BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **253,** 80–84 (1998)
ARTICLE NO. RC989747

Expression of Spliceosome-Associated Protein 49 Is Required for Early Embryogenesis in *Caenorhabditis elegans*

Masaki Fujita, Taizo Kawano, Kazuhiro Terashima,[1] Yoshimi Tanaka,[2] and Hiroshi Sakamoto[3]
Department of Biology, Graduate School of Science and Technology, Kobe University, 1-1 Rokkodaicho, Nadaku, Kobe 657-8501, Japan

Received October 30, 1998

Spliceosome-associated protein 49 (SAP 49) is a subunit of the splicing factor SF3b, which is involved in 3′ splice site recognition in pre-mRNA splicing. Here we show the *Caenorhabditis elegans SAP 49* gene is located in a gene cluster that is transcribed polycistronically with three other upstream genes. Transgenic analysis of expression of the gene cluster under the control of its native promoter revealed that in spite of its predicted essential function for all cells, the *C. elegans SAP 49* expression is limited to specific cells in both larval and adult stages. When the endogenous *SAP 49* expression was inhibited by microinjection of an antisense RNA, embryos laid by the injected worms ceased to develop at a specific stage of early embryogenesis, indicating that SAP 49 plays an essential role in the development of *C. elegans.* These results raise the possibility that SAP 49 is a cell-specific, not constitutive, splicing factor.

In metazoans, almost all protein-coding genes contain introns that are removed posttranscriptionally by splicing. Splicing occurs in multimolecular complexes which consist of pre-mRNA, small nuclear ribonucleoprotein particles (snRNP) and many other auxiliary factors (for reviews, see refs. 1,2). Human SAP 49 is a subunit of the U2 snRNP-associated auxiliary factor SF3b, which is required for recognition of the branch site of pre-mRNA (3,4) and contains two copies of the RNA recognition motif (RRM) at the amino terminus and a proline-rich domain at the carboxy terminus. We previously isolated the *C. elegans* SAP 49 homologue, which shows extensive amino acid similarity to the human counterpart (5). The two RRM regions and the proline-rich domain are conserved between the human and *C. elegans* SAP 49s, and in particular, the RRM regions of these proteins exhibit 82% identity. The *C. elegans SAP 49* gene is transcribed polycistronically together with at least two upstream genes, and then the primary transcript is processed into mature single mRNAs by the trans-splicing mechanism (5–7).

The function of SAP 49 *in vivo* is predicted to be essential because it is involved in specification of the 3′ splice sites *in vitro* (3,4). Consistent with this, the yeast *SAP 49* gene has recently been shown to be essential for cell viability (8), although the yeast homologue differs from those of humans, flies and *C. elegans* in that it does not contain the conserved carboxy-terminal proline-rich domain. The questions of whether SAP 49 is an essential splicing factor in multicellular organisms and of whether it is expressed ubiquitously, as suggested from its *in vitro* function, have not yet been addressed. We therefore examined expression of the *C. elegans SAP 49* transgene fused with the *LacZ* reporter. In addition, we applied the RNA interference method (9–11) to examine whether the function of the *SAP 49* gene is essential for the development of *C. elegans.*

EXPERIMENTAL PROCEDURES

Preparation of RNA and RT-PCR analysis. Total RNA (1 μg) isolated from mixed stages of worms with a QuickPrep total RNA extraction kit (Pharmacia) was reverse transcribed using either random or oligo d(T) primers. After reverse transcription, the reaction mixtures were subjected to PCR using the following specific primers: FW, 5′-ATC TGC AGC CAT TGC TGA TGA GGA TT-3′; RV, 5′-ATG GAT CCG CGC TGA TCT TCA CTT CG-3′. As control reactions, the reaction mixtures without reverse transcriptase were subjected to PCR using the same specific primers.

[1] Present address: Osaka Research Laboratories, Wako Pure Chemical Industries, Ltd., 6-6 Takadacho, Amagasaki, Hyogo, Japan.
[2] Present address: Department of Molecular Genetics, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita 565, Japan.
[3] Corresponding author: Department of Biology, Graduate School of Science and Technology, Kobe University, 1-1 Rokkodaicho, Nadaku, Kobe 657, Japan. Fax: +81-78-803-0554. E-mail: hsaka@kobe-u.ac.jp.

