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Cytoplasmic ribosomal protein S15a from *Brassica napus*: Molecular cloning and developmental expression in mitotically active tissues

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Abstract

We have isolated two cDNA clones which appear to encode the 40S ribosomal subunit protein S15a from *Brassica napus* (oilseed rape). The open reading frame in both clones contains 390 bases, encoding a deduced polypeptide sequence of 130 amino acids (100% homology between clones) with 76% sequence identity to the N-terminal 37 amino acids of the rat ribosomal protein S15a and 80% identity to the S24 polypeptide of yeast. Both the yeast and rapeseed proteins have a net positive charge of +9 and the rapeseed S15a protein has a molecular mass of 14778 Da compared to 14762 Da for the yeast protein. The rapeseed ribosomal protein S15a is encoded by a small multi-gene family with at least two actively transcribed members. A single transcript of ca. 1.0 kb, corresponding to ribosomal protein S15a, is abundant in actively dividing tissues such as apical meristem, flower buds and young leaves and less abundant in mature stem and fully expanded leaves.

Introduction

Protein synthesis in all organisms requires the co-ordinate expression and assembly into functional ribosomes of 3 to 4 ribosomal RNAs (rRNA) and 70–80 different ribosomal proteins (r-proteins). Regulation of rRNA and r-protein synthesis in *Escherichia coli* is largely dependent upon growth conditions or nutrient availability [1]; however, fine control of r-protein synthesis is achieved by autogenous translational feedback of the various r-protein operons [2]. The prokaryotic nature of the chloroplast, coupled with the recent sequencing of three plastid genomes [3–5], suggests that feedback mechanisms similar to those found in *E. coli* may be involved in the regulation of plastid r-protein gene expression [6, 7]. The majority of work on eukaryotic cytosolic r-protein gene expression has concentrated on animals and yeast, both of which demonstrate some co-ordinate regulation of rRNA and r-protein gene expression during development. The manner in which r-protein synthesis is regulated varies not only from organism to organism but also between different developmental stages of a single organism. For example, during

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X59983 (pPCB8) and X59984 (pA813).

spore germination in *Dictyostelium discoideum*, synthesis of the majority of r-proteins is regulated transcriptionally, whereas this changes in vegetative amoeba where the synthesis of at least three r-proteins is regulated translationally [8]. Studies in *Xenopus leavis* have shown that r-protein levels during embryogenesis are controlled translationally, post-translationally and post-transcriptionally by r-protein feedback on the processing and stability of their mRNA transcripts [9–12]. Furthermore, alterations to the gene number of r-proteins L1 [9], S6 [10] and L14 [13] by microinjection into *Xenopus* oocytes shows that r-protein in excess of that incorporated into ribosomal subunits is rapidly degraded. Individual synthesis of each of the 70–80 r-proteins in *X. laevis* [12] and *Drosophila melanogaster* [14] does not appear to be coupled to mRNA, rRNA or other r-protein accumulation. However, recent work with *Neurospora crassa* suggests that the synthesis of r-protein S14 is co-ordinated with the transcription of both 5S and 40S rRNA genes [15].

In contrast to animals, very little work has been reported on cytosolic r-protein gene expression in plants. A number of genes or cDNA clones for cytosolic r-proteins in soybean [16], pea [21], maize [17, 18], rice [19], barley [20] and *Arabidopsis thaliana* [21, 22] have been isolated and some have been used to study r-protein expression in plant development. A 3–8-fold coordinated increase in nine r-protein mRNAs has been reported in radially enlarged and proliferating cells of soybean hypocotyls, after a 24 h application of the synthetic auxin 2,4-dichlorophenoxy acetic acid [16]. High levels of endogenous auxin are indicative of rapidly dividing cells, with lower levels observed in quiescent tissues. Transcript levels of maize S14 [17] and S11 [18] and barley L17 [20] were found to be highest in seedling shoot (S14 and S11), developing endosperm (S14) and root meristem and young leaf cells (L17): all tissues undergoing rapid cell division. We are currently investigating the gene expression of mitotically active apical meristem tissue of *Brassica napus*. We report here the isolation and characterisation of two cDNAs which appear to encode the 40S ribosomal subunit protein S15a. Both clones were further used to investigate tissue specificity of expression as well as their phylogenetic relationships within *B. napus*, *B. oleracea* and *B. campestris*.

Materials and methods

Plant material

Plants of *Brassica napus* cv. Westar, used in the construction of cDNA libraries, were cultivated in growth chambers under a 13 h/11 h light/dark cycle and a 25 °C/20 °C day/night temperature cycle for 27 days. *B. napus* and *B. campestris* cv. Tobin plants used for DNA extraction were cultivated under a 16 h/8 h light/dark cycle with a 23 °C/19 °C day/night temperature cycle for 21 days prior to harvesting leaf material. *B. oleracea* DNA was extracted from broccoli florets obtained from the local grocery.

