

Priority Area:

3. Modern Biology and Biotechnology

Coordinators:

(Japanese)

Akira Ishihama

Research Leader

Nippon Institute for

Biological Science

(Indian)

D.Balasubramanian

Director of Research

L.V. Prasad Eye Institute

India-Japan Cooperative Science Programme
Activity Report in the Area: Modern Biology and Biotechnology

Overview and Future Plan

(1) FY2001-2002 Overview

The priority area “Modern Biology and Biotechnology” of the Japan-India Cooperative Science Programme was initiated in FY1993. Three previous coordinators (Drs. Tokindo OKADA, Ikuo TAKEUCHI and Norio MURATA) from Japan devoted their efforts to develop fruitful collaborations between two countries, in three subareas, molecular bacterial genetics, animal developmental biology, and plant stress biology. In FY2002, Dr. Akira ISHIHAMA took over the coordinator task and have organized new collaborative teams in subareas, ocean microbiology, insect genome biology, and eukaryotic molecular genetics.

JOINT RESEARCH

In FY2001, three on-going projects by Murata-Shivaji, Ishihama-Chatterji and Asashima-Modak teams were continued under the support of Japan-India Cooperative Science Programme. In FY2002, a total of 9 joint research projects have been accepted as listed below, of which one was cancelled and two (projects 7 and 8) were extension of the projects 1 and 2. Overall the collaborative research was carried out by six groups. Three groups (Murata-Shivaji group, Asashima-Modak group, and Ishihama-Chatterji group) carried out the continuation of on-going projects, while three newly organized groups (Kogure—Karunasagar group, Shimada-Nagaraju group, and Horikoshi-Kundu group) initiated new projects.

FY2001	FY2002	FY2003	FY2004	FY2005	FY2006
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(1) Group-1 projects

Murata-Shivaji

----- project 1

----- project 7

Asashima-Modak

----- project 2

Ishihama-Chatterji

----- project 3

----- project 8

(2) Group-2 projects

Kogure-Karunasagar

----- project 4

Shimada-Nagaraju

----- project 5

Horikoshi-Kundu

----- project 6

1) Group-1 projects (Extension of on-going projects)

Project 1 (molecular bacterial genetics)
Polyunsaturated fatty acids and desaturases in antarctic cyanobacteria
2001-2002 (two years)

Project 7 (Extension of Project 1)
Molecular basis of cold adaptation
2002-2005 (three years)
Norio MURATA (Nat. Inst. Basic Biol., Okazaki)
Sisinthu SHIVAJI (CCMB, Hyderabad)

Project 2 (animal developmental biology)
Molecular approaches of organogenesis in vitro in animal development
2001-2004 (three years)
Makoto ASASHIMA (Sch. Arts Sci., Univ. Tokyo, Tokyo)
Sopha P. MODAK (Karmatak Univ., Karmatak)

Project 3 (plant stress biology)
Global regulation of gene transcription under various environments
2001-2003 (two years)

Project 8 (Extension of Project 3)
Global regulation of gene transcription in bacteria under various environments
2002-2007 (five years)
Akira ISHIHAMA (Nat Inst. Genet., Mishima)
Dipankar CHATTERJI (Ind. Insti. Sci., Bangalore)

2) Group-2 projects (Newly initiated projects)

Project 4 (New project) (ocean microbiology)
Microbial study on mortality of natural zooplankton
2002-2005 (three years)
Kazuhiro KOGURE (Ocean Res. Inst., Univ. Tokyo)
I. Karunasagar (Univ. Agr. Sci.,)

Project 5 (New project) (silkworm genome biology)
Identification, characterization and physical mapping of Z-chromosome linked genes of the silkworm,
Bombyx mori.
2002-2005
Toru SHIMADA (Grad. Sch. Agr. Life Sci., Univ. Tokyo)
Javaregowda NAGARAJU (CDFD, Hyderabad)

Project 6 (New project) (eukaryotic molecular genetics)
Chromatin-mediated transcription

2002-2005 (three years)

Masami HORIKOSHI (Inst. Mol. Cell. Biosci., Univ. Tokyo)

Tapas Kumar KUNDU (JNC Adv. Sci. Res., Bangalore)

3) Cancelled project

Molecular mechanisms of pattern formation, development and differentiation

2001-2002 (one year)

Kunio YASUDA (Nara Inst. Sci. Technol., Nara)

D. Balasubramanian (L.V. Prasad Eye Inst., Hyderabad)

SEMINAR

The fundamental mechanism of the stress response in biological systems has been the common target of two joint research groups since the establishment of the Modern Biology and Biotechnology Area of Japan-India Co-operative Science Program in FY1993. One group headed by Drs. Norio Murata (Nat. Inst. Biol., Japan) and Shishinty Shivaji (CCMB, India) has focussed on the stress response in plants and cyanobacteria with photosynthesis activity while another group organized by Drs. Akira Ishihama (Nat. Inst. Genet., Japan) and Dipankar Chatterji (Ind. Inst. Sci., India) has analyzed the molecular mechanisms of stress response in bacteria. In order to exchange the accumulated data between these two groups and to exchange the knowledge of the fundamental mechanisms of the stress response between plants and bacteria, two groups gathered together to have a discussion meeting in FY2002. Nine active participants from each country gave a total of 20 reports.

