Title of Project : Molecular dissection of epigenetic regulations supporting gene regulations

Shigeaki Kato
(The University of Tokyo, Institute of Molecular and Cellular Biosciences, Professor)

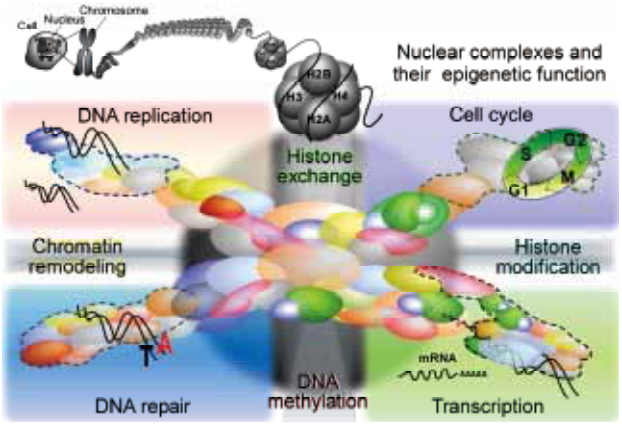
Research Area : Biology

Keyword : Genomic function and expression, Molecular genetics

【Purpose and Background of the Research】

Gene expression is in general suppressed by contacting with histone octamers in eukaryotic chromatin. Hence, chromatin reorganization is indispensable for gene regulations. Recently, certain combinations of histone chemical modifications have been shown to direct chromatin state, and these combinations are hypnotized as “histone code”. Histone code is supposed to play a central role in epigenetic controls.

We have studied the molecular basis of transcriptional controls by means of nuclear steroid hormone receptors, and have shown that transcriptional co-regulators mediate ligand-dependent transcriptional controls by nuclear receptors. Further characterization of such transcriptional co-regulators have uncovered that the prime function of identified co-regulators is associated with chromatin reorganization as histone modifying enzymes and chromatin remodelers, and they often form multisubunit complexes. However, the species of such complexes and their complex components largely remain to be studied. In the present study, to clarify the molecular basis of transcriptional controls at chromatin levels, we try to identify novel complexes supporting transcriptional controls through chromatin reorganization, and uncover their roles in chromatin reorganization.



【Research Methods】

Co-regulators/co-regulator complexes are tried to be identified by biochemical purification using DNA binding transcription factors as purification bait. Besides of nuclear receptors, several transcription factors primarily controlling cell fate decisions are also the targets. Prepared nuclear extracts from cultured cells endogenously expressing given transcription factors are used for biochemical purification, and the factors will be identified by mass-spectorometric finger printing. Genetic screening will be performed in transgenic flies expressing transcription factors. The physiological impact of identified factors will be verified by fly and mouse genetics.

【Expected Research Achievements and Scientific Significance】

This study will uncover the molecular basis of transcriptional controls with chromatin reorganization in terms of epigenomic regulations.

【Publications Relevant to the Project】

・Kim, M., Kondo, T., Takada, I., Youn, M., Yamamoto, Y., Takahashi, S., Matsumoto, T., Fujiyama, S., Shirode, Y., Yamaoka, I., Kitagawa H., Takeyama, K., Shibuya, H., Ohtake, F., **Kato, S.**: DNA demethylation in hormone-induced transcriptional derepression. ***Nature***, 461, 1007-1012, 2009.

・Fujiki, R., Chikanishi, T., Hashiba, W., Ito, H., Takada, I., Roeder, R. G., Kitagawa, H., **Kato, S.**: GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. ***Nature***, 459, 455-459, 2009.

