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FULL PAPER

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Positional differentiation for development along the sporangiophore in *Phycomyces blakesleeanus*

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Abstract Small segments of *Phycomyces* sporangiophores regenerate various structures on incubation in a moist chamber. We tested the regeneration capacities of middle sporangiophore segments whose protoplasm had been totally or partially removed and replaced with protoplasm from various segments of a genetically different strain. The structures that were regenerated depended on the source of the injected protoplasm (sporangia of various sizes and segments from middle and basal parts of the sporangiophores), implying a positional differentiation of protoplasm along the sporangiophore axis. Protoplasm from various sources showed a high affinity; that is, they mixed successfully and led, in most cases, to the formation of heterokaryotic regenerating structures. The highest affinity was seen when mixing protoplasm from the middle segments of two different strains.

Key words Differentiation of protoplasm · Membrane wall system · *Phycomyces* sporangiophores · Regeneration

Introduction

The zygomycete *Phycomyces blakesleeanus* is a single, large, multinucleated cell. Its vegetative and sexual structures, such as hyphae, sporangiophores, sporangia, progametangia, and their spheroplasts, show a remarkable capacity for regeneration (Binding and Weber 1974; Suárez et al. 1985; Galland and Ootaki 1987; Fukui et al. 2001). Sporangiophores about 0.1 mm in diameter and up to several centimeters in length grow out of the basal mycelium into the air and develop apical sporangia. They stop growing when deprived of their sporangia or separated from the mycelium but resume growth or regenerate when supplied with enough water (Götze 1918; Kirchheimer 1933; Gruen 1968; Gruen and Ootaki 1970, 1972; Ootaki and Gruen 1970). Segments less than 5 mm in length that are cut from sporangiophores have the capacity to regenerate full sporangiophores and other structures (Gruen and Ootaki 1970; Ootaki and Gruen 1970). These characteristics of *Phycomyces* are of potential practical value in the study of the physiology and the genetics of this fungus because isolated or regenerated sporangiophores are sensitive to environmental stimuli such as light and gravity (Gruen 1959). A high regenerative capacity makes it possible to microinject organelles and chemicals, including genes, and to obtain heterokaryons by grafting two genetically marked sporangiophores (Zalokar 1969; Villet 1970, 1972; Ootaki 1973, 1987; Ootaki et al. 1991).

The sporangiophores at stage II–III (Errera 1884; Castle 1942) are composed of a cylindrical stalk and a spherical sporangium, but remain unicellular, with protoplasm streaming from the basal hyphae to the sporangium (Pop 1938; Cohen and Delbrück 1959; Gruen and Ootaki 1972). If the sporangia are excised when they are smaller than 0.2 mm in diameter, they usually regenerate sporangiophores, but larger ones develop spores. Segments from the middle region of the sporangiophores regenerate sporangiophores exclusively at their apical cut ends. Basal sporangiophore segments less than 5 mm long produce mostly hyphae, sometimes accompanied by sporangiophores. Hyphal pieces cut from the mycelia regenerate hyphae alone (Gruen and Ootaki 1970, 1972; Ootaki and Gruen 1970; Galland and Ootaki 1987). These facts imply the existence of positional differentiation in *Phycomyces*.

We have investigated the regeneration of excised segments whose protoplasm had been replaced with protoplasm from various parts of another organism to elucidate whether the protoplasm or the membrane wall system

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determine regenerative development. We have tested the regenerating structures for heterokaryosis to determine the affinity, or functional compatibility, of protoplasm from different sources.

Materials and methods

Strains and culture conditions

This work was carried out with the β-carotene-deficient mutant strains of *Phycomyces blakesleeanus* C2, genotype *carA5(−)*, and C9, genotype *carR21(−)*. The albino strain C2, which contains very little β-carotene, and the red strain C9, which contains large amounts of lycopene (Meissner and Delbrück 1968), were isolated after treating spores of the standard wild type, NRRL1555(−), with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (Heisenberg and Cerdá-Olmedo 1968).

Phycomyces was aseptically cultured in 4.5-cm-diameter Petri plates containing glucose asparagine agar medium (SIV medium; Sutter 1975) supplemented with yeast extract (Difco, Detroit, MI, USA; 1 g/l) and Bactocasitone (Difco; 1 g/l) (SIVYC medium). The plate with *Phycomyces* was placed in a 9-cm-diameter Petri plate and covered with an inverted 200-ml beaker to allow adequate clearance for aerial sporangiophores. The cultures were kept at 20°C under overhead continuous white light (cool white fluorescent light; FL40SD/38, Toshiba, Tokyo, Japan) at 0.1 W/m^2 at the level of the mycelia.