0006-291X/98 $25.00

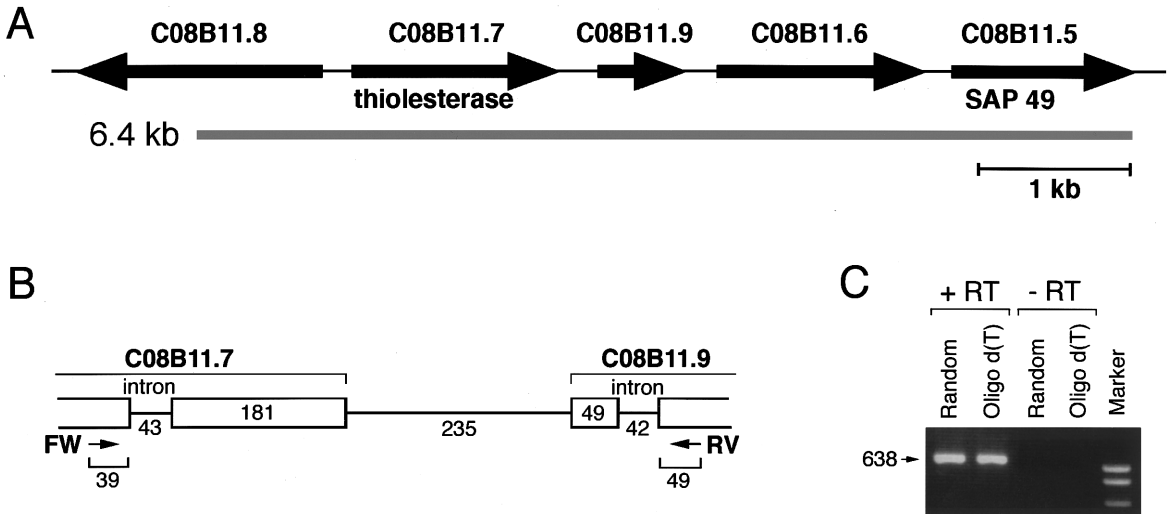

FIG. 1. Detection of the polycistronic primary transcript containing C08B11.7 and C08B11.9 coding regions. (A) Genomic organization of the gene cluster containing the SAP 49 gene. Arrows show orientation and length of each gene. The shaded bar represents the genomic fragment used for the *LacZ* reporter analysis. (B) Schematic representation of the genomic region between the C08B11.7 and C08B11.9 genes. Protein-coding regions are boxed. The gene-specific primers (FW and RV) and the nucleotide length (bp) of each portion are shown. (C) Total RNA (1 μg) was reverse transcribed using either random or oligo d(T) primers, followed by PCR using the specific primers FW and RV (+ RT). The PCR products were electrophoresed on a 1.5% agarose gel. No PCR product was detected in the reactions without reverse transcriptase (− RT), indicating that the PCR product was not produced from contaminating genomic DNA in the total RNA preparation. MspI-digested pBR322 DNA fragments (622, 527 and 404 bp) were used as size markers.

Transformation and LacZ reporter analysis. The 6.5-kb genomic fragment was cloned into the *LacZ* reporter plasmid, pPD22.04, which was developed by A. Fire and his colleagues (12), so that the translational reading frame of *SAP 49* was fused with that of the *LacZ* portion. The plasmid was injected into the gonads of the wild type N2 strain as described (13). *LacZ* expression of the transformants was analyzed as described (12). The stained worms were viewed with Nomarski optics.

RNA interference. Antisense RNAs from the *mom-2* and *SAP 49* genes were generated by *in vitro* transcription of the cDNAs cloned in the Bluescript plasmid. The antisense RNA was injected into the gonads of wild type N2 strain as described (13).

