Nuclear reprogramming in Bryopsis plumosa as demonstrated by artificial fusion of gametophytic and sporophytic protoplasts

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論文題目 Nuclear reprogramming in Bryopsis plumosa as demonstrated by artificial fusion of gametophytic and sporophytic protoplasts
（配偶体－胞子体プロトプラストの融合により示されたハネモにおける核の再編成）
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It was Hustede (1964) who first described that *Bryopsis plumosa* (Caulerpales, Ulvophyceae) have a heteromorphic biphasic life-cycle, namely, a large *Bryopsis* phase (gametophyte) and a microscopic creeping filamentous phase (sporophyte). The body of a gametophyte is composed of a large cylindrical coenocytic thallus with beautiful pinnae. The gametophyte of *B. plumosa* contains many small haploid nuclei. When it matures, the pinnae become either male or female gametangia, and a number of male or female gametes are generated within these gametangia. By contrast, a sporophyte is composed of a small, creeping, sparsely branched cell of 5 - 10 mm in length and about 100 µm in diameter, which contains a single large, diploid nucleus. When maturation of the sporophyte is induced either spontaneously or experimentally, the giant nucleus undergoes meiosis, produces many small nuclei by the following mitosis, and finally forms a number of zoospores. Protoplasts of the marine coenocytic macrophyte *B. plumosa* can easily be obtained by cutting gametophytes or sporophytes with sharp scissors. When a protoplast isolated from a gametophyte was fused with a protoplast isolated from a sporophyte of this alga (Fig. 1), it germinated and developed into either one of two completely different forms.

**Figure 1.** Left: Method for the fusion of gametophytic and sporophytic protoplasts. Right: Fusion between gametophytic and sporophytic protoplast. **a – c:** A series of photographs shows the process of cell fusion between a protoplast of a gamete (upper half) and a protoplast of a sporophyte (lower half) 2 min (a), 3 min (b) and 5 min (c) after the contact. **d, e:** DIC and an epifluorescence photomicrograph of a SYBR Green I stained fused protoplast (d and e are the same specimen). Arrows indicate the single giant nucleus of sporophyte origin, and arrowheads indicate small nuclei of gametophyte origin. Bars represent 100 µm (a - c) or 50 µm (d, e).
One, named Type G plant, appeared quite similar to a gametophyte, and the other, named Type S plant, looked similar to a sporophyte (Fig. 2). While the Type G plant contained many small nuclei of gametophyte origin together with a single giant nucleus of sporophyte origin, the Type S plant contained many large nuclei of uniform size (Fig. 3).

**Figure 2.** Germination and development form the fused protoplasts.

This large nuclei in the Type S plant were metamorphosed from the gametophytic nuclei, not formed through division of the giant nucleus of sporophyte origin. Fragments of the Type S plant, each having such a large nucleus, developed into creeping filaments that look very similar to sporophytes. While cell walls of gametophytes and Type G plants were stained by Congo-red, those of the thalli of regenerated Type S plants and sporophytes were not stained by the dye. This indicated that the large nuclei of the Type S plant did not express genes for xylan synthesis, which are characteristic of gametophytes. Two-dimensional gel

**Figure 3.** Nuclei of Type G and Type S plants.

A Type G plant (a and b) and Type S plants (c - d) were fixed about 30 d after germination and stained with SYBR Green I, and observed with DIC optics (a and d) or epifluorescence photomicrography (b, c and e). c: Two large nuclei are seen in thalli of a Type S plant. In size and morphology, they resemble the giant nucleus of a sporophyte, not those of a gametophyte. Bar in a representing 50 µm is for (a, b, d and e). Bar in c represents 100 µm.
electrophoretic analysis revealed that most of the proteins synthesized in the Type S plant were identical to those of sporophytes. These results are explained in terms of nuclear reprogramming. Regeneration and development of the Type S plant from the protoplast fused between the gametophyte and sporophyte probably result from the activation of genes specific to sporophyte generation and simultaneous silencing of some other genes specific to gametophyte generation through the influence of cytoplasmic factors produced by the coexisting giant sporophytic nucleus. Also, in the Type G plant, gene expression of many gametophytic nuclei may supersede that of the sporophytic giant nucleus, because we found that the larger the gametophytic protoplast, the more Type G plants which were regenerated. Despite morphological similarity, however, the regenerated Type S plant could not produce zoospores, because its large nuclei did not divide normally (Fig. 4). The transformed large nuclei of gametophyte origin seem to still be in the haploid state.