2. FY2003-FY2004 Future Plan

The fruitful collaboration in subarea, molecular bacterial genetics, animal developmental biology and plant stress biology, will be continued in the projects 2, 7 and 8. On the other hand, the newly initiated collaborative teams, organized in the projects 4, 5 and 6, constructed the research basis in FY2002 for collaborations, and will be extended in FY2003.

(2) Summary

In FY2003, the collaborative research in the Molecular Biology and Biotechnology area was expanded two fold by including three new collaboration teams in three new subareas, ocean microbiology, insect genome biology and eukaryotic molecular genetics.

Modes of Cooperation:

1. Joint Research Projects
2. Seminars
3. Visiting Scientists for Information Exchange

Report

1. Joint Research Projects

FY2001- 2003

Project No.1

Title: Polyunsaturated Fatty Acids and Desaturases in Antarctic Cyanobacteria

Objectives:

Poikilothermic organisms including cyanobacteria respond to low temperature by desaturating (conversion from a single C-C bond to a double C=C bond) fatty acids in membrane lipids, and desaturases catalyze these reactions. This phenomenon is regarded to compensate a decrease in membrane fluidity at low temperature by fluidization of membranes. The objective of this research is to study such a phenomenon in cryophilic cyanobacteria in Antarctic which have been adapted to extremely low temperature.

Project Coordinators:

(Japanese)

Norio Murata

Professor

National Institute for Basic Biology

Okazaki National Research Institutes

(Indian)

Sisinthi Shivaji

Scientist F

Centre for Cellular and Molecular Biology

Date of Commencement: April 1999

Date of Completion: 31 March 2002

Accomplishment Status:

The major findings of the joint study using psychrophilic *Nostoc* were:

1. Increase in the concentration of unsaturated fatty acids was observed in the four lipid fractions of *Nostoc* when grown at 9 °C compared to 25 °C.
2. The desaturase genes (*desA*, *desB* and *desC*) exhibited > 95% homology with *des* genes of mesophilic *Nostoc punctiforme*.
3. The *des* genes are constitutive in their expression.
4. The desaturase enzymes did not exhibit any characteristic features of cold active enzymes.
5. Phylogenetically psychrophilic *Nostoc* was similar to mesophilic *Nostoc*.

Exchange Visits Undertaken:

FY2001

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Sisinthi Shivaji	Polyunsaturated Fatty Acids and Acyl-Liquid	Norio Murata	20 Nov.2001-
Scientist F	Desaturases of Psychrotropic Cyanobacteria	Professor	19 Jan.2002
Centre for Cellular	from Antarctica	National Institute for Basic	(61 days)
and Molecular		Biology	
Biology			

Project No.2

Title: Molecular Approaches of Organogenesis in Vitro in Animal Development

Objectives:

1. To study the role of cell cycle in defining the induced organogenesis in vitro.
2. To examine by quantitative Real-time Polymerase Chain Reaction the chronological relationship for the expression of genes specific for Activin-A induced organogenesis in *Xenopus laevis* animal caps in vitro.

Project Coordinators:

(Japanese)

Makoto Asashima

Professor

School of Arts and Science

University of Tokyo

(Indian)

Sophan P. Modak

Professor

Karnatak University

Date of Commencement: 1 April 2001

Date of Completion: 31 March 2004

Accomplishment Status:

1. Eight experiments were set up to promote hormonally induced ovulation and fertilization in *Xenopus laevis*. *Xenopus laevis* females and males were injected intradermally 0.4ml and 0.2ml, respectively, of Progesterone and animal left in pairs in boxes at 20 °C. Next day, 7 gave fertilized eggs with embryos undergoing early embryonic development
2. Four experiments were conducted to learn the Animal Cap extirpation technique on stages 8.5 and 9 of *Xenopus laevis*. In these, a total of 25, 50, 60, and 65 caps were isolated and treated with low (1ng/ml), medium (10ng/ml) and high (100ng/ml) Activin A and survivals were examined. With these experiments, Prof. Modak has learnt the Animal Cap model and mastered the surgical technique.
3. In Two more experiments Animal caps were treated with 10ng/ml dose of Activin A

Expt. no.	Total no of caps	Activin A Treatment	Chase schedule
I	60	10ng/ml for 1h	1h, 3h, 6h, 9h 21h, Control 0h & 21h
II	70	10ng/ml for 1h	0.5, 1,2,3,4,6h
		Control	0h, 6h

Total RNA is extracted by Isogen protocol and quantified on UV spectrophotometer. RNA was reverse transcribed to obtain single stranded cDNA using oligo-dT primer, treated with RNase I (DNase-free) and readied for Polymerase chain reaction.

