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Morphological transition of the nucleus during the whole life cycle of *Acetabularia calyculus* QUOY et GAIMARD

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Structural changes of the nucleus were followed throughout the life cycle of *Acetabularia calyculus* using epifluorescent microscopy with 4′-6-diamidino-2-phenylindole (DAPI) staining. This giant unicellular alga had a life cycle of 10 weeks when cultured at 22°C with 2,000 lux of illumination in Müller's synthetic medium. The correlation between cell development during the life cycle and structural transition of the nucleus was studied with special attention paid to the chromosomes and nucleoli. Morphological change of the chromosomes in diploid nuclei (primary nuclei) and the transitional process to haploid nuclei (secondary nuclei) were observed. The development and degeneration of the nucleolus in a primary nucleus were visually observed. Nucleoli were found to develop again in the haploid nuclei during cyst formation and gametogensis in the caps. Color prints of the nuclear stages in the development and differentiation during the life cycle are presented.

Key Index Words: Acetabularia; *chromosome*; *nucleolus*; *epifluorescent microscopy*; *life cycle*.

Acetabularia, a subtropical green alga, has been a useful biological material for studying nucleocytoplasmic relationships since HÄMMERLING perceived the characteristic structure of its thallus, a single giant uninucleate cell (1931), and observed the morphogenesis of an anucleate part of this cell (1932). Observation on the chromosomes were reported by SCHULZE in 1939. More recently, many cytological (PUISEUX-DAO 1966 1970), genetical (GREEN 1973), and biochemical (SPRING *et al.* 1974) studies have been conducted to elucidate the behavior of the nucleus during the *Acetabularia* life cycle. In 1979, KOOP reviewed what was known and proposed a scheme for the behavior of its nucleus during the life cycle.

In the past few years, visual observation of the nuclear behavior of this alga has become possible using epifluorescent microscopy with 4′6-diamidino-2-phenylindole (DAPI) staining (SHIHIRA-ISHIKAWA *et al.* 1982). This technique was extremely helpful for observing chromosome behavior in the primary nucleus (SHIHIRA-ISHIKAWA 1984) and for following the transitory morphology of the nuclei during the life cycle. For the present study in which nuclear behavior was to be observed throughout the life cycle, *Acetabularia calyculus* was chosen because its life cycle is much shorter than that of *A. mediterranea*, the species usually used for *Acetabularia* research (YAMAOKA-YANO 1980).

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This is the first report of visual observation of *Acetabularia* nuclear behavior over its entire life cycle, which supports the hypothetical scheme summarized by KOOP. The correlation of the development with nuclear morphology and cell appearance was elucidated and the time required for the development under definite culture conditions was also clarified.

Material and Methods

Acetabularia calyculus (Fig. 1) was first collected from Notojima Island in Ishikawa Prefecture (SANO *et al.* 1981) in August 1980. Gametes liberated after collection were cleaned of other microorganisms, and the zygotes were cultured in the laboratory with PES*-supplied synthetic sea water** under 2,000 lux continuous illumination from a fluorescent lamp at 22°C. Zygotes germinated in a few days and caps were formed in 7 weeks after germination. Under these conditions, 10 weeks were allowed for one passage through the life cycle, but the rate of growth and differentiation could be controlled by varying the culture conditions.

The cells used in this study, descendants of several generations of cells growing in this laboratory, were cultured in MÜLLER's synthetic medium (MÜLLER 1962) at 22°C under fluorescent illumination of 2,000 lux for 12 hours a day.

The cells in each stage of the life cycle were put on a slide glass and glutaraldehyde-phosphate buffer (0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0) were dropped onto the slide. For young cells (A–F in Fig. 2), an equal amount of DAPI-staining solution (NISHIBAYASHI *et al.* 1980) with glutaraldehyde was added and a coverglass was placed on them and gently pressed. For the middle-aged cells (G–K in Fig. 2), the rhizoidal part of the cells was cut with Weckel's scissors under a dissecting

* Stock solution of PROVASOLI's enriched sea water (PROVASOLI 1968).

** Synthetic sea water, "Jamarin", manufactured by Jamarin Laboratory, Osaka, Japan.

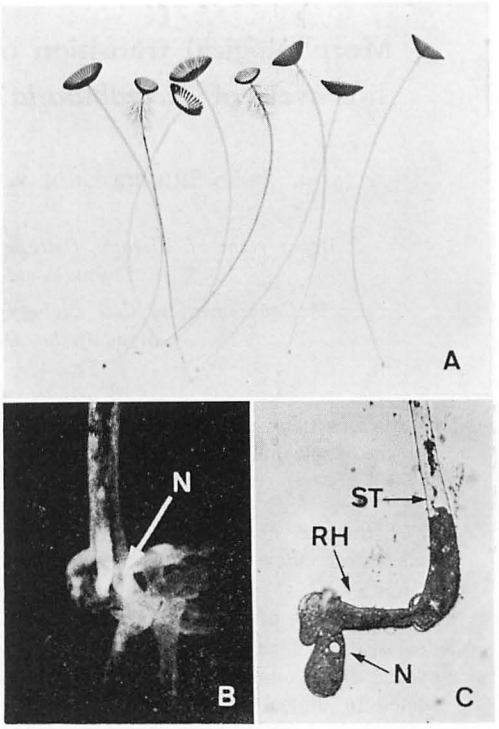

Fig. 1. A. Whole cells cultured in the synthetic medium. ×1.2; B. Rhizoidal part of the cell. ×25; C. Basal part of germling. ×100; N: nucleus, ST: stalk, RH: rhizoid.

