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Development of division asynchrony and bilateral symmetry in the first quartet of micromeres in eggs of *Lymnaea*

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SUMMARY

Experiments were undertaken to investigate the cleavage pattern of eggs of *Lymnaea* developing into exogastrulae after a treatment for 2 h with 5×10^{-2} M-LiCl at 6 °C at the beginning of second cleavage. It appeared that the 16-cell stage is reached normally, but that in the vegetative cells the following division is considerably retarded. It is assumed that this delay is associated with the suppression of division asynchrony in the first quartet of micromeres, which normally adumbrates bilateral symmetry in the animal hemisphere, and with suppression of gastrulation.

INTRODUCTION

In *Lymnaea* the first sign of bilateral symmetry in the first quartet of micromeres (the future head region of the snail) is supposed to become apparent at the division of the basal cell of the dorsal arm of the molluscan cross, $1d^{121}$ (Verdonk, 1965, 1968). However, at the end of the resting stage of the 24-cell embryo a transient sign of bilateral symmetry can be observed during the second division of the cells of the first quartet of micromeres. The dorsal cell $1d^1$ divides $\frac{1}{2}$ h later than the corresponding cells $1a^1$, $1b^1$ and $1c^1$. The primary trochoblasts $1c^2$ and $1d^2$, which are located interradially on either side of the cell $1d^1$, divide about 1 h later than the corresponding cells $1a^2$ and $1b^2$ at the future ventral side of the embryo (van den Biggelaar, 1971*b*). The first sign of bilateral symmetry at the vegetative pole of the embryo is the central position of the macromere *3D* in the 24-cell embryo and the division of this cell into the macromere *4D* and the primary mesoblast *4d* or M (Verdonk, 1965).

In *Ilyanassa* the presence of the vegetative polar lobe at the trefoil stage is essential for the development of bilateral symmetry both at the animal and vegetative pole of the embryo (Clement, 1952). The experimental studies of Clement did not allow a definite answer to the question whether the polar lobe influence reaches the animal cell 'by a process of cytoplasmic segregation, or whether the lobe effect is one of embryonic induction'. Verdonk (1965, 1968) demonstrated that in *Lymnaea* the development of bilateral symmetry in the

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first quartet of micromeres at the animal pole of the embryo can be repressed with LiCl, whereas the appearance of bilateral symmetry at the vegetative pole remains unaffected. Verdonk also observed that bilateral symmetry was not only expressed in the cells of the first quartet of micromeres that form the dorsal arm of the cross (derivatives of the cell *1d*), but also in the lateral arms (derived from the cells *1a* and *1c*). He concluded that bilateral symmetry at the animal pole of the *Lymnaea* egg is induced by a factor for bilateral symmetry present in the vegetative part of the D quadrant, presumably in the macromere *3D* or the primary mesoblast *4d*. The observation that division asynchrony in the first quartet of micromeres is likewise not restricted to derivatives of the blastomere *1d* but appears in other cells that are situated at the future dorsal side of the embryo (van den Biggelaar, 1971 *b*) provides further evidence for this explanation.

A complete radial organization of the cell pattern of the first quartet of micromeres has been observed in future exogastrulae only (Verdonk, 1965, 1968). If division asynchrony within the first quartet of micromeres adumbrates bilateral symmetry in the future head region, it may be assumed to be repressed in future exogastrulae.

It was decided to test this assumption by exposing eggs to LiCl in such a way that almost 100 % of exogastrulation is obtained. This can be achieved if eggs are exposed to a solution of 5×10^{-2} M-LiCl at 6 °C for a period of 2 h starting at the onset of the 4-cell stage (Geilenkirchen, 1967). With this method the treatment with LiCl is restricted to a well-defined stage, and uniform deviations of cell division are to be expected.

