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Cell architecture in the morphogenesis of coenocytic alga *Vaucheria sessilis.*

I. The morphology of germination and the behaviour of nuclei

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Summary

Four stages in the germination of aplanospores in *Vaucheria sessilis* were identified based on morphological pecularities of the thallome and the position of nuclei. Nuclear division starts before the appearance of germinative changes, and mitoses occur until the formation of vegetative thallomes takes place. Two populations of nuclei are formed during the germination: a) the apically positioned cluster of non-dividing nuclei with constant locations and number, and b) randomly distributed, dividing nuclei which can migrate throughout a basal part of the branch.

Key words: morphogenesis, *Vaucheria sessilis*, tip growth, germination, karyokinesis

Introduction

Vaucheria sessilis (Vauch.) D.C. is a siphonaceous alga belonging to *Xanthophyceae*. Life cycle of *V. sessilis* includes the respective stages of sexual, asexual, and vegetative reproduction. Vegetative reproduction occurs by fragmentation of vegetative filaments, while sexual reproduction is performed by conjugation between spermatozoids and the egg cell. Asexual reproduction is due to the germination of either aplanospores or zoospores. Vegetative thallome consisting of the branching tubular filaments 42-120mm in diameter, with no septae, is formed as a result of the aplanospore germination. The thallome exhibits tip, or apical, growth (Oliveira and Fitch, 1988). In contrast with the diffuse growth, a site of expansion in tip-growing cells is associated with dome-shaped apex of the filament, that results in a characteristic tubular morphology. Apical growth is characterized by the highly determined localization and movement of organelles, as well as by polarization of the synthesis and secretion of cell wall precursors. Tip growth is a cornerstone of the morphogenesis in pollen tubes (Steer and Steer, 1989; Pierson and Cresti, 1992), root hairs, algal rhizoids (Kropf, 1994), and fungi (Hearth, 1990; Gow, 1995). In such cells, taxa-specific morphogenesis is connected with a deep transformation of cell architecture, directed movement of organelles, and the specific planar orientation of nuclear and organellar divisions (Fowler and Quatrano, 1997). However, there are general aspects of all these cell types, namely, what the source and nature of signals which target the morphogenesis is, what the developmental consequences of these events for cell differentiation are, and how the sites for cell (nuclear) division and cell expantion get coordinated Complex study of the germination in a siphonaceous alga *V. sessilis* would create a basis for broad comparison of the morphogenetic cascades in different phylogenetic groups, and could serve for the understanding of basic principles of the morphogenesis in apicaly growing cells.

Material and Methods

Culture procedures

The object of investigation was V*aucheria sessilis* (Vauch.) D.C. CALU1024 obtained from the algae col-

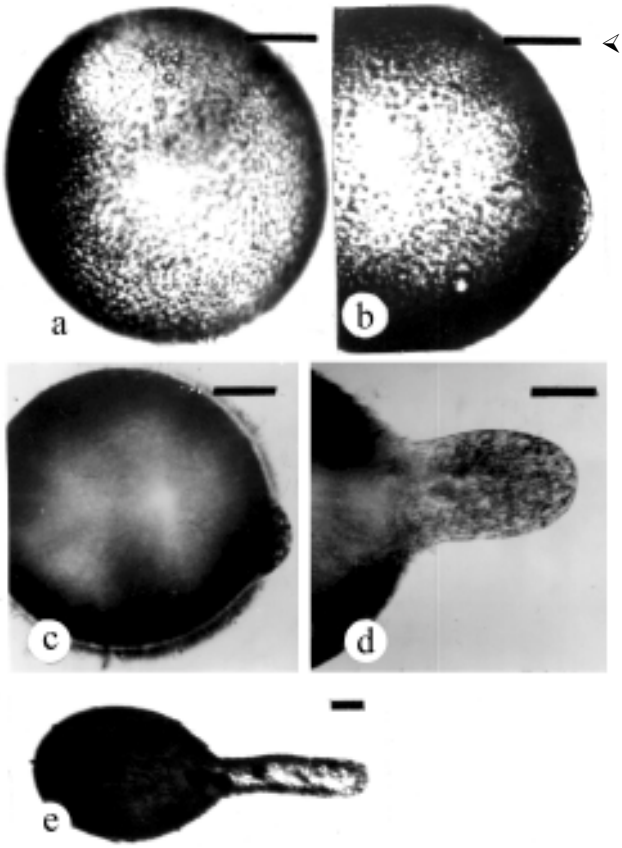

Fig. 1. Stages of aplanospore germination of *V. sessilis.* **a** – non-germling aplanospore. **b** – Phase I; the formation of hyaline cap. **c** – Phase II; the formation of primary filament. **d** – Phase III; the formation of secondary filament. **e** – Phase IV; the formation of vegetative branch. Scale bar 10µm