microscope and the giant primary nucleus was pushed out from the rhizoidal part. After isolation of the primary nucleus from the cytoplasm, DAPI-staining solution was added. In the later stages, cytoplasm including the nuclei was pushed out from the stalk, cap and cyst, and the DAPI-staining solution was added. The nuclei were observed with an Olympus BH2-RFK fluorescence microscope, equipped with a high-pressure mercury vapor lamp (HBO, 100W), a 340-nm excitation filter and a 420-nm suppression filter. Fluorescent micrographs were taken with Ektachrome ASA400 color positive films.

Results

The relationship between the time elapsed and the morphological variation of cell appearance is shown in Fig. 2. Gametes (A) being liberated from one cap conjugated at

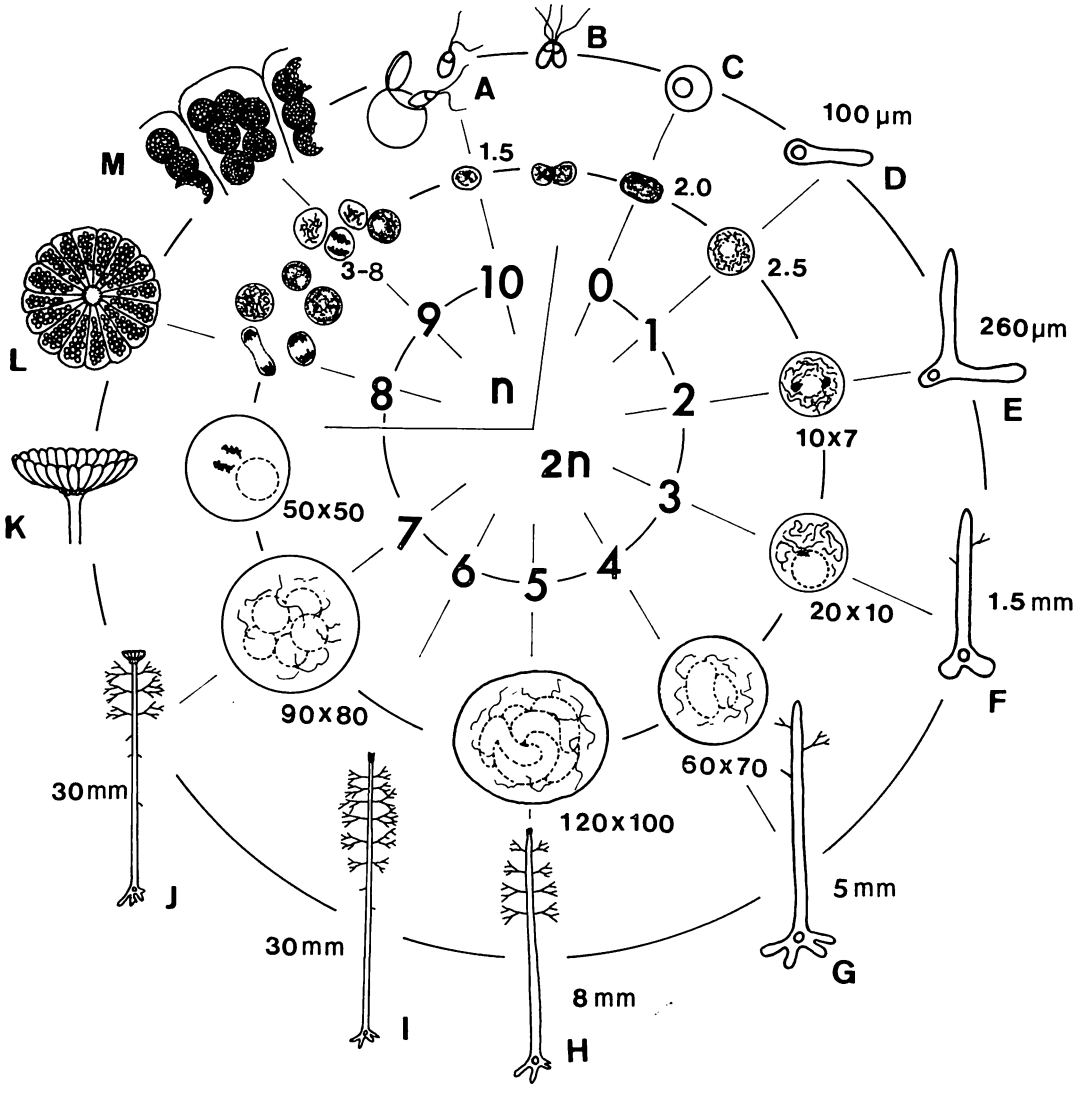

Fig. 2. Schematic presentation of the *Acetabularia calyculus* life cycle showing the correlation between the time of culture and morphological variation of the thallus (outer circle) and the nucleus (middle circle). Numbers of the inner circle indicate the number of weeks elapsed after zygote formation. Numbers of the middle circle show the nucleus size in μm.