RESULTS AND DISCUSSION

SAP 49 gene is transcribed polycistronically with three other genes. We previously showed that *C. elegans SAP 49* (referred to as C08B11.5 in the *C. elegans* database) is transcribed polycistronically as a gene cluster with at least two other upstream genes, C08B11.6 and C08B11.9 (Fig. 1a) (5). Close inspection of the genomic organization near the *SAP 49* gene in the database revealed that a predicted gene, C08B11.7, encoding a thiolesterase-like protein resides in the same orientation just upstream of the previously identified gene cluster (Fig. 1A). Since adjacent genes that have the same orientation and are separated by short intervals are often transcribed polycistronically in *C. elegans* (6,7), we assumed that the C08B11.7gene is a member of the polycistronic *SAP 49* gene cluster. To confirm this, we tested for the presence of a primary transcript containing both the C08B11.7 and the previously identified gene cluster regions. Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed using primers specific for the C08B11.7 and C08B11.9 genes (Fig. 1B, C). A cDNA product of the expected length (638 bp) indicating such a primary transcript was amplified when either the random- or oligo d(T)-primed first strand cDNAs were used. Since no PCR product was detected in the control reactions without reverse transcription, the amplified DNA should have arisen from RNA molecules, and not from genomic DNA contaminating the preparation of total RNA. Sequence analysis of the amplified cDNA revealed that it indeed contains the region covering the C08B11.7 and C08B11.9 genes (data not shown). These results indicate that the *SAP 49* gene is transcribed polycistronically with the three other genes as a gene cluster.

SAP 49 gene is expressed in specific cells in C. elegans. To examine where and when the *SAP 49* gene is expressed in *C. elegans*, we performed a transgenic analysis in which *LacZ* reporter expression was used as an indicator of *SAP 49* expression. Based on the finding that the C08B11.7 gene is positioned at the 5′-end of the gene cluster, we assumed that the region between the C08B11.8 gene and the *SAP 49* gene would contain sufficient information for transcriptional regulation of *SAP 49* expression. Therefore, we cloned a 6.4-kb genomic fragment which contains a portion of the C08B11.8 gene and all of the four other predicted genes, into a promoter-less *LacZ* reporter plasmid so that the translational reading frame of the

