Theor. Appl. Genet. 64, 269–273 (1983)

The Endosperms of Common Cereals Contain Related Poly A$^+$RNA Sequences

D. Bartels and R. D. Thompson
Cytogenetics Department, Plant Breeding Institute, Trumpington, Cambridge (England)

Summary. mRNA has been isolated from the developing endosperms of four *Triticeae* species. Wheat endosperm mRNA has been used as a template to construct a bank of cDNA plasmid clones. More than 95% of these clones hybridized strongly to endosperm poly A$^+$RNA but not to shoot poly A$^+$RNA. ^{32}P-labelled mRNA from species related to wheat was used in filter hybridizations to detect inter-species sequence homologies. The wheat cDNA clones can be grouped into 4 classes: clones hybridizing to wheat RNA only, clones cross-hybridizing to barley endosperm poly A$^+$RNA, clones cross-hybridizing to rye endosperm poly A$^+$RNA, and clones showing homology to both barley and to rye RNA. Some of the sequence homology has been assigned to storage protein mRNA sequences.

Key words: Cereal – mRNA – Prolamin – Homology – Endosperm

Introduction

Developing cereal endosperm is the site of synthesis of major grain storage proteins. These proteins, termed prolamins and glutelins, are synthesized on membrane-bound polysomes (Brandt et al. 1978; Mathews and Miflin 1980; Forde et al. 1981; Greene 1981; Luthe and Peterson 1977), deposited in protein bodies during grain maturation (Shewry et al. 1980 b), and provide an important source of dietary protein. Together they constitute the majority of the cell-free translation products of RNA isolated from the developing endosperm. Prolamins and glutelins occur as complex families of polypeptides which have similar physical properties and amino acid compositions, but which are heterogeneous. Structural studies on the prolamins from wheat, barley and rye have identified amino-terminal sequence homologies between species. These homologous groups may be the products of gene families derived from common ancestral genes as proposed by Kasarda (1980) for the gliadins; however the extent of relatedness cannot be assessed without complete protein sequences. In order to assess the extent of sequence conservation in mRNAs coding for storage proteins, and as an aid to assigning cDNA clones to sequence related groups, we have tested by hybridization a bank of wheat cDNA clones derived from developing endosperm mRNA with poly A$^+$RNA probes from related cereals. We report the presence of sequence homology to different extents in all members of the *Triticeae* tested. Part of the homology is shown to be in mRNAs related to B-hordein (Forde et al. 1981).

Materials and Methods

Poly A$^+$ RNA Preparation

Developing endosperms were isolated from 2–3 week post-anthesis plants according to O'Dell and Thompson (1982). Poly A$^+$RNA was extracted according to Apel and Kloppstech (1978). Forty g of endosperm tissue was ground to a fine powder, suspended in 300 ml extraction buffer and extracted with phenol chloroform. The RNA was purified by one cycle of oligo dT-cellulose (Kloppstech and Schweiger 1976) and stored under liquid nitrogen. Shoot RNA was from 4 day-old etiolated shoot tips.

Preparation of cDNA Clone Bank to Wheat Poly A$^+$ RNA

Double-stranded cDNA was inserted into the Pst_1 site of pBR322 using homopolymeric GC tailing. A manuscript covering the detailed characterization of this clone bank is in preparation. Plasmid DNAs were prepared by lysis with Triton and CsCl-ethidium bromide equilibrium centrifugation.

α-^{32}P ATP End-labelling of Poly A$^+$ RNA

RNA samples in 25 µl of 50 mM Tris HCl pH 9.0, 10 mM $MgCl_2$, 5 mM dithiothreitol and 5% glycerol were partially hydrolysed by incubation at 100 °C for 1 min. The sample was

diluted with 25 µl of the same buffer and incubated with 20 µCi $-^{32}$P ATP (2000 Ci. mmol^{-1}, Amersham) and 4 units T4 polynucleotide kinase (BRL), at 37 °C for 30 min. Unincorporated ATP was removed by gel-filtration on G-100 Sephadex in 10 mM Tris HCl pH 8.0, 1 mM EDTA, 0.1% sodium lauryl sulphate (SLS).