a ratio of less than 10%. Fused gametes (B) were immediately transformed into a round immobile zygote (C). In a few days, the zygote started elongation and the cell length reached about 100 μm in a week (D), with the nucleus remaining in the orignal portion. When the germinant tube reached more than 200 μm, a vertical branch appeared (E), which developed later into a stalk, while the original tube developed into a rhizoid including a nucleus. The nucleus remained at the base of the rhizoid while the stalk and rhizoid grew and the whorls and a cap were formed (F-J). Whorl differentiation began at the 3rd week of development (F) and all whorls were shed after the initiation of cap formation (J). The cap reached maximum size in a week (K) and immediately after that numerous cysts were differentiated in the cap rays (L). Gametes were produced

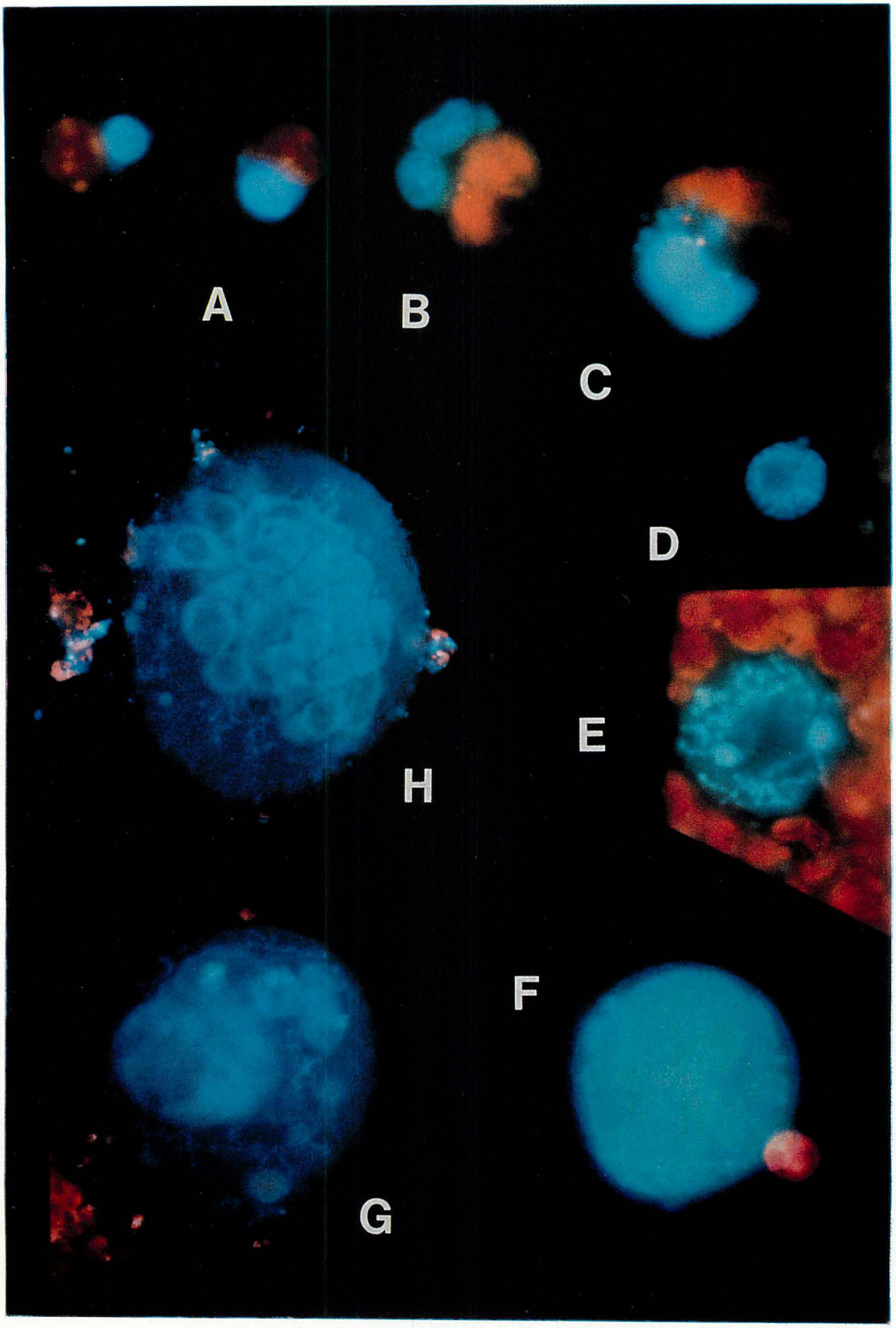

Fig. 3. Morphological transition of nuclei observed by epifluorescent microscopy with DAPI staining. See the explanatory diagram, Fig. 4.