We will use as probes cyclin B1, Cyclin E2, cdc2 kinase)cell cycle specific, X-bra (mesoderma), N-CAM, goosecoid (neural cell lineage specific and Ornithine decarboxylase gene (internal control). The real-time PCR allows quantifying the number of gene-specific transcripts represented in the cells which would reflect the relationship between cell cycle and expression of mesodermal or neural cell lineages induced by activin A.

Future Plan:

1. To complete the quantitative PCR analyses and Microarrays on cell cycle specific transcripts in relation to mesoderm specific (X-bra, myoD, chordin etc.), ectoderm-specific (epi 1) and neural lineage specific (goosecoid, N-CAM sequences in Xenopus animal caps.
2. To examine the status of cell cycle specific, mesoderm-specific and neural cell lineage-specific transcripts in chick embryo grafted with neural inducing Hensen's node or caudalizing postnodal fragments.
3. To examine the chronology of sequences expression neural induction in chick embryos having limited the duration of contact between the inductor and competent ectoblast for 0.5, 1,2,3,6,9, and 12hrs using real-time PCR and Microarray techniques.
4. To organize a training workshop on Techniques in Molecular Embryology of amphibia in conjunction of visits by two collaborators from Japan
5. As the area of molecular embryology is moving very rapidly a visit of somewhat longer duration by Prof. Modak to Tokyo in order to continue the project to a fruitful result in form of joint publications. Future Plan:

Exchange Visits Undertaken:FY2001

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Surendra Ghaskadbi Scientist E1 Agharkar Research Institute	In Vitro Control of Cell Differentiation Using Xenopus Laevis Embryos	Makoto Asashima Professor University of Tokyo	25 Feb.2002- 26 Mar.2002 (30 days)

FY2002

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Surendra Ghaskadbi Scientist E1 Agharkar Research Institute	Modulation of Gene Expression in Xenopus Embryos Developing under Conditions of Altered Salinity	Makoto Asashima Professor University of Tokyo	22 Jan.2003- 19 Feb.2003 (29 days)
Sohan P. Modak Professor Karnatak University	Molecular Signals Responsible for Transfer of Inductive Stimulus during the Establishment of Neural Cell Lineage and the Body Axis in Chick Embryos and Amphibian Animal Caps	Makoto Asashima Professor University of Tokyo	12 Feb.2003- 3 Mar.2003 (20 days)

Project No.3

Title: Global Regulation of Gene Transcription under Various Environments

Objectives:

Global regulation of gene transcription is the research subject of this collaborative research team between Japan and India, headed by Prof. Akira ISHIHAMA, National Institute of Genetics (NIG), Mishima, Japan (current address: Nippon Institute for Biological Science (NIBS), Ome, Tokyo, Japan) and Prof. Dipankar CHATTERJI, Indian Institute of Science (IIS), Bangalore, India, including Dr. J. Gowrishankar, Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India (current address: Centre for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad, India). The research aim of this group is to reveal the molecular mechanisms underlying the global regulation of gene transcription in bacteria in response to changes in environment such as nutrient starvation, osmolarity change, and heat- or cold-shock.

Project Coordinators:

(Japan)

Akira Ishihama

Professor, Head and Vice Director

Department of Molecular Genetics

National Institute of Genetics

and

Head, Division of Molecular Biology

Nippon Institute for Biological Science

(India)

Dipankar Chatterji

Professor and Head

Biophysics Unit

Indian Institute of Science

Date of Commencement: 1 April 2001

Date of Completion: 31 March 2003

Accomplishment Status:

Bacteria can survive for long periods under various stressful environments in nature. Gram-positive bacteria can survive by forming spores. Non-spore forming Gram-negative bacteria such as *Escherichia coli* are still able to survive by forming non-growing resting states in the stationary phase. The adaptation of *E. coli*, the model organism in molecular biology, to environmental changes has just begun to be investigated at the molecular level. D. Chatterji performed biochemical analyses of the functional and structural modulation of transcription apparatus in response to nutrient starvation, and found that an alarmone, ppGpp, for stringent starvation binds directly to the β -subunit of RNA polymerase to modulate its promoter recognition specificity. J. Gowrishankar analyzed the response of *E. coli* to high osmolarity and found that the transcription apparatus is modified by replacement of sigma subunit from σ^{70} to σ^S , thereby to recognize the promoters for the high-osmolarity

response genes. The group in Japan performed a comprehensive analysis of the structural and functional alteration of the transcription apparatus in response to various stresses.

Future Plan:

The total number of RNA polymerase core enzyme in *E. coli* is about 2000 molecules and less than that of genes predicted on its genome. The core enzyme is converted into various forms of the transcription apparatus after interaction with the sigma factors and the transcription factors. This collaborative team has started a systematic study for identification of the transcription factor, which is induced by each stress, and the genes under the control of each factor.