Southern Filter Hybridizations

Plasmid DNA samples as indicated in the figure legends were fractionated by electrophoresis on 1% agarose gels and transferred to nitrocellulose filters according to Southern (1975). The hybridization conditions were 65 °C, 2×SSC, 0.1% SLS, 50 µg ml^{-1} poly r(A), 50 µg ml^{-1} tRNA for 18 h. Under these conditions, the proportion of acid-precipitable probe remaining in solution after hybridization was greater than 95%, and control filters loaded with cDNA clones to high and low abundance sequences showed that the amount of probe hybridized was in direct proportion to plasmid DNA bound, up to 1 µg plasmid DNA.

Results

A bank of over 500 cDNA clones was initially screened to identify those homologous to mRNAs in high concentration in endosperm but not shoot poly A$^+$ RNA of wheat. This screen also eliminated clones of ribosomal RNA sequences which occurred at low frequencies. This was done in two ways, (a) by the method of Grunstein and Hogness (1975) using ^{32}P-labelled poly A$^+$ RNAs from developing endosperm and from four-day old shoots as hybridization probes and (b) by hybridization with the two ^{32}P-labelled RNA probes to purified, filter-bound plasmid DNA samples. Over 95% of the cDNA clones hybridized only to endosperm poly A$^+$ RNA. This indicates that most of the poly A$^+$ RNA. This indicates that most of the poly A$^+$ RNA in the developing endosperm is absent from or at very low levels in shoot cells.

One hundred cDNA clones containing endosperm-specific sequences were hybridized with ^{32}P-labelled poly A$^+$ RNA from endosperm of a hexaploid wheat (*Triticum aestivum* var. 'Chinese Spring', genome type AABBDD), a diploid wheat (*Triticum monococcum* ssp. *boeoticum,* genome type AA) (Johnson 1975), rye (*Secale cereale* var. 'Lovasz patonai'), barley (*Hordeum vulgare* var. 'Sundance'), and oats (*Avena sativa* var. 'Maris Tabard'). The results for twenty representative clones are shown in Fig. 1. There was considerable variation between clones in the extent of hybridization to poly A$^+$ RNA from wheat which was not a function of insert size. This presumably reflects the variation in the concentration of different RNA sequences in the total wheat endosperm poly A$^+$ RNA.

The extent of hybridization of individual clones to wheat (Fig. 1 a) and *T. monococcum* (Fig. 1 b) poly A$^+$ RNA was similar. Genetic analysis of the major storage proteins of hexaploid wheat has shown that all three genomes (A, B and D) specify storage proteins. The similar levels of hybridization of the clones to poly A$^+$ RNAs from the diploid and hexaploid species therefore suggest the poly A$^+$RNAs from the A, B and D genomes are closely related and produced in similar proportions.

The diploid wheat poly A$^+$RNA complement is more similar to that of hexaploid wheat than to rye or barley poly A$^+$RNA. Hybridization of rye poly A$^+$ RNA was detected to only 7 of the 20 clones (Fig. 1 c) and of barley poly A$^+$RNA to 5 of the 20 clones (Fig. 1 d). Three other clones which hybridize strongly to wheat or *T. monococcum* poly A$^+$RNA hybridized weakly or not at all to barley and rye poly A$^+$RNAs. This indicates that a major RNA sequence in wheat and *T. monococcum* does not have a close relative in similar concentration in barley or rye.

These experiments have recently been repeated with 100 wheat cDNA clones, 12% of which hybridized to rye only, 12% to barley only, 25% to rye and barley, and 50% to wheat but not rye or barley RNAs.

There was no detectable hybridization of the wheat cDNA clones with poly A$^+$RNA from endosperm of oats, a more distant relative of wheat which synthesizes globulins rather than prolamins as the predominant storage proteins.

The most abundant mRNAs in developing barley endosperm are those coding for B-group hordeins. We therefore hybridized the series of 20 wheat clones with a B-hordein cDNA probe, obtained by labelling the insert of pC179 (Forde et al. 1981). Two of the wheat cDNA clones which cross-hybridized to barley clones showed strong homology with the B-hordein cDNA (Fig. 1 f).

Sequences showing cross-hybridization are not necessarily identical. In order to determine the relatedness of the hybridizing sequences, further cross-hybridization experiments were carried out varying the washing criterion (Kafatos et al. 1979). Under high stringency washing conditions, only nearly identical sequences are retained as hybrids. Hybrids stable only at low stringency have shorter regions of homology.