DNA extraction and analysis

Plant DNA was extracted, digested, separated on a 0.6% agarose gel and blotted onto Hybond N (Amersham) membrane as described in Wiersma *et al.* [23]. The nylon membrane was prehybridised at 65 °C in 6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate pH 7.0), 1% SDS, 5 × Denhardt's solution (1 × Denhardt's solution = 0.02% PVP, 0.02% Ficoll, 0.02% BSA), 0.2 mg yeast tRNA, 0.2 mg poly(A) and 0.2 mg herring sperm DNA for 6 h. ^{32}P-labelled probe was added to the prehybridisation solution and the incubation was continued at 65 °C for 18 h. The filter was washed once in 5 × SSC with 0.1% SDS, once in 2 × SSC with 0.1% SDS and once in 1 × SSC with 1.0% SDS at 65 °C for 30 min each. Filters were exposed to Kodak X-OMAT AR X-ray film at −70 °C with intensifying screens.

RNA extraction and analysis

Total RNA was extracted from various *B. napus* tissue samples by the method of Logemann *et al.*

[24]. Poly(A)$^+$ RNA was prepared by oligo(dT) cellulose chromatography following the manufacturer's instructions (Pharmacia). 10 μg of Poly(A)$^+$ RNA (unless otherwise stated) was separated on a 0.66 M formaldehyde gel [25] and transferred to GeneScreen Plus nylon membranes (NEN) by capillary blotting in 20 × SSC. The membrane was fixed for 5 min on a UV transilluminator (Spectroline, model TC-302) and baked for 2 h at 80 °C before prehybridising in 6 × SSPE (1 × SSPE = 0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA pH 7.4), 1% SDS, 2.5 × Denhardt's, 50% deionised formamide, 0.2 mg yeast tRNA and 0.2 mg herring sperm DNA at 43 °C for approximately 6 h. Filters were hybridised in the same prehybridisation solution supplemented with ^{32}P-labelled probe, 0.2 mg yeast tRNA and 0.2 mg herring sperm DNA, at 43 °C for 16–18 h. After hybridisation the filters were washed and filmed as described above for DNA analysis.

Synthesis and cloning of cDNA

Two procedures for cDNA synthesis were used.
First library. cDNA was synthesised from 1 μg of apical meristem poly(A)$^+$ RNA by the procedure of Bellemare *et al.* [26]. Single-stranded plasmid-cDNA was electroporated into DH5α cells as described in Dower *et al.* [27]. The clone pPCB8 was isolated from the resulting partial library and subsequently recloned into pBSKS + (Bluescript, Stratagene) for sequencing.
Second library. First- and second-strand cDNA were synthesised by a modification of the procedure of Steikema *et al.* [28]. Poly(A)$^+$ RNA (10 μg) and oligo-dT$_{(12\text{-}18)}$ (2 μg), in a final volume of 17 μl, were incubated for 15 min at 65 °C followed by 15 min on ice. 30 μl of mixture I (100 mM Tris-HCl pH 8.3, 10 mM $MgCl_2$, 150 mM KCl, 20 mM DDT, 1 mM each of dATP, dGTP, dCTP, dTTP) and 60 U AMV reverse transcriptase (Pharmacia) were added and first-strand synthesis was carried out at 42 °C for 75 min. To the first-strand reaction mixture was added 150 μl of ice-cold mixture II (40 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 75 mM KCl, 25 μg/ml BSA), followed by incubation at 14 °C for 5 min. Second-strand synthesis was carried out at 14 °C for 60 min after the addition of 40 U DNA polymerase I (Pharmacia), 2 U RNAse H (Pharmacia), 32 μM dATP, dGTP, dCTP, dTTP and 2 μl α-^{32}P-dATP (6000 Ci/mmol = 220000 GBq/mmol; Amersham). The second strand was ligated by the addition of 2 μl of 20 mM Na_2ATP and 1 U T4 DNA ligase (Pharmacia) at 14 °C for 2 h. The double-stranded cDNA was end-filled, in the presence of 50 μM dNTPs and 5 U T4 DNA polymerase, at room temperature for 60 min. Methylation was carried out with the addition of 24 μM S-adenosyl-methionine, 12 mM EDTA and 80 U *Eco* RI methylase, for 60 min at 37 °C followed by 65 °C for 30 min. After separation on a 3 ml P60 Biogel (BioRad: 100–200 mesh) column, the cDNA fraction was freeze-dried, resuspended in water and added to 250 mM Tris-HCl pH 7.5, 50 mM $MgCl_2$, 25% PEG_{8000}, 5 mM Na_2ATP, 5 mM DTT and 1 U T4 DNA ligase for *Eco* RI linker addition. cDNA was size fractionated on a 1% agarose gel and cDNA more than 500 bp in length was electroeluted onto NA 45 paper (Schleicher and Schuell) following the manufacturer's protocol. The resulting double-stranded cDNA was ligated into λZAP II (Stratagene) and packaged using the Gigapack Gold system (Stratagene) following the procedures recommended by the manufacturer. Amplification of the resulting library was carried out in *E. coli* strain XL1-Blue (Stratagene).

pPCB8 was used to screen the second library and after 'Bluescript' plasmid rescue, two clones, pA613 and pA813, were analysed further. Subsequent sub-cloning and sequencing of these clones was carried out using pBSKS + / – and XLI-Blue.

