Protoplasma 113, 132–136 (1982)

PROTOPLASMA

© by Springer-Verlag 1982

Change in the Rate of Organelle Movement During Progression of the Cell Cycle in *Adiantum* protonemata

MASAMITSU WADA[1], YOSHINOBU MINEYUKI, MASAKI FURUYA*

Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo

Received March 5, 1982
Accepted May 4, 1982

Summary

The rate of organelle movement during progression of the cell cycle in single-celled protonemata of the fern *Adiantum capillus-veneris* is determined microscopically with a time-lapse video system. Under red light organelle movement is very slow (1.8 μm/min) in early G_1 in the apical 100-μm region. The rate of organelle movement becomes higher in proportion to distance from the nuclear region, reaching a plateau in the neighborhood of 300 μm from the tip. Organelle movement during the progression of G_1 and S phases in the dark does not show a significant difference from that in early G_1 under red light. In M phase, however, organelle movement in the nuclear region slows down a few minutes after nucleolar disappearance and then stops until the beginning of cell plate formation. Organelle movement in the basal region of the protonema slows down, but does not stop, shortly after movement in the nuclear region has ceased. This indicates that a message is sent from the nuclear region to the basal region.

Keywords: *Adiantum*; Cell cycle; Fern protonema; Organelle movement; Protoplasmic streaming.

1. Introduction

It has been known that cytoplasmic viscosity changes during cell division in terms of the Brownian motion of cytoplasmic particles (CHAMBERS 1917, LEBLOND 1919, SEIFRIZ 1920), and that protoplasmic streaming is not observable in a dividing cell (SCHAEDE 1925). Changes in protoplasmic streaming, however, have not been determined with precision throughout the cell cycle.

A single-celled protonema of the fern *Adiantum* offers two advantages for analysis of changes in protoplasmic streaming during progression of the cell cycle. Progression of the cell cycle in the protonema can be controlled experimentally by light (FURUYA *et al.* 1980), and the duration of each component phase of the cell cycle is determined by experimental light conditions (MIYATA *et al.* 1979). The speed of organelle movement of fern protonemata is extremely slow, however, so that a custom-made time-lapse video system was used for observations. Illumination was provided by infrared light, which was without photomorphogenic influence (FURUYA *et al.* 1980). In the present study, the speed of organelle movement was analysed in connection with the stages of the cell cycle and with the nuclear position of *Adiantum* protonemata.

2. Material and Methods

2.1. Plant Material and Aseptic Culture

Spores of *Adiantum capillus-veneris* L. were collected in a greenhouse in the Botanical Gardens of the University of Tokyo, Koishikawa, Tokyo in 1977 and stored in a cold room at about 5 °C. The methods of aseptic culture were basically the same as those described by WADA and FURUYA (1970). The culture medium consisted of 1/10-strength MURASHIGE and SKOOG (1962) mineral salt solution with 0.5% agar as modified by WADA and FURUYA (1970). Spores were sown on the surface of the solidified medium using ITO's (1969) aseptic technique. The spores were allowed to imbibe for 1 day in the dark, and then cultured for 7 days at 25 °C under continuous red light of about $0.5\,Wm^{-2}$ provided by a fluorescent tube (Toshiba FL 40 SD/NL, Tokyo Shibaura Electric Co., Kawasaki) behind a 3-mm-thick red

[1] Present address: Biology Department, Faculty of Science, Tokyo Metropolitan University, Fukazawa, Tokyo 158, Japan.

* Correspondence and Reprints: Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan.