Exchange Visits Undertaken:

FY2001

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Dipankar Chatterji Professor and Head Indian Institute of Science, Bangalore	Subunits of <i>E. coli</i> RNA Polymerase and Their Function	Akira Ishihama Professor, Head and Vice Director National Institute of Genetics	20 Feb.2002- 6 Mar.2002 (15 days)
Jayaraman Gowrishankar Staff Scientist Centre for DNA Fingerprinting and Diagnostics	In vitro Studies on Functional Activity of Sigma S Proteins with N- and C- terminal Deletions	Akira Ishihama Professor, Head and Vice Director National Institute of Genetics	10 Jan.2002- 14 Feb.2002 (36 days)

Project No.4

Title: Microbial Study on Mortality of Natural Zooplankton

Objectives:

Most marine organisms are healthy. This is because weak or unhealthy individuals are easily trapped and eaten by predators. The exceptions are the last stage of red tide or cultured marine organisms. The fishes or shrimps in the culture ponds are quite often suffered from appearance of mass death due to pathogenic microorganisms and viruses.

Recent observation on zooplankton in the deep sea, however, indicates that some natural zooplankton seem to be not healthy. This suggests that the general idea stated above may not be true and/or influence of human activities may be expanding to natural environments. The objective of the present projects is to clarify the cause of mortality of natural zooplankton with special emphasis on the contribution of bacteria and viruses.

Project Coordinators:

(Japanese)

Kazuhiro Kogure

Professor

Ocean Research Institute

The University of Tokyo

(Indian)

I. Karunasagar

University of Agricultural Sciences

Date of Commencement: 1 July 2002

Date of Completion: 30 June 2005

Accomplishment Status:

Unfortunately, Dr. A. S. Pradeep RAM was not able to come to Japan in 2002, because he has to be in the US from Dec. 2002 to Feb. 2003. Therefore, we could not exchange people.

The works are summarized as follows:

As a typical pathogenic bacterial group, we have concentrated on luminescent bacteria. During two cruises by R/V Tansei-maru, Ocean Research Institute, The University of Tokyo (ORI-UT) and several field surveys, about 100 luminescent bacteria were isolated from the seawater, zooplankton and fishes. Some zooplankton samples have been treated individually and preserved in the deep freezer for further work on attached microbes and viruses at genetic level. Dr. Nayak, ORI-UT also isolated about 10 luminescent bacteria from West coast of India in Feb. 03.

We are analyzing their physiological characteristics and 16S rDNA sequences for identification. As for *Photobacterium leiognathi*, we have also sequenced lux a gene to characterize their taxonomical positions and functional differences. In order to specifically identify pathogenic strains, we are currently trying to sequence the 23S-16S intergenic spacer region (ISR).

Future Plan:

From the works accomplished in 2002, we realized that there may be various types of strains to each luminescent bacterial species. The presence of such variants seems to be dependent on geographical regions and the host, i.e., zooplankton or fish. It is necessary to clarify such factors before the work of particular pathogenic strains.

Our future plan is as follows:

1. Isolate luminescent bacteria from coastal environments near Japan and also India. In addition to the water samples, the zooplankton or fishes samples will be collected and treated individually to see the specific association of particular luminescent strains.

2. Characterization of luminescent bacteria by sequencings of 16S rDNA, lux a and ISR. After that, some genes possibly involved in the pathogenicity and association with zooplankton will be further analyzed.

3. The presence WSSV (White spot syndrome virus) in zooplankton samples will be also examined at genetic level to clarify their possible role in mortality of natural zooplankton populations.

Project No.5

Title: Identification, characterization and physical mapping of Z-chromosome linked genes of the silkworm, *Bombyx mori*.

Objectives:

The chromosome constitution of the silkworm, *Bombyx mori*, is ZW in females and ZZ in males. Although the Z chromosome has important function in development and cocoon production, only very little information about the molecular structure of the Z chromosome is available. We planned to determine a several portion of the Z chromosome and apply the information for the genotyping and breeding of Indian races.

Project Coordinator:

(Japanese)

Toru Shimada

Associate Professor

Laboratory of Insect Genetics and Bioscience

Department of Agricultural and Environmental Biology

Graduate School of Agricultural and Life Sciences

The University of Tokyo

(Indian)

Javaregowda Nagaraju

Staff Scientist & Chief

Laboratory of Molecular Genetics

Centre for DNA Fingerprinting and

Diagnostics

Date of Commencement: 1 June 2002

Date of Completion: 31 March 2005

Accomplish Status:

We determined 830 kbp nucleotide sequence of the Z chromosome, and found 24 novel protein-coding genes. We obtained a surprising result that the Z-linked genes generally did not show dosage compensation. The results were partially published in the following papers:

