Developmentally and environmentally regulated expression of gamone 1: the trigger molecule for sexual reproduction in *Blepharisma japonicum*

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Summary

Sexual reproduction (conjugation) in protozoan ciliates is induced by specific cell-cell interactions between cells of complementary mating types. The ancestral ciliate *Blepharisma japonicum* has two mating types, I and II. The substances that act as signaling molecules in this extracellular interaction for conjugation are called gamones. The glycoprotein gamone 1, produced by mating type I cells, is a key factor that triggers this interaction. We have previously isolated gamone 1 and determined its complete amino acid sequence. To elucidate the mechanism of initiation of conjugation in ciliates, we investigated the transcription of the gamone 1 gene and found that it is controlled by various internal and external factors. The *gamone 1* gene transcript appeared specifically when sexually mature mating type I cells were starved. It was not detected in immature cells, mating type II cells or proliferating cells. The level of transcription was markedly increased in type I cells when they were stimulated with gamone 2, which is secreted by type II cells. This is the first report that the transcription of gamone genes in ciliates is strictly regulated by developmental and environmental factors. This study suggests that the onset of transcription of *gamone 1* is linked to the switching mechanism that converts mitotically proliferating cells to differentiated preconjugants, the mechanism of differentiation from immature to mature cells in clonal development, and the mechanism that ensures mating type-specific gene silencing.

Key words: Gene regulation, Cell-cell interaction, Ciliate pheromones, Mating types, Sexual maturation, Differentiation

Introduction

Conjugation-inducing substances in protozoan ciliates are called gamones, mating pheromones or mating substances and they are key factors in initiating sexual reproduction or conjugation. In many ciliates, cells undergo mitotic proliferation (binary fission) if provided with sufficient food and by repeating the cell divisions they proceed with clonal aging. However, if sexually matured cells are moderately starved, they cease dividing, the cell cycle is arrested and cells differentiate into preconjugants, which can undergo sexual interaction. Preconjugants of complementary mating types interact when stimulated with conjugation-inducing substances, they gain the capacity to unite and form conjugant pairs. Following preconjugant interaction, conjugation consisting of a series of nuclear events (meiosis, exchange of gametic nuclei and fertilization) takes place in each of the conjugants. After conjugation, cells then enter a period of immaturity during which they cannot become preconjugants (Miyake, 1981a; Miyake, 1981b; Hiwatashi and Kitamura, 1985; Luporini and Miceli, 1986; Miyake, 1996).

The mechanism of induction of conjugation in ciliates has been studied for nearly a century and the conjugation-inducing substances have been isolated from three species: *Blepharisma japonicum*, *Euplotes raikovi* and *Euplotes octocarinatus* (reviewed by Miyake, 1996; Sugiura and Harumoto, 2001). However, the molecular mechanisms relevant to the phenomenon of induction of conjugation have not been elucidated. *B. japonicum* is an ancestral ciliate that has two complementary mating types, I and II. Although each mating type (I or II) is basically maintained unchanged during asexual reproduction, *B. japonicum* cells can spontaneously change mating type (from I to II, or from II to I) in clones when clonal age has progressed significantly through repeated divisions (Miyake, 1981b; Miyake and Harumoto, 1990). This observation indicates that type I and type II cells carry genes responsible for both mating types. Conjugation in *B. japonicum* is induced by the cell-cell interaction between complementary mating types promoted by two conjugation-inducing pheromones, gamone 1 and gamone 2, which are excreted by type I and II cells, respectively (Miyake, 1968; Miyake and Beyer, 1973; Miyake, 1981b). Both gamones are soluble and they have been isolated and characterized. Gamone 1 is the only glycoprotein that has ever been isolated in ciliate pheromones (Miyake and Beyer, 1974) consisting of 272 amino acids and six sugar residues (Sugiura and Harumoto, 2001). Gamone 2 is a small molecule derived from tryptophan,
identified as calcium-3-(2′-formylamino-5′-hydroxybenzoi) lactate (Kubota et al., 1973), which has been chemically synthesized (Tokoroyama et al., 1973; Tokoroyama et al., 1978; Entzeroth and Jaenicke, 1981; Entzeroth et al., 1983). Gamone 2 is the only non-peptide substance identified in ciliates and is considered to be a primitive form of pheromone (Miyake, 1996; Sugiura and Harumoto, 2001). The expression of gamone 1 by type I cells is the first essential event in the initiation of conjugation in B. japonicum. It is thought that gamone 1 is specifically recognized by putative gamone 1 receptors in type II cells. Upon detecting gamone 1, type II cells start producing and excreting gamone 2. It is clear that mating type II cells do not autonomously produce gamone 2. Gamone 1 is therefore a trigger molecule for the interaction between complementary mating type preconjugants in B. japonicum.