DNA probes

DNA probes, approximately 20 ng, were labelled by the random-primer synthesis procedure [29] to $> 10^9$ dpm/μg DNA.

DNA sequencing

pPCB8 and pA813 were sequenced by the dideoxy chain termination method [30] using either T7 DNA polymerase (Pharmacia) or Sequenase (USB). Three oligonucleotides were used in sequencing both clones (Fig. 1): (1) 5′-GCACGGTTACATAGGTGAGT-3′ (Regional DNA Synthesis Laboratory, University of Calgary, Calgary, Alberta); (2) 5′-AGGCAGGTCATGATCAGGCCT-3′; and (3) 5′-ATCAAAACGTCCGCTGATAA-3′ (Cyclone, Milligen). Computer analysis of the sequence information was carried out with Mac Vector release 3.0 (IBI) and GenBank release 59.0.

Results

Sequence analysis of pPCB8 and pA813-rps15a *cDNAs*

Using the procedure of Bellemare *et al.* [26] we synthesised a partial cDNA library from apical meristem poly(A)$^+$ mRNA. During a search in the library for differentially expressed or apex-specific members, the clone pPCB8 (835 bp) was isolated. This clone was subsequently used to screen a second more complete cDNA library, from which pA813 (655 bp) and pA613 (579 bp) were isolated. The two clones pA613 and pPCB8 were found to be identical except that: (i) pA613 is 211 nucleotides shorter than pPCB8 at the 5′ end, (ii) the 3′ end of pA613 is extended by 33 nucleotides (ACATTTTTCTTTTGGTCTCAAGTTCTTGAGAGCC) and (iii) pA613 lacks a poly(A) tail. A restriction map of pPCB8 and pA813 is shown in Fig. 1. A search of GenBank release 59.0 demonstrated significant similarity (40%) between the nucleotide sequence of the rapeseed clones and that of yeast cytoplasmic r-protein S24 [31]. Due to the heterogeneity of r-protein nomenclature, it has been proposed that all cytoplasmic r-proteins be named with reference to the well characterized rat r-protein system [33]. Wool *et al.* [33] propose that the yeast r-protein S24 (YS22, RP50) be renamed YS15a

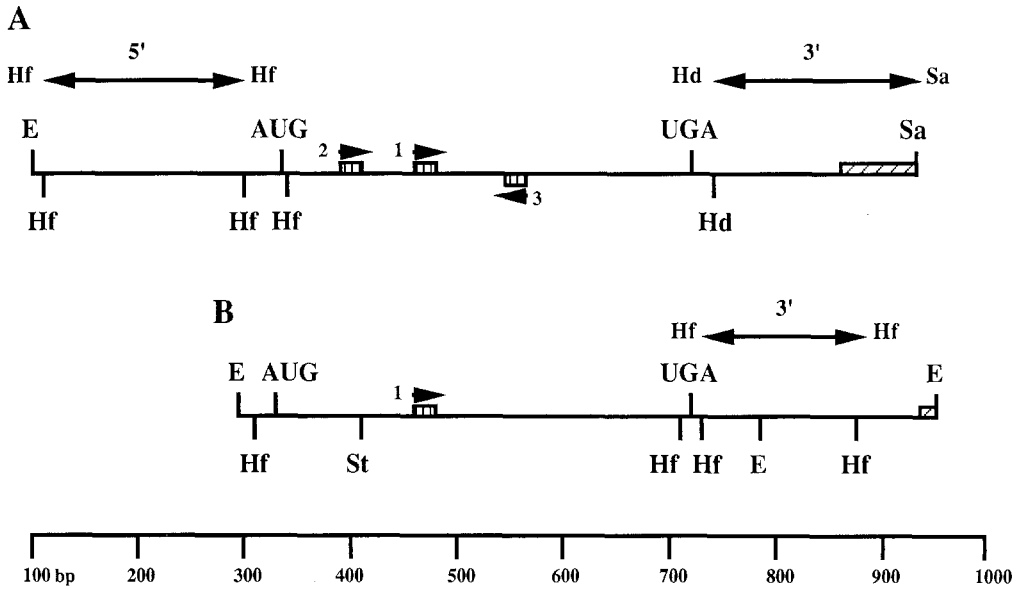

Fig. 1. Restriction enzyme maps for clones pPCB8 (A) and pA813 (B) containing the ORF for r-protein BnS15a from *Brassica napus*. E, *Eco* RI; Hd, *Hind* III; Hf, *Hinf* I; Sa, *Sal* I; St, *Stu* I. The oligonucleotides used in the sequencing of the 2 clones are designated 1, 2 and 3 (see Materials and methods for sequences) and their functional direction is indicated by arrowheads. 5′ and 3′ specific probes are indicated by two-headed arrows. The cross-hatched box at the 3′ end of each clone indicates the length of the poly(A) tail.