1. Hiroaki Abe, Toshiyuki Sugasaki, Tomoko Terada, Mariko Kanehara, Fumi Ohbayashi, Toru Shimada, Shinya Kawai, Kazuei Mita, and Toshikazu Oshiki (Aug. 2002) Nested retrotransposons on the W chromosome of the wild silkworm *Bombyx mandarina*. *Insect Molecular Biology* 11(4): 307-314.
2. Yoshiko Koike, Kazuei Mita, Masataka G. Suzuki, Susumu Maeda, Hiroaki Abe, Kazutoyo Osoegawa, Pieter J. deJong, Toru Shimada (2003) Genomic sequence of 320 kb containing a kettin orthologue on the Z chromosome in *Bombyx mori*. *Molecular Genetics and Genomics*, in press.
3. Fumi Ohbayashi, Masataka G. Suzuki and Toru Shimada (Sep. 2002) Sex determination in *Bombyx mori*. *Current Science (Bangalore)* 83 (4): 466-471.

4. Kazuei Mita, Mitsuoki Morimyo, Kazuhiro Okano, Yoshiko Koike, Junko Nohata, Masataka G. Suzuki, and Toru Shimada (Sep. 2002) Construction of an EST database for *Bombyx mori* and its applications. Current Science (Bangalore) 83(4): 426-431.

Future Plan:

Dr. Nagaraju and Shimada are planning to construct a more precise molecular linkage map to understand the overall structure of the Z chromosome. Since more than 10 sex-linked DNA markers have been already found in Dr. Nagaraju's lab, we will be able to extend the physical map of the Z chromosome drastically by our intimate collaboration. Thus we need at least one-year for our official collaboration which will be supported by JSPS-DST.

Exchange Visit Undertaken:

FY2002

Japan to India

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Toru Shimada Associate Professor University of Tokyo	Identification, characterization and physical mapping of Z-chromosome linked genes of the silkworm, <i>Bombyx mori</i> .	J.G. Nagaraju Staff Scientist and Chief Centre for DNA Fingerprinting and Diagnostics	9 Sep.2003- 13 Sep.2003 (7 days)

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
J.G. Nagaraju Staff Scientist and Chief Centre for DNA Fingerprinting and Diagnostics	Identification, characterization and physical mapping of Z-chromosome linked genes of the silkworm, <i>Bombyx mori</i> .	Toru Shimada Associate Professor University of Tokyo	1 Mar. 2003 - 8 Mar. 2003 (8 days)

Project No.6

Title: Chromatin-Mediated Transcriptional Regulation

Objectives:

Eukaryotic genome is organized in a highly complex nucleoprotein structure, the unit of which is nucleosome. Nucleosomes are composed of eight molecules of 4 different histones and 146 bp of DNA wrapped around it. The beads of nucleosome are organized in a 10nm filament, which further compacts into 30nm filament and finally the chromatin loop structure. Significantly this structural organization of

chromatin is highly dynamic. Apart from core histones, histone H1 and several non-histone proteins, histone chaperones and chromatin remodeling system are involved in the structural and functional organization of chromatin. In the present project we will address the role of three different histone chaperones in chromatin organization and function.

Project Coordinators:

(Japanese)

Masami Horikoshi

Associate Professor

Institute of Molecular and Cellular Biosciences

The University of Tokyo

(Indian)

Tapas Kumar Kundu

Faculty Fellow

Jawaharlal Nehru Centre for Advanced

Scientific Research

Date of Commencement:

1 October, 2002

Date of Completion:

30 September, 2005

Accomplishment Status:

1. Technology of yeast two-hybrid binding assay has been accomplished by Kundu with help from Horikoshi's group.
2. Yeast two-hybrid screening has been under way to identify the interacting proteins for these chaperones.
3. Several new ideas have been brought about as a result of discussion between both coordinators.

Future Plan:

1. Interactions of histone chaperones NAP1, CIA and Nucleophosmin(B23) with mono- and oligo-nucleosomes will be studied. Their role in the structural change of nucleosome and transcription factor binding to nucleosome will be investigated.
2. Yeast two hybrid system will be used to identify the interacting proteins for these chaperones.
3. Yeast homolog of nucleophosmin will be used for some comparative studies, and to find out the role of it in transcription regulation (*in vivo*).
4. The promoter specificity of this chaperones will be studied by Chromatin immunoprecipitation (Chip) assay.
5. Mechanism of transcriptional activation by these proteins will be elucidated using *in vitro* reconstituted chromatin transcription system.

Exchange Visit Undertaken:

FY2002

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Tapas Kumar Kundu	Role of Histone Chaperones in Structural	Masami Horikoshi	16 Jan. 2003 -
Assistant Professor	Organization of Chromatin and its Functional	Associate Professor	5 Feb. 2003
Jawaharlal Nehru	Significances	Institute of Molecular and	(21 days)
Center Advanced		Cellular Biosciences	
Scientific Research,		The University of Tokyo	
Bangalore			

Project No.7

Title: Molecular Basis of Cold Acclimation

Objectives:

Poikilothermic organisms including bacteria and Cyanobacteria respond to changes in temperature by regulating the level and the quality of unsaturation of fatty acids in membrane lipids. The objective of this research is to investigate the molecular mechanisms for the regulation of desaturation and *cis-trans* isomerization of fatty acids in Antarctic bacteria and cyanobacteria, as model systems.