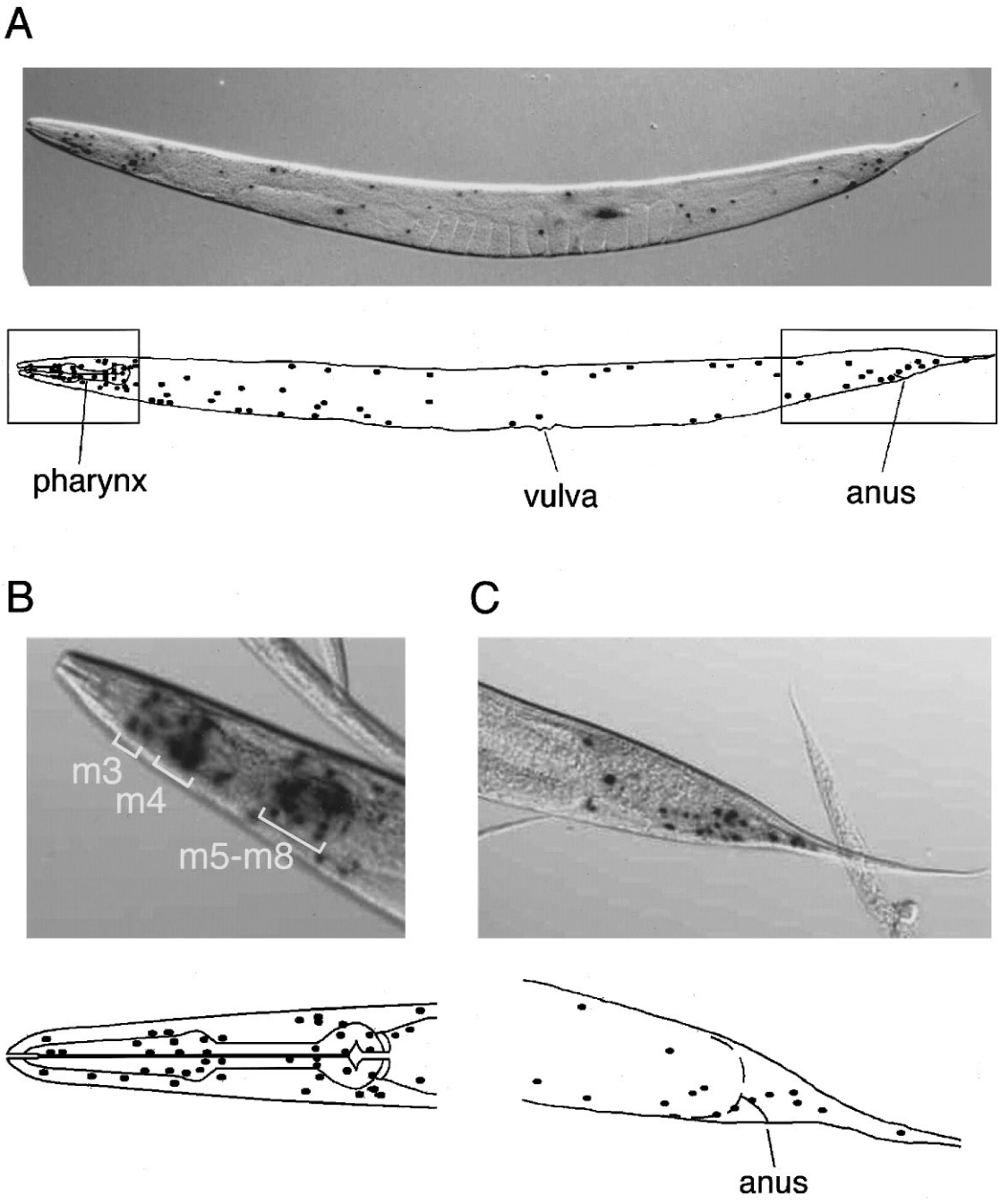

FIG. 2. *LacZ* expression pattern in F1 transformants containing the *SAP 49* gene fused with the *LacZ* gene. (A) *LacZ* expression in an adult worm was viewed with Nomarski optics. A compilation of the positions of *LacZ*-positive cells (dots) determined by observation of F1 transformants (n=41) is schematically shown below. (B) Enlarged view of the pharyngeal region of a transformant. The pharyngeal muscle cells are indicated (m3-m8). (C) Enlarged view of the posterior region of a transformant.

SAP 49 gene was fused with that of the *LacZ* portion (see Fig. 1A). After microinjection of this construct, transgenic F1 transformants were analyzed for their *LacZ* expression. Unexpectedly, *LacZ* expression in the transformants was detected only in specific cells during most developmental stages after hatching (Fig. 2). This expression pattern was observed reproducibly in independent F1 transgenic lines, although there was some fluctuation of the pattern of *LacZ*-positive cells in transformants, which was possibly due to the loss of the transgene. No *LacZ* expression could be observed with transformants that were established using the reporter plasmid alone (data not shown). A similar LacZ expression pattern was also observed with the F2

germline transformants (data not shown). Although we could not fully determine the identity of these *LacZ*-positive cells, some were identified as the pharyngeal muscle cells (m3-m8 cells) based on their characteristic arrangement (Fig. 2B). This is consistent with the fact that the possible promoter region of the *SAP 49* gene cluster contains an NK-class core consensus sequence (TTTAGTG), which is responsible for the expression of the *myo-2* gene in the pharyngeal muscle cells (14). These results suggest that expression of the *SAP 49* gene is very limited and/or active in particular cells, including some types of muscle lineage cells.

SAP 49 function is required for early embryogenesis. To examine whether SAP 49 is an essential splicing factor in *C. elegans,* we used the RNA interference method, by which a given gene function of *C. elegans* is inhibited effectively and specifically (9–11). As shown in Table 1, when an antisense RNA against the essential gene *mom-2,* which is involved in the *Wnt* signaling pathway (15), was injected, 98% of the embryos laid by the injected worms (n=696) could not hatch, indicating embryonic death. A similar high frequency of embryonic death (97%, n=735) was observed when an antisense RNA against the *SAP 49* coding region was injected. Most of the embryos laid by the anti-*SAP 49* RNA-injected worms (80%, n=48) ceased to develop at the end of the cell proliferation stage and failed to undergo any morphogenesis (Fig. 3A). The rest of the embryos (20%) showed signs of the beginning of morphogenesis (Fig. 3B). These results strongly suggest that the *SAP 49* function is essential for the morphogenesis stage of embryogenesis.

