

Conjugation Process in *Spirogyra varians* Monitored with FITC-lectins (Zygnemataceae, Chlorophyta)

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The conjugation processes of a filamentous freshwater green alga *Spirogyra varians* were examined using FITC-lectins. Conjugation comprised five steps: 1) aligning with adjacent filaments, 2) formation of conjugation protrusion (papilla), 3) fusion of the protrusions, 4) formation of conjugation tube, and 5) formation of zygotes. Three lectins, ConA, RCA and UEA, showed considerable labeling during the progression of conjugation. FITC-ConA labeled the surfaces of filaments throughout the whole conjugation processes. FITC-RCA labeling was observed at the conjugation protrusions only after the papilla formation. Strong labeling continued until formation of zygotes at the contacting area where the conjugation tube developed, but no labeling was detected on the surface of vegetative filaments. The labeling decreased gradually over time and disappeared when zygotes were formed. FITC-UEA showed similar labeling pattern with FITC-RCA except that weak labeling remained after zygote formation. Inhibition experiments using RCA, UEA which are complementary to sugars L-fucose and D-galactose, showed considerable decrease of conjugation (< 32% vs. 70% in control). These results suggested that the lectin-carbohydrate recognition system might be involved in the conjugation of *Spirogyra varians*.

Key Words: conjugation, FITC-RCA, FITC-UEA, lectin-carbohydrate recognition, *Spirogyra varians*

INTRODUCTION

In *Spirogyra*, two compatible filaments align parallel to each other making numerous papillae, which connect the opposite cells during conjugation and serve as a passage for a male cytoplasm to move into the female cell (Hoek *et al.* 1995; Sze 1998; Kim *et al.* 2007). Conjugation process involves a complex series of events but it always begins with the contact between two papillae protruded from each opposite cell. Therefore, recognition factors are expected along their surfaces.

Although there are few studies on the environmental factors inducing conjugation in the zygnematacean species (Grote 1977; Simons *et al.* 1984; Stabenau and Saeftel 1989; Kato and Ooshima 1997), the key factors which trigger the conjugation and the mechanism of recognition between the compatible filaments are still unknown (Kim *et al.* 2007).

Cell surface carbohydrates are highly suitable for encoding biological information because of their com-

plexity and structural diversity, and hence they have been reported as primary markers for cell-cell recognition events in many organisms (Sharon and Lis 1993, Kim and Kim 1999). Such recognition systems depend on the complementary binding between carbohydrate moieties of a glycoconjugate on one cell with a lectin protein on another cell (Sharon and Lis 1989; Chrispeels and Raikhel 1991; Kim and Kim 1999; Kim *et al.* 2005). Because of their sugar-binding properties, fluorescein-labeled lectins have been used as a powerful tool to analyze the characteristics and the distribution of cell surface carbohydrates in many organisms (*e.g.* Wassarman 1987; Sharon and Lis 1989; Karlsson 1991; Kim and Fritz 1993a, b; Kim *et al.* 1996; Kim and Kim 1999).

Cheli and De Vecchi (1989) showed that the extracellular mucilage of *Spirogyra* filaments disappeared during conjugation and some glycoproteinaceous materials were accumulated in the cell wall of the developing conjugation papilla, suggesting that the chemical composition in cell surface changed during the process. Our previous study on the conjugation process of *Zygnema cruciatum* also showed that the surface carbohydrate moiety changes during the conjugation and newly appeared gly-

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coconjugate on the surface of developing papillae are responsible for the recognition between the compatible filaments (Kim *et al.* 2007). Surface carbohydrates on some vegetative cells and differentiating rhizoid cells were observed in several species of *Zygnema* and *Spirogyra* by the use of FITC-lectins (Sengbusch *et al.* 1982; Inoue *et al.* 1999), but there is little analytical study on the changes of surface carbohydrates during the conjugation in zygnematacean species.

In this study, we examined the changes of cell surface carbohydrates during conjugation process of *Spirogyra varians* using various FITC-lectins. Results show that there are significant changes of surface carbohydrates during the conjugation process and some signaling glycoconjugates are secreted from the apical portion of the developing papilla.