Project Coordinators:

(Japanese)

Norio Murata

Professor

National Institute for Basic Biology

Okazaki National Research Institutes

(Indian)

Sisinthu Shivaji

Scientist F

Centrefor Cellular and Molecular Biology

Date of Commencement: 1 April 2002

Date of Completion: 31 March 2005

Accomplishment Status:

1. We isolated *cti* gene for *cis-trans* isomerase from the Antarctic bacterium *Vibrio syringe*, and mutated this gene in this bacterium. Biochemical and physiological examination of the *cti* mutant cells indicated that they did not produce *trans*-unsaturated fatty acids under any growth conditions and were sensitive to high temperature.
2. We isolated from the Antarctic cyanobacterium *Nostoc* specis the *desC1* and *desC2* genes for 9-desaturases at the *sn-1* and *sn-2* positions, respectively, of the glycerol moiety of polar glycerolipids. We characterized differences in the amino acid sequence between the DesC1 and DesC2 desaturases.

Future Plan:

1. To study the molecular mechanism for the temperature-dependant *cis-trans* isomerization of fatty acids, we will examine the temperature-dependant expressing of the *cti* gene in *Vibrio syringe* by Northern blotting and RT-PCR analysis.
2. To further characterize the *desC2* gene and DesC2 desaturase of *Nostoc* sp., We will transfom, with the *disC2* gene, another cyanobacterial strain, *Synechocystis* sp. PCC 6803, Which does not contain this gene. The fatty acid analysis of this transformant will answer whether DesC2 catalyzes the desaturation of fatty acids at the *sn-2* position specifically.

Exchange Visit Undertaken:

FY2002

Japan to India

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Hidetoshi Okuyama	Experiment on Expression of Active Cti	Sisinthi Shivaji	19 Mar. 2003-
Associate Professor	Protein in E. Coli Cells	Deputy Director	25 Mar. 2003
Hokkaido University		Center for Cellular and Molecular Biology	(7 days)

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Sisinthi Shivaji	Molecular Basis of Cold Adaptation: Antarctic	Norio Murata	1 Feb. 2003 -
Deputy Director	Cyanobacteria and Bacteria as Model Systems	Professor	15 Mar. 2003
Center for Cellular and Molecular Biology		National Institute of Basic Biology, Okazaki	(43 days)

Project No.8

Title: Global Regulation of Gene Transcription in Bacteria under Various Environments

Objectives:

This project is an extension of the project 3 that was carried out by the project team headed by Prof. Akira Ishihama, Nippon Institute for Biological Science, Ome, Tokyo, Japan, and Prof. Dipankar Chatterji, Indian Institute of Science, Bangalore, India. For elucidation of the whole set of stress-response genes, a newly developed technique of the microarray assay with the *E. coli* DNA chip was employed. In parallel, this research group has started to isolate all members of the transcription factor from *E. coli* for identification of the genes under the control of each factor. Moreover, the antibodies against the purified transcription factors are being produced, which can be used to measurement of the intracellular concentration of each factor under various environments.

Project Coordinators:

(Japan)

Akira Ishihama

Head, Division of Molecular Biology

Nippon Institute for Biological Science Biophysics Unit

Ome, Tokyo

(India)

Dipankar Chatterji

Professor and Head

Indian Institute of Science

Bangalore

Date of Commencement: 1 April 2002

Date of Completion: 31 March 2007

Accomplishment Status:

The RNA polymerase in *E. coli* is composed of the core enzyme with the catalytic activity of RNA synthesis and one of the sigma subunits with promoter recognition activity. The holoenzyme is functionally differentiated into various forms of the transcription apparatus after interaction with various transcription factors. The research group initiated a comprehensive analysis of the structural and functional alteration of the transcription apparatus in response to various stresses. For identification of the genes that are induced by each stress, the whole set of mRNA is being analyzed by using the microarray assay with the *E. coli* DNA chip. To identify the transcription factor, which is involved in expression of each set of the stress-response genes, the purification of the whole set of the transcription factors from *E. coli* is being carried out.

Future Plan:

The principal investigator A. Ishihama proposes that the total number of transcription factor species in *E. coli* is about 260. About 2000 molecules of the RNA polymerase core enzyme must be converted into various forms of the transcription apparatus after interaction with the sigma factors and the transcription factors. A systematic study of the identification of the transcription factor, which is induced by each stress, and of the genes under the control of each factor is being carried out in this group.

Exchange Visit Undertaken:**FY2002**

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
Dipankar Chatterji	Microchip Analysis of Transcription	Akira Ishihama	12 Mar. 2003-
Professor and Head	Regulation in Escherichia coli	Head, Division of	28 Mar. 2003
Indian Institute of		Molecular Biology	(17 days)
Science		Nippon Institute for	
		Biological Science	

FY2003**Project No.9**

Title: The Relationship between Function and Structure of Influenza Virus RNA Polymerase.