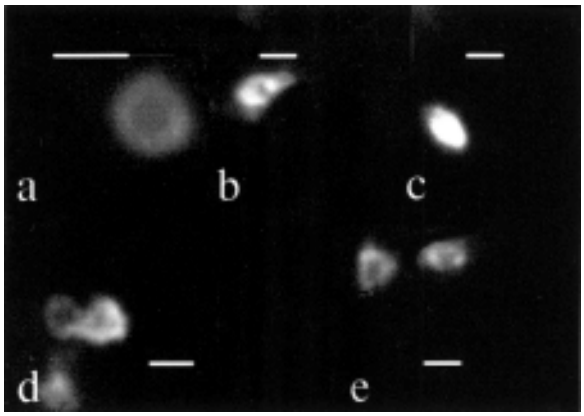

Fig. 2. Stages of karyokinesis of *V. sessilis.* **a –** interphase spherical nuclei in vegetative filament; the dark spot in the central zone is a nucleolus; **b –** prophase nucleus with nucleolus; **c –** metaphase nucleus; **d –** anaphase nuclei; **e –** telophase nuclei; nucleolus reassembled. Scale bar 3 mm.

lection of Göttingen University. Cultures were maintained in standard mineral medium (Gromov, 1965) containing 0.1% soil extract, and solidified with 1.5% agar (Difco), under continuous illumination of 10^3 lux.

Fresh liquid medium was added to the culture before the aplanospore induction which was triggered by alternating light/dark periods of 24h. At the end of a dark period, aplanospores were carefully collected with a Pasteur pipette under visual control under a BIOLAM-10 binocular. Growth rate and the morphology of thallome were observed in a PZO microscope.

Observation of nuclei

The material was fixed with 1.2% glutaraldehyde in 0.1M Na-phosphate buffer, pH 7.0 for 1 h. To ensure the permeabilization, DMSO (1%) was added, that is known not to effect cell structure and cytoplasmic stream (Peat and Oliveira, 1994). Nuclei were stained with 5µg ml^{-1} DAPI (4', 6'-diamidino-2-phenylindole) for 1h at room temperature, or overnight at 4ºC. Between individual steps of the procedure, 0.1M Na-phosphate buffer, pH 7.0, was used as a rinsing solution. The material was examined under a Leica DMRXA microscope.

Results

Round aplanospores of 50-70 µm in diameter (Fig. 1a) are formed at the tip of sporangium. After maturation, a deep green aplanospore, which demonstrates dense packing of chloroplasts and nuclei (Fig. 3a), is released from the sporangium. Spherical nuclei in vegetative filaments are 3 mm in diameter; in the central zone dark spot which corresponds to the nucleolus is observed (Fig. 2a). Nuclei are randomly scattered througout the aplanospore. Their shape is irregular, and their size is 2-3 times less than that of the nuclei of vegetative filaments. The amount of nuclei in mature aplanospore is about 2000 (Fig.3a). Oval-shaped nuclei with nucleoli, elongated nuclei with no detectable nucleoli, as well as triangular nuclei were detected. These nuclear patterns are typical of the karyokinesis. The absence of rounded nuclei with pronounced nucleoli testifies that all nuclei are involved in the process of karyokinesis. All nuclei divide simultaneously, individual mitotic stages coincide in time. Mitotic spindle is completely closed, nuclear envelope remains intact until the late telophase (Ott and Brown, 1972). In prophase, the nucleoli are preserved; nuclei became elongated and oval in shape (Fig. 2b). The nucleolus gets fragmented during metaphase and becomes invisible. The volume of

