Isolation and Characterization of a Novel GRAS Gene That Regulates Meiosis-associated Gene Expression*

Kengo Morohashit, Masayoshi Minamis, Hisabumi Takase§, Yasuo Hottat, and Kazuyuki Hiratsukat

From the tDivision of Plant Biotechnology, Graduate school of Environment and Information Sciences, Yokohama National University, Yokohama 240-8501, §Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma 650-0101, and ¤Department of Health and Nutrition, Niigata University of Health and Welfare, Shimami-cho, Niigata-shi 950-3198, Japan

GRAS protein is a family of plant-specific proteins that plays a role in various developmental processes. Here we report a novel GRAS protein from lily, designated LISCL (Lilium longiflorum Scarecrow-like), dominantly expressed at the premeiotic phase within anthers. The LISCL protein has two highly basic regions, and transient expression analyses of dissected GFP-LISCL fusion proteins showed that both basic regions are important for the nuclear localization. A series of transcriptional activation experiments demonstrated that truncated LISCL proteins fused to the yeast GAL4 DNA-binding domain clearly demonstrated that the amino terminus of the LISCL protein has a strong activity of transcriptional activation in the yeast as well as in the plant cell. Further investigation on the effect of the LISCL protein on the transcriptional activity of the meiosis-associated promoter revealed that in pollen mother cells of the lily, the activity of the meiosis-associated promoter is specifically enhanced by LISCL protein co-expression. These results suggest that LISCL is involved in transcriptional regulation during microsporogenesis within the lily anther.

All sexually reproducing organisms have a specialized developmental pathway for gametogenesis, in which diploid cells undergo meiosis to produce haploid germ cells. In plant male gametogenesis, archesporial cells differentiate into pollen mother cells (PMCs), and the PMCs give rise to the production of tetrads of four haploid germ cells with two nuclear divisions. Subsequently, the haploid germ cells develop into mature pollen through the cytological and molecular events involved in the pollen developmental pathway. Meiosis organizes the transition from diploid to haploid and thus is one of the most complex events to occur during gametogenesis. The complexity of events suggests that many genes are tightly regulated to ensure each successful meiotic division. In yeast, characterization of temporal and spatial gene expression at meiosis has contributed toward a greater understanding of the mechanism of the meiotic gene (1). In higher eukaryotes, however, only a limited number of meiotic genes have been reported because of the lack of appropriate analytical techniques. The monocotyledonous plant Lilium longiflorum has been used for decades to study meiosis because of the accessibility of the male gametophyte (microsporocytes and pollen grains) and the synchronous development among sporogenous cells within anther (2, 3). By using the subtractive method, Kobayashi et al. (4) isolated 18 cDNAs (LM1–LM18 genes), which are preferentially expressed during the premeiotic phase of microsporogenesis in L. longiflorum. LM15 gene, which shows a similarity to the DNA recombination gene RecA, has been shown to be involved in meiosis in plants (5, 6). These results suggest that genes induced at meiosis have a function associated with meiotic events. However, mechanisms involved in meiosis-specific gene expression are not necessarily clear, and factors that regulate gene expression specific to microsporogenesis have not been identified yet. In the view of gene expression at microsporogenesis, identification of the factors that regulate specific gene expression should provide important clues to elucidate mechanisms involved in meiotic events in higher plants.

In an attempt to obtain information for genes expressed during microsporogenesis and meiosis, we previously carried out a large scale sequencing project using a lily zygotee cDNA library (7). One of the sequenced clones, M1125, has homology with a plant-specific putative transcription factor, Scarecrow (SCR). SCR is required for asymmetric cell division in an Arabidopsis root and encodes a novel putative transcription factor (8). Recently, SCR-like genes were reported in various plant species such as maize and pea (9, 10). SCR-like gene functions are not restricted to the asymmetric cell division. Although Repressor of Ga1–3 (RGA) and gibberellin-insensitive (GAI) genes show a similarity to SCR in their amino acid sequences, they play important roles in the gibberellin signal transduction of Arabidopsis but not in asymmetric cell division (11, 12). In addition, PAT1 protein, which shows a similarity to SCR protein, has been shown to be involved in the phytochrome A signal transduction of Arabidopsis (13). In other species, various functions of SCR-like genes were reported; the Lateral suppressor (Ls) gene in tomato has crucial functions in the formation of lateral branches, and SLR1 of rice has been iden-

Received for publication, February 19, 2003, and in revised form, March 24, 2003
Published, JBC Papers in Press, March 25, 2003, DOI 10.1074/jbc.M301712200

* This work was supported by Grant-in-aid 14360205 for Scientific Research (B) from the Japanese Society for the Promotion of Science, by Research for the Future Program JSPS-RFFT 00L10604, and by Grant-in-aid 12052217 for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB106274.