Wheat, barley and rye poly A$^+$RNAs were hybridized to filter-bound DNA from three wheat cDNA clones, selected for their distinct hybridization properties, including a clone which showed homology to a B-hordein gene (clones 1–3 in Fig. 1), to barley B-hordein clone pC179 (Forde et al. 1981), and as a control to the plasmid vector pBR322 (Fig. 2).

The increasingly stringent washing conditions showed some discrimination between the within-species and between-species hybrids. A higher proportion of the within-species DNA duplexes (wheat RNA- wheat clones and barley RNA-pC179) than the between-

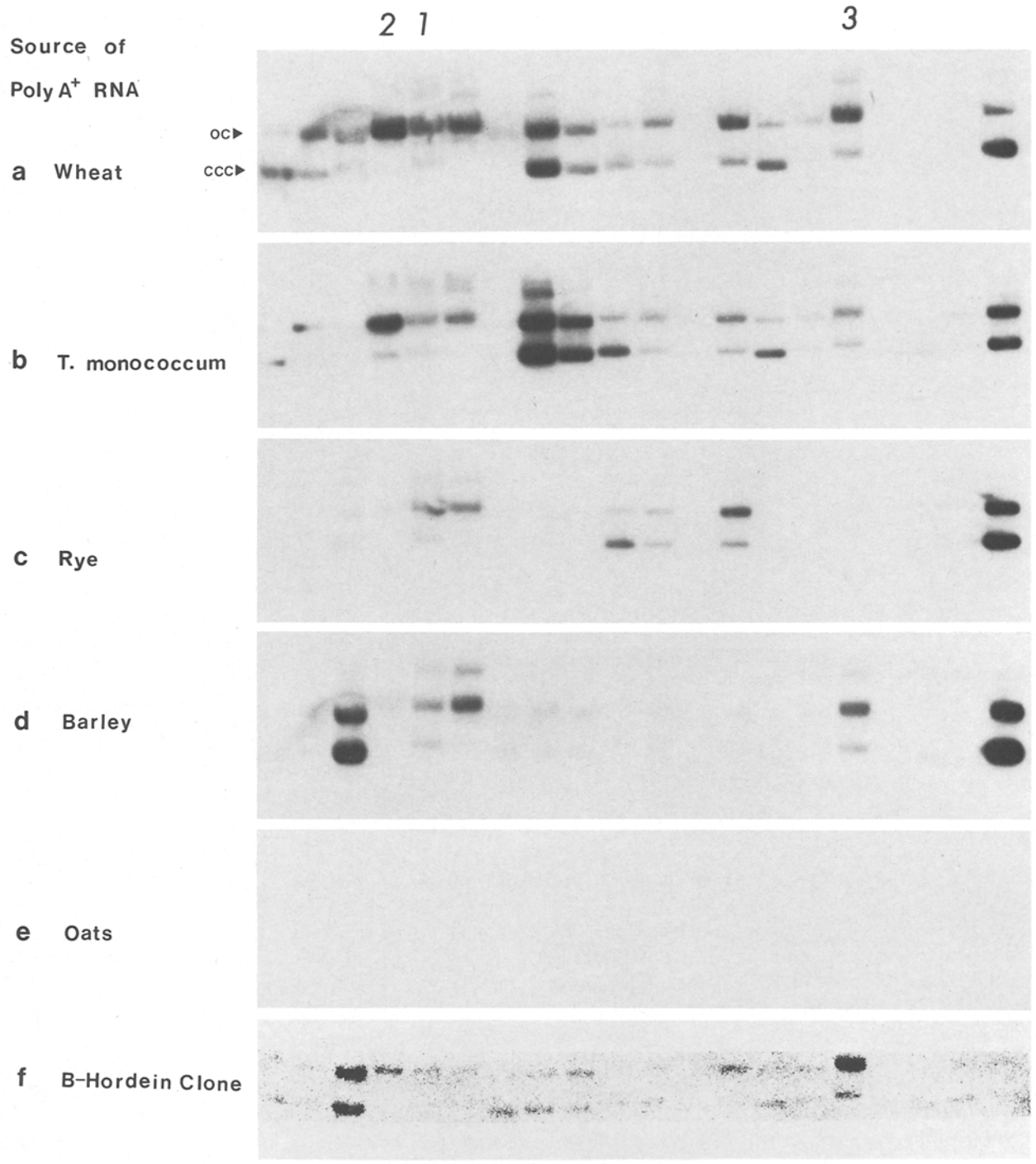

Fig. 1. Wheat cDNA clone hybridizations to cereal poly A+ RNAs (*a* – *e*) and a B-hordein cDNA clone (*f*). 0.4 μg undigested plasmid DNA of 20 endosperm-specific wheat cDNA clones were fractionated by electrophoresis on 1% agarose and transferred to nitrocellulose according to Southern (1975). Six identical filters were prepared and five were hybridized each with 2 μg of ^{32}P-labelled poly A+ RNA from (*a*) wheat. *T. aestivum*, (*b*) *T. monococcum*, (*c*) rye, (*d*) barley, (*e*) oats. The sixth filter was probed with 0.1 μg isolated insert of the B-hordein clone pC179 (Forde et al. 1981), which was labelled by nick-translation (Maniatis et al. 1975). The strong upper and lower bands represent the closed circular (CCC) and the open circular (OC) forms of the plasmid DNA. A faint higher third band in some samples is due to the presence of the dimer

