of the eye, and such an approach is possible with several kinds of suitable material. However, biochemical methodology is still limited for work on the cellular level, particularly for investigations of the macromolecular properties of RNA. We have therefore developed techniques for (1) preserving tissue for micro-dissection avoiding RNA degradation (Edström & Daneholt, 1967), (2) extracting and purifying RNA on the cellular level (Egyházi, Ringborg, Daneholt & Lambert, 1968) and (3) separating the isolated RNA by micro gel electrophoresis (Egyházi *et al.* 1968; Ringborg, Egyházi, Daneholt & Lambert, 1968). The two first steps can also be combined with large-scale separation procedures if RNA is labelled with radioactive precursors.

A suitable material in which it is possible to study questions relating to the formation and intranuclear processing of RNA has been found in the salivary-gland cells of the midge *Chironomus tentans*. Here the cells contain somatically paired polytene chromosomes that have undergone up to 13 replications and therefore furnish units equivalent to 16000 interphase chromatids (Daneholt & Edström, 1967) with a good linear representation of the different chromosomal units. Individual chromosomes or segments of chromosomes, nucleoli, nuclear sap and cytoplasm can easily be isolated in these cells by micro-manipulation.

With the techniques developed for microanalysis of RNA we have found that the nucleoli produce a 38s ribosomal precursor that is converted into one 30s and one 23s (previously designated 20s) fraction in the nucleoli, both of which may be precursors to the two finished ribosomal RNA components. After 90–180 min. components migrating like the finished ribosomal RNA components begin to appear on the chromosomes. This had already been observed by C. Pelling (unpublished work) for the 28s RNA fraction. The postulated conversion of the 30s fraction into 28s RNA occurs in the nucleolus, but the conversion of the 23s RNA fraction into 18s RNA takes place on the chromosomes, which may contain either or both components, in contrast with the nucleolus, which contains only 23s RNA.

There are pronounced peaks of ribosomal RNA in the nuclear sap. These are of the 28s and 18s size and are therefore likely to have passed the chromosomal stage if it is assumed that there is only one route for processing of ribosomal RNA. If, however, the chromosomes represent a common path in the transport of ribosomal RNA to the cytoplasm the time spent there must be of relatively short duration, since ribosomal components are found in the cytoplasm at about the same time as they appear on the chromosomes. The findings of an association of ribosomal RNA with chromosomes

open the possibility that this is a phenomenon, not only characteristic for bacteria, but possibly of general occurrence and significance.

Apart from ribosomal RNA the chromosomes contain polydisperse RNA with migration and sedimentation properties indicating a wide range of molecular weights up to more than 10^7 daltons. This RNA is probably synthesized locally, since it appears after short labelling periods, in contrast with the ribosomal RNA. Heterogeneity of the RNA is not caused by the fact that it originates from hundreds of chromosome bands, since the RNA from 'puffs', which originate from single bands, also shows a high degree of heterogeneity. However, the base composition of the stable as well as the rapidly labelled RNA is characteristic for a certain 'puff'. We have found no evidence for a decrease in size of this RNA in the nucleus or in the cytoplasm. It is transcribed from a fraction of DNA, the base composition of which differs markedly from average composition. Most of the DNA has a base composition similar to that of the chromosomal RNA, but there is a large fraction of very A+T-rich DNA distributed in pieces that are interspersed among this DNA.

There are several low-molecular-weight RNA species in the nucleus, and 4s and 5s RNA have been found in all three nuclear compartments (Egyházi, Daneholt, Edström, Lambert & Ringborg, 1969). In the chromosomes 4s RNA appears first as material migrating in the 4–5s range and seems to undergo a maturation process, whereas in the nucleolus there is a 4s RNA species with migration properties not changing with time. Methylation occurs locally in both types of RNA, but in the chromosomes only after the definite migration rate has been reached.

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Daneholt, B. & Edström, J.-E. (1967). *Cytogenetics*, **6**, 350.
Darnell, J. E. (1968). *Bact. Rev.* **32**, 262.

Edström, J.-E. & Daneholt, B. (1967). *J. molec. Biol.* **28**, 331.

Egyházi, E., Daneholt, B., Edström, J.-E., Lambert, B. & Ringborg, U. (1969). *J. molec. Biol.* (in the Press).

Egyházi, E., Ringborg, U., Daneholt, B. & Lambert, B. (1968). *Nature, Lond.*, **220**, 1036.

Ringborg, U., Egyházi, E., Daneholt, B. & Lambert, B. (1968). *Nature, Lond.*, **220**, 1037.

