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  maize  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 553
   rice  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 551
  wheat  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 551
tobacco  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 560
 rubber  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 562
 carrot  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . 547
 chlamy  E E D F K A E A I S   S E N M V L N E K G   E K V P L P K K 574
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Fig. 1. continued.

EXPRESSION PATTERN OF THE WHEAT MITOCHONDRIAL atp β cDNA

The expression of the *atp* β was determined along the length of a 7-day-old wheat leaf. The leaf contains a linear progression of cellular age, and differentiation from small densely cytoplasmic nonvacuolated meristematic cells at the leaf base to vacuolated actively photosynthesizing expanded cells at the distal tip. This sequence of cellular differentiation is paralleled by a functional differentiation in which the energy supply changes from oxidative phosphorylation in meristematic cells to a combined dependence on oxidative-and photo-phosphorylation in the photosynthesizing cells. Topping and Leaver (1980) have shown that mitochondrial gene expression decreases during wheat development. The study of the nuclear encoded wheat mitochondrial *atp* β revealed a decrease in abundance of the transcripts along the leaf (Figs. 2, 3).

The relative steady-state abundance was calibrated according to the ribosomal RNA present in each lane and the quantitation of the RNA-hybridization was performed with a phosphoimager. The abundance of *atp* β in the basal first centimeter of the leaf was 5–10-fold higher than in the distal sections (Fig. 3). Similar results have been reported for the nuclear-encoded mitochondrial protein ADP/ATP translocator of maize (Bathgate et al.,1989). The basal meristematic tissue of cereals is not photosynthetically active. We assume that as the tissue matures and becomes photosynthetically competent, expression of mitochondrial genes and nuclear genes encoding mitochondrial products decreases parallel with a decrease in mitochondrial number.

To correlate the expression of a nuclear-encoded mitochondrial protein to mitochondrial-encoded proteins, the steady-state level of *atp* β was compared to the transcript abundance of the mitochondrial-encoded *atp* α subunit (Figs. 2, 3). Although a decline in the abundance of *atp* α was observed, it was only 2.5-fold higher in the basal first centimeter as compared to the distal sections.

The higher level of *atp* α as compared to *atp* β (Fig. 2) may be explained by additional functions of *atp* α (i.e., chaperone activity) (Avni et al.,1992; Yuan and Douglas, 1992; Alconada et al., 1994). Although mitochondrial biogenesis appears to be a major factor affecting the steady-state level of mitochondrial transcripts, additional factors general to all organisms, such as oxygen concentration and cell type, or specific factors, such as carbon source (in yeasts), light (in plants), or hormones (in animals), are probably involved in the regulation of the genes encoding the F1-F0 ATPase complex (Gemel and Randall, 1992; Winde and Grivell, 1992; Heddi et al., 1993; Haraguchi et al., 1994).