Project Coordinators:

(Japanese)

Kazufumi Shimizu

Professor

Nihon University

(Indian)

Chattopadhyay Dhrubajyoti

Professor

Calcutta University

Date of Commencement:

1 April 2003

Date of Completion:

31 March 2007

2. Seminar

FY 2001

Seminar No.1

Title: Fundamental Mechanisms of Stress Response

Objective:

The fundamental mechanism of the stress response in biological systems has been the common target of two joint research groups in the Modern Biology and Biotechnology Area of Japan-India Co-operative Science Program. One group headed by Drs. Norio Murata (Nat. Inst. Biol., Japan) and Shishinty Shivaji (CCMB, India) has focussed on the stress response in plants and cyanobacteria with photosynthesis activity while another group organized by Drs. Akira Ishihama (Nat. Inst. Genet., Japan) and Dipankar Chatterji (Ind. Inst. Sci., India) has analyzed the molecular mechanisms of stress response in the model organism *Escherichia coli*. In order to exchange the accumulated data in these two groups and to exchange the knowledge of the fundamental mechanisms of the stress response between plants and bacteria, two groups gathered together to have a discussion meeting.

Seminar Organizers:

(Japan)	(India)
Akira Ishihama	Shishinty Shivaji
Professor, Head and Vice Director	Scientist F
Department of Molecular Genetics	Centre for Cellular and Molecular Biology
National Institute of Genetics	Hyderabad, India
Mishima, Shizuoka, Japan	

Period: 8-10 November 2001

Place: Hyderabad, India

Accomplishment Status:

Research of the fundamental mechanisms underlying stress responses in biological systems is a frontier in the modern biology. From the beginning of the Modern Biology and Biotechnology Area of the Japan-India Co-operative Science Program, collaborative studies related to the stress response have always been performed using various organisms. Results of these studies have been published in widely-distributed journals. The research on the stress response should be supported continuously, because the knowledge obtained in these studies will be useful to solve increasing disorders in the ecosystem.

Programme

9 November

- 9:00-9:20 Opening Remarks
- 9:20-11:00 Session 1
Co-Chairpersons: Dr. D Chatterji, IISc, Bangalore
Prof. K. Tanaka, University of Tokyo
- 9:20-9:55 Sensors for Low Temperature Signals in Synechocystis- From Systematic Genomics of Signal Sensors
Dr. I. Suzuki, NIBB, Okazaki
- 9:55-10:30 Physiology of Growth at Low Temperature in Cyanobacteria
Dr.T. Sakamoto, Kanazawa Univ.
- 10:30-11:05 Cloning and Sequencing of the Fatty Acid Desaturase Genes of a Psychrotropic Nostoc from Antarctica
Dr. S. Shivaji CCMB, Hyderabad
- 11:05-11:35 Tea Break
- 11:35-12:45 Session 2
Co-Chairpersons: Prof.T.Mizuno, Nagoya Univ.
Dr. Nagaraja, IISc, Bangalore
- 11:35-12:10 A Core RNA polymerase II subunit plays a vital role in regulation of stress response in yeast: Clues from whole genome analysis
Dr. P. Sadhale, IISc, Bangalore
- 12:10-12:45 Transcriptional Regulation through the Acetylation of Nonhistone Chromatin Proteins
Dr. T.K.Kundu, JNCASR, Bangalore
- 12:45-13:20 Collection of TA and DA forms and Confirmation of Travel Plans
- 13:20-14:30 Lunch
- 14:30-15:40 Session 3
Co-Chairpersons: Prof. Norio Murata, NIBB, Okazaki
Prof. P. Mohanty, JNU
- 14:30-15:05 Differentiation of Escherichia coli: Discontinuous Phenotype Changes during Growth Transition into Stationary Phase
Prof. A. Ishihama, NIG
- 15:05-15:40 Regulation of the RpoS-Controlled P1 Promoter of the Osmotically Induced pro U Operon of Escherichia coli and Salmonella Enterica
Dr. J. Gowrishankar, CDFD
- 15:40-16:40 Tea Break
- 16:10-18:15 Session 4
Co-Chairpersons: Prof. N. Murata, NIBB
Prof. Pr.Mohanty,JNU
- 16:10-16:45 Factors Involved in Cell Viability at the Stationary Phase of Cell Growth of Escherichia coli
Prof. Kazuei Igarashi, Chiba University
- 16:45-17:20 Regulation of RNase E-mediated Degradation of Glucose Transporter mRNA by Glycolytic Flux in Escherichia coli
Prof. H.Aiba, Nagoya Univ.
- 18:15-23:00 Dinner