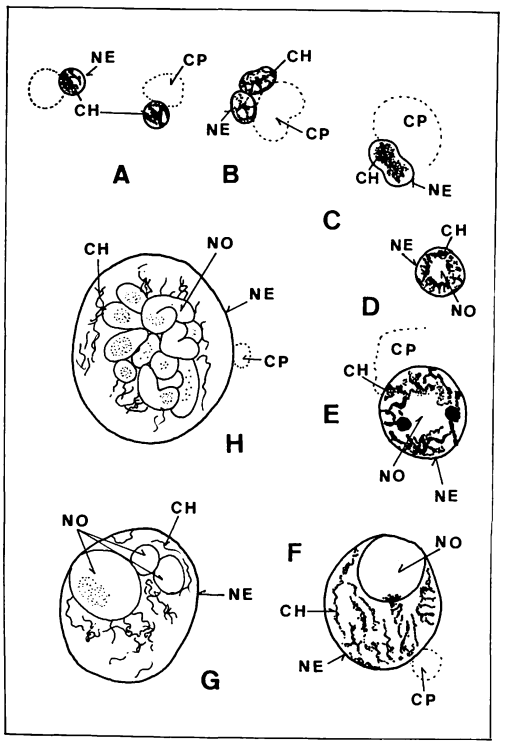

Fig. 4. Explanatory diagram of Fig. 3. A: Gamete with chromatin patches. ×5000; B: Conjugated gametes. ×5000; C: Zygote with a fused nucleus. ×5000; D: Isolated nucleus with a clear center zone of 1-week-old germling. ×5000; E: Nucleus of 2-week-old cell with DNA lumps. ×2500; F: Nucleus with matrix accumulation in center zone of 3-week-old cell. ×2000. G: Nucleus with developing nucleoli with thin chromonemata in nucleoplasm, 4-week-old cell. ×750; H: Complete giant primary nucleus with sausage-shaped nucleoli and twining chromonemata, 5-week-old cell. ×500; NE: nuclear envelope, NO: nucleolus, CH: chromatin or chromonema, CP: chloroplast; small spots are chroloplast nucleoids.

in the cysts (M) and were liberated through a circular opening one week after cyst formation (A).

Morphological changes of the nuclei observed with an epifluorescent microscope are shown in Figs. 3, 4, 5, 6, 7 and 8 while the correlation of the development with nuclear morphology and cell appearances is shown in Fig. 2.

A single nucleus, 2-3 μm in diameter, was located at the anterior portion of the cell and contained a chromatin structure (Fig. 3-A). No nucleolus could be identified. Nuclear fusion occurred after gamete fusion (Fig. 3-B) and the fused nuclei became elliptic (Fig. 3-C) at the time of zygote formation. In the nucleus of a 1-week-old cell, a chromatin-free center zone appeared and chromatin was located in the outer area (Fig. 3-D). The nucleus enlarged and the chromonemata became clearly visible. At the border of the center area appeared two fluorescent lumps which seemed to consist of amplified r-DNA produced by the nucleolar organizer (NOR) of the chromosomes (Fig. 3-E). The matrix accumulated in the clear zone and the DNA lumps decreased in size and finally disappeared (Fig. 3-F), suggesting that the r-DNA fibers had come loose from the packed structure of the lumps and spread out in the matrix. This r-DNA-containing matrix zone increased in volume and developed into a few blocks with enlargement of the nucleus (Fig. 3-G). Chromonemata became extremely difficult to detect because the nucleus volume increased by more than 10^5 times, though the DNA volume in the nucleus did not increase more than twofold. The nucleoli, the matrix zone containing developed r-DNA, assumed sausage-like shapes and frequently occupied more than 80% of the nucleoplasm (Fig. 3-H). Thin chromonemata twined about the nucleoli and spread out in the nucleoplasm (Fig. 3-H).

Seven weeks after the zygote stage, cap formation was initiated. The giant primary nucleus containing the sausage-shaped nucleoli remained for two weeks before cap formation. Soon after the initiation of cap formation, the nucleolus separated into numerous spherical units (Fig. 5-J-1), fusing with each other and finally becoming a single large spherical body (Fig. 5-J-2). Thin chromonemata (Fig. 5-J-1) became thicker (Fig. 5-J-2) and condensed on the surface of the nucleolus forming a lump. Fig. 5-J-3 shows the loosening of the thick chromosomes from the lump. The chromosomes became distributed in the nucleoplasm (Fig. 5-J-4) and became shorter in 24 hrs (Fig. 5-J-5). The peculiar chromosome shapes suggest the occurrence of chiasmata. The

