

that the CO<sub>2</sub> programs originated from a few preliminary experiments, it seems likely that they can be improved. In this way better synchronized *Tetrahymena* cultures may be obtained so that analysis of the cell cycle with improved resolution in time is facilitated.

Apart from this, alternative methods of synchronization are of interest in themselves because they allow comparative studies of the mechanisms underlying synchronization; these mechanisms are generally believed to be of importance for cell division itself.

The effect of CO<sub>2</sub> on *Tetrahymena* may also be of interest in cases when synchronization is not attempted. If aeration of cultures is low, the concentration of the CO<sub>2</sub> developed by the cells may perhaps reach a level at which it affects the cells.

Finally, the possibility for synchronization of other cells by CO<sub>2</sub> may be worth considering, especially in cases when synchronization has been difficult to obtain by other means.

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## RNA synthesis during cleavage of the *Lymnaea* egg

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### Summary

In eggs of *Lymnaea* RNA synthesis can be detected autoradiographically from the 8- to the 16-cell stage. From the 16- to the 24-cell stage distinct nucleoli reappear which are immediately engaged in RNA synthesis.

Following the study of DNA synthesis during the early development of *Lymnaea* [1] a first attempt has been made to investigate RNA synthesis during the same period. Up to the 16-cell stage cleavages succeed each other rapidly. The greater part of each cell cycle is taken up by DNA synthesis (about 25 %) and mitosis (50 to 55 %) [1], and the time available for RNA synthesis is limited. Moreover, it may be assumed that part of the genetic information of the nuclei that is transcribed into messenger RNA is linked to the reduplication of nuclear proteins, as demonstrated for sea urchins by Kedes et al. [3] and by Nemer & Lindsay [4]. For this reason, it may be assumed that lengthening of the cell cycles is a prerequisite for the participation of the nuclei in cell differentiation by transcription of genetic information for the synthesis of specific cytoplasmic proteins.

To test a possible relation between the rate of RNA synthesis and the duration of the cell cycle, the incorporation of labelled uridine has been studied from the 1- up to the 24-cell stage. From earlier experiments with cytidine [1] it has become evident that up to the 8-cell stage little or no RNA synthesis takes place. This cannot be explained by an impermeability of the egg for the isotope as the label was incorporated into the DNA. The rate of incorporation of cytidine and thymidine into DNA increases

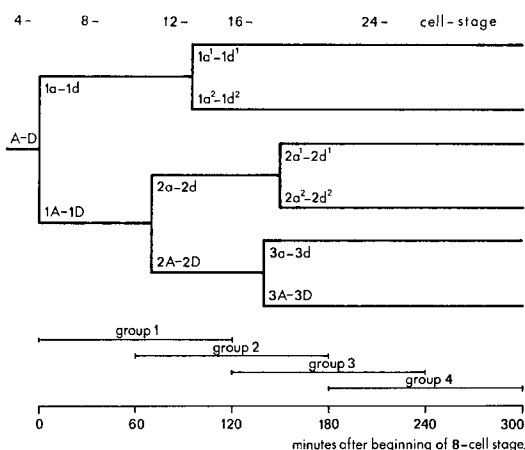


Fig. 1. Cell-lineage and division chronology of the *Lymnaea* egg from the 4- to the 24-cell stage. Nomenclature of the blastomeres: at the 4-cell stage the blastomeres are called A, B, C and D. In an animal-vegetative direction three quartets of micromeres (1a-1d; 2a-2d and 3a-3d) are split off from the cells A-D. The coefficient (1, 2 or 3) before the cells A-D designates the number of quartets that have been split off. In the case of the micromeres each exponent denotes a subsequent division; 1 designating the cell nearer the animal pole, 2 the sister cell nearer the vegetative pole. The intervals during which the eggs were exposed to  $^3\text{H}$ -5-uridine are indicated above the time scale. Rates of incorporation are listed in table 1.

considerably from the 2- to the 4-cell stage without concomitant changes in the rate of uptake of nucleosides [1].

### Materials and Methods

Egg masses were divided into synchronously dividing groups of eggs as described elsewhere [1]. For periods of 2 h, the eggs were exposed to 25  $\mu\text{Ci}$   $^3\text{H}$ -5-uridine (spec. act. 27.1 Ci/mM, The Radiochemical Centre, Amersham) per ml tapwater. Fixation was performed for 1 h in alcohol 80%, formol 40%, acetic acid (85-5-10). Prior to the application of the Kodak AR10 stripping film, some slides were prepared with RNase (0.2  $\mu\text{g}/\text{ml}$  distilled water) for 1 h at 37°C. The exposure time of the autoradiographs was 3 months.