Injection of protoplasm

Stage II–III sporangiophores of the white mutant, about 3 cm in length (Fig. 1A), were isolated from mycelia by grasping them at their bases with fine tweezers, and 5-mm segments were cut from the middle region with fine iris scissors sterilized in alcohol and washed in sterile water. The segments were laid across a plain agar (10 g/l) block 3 mm wide placed on a glass slide, which was laid on a U-shaped glass rod support in a 9-cm-diameter Petri plate with a few milliliters of sterile water to maintain high humidity. The red mutant sporangiophores were cut into sporangia, 5-mm middle segments, and 3-mm basal segments, and these structures were laid across another agar block. The basal segments were obtained by cutting the sporangiophores 3 mm above the basal hyphae. The open cut ends of these structures were left projecting in the air from the agar blocks to avoid a loss of cell contents (protoplasm) onto the agar surface. Protoplasm does not leak out of the open cut ends into the air because the cell wall is hard enough to maintain the original shape and the sporangiophore is thin enough to maintain the surface tension at the cut ends, resulting in prompt formation of protoplasmic plugs.

The protoplasm of sporangia and middle and basal segments of the red mutant was injected into the middle segments of the white mutant (Fig. 2). The protoplasm of the white mutant middle segments was first removed by absorption at the basal cut ends with a small piece of sterilized filter paper, and then one end of a red mutant structure was inserted slightly into the apical cut end of the ghost segment (Fig. 3). Several pieces from the donor were needed to fill up a ghost middle segment of the recipient. The Petri plates carrying the treated segments were covered and placed under standard culture conditions. The types and frequencies of regeneration on the segments were inspected after 72 h.

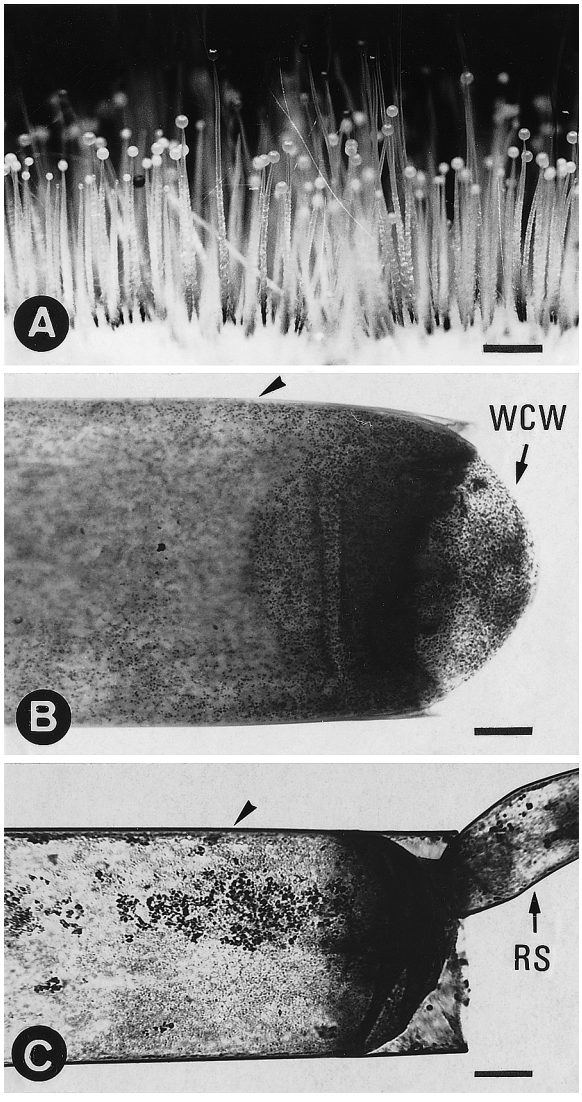

Fig. 1. **A** Stage II–III sporangiophores of a white mutant (C2) of *Phycomyces*, carrying white sporangia. Sporangiophores carrying black sporangia are at stage IV. **B** Apical end of the middle segment, the protoplasm of which was replaced by protoplasm of the middle segment of a red mutant (C9), at about 6 h after protoplasm replacement. *WCW*, wound closure wall formed at the cut end. **C** The middle segment, which regenerated sporangiophores about 36 h after protoplasm replacement. *RS*, regenerated sporangiophore. The relationship between donor protoplasm and the membrane and wall of the recipient appeared normal (*arrowheads* in **B** and **C**). *Bars* **A** 1 cm; **B** 20 μm; **C** 30 μm