MATERIALS AND METHODS

Plant material and laboratory culture

Algal materials were collected from the ponds in Kongju, Korea, from February to April 2004-7. The plants were washed three times with BBM medium (Bolds basal medium; Bischoff and Bold 1963) and kept in the same medium at 20°C, 16:8 h LD cycle under > 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by cool-white fluorescent lamps. The spores and fragments of sexual filaments were isolated from conjugating plants and kept separately. GeO_2 was added to the medium (final concentration 1 mg L^{-1}) for two weeks to eliminate diatoms. Three month later, the zygospores germinated and a unialgal culture was established in the same condition. The cultured materials were exposed to the various combinations of temperature (10, 15, 20, 25, 30°C), irradiance (10, 20, 50, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and light-dark regime (10:14, 12:12, 14:10 h LD) to induce conjugation. Nitrogen (N) depleted BBM medium was used in combination with the above environmental factors.

Application of fluorescein-labeled lectins

FITC-labeled lectins (Fluorescent Lectin Kit-2100, Vector Laboratories, Burlingame) were diluted in phosphate buffer to a final concentration of approximately 10 $\mu\text{g mL}^{-1}$. Phosphate buffer of pH 7.4 consisted of KCl 0.2 g, Na_2HPO_4 1.44 g, KH_2PO_4 0.24 g, ddH₂O 1 liter. The lectins were added to the plants and were incubated for 1 h at room temperature. The unbound lectin was removed by washing the plants with BBM medium (3 times) and material was examined with confocal laser

scanning microscope (Fluoview, v. 2.0.28, Olympus). Fluorescence was detected with the filter set of BA510IF and BA510-540 (Ex. $\lambda = 490 \text{ nm}$, Em. $\lambda = 525 \text{ nm}$). No auto-fluorescence of the material was observed in this setting. Sugar specificity of the lectin-carbohydrate interactions was assayed as described by Kim and Fritz (1993b).

Blocking of conjugation with lectins

Each lectin was dissolved in BBM medium to a final concentration of 25 $\mu\text{g mL}^{-1}$. About 20 fragments of the male and female filaments were placed together in a glass tube containing 5 mL of the lectin solution and were kept at the same conditions as described above. The number of conjugating filaments (those with conjugating tubes and spores) was counted after 3 days. Each experiment was repeated 7-10 times and the percentage in control (BBM medium without lectins), the average means and standard deviations were calculated.

Microscopy

For time-lapse video-microscopy, the conjugating filaments were placed on a glass slide and a cover slip was lowered and sealed with VALAP (1:1:1; Vaseline : Lanolin : Paraffin) melted on a hot plate at 70°C. The slide preparations were examined on an Olympus BX-51 microscope under x20 objective lens and recorded on a Digital Imaging Time-Lapse Recorder (DITRS, TCS Korea, Daejeon, Korea).

RESULTS

Environmental factors for conjugation

Spirogyra varians bloomed in several ponds in Kongju area from February to April 2005-7. The water temperature of the ponds ranged from 10-17°C and the light-dark regime was about 12:12 h LD. Some (< 10%) of the field materials were already undergoing conjugation. Almost all plants began conjugation in two days when they were moved to the laboratory and kept in BBM medium at 20°C in 16:8 h LD cycle at > 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 1). Conjugation of *S. varians* started with protrusion of papilla on the male plants, followed by the development of female papilla on the opposite filament (Fig. 1A). Two papillae grew towards each other and fused to form a conjugation tube (Fig. 1B). The male cytoplasm migrated into female cell through the conjugation tube (Figs 1C-D) and the zygotes began to develop in female plant (Fig. 1D).