†To whom correspondence should be addressed: Division of Plant Biotechnology, Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama 240-8501, Japan. Tel.: 81-45-339-4419; Fax: 81-45-339-4413; E-mail: hiratsku@ynu.ac.jp.

‡The abbreviations used are: PMC, pollen mother cell; UAS, upstream activating sequence; 5′-RACE, 5′-rapid amplification of cDNA ends; GFP, green fluorescent protein; CaMV, cauliflower mosaic virus; NLS, nuclear localization signal; BR, basic region; DB, DNA-binding domain; AD, activation domain; STAT, signal transducers and activators of transcription.

This paper is available on line at http://www.jbc.org

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
Characterization of a Novel GRAS Gene from Lily

EXPERIMENTAL PROCEDURES

Plant Materials—Flower buds of L. longiflorum cv. Hinomoto were categorized according to their length, which was calculated from the base of the pedicel to the tip of the sepals (ranging from 10 to 35 mm). Estimation of the stages of the microsporogenesis was based on the comparison of the length and the thickness of the meiotic stages by cytological examination according to published methods (2). Antlers were isolated from the dissected buds. Leaves were collected from fresh lilies. All materials were soaked in liquid nitrogen and stored at −80 °C.

DNA Sequencing—DNA sequencing was performed using a Perkin-Elmer dye primer cycle system according to the manufacturer’s instructions with a ABI 373 Stretch sequencer (Applied Biosystems Inc.). Homology searches were performed in the GenBank™ data base using the BLAST program (19).

RNA Isolation and Gel Blot Analysis—RNA was prepared from samples kept at −80 °C using the auranintricarboxylic acid method as described previously (20). Each lane was loaded with 10 µg of total RNA, which was then fractionated on 1% agarose-formaldehyde gels and blotted onto Hybond N+ membrane according to the manufacturer’s protocol (Amersham Biosciences). Hybridization of 32P-labeled DNA with GFP at the amino-terminal portion and with LlSCL at the carboxyl-terminal portion. Onion epidermal cells were prepared and bombarded with 1.6 µm gold particles (Bio-Rad PDS-1000/He) coated with the control plasmid DNA. Samples were kept at room temperature for 8 h, and the GFP localization was visualized using a fluorescent microscope E800 (Nikon) equipped with a color charge-coupled device camera system (CCD, Hamamatsu Photonics).