0033-183X/82/0113/0132/$ 01.00

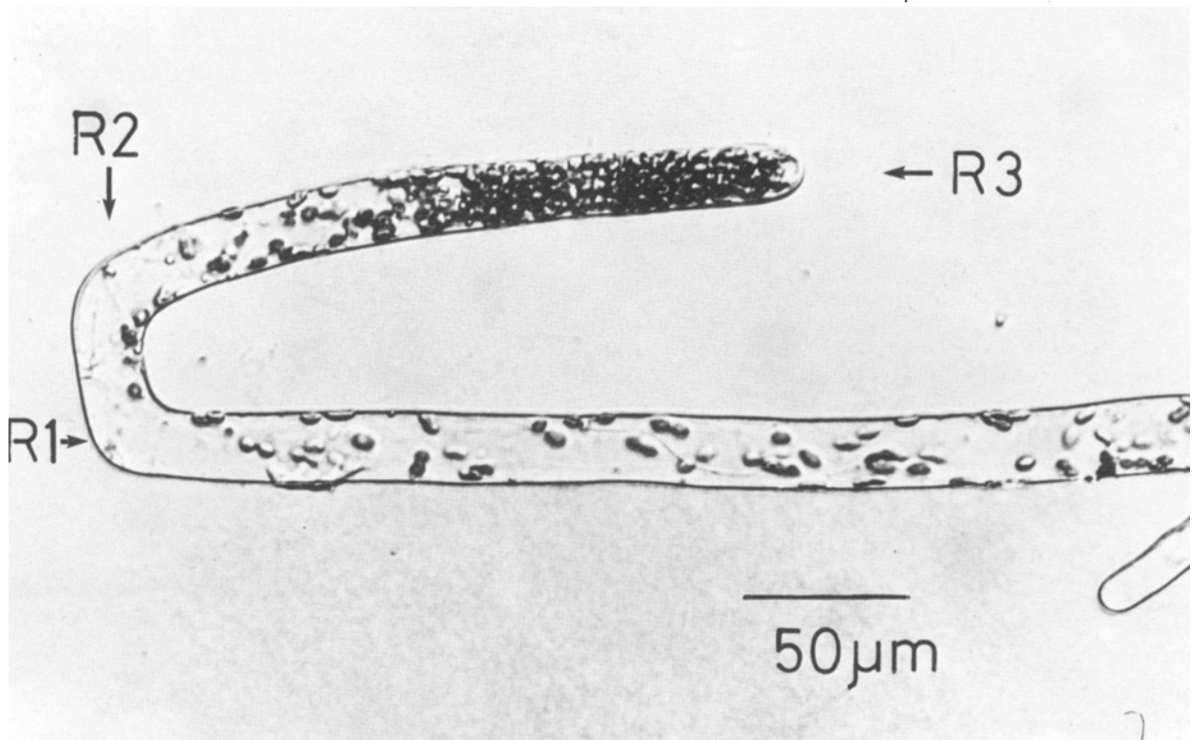

Fig. 1. An *Adiantum* protonema cultured under continuous red light for 7 days. Red light (0.5 Wm^{-2}) was given from the direction of R 1 for 6 days, then from R 2 for 4 hours and finally from R 3 for 1day

plastic plate (Torayglas 130, Toray Co. Ltd., Osaka). The light was provided in a horizontal direction. As a result, single-celled protonemata grew horizontally at the apex towards the red light source. The direction of red light irradiation was changed twice to get U-shaped protonemata (Fig. 1), which made it possible to observe simultaneously on one television screen not only the apical region of a protonema but also the basal region. When protonemata, for which the cell cycle was arrested in early G_1 by red light, were transferred to the dark, cell division occurred 30–50 hour after transfer to the dark (MIYATA *et al.* 1979). All procedures were aseptically carried out at 25 °C.

2.2. *Observation of Cell Division and Organelle Movement Under Infrared Light*

Progression of the cell cycle and organelle movement in individual protonemata was monitored and recorded continuously through a microscope. Illumination was provided by wavelengths longer than 780 nm. Images were recorded with a time-lapse video recorder coupled with a video camera equipped with an infrared sensitive tube (for detail, see FURUYA *et al.* 1980). The video system is controlled by a time-lapse controller and video timer. Real time can be recorded and shown on the video screen at 10-millisecond or longer intervals. Position of the cell tip and nucleus and the rate of organelle movement on the video screen were determined with the recorded images of cells in a stop-motion mode at appropriate intervals of time. Rate of streaming was measured by an observing on the screen of a monitor television the distance moved by organelle for 1 minute. Each point was obtained from 7–10 organelles.

3. Observations

3.1. *Pattern of Organelle Movement in Filamentous Protonemata Under Red Light*

Such organelles as reflecting round-shaped oil-drops and big spindle-shaped chloroplasts are distinguishable in the present material on the screen of a monitor television, but other organelles are difficult to identify. The rate of movement of individual organelles (excluding nuclei and chloroplasts which usually do not move) is not constant. Organelles around the cytoplasmic strand move very fast (maximum 25 µm/min, average 16.9 ± 2.6 µm/min) but those out of the cytoplasmic strand or those that hit upon a chloroplast or some other large organelle move very slowly (average 6.6 ± 3.7 µm/min). Rarely, chloroplasts are observed to move rapidly (9.0 µm/min) along the cytoplasmic strand.