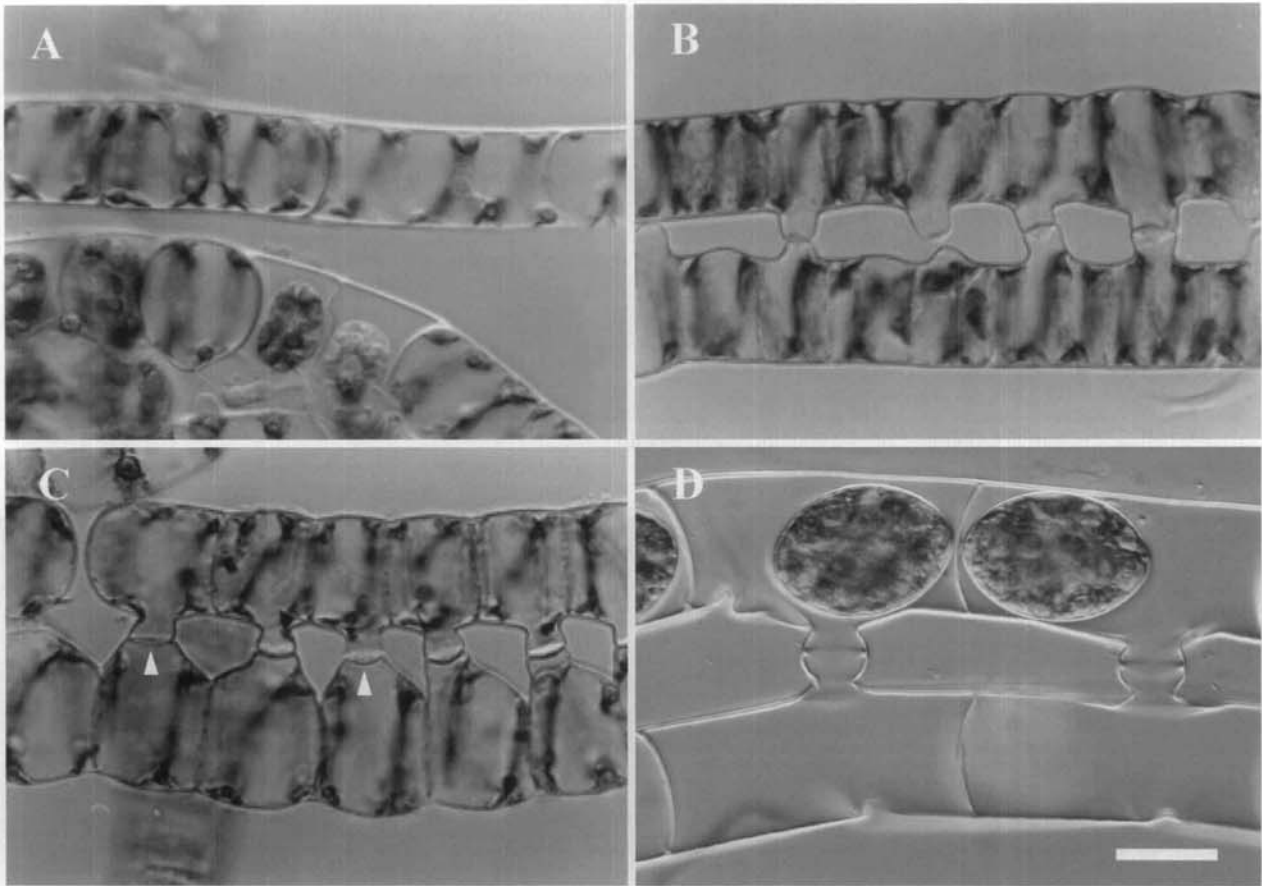


Fig. 1. The conjugation events leading up to zygote formation in *Spirogyra varians*. (A) Male and female papillae contacted each other. (B) The conjugation tube developed. (C) The center of conjugation tube opened. (D) Male protoplasm moved into the female filament. Scale bar, 30 μm .

The cultures from germinating zygospores grew well in the laboratory condition, but conjugation was not induced when we repeated the same environmental conditions described above. The cultured plants were subjected to various environmental conditions including N depletion, changing temperature, light and dark cycle, and light intensity, but conjugation was not induced in any of these conditions.

Lectin labeling during conjugation

Most of FITC-lectin labeling experiments were performed with field material washed three times with BBM medium. The labeling pattern of seven FITC-lectins during conjugation of *S. varians* is listed in Table 1. Three lectins, ConA, RCA and UEA bound to either vegetative or reproductive cells (Figs 2, 3 and 4). Other lectins had no labeling during conjugation (Table 1).

