Research Achievement; Shuichi Hashimoto

(1) Nagoya University Graduate School of Science, Molecular Biology Research Facility (Doctoral Program Student and Research Facility Staff Member)

Research objectives and overviews: Research on the Structure and Function of Transfer RNA.

Determined the complete structure of tyrosine tRNA from Torula yeast. Based on this structure, obtained enzymatically cleaved fragments of tyrosine tRNA and demonstrated that reconstituting these fragments restored activity. Applied this finding to advance research identifying the active site of tRNA. Throughout this series of studies, concurrently established RNA analysis methods, leading to subsequent research frequently utilizing RNA as a research material.

Publication and Paper

1. Takemura, S., Miyazaki, M., Kawata, M., Mizutani, T., Hashimoto, S., and Murakami, M. (1967) Nucleotide sequences in the valine, alanine, phenylalanine, and other transfer RNAs from Torulopsis utilis. 7th International Cong. Biochem. Symp., Tokyo, Symp. Abs. p49-52.

2. Hashimoto, S., Miyazaki, M., and Takemura, S., (1969) Nucleotide sequence of tyrosine transfer RNA from Torulopsis utilis. J. Biochem. 65, 659-661.

3. Hashimoto, S., Kawata, M., and Takemura, S. (1969) Recovery of tyrosine acceptor activity by combining 3'-half molecule with stepwise degradation products of 5'-half molecules from tyrosine transfer RNA. Biochem, Biophys. Res. Commun. 37, 777-784.

4. Takemura, S., Kawata, M., Hashimoto, S., and Murakami, M., (1970) Reconstitution of amino acid acceptor molecules from fragments of torula yeast transfer RNA. 7th International Symp. on the Chem. of Natural Products. Riga, USSR, B4 p193-194.

5. Takemura, S., Hashimoto, S., and Miyazaki, M. (1972) Complete digestion of tyrosine transfer RNA from Torulopsis utilis with pancreatic- and T1-ribonucleases. J. Biochem. 72, 111-121.

6. Hashimoto, S., Takemura, S., and Miyazaki, M. (1972) Partial digestion with ribonuclease T1 and derivation of the complete sequence of tyrosine transfer ribonucleic acid from Torulopsis utilis. J. Biochem., 72, 123-134.

7. Hashimoto, S., Takemura, S., Yabuki, S., Konishi, K., and Samejima, T. (1972) Physicochemical studies on conformation of a complex reconstituted from half molecules Torulopsis utilis tyrosine transfer ribonucleic acid. J. Biochem. 72, 1185-1195.

8. Hashimoto, S., Kawata, M. and Takemura, S, (1972) Reconstitution of an active acceptor complex which lacks the anticodon of Torulopsis tyrosine transfer ribonucleic acid. J. Biochem. 72, 1339-1349.

Reviews, Books (in Japanese)

1. Shosuke Takemura, Shuichi Hashimoto (1968) On the Determination of the Chemical Structure of Transfer RNA, Chemistry (Kagaku-Dojin), 23, 15-23.

2. Shuichi Hashimoto (1969) Recent Studies on the Structure and Function of Transfer RNA, Biological Sciences (Iwanami Shoten), 21, 49-57.

3. Shosuke Takemura et al. (contributing authors), (1970), Base Sequences of RNA, Handbook of Biophysical Experiments (Yoshioka Shoten), 218-222.

4. Shosuke Takemura et al. (contributing authors), (1971), Nucleic Acids and Related Substances, Handbook of Analytical Chemistry (Maruzen), 1261-1281.

(2) Cancer Research Institute, Biochemistry Department (1 year) and Tokushima University School of Medicine, Department of Biochemistry. Instructor, Research staff.

