Alterations in Gene Expression Specific to Autogamy in *Paramecium tetraurelia*

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1 Introduction

The ciliated protozoan *Paramecium tetraurelia* undergoes two forms of sexual reproduction, both of which include meiosis, fertilization, and macronuclear development. One, conjugation, develops from surface interaction between sexually competent cells of complementary mating types. The other, autogamy, is an intracellularly initiated process of self-fertilization in single cells which is triggered by food deprivation. Termination of the *Paramecium* clonal life span can be defined by either clonal death or entry into these sexual pathways. Paramecia count a definite number of cell fissions to traverse the period from the clonal origin to clonal death. Likewise, undergoing sexual reproduction requires a certain number of fissions after the preceding fertilization, the duration of which is called the immaturity period. Conjugation and autogamy have their own periods for immaturity, sexual immaturity, and autogamy immaturity, respectively, and thereafter paramecia become mature, that is, capable of entering each sexual pathway.

A positive correlation has been established between the ciliate clonal life span and the length of the sexual immaturity period. The clonal age of parent cells has an adverse effect on the duration of sexual immaturity and of autogamy immaturity in their progeny. Autogamy immaturity is similarly affected by parental cultural age as well. In the *jumyo* mutant of *P. tetraurelia* which has an extremely short life span, the immature period for autogamy is also shortened. These findings allow the speculation that the mechanism for setting the clonal life span and that for initiating sexual reproduction are tightly linked.

The molecular aspects of the control of the *Paramecium* sexual reactivity have been elucidated to some extent. Immaturin, a small cytoplasmic protein, was identified as the factor responsible for sexual immaturity. There seems to exist the autogamy version of immaturin. Transplantation of macronuclear material or the entire macronuclei between autogamy-immature and mature cells significantly influences the occurrence of autogamy in *P. tetraurelia*. Together with the cell-cycle-stage-dependence of commitment to autogamy, these experiments indicate that a nuclear factor(s) is directly involved in the regulation of the autogamy pathway and hence particular alterations in gene expression must be associated with this sexual event. We have compared gene expression profiles between the short-lived *jumyo* mutant and its wild-type parent stock and isolated differentially expressed genes for the final purpose of identifying the causal determinant of the *Paramecium* clonal life span.

In view of the above argument, investigation of autogamy-specific gene expression would be another important approach to our goal. This paper describes an initial step in the second approach.

2 Materials and Methods

Culture of paramecia *P. tetraurelia* wild-type stock 51 was used in all experiments. Daily isolation line culture was developed from exautogamous as a source of cell mature for autogamy. To obtain preautogamous, autogamy-committed, and postautogamous cells at the age of about 35 fissions, mass cultures derived from the isolation culture were allowed to reach 400-500, 1,200-1,500, and 4,000-5,000 cells/ml, respectively. The clonal age and population density of each autogamy stage were determined on the basis of kinetic experiments by Berger and Rahmatullah. The occurrence of autogamy at the last stage was ensured by cell staining. Other cultivation conditions were described in our earlier paper.

Construction and screening of subtractive libraries

Subtracted cDNA libraries were generated through
suppression subtractive hybridization,²⁰,²¹ an improved version of cDNA subtraction. Figure 1 presents a brief overview of the procedure. A tester (the population containing cDNAs of interest) and a driver (the reference population) cDNAs were synthesized from their respective mRNA populations and digested with Rsal. An age-synchronized cell population of stock 51 (about 35 fissions old) in a preautogamous state and those committed to autogamy were used as sources of the starting mRNA materials. Two different adaptor DNAs were separately ligated to the tester cDNAs. After two rounds of hybridization of the tester with excess of the driver through which tester cDNA species shared by the driver were subtracted against the driver cDNAs, the resulting subtractive tester cDNAs were selectively amplified by PCR using primers complementary to the sequences of the adaptors and inserted into the vector pBluescript (Stratagene).

We carried out subtractions in both directions: in the forward subtraction for leaving cDNAs specific to preautogamy, cDNAs derived from stock 51 before autogamy were used as testers and cDNAs from autogamy-commitment paramecia served as drivers. The reverse subtraction, a second subtraction done in reverse, was designed to obtain cDNAs expressed in autogamy-commitment (tester) but not in preautogamy (driver). The resulting subtractive cDNA pools were greatly enriched for sequences found exclusively in the tester population, from which two subtractive cDNA libraries were constructed: the forward-subtracted library rich in non-autogamy-specific clones and the reverse-subtracted library dominated by autogamy-specific ones. These libraries were then subjected to differential screening to remove cDNA species common to tester and driver which were still present after subtraction: 200 cDNA clones randomly picked from each library were dotted (100 ng per spot) onto two pieces of nylon membranes for duplicate screening. Each membrane was probed with either of the two kinds of the subtracted cDNA pool: one piece with the forward-subtracted cDNA pool and the other with the reverse-subtracted one. The filters were then washed under stringent conditions and exposed to signal detection. Further screening was carried out by partial DNA sequencing for the removal of redundant clones and Northern analysis to confirm expression specificity.

