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Generation of Expressed Sequence Tags from Low-CO_2 and High-CO_2 Adapted Cells of *Chlamydomonas reinhardtii*

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Abstract

To characterize genes whose expression is induced in carbon-stress conditions, 12,969 and 13,450 5′-end expressed sequence tags (ESTs) were generated from cells grown in low-CO_2 and high-CO_2 conditions of the unicellular green alga, *Chlamydomonas reinhardtii*. These ESTs were clustered into 4436 and 3566 non-redundant EST groups, respectively. Comparison of their sequences with those of 3433 non-redundant ESTs previously generated from the cells under the standard growth condition indicated that 2665 and 1879 EST groups occurred only in the low-CO_2 and high-CO_2 populations, respectively. It was also noted that 96.2% and 96.0% of the cDNA species respectively obtained from the low-CO_2 and high-CO_2 conditions had no similar EST sequence deposited in the public databases. The EST species identified only in the low-CO_2 treated cells included genes previously reported to be expressed specifically in low-CO_2 acclimatized cells, suggesting that the ESTs generated in this study will be a useful source for analysis of genes related to carbon-stress acclimatization. The sequence information and search results of each clone will appear at the web site: http://www.kazusa.or.jp/en/plant/chlamy/EST/.

Key words: *Chlamydomonas reinhardtii*; cDNA; EST; CO_2 stress

Chlamydomonas reinhardtii, a single-celled green alga, has been recognized as a model system to perform genome-wide studies on photosynthesis, flagellar assembly, mating processes, maternal inheritance, cell wall biogenesis, gametogenesis, phototaxis and stress responses. *Chlamydomonas* cells can adapt to various kinds of environmental stresses such as nutrient limiting conditions of carbon,[1] nitrogen,[2] sulfate,[3] and phosphate[4] by inducing series of genes which function to acclimatize to the conditions. Among these conditions, carbon dioxide concentration in an autotrophic culture medium is one of the essential rate-limiting factors which determine the photosynthetic rate and growth. With the aim of identifying genes related to carbon-stress acclimatization, we performed a large scale EST analysis using cDNA libraries from the cells cultured under low-CO_2 and high-CO_2 conditions, and the EST sequences obtained were compared. Comparison was also made with those previously generated under the photoautotrophic condition.[5]

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1. Construction and Qualification of cDNA Libraries

The *C. reinhardtii* Dangeard C-9 (mt^-) strain was obtained and maintained as described previously.[5] Cells were photoautotrophically grown in HSM minimal liquid medium under continuous illumination at 100 $\mu E \cdot m^{-2}s^{-1}$ at 28°C with constant bubbling. To obtain high-CO_2 adapted cells, cultures were bubbled with CO_2-enriched air containing 5% CO_2. In contrast, to obtain CO_2-acclimatized cells (low-CO_2 cells), the gas was changed to ordinary air containing 0.04% CO_2 after supplying bubbling air containing 5% CO_2.

Purification of poly(A)$^+$ RNA was performed as described.[5] Two types of cDNA libraries, normalized and size-selected libraries, were generated as described previously.[5,6] Sixty six percent and 64.9% of the clones from normalized libraries of low-CO_2 and high-CO_2 treated cells contained the inserts of 0.5 to 1.5 kb, whereas 69.8% and 78.3% of the size-selected libraries had the inserts longer than 2.0 kb.

The quality of the libraries with respect to the intactness of cDNA was assessed by comparison of the translated 5′-end sequences to known protein sequences. Among 100 randomly chosen 5′-end sequences from nor-

Table 1. Number of 5′-end ESTs generated from cDNA libraries of cells grown in low-CO_2 and high-CO_2 conditions.

	Low-CO_2	High-CO_2	Standard[5]
Number of ESTs			
Normalized library	7113	7694	7310
Size-selected library	5856	5756	4261
Total	12969	13450	11571
Number of non-redundant groups[a]	4436	3566	3433
Number of specific groups[b]	**2665**	**1879**	**1753**
Number of common groups[c]		**2117**	

a) Number of groups occurred in each of the low-CO_2, high-CO_2 and standard conditions. b) Number of groups occurred only in the low-CO_2, high-CO_2 or standard conditions. c) Number of groups commonly occurred in two or three conditions among the low-CO_2, high-CO_2 and standard conditions.