10 November

- 9:00-10:45 Session 1
Co-Chairpersons: Dr J Gowishankar, CDFD, Hyderabad
Prof. A. Ishihama, NIG
- 9:00-9:35 Transcription Factor Sigma in Chloroplasts: A Bacteria Life in Plants
Prof. K. Tanaka, Univ. of Tokyo
- 9:35-10:10 Low Temperature Response of Outer Membrane in Bacteria
Dr. M. K. Ray CCMB, Hyderabad
- 10:10-10:45 Stress Response and Transcription Network in Mycobacteria
Dr. D. Chatterji, IISc, Bangalore
- 10:45-11:15 Tea Break
- 11:15-13:00 Session 2
Co-Chairpersons: Prof. H. Aiba, Nagoya Univ.
Dr. S.E. Hasnain, CDFD, Hyderabad
- 11:15-11:50 Molecular Biological Basis and Physiological Significance of Extraordinary High Catalase Activity in *Vibrio Rumeiensis* S-1
Prof. H. Okuyama, Hokkaido Univ.
- 11:50-12:25 Stress Responsive RNA- Binding Proteins in Cyanobacteria and Plants – Molecular and Evolutionary Aspects.
Prof. N. Sato, Saitama Univ.
- 12:25-13:00 Many Facets of Regulation of *gyr* Operons in Mycobacteria
Dr. V. Nagaraja, IISc, Bangalore
- 13:00-14:10 Lunch
- 14:10-15:20 Session 3
Co-Chairpersons: Dr. S. Shivaji, CCMB, Hyderabad
Prof. K. Igarashi, Chiba Univ.
- 14:10-14:45 Analysis of Global Gene Expression in *Synechocystis* with DNA Microarray
Prof. N. Murata, NIBB, Okazaki
- 14:45-15:20 Molecular Analysis of the *kdp* Operon from the Cyanobacterium *Anabaena* L-31
Dr. Anand ballal, BARC, Mumbai
- 15:20-15:50 Tea Break
- 15:50-17:35 Session 4
Co-Chairpersons: Dr. S. Shivaji, CCMB, Hyderabad
Prof. K. Igarashi, Chiba Univ.
- 15:50-16:25 AcNPV Polyhedrin Gene Transcription A Canny Virus Signs on Host Recruits
Dr. S. E. Hasnain, CDFD, Hyderabad
- 16:25-17:00 Two- Component Signal Transduction System in *E. coli*
Prof. T. Mizuno. Nagoya Univ.
- 17:00-17:35 On the Existence of an Early Intermediate of Inactive Photosystem II Centers During Photo Inhibition at Low Temperature
- 19:30- Dinner

List of Participants:

From Japan

H. Aiba	Professor, Nagoya University
K. Igarashi	Professor, Chiba University
Kan. Tanaka	Associate Professor, University of Tokyo
Takeshi Mizuno	Professor, Nagoya University
Norio Murata	Professor, National Institute for Basic Biology
H. Okuyama	Associate Professor, Hokkaido University
Toshio Sakamoto	Assistant, Kanazawa University
Iwane Suzuki	Assistant, National Institute for Basic Biology
N. Sato	Professor, Saitama University

From India

J. Gowrishankar	Scientist E, Centre for Cellular and Molecular Biology, Hyderabad)
Parag Sadhale	Associate Professor, Indian Institute of Science, Bangalore
Tapas K. Kundu	Lab Chief, JN Centre for Advanced Scientific Research, Bangalore)
D. Chatterji	Professor, Indian Institute of Science, Bangalore
M.K. Ray	Scientist C, Centre for Cellular and Molecular Biology, Hyderabad
V. Nagaraja	Professor, Indian Institute of Science, Bangalore
A. Ballal	Researcher, Bhabha Atomic Research Centre, Bombay
S.E. Hasnain	Director, Centre for DNA Fingerprinting and Diagnostics, Hyderabad
Prasanna Mohanty	Professor, Jawaharlal Nehru University

3. Visiting Scientists for Information Exchange

FY2002

India to Japan

Name and Affiliation	Research Subject	Main Host	Period
P.T. Manoharan Institute Emeritus Professor Indian Institute of Technology, Chennai	Collaborative Research for Bioinorganic Chemistry	Teizo Kitagawa Professor Institute for Molecular Science, Okazaki	2 Mar.2003- 29 Mar.2003 (28 days)
Shyamalava Mazumdar Associate Professor Department of Chemical Sciences	Resonance Raman Studies of Metalloproteins	Teizo Kitagawa Professor Institute for Molecular Science, Okazaki	4 Mar.2003- 11 Mar.2003 (8 days)

FY2003

India to Japan

<u>Name and Affiliation</u>	<u>Research Subject</u>	<u>Main Host</u>	<u>Period</u>
D. Balasubramanian Director, L.V. Prasad Eye Institute	Molecular and Cell Biology of Eye Diseases	A. Ishihama Division Director, Department of Molecular Biology Nippon Institute for Biological Science	10 Jan.2004- 25 Jan.2004 (15 days)