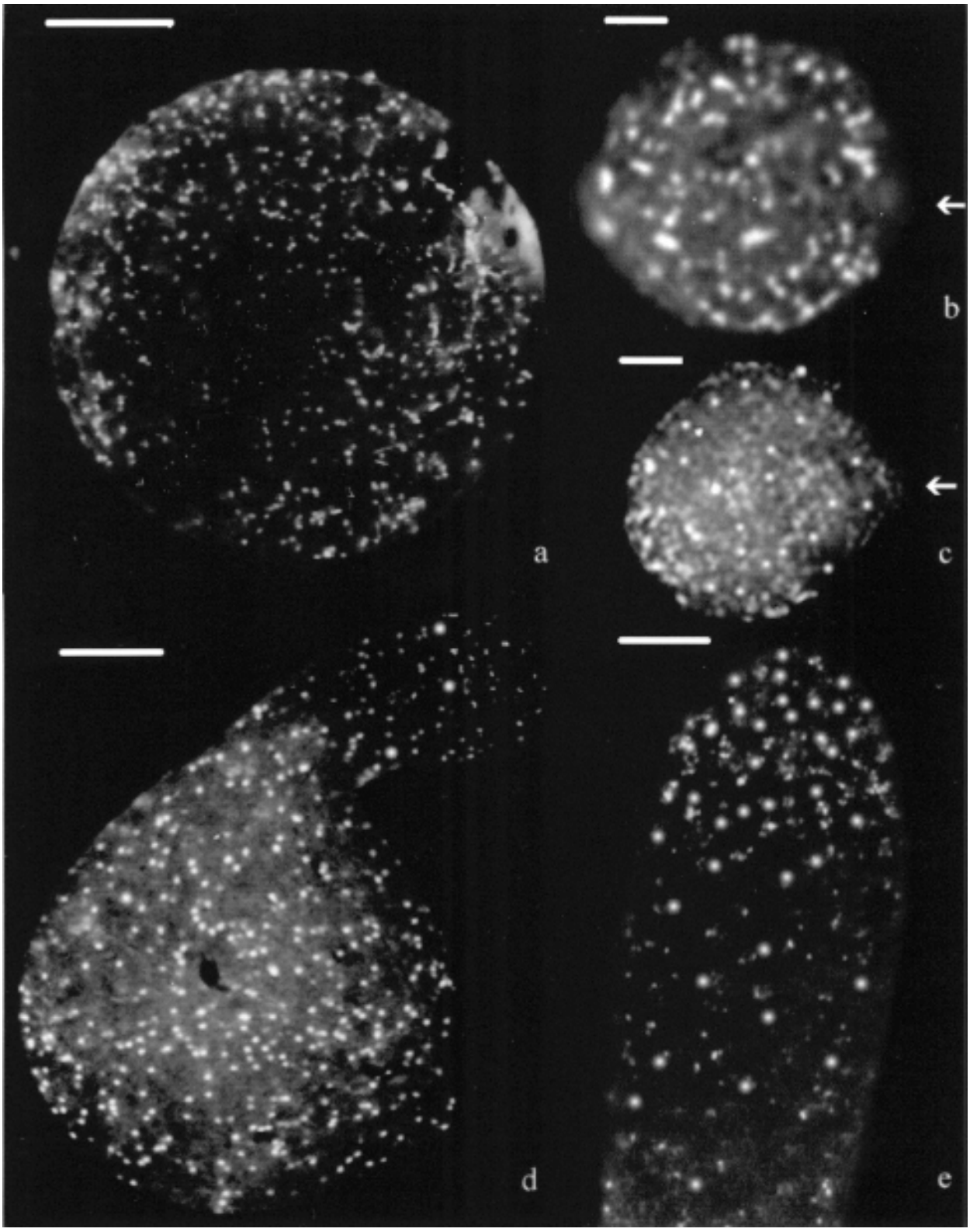

Fig. 3. Nuclei distribution in course of germination of *V. sessilis*. Scale bar 10 mm

a – non-germling aplanospore; nuclei are randomly scattered througout the aplanospore.

b – Phase I of germination; the distribution of nuclei at different stages of the division is the same as in the non-germling aplanospore; arrow- position of hyaline cap.

c – Phase II of germination; arrow - position of primary filament.

d – Phase III of germination; nuclei migrate into the forming filament.

e – Phase IV of germination; nuclei cluster in apical zone, nuclei randomly distributed in basal part of the branch.

nuclei increases twice, the condensed chromatin demonstrates bright fluorescense (Fig. 2c). In anaphase and telophase, the nuclei are easily discerned because of their strongly elongated and angular shape. In anaphase, daughter nuclei still closely assosiate with each other (Fig. 2d). In telophase, nuclei are interconnected via a thin bridge of interzonal microtubules (Ott and Brown, 1972), the length of which is up to 2 µm. Nucleoli are reassembled, and they can be easily detected (Fig. 2e).