Conjugation usually occurs when cells are exposed to specific conditions such as food deprivation. Cells differentiate into preconjugants in response to internal and external factors. We hypothesized that to prevent unsuccessful conjugation, the expression of conjugation-inducing pheromones should be strictly controlled by these factors. In this study, we examined the regulation of gamone 1 gene expression in B. japonicum by internal factors (developmental stage and mating type) and external factors (nutritional conditions and treatment with gamone 2) to elucidate the molecular mechanism of the initiation of conjugation in ciliates.

Materials and Methods

Cells and cell culture

B. japonicum strain R1072 (mating type I) and strain T121 (mating type II) were used. Cells were cultured in WGP (Wheat Grass Powder, Pines) medium (Sugiura and Harumoto, 2001), concentrated by low-speed centrifugation, washed with physiological balanced solution SMB– (synthetic medium for Blepharisma) and suspended in SMB– at a density of 2000-5000 cells/ml. T121 (mating type II) cells were also used to assay for gamone 1 activity, suspended in SMB– at a density of 1500-2000 cells/ml. Cultures were maintained at 25°C.

Cisroles and culture of progeny

Strain R1072 and strain T121 were crossed. Exconjugants were isolated, and after 2 days, caryonides were isolated in 0.8 ml WGP medium and cultured. The cultures were grown to early stationary phase in depression slides (approximate clonal age/number of cell fissions ~6-7). They were then transferred to 100 ml fresh medium in 300 ml flasks. When the culture had reached the stationary phase, a part of the culture was transferred to fresh medium. This was repeated, and samples were collected at different estimated clonal ages. The level of maturation of each sample was graded using a maturation index (−, ±, +, ++, +++ or ++++) measured by mixing samples with sexually matured tester cells (mating type I or II). The maturation index in each sample was judged based on the frequency of mating pairs formed.

Preparation of synthetic gamone 2 solution

Synthetic gamone 2 was dissolved in SMB– (20 µg/ml) and filtered. Biological gamone activity was represented in units (U). A unit of activity was defined as the smallest amount of gamone activity that could induce at least one face-to-face pair in 750-1000 cells suspended in 1 ml of SMB– (Kubota et al., 1977; Miyake, 1981b). The average activity of synthetic gamone 2 was about 1.6×10³ U/ml. For cell activation, synthetic gamone 2 solution was added to the cell suspensions at a ratio of 1:50 (final activity was ~32 U/ml).

Isolation of total RNA

B. japonicum has red pigments (blepharismine) that are presumed to play the roles of photoreception and/or defense against predators (Giese, 1973a; Miyake et al., 1990; Harumoto et al., 1998). Because trace amounts of pigments often hamper the process of extraction of RNA, we prepared pigment-depleted cells by cold treatment as described (Sugiura and Harumoto, 2001). Cells were then suspended in TRZol reagent (Lifetech) and total RNA was isolated by the acid guanidinium-phenol-chloroform method.

Preparation of gamone 1-specific cDNA probes

To amplify the full-length sequence of gamone 1 cDNA by PCR, cDNAs from mating type I cells in early stationary phase were prepared as described (Sugiura and Harumoto, 2001) and used as a template for PCR. The oligonucleotides based on the gamone 1 sequence (DDBJ accession no. AB056696) (Sugiura and Harumoto, 2001), Gm1_for (5′-GAAAATCTTGAATGATGAA-3′) and Gm1_rev (5′-TTATTACCAATGACGTA-3′) were used as 5′ and 3′ primers, respectively. The PCR products were fractionated by electrophoresis on a 0.8% agarose gel and purified and cloned into the vector pCR2.1-TOPO vector with the TOPO TA cloning kit (Invitrogen). After confirmation by sequencing, plasmids bearing full-length or partial gamone 1 (Sugiura and Harumoto, 2001) were digested with EcoRI to excise the inserts and electrophoresed in a 0.8% agarose gel. EcoRI-digested fragments were purified and labeled with a DIG (digoxigenin) DNA Labeling kit (Roche Molecular Biochemicals) according to the conditions suggested by the supplier.