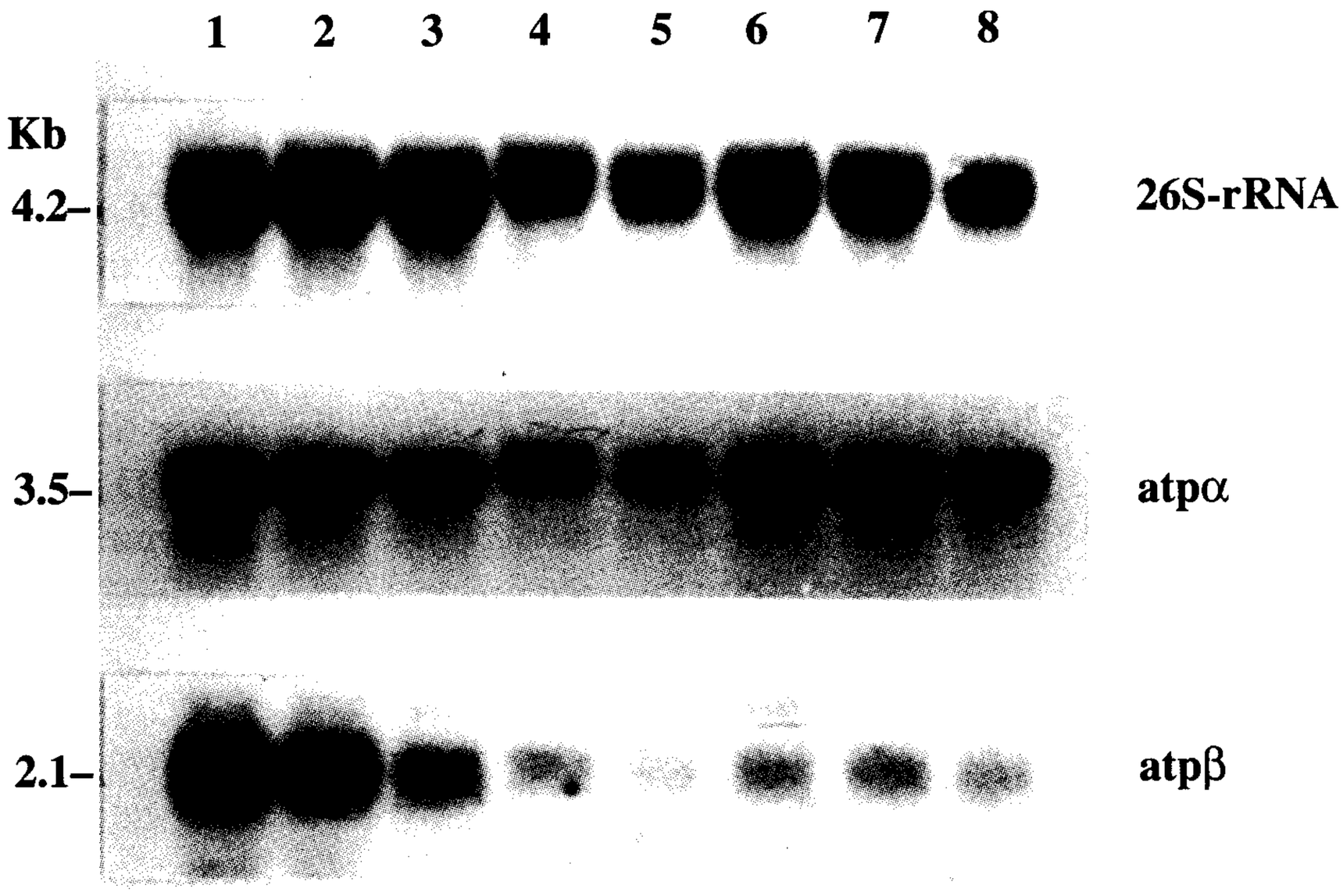

Fig. 2. Autoradiography of Northern blot representing the abundance of *atp* β, *atp* α relative to the the 26S rRNA along the leaf. Wheat leaves were cut at the given distances from the leaf basis. Lane 1 (0–0.5 cm), lane 2 (0.5–1 cm), lane 3 (1–1.5 cm), lane 4 (1.5–2 cm), lane 5 (2–2.5 cm), lane 6 (2.5–3 cm), lane 7 (3–4 cm), lane 8 (6–7 cm).