### Results

In two experiments eggs were incubated in labelled uridine for a period of 2 h prior to the 8-cell stage. No positive autoradiographs were obtained, which confirms earlier

experiments with labelled cytidine [1]. In one experiment RNA synthesis was investigated from the 8- up to the 24-cell stage. At successive intervals of 1 h, the eggs were exposed to  $^3\text{H}$ -5-uridine for a period of 2 h (fig. 1). Then after an equal exposure time of the autoradiographs, incorporation into RNA could be demonstrated. Owing to the results of the experiments mentioned in the introduction, it is highly improbable that the changes in the incorporation of uridine are due to changes in the permeability of the egg for the labelled uridine. The eggs of group 1 (fig. 1) were incubated from the onset of the 8-cell stage up to 25 min after the beginning of the 16-cell stage. At the moment of fixation no distinct nucleoli were present, but only a small number of nucleolar bodies, which were not labelled significantly. The nuclei were clearly labelled, whereas the labelling of the cytoplasm exceeded the background twice or thrice (fig. 2). As no distinct nucleoli were present, it may be assumed that the label has been incorporated into RNA other than ribosomal RNA. No grains could be observed over sections treated with RNase. The eggs of group 2 were fixed 30 min after the onset of the 24-cell stage. Each cell type exhibited distinct nucleoli overlain by a small number of grains (fig. 3). This increased in group 3 and 4, fixed 90 and 150 min, respectively, after the beginning of the 24-cell stage (fig. 4). This probably suggests that the nucleoli, shortly after their reappearance, become engaged in the synthesis of ribosomal RNA.

To compare the labelling intensity of nuclei, nucleoli and cytoplasm of the different cell types in the same embryo and between embryos of different groups, the total number of grains over the nuclear and nucleolar sections was determined. For the labelling intensity of the cytoplasm the number of grains over a section containing the nucleus

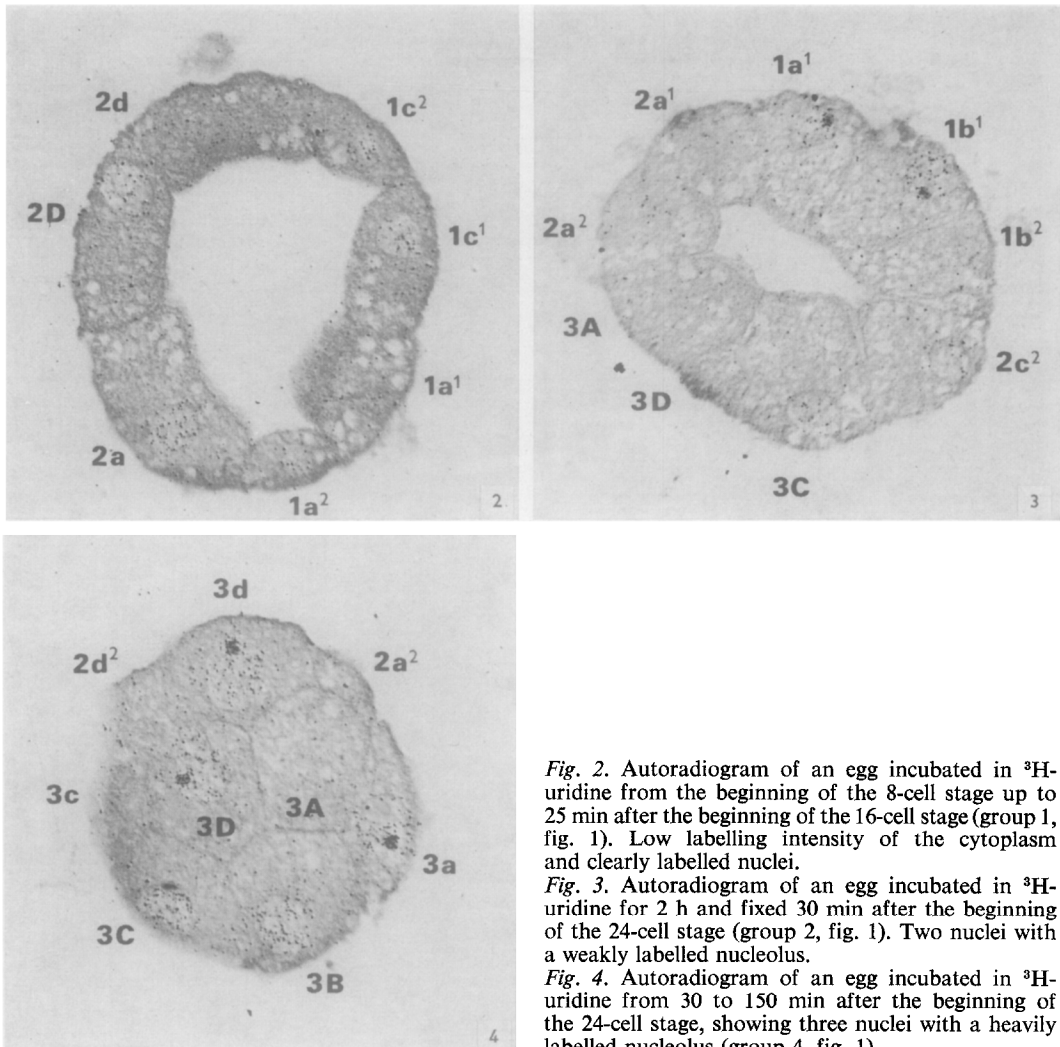


Fig. 2. Autoradiogram of an egg incubated in  $^3\text{H}$ -uridine from the beginning of the 8-cell stage up to 25 min after the beginning of the 16-cell stage (group 1, fig. 1). Low labelling intensity of the cytoplasm and clearly labelled nuclei.