Transcriptional Activation Experiments in Plant Cells—For transcriptional activation through the GAL4 target sequence, we used the firefly luciferase gene reporter plasmid containing six copies of the GAL4 target sequence fused to the ~46 region of the cauliflower mosaic virus (CaMV) 35S promoter. For the construction of the effector plasmids, the PCR-amplified LISL fragments were fused to the GAL4 DNA-binding domain and inserted into the pBI221 vector by replacing the β-glucuronidase coding region: p221DBLlSCL-FL, LlSCL-5′-AAATGCCAACAAAAGCTAAGATGGCTGGTCAGATGGAG-3′, and 1125-C (linker site is underlined); p221DBLlSCL-N, LlSCL-5′-GAGGATCCATGGTGAAAGAGCTAAAAGTAGACG-3′; and p221DBLlSCL-N(1–129), LlSCL-5′-Bam, and LlSCL587-STP (5′-GGGGATCCATGACTAACATTGAAGCTCGTG-3′); p221DBLlSCL-C, LlSCL-C-Met (5′-GGGATCCATGGTGAAAGAGCTAAAAGTAGACG-3′), and 1125-C; p221DBLlSCL-DL, LlSCL-DL-C, and 1125-C; p221DBLlSCL-DL-CdV, LlSCL-DL-CdV and 1125-C; and p221DBLlSCL-N(1–129), LlSCL-5′-Bam, and LlSCL587-STP (5′-GGGGATCCATGACTAACATTGAAGCTCGTG-3′); p221DBLlSCL-DL-N(1–202), LlSCL-DL-C, and LlSCL-DL-N(1–317), LlSCL587-Met, and LlSCL-DL-NSTOP. The yeast GAL4 activation domain was amplified from pGADT7 (Clontech) with the following primers: AD5-Bam (5′-GGGATCCATGGTGAAAGAGCTAAAAGTAGACG-3′) and AD3-Xh (5′-GGGATCCATGGTGAAAGAGCTAAAAGTAGACG-3′). These PCR-amplified DNAs were digested with appropriate enzymes and cloned into the pBI221-GAL4DB vector containing a GAL4 DNA-binding domain. For the transactivation assay of LlSCL protein in plant cells, the LlSCL-FL fragment was amplified with LlSCL-5′-Bam and 1125-C primers and then inserted into Sad-blunt/BamHI-digested pBI221 by replacing the β-glucuronidase coding region. A reporter plasmid harboring a tissue-specific promoter, pL1M1prom::221:LUC+, was constructed as described previously (21).

The BY-2 cells, the lily leaves, and PMCs were incubated at room temperature for 12 h and 24 h in the dark, respectively. Dual luciferase assay was conducted as described previously (22).

Yeast Expression Vectors, Transformation, and β-Galactosidase Assay—To construct a yeast expression vector, the PCR-amplified LlSCL fragments and yeast activation domain were inserted into the pGBK77 vector (Clontech). Constructs used in these experiments were pGB, pGBAD, pGBLlSCL-N, pGBLlSCL-N(1–129), pGBLlSCL-N(130–202), pGBLlSCL-N(203–317), pGBLlSCL-N(130–202), and pGBLlSCL-N(1–317). Transformation of budding yeast Saccharomyces cerevisiae was accomplished by the lithium acetate method. Mutagenesis was performed using the Escherichia coli strain AM1000 (Mata, trp1–901, leu2–3), 112, ura3–52, his3–200, gal4Δ, gal80Δ, lys2Δ1, GAL1::GAL::GAL1::TATA H313, GAL2::GAL2::GAL2::TATA ADE2, URA3::MELI::MELI::TATA lacZ, MEL1). The β-galactosidase liquid assay was performed with a luminescent β-galactosidase detection kit II according to the manufacturer’s instructions (Clontech).

RESULTS

Isolation of a GRAS Gene cDNA from Lily Microsporocytes—We have selected and sequenced ~400 cDNA clones from a cDNA library of lily PMCs by the self-hybridization method as described previously (7). One of these cDNA clones, designated M1125, is closely related in amino acid sequence to the SCR gene, which encodes a putative transcription factor that regulates an asymmetric cell division in the Arabidopsis root (8). By using the 5′-RACE technique, we isolated a 2.6-kb full-length cDNA of the M1125 from the total RNA at the pachyten stage of lily PMCs. The full-length cDNA contains an open reading frame with a coding capacity for 740 amino acids (Fig. 1). Comparison of the 740-amino acid sequence with the previously described cDNA sequences showed a similarity to the GA1, RGA, and SCR proteins belonging to the GRAS protein family. Comparison of the M1125 protein, designated liscl2. H. Takase, M. Minami, Y. Hotta, and K. Hiratsuka, unpublished.
FIG. 1. Nucleotide sequence and the deduced amino acid sequence of LlSCL cDNA. The amino acid sequence encoded by an open reading frame is shown below the nucleotide sequence. Basic amino acid regions are boxed, acidic amino acids are circled, and serine stretches are underlined.
(Lilium longiflorum SCARECROW-Like), with other GRAS gene products revealed the presence of highly conserved VHIID, PFYRE, and SAW motifs within the carboxyl terminus of the protein (Fig. 2). Two leucine heptad repeats, referred to as LHRI and LHRII, are also identified in the carboxyl terminus (amino acids 369–413 and 537–603, respectively). In the middle (amino acids 365–369) of the LlSCL protein, the LXXLL motif, which has been shown to mediate the binding of transcriptional coactivators to nuclear receptors (23, 24), was also identified. Although no typical nuclear localization signal (NLS) was found within the LlSCL amino acid sequence, two parts of the highly basic region, referred to as BRI and BRII, were identified (amino acids 351–359 and 697–704, respectively). The amino terminus of the LlSCL protein does not show any significant homology to known proteins (Fig. 1).