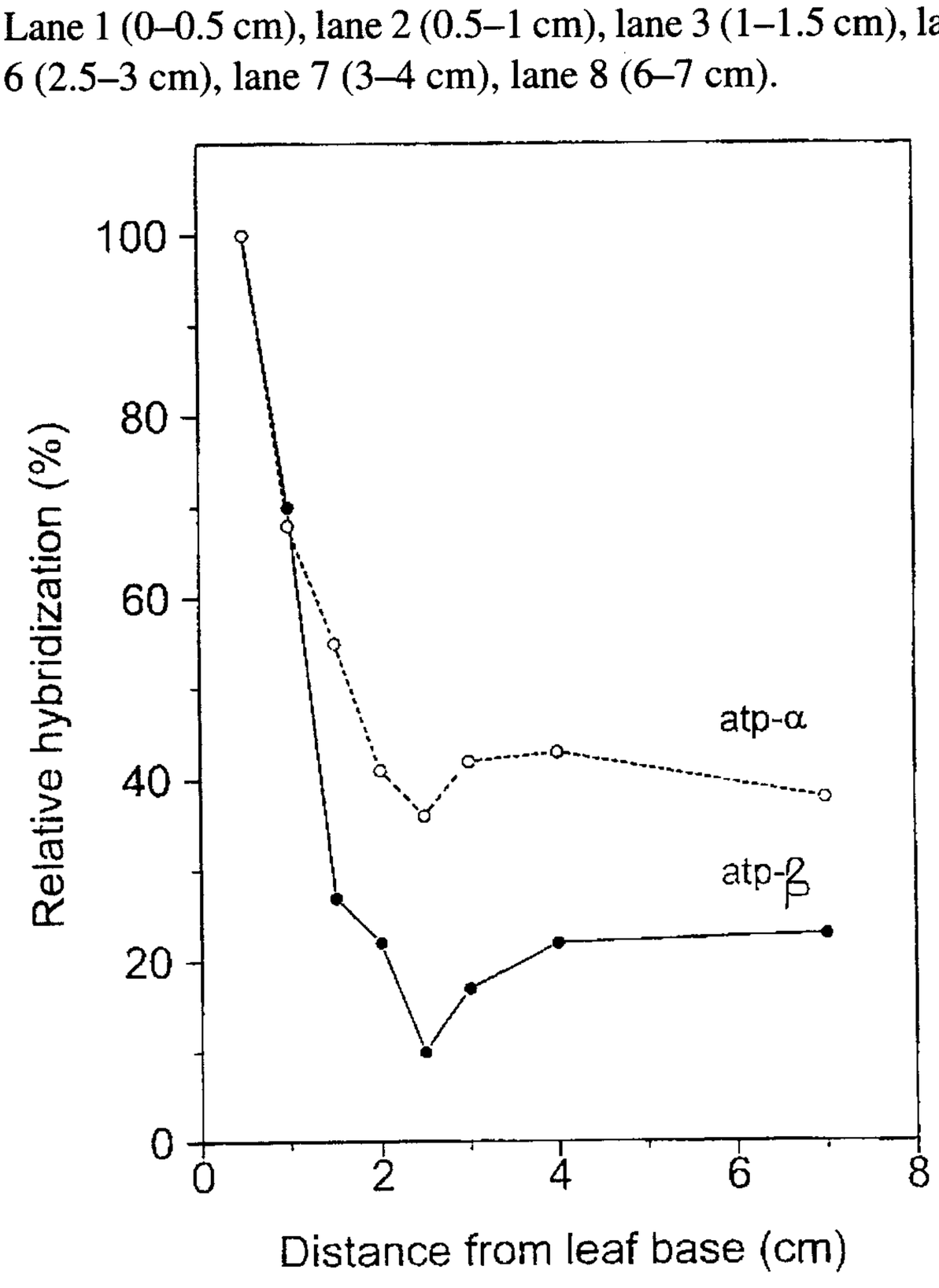

Fig. 3. Relative changes in the steady-state abundance of mRNA coding for the wheat mitochondrial *atp* β and *atp* α as compared to the rRNA (Korek, 1994) along a 7-day-old wheat leaf, based on the Northern data represented in Fig. 2.

MAPPING OF THE atp β cDNA *ON THE WHEAT GENOME BY SOUTHERN ANALYSIS*

To identify the chromosomal location of the *atp* β gene, we used the mapping system developed for wheat (Devos et al., 1992). Hybridization of the *atp* β cDNA to 21 nulitetrasomic (NT) lines, each lacking one chromosome pair, showed *atp* β to be located on the long arm of the homologous group 1 chromosome (1BL, 1DL) (Fig. 4). The *atp* β cDNA hybridizes to one major DNA fragment presumed to be the main locus in the group 1 chromosome. The other bands seen in Fig. 3 are probably due to slight cross hybridization with loci on group 3 chromosomes. Similarly, a strong and a weak band were detected in the barley lines (lanes 2–5). RFLP variation could be detected in the segregating F2 lines for the fragments located on chromosome B and D (data not shown). From these results it appears that *atp* β belongs to a low copy number gene family (one gene on each chromosome) showing a moderate degree of polymorphism.

