Theor Appl Genet (1985) 71:330–333

Characterisation by molecular hybridization of RNA fragments isolated from ancient (1400 B.C.) seeds

F. Rollo

Dipartimento di Biologia Cellulare, Università di Camerino, Via F. Camerini 2, I-62032 Camerino, Italy

Accepted May 7, 1985
Communicated by F. Salamini

Summary. The analysis of cress seeds from Thebes dated approximately 1400 years B.C. showed that fragments of RNA up to 10 bases in length were still present in the ancient seeds. After having been made radioactive at the 5'OH terminus, the RNA fragments were used as probes in a spot hybridization experiment. They were shown to hybridize to cress DNA and, to a lesser extent, to that of phylogenetically distant species. When fixed onto nitrocellulose and probed with different cloned genes, the RNA fragments were shown to originate from breakage of the 25 and 18s cytoplasmic rRNA.

Key words: Ancient seeds – rRNA – Spot hybridization – Plant evolution

Introduction

The scope of animal evolution studies has undoubtedly widened since the isolating, cloning and sequencing of DNA from a 140-year-old mummified Quagga skin was shown to be possible (Higuchi et al. 1984). The question can then be raised if nucleic acids can be recovered from old plant specimens. The exceptional state of preservation frequently shown by old seeds found in archaeological excavations makes them attractive subjects for such an investigation.

The results of an analysis performed on a sample of cress from the Thebes Necropolis, dated approximately 1400 years B.C., are presented here.

The aim of the present work was to investigate, 1) whether polymerized nucleic acids can be detected in old seeds and, 2) whether significant genetic information can be obtained from them.

Material and methods

Material for this investigation was kindly made available by S. Curto and E. Leospo of the Soprintendenza per le Antichità Egizie of Turin. The cress seeds (*Lepidium sativum* L.) (Mattirolo 1926), together with a vast collection of desiccated fruits and vegetables, belong to the funerary trappings (basket no. 8248) found in the tomb of Architect Kha and his wife Merit (West Thebes Necropolis, 1420–1375 B.C.).

RNA was labelled at the 5'OH terminus by T4 polynucleotide kinase and (γ32P) ATP according to D'Alessio (1982).

DNA was nick translated according to Maniatis et al. (1975).

Spot hybridizations were performed essentially as reported by Brandsma and Miller (1980) and Maule et al. (1983).

32P labelled synthetic oligoribonucleotides were used as size markers for polyacrylamide gel electrophoresis.

Results and discussion

As a first step, 0.5 g of seeds were ground in a mortar and extracted three times with a phenol based mixture. Following the addition of cold ethanol and sodium acetate to the supernatant of the last phenol wash, a brownish precipitate was obtained. The precipitate was desiccated, resuspended in sterile distilled water and electrophoresed on a 2% agarose gel together with the total nucleic acid extracted from viable cress seeds as a control. The result is shown in Fig. 1. It can be seen that the ancient seeds still contain nucleic acid (approx. 400 µg of NA/g of seeds), its degree of polymerization appearing, however, quite low. When characterized on the basis of its resistance to RNAse or DNAse treatment the nucleic acid turned out to be RNA (not shown).

In order to determine the size of the RNA, the latter was terminally labelled with 32P and fractionated on a 12% polyacrylamide gel containing 7 M urea. The auto-