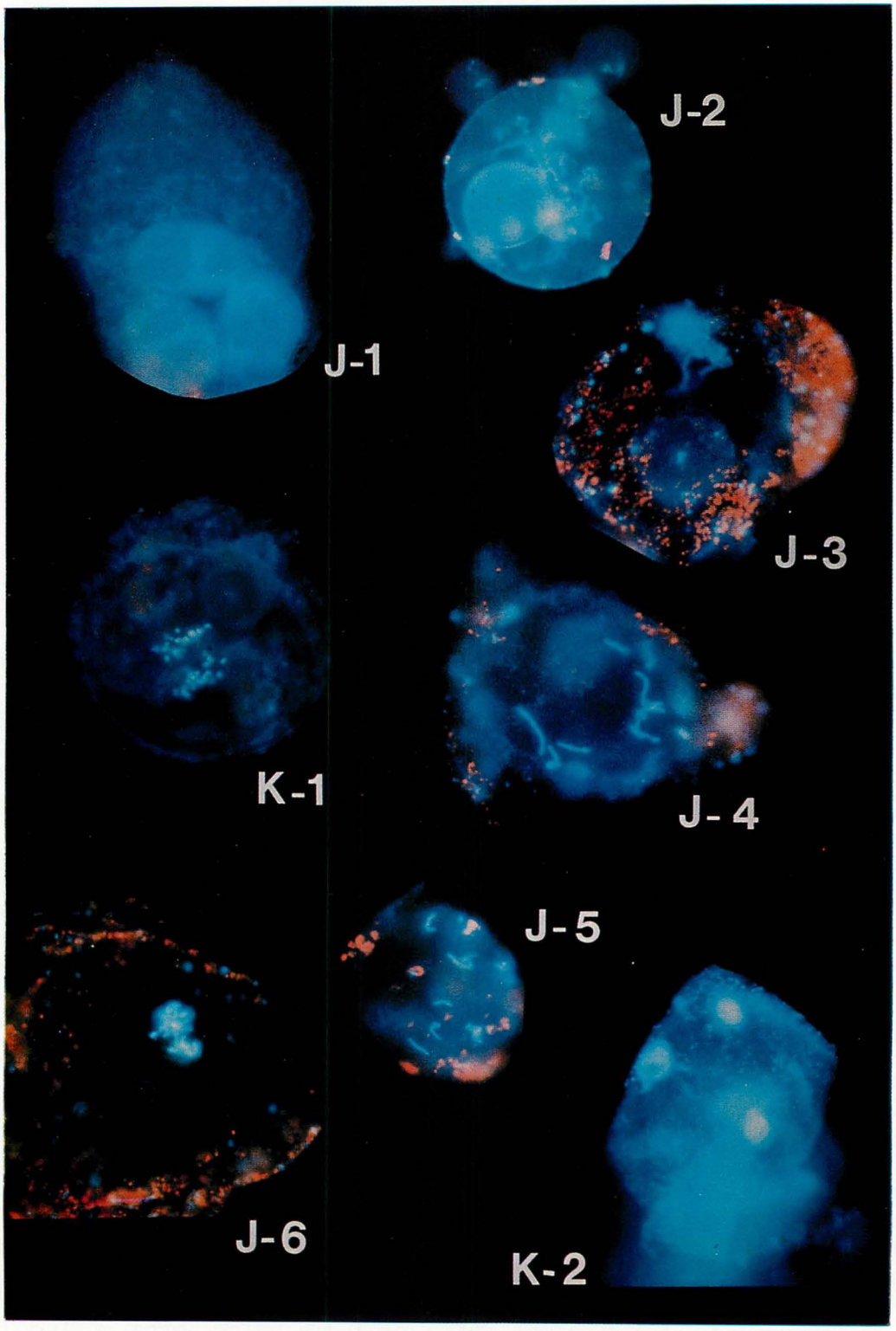

Fig. 5. Morphological transition of nuclei observed by epifluorescent microscopy with DAPI staining. See the explanatory diagram, Fig. 4.