The cell surface of vegetative plant was strongly labeled with FITC-ConA (Fig. 2A'). The labeling continued throughout the conjugation process except on the

protruding papillae (Figs 2B'-D'). No vegetative filament was labeled with FITC-RCA (Fig. 3A'). The labeling began to appear at the contacting area between male and female filaments (Figs 3B'-D'). The labeling became stronger at the gap during the conjugation process but only weak labeling appeared on the back side of the filaments (Figs 3E-E'). When conjugation completed, the labeling disappeared on the papillae and on the conjugation tube (Figs 3F-F'). Only the gap between male and female filaments was labeled with FITC-UEA too (Figs 4B'-D'). When conjugation tube was formed the labeling remained at the site where the two papillae met (Figs 4E-E'). Weak labeling remained on the cell surface when the zygospore was completely formed.

Blocking of conjugation with lectins

To determine if the ConA-, RCA-, and UEA-specific materials were involved in signaling or cell-cell recognition during conjugation of *S. varians*, an inhibition experiment using various lectins was performed (Table 2).

Table 1. Lectin binding pattern of the vegetative and reproductive cells of *Spirogyra varians* during the conjugation

Lectin	Specificity	Vegetative filament	Papillae	Contact area between male and female papillae
<i>Ulex europaeus</i> agglutinin (UEA)	L-fucose	-	+	+
Soybean agglutinin (SBA)	N-acetyl-D-galactosamine	-	-	-
Peanut agglutinin (PNA)	D-galactose (1->3), N-acetyl-D-galactosamine	-	-	-
Concanavalin A (ConA)	D-mannose, D-glucose	+	+	+
<i>Ricinus communis</i> agglutinin (RCA)	D-galactose	-	+	+
<i>Dolichos biflorus</i> agglutinin (DBA)	N-acetyl-D-galactosamine	-	-	-
Wheat germ agglutinin (WGA)	N-acetyl-D-glucosamine, Neuramic acid	-	-	-

+ positive labeling; - negative labeling; * occasional and weak labeling.

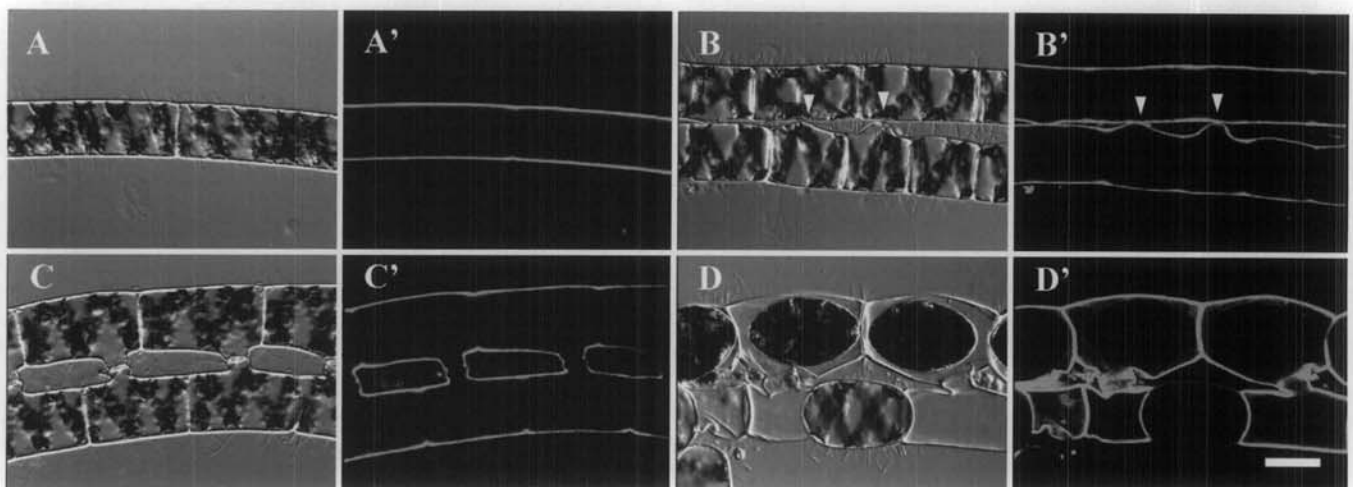


Fig. 2. ConA labeling patterns during conjugation in *Spirogyra varians*. Differential interference micrographs of the filaments (A-D) and fluorescence micrographs of FITC-lectin labeling of the same filaments (A'-D'). (A-A') Vegetative filaments showing dense labeling of ConA on the surface. (B-B') Initial stage of conjugation. Arrowheads point to papilla protruding from the male filament. (C-C') Protruding papillae are not labeled. (D-D') Strong labeling appeared on the surface of developing zygospore. Scale bar, 30 μm .