3 Results and Discussion
Subtractive cloning of genes expressed differentially during autogamy In the conviction that underlying transcriptional changes precede the prosecution of autogamy, we isolated genes up- or down-regulated on conversion from preautogamous to autogamy-committed stages by means of cDNA subtraction (Fig. 1). In this experiment, mRNAs prepared from matured cells in a

![Probe](NS AS Cont. 1 2 3)

Fig 2. Northern blot analysis of autogamy-dependent gene expression in Paramecium tetraurelia.
mRNA samples (1 µg each) derived from preautogamous (lane 1), autogamy-committed (lane 2), and postautogamous (lane 3) mature cells of stock 51 were separated by electrophoresis and transferred onto nylon membranes to prepare a triplicate blot. Each blot was hybridized with a cDNA probe for NS, AS, or control for mRNA load (from top to bottom). The control gene has proven to be transcribed independent of the autogamy stage in a preliminary experiment.
preautogamous state and those committed to autogamy were
used as the starting materials. The cell densities of 400-500
and 1,200-1,500 cells/ml are those estimated before and at
the median time of initial commitment to autogamy,
respectively, for 35-fission old paramecia.\textsuperscript{2}\textsuperscript{23} The forward
and the reverse subtractions were designed to enrich genes
expressed preferentially in the preautogamous and the
autogamy-committed cells, respectively.

Following a series of screening steps, two cDNA clones
were finally isolated and tentatively named NS and AS,
which were derived from the forward and the reverse
subtractions, respectively. Figure 2 depicts their transcription
patterns during the development of autogamy. The
expression of the non-autogamy-specific NS clone declined
abruptly to a basal level when cells were committed to
autogamy. On the contrary, the AS clone from autogamy­
committed stage is positively regulated over the course of
the autogamy process. Its transcript level remained elevated
until the completion of autogamy.

Identification of autogamy-related genes A search of
the DDBJ database revealed that the two clones were
identical to the $a$ -51D gene and M2S. These genes are
the ones increasing expression in the short-lived
jumyo mutant than in its wild-type parental strain as well as
increasing expression during clonal aging\textsuperscript{18}; We found the
nucleotide sequence of the NS clone in the gene that encodes
the $a$ -51D surface protein specifying the D serotype of P.
tetraurelia.\textsuperscript{22} The unique multiply banded profile of the AS
transcripts (Fig. 2) is reminiscent of the MS2 gene with the
accession number AB059619. Indeed, both DNA sequences
were aligned with a perfect homology.

Since aged paramecia are prone to undergo autogamy
even when they are well fed and the same is the case for the
jumyo mutant,$^{26}$ it was conceivable that the $a$ -51D gene
was up-regulated in these cells is similarly regulated in the
autogamy process. However, Figure 2 shows a result to the
contrary. Although the observed autogamy-dependent repression of this serotype gene is difficult to reconcile with
its aging-dependent activation, one possible explanation for
the two opposite expression patterns is that the aging
pathway and the autogamy pathway involve their respective
independent steps, to each of which $a$ -51D contributes in
a different manner. In any case, it is likely that this cellular
surface protein receives adverse environmental stimuli as
food restriction and regulates the subsequent intracellular
transmission of the external signals. This notion is supported
by a structural analysis indicating that Paramecium surface
proteins are potentially capable of intermolecular protein
interactions.\textsuperscript{29} The MS2 gene with little known function is
worth noting in that its transcription mode in the course of
autogamy (AS in Fig. 2) is parallel with that during clonal
aging.\textsuperscript{20} This close transcriptional association between the
two events could reflect their intimate interrelation\textsuperscript{39} as
introduced above, in which the MS2 gene product may play
an important role. Further study for the underlying aspect
common to autogamy and clonal aging is currently in
progress.

4 Synopsis

Autogamy is a sexual process of meiosis and self-
fertilization which occurs in unpaired Paramecium cells and
is considered to be coupled to the Paramecium clonal life
span. In order to identify the gene expression which may
underlie the initiation of autogamy, we screened for genes
differentially expressed before and after the onset of
autogamy by using a subtraction strategy and obtained two
species of difference genes. Both genes were found to be
the ones increasingly expressed with clonal age that we have
cloned previously. One was repressed upon commitment to
autogamy and identified to be the $a$ -51D surface protein
gene. The other, progressively activated while paramecia
traversed the autogamy pathway, was identical to the gene
referred to as MS2. The present result may be the first report
of the molecular basis of the functional correlation between
autogamy and clonal life span.

References

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ヨツヒメソウリムシの自家生殖に特異的な遺伝子発現変化

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要  旨

ソウリムシの自家生殖であるオートガミーは未接合細胞内で起きる減数分裂と自家受精から成る有性生殖法であり、ソウリムシの寿命と関連していると考えられている。オートガミーを引き起こす遺伝子の同定を目的として、オートガミー開始の前後で差次的に発現する遺伝子をサプラクション法により選択した結果、2種の遺伝子が得られた。これらは両方とも著者らが以前クローン化した、老化過程で発現性が高まる遺伝子と同じものであった。一方はオートガミーのコミットメント（方向づけ）の際に発現が抑制され、これはα-51D 表層抗原の遺伝子であった。他方はオートガミーの進行に伴い発現が上昇するもので、これは著者らがMS2 と命名した遺伝子であることがわかった。この結果はオートガミーとソウリムシの寿命との機能的な結びつきを分子レベルで初めて説明するものになるかもしれない。