LlSCL Gene Is Strongly Expressed at the Premeiotic Phase—To characterize the expression pattern of the LlSCL gene, we conducted RNA gel blot analysis. Fig. 2 shows the results obtained by probing a blot of total RNA prepared from whole anthers collected at various stages of anther development with the radiolabeled 5′-region of the LlSCL gene. A 2.6-kb LlSCL transcript was found to be differentially regulated during the course of microsporogenesis. The maximal level of LlSCL mRNA was detected in anthers containing sporogenous cells before meiosis. During the meiosis of microsporogenesis, the transcript was detected in a slightly lower level throughout microspore development, and then it decreased to an undetectable level in mature pollen (Fig. 3). In vegetative tissues, LlSCL gene expression levels were barely detectable (data not shown).

Nuclear Localization of LlSCL Protein—SCR and related proteins have been suggested to be involved in the transcriptional regulation (8). Thus, they are thought to have nuclear localizing activity. In fact, a previous report indicated that RGA-GFP fusion proteins are located in the nucleus of the onion epidermal cell (11). To investigate the intracellular localization of LlSCL protein, chimeric genes encoding the LlSCL protein fused to the GFP were introduced into onion epidermal cells using a particle bombardment system and expressed under control of the CaMV 35S promoter (Fig. 4). Eight hours after bombardment, green fluorescence derived from GFP fusion proteins were examined by fluorescent microscopy. The fluorescent signals derived from the control plasmid vector expressing GFP alone were observed in both the nucleus and cytoplasm, the signals in the nucleus were stronger than those in cytoplasm (Fig. 3C). On the contrary, the signals from the GFP-LlSCL-N fusion proteins strongly aggregated in cytoplasm (Fig. 5G). Although GFP signals of the pGLlSCL-CdBI construct were observed in both the nucleus and cytoplasm, the signals in the nucleus were stronger than those in cytoplasm (Fig. 5C).
were located exclusively in the nucleus (Fig. 5B). Interestingly, the signals from the construct pGLISSCL-CdBII were observed in the cytoplasm, even though the pGLISSCL-CdBII construct expressed a fusion protein including the BRI region (Fig. 5F). These results indicate that both BRI (amino acids 351–359) and BRII (amino acids 697–704) are important for the nuclear localization of LlSCL protein. It is necessary to confirm the requirement of basic regions in the context of full-length protein; however, we could not carry out a mutational analysis using full-length protein because of our inability to detect fluorescent signals from samples transfected with pGLISSCL-FL.

The LlSCL Protein Contains a Transcriptional Activation Domain—To evaluate the function of the LlSCL protein as a transcription factor, we performed transcriptional activation experiments by transient assay in tobacco BY-2 cells. The reporter gene was the firefly (Photinus pyralis) luciferase gene preceded by a promoter containing six repeats of the GAL4 target sequence (UAS) fused to the CaMV 35S promoter TATA-box region. The effector genes were designed to express fusions of various parts of the LlSCL protein with the DNA-binding domain (DB) of yeast GAL4. The activation domain (AD) of yeast GAL4 protein was used as a positive control. The plasmid constructs used and the results are shown in Fig. 6. The full-size LlSCL protein fused to GAL4-DB, DB-FL, was able to raise the activation level the UAS promoter 2.5-fold higher than the activation level of GAL4-DB alone. However, three truncated LlSCL proteins, LlSCL-C, LlSCL-CdL, and LlSCL-CdV, fused to GAL4-DB did not activate the UAS promoter. On the other hand, the amino-terminal portion of the LlSCL protein fused to GAL4-DB strongly activated the UAS promoter. The transcriptional activation levels of GAL4-DB-LlSCL fusion protein derivatives containing the amino-terminal region were equivalent to the level of the GAL4-AD. These results suggest that the amino terminus of the LlSCL protein possesses the capability of strong transcriptional activation.

