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Analysis of mRNA populations

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When studying transcription, whether in living cells or in cell-free systems, it often becomes important to know something of the composition of the RNA produced, in terms of the nucleic acid sequences that it contains. Nucleic acid reassociation methods very often offer the best approach. Those that employ copy DNA (cDNA), synthesized enzymically on an RNA template, are among the most accurate and sensitive (Bishop *et al.* 1975). According to the procedure most commonly used, the cDNA, which is radioactively labelled to a high specific activity, is annealed with a sufficient excess of RNA to make the reassociation reaction effectively first-order with respect to the RNA concentration. The incorporation of the labelled cDNA into hybrid DNA–RNA duplexes can readily be measured, for example by the use of a single-strand specific endonuclease, or by chromatography on hydroxyapatite. Inferences can be drawn from both the rate and the extent of duplex formation. The experiments described below are based on this approach.

In the course of a series of experiments designed to analyse gene expression during the development of *Drosophila melanogaster*, Marta Izquierdo made an unexpected observation. When cytoplasmic RNA from either larvae or imagos was fractionated by means of oligo(dT) cellulose, the bound fraction (putative poly(A)-mRNA) contained a very predominant component that sedimented at about 14*S*. This could readily be isolated, and proved to be a good template for reverse transcriptase, provided an oligo(dT) primer was present. Reassocation experiments gave an estimate of sequence complexity that was very close to the molecular mass (570000) measured by electrophoresis under denaturing conditions. This shows that the 14*S* RNA is a single molecular species. However, the following series of experiments show that the 14*S* RNA is not a poly(A)-mRNA; rather, it is the larger mitochondrial rRNA. First, upon crude separation of mitochondria from cytosol, the RNA that hybridizes with the 14*S*-specific cDNA is found in the mitochondrial fraction (100:1). Secondly, the 14*S*-specific cDNA forms duplexes with purified mitochondrial DNA. Thirdly, when total *Drosophila* DNA (both nuclear and mitochondrial together) is cleaved with the restriction endonuclease *Hin*d III, the 14*S* RNA is found to hybridize with a 5.3×10^6 molecular mass (8000 bases) DNA fragment. This is the precise size of the *Hin*d III fragment of mitochondrial DNA shown by Kuklas & Dawid (1976) to form duplexes with the larger mitochondrial rRNA, itself found by these authors to have a molecular mass of 540000.

A series of experiments is being carried out by Jane Bower to determine whether the appearance of heat-shock sequences in *Drosophila* (Tissières, Mitchell & Tracy 1974; McKenzie, Henikoff & Meselson 1975) is due to a quantitative or a qualitative change in transcription. Poly(A)-RNA can quite readily be recovered in quantity from the polyribosomes of normal cells, but now two components are observed that differ in abundance by a factor of about 10^3. The more abundant of these (corresponding to one-third of the cDNA) are among the most

abundant sequences in the normal cell, while the remainder are among the rarest. A very similar pattern is observed when the heat-shock cDNA is annealed with poly(A)-RNA from either eggs + embryos (less than 16 h after laying) or 3rd instar larvae. Thus, in part at least, the appearance of the heat-shock proteins appears to be due to a quantitative change. We have found no evidence of any qualitative changes in transcription.

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