***Japan Academy Prize to:***

Tsuneyoshi KUROIWA
Professor, The Department of Life Sciences, and
Director, Research Information Center for
Extremophile (RICE), Rikkyo University
Professor Emeritus, The University of Tokyo

for "Discovery of Fundamental Mechanisms
for Division and Inheritance of Mitochondria
and Chloroplasts"

***Outline of the work:***

Plant and algae cells produce the energy that is essential for the maintenance of life using chloroplasts (plastids) and mitochondria. Chloroplasts synthesize carbohydrates from atmospheric carbon dioxide and water by photosynthesis using the energy of sunlight, release oxygen and deliver the products to the host cell as food. Mitochondria consume oxygen and organic fuel to produce most of the ATP by cellular respiration. It is thought that chloroplasts and mitochondria originated as symbiotic bacteria that were engulfed by ancestral eukaryotic cells 1.5–2 billion years ago. Although these organelles have their own genomes that divide and segregate, very little is known about the underlying mechanisms. Dr. Tsuneyoshi Kuroiwa discovered the mitochondrial and chloroplast division machineries, and resolved key issues concerning the fundamental mechanisms of their division, and of mitochondrial and chloroplast maternal inheritance, which had long been elusive. To advance the field from the descriptive to the molecular level, he used the red alga *Cyanidioschyzon merolae* as a model organism to study organelle division. He and his colleagues determined the full nucleotide sequence of this algal genome; this was the first eukaryotic genome to be fully sequenced. A summary of his major achievements is as follows.

**1. Discovery of the mitochondrial and chloroplast division machineries**

Until 1973, it was generally considered that the nuclear DNA within a cell was organized into chromosomes by proteins called histones, while the mitochondrial and chloroplast DNA was naked within the organelles. Dr. Kuroiwa first demonstrated that the mitochondrial DNA in slime mold was organized with basic proteins to form an organelle nucleus (nucleoid). This idea has now been applied to mitochondrial DNA in animals and plants and to chloroplast DNA in plants. Moreover, Dr. Kuroiwa discovered that the mitochondria divided using ring-like structures at the division site after mitochondrial nuclear division. However, it proved very difficult to study the cellular and molecular mechanisms of organelle division because, in general, eukaryotic cells contain many organelles that are irregularly shaped and divide randomly. Thus, Dr. Kuroiwa searched for the simplest suitable organism for the study of organelle division. Finally, he selected the red alga *C. merolae*. This alga is a small unicellular organism that inhabits sulfate-rich hot springs. The cells contain one nucleus, one mitochondrion and one chloroplast. Dr. Kuroiwa discovered the large mitochondrial division (MD) ring and the chloroplast division (PD) ring at the division site. Later, similar small rings were identified in many eukaryotes. He revealed that the MD and PD division machineries exist as a chimera of the bacterial filamentous temperature sensitive Z (FtsZ) ring in the inner stroma and the eukaryotic mechanochemical PD/MD and dynamin rings in the outer cytoplasm. These division machineries are similar to each other. Dynamin enables the sliding of the PD/MD ring filaments, 5–7 nm in diameter, and causes the contraction required for organelle division. Finally, dynamin pinches off the membranes between daughter organelles. To elucidate the complete set of proteins

and other components required for organelle division, Dr. Kuroiwa isolated the organelle division machineries from highly synchronized cells. Using the complete *C. merolae* genome sequence and the matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF-MS) analysis, he identified 30–40 essential proteins, including FtsZ and dynamin. Dr. Kuroiwa proposed that the organelle division machineries play an important role in controlling the division and multiplication of endosymbiotic bacteria and organelles.

**2. Discovery of the fundamental mechanisms of the maternal inheritance of mitochondrial and chloroplast genomes**

It is well established that the mitochondrial and chloroplast genomes are transmitted to the progeny in a maternal fashion among the diverse taxa of sexually reproducing eukaryotes. The maternal inheritance of organelle genomes was thought to occur simply by dilution of the paternal contribution, because the paternal gametes (sperm) are much smaller than the maternal gametes (eggs) and therefore contribute only a small proportion of the progeny cytoplasm. However, Dr. Kuroiwa discovered that, in young zygotes of isogamous alga, the active, preferential digestion of chloroplast DNA of male (mating type -) origin was responsible for maternal inheritance. This overturned the conventional theory. Furthermore, Dr. Kuroiwa revealed that the inheritance of organelle DNA in algae, plants and animals involves the active digestion of the male DNA by nucleases. It is now generally accepted that maternal inheritance is not caused by the difference in size between the male and female gametes, but rather by the active digestion of male-origin DNA.

**3. Identification of *C. merolae* as a model organism, and sequencing of the complete algal genome**

A hallmark of Dr. Kuroiwa's scientific contribution has been consistent, highly innovative approaches towards the better visualization of organelle DNA. He developed various new techniques and instruments, including a high-resolution fluorescence microscope, which was later marketed by Olympus. To identify all the proteins present in the organelle division machineries, he and his colleagues sequenced the entire nuclear genome of the primitive red alga *C. merolae*, adding to the previously sequenced mitochondrial and chloroplast genomes. The *C. merolae* genome was the first algal genome to be sequenced, and at present is the only eukaryotic genome for which 100% has been sequenced. This complete genomic information provides an excellent opportunity to address basic questions, such as the mechanisms of organelle division and inheritance in eukaryotes, using microarray and proteome analyses. *C. merolae* contains a minimal set of genes with straightforward organization and few introns. *C. merolae* was thus an experimentally tractable model system for the analysis of organelle division and other aspects of organelle biology.

Dr. Kuroiwa's findings are cited in international molecular biology textbooks and have become part of the fundamental basis of cell biology and genetics. *C. merolae*, which played a key role in Dr. Kuroiwa's discoveries, is now utilized in many institutes throughout the world. For example, the Center for Eukaryotic Structural Genomics at the University of Wisconsin has adopted *C. merolae* as a model organism for structural biology. In addition, *C. merolae* can be useful not only for the study of eukaryotic heredity but also of the environment, medical treatments and food.

Below are the key publications among the 390 that Dr. Kuroiwa has published.

**Original papers**

1. Kuroiwa, T. and Tanaka, N. (1971) Fine structures of interphase nuclei IV. The behavior of late replicating chromatin during a late portion of the S period as revealed by electron microscopic autoradiography. *J. Cell Biol*. **49**, 939–942.
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13. Kobayashi, T., Takahara, M., Miyagishima, S., Kuroiwa, H., Sasaki, N., Ohta, N. and Kuroiwa, T. (2002) Detection and localization of a chloroplast-encoded HU-like protein that may organize chloroplast nucleoids. *Plant Cell* **14**, 1579–1589.
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15. Miyaghishima, S., Nishida, K., Mori, T., Matsuzaki, M., Higashiyama, T., Kuroiwa, H. and Kuroiwa, T. (2003) A plant-specific dynamin-related protein forms a ring at the chloroplast division site. *Plant Cell* **15**, 655–665.
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