The amino terminus of LlSCL protein has two acidic regions. The domain responsible for transcriptional activation often belongs to the sequence that is rich in acidic residues (25, 26). Therefore, we dissected the amino-terminal region of LlSCL protein to investigate the activities of transcriptional activation with respect to these acidic regions. As shown in Fig. 7, DB-N(1–129), which includes the first acidic domain (amino acids 4–110), showed strong transcriptional activation equivalent to that of DB-N(1–317). The neutral domain fused to GAL4-DB, DB-N(130–202), had up to 3-fold higher activity than GAL4-DB alone. On the other hand, DB-N(203–317) and DB-N(130–317) showed no transcriptional activity, even though both constructs included the second acidic domain. Interestingly, the highest activity was obtained by the expression of DB-N(1–202) harboring the first acidic domain and the neutral domain. These results suggest that the region containing the first acidic domain and the neutral domain but not the second acidic domain of LlSCL protein is responsible for the transcriptional activation.
The Amino Terminus of LlSCL Protein Functions as a Transcriptional Activator in Yeast Cells

To investigate whether the LlSCL protein activates transcription through a plant-specific mechanism, we also examined the activity of transcriptional activation of LlSCL protein in yeast cells. We constructed various plasmids expressing the GAL4 DNA-binding domain fusions of different regions of LlSCL protein in yeast. These plasmids were introduced into yeast cells carrying the lacZ reporter gene under the control of the GAL1 promoter. (Fig. 8). The results of the β-galactosidase assay indicated that the properties of transcription activation of LlSCL protein in yeast cells were the same as those in plant cells. The region covering the first acidic domain and the neutral domain of the amino terminus of LlSCL protein caused transcriptional activation in the yeast as well as in the plant cell. Interestingly, the transcriptional activation levels of the GAL4-DB fusion proteins containing the LlSCL acidic domain were higher than that of GAL4-DB-AD fusion in yeast cells. These results suggest that plant-specific factors are not required for the strong activity of transcriptional activation of LlSCL acidic domain.

**Fig. 6.** Identification of transcriptional activation domain of LlSCL in tobacco BY-2 cells. The upper panel indicates the reporter construct. The luciferase gene was placed downstream of six copies of the GAL4-binding site. The lower panel shows the effector constructs containing DNA sequence encoding the GAL4 DNA-binding domain and distinct regions of LlSCL proteins. Each motif is described below the lower panel. The numbers refer to the positions of residues at the ends of the LlSCL regions fused to GAL4-DB. The following were used as effector plasmids: DB, p221DB; DB::AD, p221DBAD; DB::FL, p221DBLlSCL-FL; DB::N, p221DBLlSCL-N; DB::C, p221DBLlSCL-C; DB::CdL, p221DBLlSCL-CdL; DB::CdV, p221DBLlSCL-CdV. Relative luciferase activities in triplicate samples are shown after normalization with Renilla luciferase activity from the reference plasmid. Error bars show standard deviations.

**Fig. 7.** Transcriptional activation of the amino terminus of LlSCL in tobacco BY-2 cells. The upper panel indicates the reporter construct. The luciferase gene was placed downstream of six copies of the GAL4-binding site. The lower panel shows the effector constructs containing DNA sequence encoding the GAL4 DNA-binding domain and distinct regions of LlSCL proteins. The D/E-rich region is indicated by a black box. The numbers refer to the positions of residues at the ends of the LlSCL regions fused to GAL4-DB. Both reporter plasmid and effector plasmid were transiently expressed in tobacco BY-2 cells with a reference plasmid. The following were used as effector plasmids: DB, p221DB; DB::AD, p221DBAD; DB::N(1–317), p221DBLlSCL-N(1–317); DB::N(1–129), p221DBLlSCL-N(1–129); DB::N(130–202), p221DBLlSCL-N(130–202); DB::N(203–317), p221DBLlSCL-N(203–317); DB::N(1–202), p221DBLlSCL-N(1–202); DB::N(130–317), p221DBLlSCL-N(130–317). Relative luciferase activities in triplicate samples are shown after normalization with Renilla luciferase activity from the reference plasmid. Error bars show standard deviations.
Characterization of a Novel GRAS Gene from Lily