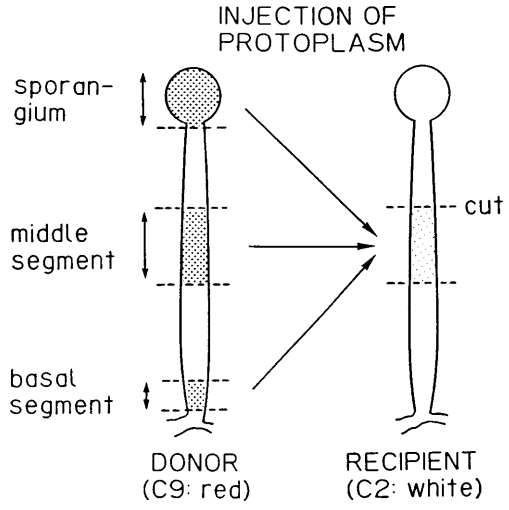

Fig. 2. Schematic diagram of stage II–III sporangiophores of two β-carotene-deficient mutants of *Phycomyces*. The protoplasm of the sporangium and middle and basal segments of a red mutant (C9) sporangiophore was injected into the middle segments of a white mutant (C2) sporangiophore

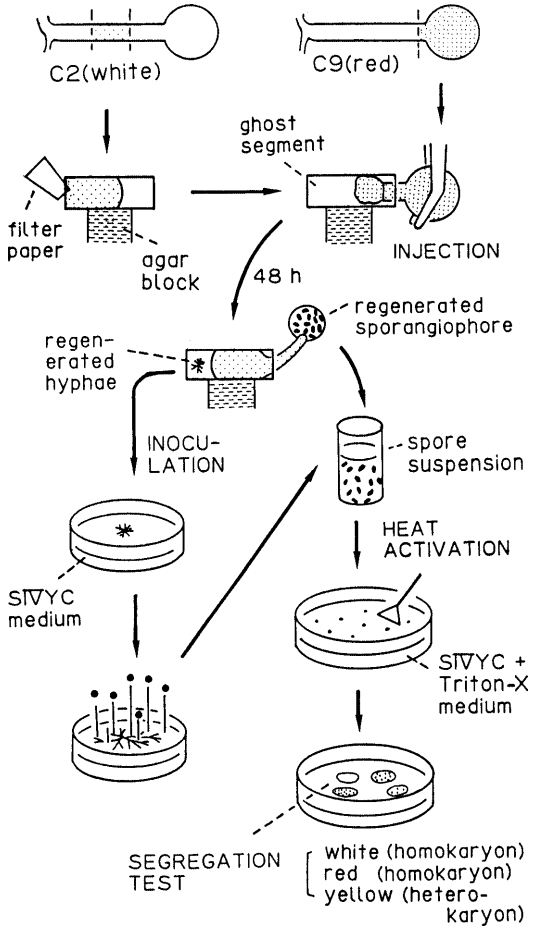

Fig. 3. Schematic diagram of the procedures for injection of the protoplasm of the sporangium of a red mutant (C9) into the middle segment of a white mutant (C2) and for genetic analysis of the regenerates

Segregation test and statistical analysis

The segregation test was applied to decide whether the regenerates were homokaryotic or heterokaryotic and whether the recipient protoplasm had been removed completely and replaced by the donor protoplasm. This approach permitted us to determine the affinity of protoplasm from various sources, that is, whether the nuclei could live together and produce heterokaryons.

Spores were collected from the sporangia developing on the regenerates with tweezers carrying a drop of water between the tips, heat activated, and streaked on SIVYC medium with 2 ml/l Triton X-100 (polyethylene glycol mono-ρ-isooctylphenyl ether) (Fig. 3). When hyphae regenerated, they were transplanted on agar medium to allow them to form sporangiophores and sporangia. When more than two identical or different structures regenerated, each structure was inspected for heterokaryosis after subculturing. The detergent plates, which allowed colonies to be compact and recognizable, were incubated for 5 days before inspection for mycelial colors. Homokaryons produced either red or white colonies. Heterokaryons produced yellow colonies in addition to homokaryotic white and red ones because of complementation of the allelic mutations, with various color tints depending on the nuclear proportions of two mutants (Heisenberg and Cerdá-Olmedo 1968; Meissner and Delbrück 1968; Cerdá-Olmedo and Reau 1970; Ootaki 1973).

For statistical comparison, the χ^2 test at $P = 0.05$ was used.