3.2. *Organelle Movement Along the Growing Axis of Filamentous Protonemata Under Red Light*

Organelle movement in the apical region of the protonema is very slow (average 1.8 ± 1.1 µm/min) (Fig. 2). The rate of movement of organelles in the

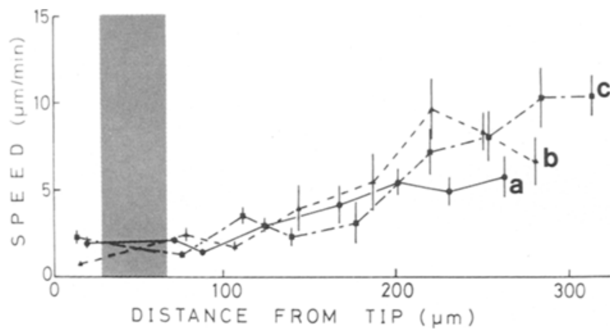

Fig. 2. Rate of organelle movement in a filamentous protonema cultured under continuous red light. The rate was determined 3 times at 1 (*a*), 5 (*b*) and 11 (*c*) hours after the onset of time-lapse recording. Mean and standard error of each point was obtained from 7 to 10 organelles. ■ nuclear region

nuclear region was also relatively slow and was very difficult to determine, because not only were many organelles accumulated around the nucleus, but also their movement was not directional but irregular. Once an organelle moves away from the nuclear region, its rate of movement is more rapid the further it is from the nucleus until it reaches a maximum at about 300 μm from the tip of the protonema (Fig. 2).

3.3. Organelle Movement During Growth Under Red Light

Organelle movement in filamentous protonemata in early G_1 under red light was recorded for 3 days. The rate of organelle (except chloroplast) movement was measured at a fixed position 200 μm from the tip of the protonema at the beginning of the experiment and 470 μm from tip at the end of the experiment (Fig. 3).

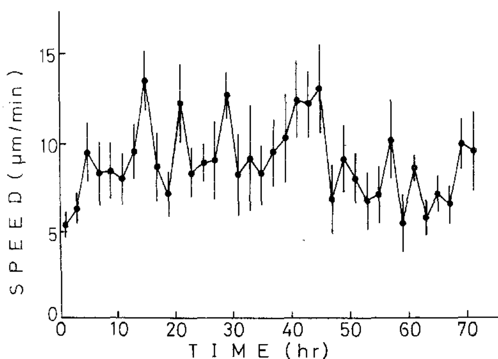

Fig. 3. Time course study on the rate of organelle movement in an early G_1-arrested protonema under red light. The rate was determined at a fixed position of 200 μm from the tip of the protonema at the beginning of the experiment and 470 μm at the end. Mean and standard error of each point were obtained from 7 to 10 organelles. There is no significant difference among points at the 5% level as judged by an F-test ($F^{35}_{287} = 1.480$)

The rate of movement was 8.9 ± 5.5 μm/min. No significant difference among measurements was detected at the 5% level of an F-test.

3.4. The Change in Organelle Movement During Progression of the Cell Cycle

When a protonema grown under red light was transferred to the dark, the cell cycle was initiated and cell division took place within 52 hours after transfer. The rate of organelle movement during progression of the cell cycle could thus be determined (Fig. 4).

The rate of movement was measured throughout the cell cycle at positions of 35 to 65 μm away from the

