

Chattopadhyay, S. K. and S. K. Dutta. A method for isolation of **pure** DNA and RNA **simultaneously**.

Our **attempts** to isolate DNA and RNA from **mycelia** of N. crassa using their technique were not successful. Their method is more suitable for the isolation of combined nucleic **acids, and necessitates** the use of **a considerable amount** of DNase or RNase and more time in order to isolate RNA and DNA separately.

Bernardi and others (1969 Biochim. Biophys. **Acta 175:423**) have described the **fractionation** of DNA and RNA **separately** through **hydroxyapatite**. The entire principle lies in the **fact that at** different **molarities** of phosphate buffer different nucleic **acids** can be **adsorbed and eluted**. Based on this principle **Britten** (1969, personal **communication**) has developed **a** new technique of isolation of very pure DNA from **many organisms**. His method involves **lysing** the cells with **a** mixture of urea, **ethylenediamine tetraacetate** (EDTA), and **phosphate** buffer (PB). DNA can be **isolated** from the cell **lysate** by **chromatography** on **hydroxyapatite**. This method, however, is **meant** for the **isolation** of DNA **alone**.

This paper describes **a** procedure which permits **simultaneous isolation** of very pure DNA and RNA from N. crassa, by combining the underlying principles of these **methods**. One **gram** of well squeezed **mycelium** (wet wt. ) from the **exponential** growth **stage** (16 hours) is taken in **a** glass tissue grinder placed in ice. This **tissue** is then homogenized with 3 ml of **0.05 M** Tris HCl buffer (**pH** 7.6) containing 1% Sodium **lauryl sulphate**, 3.33% **diethyl pyrocarbonate** (**Eastman Kodak** ) and 5 mM **MgCl<sub>2</sub>**. An **extra** 3 ml of the same solution may be **added** in order to suspend the **paste** thus made. The **homogenate** is incubated **at 37°C**

Solymosy et al. (1968 **European J. Biochem.** 5:420) have isolated and **characterized** a mixture of pure **undegraded** DNA and RNA from fresh **leaves** of **higher plants**. They used **diethyl pyrocarbonate, a nuclease** inhibitor, in their **isolation** technique to inhibit **all nucleases**.

for 10 minutes and is again homogenized in the tissue grinder and then centrifuged at  $8000 \times g$  at room temperature. To this supernatant fluid, 0.6 g of NaCl is added and dissolved by breaking the NaCl crystals in the tissue grinder, after which it is again incubated at  $37^\circ C$  for 10 minutes and then centrifuged at  $10,000 \times g$  for 20 minutes at  $4^\circ C$ . The supernatant is then treated with 2.5 vols. of 9% chilled ethyl alcohol and kept in the cold ( $4^\circ C$ ) for 2 hours. The resulting precipitate is then dissolved in 0.05 M phosphate buffer (pH 7.4) with 0.4 M NaCl and dialyzed against the same buffer overnight. This solution is then dialyzed again 0.035 M phosphate buffer (pH 6.8) for 12 hwn and centrifuged at  $20,000 \times g$  for 20 minutes at  $4^\circ C$ . The supernatant is then passed through a column of 10 cc hydroxyapatite (Bio Gel-HTP, BioRAD) prepared with 0.035 M (pH 6.8) phosphate buffer.

The amount of hydroxyapatite used for the preparation of the column depends upon the quantity of DNA it has to adsorb. The rate of adsorption varies from batch to batch of hydroxyapatite. It should be ascertained first for a new batch of hydroxyapatite (Kohne 1969 Biophys. J. 8: 1104). As an average, 1 c.c. of hydroxyapatite adsorbs 70-90 micrograms of DNA. At a molarity of 0.035 phosphate buffer, both RNA and DNA are adsorbed in the column. After several washing with the 0.035 M phosphate buffer (pH 6.8), the RNA molecules are eluted with 0.18 M phosphate buffer (pH 6.5).

Thereafter the column is washed with approx. 300 ml of a mixture of urea and phosphate buffer (8 M urea and 0.24 M PB at pH 7.6). Finally, the DNA is eluted with 0.048 M (pH 6.5) phosphate buffer after washing out the urea with 0.035 M PB, pH 6.8. For the removal of unincorporated phosphate which still remains associated with DNA and/or RNA, particularly in the case of  $^{32}P$  labelling, several alcohol precipitations are required, after dialysis against 0.14 M NaCl. With this procedure it is possible to isolate 140 to 160  $\mu g$  of DNA from one gram wet (well-squeezed) mycelium, in comparison to 50-70  $\mu g$  of DNA isolated from the same quantity of mycelium using other techniques (Dutta et al, 1967 Genetics 57: 719).

Isolation of very pure and undegraded DNA and RNA is essential for any predictable result in nucleic acid hybridization. Our criteria of purity of DNA were as follows: (a)  $COT$  at  $260 m\mu = 2 \pm 0.1$ , (b) hyperchromicity of at least 26%, (c) no

rise in OD at  $260 m\mu$  below  $78^\circ C$  during denaturation, (d) two-step melting curve for whole cell DNA (Dutta and Kohne 1969 Proc. XI Intl. Botan. Congress 1969: SO), (e) no hybridization at 0 hour of incubation and 95% hybridization at a Cot value of approx. 377 (Cot = OD at  $260 m\mu / 2 \times$  hours of incubation; Britten and Kohne 1968 Science 161: 529). More accurate tests of purity are routinely done with  $^{32}P$  labelled DNA by verification of less than 1% acid solubility, RNase lability, or alkaline lability and 95% or more DNase lability. Tests of purity of RNA were primarily these last four tests, including at least 99% RNase lability and alkaline lability and no DNase lability. Color tests of DNA (diphenylomine) and RNA (orcinol) are not sensitive enough to detect less than 10% contamination of DNA or RNA.

Isolation of RNA alone may be accomplished with or without the use of a hydroxyapatite column. Following the same procedure as described in paragraph three, up to the dialysis against 0.05 M phosphate buffer with 0.4 M NaCl (pH 7.6). it is again dialyzed against 0.4 M NaCl and is precipitated down by alcohol. The precipitate is dissolved in 0.14 M NaCl followed by shaking with phenol saturated with 0.14 M NaCl (pH 5.0). Further purification is done by repeated alcohol precipitations.

For the isolation of DNA alone, the 0.18 M PB step during fractionation through hydroxyapatite can be omitted. Instead, first p- $\alpha$ -MUP solution (8 M urea plus 0.24 M phosphate buffer, pH 7.6) through the column until there is no OD or isotope count, followed by thorough washing with 0.035 M phosphate buffer (pH 6.8). DNA is then eluted with 0.48 M phosphate buffer (pH 6.5).

These procedures allow almost quantitative isolation of undegraded nucleic acids (with essentially no denaturation of DNA). We have been able to label DNA or RNA molecules with  $^{32}P$  at a level of more than 100,000 cpm per  $\mu g$ . This procedure is more economical when  $^{32}P$ -labelled DNA and RNA are required from the same material by several workers in the same laboratory and at the same time. Furthermore, the isolation of large quantities of very pure DNA and RNA with these procedures, including the urea method developed by Britten (1969, personal communication), from *Neurospora* has enabled the studies of kinetics of DNA reassociation and isolation of ribosomal RNA cistrons, hitherto unaccomplished.

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