Results

Regeneration on excised sporangia, middle segments, and basal segments

Regeneration types and frequencies of sporangia and middle and basal segments excised from stage II–III sporangiophores of the red mutant, were first inspected. The regeneration of excised sporangia depended on their diameter (Table 1); most sporangia smaller than 200 μm in diameter regenerated sporangiophores alone and only one produced spores in situ. Nearly all sporangia larger than 401 μm, however, developed spores alone. The sporangiophores regenerated together with spores were usually hairy and appeared around the cut ends.

All the middle segments cut from both red and white mutants regenerated sporangiophores alone (Table 1). Most of these appeared at the apical cut ends of the segments with an intense apicobasal polarity (90% and 81% for the red and white mutants, respectively). Failure in regeneration was mainly a result of accidental leakage of protoplasm out of the cut ends before formation of the wound closure wall.

The most common regenerate on basal segments was hyphae, sometimes with sporangiophores, and only a few basal segments regenerated sporangiophores alone (see Table 1).

Table 1. Regeneration on sporangia and middle and basal segments cut from red mutant sporangiophores of *Phycomyces* at stage II–III

Excised structures	No. of structures observed	Percent regeneration (%)	Type and frequency of regenerates				
			Spores alone (%)	Spphs+ spphs (%)	Spphs alone (%)	Spphs+ hyphae (%)	Hyphae alone (%)
Sporangia							
100–200 μm	29	93	4	30	67	0	0
201–400 μm	44	95	79	12	10	0	0
401–600 μm	38	100	97	3	0	0	0
Middle segments							
Red mutant	50	98	0	0	100	0	0
White mutant[a]	57	91	0	0	100	0	0
Basal segments	51	94	0	0	10	25	65

spphs, sporangiophores
[a] Regeneration on the middle segments of the white mutant was also represented as a control

Table 2. Regeneration on middle segments of white mutant containing protoplasm of sporangia and middle and basal segments of red mutant

Donor of proto plasm	No. of middle segments tested	Percent regeneration (%)	Type and frequency of regenerates					
			Spores+ spphs (%)	Spores+ spphs+ hyphae (%)	Spores+ hyphae (%)	Spphs alone (%)	Spphs+ hyphae (%)	Hyphae alone (%)
Sporangia								
100–200 μm	16	100	19 (Hm = 67, Hm + Ht = 33)[a]	0	0	81 (Hm = 92, Ht = 8)	0	0
201–400 μm	54	98	28 (Hm = 87, Hm + Ht = 13)	4 (Hm = 50, Hm + Ht = 50)	2 (Hm + Ht = 100)	66 (Hm = 89, Hm + Ht = 3, Ht = 9)	0	0
401–600 μm	42	98	20 (Hm = 88, Hm + Ht = 13)	42 (Hm = 82, Hm + Ht = 18)	15 (Hm = 83, Hm + Ht = 17)	24 (Hm = 80, Hm + Ht = 10, Ht = 10)	0	0
Middle segments	132	96	0	0	0	98 (Hm = 97, Ht = 3)[b]	2 (Hm = 67, Hm + Ht = 33)	0
Basal segments	104	93	0	0	0	60 (Hm = 88, Hm + Ht = 5, Ht = 7)	37 (Hm = 89, Hm + Ht = 6, Ht = 6)	3 (Hm = 67, Ht = 33)

The protoplasm of a red mutant was injected into the middle segment of a white mutant from which the protoplasm was removed
Hm, homokaryotic; Ht, heterokaryotic
[a] When more than two identical or different structures regenerated on a recipient segment, each structure was inspected for heterokaryosis after subculturing and spore harvesting; in this case, both regenerated spores and sporangiophores (spphs) were homokaryotic (Hm) in 67% of the segments, but the remaining 33% formed homokaryokic spores and regenerated heterokaryotic sporangiophores and scored as Hm + Ht = 33 (homokaryons produced red colonies alone in spore segregation, but heterokaryons produced yellow colonies in addition to homokaryotic red or white colonies)
[b] At random sample of 30 regenerating sporangiophores were tested for nuclear segregation in the spores

These results confirmed results obtained previously with the wild types (Gruen and Ootaki 1970, 1972; Ootaki and Gruen 1970; Galland and Ootaki 1987).

