Table 1. Effect of brief exposure to hypo-osmotic media on the respiratory control ratio

Respiratory control ratios of liver mitochondria isolated from rats of various ages with succinate as substrate. The values are the means±S.E.M. of separate experiments with mitochondria isolated from three animals in each age group. The details of the technique of brief exposure to hypo-osmotic media and the measurement of the respiratory control ratio are described in the text.

| Osmotic strength of suspension medium during 20s treatment (mosmol/l) | Age (months) | Respiratory control ratios |
|---|---|---|---|---|
| | ... | 3–4 | 10–11 | 19–20 | 27–30 |
| 265 | 4.6 ± 0.21 | 4.5 ± 0.25 | 4.5 ± 0.15 | 4.6 ± 0.20 |
| 75 | 3.7 ± 0.30 | 3.6 ± 0.31 | 3.6 ± 0.20 | 3.8 ± 0.29 |
| 64 | 2.9 ± 0.25 | 2.9 ± 0.11 | 3.0 ± 0.18 | 3.1 ± 0.20 |
| 52 | 2.7 ± 0.16 | 2.7 ± 0.20 | 2.8 ± 0.20 | 2.1 ± 0.11* |
| 40 | 2.4 ± 0.15 | 2.3 ± 0.12 | 2.4 ± 0.15 | 1.4 ± 0.10* |
| 27 | 1.7 ± 0.17 | 1.9 ± 0.20 | 1.8 ± 0.17 | 1.1 ± 0.10* |
| 21 | 1.4 ± 0.20 | 1.4 ± 0.15 | 1.3 ± 0.19 | 1.05 ± 0.05* |

* Significantly different from 19–20-month age group; P < 0.01 by Student’s t test.

membrane properties, show that, although mitochondria isolated by normal procedures from livers of young and senescent rats are similar in terms of oxidation of succinate and the associated phosphorylation of ADP under iso-osmotic conditions, in agreement with other workers (Weinbach & Garbus, 1959; Chen et al., 1972; Inamdar et al., 1974; Wilson et al., 1975), there are certain age-related differences in membrane properties which are only apparent after the mitochondria are subjected to abnormal treatment such as brief exposure to hypo-osmotic media.

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Synthesis of 50S Ribosomes in a Mutant of Escherichia coli

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Intermediates in the assembly of bacterial 50S ribosomes in vivo are presently ill-defined. Pulse-labelling originally detected two precursors with nominal sedimentation coefficients of 32S and 43S (Mangiarotti et al., 1968; Osawa et al., 1969). Lindahl (1975) showed that there is a third (and possibly major) intermediate that cannot be distinguished from the mature native subunit by sedimentation. Only about 2% of the total 23S RNA of growing organisms is in the three precursor particles so that their isolation for detailed study presents difficulties.
Inhibition of growth by selective means can transiently increase the concentration of ribosome precursors (see, e.g., Blundell & Wild, 1969; Osawa et al., 1969), but assembly is then being studied under abnormal conditions. In addition, ribonucleoprotein particles, distinct from ribosomes, are found in large amounts in some mutant strains of Escherichia coli. Such mutants are either conditional (see, e.g., Nashimoto et al., 1971) or have a lesion that leads to the over-production of particles during exponential growth (Lewandowski & Brownstein, 1969; Buckel et al., 1972). The relationship of these putative precursors to those in wild-type organisms is often not clear, but the use of mutants has the advantage that material is more readily available for characterization.

We are studying the assembly of ribosomes in E. coli 15–28, a mutant derived from E. coli 15 thy– pro– ('15TP'). MacDonald et al. (1967) showed that E. coli 15–28 has 70S ribosomes that are about half as efficient as those of E. coli 15TP in cell-free protein synthesis and that, in addition, extracts of E. coli 15–28 contain an excess of a ribonucleoprotein particle with a sedimentation coefficient rather less than that of 50S ribosomes. We have confirmed this latter observation.

In 10 mM-Tris/HCl/10 mM-magnesium acetate/100 mM-KCl, pH 7.4, and dilute (30–60 µg/ml) solution, the sedimentation coefficient at 20°C of the particles isolated from E. coli 15–28 is 47S whereas that of derived 50S ribosomes from both mutant and parent strains is 53S. ‘47S particles’ are precursors of 50S ribosomes. Cultures of E. coli 15–28 were grown for several generations with both [3H]uracil and [14C]uracil present. The [14C]uracil was then removed, and the redistribution of radioactivity was followed by gradient centrifuging of extracts made at intervals during continued growth. No radioactivity was lost from the cells; a decrease in [14C] radioactivity in 47S particles was matched by an increase in 70S ribosomes. Further, 47S particles have some other of the general properties of ribosome precursors; their sedimentation coefficient is susceptible to changes in ionic conditions. In 10 mM-Tris/HCl/0.1 mM-magnesium acetate, pH 7.4, they unfold and their sedimentation coefficient becomes 36S, whereas the sedimentation velocity of 50S ribosomes is unaltered. Isolated 47S particles are more susceptible to degradation by pancreatic ribonuclease in both the buffers above than are 50S ribosomes.