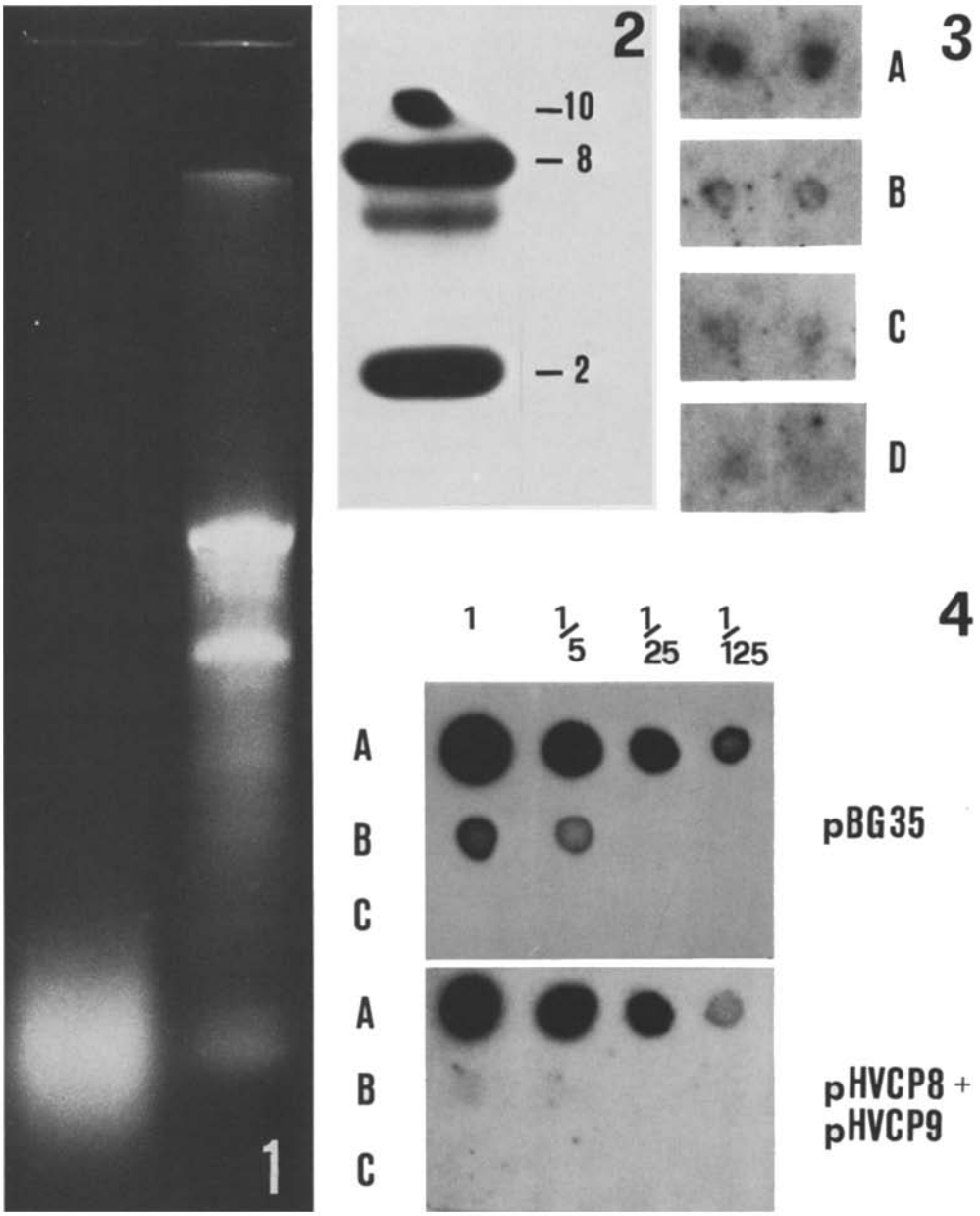

Fig. 1. Agarose-gel electrophoresis of nucleic acids extracted from ancient (*left*) and viable (*right*) cress seeds

Fig. 2. Analysis by electrophoresis on polyacrylamide gel of RNA extracted from ancient cress seeds. The figures on the right indicate the size in bases of the major fragments

Fig. 3. Hybridization of 5′OH end-labelled RNA isolated from ancient cress seeds to various DNAs. *A*: 0.5 μg cress DNA; *B:* 0.5 μg oats DNA; *C:* 0.5 μg calf thymus DNA; *D:* 0.5 μg pUC8 DNA (all samples in duplicate)

Fig. 4. Hybridization of pBG35 (25 and 18s rRNA flax cytoplasm genes) and pHVCP8 + pHVCP9 (23, 16 and 5s rRNA barley chloroplast genes) to the RNA extracted from the ancient cress seeds. *A:* rRNA from cress (0.1 μg); *B:* RNA from ancient cress seeds (2 μg); *C:* phage MS2 RNA (0.1 μg)

radiograph of the gel reveals the presence of fragments distributed in three major bands, corresponding respectively to 2, 8 and 10 nucleotides in length (Fig. 2).

In a preliminary attempt to characterize the fragments these were used as probe in a spot-hybridization (Brandsma and Miller 1980; Maule et al. 1983) experiment where cress, oats, calf thymus and pUC8 DNA were fixed onto the nitrocellulose. The result (Fig. 3) shows that the RNA extracted from the ancient seeds hybridize to cress DNA, to a minor extent to that of oats and calf thymus while a very poor signal, if any, is observed with pUC8 DNA. In a similar experiment, the same probe was shown to hybridize with mustard DNA and not to phage lambda DNA (not reported).

As was to be expected considering the shortness of the RNA fragments, the selectivity shown by the probe is modest. However, this could also be attributable to the presence of degradation products of the 25s and 18s ribosomal RNA amongst the fragment population. These are known to show a large degree of homology between different plant species and are by far the most abundant nucleic acid found in the cell (Maggini et al. 1976). To test this hypothesis, the RNA extracted from the ancient cress seeds was spotted onto nitrocellulose, together with RNA extracted from cress ribosomes and phage MS2 as controls, and probed with cloned genes for flax cytoplasm 25 and 18s rRNA (plasmid pBG35 Goldsbrough and Cullis 1981) or with cloned genes for barley chloroplast 23, 16 and 5s rRNA (plasmid pHVCP8 and pHVCP9, courtesy of Anil Day, John Innes Institute, Norwich). It can be observed (Fig. 4) that the first probe strongly hybridizes with the RNA extracted from the ancient cress seeds while the second doesn't seem to bind appreciably to the same sample. Under the same conditions cauliflower mosaic virus DNA does not bind detectably to the RNA extracted from the ancient cress seeds or to the control RNAs.