【Term of Project】 FY2010-2014

【Budget Allocation】 605, 300 Thousand Yen

【Homepage Address and Other Contact Information】

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Title of Project : Molecular mechanism of the engulfment and degradation of dead cells by macrophages

Shigekazu Nagata
(Kyoto University, Graduate School of Medicine, Professor)

Research Area : Medicine, dentistry, and pharmacy

Keyword : Medical Chemistry

【Purpose and Background of the Research】

Apoptosis plays an important role in maintaining homeostasis of animals. However, due to few experimental systems, its molecular mechanism and physiological roles had been elusive. We showed Fas, a protein of the TNF receptor family, transduces an apoptotic signal. We then in collaboration with Dr. Golstein identified Fas ligand (FasL) that kills the cells, establishing a concept of "death factor". We found that two mouse mutations, *lpr* and *gld* are mutations of Fas and FasL, respectively. FasL works as an effector of CTL and NK cells. Administration of FasL rapidly killed the mice by causing acute hepatitis, suggesting that Fas-mediated apoptosis is responsible for the CTL-induced tissue destruction.

We found that a protease (caspase) cascade is activated in FasL-induced apoptosis, and identified an enzyme (CAD) for the apoptotic DNA fragmentation. We then showed that DNA of apoptotic cells and erythroid precursors is digested by DNase II in macrophages after they are engulfed. *DNase II*$^{-/-}$ mice suffer from lethal anemia in embryos, and polyarthritis in adults due to the IFNβ and TNFα produced from the macrophages. IFNβ gene induction in *DNase II*$^{-/-}$ macrophages is TLR-independent. We found that Eya, a Janus phosphatase, regulates the IFNβ gene expression.

Using the knowledge of the DNA degradation of dead cells, we established an assay for phagocytosis of apoptotic cells, and identified a soluble factor (MFG-E8) and a membrane protein (Tim-4) that stimulate the engulfment. MFG-E8 and Tim-4 bind phosphatidylserine (PS) on dead cells. MFG-E8 is expressed in the tingible-body macrophages in the spleens. Many apoptotic cells are left unengulfed in *MFG-E8*$^{-/-}$ tingible-body macrophages, and the mice develop SLE-type autoimmune diseases, confirming that apoptotic cells must be swiftly cleared to prevent the release of cellular components from dying cells. We also showed that nuclei from erythroid cells are phagocytosed in a PS-dependent manner.

【Research Methods】

In this project, we want to elucidate the molecular mechanism how apoptotic cells are engulfed by macrophages and how dead cells are degraded. In particular, we will study (1) how PS is exposed on the surface of apoptotic cells. Our goal is the identification of the enzyme(s) that mediates the PS exposure. (2) How Tim-4, a PS-receptor, and MFG-E8, a soluble protein that binds to PS, works for the engulfment of apoptotic cells. Or, does Tim-4 associate with other molecules for engulfment of apoptotic cells? What kinds of molecules are involved in engulfment of apoptotic cells? (3) How Eya is involved in the intracellular pathogens- induced IFN gene activation? We will determine the active site of its threonine-phosphatase domain in Eya and its tertiary structure. We are planning to determine the targets of the Eya phosphatase.

【Expected Research Achievements and Scientific Significance】

Dead cell generated during apoptotic cell death are engulfed and degraded by macrophages. We will elucidate the molecular mechanism of engulfment and degradation of dead cells. The outcome of this project will help our understanding of human diseases, in particular autoimmune diseases.

【Publications Relevant to the Project】

1. Okabe, Y., Sano, T. and Nagata, S.: Regulation of the innate immune response by threonine phosphatase of Eyes absent. *Nature* **460**: 520-524. 2009
2. Nagata, S., Hanayama, R. and Kawane, K.: Autoimmunity and the Clearance of Dead Cells. *Cell* **140**, 619-630, 2010

【Term of Project】 FY2010-2014

【Budget Allocation】 318,700 Thousand Yen

【 Homepage Address and Other Contact Information】

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Title of Project : Differentiation of endomembrane system for defense strategy in higher plants

Ikuko Hara-Nishimura
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Research Area : Biology, Basic biology, Plant molecular biology/plant physiology
Keyword : Organelles, Response to environmental factors, Plant-microbe interaction, Plant molecular function