Northern hybridization analysis

Total RNA was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to positively charged Nylon membranes (Roche Molecular Biochemicals). After UV-crosslinking, membranes were prehybridized in standard hybridization buffer [5×SSC; 0.1% (w/v) N-lauroylsarcosine; 0.02% (w/v) SDS; 1% blocking reagent] at 60°C for 30 minutes. Denatured DIG-labeled gamone 1 probe was then added to the standard hybridization buffer and the membranes were incubated at 60°C overnight. The hybridized membranes were washed twice with 2×SSC, 0.1% SDS at room temperature for 5 minutes, and then washed twice with 0.1×SSC, 0.1% SDS at 60°C for 15 minutes with shaking. DIG was detected using a DIG Luminescent Detection kit (Roche Molecular Biochemicals).

Preparation of cell-free fluid (CFF) and gamone activity assay

CFF was obtained by removing cells from suspension by low-speed centrifugation and filtration through a Nylon net (5 m). Gamone activity was represented by units (U) (Kubota et al., 1977; Miyake, 1981b) (see above) and by the index of pair formation (0-5) (Sugiura and Harumoto, 2001).

Protein electrophoresis

SDS-polyacrylamide gel electrophoresis (PAGE) was performed on a 15% polyacrylamide separation gel. Samples were boiled for 5 minutes in sample buffer [0.1 M Tris-HCl (pH 6.8), 4% SDS, 12% β-mercaptoethanol, 20% glycerol, Bromophenol Blue] and then subjected to electrophoresis. Proteins were visualized by silver staining.
Results
Detection of gamone 1 transcript
To prepare probes corresponding to the sequence of full-length gamone 1 cDNA, we performed RT-PCR using gamone 1-specific primers. We cloned a product of about 930 base pairs and confirmed that its sequence was coincident with the sequence of gamone 1 cDNA. This product and the 5' region of gamone 1 (about 500 bp) were labeled with DIG, and used as probes for northern hybridization (Fig. 1A). Total RNA prepared from moderately starved mating type I cells in early stationary phase was examined, and the gamone 1 transcripts were detected with probe 1 (Fig. 1B, lanes 1-4) and probe 2 (lane 5). B. japonicum has a single gene encoding gamone 1 (data not shown) and we detected a single band with a size of ~1000 bases in each lane. This result indicates that there is only one transcript from the gamone 1 gene, which corresponds to the precursor of gamone 1 (pre-pro-gamone 1) consisting of 305 amino acids.

Gamone 1 expression correlates with mating type differentiation
It has been assumed that type I and II cells carry the genes responsible for both mating types but that they alternatively express mating type I or II (Miyake, 1981b; Miyake and Harumoto, 1990). We detected the band of a gamone 1-specific sequence by PCR using gamone 1-specific primers and genomic DNA as a template in both mating types (data not shown), indicating that type II cells do carry the gamone 1 gene. To examine the appearance of transcription of gamone 1 in both mating types, we prepared total RNA from type I and type II cells that had been moderately starved in early stationary phase, and carried out northern hybridization with the gamone 1 probe 2 (Fig. 2A). A single band corresponding to the gamone 1 mRNA was detected only in type I cells. To detect the secreted mature gamone 1 protein, we performed SDS-PAGE and a biological activity test with CFF prepared from cell suspensions (Fig. 2B,C). It is presumed that gamone 1 mRNA is translated as pre-pro-gamone 1 and processed proteolytically before being secreted as mature gamone 1, with a putative molecular mass of 30 kDa (Sugiura and Harumoto, 2001). The mature gamone 1-specific band was detected only in CFF derived from type I cells, consistent with the results of northern blot analysis (Fig. 2B). The gamone 1 activity tests, showed that CFF from type II cells did not induce pair formation in tester cells, unlike CFF from type I cells (Fig. 2C). The gamone 1 activity index was level 1 (4,096 U) in CFF collected from type I cells. The results of biological activity tests were consistent with those from the northern blot and SDS-PAGE experiments. These results indicate that gamone 1 expression is regulated by a mating type-specific system.
gamone 1 gene expression in type I cells correlates with sexual maturation