A mature sporophyte of *B. plumosa* forms a huge number of zoospores (stephanokontic zoids) in its cell continuum. Zoospore formation starts with the division of a single giant nucleus and subsequent repeated mitosis. I found that an elevation of photosynthetic activity triggered the division of mature giant nucleus. Transfer to short-day condition was not necessary. Giant nuclei did not divide in darkness or in the presence of 1 μM DCMU. Giant nuclei of as many as 90% of sporophytes started to divide by the addition of 5 mM NaHCO₃ to medium under continuous white light (6 – 12 W m⁻²). Frequency of nuclear division

![Figure 4. Fate of nuclei in Type S plant after induction of maturation.](image)
increased with increased light intensity. Combining parameters that promoted the division of giant nuclei, we have developed the “two-step culture method” which is composed of preliminary and main cultures. This new method guarantees that giant nuclei of more than 90% sporophytes synchronously divide between 72 and 96 h after the transfer to the main culture [continuous white light of 12 Wm$^{-2}$ in Provasoli’s Enriched Sea water (PES) medium supplemented with 5mM NaHCO$_3$].

By this method, it was indicated that sporophytes of about 80% passes through the point of no return for zoosporogenesis between 24h and 48h of main culture. When protoplasts from sporophytes prepared 12h and 24h after transfer to main culture condition were fused with gametophytic sporophytes, the relative yield of Type S plants was as many as 40%. On the other hand, when sporophytic protoplasts prepared 48h and 60h after the transfer were fused, type S plants never developed. These results indicate that some factors to reprogram gametophytic nuclei to sporophytic nuclei were lost in the maturing sporophytes (24h to 48h after transfer).
論文審査結果の要旨

植物の生活環回転に伴って遺伝子発現パターンの劇的な変化、すなわち核の再編成が予想されるが、種子植物では配偶体世代は極度に退化しているため、その機構の研究是不可能である。この論文は、配偶体世代と胞子体世代が全く異なる形態をもつ海産の緑藻多核細胞ハネモ（Bryopsis plumosa）を用いて、植物でも核の再編成が起きていることを実証することを目的としている。

ハネモはその美しい配偶体の形態が好まれ、1980年代までに光屈性や細胞骨格に関するいくつかの優れた研究がなされたが、現在その研究者はほとんどいない。山岸隆博君は山形大学での修士課程中に、画期的なハネモ胞子体の成熟誘導法を開発した。高光強度と炭酸水素塩の添加によって希望時間に90％以上の高率で胞子体核の減数分裂を誘導できたのである。山岸君は修士の段階で既に自分の実験系を確立していたといえる。

2001年本大学院生命科学研究科博士課程に編入後、山岸君の研究は細胞生理学に向く。まず、ハネモの配偶体を切断して得たプロトプラスト1個と、胞子体からその1個の2nの巨大核を含むよう切断して得たプロトプラスト1個を融合させ、その後の運命を追跡した。配偶体プロトプラストには多数の小さなnの核が含まれている。融合プロトプラストの最大40%が胞子体に似た形態（Type S）に育つ。Type S細胞に含まれる配偶体核は徐々に大きくなり、10日後には胞子体由来の巨大核と区別できなくなる。その細胞壁も配偶体性のキシランから胞子体と同じマンナンに置き換わり、蛋白組成も胞子体と酷似するようになる。これからのことは、配偶体由来のnの核が共存する胞子体の2nの核が作る因子によって胞子体様の巨大な核となり、胞子体特有の遺伝子発現をするよう再編成されたことを示す。しかし、Type S細胞を成熟誘導すると、配偶帯由来の核は巨大となってもnのままなので正常な減数分裂ができず、游走子もできない。

本研究の一部はJ Plant Res.に発表され、Plantaに印刷中である。Plantaの編集長はこの論文を高く評価している。山岸君は現在胞子体核の成熟に伴う蛋白発現の消長を解析中である。これらのことは山岸隆博君が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、山岸隆博提出の論文は、博士（生命科学）の博士論文として合格と認める。