Quantitative Analysis of Transcripts from Two Zygote-specific zys1 Genes in Chlamydomonas reinhardtii

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Accepted January 18, 2001

Summary  Primer extension analysis using 10 µg of polyA RNA extracted from zygotes of Chlamydomonas reinhardtii 10 min after mating was carried out to elucidate relative amounts of transcripts of early zygote-specific (zys1) genes. A novel minor extension product as well as a major product for each of the zys1A and zys1B genes was detected. Quantification of band intensity showed that relative accumulation amounts of zys1A major, zys1A minor, zys1B major and zys1B minor transcripts were in the ratio of 1.78 : 0.17 : 1.00 : 0.73. This result suggests that the zys1A promoter is slightly stronger than that of zys1B at an initial stage of gene expression.

The unicellular green alga Chlamydomonas reinhardtii has been widely used as a model organism. Because of the large amount of accumulated mutants and the well established mating system (Harris 1989), this alga has been widely used for genetic and biotechnology studies. Nuclear transformation of the alga was first attained by rescuing auxotrophic mutants with corresponding wild-type genes (Debuchy et al. 1989, Kindle et al. 1989). The introduction of drug resistance markers to wild-type strains of C. reinhardtii further developed the transformation system (Stevens et al. 1996, Cerutti et al. 1997a). Though foreign genes integrated into the nuclear genome, they were often not expressed (Cerutti et al. 1997b). To overcome this problem, codon bias change (Fuhrmann et al. 1999), or intronic enhancer was adopted (Lumbreras et al. 1998).

Another strategy that would aid in efficient expression of transgenes, for example reporter genes, would be to drive expression of these genes with an inducible promoter, which would be silent during genome integration, and would respond only to external stimuli. Such candidate promoters include the promoters of zygote-specific genes, carbonic anhydrase genes, or heat shock protein genes. Of these candidates, zygote-specific promoter is easily induced and the corresponding stimulus does not damage cells or gene products. The zygote-specific genes named as zys1 to zys4 have been isolated from zygotes 10 min after mating (Uchida et al. 1993). Though quantitative analysis of zys1, zys2, zys3 and zys4 transcripts is yet to be analyzed, the zys1 transcripts (expression of zys1A and zys1B genes is similar in temporal pattern after mating) accumulate earliest of all the zys gene transcripts. A foreign gene product driven by zys1 promoter, if successfully expressed, might be modified by transgenes expressed using the other later expressing zys promoters.

In order to estimate which of zys1A and zys1B promoter is suited better for driving foreign gene, quantitation of 2 endogenous zys1 gene transcripts was performed using polyA RNA which was extracted from young zygotes formed in highly synchronized mating. In this study, we show that a novel minor band and a major band in extension products for each zys1 gene were detected using 10 µg of polyA RNA that was isolated from cells of C. reinhardtii 10 min after mating. Relative amounts of each product were also determined from intensity of bands on an autoradiogram.

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Materials and methods

The wild-type strain 137c of *Chlamydomonas reinhardtii*, which originated from the *Chlamydomonas* Genetic Center, Duke University, was used in this study. Mating condition was the same as in a previous paper (Uchida *et al.* 1992).

PolyA RNA was isolated from cells 10 min after mixing the 2 mating types of gametes. Primer extension was performed using Primer Extension System™ (Promega, Madison, WI, U.S.A.). SuperScript™ RNase− Reverse transcriptase was also provided with this kit. In each extension reaction, 10 μg of polyA RNA was used. Nucleotide sequence of the primer, which anneals to the 2 genes, is complementary to *zys1A* sequence (Genbank accession number of AB001485) at +171 to +140, and to *zys1B* sequence (Genbank accession number of AB001486) at +179 to +148. The primers specific for each gene are mentioned in the previous report (Uchida *et al.* 1999). An imaging analyzer, BAS2000 (Fuji Film, Tokyo, Japan), was used to monitor images of sequence ladders and primer extension products.