Table 2. Inhibition of conjugation in *Spirogyra varians* by lectins (25 $\mu\text{g mL}^{-1}$)

Treatment	Control*	ConA	RCA	WGA	DBA	UEA	PNA	SBA	RCA + UEA
Percentage (%)	70 \pm 4.6	64.0 \pm 6.0	31.9 \pm 4.9	65.5 \pm 5.8	67.4 \pm 4.7	28.1 \pm 4.9	57.0 \pm 5.8	58.0 \pm 6.6	30.2 \pm 4.7

Data are means \pm standard deviation.

* BBM medium without lectins.

When the filaments were placed in the solution containing 25 $\mu\text{g mL}^{-1}$ of lectins, only two lectins, RCA and UEA, inhibited conjugation (Table 2). Especially, UEA showed about 28% of conjugation filaments which was 2.5 times less than the control. The inhibition effect did not increase significantly (below 30%) even when we treated two lectins together (Table 2).

DISCUSSION

Our results indicate that there are significant changes in the composition of cell surface carbohydrates during the conjugation process of *Spirogyra varians*. Some glycoconjugates specific to lectin RCA and UEA were secreted from the tip of the developing papillae and accumulate at the gap between male and female filaments. The

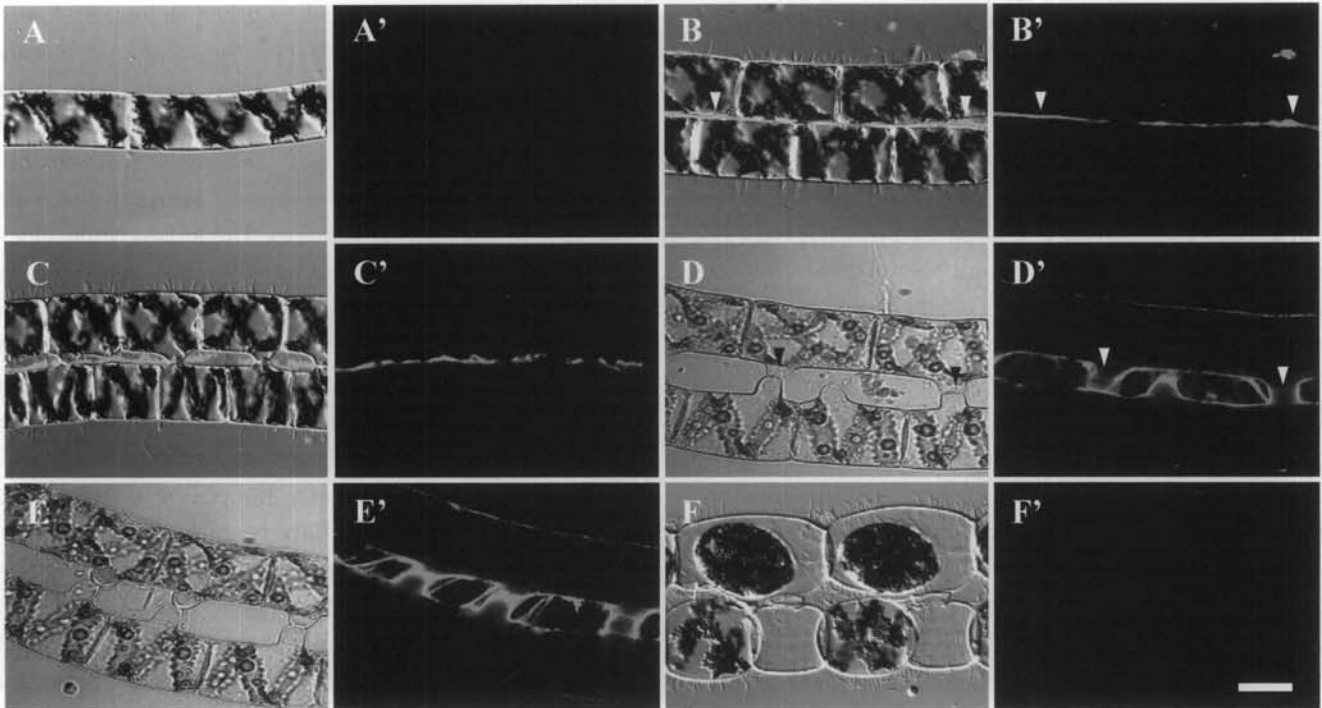