Research objectives and overview: Research on Ribosomal RNA Biosynthesis

I moved from Nagoya University to the Biochemistry Department at the Cancer Research Institute in Tokyo as a research fellow. I began working in Dr Masami Muramatsu's group. This was around the time Dr. Muramatsu had returned from the United States, where he had achieved significant accomplishments in studying ribosomal RNA biosynthesis in animal cells. At that time, it was becoming understood that animal cells possess several hundred ribosomal genes. As a cancer researcher, I considered the possibility that different ribosomal genes might be used in normal cells versus cancer cells. It was also an era when it was becoming understood that ribosomal RNA in animal cells undergoes methylation modification, and interest in its significance and function was high. We pursued research in these areas. After about a year of research at the Cancer Institute, Professor Muramatsu was appointed Professor of the Department of Biochemistry at Tokushima University School of

Medicine. Consequently, the entire laboratory relocated to Tokushima. I also moved there as an Instructor and Research Staff in the same Department of Biochemistry.

Publication and paper

1. Hashimoto, S. and Muramatsu, M. (1973) Differences in nucleotide sequences of ribosomal RNA between the liver and a hepatoma C3H/He mice. Eur. J. Biochem. 33, 446-458.

2. Takai. K., Hashimoto, S., and Muramatsu, M. (1975) Oligonucleotide sequences of pancreatic and T1 ribinuclease digests of 5S RNA from mouse. Biochemistry 14, 536-

542.

3. Hashimoto, S., Sakai, M., and Muramatsu, M. (1975) 2'-O-methylated oligonucleotides in ribosomal 18S and 28S RNA of a mouse hepatoma, MH134. Biochemistry 14. 1956-1964.

Review (in Japanese)

1. Shuichi Hashimoto, Masami Muramatsu (1973) Primary Structural changes

In ribosomal RNA in Cancer Cells. Kagaku (Kagaku-Dojin), Kagaku Special Issue 60. pp. 63-74.

(3) St. Louis University School of Medicine. Institute for Molecular Virology

Research objectives and overview: Research on biosynthesis of adenovirus early mRNA

Under the leadership of Dr. Maurice Green as Director, the Molecular Virology Institute was a vibrant hub where researchers from over ten countries pursued research. Adenovirus research was globally recognized as a potential model for viral replication and viral carcinogenesis studies, making it a highly competitive and dynamic field of investigation. Within this environment, I was assigned to study adenovirus gene expression, specifically focusing on the transcription mechanism. Concurrently, rumors circulated that during transcription in nucleated cells, a cap structure was synthesized at the 5'-end of mRNA during transcription initiation. Consequently, I also pursued research from this angle. Beyond adenovirus-related work, I conducted research as part of postdoctoral and graduate student training and supervision.

Publication

1. Bondurant, M., Hashimoto, S., and Green, M. (1976) Methylation pattern of genomic RNA from Moloney murine leukemia virus. J. Virol. 19, 998-1005.

2. Hashimoto, S., and Green M. (1976) Multiple methylated cap sequences in adenovirus 2 early mRNA. J. Virol. 20, 425-435.

3. Birenbaum, M., Schlessinger, D., and Hashimoto, S. (1978) RNase III cleavage of Escherichia coli rRNA precursors: Fragment release and dependence on salt concentration. Biochemistry 17, 298-307.

4. Hashimoto, S. and Green, M, (1979) Methylated 5'-terminal caps of adenovirus 2 early mRNA – evidence for at least six 5'-termini. Virology 94, 254-272.

5. Hashimoto, S., Pursley, M.H., Wold, W.S.M., and Green, M. (1980) Characterization of distinct 5'-terminal cap structures adenovirus type 2 early mRNA and KB cell mRNA. Biochemistry 19, 294-300.

6. Hashimoto, S, and Green, M. (1980) Adenovirus 2 early mRNA – genome mapping of 5'-terminal RNase T1 oligonucleotides and heterogeneity of 5'-termini. J. Biol. Chem., 255, 6780-6788.

7. Brackmann, K.H., Green, M., Wold, W.S.M., Cartas, M., Matsuo, T., and Hashimoto, S. (1980) Identification and peptide mapping of human adenovirus type 2-induced early polypeptides by two-dimentional gel electrophoresis and immunoprecipitation. J. Biol. Chem. 255, 6772-6779.

8. Green, M., Wold, W.S.M., Brackmann, K.H., Matsuo. T., Hashimoto, S., and Symington, J., (1980) The integration and expression of human DNA tumor virus genes. Studler Symp. 12. 171-200.