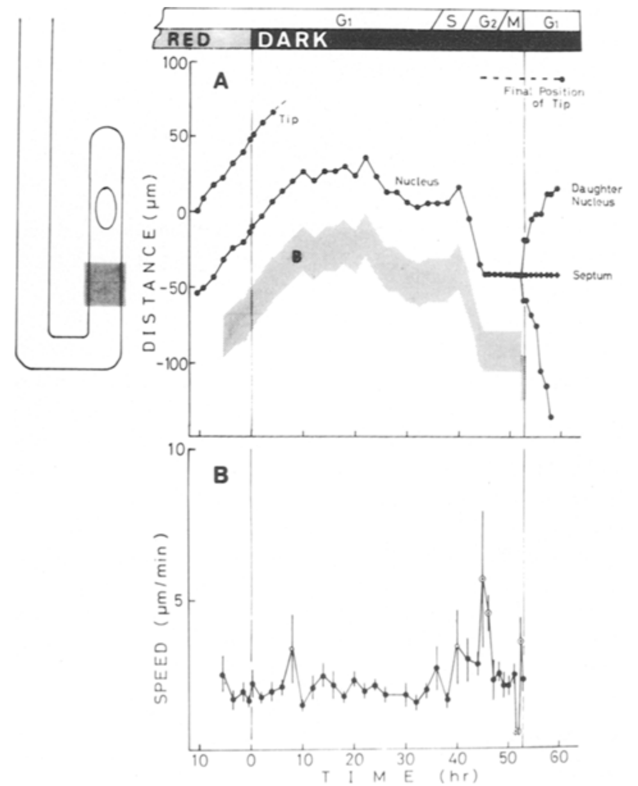

Fig. 4. Time course of nuclear migration and change in rate of organelle movement during the cell cycle in a protonema of *Adiantum*. The protonema was cultured under red light for 7 days (▦) and then transferred to the dark (■) so that cell division was induced. (A) Apical growth, nuclear migration, and the region (▦ B) where the speed of organelle movement was measured. The average duration of G_1, S, G_2 and M phases in the cell cycle has been estimated from the data of Miyata (1979). B region is the region of from 35 to 65 μm in a basipetal direction from the center of the moving nucleus. (B) The change in rate of organelle movement in the B region during the progression of the cell cycle. ⊙, ○: These symbols indicate significant differences from the rate in early G_1 phase at 5% and 1% levels, respectively, by a t-test

center of the moving nucleus (B in Fig. 4 A). In this case, the speed of streaming during G_1 and S phases showed no significant difference with that in early G_1 under red light (Fig. 4 B). During G_2 many organelles, including chloroplasts, gathered into the nuclear region and accumulated around the nucleus.

The rate of organelle movement in the M phase decreased throughout the protonemal cell (Table 1), particularly in the nuclear region where movement stopped completely a few minutes after nucleolar disappearance. Movement did not resume until the beginning of cell plate formation. The rate of organelle movement in the region between 35 to 65 μm from the center of the nucleus (B in Fig. 4 A) slowed to 0.7 μm/min about 6 minutes after movement in the nuclear region had stopped. In the region further from the nucleus (215 to 245 μm from the center of nucleus) organelle movement also slowed to some extent, but the timing at which it slowed was later than that in the region of 35 to 65 μm from the nucleus.

Table 1. *Changes of the timing of retardation and acceleration of organelle movement during the mitotic phase in different regions of the cell relative to the nucleus*

Position	Movement Retardation		Timing of
(μm from center of nucleus)	Timing (min)	Speed (μm/min)	Acceleration (min)
Nuclear region	0*	0.0	45
35–65	6	0.7	46
215–245	36	1.3	46

Organelle movement during the mitotic phase in one protonema was observed continuously. The timing of the retardation and the acceleration of the movement and the rate of the movement during the retardation were measured in proportion to the distance from the nucleus.

* 0 time is 51 hours 24 minutes after transfer from the red light condition to darkness.

Just before the end of cell plate formation, the rate of organelle movement recovered very quickly (Fig. 4 B) throughout the cell. At the beginning of migration of the daughter nucleus toward the center of the apical cell, chloroplasts that had been spread over the apical cell gathered quickly around the nucleus. The nucleus then migrated slowly toward the center of the cell.

4. Discussion

The rate of organelle movement slowed during the M phase in the filamentous *Adiantum* protonema, especially in the nuclear region. This phenomenon may be the same as the "fixed tension" state in tobacco cells reported by JONES *et al.* (1960). They reported that "the change from fluid state into the 'fixed tension' started in the prophase strands near the nucleus and progressed toward the distal end of the dividing cell". In our material, retardation of organelle movement starts in the nuclear region and extends towards the basal region. These facts indicate that some message to stop organelle movement arises from the nuclear region.

Comparing our observations with those of organelle movement in somatic tobacco cells (JONES *et al.* 1960), two different results on the timing of retardation (or change into "tension" state) and on the recovery of organelle movement are evident. First, "all of the cytoplasm was under 'tension' by late prophase" in tobacco cells, but the timing of retardation is several minutes after nucleolar disappearance, even in the nuclear region in the protonema of *Adiantum*. Namely, in the latter, the tension state begins not in prophase, but in metaphase. Second, "reversion started at the distal end and progressed toward the new cell wall and was completed within 1–2 hours" in tobacco cells, whereas organelle movement recovered very quickly everywhere in the fern protonemal cell. In addition, the time of recovery is just before the end of cell plate formation.