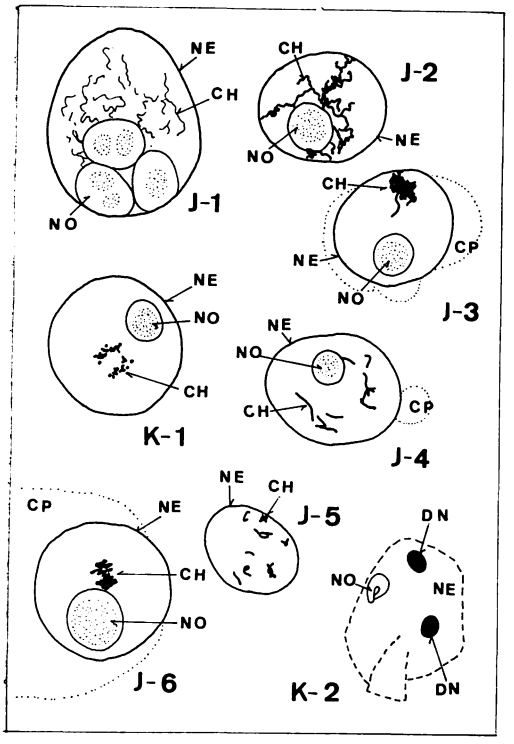

Fig. 6. Explanatory diagram of Fig. 5. Process of "meiotic division" of the primary nucleus. J-1: Nucleus 2 days after cap initiation, showing separate spherical nucleoli. ×500; J-2: Nucleus 24 hrs after J-1 with a fused nucleolus and tangled, thicker chromosomes. ×450; J-3: Chromosome lump loosened from surface of the spherical nucleolus, soon after J-2. ×500; J-4: Thick chromosomes distributed in the nucleoplasm, 24 hrs after J-2. ×500; J-5: Shortened chromosomes. ×480; J-6: Chromosomes arranged at metaphase plate. ×750; K-1: Chromosome segregation, 12 hrs after J-4. ×750; K-2: Daughter nuclei and degenerated nucleus remaining on the broken envelope of the primary nucleus, immediately after nuclear division. ×1000; NE: nuclear envelope, NO: nucleolus, CH: chromo some, CP: chloroplast; small spots are chloroplast nucleoids.

process from J-1 to J-5 is a prophase of "meiosis". Next, the chromosomes again assembled near the nucleolus and arranged themselves at the metaphase plate (Fig. 5-J-6). The chromosomes then became segregated and moved towards the poles (Fig. 5-K-1). Two daughter nuclei on the broken piece of nuclear envelope can be seen in Fig. 5-K-2. The nucleolus entirely degenerated and became fixed on the broken envelope (Fig. 5-K-2). We seldom observed four daughter nuclei which would result from successive secondary meiotic division. At the nuclear stage of K, the cap size reached its maximum dimensions. Four days were allowed for the passage through the "meiotic division".

After "meiotic nuclear division" of the primary nucleus, numerous secondary nuclei were produced as a result of repeated mitotic division in the rhizoid and stalk, moving towards the caps (Fig. 7-L-1). These nuclei entered each cap ray and became arranged in the cytoplasmic layer located underneath the cell membrane leaving spaces between the nuclei. These nuclei stopped dividing for about a day and increased in size to 7-8 μm in diameter (Fig. 7-L-2). Several small nucleoli were detected in the surface area. The process of cyst formation observed under a dissecting microscope is shown in Fig. 9. Cytoplasm gathered around the nuclei and formed same-sized spherical cysts, originally including a nucleus in each. Cysts were formed within 48 hr after the first secondary nuclei production (Fig. 2). In the cysts, mitotic division of the secondary nuclei was again repeated (Fig. 7-M-1), increasing the number of nuclei (Fig. 7-M-2, M-3) until approximately one thousand gametes were produced in each cyst. In the prophase and metaphase of this mitosis, a single large nucleolus was observed, suggesting a considerable ribosomal production in the gametogenesis.

Discussion

Chromosome behavior in the primary nuclei of *Acetabularia* had remained unknown for more than 40 years since Hämmerling's discovery of the special characteristics of the nuclei. Recently, some observations have been made using phase-contrast and electron microscopy by SPRING *et al.* (1974 1975 1978), KOOP (1979) and TIKHOMIROVA (1979). Lampbrush-like chromosomes, spindles and the nucleolar structure have been reported, but