To examine the expression of the gamone 1 gene during clonal development, we isolated the progeny of crossed type I and II cells by culturing and harvesting samples at clonal ages (number of cell fissions after conjugation) of 10, 18, 25 and 39 (Fig. 3). After conjugation, B. japonicum cells enter a period of immaturity. One clone did not form mating pairs with either type of tester cell until the clonal age of 18 (Fig. 3A; maturation index, –). This indicates that they were still sexually immature at this stage. At the clonal age of 25, they formed a few pairs (maturation index, +) and more pairs were detected at clonal age 39 when they were mixed with type II cells (maturation index, ++). These results indicated that this clone differentiated into mating type I, and became mature at the clonal age of about 25 with continuing maturation thereafter, although their maturation index was still lower than that of control cells (mature type I, of an age of more than several hundred fissions) (maturation index, ++++). This is probably because this progeny clone was still making the transition from an immature to mature stage. Other progeny clones also reached maturity at the clonal age of 25-30. To examine the expression of gamone 1 at different clonal ages, RNA was prepared from cells of each age and from mature type I cells (Fig. 3, mature). Total RNA was loaded (clonal age 10 at 2.2 \( \mu \)g/lane and all others at 10 \( \mu \)g/lane) and subjected to northern blot analysis (Fig. 3A). Gamone 1 mRNA was clearly detected in mature type I cells.

Promotion of the transcription of gamone 1 by the complementary mating pheromone, gamone 2

To study the influence of gamone 2 on the transcription of the gamone 1 gene in the mating type I and II cells, total RNA from mature cells that had been incubated in gamone 2-containing SMB− for 20 hours, was extracted and subjected to northern blot analysis (Fig. 4A, G2+). Type I cells incubated in gamone 2-containing SMB−, formed homotypic pairs, and the level of transcription of gamone 1 gene increased remarkably compared with that of untreated cells (G2−). In contrast, gamone 1 mRNA was not detected in type II cells irrespective of the presence of gamone 2. These results indicated that mature type I cells that were moderately starved in the early stationary phase begin transcribing the gamone 1 gene and that transcription is dramatically enhanced in response to the presence of gamone 2. A band of 30 kDa corresponding to secreted mature gamone 1 was detected only in CFF derived from type I cells, consistent with the results of northern blot analysis (Fig. 4B). The intensity indices of gamone 1 activity were level 1 in CFF from unstimulated type I cells [I (–G2)] and level 2 in CFF from type I cells stimulated with gamone 2 [I (+G2)] (Fig. 4C). These results show that the expression of the gamone 1 gene in starved type I cells is

**Fig. 3.** Influence of sexual maturation on the expression of gamone 1 during development. (A) The level of transcription of gamone 1 was examined in a progeny clone at each clonal age and in sexually mature type I cells (mature). The relative amounts of gamone 1 mRNA are indicated in the graph. For cells of clonal age of 10, 2.2 \( \mu \)g/lane was loaded, and for the others, 10 \( \mu \)g/lane. The extent of maturation at each clonal age is represented by the maturation index. These results suggested that the transcription of gamone 1 and maturation were strictly linked. (B) The biological activity of secreted mature gamone 1, represented in units.

**Fig. 4.** Influence of the complementary mating type pheromone, gamone 2, on the expression of gamone 1. (A) The transcriptional level of gamone 1 was examined by northern blotting of the moderately starved cells of both types stimulated with gamone 2 (+G2) or unstimulated (–G2). Total RNA was prepared from each cell type (6 \( \mu \)g/lane). The level of the transcription of gamone 1 was increased when starved type I cells were stimulated with gamone 2. Type II cells did not transcribe gamone 1 mRNA, irrespective of the presence of gamone 2. The graph shows the relative amounts of gamone 1 mRNA. (B) The secreted mature gamone 1 protein was detected by SDS-PAGE and silver staining. (C) The biological activity of secreted mature gamone 1 was measured and is represented by the index of pair formation.
promoted by gamone 2, and that this enhanced expression probably increases the opportunity for conjugation during the interaction between cells of complementary mating type.

To examine the influence of gamone 2 on the expression of the gamone 1 gene more precisely, we measured the transcription levels of gamone 1 in type I cells that had been incubated in gamone 2-containing SMB\(^+\) for 0, 2, 4, 6, 8 and 20 hours (Fig. 5A). Cells started forming homotypic pairs 2 hours after the addition of gamone 2 and the frequency of cell pairs increased gradually. Transcription of the gamone 1 gene was enhanced in cells treated with gamone 2 for 2 hours. The levels of mRNA for gamone 1 reached a peak at 4-6 hours and then gradually decreased. Gamone 1 in CFF obtained from each cell suspension was detected by SDS-PAGE and the activity of gamone 1 was measured. These results were consistent with that of the northern blot analysis (Fig. 5B, C) and suggest that transcribed gamone 1 mRNA is translated and secreted as mature gamone 1 in a relatively short time.