MATERIALS AND METHODS

According to the method described by Geilenkirchen (1967), eggs were exposed to 5×10^{-2} M-LiCl at 6 °C for a period of 2 h starting at the onset of the 4-cell stage. After exposure and washing the eggs for a period of 2 h in tap-water of 6 °C the eggs were transferred to tap-water of 25 °C. One control group was kept for 4 h in distilled water of 6 °C and then transferred to tap-water of 25 °C; a second control group was kept in tap-water of 25 °C. One group of Li-treated eggs was used to check if 90–100 % of them developed into exogastrulae, if not, the experiments were discarded. At regular intervals after the formation of the second quartet of micromeres whole mounts were prepared from the remaining eggs as described elsewhere (van den Biggelaar, 1971 *a*) and analysed for cell division. Earlier stages were studied in living embryos.

RESULTS

The chronology of division of eggs treated with LiCl is shown in Fig. 1. For comparison, the division chronology of normal eggs is shown in Fig. 2. After the eggs were transferred to tap-water of 25 °C it took 120 and 150 min in the controls and Li-treated eggs, respectively, before the 8-cell stage was reached. Both in normal and Li-treated eggs the development up to the 16-cell stage was

normal. Beyond this stage it was no longer possible to follow the successive divisions in the living embryos, and division chronology was reconstructed by means of 209 whole mounts of eggs. A comparison between the division chronology of normal and Li-treated eggs clearly demonstrates the obtained results. The divisions of the macromeres *2A–2D* and the micromeres *2a–2d* were

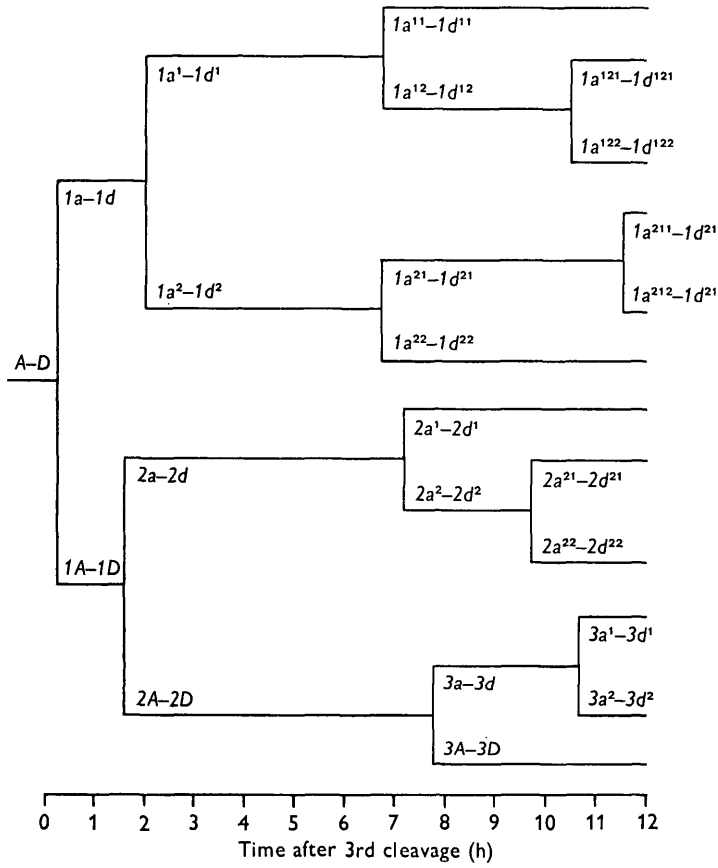

Fig. 1. Division chronology of eggs developing into exogastrulae after a treatment with LiCl at 6 °C for a period of 2 h starting at the beginning of the 4-cell stage. Results from four experiments including 209 eggs.

conspicuously delayed, whereas the divisions of the cells $1a^1$–$1d^1$ and $1a^2$–$1d^2$ were almost unaffected. The second division of the cells of the first quartet of micromeres was synchronized. The disappearance of division asynchrony showed that the first indication of bilateral symmetry in the first quartet of micromeres was repressed (Fig. 3). At least one additional division was observed in the trochoblasts $1a^{21}$–$1d^{21}$, which normally stop dividing. In certain cell-lines the divisions were remarkably more delayed than in others, and as a result the 48-cell Li embryos consisted of 24 cells derived from the first quartet of

micromeres and 24 cells derived from the macromeres *1A–1D*. A 49-cell normal embryo has only 16 cells derived from the first quartet of micromeres. It is remarkable that the chronology of divisions in the first quartet of micromeres was repeated by the remaining cells of the embryo.