Table 2. Classification of 5′-end ESTs appeared in common or only in low-CO_2 or high-CO_2 EST populations.

	Low-CO_2		**High-CO_2**		**Common**	
Similarity	Clones	Non-redundant ESTs	Clones	Non-redundant ESTs	Clones	Non-redundant ESTs
Genes of known function[a)]	648	384	363	269	14846	726
Hypothetical genes[b)]	139	91	64	50	831	105
No similarity[c)]	2647	2190	1740	1560	5141	1286
Total	3434	2665	2167	1879	20818	2117

a) Number of genes showed similarity to genes of known function, b) Number of genes showed similarity to hypothetical genes that have no definition of function, c) Number of genes showed no similarity.

malized and size-selected libraries of low-CO_2 treated cells, 74 (74%) and 67 (67%) were found to contain a translation initiation codon (data not shown). The result suggests that the libraries generated in this study contain intact cDNA species abundantly.

2. Features of Generated ESTs

The number of clones indicated in Table 1 were randomly chosen from the normalized and size-selected libraries of cells treated in low-CO_2 and high-CO_2 conditions, respectively, and their 5′ ends were sequenced. Grouping of the EST sequences was performed as described;[5] the low-CO_2 ESTs (12,969) were clustered into 4436 non-redundant groups and the high-CO_2 ESTs (13,450) into 3566 non-redundant groups. Comparison was made among the EST groups generated under the low-CO_2 and high-CO_2 conditions and those of 3433 non-redundant EST groups obtained from the cells grown under the standard condition.[5] According to the results, 2117 groups were classified as ESTs that commonly occurred in two or three conditions among the low-CO_2, high-CO_2 and standard conditions, whereas 2665, 1879 and 1753 occurred only in low-CO_2, high-CO_2 or standard EST populations, respectively (Table 1). Therefore, the number of non-redundant ESTs generated in this study is 6661, and the total number of non-redundant ESTs isolated from this alga by our group becomes 8414.

More than 20,000 *C. reinhardtii* EST sequences have been deposited in the public EST database in GenBank at the time of this writing. To identify the novel cDNA species newly isolated in this project, a similarity search was performed against all the entries in the EST database using the BLASTN program.[7] Eighty-four (3.8%) of the 2665 groups occurred only in the low-CO_2 population and 75 (4.0%) of the 1879 groups occurred only in the high-CO_2 population showed similarity to sequences deposited in the database. This result apparently indicates that a large number of condition-specific gene candidates were newly identified in this study.

The 6661 non-redundant EST groups were similarity searched against the public protein databases. As a result, 475 groups appeared in the low-CO_2 condition, 319 groups in the high-CO_2 condition and 831 groups which appeared commonly in the low-CO_2, high-CO_2 and standard conditions, had significant similarity to registered sequences, and the remaining 5036 groups, were novel. Among the EST groups with significant similarity, the number of groups which showed similarity to genes with known function and to hypothetical genes in each EST population are shown in Table 2. The search results of the individual clone as well as the classifica-

tion of functional categories will appear at the web site, http://www.kazusa.or.jp/en/plant/chlamy/EST/.