In conclusion, our results demonstrate that the *C. elegans SAP 49* gene is located in a polycistronic gene cluster with three other genes and strongly suggest that it is expressed only in a limited population of cells during the larval and adult stages. The limited expression leads us to speculate that the *SAP 49* function is not needed for all cell types in *C. elegans.* Nevertheless, our RNA interference experiment has shown that the *SAP 49* function is essential for *C. elegans* embryogenesis. A simple interpretation to reconcile these seemingly contradictory findings is that SAP 49 may be required for all cells in the developing embryos, especially at the beginning of morphogenesis, whereas its requirement may be limited to specific cells during subsequent developmental stages after hatching. Alternatively, SAP 49 may be required in specific, not all, cells in embryos as well as in later stages, and its absence may disturb proper cell lineage and thereby cause embryonic death. Since the embryos in which *SAP 49* expression was inhibited could undergo most cycles of cell cleavage but failed to enter the morphogenesis stage, SAP 49 may be required for the expression of some specific genes that are involved in the initiation of morphogenesis. In any case, since human SAP 49 is likely to be an essential splicing factor, at least *in vitro,* the limited expression of the *C. elegans SAP 49* gene in larval and adult stages raises the possibility that some other protein(s) sharing redundant function with SAP 49 may exist in *C. elegans.*

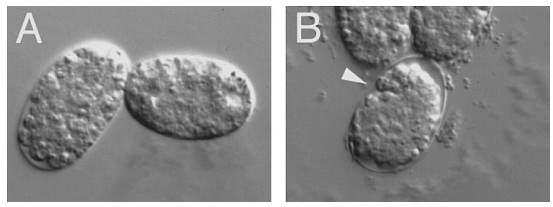

FIG. 3. Inhibition of *SAP 49* expression causes embryonic death of *C. elegans.* Twenty hours after microinjection of the *SAP 49* antisense RNA, the worms were transferred to fresh plates. Embryos laid by the injected worms during the subsequent 10 hr were examined after 24 h with Nomarski optics. (A) Embryos that ceased to develop at the end of the cell proliferation stage. (B) An embryo that ceased to develop after forming a faint furrow structure (arrowhead), a sign of the beginning of the comma stage.

TABLE 1

Scoring for Embryonic Lethality in RNA Injections

Molecules injected	Number of embryos laid[a]	Number of dead embryos[b]	Percent embryonic lethality
Control (injection buffer)	303	15	5
SAP 49 antisense	735	713	97
mom-2 antisense	696	688	98

[a] Embryos laid during 10 hr (20–30 hr after RNA injection) were counted.

[b] Unhatched embryos were counted 54 hr after RNA injection.

ACKNOWLEDGMENTS

We thank A. Fire for the *LacZ* reporter plasmid and H. Kawai for technical advice on microscopy. We also thank E. Nakajima for critical reading of the manuscript. This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture of Japan and the Yamanouchi Foundation for Research on Metabolic Disorders to H. S.

REFERENCES

1. Moore, M. J., Query, C. C., and Sharp, P. A. (1993) *in* The RNA World (Gesteland, R. F., and Atkins, J. F., Eds.), pp. 303–357, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Baserga, S. J., and Steitz, J. A. (1993) *in* The RNA World (Gesteland, R. F., and Atkins, J. F., Eds.), pp. 359–381, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Champion-Arnaud, P., and Reed, R. (1994) *Genes Dev.* **8,** 1974–1983.
4. Gozani, O., Feld, R., and Reed, R. (1996) *Genes Dev.* **10,** 233–243.
5. Tanaka, Y., Ohta, A., Terashima, K., and Sakamoto, H. (1997) *J. Biochem.* **121,** 739–745.

6. Zorio, D. A., Cheng, N. N., Blumenthal, T., and Spieth, J. (1994) *Nature* **372,** 270–272.
7. Blumenthal, T. (1995) *Trends Genet.* **11,** 132–136.
8. Igel, H., Wells, S., Perriman, R., and Ares, M. (1998) *RNA* **4,** 1–10.
9. Fire, A., Albertson, D., Harrison, S. W., and Moerman, D. G. (1991) *Development* **113,** 503–514.
10. Guo, S., and Kemphues, K. J. (1995) *Cell* **81,** 611–620.
11. Fire, A., Xu, S. Q., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998) *Nature* **391,** 806–811.
12. Fire, A., Harrison, S. W., and Dixon, D. (1990) *Gene* **93,** 189–198.
13. Mello, C., and Fire, A. (1995) *Methods Cell Biol.* **48,** 451–482.
14. Okkema, P. G., and Fire, A. (1994) *Development* **120,** 2175–2186.
15. Thorpe, C. J., Schlesinger, A., Carter, J. C., and Bowerman, B. (1997) *Cell* **90,** 695–705.