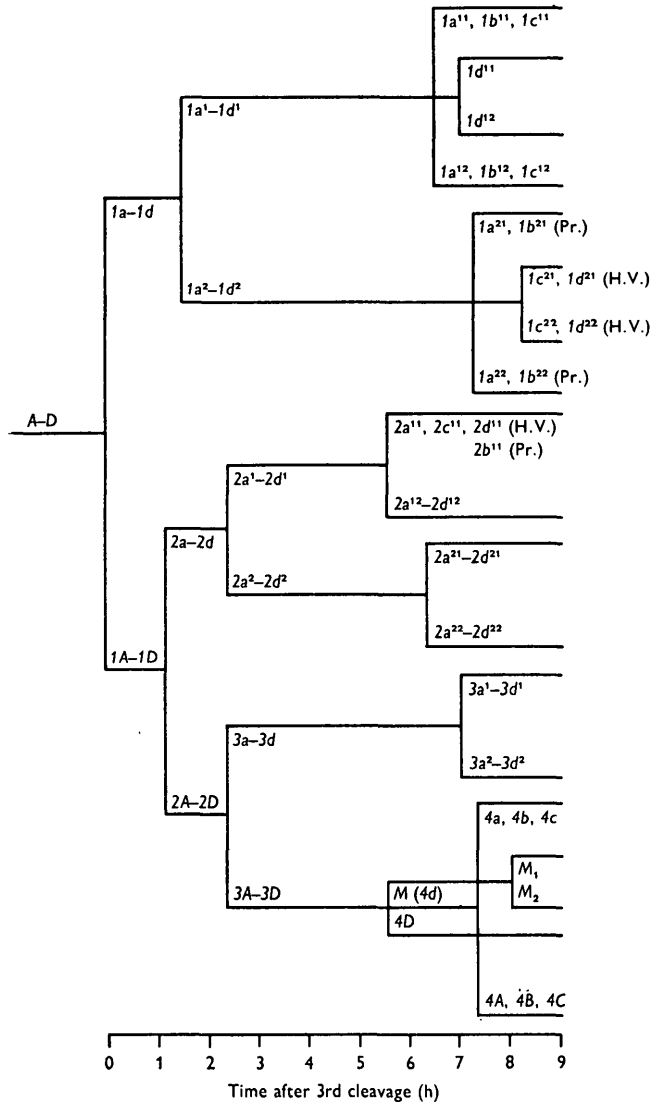

Fig. 2. Division chronology in normal development. Cells of the head vesicle (H.V.) and prototroch (Pr.) stop dividing.

From these observations it emerges that Li disturbs the normal cleavage pattern at first particularly at the vegetative side, and in such a way, that the second division in the first quartet of micromeres takes place before bilateral symmetry in the vegetative part has become apparent by the position of the

macromere *3D* or the formation of the primary mesoblast *4d*. As a result division synchrony is maintained in the first quartet of micromeres.

No definite answer was obtained about the appearance of bilateral symmetry at the vegetative part of Li-treated eggs. Up to the 48-cell stage the cleavage pattern was completely radially symmetrical; with the method used, however, it was no longer possible to reconstruct division chronology of older embryos.