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**Fig. 1.** Differential-interference-contrast images of cells forming zygotes. Cells before (a, b) and 1 (c, d), 2 (e), 3 (f), 5 (g), and 10 (h) min after mixing 2 types of gametes. Images (a, c) and (b, d) show mt′ and mt′− gametes, respectively. Arrowheads and an arrow designate gametic cell walls and a fertilization tubule, respectively. Bar represents 5 μm.
Results and discussion

Mating of Chlamydomonas

In a previous preliminary analysis of transcription initiation sites of zys1 genes, polyA RNA was extracted from 10-min old zygotes (Uchida et al. 1999). In this analysis little attention was paid on synchronizing mating of cells from which the RNA was extracted. In the present study, in order to check synchronous mating of cells, aliquots from cell suspension mixture were withdrawn successively and morphology of cells was monitored under light microscopy. After suspension of mt+ (Fig. 1a) and mt− (Fig. 1b) gametes were mixed, cell wall was lysed in either type of cells (Fig. 1c, d). In this stage, fully developed mating tubule was observed at the anterior of the mt+ gamete (Fig. 1c). Pairing of opposite types of gametes is initiated by flagellar tipping (Fig. 1e). Cell fusion was performed to produce quadriflagellate zygotes (Fig. 1f, g, h). These morphological changes observed were the same as in previos reports (Harris 1989), and 0%, 82% and 90% of cells formed to be quadriflagellated zygotes in 0, 8 and 10 min after the mixing, respectively.

Primer extension analysis

A previous report showed that a transcription initiation site was detected 22 bp and 24 bp downstream of a TATA box-like sequence for zys1A and zys1B genes, respectively (Uchida et al. 1999). In this analysis, this major extension product was detected, when primer extensions were performed using 2 μg of polyA RNA. Further analysis using 10 μg of polyA RNA was also carried out. As a result, we recognized a novel minor extension product for each gene (Fig. 2). When a zys1A-specific primer was used, a minor band was detected 5 bp downstream of the major band. When a zys1B-specific primer was used, a novel minor band was detected 4 bp upstream of the major band. No other bands were detected in the entire region on the autoradiograms analyzed for both genes. The same results were obtained using the same amount of RNA in another experiment. These results showed that each of zys1A and zys1B gene transcription starts from 2 adjacent positions located about 20–30 bases downstream of the TATA box-like sequence (Fig. 3). Multiple transcription initiation sites has been suggested in Chlamydomonas zygote-specific class IV gene (Woessner and Goodenough 1989). Multiplicity of transcripts might be due to evolution of different function, as indicated in higher plant genes (Dehesh et al. 1994). Hereafter, transcripts corresponding to a major, or a minor band was referred to as a major, or a minor transcript.

In order to examine the expression of zys1A and zys1B genes at an early stage of expression, further analysis was performed using the primers which anneal with the 2 zys1 genes. Using 10 μg polyA RNA isolated from zygotes 10 min after mating, the major and minor bands were again the
only products detected and located at positions mentioned above, and no other bands were detected in the entire region. Relative accumulation amounts of major and minor zysl transcripts 10 min after mating were estimated from intensities of the bands on an autoradiogram using an image analyzer (Table 1). When the major zyslB transcript amount was set to 1.00, the relative amounts of zyslB minor, zyslA major and zyslA minor transcripts were calculated as 0.730, 1.78 and 0.171, respectively. These results show that total amount of transcripts driven by the zyslA upstream region (zyslA major plus zyslA minor transcripts) is more than that of zyslB 10 min after mating. In this context, the zyslA promoter is stronger and would be better suited for driving foreign gene than that of zyslB. We are now investigating whether this promoter power difference might be related with positive effect of cycloheximide on zyslA transcript accumulation in young zygote, or distinct nucleotide sequence difference in 3' UTR of the 2zysl genes (Uchida et al. 1993).

### Acknowledgments

The authors appreciate the kindness of Prof. Dr. Tsuneyoshi Kuroiwa, University of Tokyo, for his encouragement, and Dr. Joe Zuccarello, University of New South Wales, for his critical reading of the manuscript. This work was partly supported by a grant for the Regional Joint Research Pro-

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**Table 1.** Relative amounts of zysl transcripts detected in the primer extension reaction using polyA RNA extracted from cells 10 min after mating

<table>
<thead>
<tr>
<th>Extension products</th>
<th>Mean (n=4)±S.D.</th>
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<tr>
<td>zyslA major</td>
<td>1.78 ±0.11</td>
</tr>
<tr>
<td>zyslA minor</td>
<td>0.171±0.044</td>
</tr>
<tr>
<td>zyslB major</td>
<td>1.00†</td>
</tr>
<tr>
<td>zyslB minor</td>
<td>0.730±0.062</td>
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</tbody>
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†Mean values in 4 reactions, 2 extension reactions were performed using 2 independent RNA samples.

The relative intensity of zyslB major band was defined as 1.00.
ject of Yamagata Prefecture from Japan Science and Technology Corporation.

References