【Purpose and Background of the Research】

Our recent studies indicate a novel defense strategy, which is associated with the fusion of vacuolar membrane and plasma membrane, resulting in releasing anti-bacterial defense proteins to abolish propagation of bacteria on the outside of cells[1] (Fig. 1). This novel defense system to attack bacterial pathogens outside the cells is contrasted with a VPE-mediated vacuolar-collapse system to kill viral pathogens inside the cells (***Science***, 2004). We also discovered a secretory signaling factor responsible for development of stomata, through which bacteria invade[2]. The aim of this project is to understand the molecular mechanism underlying the differentiation of the ER and vacuolar membranes in response to pathogen and environmental stresses.

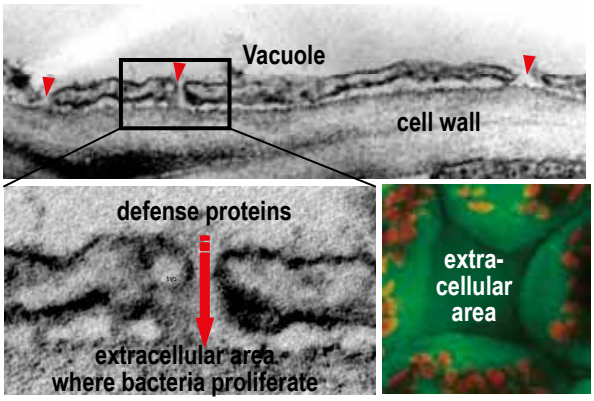

Figure 1. Membrane fusion-mediated plant immunity. Bacteria infection induces fusion of vacuolar membrane with plasma membrane (EM pictures). Membrane fusion triggers discharge of vacuolar content outside the cells (lower right).

【Research Methods】

This project focuses on four subjects (Fig. 2). Specific aims are to genetically and biochemically identify factors that participate in the inducible differentiation of the vacuolar membranes and endoplasmic reticulum and to address how the membrane dynamics support the defense strategy against various pathogen and environmental stresses.

【Expected Research Achievements and Scientific Significance】

Evidence from our studies provides an idea that plants have evolved the ER and vacuolar system as a defense strategy against pathogen and environmental stresses. This project is based on our findings on the diversity of plant endomembranes as described above. We believe that this research will give novel and valuable insights into the field of plant science.

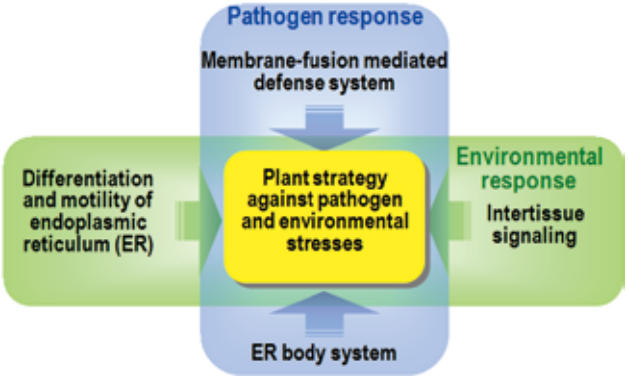

Figure 2. Conceptual diagram of this project.

【Publications Relevant to the Project】

[1] Hatsugai, N., Iwasaki, S., Tamura, K., Kondo, M., Fuji, K., Ogasawara, K., Nishimura, M., and *Hara-Nishimura, I. (2009) A novel membrane-fusion-mediated plant immunity against bacterial pathogens. ***Gene. Dev.***, 23: 2496-2506.