in recognition of its homology to the rat r-protein RS15a. For this reason the *Brassica napus* gene product will be referred to as BnS15a for the purposes of this discussion.

An open-reading frame (ORF) of 390 bases (comparable to that for yeast r-protein S24 (YS15a) [31]) was identified in both clones (Fig. 2). In pA813 the ORF is followed by a 3′ non-coding sequence of 227 bases, which includes a 16 nucleotide poly(A) tail starting at position 605. pPCB8 contains a 213 base 3′ non-coding sequence which includes a 78 nucleotide poly(A) tail at position 529. The consensus polyadenylation signal AATAAA, which is found 5′ to the poly(A) tail in the majority of plant mRNAs [32], is not present in the 3′ non-coding region of either clone. The absence of a polyadenylation signal has recently been reported in maize [18], *Arabidopsis thaliana* [21] and pea [21] cytoplasmic r-protein S11 cDNAs. However, pA813 does contain possible polyadenylation signals: AAGAA and AATAT, 39 and 89 nucleotides 5′ to the poly(A) tail, respectively. The predicted ATG initiation codon of both clones is the 5′ proximal ATG which is preceded by an in-frame TGA stop codon at position −6 in pA813 and an in-frame TAG stop codon at position −42 in pPCB8. Furthermore, the sequences flanking this

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pPCB8                                        GACAGTCTCGACTCTCTTAGCTCCCGC  -202
ATCAAAACTTTTTCGGCATATTCTTGTCTGAAGATTTTAGTGTTGCTGGTTAGCTTTACATAGGCGA  -135
ACTTAGGCATAGCTTGTTATTAGACTTAATATCAATCCTGCTTTGACTTTTGTTGCTTAACAGAGAG  -68
CTGTTGTGTTGTTCTCGGTGTGATTTAGTTATTTAGCAGAGGCTTTGAGTCTTTTTTTGGGAGAAAA  -1
pA813                                  GT**G*CT**CA*TTCGA**CCAAGCTCT***G*

MET val arg ile ser val leu asn asp ala leu lys ser met phe asn ala  17
ATG GTG AGA ATC AGT GTG CTC AAC GAT GCT CTC AAG AGC ATG TTC AAT GCC  51
*** *** **G *** *** *** **T *** *** *** *** *** **T *** *** *** **G

glu lys arg gly lys arg gln val met ile arg pro ser ser lys val ile  34
GAG AAG CGT GGG AAG AGG CAG GTC ATG ATC AGG CCT TCT TCC AAA GTC ATC  102
*** *** A*A *** *** *** *** **T *** *** *** *** *** *** *** *** ***

ile lys phe leu ile val met gln lys his gly tyr ile gly glu phe glu  51
ATC AAG TTC CTC ATC GTC ATG CAG AAG CAC GGT TAC ATA GGT GAG TTT GAG  153
*** *** **T *** **T *** *** *** **A *** *** *** **T *** *** **C ***

tyr val asp asp his arg ser gly lys ile val val glu leu asn gly arg  68
TAT GTT GAT GAC CAC CGA TCC GGC AAA ATC GTT GTC GAA TTG AAC GGG AGG  204
**C *** *** *** *** *** *** *** *** *** **T **G C*C **T **A **A C*T

leu asn lys cys gly val ile ser pro arg phe asp val gly val lys glu  85
TTG AAC AAG TGT GGC GTT ATC AGC CCA CGT TTT GAT GTT GGT GTC AAG GAG  255
*** *** *** *** **T *** **T **T **T *** *** *** **C **A **T *** ***

ile glu gly trp thr ala arg leu leu pro ser arg gln phe gly tyr ile  102
ATT GAA GGT TGG ACT GCC CGT CTG CTT CCT TCC AGA CAG TTT GGG TAC ATT  306
*** **G *** *** **C **T *** **T *** *** *** C** *** *** **C *** ***

val leu thr thr ser ala gly ile met asp his glu glu ala arg arg lys  119
GTG CTG ACT ACT TCC GCT GGC ATT ATG GAC CAT GAA GAA GCG AGG AGA AAG  357
**T **T **A **C *** *** **T **C *** *** **C *** *** **A *** **G ***

asn val gly gly lys val leu gly phe phe tyr                          130
AAT GTT GGA GGC AAG GTT CTT GGC TTC TTC TAT TGA AAAGCTTTATGGGGAAAGA  412
*** *** **T *** *** *** *** **A *** **T **C *** *G*AGGCA*GA*ACTCTTC