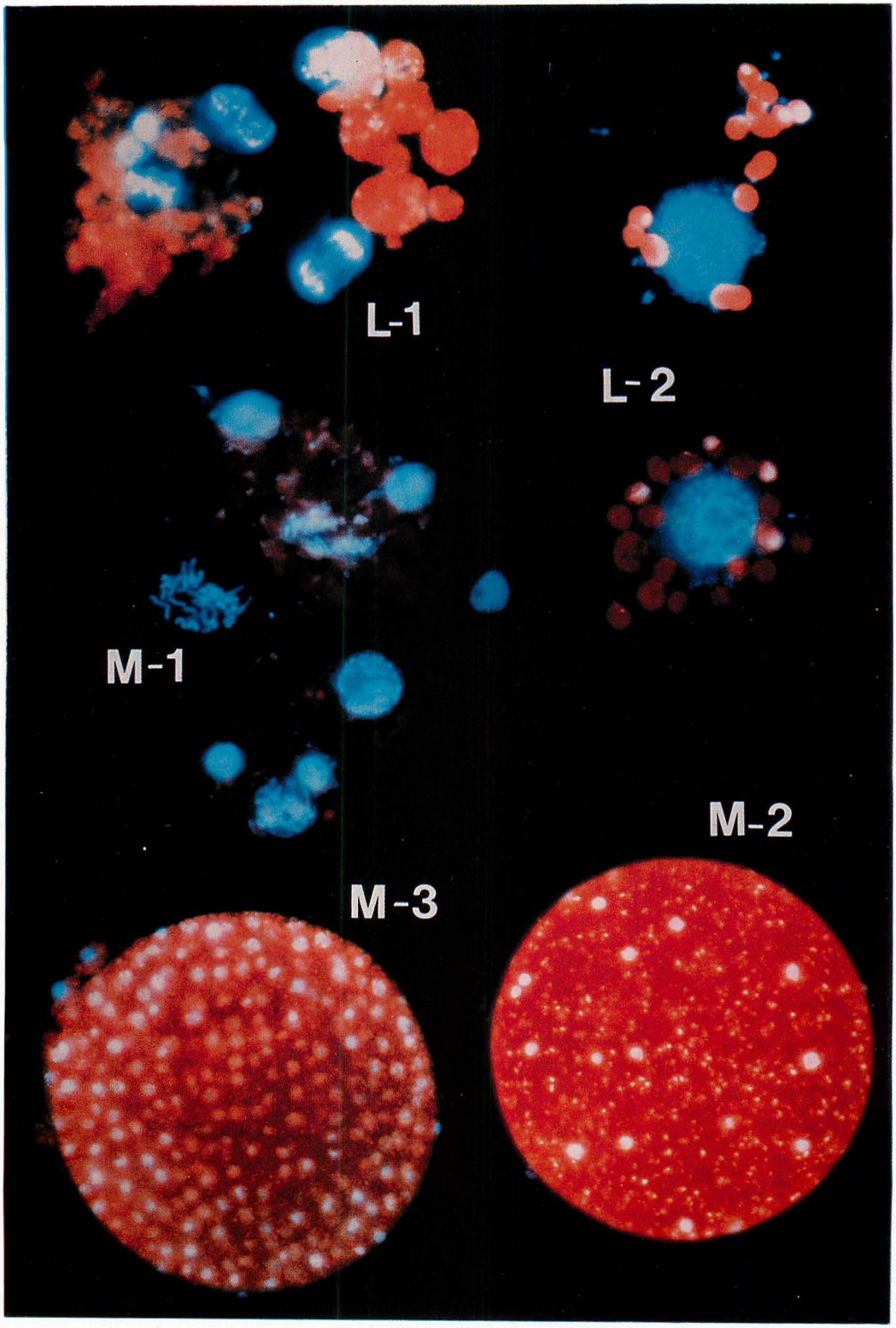

Fig. 7. Morphology of secondary nuclei observed by epifluorescent microscopy with DAPI staining. See the explanatory diagram, Fig. 8.

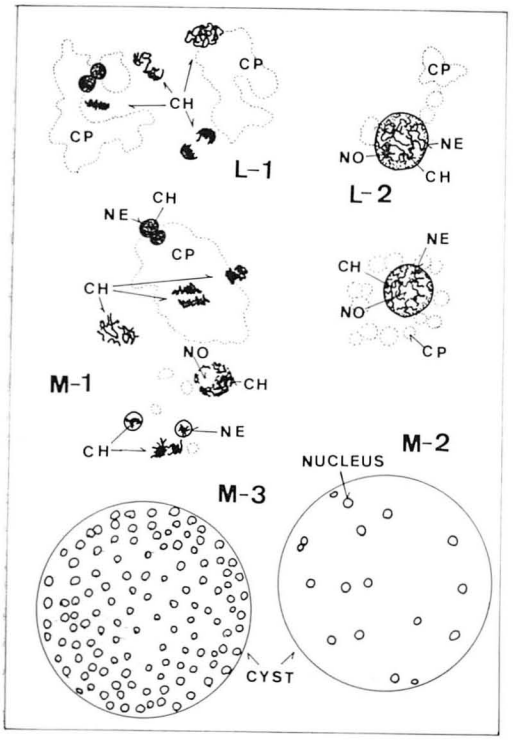

Fig. 8. Explanatory diagram of Fig. 7. L-1: Secondary nuclei in the rhizoid, dividing in mitosis, immediately after K-2. ×1600; L-2: Secondary nuclei in the caps, after translocation from rhizoid to caps. Mitotic division slowed down and the nuclei stopped movement. Cyst formation began 24 hrs after the end of "meiotic division". ×1600; M-1: Mitotic division of secondary nuclei in the cyst in gametogenesis. ×1600; M-2: Young cyst containing some nuclei resulting from repeated mitotic division in the cyst. ×200; M-3: Numerous nuclei in a cyst in which the cytoplasm was later transformed into gametes including a nucleus. Gametes were released from the cysts 7 weeks after cyst formation. ×200; NE: nuclear envelope, NO: nucleolus, CH: chromosome or chromatin, CP: chloroplast; small spots are chloroplast nucleoids.

the consecutive behavior of the chromosomes had not been observed. This paper presents the transition of the chromosomal and nucleolar appearance during the life cycle of *Acetabularia calyculus*. Structural details will be reported in other papers. Chromosome behavior in "meiotic division" in a primary nucleus has been reported (SHIHIRA-ISHIKAWA 1984), but the period of DNA