RNA was extracted from 47S particles and 70S ribosomes of E. coli 15–28, derived 50S ribosomes from E. coli 15TP and ‘chloramphenicol particles’. These last, isolated from extracts of cultures of E. coli 15TP that had been inhibited by 100 µg of chloramphenicol/ml for 30 min, contain precursor forms of both 16S and 23S RNA (Hecht & Woese, 1968). Electrophoresis in slabs of polyacrylamide gel, made with a gradient (2–7%) of acrylamide, resolved the precursor 23S RNA of chloramphenicol particles from the ‘mature’ 23S RNA of 50S and 70S ribosomes. The 23S RNA from 47S particles had the same mobility as the mature species. The proteins of derived 50S ribosomes from E. coli 15TP and E. coli 15–28, and those of 47S particles, were extracted with acetic acid (Kaltschmidt & Wittmann, 1972) and subjected to two-dimensional gel electrophoresis (Kalschmidt & Wittmann, 1970). There were no differences between the patterns of proteins from the 50S ribosomes of E. coli 15TP and E. coli 15–28. However, 47S particles lacked proteins L16, L28 and L33.

These results suggest that 47S particles are an unusual precursor of 50S ribosomes. Other intermediates in assembly contain precursor forms of 23S RNA, as judged by gel electrophoresis (Lindahl, 1975). ‘43S particles’, isolated from E. coli A19 as material present in low concentration and sedimenting between 30S and 50S ribosomes (Nierhaus et al., 1973), contain protein L33 and lack not only L16 and L28 but also at least six other proteins present in 47S particles. Either 47S particles are a hitherto undetected stage in the production of 50S ribosomes or, more likely, assembly in E. coli 15–28 differs markedly from that in wild-type strains. This difference may produce completed 50S ribosomes that have a slightly altered topography and so function inefficiently in protein synthesis.

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Gene Expression and its Modification*

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Changes in gene expression arise from introduction, substitution or deletion of genes in the DNA genome. This has been expressed in the 'Central Dogma' (Crick, 1958, 1968, 1970), which states that the direction of flow of genetic information is

\[
\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}
\]

which signifies that, although DNA makes RNA, and RNA makes protein, some RNA species, using reverse transcriptase, are responsible for biosynthesis of DNA, but that protein is never responsible for RNA formation nor for DNA formation (for extensive references see Ycas, 1969; Wolstenholme & Knight, 1969; Lewin, B., 1974).

The switching on and off of transcriptional activities in DNA cistrons follow dynamic processes which comprise (a) unmasking of specific genetic sites on the double helix, and/or (b) unwinding of specific genetic sites required by RNA polymerase for expression of the genetic potential of DNA.

For mechanisms to be biologically admissible, they must be stereochemically and energetically permissible. A guide to stereochemical permissibility can be obtained from the construction of correctly proportioned space-filling molecular models (e.g. Lewin, 1968, 1970, 1974a,b, 1975a,b). Energetic permissibility can be evaluated from calculations based on graded interfacial-tension changes on specific sequences of hydrophobic-group de-adherances (Lewin, 1974a,b, 1975a,b).

Unmasking of specific sites on the double helix may arise from hinged movements of nuclear proteins (Lewin, 1968, 1970, 1975b) or from contraction of these proteins as a result of random chain segments undergoing conformational change to the \(\alpha\)-helix (since the area covered efficiently by an \(\alpha\)-helix form is smaller than the corresponding random coil).

Thermal unwinding of the double helix presents difficulties, since the \(T_m\) values of DNA molecules and synthetic polydeoxyribonucleotide double helices are far too high to allow unwinding at the physiological temperature, pH values and ionic strength. The difficulties can be avoided if one invokes the assistance of compressor/repressor proteins and extender/operator proteins in the dynamic processes of unwinding and rewinding of the double helix (Lewin, S., 1974a,b, 1975a,b). Unwinding of the double helix can be enforced as a result of conformational change, from \(\alpha\)-helix to \(\beta\)-conformation, in compressor/repressor proteins which are attached to DNA at respective endings of complementary genes (Lewin, S., 1974b, 1975b). Extension of the protein, after interaction with its complementary extender/operator protein to form a \(\beta\)-conformation, results in corresponding enforced extension of the DNA in which the double helix is unwound (see, e.g., Lewin, S., 1974a). The reverse process results in rewinding the DNA into the double helix.

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