GACTTCTTCATTCTGTTTTTTTTTTTTTTTAAACTTTTGTCATCCATTTTCTTTGAGACTTCCTTCT  479
TTTG**CCAG*ATAC***GC**C*CC****GGTGAGGC*AGGAATTCGAAT*GA*CTCAAAGG*CAA

CCCTTTTTGTTTCGAATTTGTGGTAACTATGTTGGATAGTTCTTAAACTA78                528
AGAGGG*A**AGAGTT****AT**TTGGT*TC*TAG*TAA*ATGCC*T*TTGTTTGTTTTGCGCAAA  545

AGACTCGCAAATGTAGACGTAAGAAGTGCTTATTTTCAGACATTTTTTTATCTTCACTTA16      604
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Fig. 2. DNA sequence and deduced amino acid sequence of the two clones pPCB8 and pA813 encoding r-protein BnS15a from *Brassica napus*. Identical nucleotides are indicated by an asterisk(*). In-frame termination codons 5′ to the predicted ATG initiation codon are double-underlined and possible polyadenylation signals for pA813 are single-underlined.

ATG codon (AAAAATGGT in pPCB8 and AAGAATGGT in pA813) fit the consensus initiation sequence of AACAATGGC reported for plant genes [32]. Only 34 nucleotides of 5′ non-coding sequence is present in the pA813 clone, whereas pPCB8 contains 228 nucleotides of 5′ non-coding sequence. However, comparing the approximate size of the *rps15a* transcript (see below) with that of the two clones, it is probable that both clones are incomplete in 5′ non-coding sequence. At the nucleotide level there is 87% (342 of 390 bases) sequence identity within the two identified ORFs and with 44 of the 48 base differences occurring at the third base, or 'wobble' position, this translates into 100% identity at the amino acid level (Fig. 2). pA813 demonstrates a preference for T residues in 21 (48%) of these 'wobble' positions. In 14 (66%) of these positions, the T residue in pA813 has replaced a C residue in pPCB8. The 3′ and 5′ non-coding sequences of both clones show very little similarity suggesting that these two transcripts are the products of two different family members. The A + T content of both clones is highest in the 5′ (62% for pPCB8) and 3′ (70% for pPCB8 and 63% for pA813) non-coding regions, with these levels decreasing to 52% (pPCB8) and 54% (pA813) in the coding region.

The deduced amino acid sequence of the two rapeseed clones shows 80% sequence identity (104 of 130 amino acids) with that of the predicted yeast r-protein S24 (YS15a) sequence (Fig. 3). This level of identity increases to 89% if conservative exchanges are taken into account. Two blocks of four amino acids at positions 12 to 15 and 82 to 85 show no identity between rapeseed and yeast. The yeast *rps24* (*rps15a*) sequence codes for a protein of 14762 Da with a net positive charge of + 9 [31] whereas the rapeseed sequence, which also has a positive charge of + 9, codes for a protein of 14778 Da (Fig. 3).

Hydrophilicity profiles of the rapeseed and yeast proteins deduced from the algorithm of Kyte and Doolittle [34] show a very similar distribution of polar domains (Fig. 4). A hydrophilic region is present at both N- and C-terminals of the proteins, with the N-terminal hydrophilic region

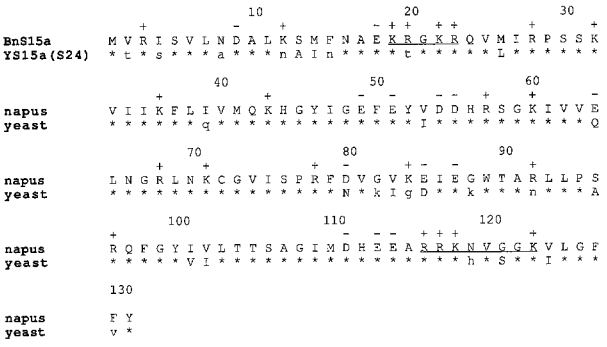

Fig. 3. Comparison of the deduced amino acid sequence of *Brassica napus* r-protein BnS15a with that of yeast r-protein S24 (YS15a) [31]. Identical residues are indicated by an asterisk (*), conservative differences are shown by upper-case letters and non-conservative exchanges by lower-case letters. For determining conservation, the following groups of amino acids were used: PAGST, neutral or weakly hydrophobic; QNED, acidic; HKR, basic; LIVM, hydrophobic; FYW, hydrophobic aromatic; C, cross-link-forming. Charged amino acids are indicated (+ or −) and putative nuclear localising sequences are underlined.