3. Identification of Genes Specific to Low-CO_2 and High-CO_2 Conditions

According to the result of similarity search, gene sequences encoding periplasmic carbonic anhydrase,[8] mitochondrial carbonic anhydrase,[9] and chloroplast membrane protein LIP-36[10] were included in the EST groups appeared only in the low-CO_2 condition. Five carbonic anhydrase genes, *Cah1*, *Cah2*, *Cah3*, *Mca1* and *Mca2*, have been reported in *C. reinhardtii*; *Cah1* and *Cah2* encode periplasmic carbonic anhydrase,[8] *Cah3* encodes chloroplastic (alpha-) carbonic anhydrase,[11] and *Mca1* and *Mca2* encode mitochondrial carbonic anhydrase.[12] Among these genes, *Cah1*, *Mca1* and *Mca2* appeared only in the low-CO_2 EST populations, and *Cah2* and *Cah3* were found in both the low-CO_2 and high-CO_2 populations. These results are consistent with previous reports[8–12] that genes specifically expressed in low-CO_2 and high-CO_2 conditions are included in the respective condition-specific EST populations. These results suggest that the ESTs generated in this study will be a useful source for identification of genes related to carbon-stress acclimatization, though further expression analyses using DNA arrays and Northern hybridization are necessary to support the specificity of these clones.

The EST sequences reported in this paper appear in the GenBank/EMBL/DDBJ databases with accession numbers AV618893-AV627335 and AV627337-AV645312.

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References

1. Spalding, M. 1998, CO_2 acquisition. Acclimation to changing carbon availability. In Rochaix et al. (Eds): The molecular Biology of chloroplast and mitochondria in *Chlamydomonas*, Kluwer Academic Press, pp. 529–547.
2. Menacho, A. and Vega, J. M. 1989, Effect of nitrogen starvation on ammonium assimilation by *Chlamydomonas reinhardtii*, *Physiologia Plantarum*, **75**, 285–289.
3. Davies, J. P., Yildiz, F. H., and Grossman, A. R. 1999, Sac3, an Snf1-like serine/threonine kinase that positively and negatively regulates the responses of *Chlamydomonas* to sulfur limitation, *Plant Cell*, **11**, 1179–1190.
4. Wykoff, D. D., Grossman, A. R., Weeks, D. P., Usuda, H., and Shimogawara, K. 1999, Psr1, a nuclear localized protein that regulates phosphorus metabolism in *Chlamydomonas*, *Proc. Natl. Acad. Sci. USA*, **96**, 15336–15341.
5. Asamizu, E., Nakamura, Y., Sato, S., Fukuzawa, H., and Tabata, S. 1999, A Large Scale Structural Analysis of cDNAs in a Unicellular Green Alga, *Chlamydomonas reinhardtii*. I. Generation of 3433 Non-redundant Expressed Sequence Tags, *DNA Res.*, **6**, 369–373.
6. Bonaldo, M. F., Lennon, G., and Soares, M. B. 1996, Normalization and subtraction: two approaches to facilitate gene discovery, *Genome Res.*, **6**, 791–806.
7. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990, Basic local alignment search tool, *J. Mol. Biol.*, **215**, 403–410.
8. Fukuzawa, H., Fujiwara, S., Yamamoto, Y., Dionisio-Sese, M. L., and Miyachi, S. 1990, cDNA cloning, sequence, and expression of carbonic anhydrase in *Chlamydomonas reinhardtii*: Regulation by environmental CO_2 concentration, *Proc. Natl. Acad. Sci. USA*, **87**, 4383–4387.
9. Eriksson, M., Karlsson, J., Ramazanov, Z., Gardestrom, P., and Samuelsson, G. 1996, Discovery of an algal mitochondrial carbonic anhydrase: molecular cloning and characterization of a low-CO_2-induced polypeptide in *Chlamydomonas reinhardtii*, *Proc. Natl. Acad. Sci. USA*, **93**, 12031–12034.
10. Chen, Z. Y., Lavigne, L. L., Mason, C. B., and Moroney, J. V. 1997, Cloning and overexpression of two cDNAs encoding the low-CO_2-inducible chloroplast envelope protein LIP-36 from *Chlamydomonas reinhardtii*, *Plant Physiol.*, **114**, 265–273.
11. Karlsson, J., Clarke, A. K., Chen, Z. Y. et al. 1998, A novel alpha-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2, *EMBO J.*, **17**, 1208–1216.
12. Eriksson, M., Villand, P., Gardestrom, P., and Samuelsson, G. 1998, Induction and regulation of expression of a low-CO_2-induced mitochondrial carbonic anhydrase in *Chlamydomonas reinhardtii*. *Plant Physiol.*, **116**, 637–641.