The Loss of Ribosomal Ribonucleic Acid during the Preparation of Nucleic Acid from certain Plant Tissues by the Detergent-Phenol Method

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(Received 19 September 1968)

During a study of RNA metabolism associated with the transition to biplanar growth in the gametophyte of *Dryopteris borreeri* (Burns & Ingle, 1969), difficulties were experienced in the preparation of total nucleic acid. The detergent-phenol method (see Fig. 1 for details of method), which has been successfully used for many animal (Kirby, 1967) and plant (Loening & Ingle, 1967) tissues, extracted the normal pattern of RNA components

from imbibed spores (Fig. 1a). However, no DNA, and a much lower yield of ribosomal RNA, was obtained from gametophytes that had been grown for 4 days (Fig. 1c), and only traces of ribosomal RNA were recovered from 9-day-old tissue (Fig. 1d). The preferential loss of the heavy-ribosomal RNA species, 1.3×10^6 and 1.1×10^6 mol.wt., can be seen in both the 4-day and 9-day preparations. The low-molecular-weight

Fig. 1. Fractionation by gel electrophoresis of total nucleic acid prepared from spores (a), 4-day (c) and 9-day (b and d) gametophyte tissue of *Dryopteris borreeri*. In (a), (c) and (d) the tissue was ground in a mortar and pestle at room temperature in a medium containing 0.05 M-NaCl, 0.01 M-EDTA, 0.01 M-tris-HCl buffer, pH 7.4, 1% tri-isopropyl-naphthalenesulphonate and 6% *p*-aminosalicylate plus sufficient phenol to solubilize the latter detergent. An equal volume of phenol, containing 10% *m*-cresol and 0.1% 8-hydroxyquinoline, was added, and the mixture was shaken and maintained at 0–4° thereafter. After centrifugation the lower phenol phase was removed to leave the interphase plus aqueous layer, to which NaCl (final concn. 0.5 M) and an equal volume of the phenol mixture were added. After shaking and centrifuging, the top aqueous layer was removed and extracted once more with the phenol mixture. Then 2 vol. of ethanol was added to precipitate the nucleic acids from the final aqueous phase. After storage at 0–4° for 16–24 hr. the precipitate was collected by centrifugation, washed once in cold 80% ethanol containing 0.5% sodium dodecyl sulphate, and partially dried under vacuum. In (b) the tissue was homogenized in the detergent medium as described above, with the difference that 6% butan-1-ol was used to solubilize the detergent. The homogenate was then maintained at 25° for 10 min. before the addition of NaCl (final concn. 0.5 M) and an equal volume of chloroform-pentan-1-ol (25:1, v/v). All manipulations were carried out at room temperature. After vigorous shaking and separation of the phases by centrifugation, the top aqueous layer was twice re-extracted with the chloroform mixture, and finally precipitated as described. The amounts of tissue used in these extractions had the same total RNA content, as determined by alkali hydrolysis, extraction and anion-exchange purification of the nucleotides (Ingle, 1963). The dried pellets were dissolved in the electrophoresis buffer to give a concentration of 1 mg./ml. based on the known RNA content of the sample, and 25 μ l. was fractionated by electrophoresis for 2 hr. (Loening & Ingle, 1967). The gels were then washed for 2 hr. and scanned at 265 m μ by using the Joyce-Loebl Chromoscan. The RNA components are referred to as their molecular weights in millions.

RNA (5s and 4s), on the other hand, was present in comparable amounts in the preparations from all ages of fern gametophyte. The low yield of the ribosomal RNA was not improved by variation of the detergent (tri-isopropyl-naphthalenesulphonate, *p*-aminosalicylate or sodium dodecyl sulphate and many of the possible combinations), detergent concentration (1–5%), ionic strength (0.05–0.5M-NaCl), temperature (0–50°) or pH (pH 5–9). However, the use of chloroform, instead of phenol, in the extraction procedure resulted in a preparation of RNA with the normal distribution of high-molecular-weight and low-molecular-weight components (Fig. 1b). The phenol preparation from 8-day gametophytes contained only 5% of the ribosomal RNA prepared by the chloroform method. If the phenol layer was removed after the first phase separation, and the aqueous layer plus interphase and pelleted material was treated with chloroform, the yield of ribosomal RNA was only increased to 15%. This indicates that either most of the RNA was removed with the phenol layer, or, if still present in the interphase material, it was not readily released by the chloroform. If the aqueous layer was removed after the first phase separation of a chloroform preparation, and further deproteinized with phenol, the yield was decreased to 80% but there was no change in the relative distribution of the components. If, however, the aqueous phase plus interphase material from a chloroform preparation was treated with phenol there was a greater decrease in the yield of ribosomal RNA, and a preferential loss of DNA and the larger ribosomal components. A phenol–chloroform (1:1, v/v) mixture released about twice as much ribosomal RNA as phenol alone.

The loss of ribosomal RNA by the phenol method was not a general phenomenon for fern gametophytes. Phenol–detergent preparations of *Dryopteris filix-mas*, which differs principally in ploidy from *D. borrieri*, and of *Polystichum* sp. were comparable with those prepared with chloroform, whereas *Athyrium filix-foemina* was similar to *D. borrieri*, giving a very low recovery of ribosomal

RNA when prepared with phenol. With all four fern gametophytes chloroform was necessary to extract the DNA. Fern sporophyte tissue yielded some ribosomal RNA when extracted by the phenol method, but the yield was improved when chloroform was substituted for the phenol. The detergent–phenol method has been successful for the preparation of nucleic acid from all the Angiosperm material so far examined (Ingle, Key & Holm, 1965; Loening & Ingle, 1967) and from a range of lower plants, species of *Pohlia*, *Marchantia*, *Selaginella* and *Equisetum*. The preparation of RNA from *Pinus sylvestris* (6-week-old seedlings), however, showed an absolute requirement for chloroform rather than phenol as the deproteinizing agent. Only a few of the tissues examined gave comparable preparations with both the phenol and the chloroform procedure. In general, tissues from which RNA could be prepared by the phenol method did not give good RNA preparations with the chloroform procedure, and vice versa.

This requirement for different deproteinizing agents in the preparative procedure presumably reflects differences in the properties of the proteins, with regard to denaturation and solubility. Although loss of the adenine–thymine-rich DNA satellite was reported when crab DNA was deproteinized with phenol rather than chloroform (Skinner & Triplett, 1967), and chloroform has been found beneficial for the preparation of natural DNA–RNA hybrids from HeLa cells (Levis, Krsmanovic & Errera, 1967), the choice of deproteinizing agent has not generally been regarded as critical in the preparation of RNA.

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