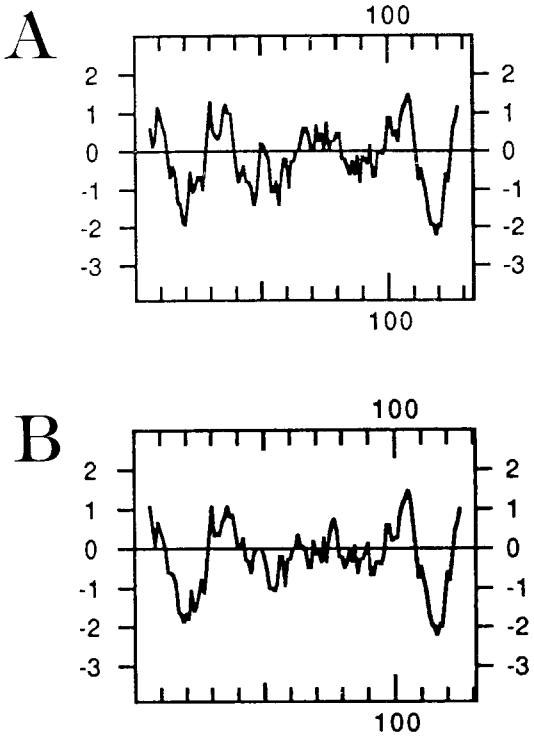

Fig. 4. Hydrophilicity index of the predicted rapeseed r-protein BnS15a (A) and yeast r-protein S24 (YS15a) (B) sequences, using the Kyte-Doolittle algorithm [34]. Negative numbers are hydrophilic and positive numbers are hydropathic.

being followed immediately by a hydrophobic region, whereas the C-terminal hydrophilic region is immediately preceded by a hydrophobic region.

Southern analysis of rps15a

On the basis of both cytogenetic evidence [35] and more recently using molecular probes [23], the amphidiploid species *B. napus* is generally regarded as being the product of an inter-species cross between the diploids *B. campestris* and *B. oleracea*. Probing a genomic Southern blot of *B. napus*, *B. campestris* and *B. oleracea* DNA with pPCB8 suggests that rapeseed r-protein BnS15a is encoded by a small multi-gene family of members arising from both *B. campestris* and *B. oleracea* (Fig. 5A). The same Southern blot, probed with the *Hin*f I-*Eco* RI 3′ non-coding region of