9. Hashimoto, S., Pursley, M.H., and Green, M. (1981) Nucleotide sequences and mapping of noval heterogeneous 5'-termini of adenovirus 2 early region 4 mRNA. Nucl. Acids Res. 9, 1675-1689.

10. Hashimoto, S., Wold, W.S.M., Brackmann, K.H., and Green, M. (1981) Nucleotide sequences of 5' termini of adenovirus 2 early transforming region E1a and E1b messenger ribonucleic acids. Biochemistry 20, 6640-6647.

11. Matsuo, T., Hashimoto, S., Wold, W.S.M., Symington, J., Rankin, A., and Green, M. (1982) Idenitification of adenovirus 2 early region 4 polypeptides by in vitro

translation and tryptic peptides map analysis. J. Virol. 41, 334-339.

12. Matsuo, T., Wold, W.S.M., Hashimoto, S., Rankin, A., Symington J., and Green, M. (1982) Polypeptides encoded by transforming region E1b of human adenovirus 2: Immunoprecipitation from transformed and infected cells and cell-free translation of E1-specific mRNA. Virology 118, 456-465.

13. Hashimoto, S., Symington, J., and Matsuo, T. (1984) Cell-free translation of adenovirus 2 E1a and E1b-specific mRNAs and evidence that E1a-related polypeptides are produced from E1a-E1b overlapping mRNA. J. Biol. Chem. 259, 7016-7023.

14. Hashimoto, S. and Green, M. (1984) Unusual heterogeneity of the 5’-termini of human adenovirus type 2 early region E2 mRNA. Nucl. Acids Res. 12, 9067-9082).

(4) Meiji Institute of Health Science (research division of Meiji Milk Co., Ltd.): (today, Meiji Innovation Center, Meiji Co., Ltd.)

Research objectives and overview: Research on Human Adenovirus Gene Function.

Research primarily focused on adenovirus gene expression and gene function, while also actively pursuing collaborative studies with other groups within the institute and external research institutions. Particularly notable achievements include:

◦ Human adenovirus E1a gene expression is observed in mouse undifferentiated cells but disappears in differentiated cells: Collaboration with Dr. Nakatsuji's group (Cell Differentiation group, now at Kyoto University).

◦ Discovery that adenovirus genes contain both genes inducing apoptosis and genes suppressing apoptosis; collaborative work with Dr. Yonehara's group (Institute of Clinical Medicine, now Kyoto University).

◦ Demonstration that adenovirus gene DNA is linear double-stranded, with the presence of a DNA replication origin and an E1 gene enhancer at the left end. We demonstrated the presence of a bend structure in this region.

◦Human adenovirus type 40 is known to cause diarrhea in children, but research has been limited due to the lack of a suitable cell culture for its propagation. We discovered that it proliferates in human lung cancer-derived A549 cells (collaborative research with Sapporo Medical University Cancer Research Institute).

◦ We identified human-derived cultured cells that produce interleukin-6 (IL-6), discovered a method to increase its production, and established a purification method. Compared to IL-6 purified from IL-6 recombinant gene-transformed E. coli, significant differences in molecular weight and activity were observed. Using purified IL-6, we advanced research on its biological activity (joint research with Dr. Konishi, Dokkyo Medical University). However, due to the high patentability of this research, patent application took priority over publication, and the patent was obtained. The paper was published in the Dokkyo Medical University Bulletin.

Publication and Paper

1. Murasugi, A., Takemori, N., and Hashimoto, S. (1987) Discrimination and quantitative analysis of wild type and point mutant early region 1b adenovirus using oligonucleotide hybridization probes. J. Biochem. 102, 627-633.

2. Kitani-Yasuda, T., Yasuda, N., and Hashimoto, S. (1988) Activation of the adenovirus 2 E2a late promoter during inhibition of protein synthesis by cycloheximide. Gene 69, 165-169.

3. Suemori, H., Hashimoto, S., and Nakatsuji, N. (1988) Presence of adenovirus E1a-like activity in preimplantation stage mouse embryos. Mol. Cell. Biol. 8, 3553-3555.

