APPENDIX

Improved Preparation of Bacteriophage T4-Infected *Escherichia coli* for the Isolation of Polynucleotide Kinase

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Scaling up the standard procedure for producing bacteriophage T4-infected cells (Richardson, 1965) presents two problems. The first is to produce a synchronous, efficient infection and the second is to stop the infection instantaneously when the enzyme titre is highest and before lysis can occur. These problems may be overcome simultaneously by using bacteriophage T4 *amN122* which gives the bonus of a greater than threefold increase in the yield of polynucleotide kinase. In a restrictive (non-suppressing) host, bacteriophage T4 *amN122* is defective in DNA synthesis and it is known that DNA synthesis is involved in the switch-off of the early phase of enzyme synthesis in bacteriophage T4 development (Hosoda & Levinthal, 1968). Consequently the synthesis of polynucleotide kinase, which is an 'early' enzyme, continues throughout the infection and high yields are obtained. Moreover, since bacteriophage development is not completed, lysis does not occur and infections can be run for long periods (up to 90 min). This removes the requirement for instantaneous stopping of the infection. The need for synchronous infection is also removed. This allows the bacteriophage to be added slowly giving a more uniform distribution of multiplicity of infection. Thus, the manipulation of the infection need not limit the scale on which it is carried out.

An amber suppressing strain of *Escherichia coli* (CR63) was used for the propagation of bacteriophage T4 *amN122* and lysates were stored over chloroform. Infections were done at a bacteriophage multiplicity

of 4 at a cell density of 8×10^8 – 1×10^9 /ml with L-tryptophan added to a concentration of $10 \mu\text{g/ml}$ shortly before infection. As a routine L broth has been used for bacteriophage propagation and infection, although medium M9 supplemented with glucose or glycerol and casein hydrolysate (Difco) [see accompanying paper (Murray, 1973)] has also been used successfully. Cells were harvested 60 to 90 min after infection. Yields of enzyme recoverable from cells infected by bacteriophage T4 *amN122* were compared with those from the wild-type bacteriophage in extracts made in parallel. The extracts were assayed quantitatively after chromatography on phosphocellulose (Richardson, 1965). The assay was based on the conversion of the dinucleoside C-U into its dinucleotide [see the accompanying paper (Murray, 1973)]. From the amounts of phosphocellulose fraction required to transfer 40% of the input ^{32}P -labelled γ -phosphate from ATP to the dinucleoside it was clear that at least three times more polynucleotide kinase activity could be recovered from cells infected with bacteriophage T4 *amN122* than from cells infected with wild-type bacteriophage T4.

References

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