Fig. 3. RCA labeling patterns during conjugation in *Spirogyra varians*. Differential interference micrographs of the filaments (A-F) and fluorescence micrographs of FITC-lectin labeling of the same filaments (A'-F'). (A-A') Single filament showing no RCA labeling on the surface. (B-B') RCA labeling appeared at the surface of contacting area of male and female filaments (arrowheads). (C-C') Labeling becomes stronger over a time course. (D-D') Papillae contact stage (arrowheads). (E-E') Weak labeling appeared at the back of the filaments. (F-F') Labeling disappeared when the conjugation completed. Scale bar, 30 μm .

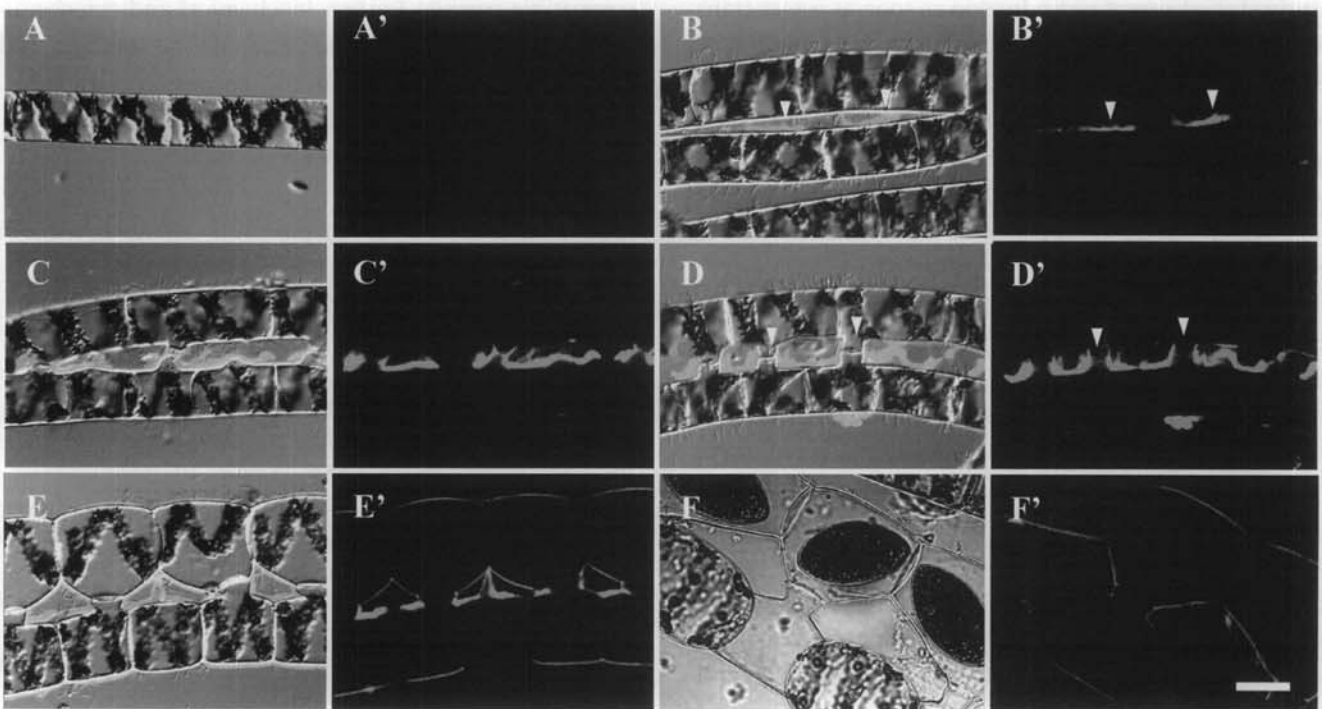


Fig. 4. UEA labeling patterns during conjugation in *Spirogyra varians*. Differential interference micrographs of the filaments (A-F) and fluorescence micrographs of FITC-lectin labeling of the same filaments (A'-F'). (A-A') Single filament showing no UEA labeling on the surface. (B-B') UEA labeling appeared at the surface of contacting area of male and female filaments (arrowheads). (C-C') Labeling becomes stronger over a time course. (D-D') Papillae contact stage (arrowheads). (E-E') Weak labeling appeared at the back of the filaments. (F-F') Labeling disappeared when the conjugation completed. Scale bar, 30 μm .