LISCL Protein Is a Transcriptional Activator of the Meiosis-associated Gene in PMCs—To study whether the LISCL gene functions as a transcriptional activator at microsporogenesis, we investigated the effect of the full-length LISCL protein expression on the transcriptional activity of the meiosis-associated promoter that directs microsporogenesis-specific gene expression. We exploited a meiosis-associated gene, LIM10, which encodes a small molecular weight heat shock protein specifically induced at the meiotic prophase (zygotene) in lily PMCs. To measure the transcriptional activation of the meiosis-associated promoter, the firefly luciferase coding region was placed downstream of the sis-associated promoter, the firefly luciferase coding region was

factor(s).

promoter during meiosis in conjunction with PMC-specific transcriptional activity of the meiosis-associated promoter was down-regulated (Fig. 9). When the LISCL protein was co-expressed together with reporter genes, the activity of the CaMV 35S promoter was enhanced by LISCL co-expression, whereas the activity of CaMV 35S promoter sequence. 

The LIM10 promoter sequence. The full-length LISCL cDNA preceded by the CaMV 35S promoter was used as the effector gene. The activity of the CaMV 35S promoter was slightly decreased in all plant cells tested. The activity of the meiosis-associated promoter was detected in lily PMCs (Fig. 9). When the LISCL protein was co-expressed together with reporter genes, the activity of CaMV 35S promoter was down-regulated (Fig. 9).

These results suggest that the LISCL protein plays a role in the transcriptional activation of the meiosis-associated (LIM10) promoter during meiosis in conjunction with PMC-specific factor(s).

DISCUSSION

A Novel Gene Encoding a Nuclear GRAS Protein Is Expressed at Microsporogenesis—In this study, we isolated and characterized a novel GRAS gene, LISCL (L. longiflorum Scarecrow-like), expressed specifically in premeiotic phase within the anthers of L. longiflorum. Because the SCR has been predicted to be a transcriptional regulator, we speculated that the LISCL gene encodes a novel transcriptional regulator involved in microsporogenesis. The molecular dissection experiments indicated that both the first and second basic regions (BRI and BRII) of the LISCL protein are important for the nuclear localization. Although the NLSs within plant transcription factors vary in sequence, organization, and number, it is noteworthy that the NLS of the LISCL protein separates the two regions by 400 residues. This kind of distant NLSs has not been reported in previously described transcription factors including GRAS gene products (27). The amino acid sequence of the BRI domain of LISCL protein shares homology with the basic region in the SCR protein, but domain identical to BRII of the LISCL protein was not found in the SCR protein. In addition, no homology was found between the putative NLS of the RGA protein and BRI or BRII of LISCL protein (11). On the other hand, the PAT1 protein has been shown to contain no putative NLS, and the protein is distributed throughout the cytoplasm (13). These data suggest the presence of various functions and mechanisms involving intracellular localization of GRAS gene products.

Amino Terminus of LISCL Protein Has a Strong Transcriptional Activity—Although the GRAS gene products share highly conserved motifs located within the carboxyl terminus, the amino termini were variable (16). The variable structures of the amino termini of GRAS gene products would be related to their functions. In fact, the DELLA domain, which is positioned in the amino termini of the RGA, GAI, RGL1, and SLR1 proteins, is required for the specific functions of those proteins in the gibberellin response (11, 18, 28). On the contrary, the SCR protein, which has a function distinct from other GRAS proteins, does not contain any DELLA domains in the amino terminus. The LISCL protein does not contain any DELLA domains and shows no similarity to the amino terminus of SCR protein. Therefore, it is speculated that the LISCL gene would have specific functions distinct from the gibberellin response and from asymmetric cell division in the root.