Regeneration of middle segments containing protoplasm from various sources

When the protoplasm of the middle segments of the white mutant was removed and replaced by the protoplasm of red mutant sporangia of diverse diameter, sporangiophore regeneration was highest (about 81%) on the segments replaced by the protoplasm of young sporangia 100–200 μm in diameter (Table 2). Most (92%) of the regenerated sporangiophores were homokaryotic. Sporangiophore regeneration frequency, however, sharply decreased with an increase in the sporangial diameter, as shown in about 24% regeneration on the segments containing the protoplasm of sporangia of 401–600 μm. In contrast, spore development frequency increased with an increase in the diameter of donor sporangia; spore development occurred on about 19% of the segments containing protoplasm of

100–200 μm sporangia but occurred on about 77% in total on the segments containing the protoplasm of sporangia more than 401 μm, in some cases together with sporangiophores and/or hyphae.

Injection of the protoplasm of the red mutant middle segments into the white mutant segments resulted in regeneration of sporangiophores in all the treated segments with or without hyphal regeneration (see Table 2). Sporangiophores regenerated only when the injected protoplasm united successfully with the membrane wall system of the ghost middle segments of the white mutant (Fig. 1B,C). Hyphae, however, emerged from a mass of protoplasm that failed to unify with membrane wall system and grew as intrasporangiophoral hyphae. A random sample of 30 regenerating sporangiophores were tested for nuclear segregation in the spores; 29 of them were homokaryotic for the red mutant, and only 1 produced a few heterokaryotic yellow colonies. This heterokaryon contained very few nuclei of the white mutant, as judged by its spore segregation. This result implies that in almost all segments the regenerated progeny carried nuclei from the donor strain only, indicating successful cleaning of the recipient protoplasm before replacement.

In 78 of 127 treated middle segments regenerating sporangiophores, regeneration occurred at the apical end alone; the others regenerated from both apical and basal ends. This value was significantly lower ($0.01 < P < 0.025$) than 42 of 52 of excised but untreated white mutant middle segments that regenerated at the apical end only; this is probably a result of disturbance of the apicobasal polarity by mechanical surgery.

About 40% of the white mutant middle segments containing the protoplasm of the red mutant basal segments regenerated hyphae alone or with sporangiophores. This frequency was much higher ($P < 0.001$) than the control in which the protoplasm of the middle segments was replaced by the protoplasm of red mutant middle segments (Table 2). In the case of injection of the protoplasm of basal segments, hyphae emerged from the injected red mutant protoplasm, even when the injected protoplasm united successfully with the membrane wall system of the recipient.

Affinity of protoplasm

To determine the affinity of protoplasm between two identical or different structures, the protoplasm of the red mutant sporangia and middle and basal segments was directly injected into the white mutant middle segments, which maintained their own protoplasm. Injection of excess protoplasm induced an overflow of protoplasm from the recipient segments, and this overflowing protoplasm, about half of the total volume of protoplasm, was removed from the basal cut ends with a piece of filter paper. Protoplasm of the donor and recipient, therefore, was mixed in the recipient white mutant segments. In this experimental system, heterokaryotic regenerates imply a high affinity of two mixed protoplasms, whereas homokaryons imply an independent regeneration, probably as a result of low affinity of two kinds of protoplasm.

Table 3 shows the types, frequencies, and nuclear conditions of the regenerates. When the protoplasm of sporangia of various sizes was injected, heterokaryons alone were formed very frequently on sporangia 100–200 μm in diameter, and their frequency decreased in larger sporangia. The difference between sporangia smaller than 200 μm

Table 3. Affinity of protoplasm of white mutant middle segments with protoplasm of sporangia and middle and basal segments of red mutant

Donor of protoplasm	No. of middle segments as recipient	Type and frequency of regenerates and nuclear condition				Nuclear condition of total middle segments treated
		Spores+ spphs (%)	Spores+ spphs+ hyphae (%)	Spphs alone (%)	Spphs+ hyphae (%)	
Sporangia						
100–200 μm	16	13 (Hm = 50, Hm + Ht = 50)	0	81 (Ht = 100)	6 (Hm + Ht = 100)	Hm = 6 Hm + Ht = 13 Ht = 81
201–400 μm	53	15 (Hm = 75, Hm + Ht = 25)	11 (Hm = 67, Hm + Ht = 33)	66 (Ht = 100)	8 (Hm = 75, Hm + Ht = 25)	Hm = 25 Hm + Ht = 10 Ht = 66
401–600 μm	41	17 (Hm = 86, Hm + Ht = 14)	37 (Hm = 47, Ht = 13, Hm + Ht = 40)	32 (Ht = 100)	15 (Hm = 50, Ht = 50)	Hm = 39 Hm + Ht = 17 Ht = 44
Middle segments	127	0	0	97 (Ht = 100)	3 (Hm + Ht = 75, Ht = 25)	Hm + Ht = 2 Ht = 98
Basal segments	97	0	0	73 (Ht = 100)	27 (Hm = 8, Hm + Ht = 58, Ht = 35)	Hm = 2 Hm + Ht = 16 Ht = 83