### Influence of nutritional conditions on the transcription of the gamone 1 gene

Next, we examined the level of transcription of gamone 1 under different nutritional conditions. Total RNA was prepared from log phase cells cultured in fresh medium for 20 hours, and was subjected to northern hybridization analysis (Fig. 6A, type I, L). No gamone 1-specific mRNA was detected in this sample, however, it was detected in samples from moderately starved cells (Fig. 6A, type I, 1D). We examined whether the transcription of the gamone 1 gene was affected by the extent of starvation (Fig. 6B). We suspended type I cells in SMB\(^-\) when they reached the early stationary phase and collected them at different stages of starvation, after 1 day (1D) and 5 days (5D). In cells suspended in food-deprived conditions for 5 days, cell bodies became thinner, and their color was bleached compared to that of 1-day cells, but they rarely engaged in cannibalism (Giese, 1973b). Northern hybridization analysis showed that the transcriptional activity of gamone 1 was raised in 5D (–G2) compared with 1D (–G2) cells. Moreover, cells starved for 5 days also promoted transcription of the gamone 1 gene in response to gamone 2 [5D (+G2)]. Therefore, the transcription was promoted in cells that had been starved longer (5D) and further accelerated by gamone 2 treatment. These results indicated that mature type I cells did not transcribe the gamone 1 gene in the log phase, and that they started transcribing it only when they were starved in the early stationary phase. The level of transcription of the gamone 1 gene is increased as starvation progresses. The level of secreted mature gamone 1 and the intensity of its activity were consistent with these results (data not shown).

### Discussion

We show in this study that B. japonicum controls the expression of gamone 1, a key factor for the initiation of conjugation in response to internal and external factors, at the level transcription, but not at the level of translation or secretion.

We detected a single band of the gamone 1 gene transcript (~1000 bases) only in stationary phase type I cells. This transcript was not expressed and was not detected in mating type II cells, although they do carry the gamone 1 gene in the genome (Fig. 2A). This result suggests that the expression of gamone 1 is controlled by a mating type-specific system at the level of transcription. In differentiated type II cells, putative

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**Fig. 5.** Effect of gamone 2 on the promotion of gamone 1 transcription. (A) We examined the transcriptional levels of gamone 1 in type I cells in the early stationary phase after the addition of gamone 2 (G2). Total RNA was prepared and separated (6 µg/lane) from cells that had been incubated with gamone 2 for 0, 2, 4, 6, 8 or 20 hours, and gamone 1 mRNA was detected by northern hybridization using probe 2. A peak of transcriptional activity was reached in cells stimulated for 4-6 hours with gamone 2. The graph indicates the relative amounts of gamone 1 mRNA. (B) SDS-PAGE analysis and (C) biological activity tests of CFFs collected from individual cell suspensions.

**Fig. 6.** Influence of nutrient deprivation on the expression of the gamone 1 gene. (A) Total RNA was extracted from type I cells in the log phase [I (L)] and in the early stationary phase [I (1D)]. Northern hybridization showed that cells in the log phase did not transcribe the gamone 1 gene, but that early stationary phase cells started to transcribe it when they were moderately starved (1D). (B) The transcriptional level was compared for type I cells in the early stationary phase (1D) and cells starved in SMB\(^-\) for 5 days (5D). In both cells, the response to gamone 2 was also examined (G2–, untreated; G2+, treated with gamone 2). The transcription of gamone 1 was promoted in cells that had been starved longer (5D) and was accelerated by gamone 2 treatment. The graph shows the relative amounts of gamone 1 mRNA.
Fig. 7. Schematic illustration of asymmetric cell-cell interactions mediated by gamones during conjugation in *B. japonicum*. When mature type I cells receive the food-deprivation stimulus (step 1), they start transcribing the *gamone 1* gene and synthesizing gamone 1 (steps 2-3). Secreted gamone 1 is recognized by mature type II cells through the putative gamone 1 receptor (step 4), inducing them to synthesize gamone 2 from tryptophan (steps 5-6). The *gamone 1* gene is inactive in mating type II cells. Secreted gamone 2 is recognized by type I cells through the putative gamone 2 receptor (step 7). Gamone 2 signal is transmitted to the nucleus, promoting further transcription of the *gamone 1* gene (step 8). I, mating type I cell; II, mating type II cell; G1, gamone 1; G1-R, hypothetical receptor for gamone 1; G2, gamone 2; G2-R, hypothetical receptor for gamone 2; E, enzyme(s) for production of gamone 2; Trp, tryptophan.