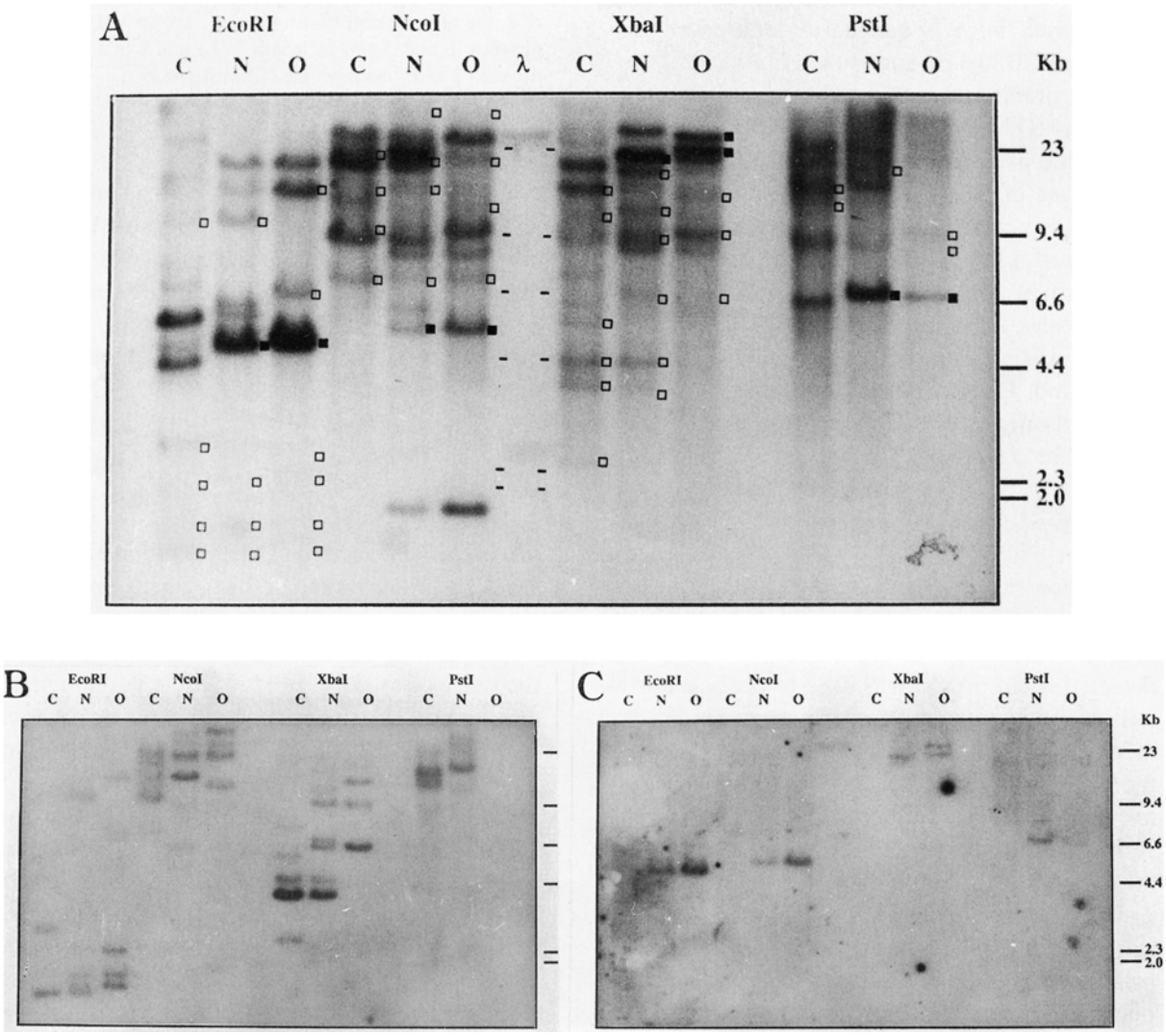

Fig. 5. Southern hybridisation of restriction enzyme digests of *Brassica campestris* (C), *B. napus* (N) and *B. oleracea* (O) genomic DNA with (A) pPCB8, (B) the pA813 3′-*Hin*f I-*Eco* RI fragment (see Fig. 1), and (C) pPCB8 3′-*Hin*dIII-*Sal* I fragment (see Fig. 1). The bands corresponding to those hybridising to the 3′ fragments of each clone are also indicated in A: □ pA813; ■ pPCB8. Genomic DNA was digested with *Eco* RI, *Nco* I, *Xba* I or *Pst* I as labelled and electrophoresed alongside λ/*Hin*dIII size markers as indicated.

pA813 (see Fig. 1) suggests that this gene in *B. napus* arose in both *B. campestris* and *B. oleracea* (Fig. 5B). However, only one band was highlighted in each of the *B. napus* lanes (Fig. 5C) when the Southern blot was probed with the *Hind*III-*Sal* I 3′ non-coding region of pPCB8 (see Fig. 1). The corresponding band was also identified in the *B. oleracea* lanes (as well as an extra band in the *Xba* I-digested lane) but no bands were found in the *B. campestris* lanes. The same pattern was produced when the Southern was probed with the *Hinf* I-*Hinf* I 5′ non-coding region of pPCB8 (data not shown).

It has previously been reported in maize, that r-proteins S11 [18] and S14 [17] are encoded by small multi-gene families of two to three members in the case of S11 and four to six members for S14. At least three of the S14 genes are actively transcribed [17]. Our results suggest that the *B. napus* r-protein BnS15a is encoded by a family of at least 2 genes, of which, pA813 was identified as arising from both parental strains *B. oleracea* and *B. campestris*, whereas pPCB8 was identified only in the *B. oleracea* parent.

Tissue-specific expression of rps15a

Expression of the r-protein BnS15a gene in various rapeseed tissues was examined. A single size of mRNA transcript of approximately 1.0 Kb was detected when pA813 was used to probe a northern blot of various *B. napus* tissues (Fig. 6). An identical pattern of expression was found when the northern blot was probed with pPCB8 (data not shown). The abundance of BnS15a transcripts in the mitotically active tissues of young flower buds, apical meristem and young leaf, compared to that observed in mature stem and expanded leaves, correlates well with the expression data previously reported for r-proteins in maize [17, 18], *Arabidopsis thaliana* [22] and barley [20]. In the latter report, L17 mRNA transcripts were detected in the basal, meristematic region of young leaves, with levels decreasing rapidly with distance (and maturity) from this region. High transcript levels were also found in young

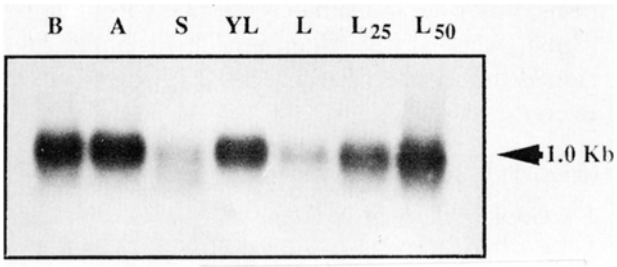

Fig. 6. Northern hybridisation of 10 μg of rapeseed poly(A)$^+$ RNA from 28-day-old plants: flowering bud (B), apical meristem and 1 cm subtending tissue (A), mature stem consisting of steele and cortical tissue from the crown (S), young leaf between 1 and 5 mm in length (YL) and expanded leaf of between 2 and 5 cm in length (L) with pA813. L_{25} and L_{50} are 25 μg and 50 μg of expanded leaf poly(A)$^+$ RNA, respectively.