The protoplasm of a red mutant was injected into the middle segment of a white mutant from which half the total volume of protoplasm was removed

spphs, sporangiophores; Hm, homokaryotic; Ht, heterokaryotic (see also legend of Table 2 for details)

and those larger than 400 μm was significant ($0.01 < P < 0.025$). When the regenerates were sporangiophores alone, these were heterokaryotic. When the regenerates were all homokaryotic, sporangiophores emerged from the protoplasm of white mutant segments and spores, hyphae, or both emerged from the injected red mutant protoplasm, implying their independent origins and low protoplasmic affinity. When the regenerates were both homokaryons and heterokaryons, the latter were always sporangiophores and the former were spores or hyphae, resulting from donor protoplasm.

A high frequency of heterokaryons, i.e., high protoplasmic affinity, was found when the protoplasm of red mutant middle segments was injected into similar white mutant middle segments; nearly all the segments regenerated heterokaryotic sporangiophores alone, and the others regenerated heterokaryotic sporangiophores in addition to homokaryotic or heterokaryotic hyphae. Homokaryotic hyphae resulted from a part of the injected protoplasm that failed to mix with the host protoplasm. The frequency of heterokaryons alone (98%) was clearly higher than those in sporangia of 201–400 μm and greater than 401 μm (both, $P < 0.001$).

Injection of protoplasm from basal segments of the red mutant led the large majority of the white mutant middle segments to regenerate heterokaryons alone (see Table 3). The regeneration of heterokaryons alone was less frequent in this case (83%) than with injection of protoplasm from middle segments (98%). Hyphae were formed more frequently in the former case than in the latter ($P < 0.001$).

Discussion

The present study confirmed the high regeneration capability of the sporangia and middle and basal segments of *Phycomyces* sporangiophores and the dependence of regeneration type on position along the sporangiophore axis (Table 1). The mature sporangia developed spores exclusively in situ, the middle segments always produced sporangiophores alone, and the basal segments regenerated hyphae at a high frequency. Such differential regeneration patterns along the sporangiophore axis may be governed by the positional differentiation of the protoplasm, membrane, and cell wall system as sources of positional information.

Positional differentiation of the protoplasm is supported (1) by dependence of regeneration type on the donor protoplasm (Table 2) and (2) by the different affinity of protoplasm from different sources with the protoplasm in the middle segments (Table 3). Such a positional differentiation of protoplasm, however, must be closely correlated with the membrane wall system, because it is also true that the donor protoplasm was often obliged to alter the development of its presumptive structure to the recipient structure (Table 2). In this case, the recipient structure appeared only when the donor protoplasm successfully united with the membrane wall system of the recipient. On the other hand, donor protoplasm that failed to unite always regenerated hyphae, if it did regenerate, as the most fundamental structure of the development.

The differentiation of protoplasm as a function of developmental stage and position along the sporangiophore axis has been intensively studied at cytological and physiological levels. Different amounts of total lipids and different lipid composition were formed among mycelia, sporangiophores, and spores (reviewed by Hilgenberg et al. 1987). Sporangiophores contained more auxin and auxin-degrading enzyme than mycelia (Hilgenberg et al. 1980; Hilgenberg and Hanke 1978). Autophagic vesicles surrounded by membrane were seen in sporangiophores but not in hyphae (Thornton 1968). Proteinaceous octahedral crystals were formed in sporangiophores but in neither hyphae nor spores (Thornton 1969; Ootaki and Wolken 1972; Schimek et al. 1999a,b; Eibel et al. 2000). Nuclear division and DNA synthesis are active in the hyphae but do not seem to occur in sporangiophores and sporangia (Bergman et al. 1969). Sporangiophores respire more actively than mycelia but less actively than sporangia (Bergman et al. 1969; Goodell 1971). Mitochondria are at least twice as abundant in the apical region of the sporangiophore than in the basal region; mitochondria in the apical region are spherical or ellipsoidal, but those in the middle and basal regions are elongated and condensed (Tu et al. 1971). The total amount of flavin in the growing zone is about fivefold higher than the average amount of flavins in the entire sporangiophore (Dohrman 1983). Potassium and sodium concentrations also vary along the sporangiophore axis (Cowan et al. 1972). Differential accumulation of organelles and endoplasmic reticulum, which may regulate intracellular Ca^{2+}, was seen with the electron microscope (Morales and Ruiz-Herrera 1989, 1993). These differences may affect growth, regeneration capacity, development of structures, and responses to environmental stimuli of the sporangiophores.