Except for the DELLA domain, the molecular functions of the amino terminus of GRAS gene products have not been reported. In this study, however, we have demonstrated that the amino terminus of LISCL protein (amino acids 1–317) is able to direct transcriptional activation equivalent to the level of the activation domain of yeast GAL4 protein. It is well known that hydrophobic residues interspersed between the acidic residues are associated with the transcriptional activation (26). This is consistent with the fact that the amino terminus of LISCL (amino acids 1–317) is highly acidic with a net negative charge of 32. The amino terminus of LISCL protein can be further divided into three domains: first acidic domain, neutral domain, and second acidic domain. Figs. 7 and 8 clearly indicate

3 M. Minami, H. Takase, and K. Hiratsuka, manuscript in preparation.
that both the first acidic and neutral domains, but not the second acidic domain, show strong activity. The transcriptional activation of both domains was observed not only in plant cells but also in yeast cells. This indicates that the mechanism of transcriptional activation by the amino terminus of LlSCL protein is evolutionarily conserved.

Silverstone et al. (11) proposed a model for gibberellin signal transduction through O-GlcNAc modification at serine/threonine-rich region(s) of GAI/RGA proteins. Because the LlSCL protein also contains serine stretches within the first acidic and neutral domains, it may be possible that the serine stretches could be O-GlcNAc modified by unknown factors in tobacco BY-2 cells and yeast cells, modulating the levels of transcriptional activation.

Compared with the high activity of transcriptional activation of the amino terminus of LlSCL protein, the full-length LlSCL protein exhibited lower activity (Fig. 6). The carboxyl terminus region of the LlSCL includes two parts, a leucine heptad repeat and an LXXLL motif, both of which mediate protein-protein interaction. Thus, it is possible that the LISCL protein interacts with factors involved in the regulation of transcription via these protein domains that modulate the level of transcriptional activation.

Richards et al. (29) proposed that GRAS gene products were related to the STAT family of proteins based on structural similarities between GRAS and STAT family proteins. Because STATs are activated by the receptor kinase (30), the LISCL protein could also be phosphorylated by an unknown protein kinase for a suitable function. Because the O-GlcNAc modification and serine/threonine phosphorylation are broadly observed in eukaryotic cells (31), it will be interesting to analyze the properties of mutated LISCL proteins with amino acid substitutions within the serine stretch region.

**LISCL Protein May Play a Role in Transcriptional Regulation during Microsporogenesis**—By the experiments of transient expression of the full-length LISCL protein in plant cells, we found that the LISCL protein may activate the expression of the meiosis-associated gene in lily PMCs (Fig. 9). To our knowl-

---

**Fig. 9. Transcriptional activation of the meiosis-associated gene by transient expression of LISCL protein.** A. Diagrams of the reporter plasmid and effector plasmid used in this experiments. B–D, effector, reporter, and reference plasmids were co-transformed into tobacco BY-2 cells (B), lily leaves (C), and lily PMCs (D) by particle bombardment. Relative luciferase activity is shown in more than three samples after normalization with Renilla luciferase activity from the reference plasmid. Error bars show standard errors (B and C, n = 3; D, n = 6).
edge, this is the first report indicating that the GRAS gene product is directly involved in regulation of a specific gene expression. Together with the result of mRNA accumulation pattern, we report here for the first time that a GRAS gene is involved in the specific gene expression during microsporogenesis. The results obtained in this study indicated that the LISCL protein requires a specific factor(s) for specific activation in PMCs, because the LISCL protein did not elevate the activities of LIM10 promoter in BY-2 and leaves. Thus, the LISCL protein functions as a co-activator for activating a gene expression in concert with the LISCL protein.

Further studies will be needed to define the biological functions and biochemical properties of LISCL protein in the microsporogenesis and anther development in lily. However, because of our inability to conduct a molecular genetics approach, studies using a lily system are rather limited. The GRAS gene, which shows a high similarity to LISCL gene, would have a similar function in the anthers from various species. Recently, we identified a gene encoding the LISCL homolog from *Arabidopsis* and rice. The availability of LISCL homologs from these species would allow further study of the function and biological implication of the LISCL gene.