internode (1–2 mm below the apical meristem) and root tissue including the root meristem (data not shown). These data suggest that expression of the genes represented by the pPCB8 and pA813 clones is developmentally regulated, with the highest transcript levels being present in actively dividing tissues.

Discussion

We have isolated two cDNA clones from a rapeseed apical meristem library which encode the cytoplasmic ribosomal small subunit protein BnS15a. The deduced amino acid sequences of the two clones show 100% identity and are highly conserved (80% identity) with the yeast r-protein S24 (YS15a) [31] and the N-terminal 37 amino acids (76% identity) of the rat r-protein S15a (I.G. Wool, personal communication). The conserved nature of r-protein S15a, between these three widely divergent species, suggests an evolutionary constraint on the structure of this protein, possibly due to an importance in the structural integrity and/or function of the ribosome. The typically basic nature of r-protein S15a, +9 in both yeast and rapeseed, could promote specific interactions between itself, rRNA, mRNA or other primary rRNA-binding r-proteins, required for ribonucleoprotein subunit structure. Two nuclear localisation sequences (NLS) have recently

been identified in the yeast r-protein L29 [36]. The sequences, KHRKHPG and KTRKHRG, resemble NLSs reported for other yeast r-proteins [37], SV40 large T antigen [38] and yeast histone H2B [39], in their basic nature. The hydrophilicity profiles of both the yeast and rapeseed proteins indicate the presence of two hydrophilic regions at 19–23 amino acids and 117–121 amino acids. It is evident that both of these hydrophilic regions have basic properties due to a predominance of lysine (K) and arginine (R) residues (KRGKR and RRKNVGGK). In the absence of definitive information on NLSs in plants, we are investigating the possibility that one or both of these motifs may act as NLSs for r-protein S15a.

Although the overall homology between the rapeseed and yeast S15a r-proteins is very high, there are two regions of four amino acids where this identity breaks down. Neither of these regions occur within the two major hydrophobic areas of the predicted proteins.

Multi-gene families of r-proteins are common to all eukaryotic organisms [40–42]. In mouse it is common for r-protein multi-gene families to contain up to 25 gene and pseudogene members with only one member being actively expressed [41, 43, 44]. The rapeseed *rps15a* gene is encoded by a small multi-gene family of at least two members, both of which we have shown to be transcriptionally active. These results correlate well with an earlier report in maize, a monocotyledonous plant, where at least three members of a six-member family, for r-protein S14, were found to be transcriptionally active [17]. As in mouse, it is possible that some of the non-transcribed family members could be pseudogenes. There is no major difference in genome size between mouse, rapeseed and maize which would account for the observed variation in size or content of the corresponding r-protein gene families. Furthermore, one would expect the larger gene family to contain more transcriptionally active members than a smaller family. In comparing mouse, rapeseed and maize, the opposite appears to be true. The presence of more than one transcriptionally active gene for r-proteins S14 in maize and S15a in rapeseed would allow for independent expression of these genes, and the subsequent formation of functionally distinct ribosomes, in a tissue-specific or developmentally specific manner.

The lack of nucleotide similarity, both 3′ and 5′ to the predicted ORF, in the two rapeseed clones has allowed us to use these two regions as specific probes. Many of the *rps15a* homologous fragments identified with both the whole clone and the 3′ non-coding region probe of pA813, in *B. napus*, have equivalent fragments in *B. campestris* and *B. oleracea*. This is consistent with the amphidiploid character of *B. napus* resulting from a recent sexual hybridisation event between *B. campestris* and *B. oleracea* [35]. However, one member of the *B. napus* S15a gene family, that encoded by pPCB8, came solely from *B. oleracea*.

Expression of both rapeseed clones was highest in mitotically active tissues such as young flower buds, apical meristems and young leaves and lowest in non-dividing tissues such as expanded leaf and mature stem. These results, as well as those previously reported in other plants [17, 18, 20, 22], suggest that ribosome r-protein levels, during development, are regulated by the mitotic activity of the cell.

In yeast, transcription of most r-protein genes appears to be regulated co-ordinately through a transcriptional activator sequence. UAS_{rpg}, located in the 5′ region of the gene [45]. In order to determine if similar elements are present in plants, the *rps15a* genes of *B. napus* are currently under investigation.

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