[2] Sugano, S. S., Shimada, T., Imai, Y., Okawa, K., Tamai, A., Mori, M., and *Hara-Nishimura I. (2010) Stomagen positively regulates stomatal density in Arabidopsis. ***Nature***, 463: 241-244. (*These two authors contributed equally to this work)

【Term of Project】 FY2010-2014

【Budget Allocation】 419, 700 Thousand Yen

【 Homepage Address and Other Contact Information】

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Title of Project : Mechanism for genome instability by activation induced cytidine deaminase induced-reduction of topoisomerase1

Tasuku Honjo
(KyotoUniversity, Graduate School of Medicine, Professor)

Research Area : Medicine, dentistry, pharmacy/Basic medicine/General medical chemistry
Keyword : Somatic hypermutation/Class switch recombination/ RNA editing/ Large scale sequence/Fact complex

【Purpose and Background of the Research】

Since E. Jenner first applied vaccination to small pox, vaccines against wide variety of diseases have been developed. As a result human beings almost completely succeeded in avoiding fatal causality by infectious diseases. Effective vaccination depends on generation of antibodies with memory. For a long time, it was a total mystery how vaccine (or antigen memory) is printed in antibodies. In 2000, we discovered that activation-induced cytidine deaminase (AID) is the enzyme that engraves antibody memory in the genome. AID introduces alterations in the immunoglobulin gene by changing the base sequence in the antibody binding site (somatic hypermutation) as well as replacing the constant region gene to diversify antigen processing mechanisms (class switch recombination). Furthermore, aberrant expression AID causes tumor by introducing genetic alterations in oncogenes. The purpose of this investigation is to understand how AID introduces DNA alterations in the immunoglobulin gene. In addition, we investigate why other oncogenes are also mutated by AID expression.

【Research Methods】

Last year, we found topoisomerase1 (Top1) that regulates the superhelical structure of DNA is reduced by AID, resulting in DNA cleavage of the immunoglobulin gene. Reduction of Top1 induces the structural alteration of the immunoglobulin gene, which causes irreversible cleavage by Top1. We will investigate how AID reduces the Top1 protein amount. Our hypothesis is AID suppresses Top1 mRNA translation. Our hypothesis is that AID edits small molecular RNA to convert C to U. The resultant small molecular RNA will change the translation efficiency of Top1 mRNA. Therefore, we investigate small molecular RNA that was edited by AID by large-scale DNA sequencing. We also isolate RNA and protein that associate with Top1 mRNA. Furthermore, we will investigate DNA sequences cleaved by AID and their DNA structure by whole genome sequencing.

【 Expected Research Achievements and Scientific Significance】

To understand the molecular mechanism of AID and elucidation of the mechanism for antibody memory generation will help not only the development of effective vaccine but also elucidation of the tumorigenesis mechanism by AID. There are reports that AID is involved in gastric cancer, hepatoma, lymphoma, and myloid leukemia. To understand the molecular mechanism for AID to cleave DNA will facilitate the discovery of the method to regulate its function and consequently to prevent tumorigenesis.

【Publications Relevant to the Project】

1) AID-induced decrease in topoisomerase1 induces DNA structural alteration and DNA cleavage for class switch recombination. Kobayashi, M, *Honjo,T. Proc. Natl. Acad. Sci. USA 106 22375-22380 (2009) refereed
2) A memoir of AID, which engraves antibody memory on DNA. *Honjo,T. Nature Immunol. 9 335-337 (2008) not refereed
3) Discovery of activation-induced cytidine deaminase, the engraver of antibody memory. Muramatsu,M., *Honjo,T. Adv. Immunol. 94 1-36 (2007) not refereed
4) *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Matsumoto,Y., Honjo,T., *Chiba,T. Nature Medicine 13 470-476(2007) refereed
5) Class switch recombination and hypermutation require activation-induced cytidine deaminase(AID), a potential RNA editing enzyme. Muramatsu,M., *Honjo,T. Cell 102 553-563 (2000) refereed

【Term of Project】 FY2010-2014

【Budget Allocation】 343,200 Thousand Yen

【 Homepage Address and Other Contact Information】

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Grant-in-Aid for Specially Promoted Research