4. Murasugi, A. and Hashimoto, S. (1988) Cloning of adenovirus terminal DNAs using the primer extension method. Nucl. Acids Res. Symposium Series No. 20, 55-56.

5. Kumai, H., Takemori, N., and Hashimoto, S, (1989) Role of adenovirus type 2 early region 1B 19K protein stability in expression of the cyt and deg phenotypes. J. Gen. Virol. 70, 1975-1986.

6. Ohyama, T. and Hashimoto, S. (1989) Upstream half of adenovirus type 2 enhancer adopts a curved DNA conformation. Nucl. Acids Res. 17, 3845-3853.

7. Hashimoto, S., Ishii, A., and Yonehara, S. (1991) The E1b oncogene of adenovirus confers cellular resistance to cytotoxicity of tumor necrosis factor and monoclonal ant-Fas antibody. Int. Immunol. 3. 343-351.

8. Hashimoto, S., Sakakibara, N., Kumai, H., Nakai, M., Sakuma, S., Chiba, S., and Fujinaga, K. (1991) Fastidious human adenovirus 40 can propagate efficiently and produce plaques on a human cell line , A549, derived from lung carcinoma. J. Virol. 65, 2429-2435.

9. Hashimoto, S., Kumai., Nakai, M., Hashimoto, K., and Nakatsuji, N. (1993)

Promotion of KB cell adhesion on fibronectin during adenovirus infection of KB cells. Exp. Cell Res. 205, 270-275.

10. Ishida, S., Fujinaga, F., Fujinaga, K., Sakamoto, N., and Hashimoto, S. (1994) Unusual splice sites in the E1A-E1B cotranscripts synthesized in adenovirus type 40-infected A549 cells. Arch. of Virol. 139, 389-402.

11. Sekiguchi, T., Nakashima, T., Hayashida., T., Kuraoka, A., Hashimoto, S,.

Tsuchida、N., Shibata, Y., Hunter, T., and Nishimoto, T. (1995) Apoptosisi is induced in

BHK cells by the tsBN463/13 mutation in the CCG1/TAFII250 subunit of the TFIID basal transcription factor. Exp. Cell Res. 230, 490-498.

12. Watanabe, M., Shirayoshi, Y., Koshimizu, U. Hashimoto, S., Yonehara, S., Eguchi, Y., Tsujimoto, Y., and Nakatsuji, N. (1997) Gene transfection of mouse primordial germ cells in vitro analysis of their survival and growth control. Exp. Cell Res. 230, 76-83.

13. Yokoyama, M., Konishi, K., Imamura, T., Konishi, K. Hirano, H., Sakamoto, N.,

Wakabayashi, A., Ashida, K., Noguchi, S., and Hashimoto, S. (2001) Production of IL-6 isomers by KB cells, Purification and The Cytotoxic Effect on Human Cells. Dokkyo Jourrnal of Medical Sciences 28(1), 603-616.

Review (in Japanese)

1. Hashimoto, S. (1989). Regulation of adenovirus E2a gene expression. Biochemistry, 61(3), 198-201.

2. Hashimoto, S., & Fujinaga, K. (1992). Adenovirus E1B gene and protein. Nucleic Acids and Enzymes, 37(10),

93-95.

3. Hashimoto, S. (1994). Control of Apoptosis by the Adenovirus Oncogene Product E1B 19kDa Protein. Medical Immunology (Kokusai Iishobo), 28(1), 65-74.

4. Hashimoto, S., & Fujiki, Y. (1994). Viral genes controlling apoptosis. Proteins Nucleic Acids Enzymes, 39(13), 46-56.

5. Hashimoto, S., (1991) Methods for Gene Introduction into Animal Cells 4. Viral Methods I: Adenovirus, Chemistry and Biology. 29(3), 184-187.

(5) Tokyo University of Science, Faculty of Pharmaceutical Science.

Research objectives and overview: Collaborative research and development activities with young researchers, Research into viral replication mechanisms for drug discovery.

Publication

1. Hashimoto, S., Uchiumi, F., Furuya, H., and Padmanabhan, R. (2026) The inevitable relationship between viruses and RNA modifications revealed through adenovirus research. Viruses (MDPI). This article is an open access article.