PHYLOGENETIC ANALYSIS OF β *ATPASE SEQUENCES*

All analyses were performed at the amino acid level. Hypervariable regions were

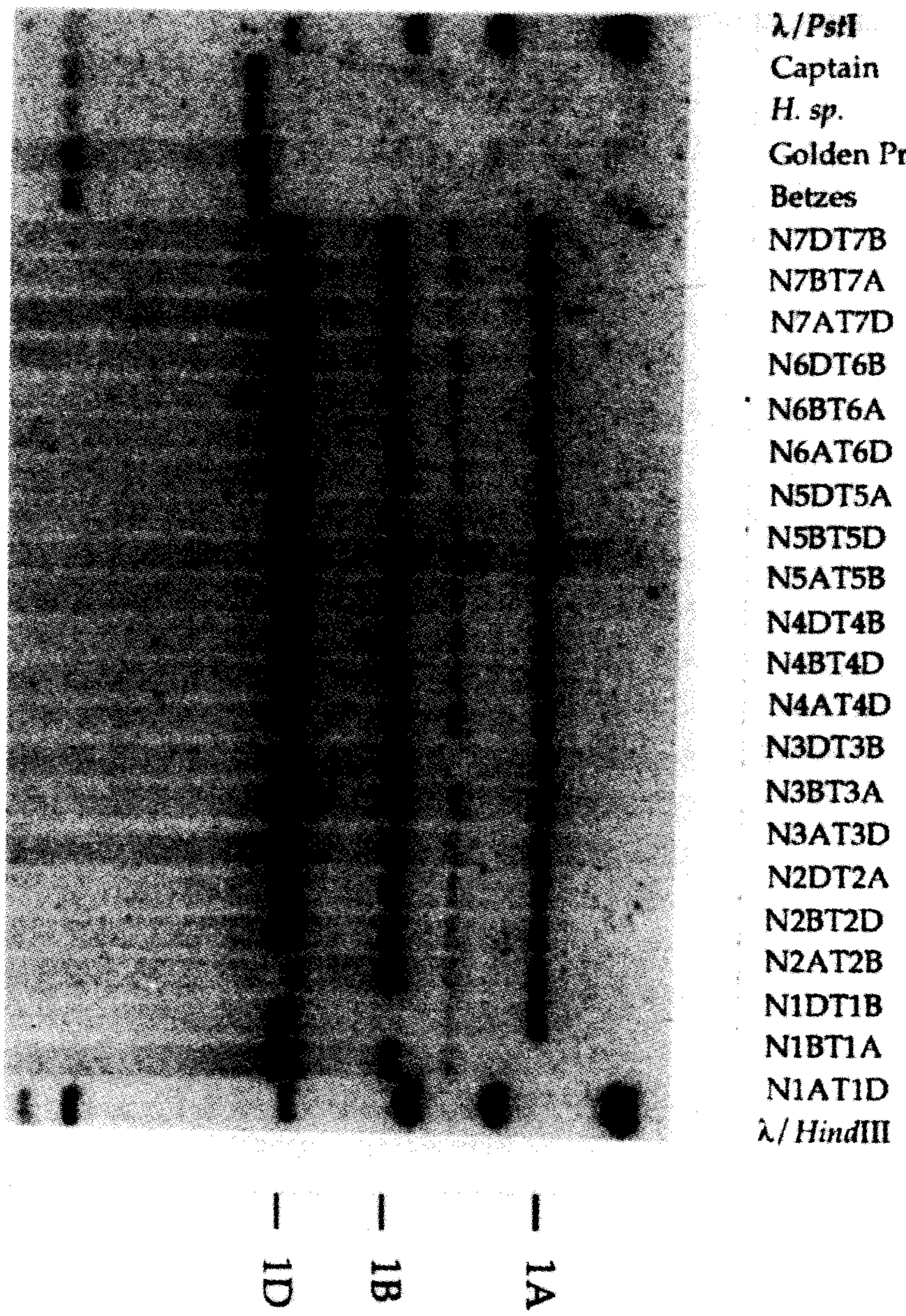

Fig. 4. Southern blot hybridization of DraI-digested DNA from nullitetra (NT) wheat individuals (lanes 6–26), barley cultivars (lanes 1, 2, 4), and wild barley *(Hordeum spontaneum)* (lane 3). Lanes 1 and 27 are size markers of λPst I and λHind III, respectively.