The present results and speculation agree with the fact that biochemical composition and physical characteristics of the membrane wall system vary between mycelia and sporangiophores and along the sporangiophore axis (reviewed by Gamow et al. 1987). The role of the membrane wall system is also supported by the fact that a mass of naked protoplasm, which was squeezed from sporangiophores, rarely regenerated and, if it did, the regenerates were always hyphae, whereas sporangiophores sometimes appeared directly on the naked protoplasm when sporangiophores with cut ends were applied (Weide 1939).

Thus, the present study clearly revealed the existence of positional differentiation of protoplasm and the membrane wall system in *Phycomyces* sporangiophores and visualized this by observing the regeneration patterns on the segments. Because sporangiophores are unicellular and their protoplasm streams continuously from the basal hyphae to the sporangium, some mechanism, which we believe to be the membrane wall system, is essential for maintaining such positional protoplasmic differentiation. A close correlation between these protoplasm and membrane wall systems may act to provide the positional information on the specific gene expression responsible for specific structure development. The gigantic sporangiophore of *Phycomyces* is one of

the most prominent materials for studying regeneration, which is a useful tool to reveal intracellular differentiation and physiological conditions.

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References

Bergman K, Burke PV, Cerdá-Olmedo E, David NC, Delbrück M, Foster KW, Goodell EW, Heisenberg M, Meissner G, Zalokar M, Dennison DS, Shropshire WJ (1969) *Phycomyces*. Bacteriol Rev 33:99–157

Binding H, Weber HJ (1974) The isolation, regeneration and fusion of *Phycomyces* protoplasts. Mol Gen Genet 135:273–276

Castle ES (1942) Spiral growth and reversal of spiraling in *Phycomyces*, and their bearing on primary wall structure. Am J Bot 29:664–672

Cerdá-Olmedo E, Reau P (1970) Genetic classification of the lethal effects of various agents on heterokaryotic spores of *Phycomyces*. Mutat Res 9:369–384

Cohen R, Delbrück M (1959) Photoreactions in *Phycomyces*. Growth and tropic responses to the stimulation of narrow test areas. J Gen Physiol 42:677–695

Cowan MC, Thain JF, Lewis BG (1972) Mechanism of translocation of potassium in sporangiophores of *Phycomyces blakesleeanus*. Trans Br Mycol Soc 58:91–102

Dohrman U (1983) *In vitro* riboflavin binding and endogenous flavins in *Phycomyces blakesleeanus*. Planta 159:357–365

Eibel P, Schimek C, Fries V, Grolig F, Schapat T, Schmidt W, Schneckenburger H, Ootaki T, Galland P (2000) Statoliths in *Phycomyces*: characterization of octahedral protein crystals. Fungal Genet Biol 29:211–220

Errera L (1884) Die grosse Wachsthumsperiode bei den Fruchtträgern von *Phycomyces*. Bot Zeit 42:497–503, 513–522, 529–537, 545–552, 561–566

Fukui J, Choi KS, Miyazaki A, Ootaki T, Oikawa T (2001) Analysis of the formation of protoplasts and regeneration of cells in *Phycomyces blakesleeanus*. J Microbiol Biotechnol 11:72–78

Galland P, Ootaki T (1987) Differentiation and cytology. In: Cerdá-Olmedo E, Lipson ED (eds) *Phycomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 281–316

Gamow RI, Ruiz-Herrera J, Fischer EP (1987) The cell wall of *Phycomyces*. In: Cerdá-Olmedo E, Lipson ED (eds) *Phycomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 223–246

Goodell EW (1971) Oxygen consumption of *Phycomyces* sporangiophores. Planta 98:76–88

Götze H (1918) Hemmung und Richtungsänderung begonnener Differenzierungsprozesse bei Phycomyceten. Jahrb Wiss Bot 58:337–405

Gruen HE (1959) Growth and development of isolated *Phycomyces* sporangiophores. Plant Physiol 34:158–168

Gruen HE (1968) Growth of isolated *Phycomyces* sporangiophores on nutrient media and simple sugars. Can J Bot 46:247–254

Gruen HE, Ootaki T (1970) Regeneration on *Phycomyces* sporangiophores. I. Induction by decapitation and basal cutting on sporangiophores attached to mycelium and after isolation. Can J Bot 48:55–65