Cytoplasmic Proteins and the Control of Nuclear Activity in Early Amphibian Development

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Experiments involving the transplantation of living cell nuclei from one cell type to another have

established that changes in nuclear activity can be rapidly induced by components or conditions of amphibian egg and oocyte cytoplasm (see review by Gurdon & Woodland, 1968). The changes include gross transitions from DNA to RNA synthesis or vice versa, as well as changes in the classes of RNA (ribosomal RNA, transfer RNA) synthesized. In all cases a nucleus changes the nature of its synthetic activity so as to conform rapidly to that characteristic of the host cell to whose cytoplasm it has been exposed. A temporal relationship has been established between a rapid nuclear enlargement (of up to 60-fold volume increase) and changes in synthetic activity induced by egg or oocyte cytoplasm. During the nuclear enlargement chromatin becomes dispersed throughout the nucleus; the staining properties of the nuclear material change, probably on account of the entry of cytoplasmic material into the nucleus.

The entry of cytoplasmic protein into nuclei induced to change synthetic activity has been demonstrated by Arms (1968), who injected adult frog brain nuclei into *Xenopus* eggs whose cyto-

plasm contained labelled proteins. Labelled macromolecules accumulated in the enlarged nuclei, and protein synthesis was suppressed by puromycin. These results were confirmed under different conditions by R. W. Merriam (unpublished work). Experiments are now in progress with fractions of saline-extracted oocyte proteins that have been iodinated before insertion into eggs or oocytes containing transplanted nuclei. It is hoped by this means to find out whether the classes of cytoplasmic proteins that enter transplanted nuclei differ according to the kind of nuclear activity induced.

The changes in nuclear activity studied in these experiments are the same as those that take place during normal embryogenesis. The conclusions are presumed to be relevant to the processes that lead to changing nuclear activity during normal development.

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Arms, K. (1968). *J. Embryol. exp. Morph.* **20**, 367.

Gurdon, J. B. & Woodland, H. R. (1968). *Biol. Rev.* **43**, 233.

COMMUNICATIONS

The Aggregation of Calf Thymus Histone Fractions in Solution

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Sedimentation-velocity experiments indicate that histone fractions F2(a), F2(b) and F3 are present as aggregates in solution in 0.1 M-NaCl, the degree of aggregation being independent of pH in the range 1–7. Dissociation of the aggregates occurs in 6 M-guanidine hydrochloride, but even under these conditions fractions F1, F2(a) and F2(b) exhibit heterogeneity. Molecular weights determined in this solvent system are comparable with those reported by other workers (Hnilica, 1967).

It has been found that the degree of aggregation can be decreased by decreasing the concentration of NaCl. Detailed studies have been made with fraction F2(b) only. The effect of concentration on rate of sedimentation has been determined for solutions containing different concentrations of NaCl. The results indicate that aggregation is at a minimum in 0.01 M-NaCl and increases with increasing salt concentration. In 0.01 M-NaCl the effect of charge on the histone accounts for nearly 90% of the decrease in sedimentation coefficient observed at finite concentration. In 0.1 M-NaCl charge effects account for 50% of the observed

change and in 0.5 M-NaCl about 10%. Application of the theory of Alexandrowicz & Daniel (1963) and Daniel & Alexandrowicz (1963) shows that the charge/mass ratio is not affected by the aggregation process.

Measurements of the weight-average molecular weight of fraction F2(b) have been made by the method of Archibald (1947). In 0.01 M-NaCl, after correction for charge effects and for bound chloride, the molecular weight calculated at infinite dilution of histone is 14 000. This value is somewhat greater than the value of 10 000–11 000 calculated from end-group analysis (Phillips, 1963). It would appear that, even under conditions minimizing aggregation, fraction F2(b) in solution exhibits some heterogeneity. With increasing salt concentration, the polydispersity of the solute, as judged by the change in the observed molecular weight with time of sedimentation, increases. In 1.0 M-NaCl the weight-average molecular weight is 87 000.

Alexandrowicz, Z. & Daniel, E. (1963). *Biopolymers*, **1**, 447.

Archibald, W. J. (1947). *J. phys. Colloid Chem.* **51**, 1204.

Daniel, E. & Alexandrowicz, Z. (1963). *Biopolymers*, **3**, 473.

Hnilica, L. (1967). *Progr. Nucleic Acid Res. molec. Biol.* **7**, 51.

Phillips, D. M. P. (1963). *Biochem. J.* **87**, 258.