The regulation of transcription of *gamone 1* is likely to reveal the mechanism of the transition from immaturity to maturity. We are now analyzing the promoter regions of the *gamone 1* gene.

We also showed that the transcription of *gamone 1* is also controlled by external factors. *Gamone 1* is not transcribed in log phase type I cells (Fig. 6A). However, we found that the transcription is induced by food deprivation and the level of transcription of *gamone 1* was increased when type I cells were incubated in nutrient-deprived condition for 5 days (Fig. 6B). We suggest that nutrient deprivation is an essential external factor in the initiation of transcription of the *gamone 1* gene and that the extent of deprivation influences transcription. Another external factor is the mating pheromone of the complementary mating type, gamone 2 (Figs 4 and 5). In moderately starved type I cells, stimulation by gamone 2 increases the level of transcription of *gamone 1*.

In the ciliate *Euplotes raikovi*, mating pheromones (euplomones) are synthesized constitutively throughout the entire clonal life cycle and in growing cells (Micieli et al., 1989; Luporini et al., 1992; Miceli et al., 1992; Vallesi et al., 1995; Luporini et al., 1995; Ortenzi et al., 2000). The gene encoding each pheromone generates at least two distinct molecules by alternative splicing, one of which is the soluble pheromone and the other a membrane-bound pheromone isoform. It has been suggested that these isoforms are pheromone binding sites or receptors for soluble pheromones. In *E. raikovi*, cells possess receptors at the cell surface that recognize autologously produced pheromones. The binding of pheromones to these receptors stimulates mitotic proliferation by binding them in an autocrine-like manner and promotes mating-pair formation for conjugation by binding them in a paracrine-like manner. In this system, pheromones must have the ability to bind both to the cells that produce them and to other cells of complementary mating type, through different receptors. In the case of *E. raikovi*, this might be possible because their pheromones have similar structure and features. However, the mechanism of conjugation in other ciliates, such as *B. japonicum*, is not readily explained by such a system. The conjugation of *B. japonicum* is remarkable among ciliates in that the cell-cell interaction is mediated by two different types of molecule, a glycoprotein (gamone 1) and a tryptophan derivative (gamone 2). In this system specific gamone receptors are thought to be produced by complementary mating type cells (Miyake, 1981b; Miyake, 1996). In this study, we confirmed that only one transcript was produced from the *gamone 1* gene in *B. japonicum* (Fig. 1), indicating that the gene encoding gamone 1 does not encode other isoforms, in contrast to the system found in *E. raikovi*. It is likely that the actual mechanism of induction of conjugation in *B. japonicum* is distinct from that postulated for *E. raikovi*.

From the results reported here and previous papers (Miyake, 1996; Sugiura and Harumoto, 2001), we postulate the following series of cell-cell interaction events promoted by gamones during conjugation (Fig. 7). When mature type I cells are starved, they start transcribing the *gamone 1* gene. *Gamone 1* mRNA is translated as pre-pro-gamone 1, which is secreted as mature gamone 1 after going through proteolytic processing and glycosylation (Sugiura and Harumoto, 2001). If cells of both mating types are mixed, the secreted gamone 1 is recognized specifically by type II cells through the putative gamone 1 receptor (G1-R), inducing them to synthesize...
Regulation of gamone 1 gene expression

It has been shown that the expression of gamone activity is regulated by various specific conditions based on studies of the conjugation-inducing activity in cell-free fluids (Miyake, 1981b). Here, we revealed that the specific expression of gamone 1 activity is regulated at the transcriptional level, and that transcription does not occur constitutively but is induced in response to various intra- and extracellular stimuli that ultimately initiate the process of conjugation. Further studies on the transcriptional regulation of the gamone 1 gene will shed light on the long-standing unsolved problems in ciliates, which concern such phenomena as mating type differentiation, the transition from mitotic proliferation to conjugation, the differentiation of preconjugant cells, and the transition from sexual immaturity to maturity.

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