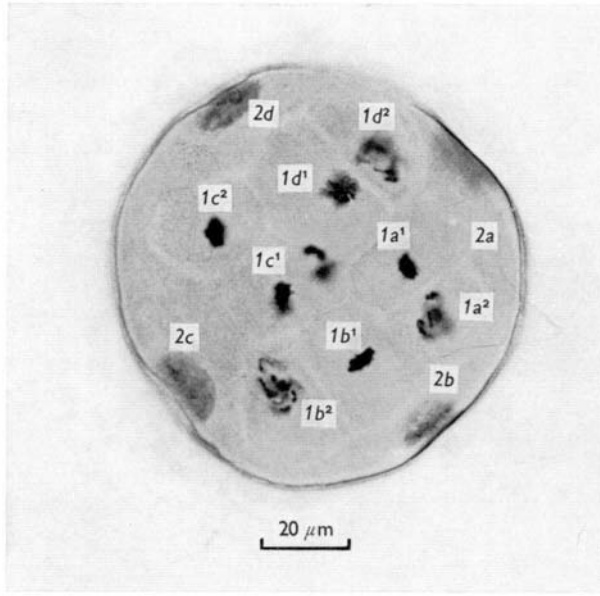

Fig. 3. Whole-mount of a Li-treated egg, the nuclei of the micromeres $1a^1$–$1d^1$ are in metaphase (two polar bodies in the centre of the animal pole), the nuclei of the micromeres $1a^2$–$1d^2$ are in prophase or in metaphase. The nuclei of the micromeres *2a–2d* are in interphase.

Finally, it should be mentioned that an aberrant cleavage pattern was not observed in experiments in which the embryos failed to develop into exogastrulae.

DISCUSSION

A complete radially symmetrical cell-pattern in the first quartet of micromeres has been observed in exogastrulae only. The data presented in this paper clearly demonstrate that in future exogastrulae division asynchrony between corresponding cells of the first quartet of micromeres – which may be regarded as the first indication of bilateral symmetry in the animal hemisphere – is completely repressed. In embryos which fail to develop into exogastrulae, division chronology is not disturbed. This suggests that during normal development bilateral symmetry in the animal hemisphere is induced at the 24-cell stage, and that this induction is associated with inductive processes preparing for gastrulation. This

assumption is supported by observations of N. H. Verdonk (to be published), who demonstrated that 7 h after third cleavage, at the end of the 24-cell stage, gastrulation can no longer be disturbed. From this point of view, a thorough study of the 24-cell stage becomes of great interest, as during this resting stage bilateral symmetry appears at the vegetative side (Verdonk, 1965), the vegetative cells come into contact with the cells of the first quartet of micromeres (Raven, 1945, 1970; Minganti, 1950), and the first indication of bilateral symmetry in the animal half seems to be induced (van den Biggelaar, 1971*b*).

The observations of Verdonk (1965, 1968) and the data presented in this paper clearly demonstrate that Li interferes with cell division. Division is stimulated in the animal trochoblasts ($1a^{21}$–$1d^{21}$), which normally stop dividing (Fig. 1). Cleavages are conspicuously more retarded at the vegetative part of the embryo than at the animal side. The question arises why Li-treatment at the onset of the 4-cell stage disturbs the cleavage pattern after the 16-cell stage. It might seem that during normal development divisions at the 16-cell stage are programmed at the beginning of the 4-cell stage. Further investigations of this problem will be important not only for understanding the effects of Li, but they will be of particular importance also for the study of the regulation of cell division.

RÉSUMÉ

Développement de l'asynchronie des divisions et de la symétrie bilatérale dans le premier quartette des micromères des œufs de Lymnaea

Des expériences ont été exécutées pour investiger la chronologie des divisions des œufs de la limnée se développant à des exogastrulas après un traitement pendant 2 h avec 5×10^{-2} M-LiCl à 6 °C au commencement du deuxième division. On observait que le développement jusqu'au stade à 16 blastomères était normal, mais que la division suivante des cellules végetatives était retardée considérablement. Il est admis que cette retardation soit associée avec la suppression de l'asynchronie des divisions dans le premier quartette des micromères (par laquelle normalement la symétrie bilatérale est annoncée), et avec la suppression de la gastrulation.

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