REFERENCES

1. Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., and Herskowitz, I. (1998) Science 282, 699–705
2. Erickson, R. O. (1948) Am. J. Bot. 35, 729–739
3. Hotta, Y., Takase, H., Stobbs, L., and Stern, H. (1985) Cell 40, 785–793
4. Kobayashi, T., Kobayashi, E., Sato, S., Hotta, Y., Miyajima, N., Tanaka, A., and Tabata, S. (1994) DNA Res. 1, 15–26
5. Terasawa, M., Shinohara, A., Hotta, Y., Ogawa, H., and Ogawa, T. (1995) Genes Dev. 9, 925–934
6. Couteau, F., Belizé, F., Horlow, C., Grandjean, O., Vezon, D., and Doutriaux, M.-P. (1999) Plant Cell 11, 1623–1634
7. Morohashi, K., Takase, H., Hotta, Y., and Hiratsuka, K. (2000) Plant Biotechnol. 17, 131–135
8. Di Laurenzio, L., Wysocka-Diller, J., Malamy, J. E., Pysz, L., Helariutta, Y., Freshour, G., Hahn, M. G., Feldmann, K. A., and Benfey, P. N. (1996) Cell 86, 423–433
9. Lim, J., Helariutta, Y., Specht, C. D., Jung, J., Sims, L., Bruce, W. B., Diehn, S., and Benfey, P. N. (2000) Plant Cell 12, 1307–1318
10. Sassa, N., Matsushita, Y., Nakamura, T., and Nyunoya, H. (2001) Plant Cell Physiol. 42, 385–384
11. Silverstone, A. L., Ciampaglio, C. N., and Sun, T.-P. (1998) Plant Cell 10, 153–169
12. Peng, J. P., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P., and Harberd, N. P. (1997) Genes Dev. 11, 3194–3205
13. Bolle, C., Koncz, C., and Chua, N.-H. (2000) Genes Dev. 14, 1269–1278
14. Schumacher, K., Schmitt, T., Rosberg, M., Schmitz, G., and Theres, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 290–295
15. Ikeda, A., Ueguchi-Tanaka, M., Sonoda, Y., Kitano, H., Koshiba, M., Futsuhara, Y., Matsuoka, M., and Yamaguchi, J. (2001) Plant Cell 13, 999–1010
16. Pysz, L. D., Wysocka-Diller, J. W., Camilleri, C., Bouchez, D., and Benfey, P. N. (1999) Plant J. 18, 111–119
17. Ogawa, M., Kusano, T., Katsumi, M., and Sano, H. (2000) Gene 245, 21–29
18. Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M., and Matsuoka, M. (2002) Plant Cell 14, 57–70
19. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. (1990) J. Mol. Biol. 215, 403–410
20. Mousavi, A., Hiratsuka, K., Takase, H., Hiratsuka, K., and Hotta, Y. (1999) Plant Cell Physiol. 40, 406–416
21. Uefuji, H., Minami, M., Takase, H., and Hiratsuka, K. (2001) Plant Biotechnol. 18, 151–156
22. Matsuoka, N., Minami, M., Maeda, T., and Hiratsuka, K. (2001) Plant Biotechnol. 18, 71–75
23. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) Nature 387, 753–756
24. Leo, C., and Chen, J. D. (2000) Gene 245, 1–11
25. Giniger, E., and Ptashne, M. (1987) Nature 320, 670–672
26. Triesenberg, S. J. (1995) Curr. Opin. Genet. Dev. 5, 190–196
27. Liu, L., White, M. J., and MacRae, T. H. (1999) Eur. J. Biochem. 262, 247–257
28. Wen, C.-K., and Chang, C. (2002) Plant Cell 14, 87–100
29. Richards, D. E., Peng, J., and Harberd, N. P. (2000) BioEssays 22, 573–577
30. Darnell, J. E., Jr. (1997) Science 277, 1630–1635
31. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335

K. Morohashi, H. Takase, and K. Hiratsuka, unpublished data.
Isolation and Characterization of a Novel GRAS Gene That Regulates Meiosis-associated Gene Expression
Kengo Morohashi, Masayoshi Minami, Hisabumi Takase, Yasuo Hotta and Kazuyuki Hiratsuka

J. Biol. Chem. 2003, 278:20865-20873.
doi: 10.1074/jbc.M301712200 originally published online March 25, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301712200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 12 of which can be accessed free at http://www.jbc.org/content/278/23/20865.full.html#ref-list-1