Concluding remarks

Seeds from ancient Egyptian tombs most often show an amazing degree of preservation. Because of this they have attracted the curiosity of the public and scientists since the last century. However, reports published over the past 40 years show that while the ancient seeds retain their original shape and even many details of the cell fine structure, they have undergone severe physiological and biochemical degradation.

Barley grains from King Tutankhamun's tomb (ca. 1350 B.C.) were found to contain riboflavin and nicotinic acid but no phytin (Barton-Wright et al. 1944). An ultrastructural investigation of the non-viable embryos of wheat grains from Thebes (3000–2000 B.C.) and from the grain silos of Feyum (ca. 4400 B.C.) showed an amazing preservation of the cell fine structure with lipid bodies, nuclei, nucleoli, chromatin, some plasmalemma and possibly mitochondrial remnants (Hallam 1973). On the other hand, neither high molecular weight nucleic acids nor RNAse activity were found in rye grains from Egyptian archaeological sites (Osborne et al. 1974). More recently, Derbyshire et al. (1977) demonstrated the presence of glutelin and zein in maize grains from a site in Canyon del Muerto, Arizona, dating from the earliest part of the first millennium to the 11th and 13th centuries. A completely different situation was found in barley grains from Egypt dating approximately 1000, 1900 and 3000 B.C. Infact, while the relative amino acid composition of the ancient seeds was remarkably similar to that found in recent grain – the main differences being decreased relative amounts of lysine and methionine – neither salt-soluble or hordein fraction proteins could be detected by electrophoresis or immunoassay (Shewry et al. 1982).

The present work shows that RNA at a low degree of polymerization still persists in 3300 year-old seeds.

The size of the RNA fragments recovered from the seeds is quite low and this situation may be general. In fact, the presence of only low molecular weight nucleic acid and nucleotides in ancient seeds from the tomb of Tuthankamun and from the silos of Fayum has been shown before by D. J. Osborne (personal communication). Nevertheless, the low size of the fragments doesn't seem to represent an obstacle to their unambiguous characterization by hybridization to cloned genes and this might indicate that a consistent fraction of the 25 and 18s rRNA nucleotide sequence is represented among the fragments.

References

Barton-Wright EC, Booth RG, Pringle WJS (1944) Analysis of barley from King Tutankhamun's tomb. Nature 153:288

Brandsma J, Miller G (1980) Nucleic acid spot hybridization: rapid quantitative screening of limphoid cell lines for Epstein-Barr viral DNA. Proc Natl Acad Sci USA 77: 6851–6855

D'Alessio JM (1982) RNA sequencing. In: Rickwood D, Hames BD (eds) Gel electrophoresis of nucleic acids. IRL Press, Oxford Washington, p 173

Derbyshire E, Harris N, Boulter D, Jope EM (1977) The extraction composition and intra-cellular distribution of protein in early maize grains from an archaeological site in N.E. Arizona. New Phytol 78:499–504

Goldsbrough PB, Cullis CA (1981) Characterisation of the genes for ribosomal RNA in flax. Nucleic Acids Res 9: 1301–1309

Hallam ND (1973) Fine structure of rye and other embryos. In: Heydecker W (ed) Seed ecology. Butterworths, London, p 115

Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC (1984) DNA sequences from the quagga, an extinct member of the horse family. Nature 312:282–284

Maggini F, De Dominicis RI, Salvi G (1976) Similarities among ribosomal RNA's of Angiospermae and Gymnospermae. J Mol Evol 8:329–335

Maniatis T, Jeffrey A, Kleid DG (1975) Nucleotide sequence of the rightward operator of phage lambda. Proc Natl Acad Sci USA 72:1184–1188

Mattirolo O (1926) I vegetali scoperti nella tomba dell'architetto Kha e di sua moglie Mirit nella necropoli di Tebe dalla Missione Archeologica italiana diretta dal Senatore E Schiaparelli. Atti R Accad Sci Torino Cl Sci Fis Mat Nat 61:545–568

Maule AJ, Hull R, Donson J (1983) The application of spot hybridization to the detection of DNA and RNA viruses in plant tissues. J Virol Meth 6:215–224

Osborne DJ, Roberts BE, Payne PI, Sens S (1974) Protein synthesis and viability in rye embrios. In: Bieleski RL, Ferguson AR, Cresswell MM (eds) Mechanism of regulation of plant growth. R Soc NZ Bull 12:805–812

Shewry PR, Kirkman MA, Burgess SR, Fenstenstein GN, Miflin BJ (1982) A comparison of the protein and amino-acid composition of old and recent barley grain. New Phytol 90:455–466