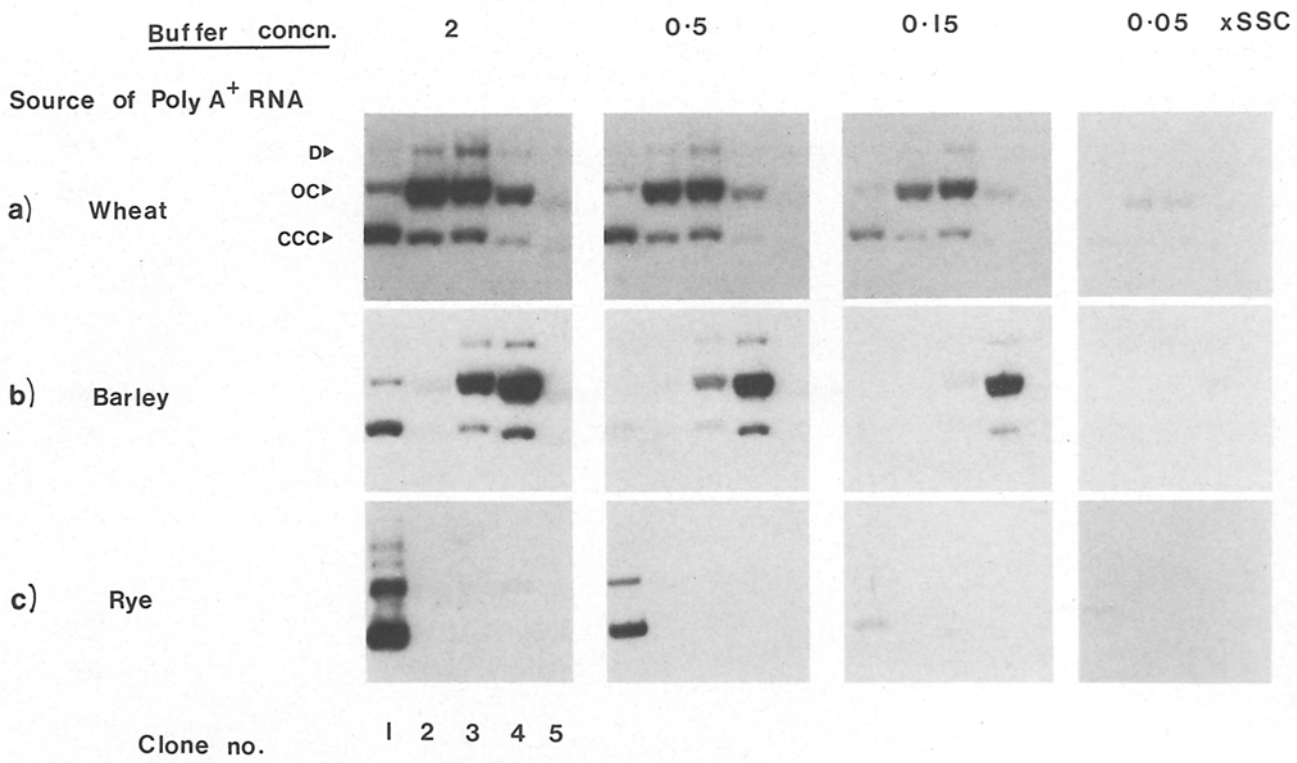

Fig. 2. Effect of different stringencies of washing on the stability of filter hybrids. Equimolar amounts of plasmid DNAs of similar insert length were bound to nitrocellulose filters by the procedure of Southern. Twelve identical filters were prepared, each carrying wheat cDNA clones *1, 2* and *3,* in addition to pC179 (No. 4, B-hordein cDNA clone) and pBR322 (No. 5). Four filters were hybridized as described with each of three probes: poly A^+ RNA from wheat (*a*), barley (*b*) and rye (*c*). After hybridization, one filter from each RNA set was washed with 2×SSC, a second with 0.5×SSC, a third with 0.15×SSC and a fourth with 0.05×SSC, with three changes of the same buffer concentration, all at 65 °C. The autoradiographs were obtained using pre-flashed film (Laskey and Mills 1977)

species duplexes were still detectable after washes with 0.15×SSC. These results indicate that closely related genes in different cereal species contain sequence differences.

The B-hordein cDNA clone pC179 (clone 4) hybridized to related sequences in wheat. pC179 did not hybridize to rye poly A^+RNA. This observation was repeated for several other B-hordein clones (RT, DB data not shown). The observation suggests the absence in rye of B-hordein-like sequences. This sequence class may correspond to the group of polypeptides having an α-type N-terminal amino acid sequence (Autran et al. 1979), which is present in α- and β-gliadins but absent from rye.

Discussion

The experiments described here enable the genes expressed in the same tissue of four *Gramineae* species to be compared. The four species evolved from a common ancestor (Feldman 1976; Smith and Flavell 1974; Vedel et al. 1980). Barley, wheat and rye are more closely related to one another than to oats. Wheat and rye are the most closely related pair, according to the distribution of repeated DNA sequences in the genomes. Our results show the presence of related abundant poly A^+RNAs in wheat, rye and barley including those homologous to storage protein RNAs. Flavell et al. (1977) demonstrated that repeated sequences in common between wheat and barley were