Gruen HE, Ootaki T (1972) Regeneration on *Phycomyces* sporangiophores. III. Grafting of sporangiophore segments, weight changes, and protoplasmic streaming in relation to regeneration. Can J Bot 50:139–158

Heisenberg M, Cerdá-Olmedo E (1968) Segregation of heterokaryons in the asexual cycle of *Phycomyces*. Mol Gen Genet 102:187–195

Hilgenberg W, Hanke R (1978) Der enzymatische Abbau der Indol-3-Essigsäure bei *Phycomyces blakesleeanus* Bgff. Z Pflanzenphysiol 86:207–215

Hilgenberg W, Sandmann G, Hofmann F (1980) Der Einfluss von Indol-3-Essigsäure auf das Wachstum von *Phycomyces blakesleeanus* und deren quantitative Bestimmung im Pilz. Z Pflanzenphysiol 97:89–94

Hilgenberg W, Burke PV, Sandmann G (1987) Metabolic pathways. In: Cerdá-Olmedo E, Lipson ED (eds) *Phycomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 155–198

Kirchheimer F (1933) Protoplasma und Wundheilung bei *Phycomyces*. Planta 19:574–606

Meissner G, Delbrück M (1968) Carotenes and retinal in *Phycomyces* mutants. Plant Physiol 43:1279–1283

Morales M, Ruiz-Herrera J (1989) Subcellular localization of calcium in sporangiophores of *Phycomyces blakesleeanus*. Arch Microbiol 152:468–472

Morales M, Ruiz-Herrera J (1993) Critical evaluation of the ultrastructure of sporangiophores of *Phycomyces blakesleeanus* (Zygomycetes) at stages I and IV. Cryptogam Bot 3:273–282

Ootaki T (1973) A new method for heterokaryon formation in *Phycomyces*. Mol Gen Genet 121:49–56

Ootaki T (1987) Heterokaryon formation. In: Cerdá-Olmedo E, Lipson ED (eds) *Phycomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 345–349

Ootaki T, Gruen HE (1970) Regeneration on *Phycomyces* sporangiophores. II. Regeneration on isolated segments from different positions along the sporangiophore axis. Can J Bot 48:95–109

Ootaki T, Wolken JJ (1972) Octahedral crystals in *Phycomyces*. II. J Cell Biol 57:278–288

Ootaki T, Miyazaki A, Fukui J, Kimura Y, Umemura S, Okazaki R, Jayaram M (1991) A high efficient method for introduction of exogenous genes into *Phycomyces blakesleeanus*. Jpn J Genet 66:189–195

Pop LJJ (1938) Protoplasmic streaming in relation to spiral growth of *Phycomyces*. Proc K Ned Akad Wet 41:661–672

Schimek C, Eibel P, Horie T, Galland T, Ootaki T (1999a) Protein crystals in *Phycomyces* sporangiophores are involved in graviperception. Adv Space Res 24:687–696

Schimek C, Eibel P, Grolig F, Horie T, Ootaki T, Galland P (1999b) Gravitropism in *Phycomyces*: a role for sedimenting protein crystals and floating lipid globules. Planta 210:132–142

Suárez T, Orejas M, Eslava AP (1985) Isolation, regeneration, and fusion of *Phycomyces blakesleeanus* spheroplasts. Exp Mycol 9:203–211

Sutter RP (1975) Mutation affecting sexual development in *Phycomyces blakesleeanus*. Proc Natl Acad Sci USA 72:127–130

Thornton M (1968) The fine structure of *Phycomyces*. I. Autophagic vesicles. J Ultrastruct Res 21:269–280

Thornton M (1969) Crystalloids of *Phycomyces* sporangiophores: mature and photosensitive accumulation. Plant Physiol 44:861–865

Tu JC, Malhotra SK, Prasad S (1971). Electron microscopy of stage IV sporangiophore of *Phycomyces*. Microbios 3:143–151

Villet RH (1970) Genetic curing of blindness in *Phycomyces blakesleeanus*. A quantitative assessment of dominance. Nature (Lond) 225:453–454

Villet RH (1972) Microinjection of *Phycomyces*. Selection of a strain for possible biological assay of photoreceptor pigment. Plant Physiol 49:273–274

Weide A (1939) Beobachtungen an Plasmaexplantaten von *Phycomyces*. Arch Exp Zellforsch 23:299–337

Zalokar M (1969) Intracellular centrifugal separation of organelles in *Phycomyces*. J Cell Biol 41:494–509