blocking experiment with their complementary sugars L-fucose and D-galactose showed that these secreted materials might be involved in signaling or recognition between male and female papillae via lectin-carbohydrate complementary system.

Many environmental factors have been reported inducing conjugation of *Spirogyra* spp. (Grote 1977; Simons *et al.* 1984; Stabenau and Saeftel 1989; Kato and Ooshima 1997; Kim and Kim 2002). N depletion and light intensity have been regarded as key factors for the induction of zygotes in *Spirogyra* spp. (Grote 1977; Simons *et al.* 1984). A high C/N ratio produced by depletion of N compounds was also found effective for induction of sexual reproduction in some *Spirogyra* (Yamashita and Sasaki 1979). On the contrary, high temperature was reported as the only important factor to induce conjugation in *S. extenuae* Jao (Kato and Ooshima 1997). Although there are numerous studies showing positive induction of conjugation by various environmental factors, the key factors that trigger this process in *Spirogyra* seem still inconclusive. In *S. varians* it was very difficult to initiate the conjugation in a controlled manner. In most cases, the cultured plants appear to lose the ability of conjugation soon after they adapt to the culture condition (Kim and Kim 2002, Kim *et al.* 2007). The material used in this study started sexual reproduction as soon as it was transferred to the laboratory, making it easy to carry out the experiment in a short time. However, when the algae were kept in culture for long time they lost ability to conjugate no matter what the condition was. Therefore, it would be essential to know the key environmental factors, which initiate conjugation of this species for further biochemical studies.

Our results showed that the composition of surface carbohydrates on conjugation papilla is different from that of external mucilage and some new glycoconjugates were produced and secreted from the tip of protruding papilla indicating that the external mucilage is not directly involved in the signaling or cell-cell recognition during the conjugation process. This suggestion is supported by an ultrastructural study by Cheli and De Vecchi (1989), which showed that the extracellular mucilaginous sheath disappeared, and a glycoproteinaceous material was accumulated in the cell wall of the developing papillae during conjugation. This result was similar to our previous conjugation study using *Zygnema cruciatum* (Kim *et al.* 2007).

Blocking experiment shows that two lectins, RCA and UEA, inhibited fusion between the male and female

papillae. In *Zygnema cruciatum* other lectins, SBA and PNA, inhibited fusion between the male and female papillae. As RCA and UEA are specific to different sugar we may conclude that two different glycoconjugates (probably glycoproteins) with the sugar residues are involved in signaling and adhesion between the male and female papillae of *S. varians*. The lectins could block the signaling either by competing with the sugar-binding receptors on female papillae or by binding with the secreted glycoconjugates making it structurally difficult to bind with female receptors (Kim *et al.* 2007). Signaling glycoproteins involved in conjugation of unicellular algae, *Closterium* spp., have been studied extensively (*e.g.* Sekimoto *et al.* 1998). Recently, the cDNA encoding one of the signal glycoproteins have been cloned and characterized in *Closterium ehrenbergii* Meneghini ex Ralfs (Fukumoto *et al.* 2003). Similar glycoprotein involved in gamete recognition has been found in the other red algal species, *Antithamnion* spp., and its secretion process from the tips of repair cells has been visualized by the use of FITC-lectins (Kim and Fritz 1993c; Kim *et al.* 1995, 1996). Conjugation process in *S. varians* shares some common features with red algal somatic cell fusion in having a glycoconjugate as a signal molecule and showing the directional elongation of a cell protrusion towards its compatible partner. Further biochemical and cytochemical studies are necessary to elucidate the function and properties of the signaling glycoconjugates in *S. varians*.

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