also common between wheat and rye. We have also shown the presence of RNA sequences common to all three species, but in addition we have identified a group of sequences common to wheat and barley but not in rye. This may be explained by repression or deletion of these sequences in rye. The group of cloned sequences specific to wheat indicates the evolution of new transcription units since the species diverged.

Several examples of N-terminal sequence homology have been reported among prolamins (Bietz et al. 1977; Shewry et al. 1980a; Autran et al. 1979). This method of comparing polypeptide sequences has two limitations. If the amino termini of two polypeptides differ, for example due to the loss of a few residues from one, they will not be scored as related although the rest of the sequence may have extensive homology. Also, the degree of homology suggested by comparison of the amino terminal regions may reflect a structural require-

ment for processing (Wall 1979), and may not extend throughout the coding sequence. However, the hybridizations reported here measure homologies throughout the coding sequences.

Because wheat, rye and barley had a common ancestor the differences in the genes expressed in the endosperms reflect the molecular changes that have occurred to the families of storage protein genes during species evolution. The widely differing amounts of different RNA sequences detected in each species may result from differential sequence amplifications, deletions or recombination mechanisms (Kasarda 1980) as has been discussed for other multigene families (Dover and Flavell 1982). Similar processes of gene amplification probably also account for the large number of allelic variants for prolamins within species. The use of poly A$^+$ RNA probes from various cereals has given an estimate of the relatedness of the sequence groups. The homology of one group of wheat cDNA clones to B-hordein sequences suggests it may code for related prolamins.

Acknowledgements

We thank Drs. T. Dyer, R. B. Flavell and P. I. Payne for helpful discussions and comments on the manuscript. D. B. is supported by the Deutsche Forschungsgemeinschaft.