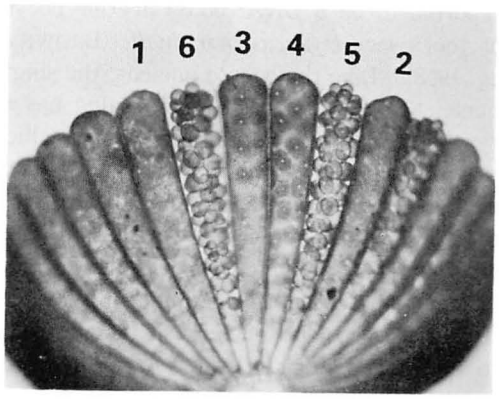

Fig. 9. Cyst formation in cap rays. ×20. Cyst formation occurred non-synchronously and was completed in all of the cap rays in a cap in 12 hrs. Each ray shows a stage in the course of cyst formation. Numbers indicate the order of the progress. White spots seen in 2, 3 and 4 are nuclei.

duplication and initiation of "meiosis" had not been elucidated.

The primary nucleus of *Acetabularia* had been suspected to be polyploid (PUISEUX-DAO 1970). Recently, quantitative analysis of the DNA content in the nucleus confirmed that the DNA volume in the primary nuclei was double that in the gametes. During "meiotic division", the approximate number of chromosomes could be counted in the primary nucleus, suggesting around 20 as a haploid, which corresponded with Yabu's results (1981) on the gametogenesis. Such a small volume of chromosomal DNA in the giant primary nucleus had posed a problem to detection by microscopy. This difficulty could be overcome by epifluorescent microscopy using DAPI staining which made thin chromonemata in the primary nucleus twining round the nucleolar structure become clearly visible.

The development of the nucleolus was observed for the first time with this alga. The lumps of DNA in the nuclei of young *Acetabularia* cells are presumed to be accumulations of r-DNA which were formed by the nucleolar organizers of the chromosomes due to excess amplification of r-DNA cistrons, as has been found in some animal oocytes (BROWN 1967). A lump of r-DNA has been

reported to be a DNA body in the oocyte of the insect *Dytiscus marginalis* (BROWN *et al.* 1968). Like the oocyte nucleus, the single nucleus in a young cell of this alga has to synthesize a large amount of ribosomes which will be required in later stages of development. *Acetabularia* is an unusual alga with a diploid phase under the regulation of a single nucleus, although its cell size is much larger than those of other unicellular algae. The increase of its nuclear size corresponds to the development of the nucleolus which results in an increase in ribosome productivity. Small DNA-containing particles, 2-3 μm in diameter, were observed in young cells beside a primary nucleus (SHIHIRA-ISHIKAWA *et al.* 1982). They may be some DNA parts which had been amplified from nuclear DNA and emitted through nuclear pores like spherules (BOLOUKHERE 1970). They may support the role of the nucleolus in extranuclear protein synthesis to construct and maintain such a huge cell, like the karyosome discussed by BARLOW (1983) in seedlings of higher plants. The degeneration of the nucleolus and the occurrence of "meiotic division" may correspond to cessation of the nucleolar activity in the primary nucleus.

A single nucleolus can be clearly identified in each of the secondary nucleus soon after "meiosis", while in a cap ray, each nucleus contains several small nucleoli. In the cyst, each prophase nucleus contains a large, distinct nucleolus and some have fluorescent lumps like those in the young primary nucleus. Thus, protein syntheses seem to occur actively in cysts independent of those derived from the primary nucleus.

This study enabled identification of the period of active r-RNA production from changes in the nucleolus appearance. However, when and how m-RNA is produced remains for elucidation by further study.

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石川依久子*・黒岩常祥：ホソエガサ（*Acetabularia calyculus*）の生活環における核形態の変遷**

巨大単細胞緑藻ホソエガサの成長と分化に対応する核の行動を DAPI 染色による DNA の螢光で観察した。合成海水，2000 lux 照射 22°C の室内単藻培養で数世代を経過した藻体を用いた。一次核は発芽後 4 週間で最大（直径 100 μm）となりカサ形成時に減数分裂をおこなって二次核に分化した。一次核内の染色体の挙動および核小体（仁）の発達退化過程がはじめて可視的にとらえられた。核小体の発達に先駆けて発芽体の核内に DNA 塊が一時的に出現した。これは昆虫の卵細胞で報告されている DNA body と同様にリボソームの DNA 一時的大量増ではないかと考えられる。二次核は有糸分裂をくり返してカサに移動しシストを形成し，更にその中に多数の配偶子を形成するが有糸分裂の前期中期の核にはかならず明瞭な核小体の発達がみられた。シストははじめ一個の二次核を中心として形成されるが，シスト形成のためにカサ内で静止した二次核は，分散した数ケの核小体をもっていた。（*豊中市待兼山 1-1 大阪大学教養部生物学教室　**岡崎市明大寺町基礎生物学研究所）