The germination of aplanospores occured without a lag period immediately after release of aplanospores from the sporangium, sometimes at the tip of sporangium. The duration of germination time varies with temperature, although it is independent on light or the composition of culture media. The germination of aplanospores in *V. sesilis* proceeds in four phases.

Phase I represents the stage of formation of a hyaline cap - the domelike opaque area free of nuclei and chloroplasts (Fig. 1b). The length of hyaline cap increases with at a rate of 39 mm/h. The distribution of nuclei at different stages of the division is the same as in the non-germinating aplanospore (Fig. 3b). The duration of these stage is very short (10-15 min).

Phase II is the stage of the formation of a primary filament (Fig. 1c). Growth rate at this stage was 43-52 mm/h. Nuclei are regulary distributed within aplanospores; most of them showing various stages of the karyokinesis (Fig. 3c). Hyaline cap is preserved, although it slightly decreases in volume. The duration of stage II is about 15 min.

Phase III is the stage of the formation of a secondary filament (Fig. 1d). Growth rate is 29mm/h. The transposition of nuclei to the forming filament was observed. Karyokinetic processes cease, and the nuclei become round (Fig. 3d). At the end of phase III, hyaline caps disappear; and main part of the nuclei population migrates into the forming filament. The duration of this stage is 1-1,5 h.

Phase IV represents the stage of the formation of a vegetative branch (Fig. 1e). During this stage, growth rate is decreased. Nuclei show the pattern typical of that in vegetative branch, namely, the cluster with a density of 74±6 per 50 mm of thallome length is observed in apical zone (Fig. 3e). In basal parts, regular distribution of nuclei with a density of 35±5 per 50 mm is observed (Gavrilova et al., 1997). The size of apical cluster is 50-70 mm; no karyokinesis in the apical zone is observed. Individual mitotic nuclei were detected in a basal part of the branch. At the end of germination, all chloroplasts and nuclei migrate from the aplanospore towards the vegetative branch.

Discussion

The karyokinesis in mature aplanospores and germlings of *Vaucheria sessilis* possibly represents starting event in the cascade of germination processes which can be detected at morphological level. The division cycle of nuclei is presumably triggered by endogeneous factors already at a period of the formation of aplanospores. The analysis of exogeneous stimuli including different light conditions, culture medium content, and physiological temperature did not influence the onset of germination. A leader position of nuclei division in the morphogenesis, documented for algae and fungi, is in favour of a hypothesis dealing with an endogeneous nature of the triggering signals which are effective at checkpoints of the cell (nuclei) cycle (Hartwill and Weinert, 1989; Harold, 1995; Lew and Reed, 1995).

It is noteworthy that the position of division plane of nuclei is not strictly determined; mitotic nuclei rotate to adjust their orientation to that of the elongation axis, as it has been demonstrated in yeasts and fucoid zygotes (Harold, 1995; Shaw and Quatrano, 1996). The position of apical zone is determined in early ontogeny of the tip growing cells by either endogeneous (Chant and Pringle, 1995) or exogeneous (Kropf, 1992) factors. A coenocytic nature of the *V. sessilis* aplanospore is possibly masking the spatial coordination between nuclear division and the formation of a filament. Taking into account that our micrographs show no regularity in the position of mitotic nuclei, crucial step in the morphogenesis of *V. sessilis* - in analogy with fungi - is suggested to be the determination of an apical zone rather than the karyokinesis proper.

However, guided migration of nuclei along the axis of elongation, described as the common character of tip-growing cells (Heath, 1994; Morris et al., 1995), was also detected in the case of *V. sessilis*. The movement of nuclei coincided with a decrease in mitotic activity, and caused the formation of a distinct population of nuclei in apical zone of the growing filament. The position of nuclei in apical cluster is extremely stable. All stress factors tested do not influence the density of nuclei in apical zone (of 74 per 50 µm, see: Gavrilova et al., 1998). This cluster is composed of non-dividing nuclei only. The absence of mitoses in the apical zone has been detected long ago (Kursanoff, 1911), although the mechanisms of nuclei segregation and maintenance of their specific physiological state remain unresolved. The existense of two different populations of nuclei was demonstrated in filamentous fungi (Bago et al., 1998; Bécard and Pfeffer, 1993). This data would suggest that, in coenocytic thallome, isolated compartments can exist in the absence of physical barriers.

To summarize, the morphogenesis in *V. sessilis*, as in other apically growing cells, is charactherized by the spa-

tial segregation of sites of cell expansion and nuclei division.

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