We have observed two types of protoplasmic movement. One is real, high speed streaming along the cytoplasmic strands which is readily observable in the basal region of protonema. The other is slow and undirectional, Brownian-motion-like movement around the nucleus. The mechanisms of these two types of movement are supposed to be different, indicating that the state of fixed tension during cell division in tobacco cells (JONES *et al.* 1960) must be a different phenomenon from the well known cessation of Brownian motion that is observed during cell division (CHAMBERS 1917, LEBLOND 1919, SEIFRIZ 1920).

The rate of protoplasmic streaming fluctuates among plant materials, between 2 (*Phycomyces*, POP 1938) and 1,350 μm/sec (*Physarum*, KAMIYA 1950). The average rate among higher plants is about 2 to 7 μm/sec (KAMIYA 1959). On the contrary, in the case of fern protonema the rate is very slow. The highest rate determined is 0.42 μm/sec and the average is about 0.28 μm/sec. The custom-made time-lapse video system

made it possible to quantify such a slow protoplasmic streaming.

In the same experimental system of *Adiantum* protonemata, the nuclear premitotic positioning in cytokinesis takes place at about 5 hours before cell plate formation (MINEYUKI and FURUYA 1980). Although it has been expected that the cessation of protoplasmic streaming or gelation of cytoplasm in the M phase is closely related in some way to cessation of nuclear migration, protoplasmic streaming (Fig. 4) stops later than does nuclear migration and stops after premitotic positioning of the nucleus.

Acknowledgements

The authors would like to thank Mr. Y. NEMOTO for his technical assistance. This research was supported in part by a Grant-in-Aid for Scientific Research to MF and MW and a Grant-in-Aid for Developmental Scientific Research to MF and MW from the Ministry of Education, Science and Culture in Japan.

References

CHAMBERS, R., 1917: Microdissection studies. II. The cell aster; a reversible gelation phenomenon. J. exp. Zool. **23**, 483–504.

FURUYA, M., WADA, M., KADOTA, A., 1980: Regulation of cell growth and cell cycle by blue light in *Adiantum* gametophytes. In: The Blue Light Syndrome (SENGER, H., ed.), pp. 119–132. Berlin-Heidelberg-New York: Springer.

ITO, M., 1969: Intracellular position of nuclear division in protonema of *Pteris vittata*. Embryologia **10**, 273–283.

JONES, L. E., HILDEBLANT, A. C., RICKER, A. J., WU, J. H., 1960: Growth of somatic tobacco cells in microculture. Amer. J. Bot. **47**, 468–475.

KAMIYA, N., 1950: The rate of the protoplasmic flow in the myxomicete plasmodium. II. Cytologia **15**, 194–204.

– 1959: Protoplasmic streaming. Protoplasmatologia. Handbuch der Protoplasmaforschung VIII/3 a. Wien: Springer.

LEBLOND, E., 1919: L'état de sol dans ses rapports avec l'activité fonctionnelle du protoplasme. Compt. Rend. Soc. Biol. **82**, 1220–1222.

MINEYUKI, Y., FURUYA, M., 1980: Effect of centrifugation on the development and timing of premitotic positioning of the nucleus in *Adiantum* protonemata. Develop. Growth Differ. **22**, 867–874.

MIYATA, M., 1979: Photoregulation of cell cycle in *Adiantum* protonemata. M. Sc. Thesis, University of Tokyo.

– WADA, M., FURUYA, M., 1979: Effects of phytochrome and blue-near ultraviolet light absorbing pigment on duration of component phases of cell cycle in *Adiantum* gametophytes. Develop. Growth Differ. **21**, 577–584.

MURASHIGE, T., SKOOG, F., 1962: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15**, 473–497.

POP, L. J. J., 1938: Protoplasmic streaming in relation to spiral growth of *Phycomyces*. Proc. Roy. Acad. Amsterdam **41**, 661–672.

SCHAEDE, R., 1925: Untersuchungen über Zelle, Kern und ihre Teilung am lebenden Objekt. Beitr. Biol. Pfl. **14**, 231–260.

SEIFRIZ, W., 1920: Viscosity values of protoplasm as determined by microdissection. Bot. Gaz. **70**, 360–386.

WADA, M., FURUYA, M., 1970: Photocontrol of the orientation of cell division in *Adiantum*. I. Effects of the dark and red periods in the apical cell of gametophytes. Develop. Growth Differ. **12**, 109–118.

– – 1972: Phytochrome action on the timing of cell division in *Adiantum* gametophytes. Plant Physiol. **49**, 110–113.