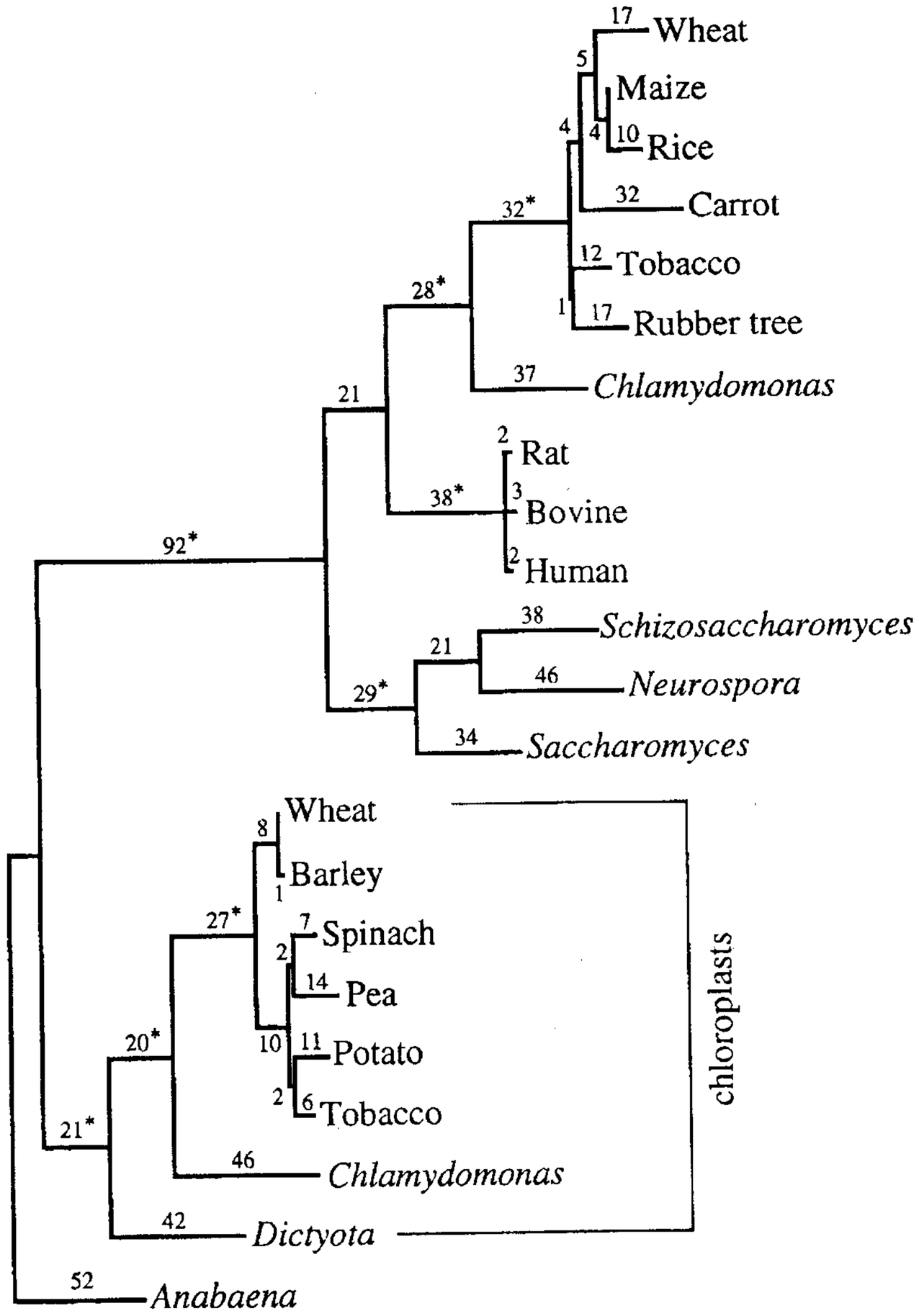

Fig. 5. Neighbor-joining phylogenic tree for a sample of β ATPase amino acid sequences. Minimum numbers of amino acid replacements are marked in the branches. Asterisks denote bootstrap values above 95%.

excluded. The sequences were taken from SWISSPROT (Bairoch and Boeckmann, 1992) and positional homology was ascertained by alignment with CLUSTAL V (Higgins et al., 1992). General heuristic searches for identifying maximum parsimony trees were performed on PAUP (Swofford, 1991). When possible, statistical significance of internal branches has been computed by bootstrap (Felsenstein, 1985). Depending on the analysis, either *Anabaena* or human has been used to root the trees.

With 22 representative taxa, two maximum parsimony trees were identified. In Fig. 5 we see that all sequences can be classified in one of two monophyletic groups: Nuclear and chloroplastic. Therefore, the duplication of the gene giving rise to the nuclear and chloroplastic sequences probably has occurred prior to the divergence among kingdoms Fungi, Plantae, and Animalia. As with other genes in plants, the nuclear sequences evolve much faster than their chloroplastic homologues (Wolfe et al., 1989). Within the nuclear sequences, three subgroups are clearly distinguished: fungi, animals, and plants. This suggests that all nuclear genes have a monophyletic origin. The wheat sequence clusters with the other monocot sequences, but no distinction is obtained between

monocots and dicots. Whether dicots are polyphyletic or monophyletic cannot be decided on the basis of this single gene.

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