Literature

Apel, K.; Kloppstech, K. (1978): The plastid membrane of barley (*Hordeum vulgare*). Light-induced appearance of mRNA coding for the apoprotein of the light-harvesting chlorophyll a/b protein. Eur. J. Biochem. **85,** 581–588

Autran, J.-C.; Lew, E.Y.-L.; Nimmo, C.C.; Kasarda, D.D. (1979): N-terminal amino acid sequencing of prolamins from wheat and related species. Nature **282,** 527–529

Bietz, J.A.; Huebner, F.R.; Sanderson, J.E.; Wall, J.S. (1977): Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem. **54,** 1070–1083

Brandt, A.; Ingversen, J. (1978): Isolation and translation of hordein messenger RNA from wild type and mutant endosperms in barley. Carlsberg Res. Commun. **43,** 451–469

Dover, G.A.; Flavell, R.B. (1982): Genome evolution. New York: Academic Press

Feldman, M. (1976): Wheats. In: Evolution of crop plants (ed. Simmonds, N.W.), pp. 120–128. London: Longman

Flavell, R. B.; Rimpau, J.; Smith, D.B. (1977): Repeated sequence DNA relationships in four cereal genomes. Chromosoma **63,** 205–222

Forde, B.G.; Kreis, M.; Bahramian, M.B.; Mathews, J.A.; Miflin, B.J.; Thompson, R.D.; Bartels, D.; Flavell, R.B. (1981): Molecular cloning and analysis of cDNA sequences derived from poly A$^+$RNA from barley endosperm: identification of B-hordein-related clones. Nucleic Acids Res. **9,** 6689–6707

Greene, F. (1981): In vitro synthesis of wheat (*Triticum aestivum* L.) storage proteins. Plant Physiol. **68,** 778–783

Grunstein, M.; Hogness, D.S. (1975): Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72,** 3961–3965

Johnson, B.L. (1975): Identification of the apparent B-genome donor of wheat. Can. J. Genet. Cytol. **17,** 21–39

Kafatos, F.C.; Jones, C.W.; Efstratiadis, A. (1979): Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res. **7,** 1541–1552

Kasarda, D.D. (1980): Structure and properties of α-gliadins. Ann. Technol. Agric. **29,** 151–173

Kloppstech, K.; Schweiger, H.G. (1976): In vitro translation of poly (A) RNA from *Acetabularia.* Cytobiologie **13,** 394–400

Laskey, R.A.; Mills, A.D. (1977): Enhanced autoradiographic detection of ^{32}P and ^{125}I using intensifying screens and hypersensitized film. FEBS Lett. **82,** 324–326

Luthe, D.S.; Peterson, D.M. (1977): Cell-free synthesis of globulin by developing oat (*Avena sativa* L.) seeds. Plant Physiol. **59,** 836–841

Maniatis, T.; Jeffrey, A.; Kleid, D. (1975): Nucleotide sequence of the right-ward promoter of phage λ. Proc. Natl. Acad. Sci. USA **72,** 1184–1188

Mathews, J.A.; Miflin, B.J. (1980): In vitro synthesis of barley storage proteins. Planta **149,** 262–268

O'Dell, M.; Thompson, R.D. (1982): The purification of immature barley endosperm. J. Sci. Food Agric. **33,** 419–420

Peterson, D.M. (1978): Subunit structure and composition of oat seed globulin. Plant Physiol. **62,** 506–509

Shewry, P.R.; Autran, Y.-C.; Nimmo, C.C.; Lew, E.J.; Kasarda, D.D. (1980a): N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. Nature **286,** 520–522

Shewry, P.R.; Field, J.M.; Kirkman, M.A.; Faulks, A.J.; Miflin, B.J. (1980b): The extraction, solubility and characterization of two groups of barley storage polypeptides. J. Exp. Bot. **31,** 393–407

Smith, D.B.; Flavell, R.B. (1979): The relatedness and evolution of repeated nucleotide sequences in the genomes of some graminae species. Biochem. Genet. **12,** 243–256

Southern, E.M. (1975): Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol. **98,** 503–517

Vedel, F.; Lebacq, P.; Quetier, F. (1980): Cytoplasmic DNA variation and relationships in cereal genomes. Theor. Appl. Genet. **58,** 219–224

Wall, J.S. (1979): The role of wheat proteins in determining baking quality. In: Recent adv. biochem. cereals (eds. Laidman, D.L.; Jones, R.G.W.), pp. 275–308. London: Academic Press

Received November 3, 1982
Communicated by R. Riley

Dr. D. Bartels
Dr. R. D. Thompson
Cytogenetics Department
Plant Breeding Institute
Trumpington, Cambridge CB2 LQ2
(England)