Fig. 3. Autoradiogram of an egg incubated in  $^3\text{H}$ -uridine for 2 h and fixed 30 min after the beginning of the 24-cell stage (group 2, fig. 1). Two nuclei with a weakly labelled nucleolus.

Fig. 4. Autoradiogram of an egg incubated in  $^3\text{H}$ -uridine from 30 to 150 min after the beginning of the 24-cell stage, showing three nuclei with a heavily labelled nucleolus (group 4, fig. 1).

was counted, and expressed as the number of grains over an area of  $550\ \mu\text{m}^2$  of cytoplasm (this surface represents approximately the sum of the surfaces of the  $2\ \mu\text{m}$  sections of one nucleus). The results of the grain counts are listed in table 1. It will be clear that these counts neither permit a definite conclusion about changes in the rate of RNA synthesis during an incubation period of 2 h prior to or at the 24-cell stage, nor about differences between the different types of cells within the same embryo. Only

the labelling intensity of the nucleoli increased significantly in the advanced 24-cell embryos (groups 3 and 4). As the eggs of group 1 were incubated during the period of rapid cell division, it may be concluded that, notwithstanding the short cell cycles, RNA synthesis becomes detectable at the 16-cell stage. However, the total amount of RNA synthesized during the long cell cycles of the blastomeres in a 24-cell embryo will finally reach a much higher level than in one of the preceding stages.

Table 1. Incorporation of  $^3\text{H}$ -5-uridine into RNA

Rate of incorporation is expressed in number of grains counted over nucleoli (Nc), nuclei (Nu) and over  $550\ \mu\text{m}^2$  cytoplasm (Cy) of two or three eggs. Exposure time of the autoradiograms was 3 months. Incubation periods of the four groups are indicated in fig. 1.

Group	Egg	Number of grains over											
		$1a^1-1d^1$			$1a^2-1d^2$			$2a-2d$			$2A-2D$		
		Nc	Nu	Cy	Nc	Nu	Cy	Nc	Nu	Cy	Nc	Nu	Cy
1	1	0	44	13	0	31	14	0	89	3	0	107	5
1	2	0	43	6	0	34	5	0	96	10	0	77	11
		$2a^1-2d^1$			$2a^2-2d^2$			$3a-3d$			$3A-3D$		
		Nc	Nu	Cy	Nc	Nu	Cy	Nc	Nu	Cy	Nc	Nu	Cy
2	1	11	104	12	3	59	9	3	52	9	5	93	7
2	2	2	80	40	4	121	26	2	99	30	2	134	24
3	1	12	138	10	3	89	13	12	160	8	20	184	3
3	2	19	94	26	12	45	25	11	87	8	13	124	15
4	1	12	61	9	3	45	7	9	64	6	12	89	1
4	2	16	149	11	15	140	10	9	124	7	15	162	14
4	3	28	276	21	19	149	37	28	300	26	35	372	25

### Discussion

The data presented in this paper are the results of a preliminary attempt to investigate RNA synthesis during the early development of *Lymnaea*. It may be concluded that little or no RNA is synthesized prior to the 8-cell stage. Incorporation of uridine into RNA has been observed first in the 16-cell embryo at a moment when no distinct nucleoli were present. As there is strong evidence that the presence of nucleoli is associated with ribosomal RNA synthesis [2], probably incorporation of  $^3\text{H}$ -5-uridine prior to the appearance of nucleoli indicates synthesis of RNA other than ribosomal RNA, i.e. transfer or messenger RNA. In the 24-cell embryo, the nucleoli become increasingly engaged in the synthesis of RNA. The presence of label in the cytoplasm probably indicates that there has been a transport of RNA from the nucleus towards the cytoplasm.

RNA synthesis within the nucleus seems

to be determined by the cytoplasm [2]. This suggests that in *Lymnaea* the properties of the cytoplasm progressively change after fertilization so that the nuclei are induced to synthesize detectable amounts of RNA from the 8- to the 16-cell stage. The longer the cell cycle, the more RNA can be synthesized. For this reason the flow of information from the nucleus to the cytoplasm will be much higher in the 24-cell embryo, when the cells stop dividing for several hours, than during earlier stages.

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