Duke of Edinburgh Prize to:

Kiyoshi KITA
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for "Studies on the Strategy of Survival and Expansion Exhibited by Tropical Pathogenic Microorganisms: Diversity in the Adaptation Mechanisms in the Parasites"

Outline of the work:

Parasitism is a typical example of biological interactions occurring in living organisms. Over time, it has been considered that most parasitic relationships were established from its free-living ancestors and adapting to the host environment throughout its evolutionary process after transition to the parasitic life, with host- and organ-specific properties. In this view, parasites are very good target organisms for the study of the mechanism of adaptation in eukaryotes, particularly in the understanding of the adaptive mechanisms and evolution of metabolic systems that exist among all organisms, such as energy conversion and the basic biological mechanisms. From this perspective, Prof. Kiyoshi Kita has been conducting biological research on the mechanism of energy metabolism occurring in the roundworm, trypanosomes, and malaria parasite and their hosts, with the aim of understanding the mechanisms of adaptation by these parasites. As a result, it was revealed that a diverse range of respiratory chain functions occurring in the mitochondria of these parasitic cells cooperates with its cellular enzymes to aid its adaptation to environmental changes. Furthermore, because the respiratory chain of the parasite is significantly different from its mammalian host, it is considered to be an excellent target for drugs, and several compounds that possess *in vivo* effects have been discovered and are under development.

1. Energy Maintenance Mechanisms of *Escherichia coli* by Respiratory Chain Switch

Prof. Kita found that *Escherichia coli* alters the respiratory chain electron transport system in response to oxygen supply and maintains the energy conservation reaction by means of oxidative phosphorylation. This implies that when sufficient oxygen is available to the cell, a cytochrome *bo* complex is induced as a terminal oxidase, and under low-oxygen conditions, a cytochrome *bd* complex having higher oxygen affinity is induced to adapt to environmental changes. Using a novel method of reconstitution in the liposomes which occurred in the year 1870, he demonstrated that the cytochrome *bo* complex has a proton pumping activity and also showed its similarity with mitochondrial cytochrome *c* oxidase proposing its evolutionary relationship. These two oxidases are quinol oxidases that oxidize the reduced form of ubiquinone, ubiquinol, which lead to the study of cyanide-insensitive oxidase in the trypanosomes.

In the course of the research carried out on *Escherichia coli* at the Graduate School of Pharmaceutical Sciences and the Graduate School of Science, The University of Tokyo, the research

concluded that these eukaryotes might have taken a strategy of energy supply maintenance through changes in the respiratory chain of the cells. Therefore, Prof. Kita conducted research on the roundworm, *Ascaris suum*, whereby its supply of oxygen from its environment changes significantly during its life cycle.

2. Energy Metabolism in Parasitic Helminthes

The energy metabolism of parasites that live in a low-oxygen environment is significantly different from that of the host. Parasites adapt to their environment using their own metabolic system. Parasites are characterized by having at least two phases of free-living, outside the host and parasitism within the host during their life cycle. The energy metabolism in the parasite in the free-living stage is aerobic, whereas during the period of infestation in its host, specific systems are developed depending on the type of parasite, the parasitic environment, and its infection mode.

Prof. Kita decided to monitor the various adaptation strategies exhibited by the parasite at the level of proteins and genes involved, and moved to Juntendo University to start his study on the parasites together with Prof. Hiroshi Oya and Dr. Shinzaburo Takamiya. Parasites that are eukaryotes are classified into multicellular helminthes and unicellular protozoa. *A. suum*, a representative helminthes, lives in the small intestine of its host, which has a very low-oxygen tension. It is large in size, easy for biochemical analysis and the environment changes significantly during its life cycle.

Prof. Kita took advantage of the characteristics of this roundworm. It was revealed that considerable changes occur in the mammalian type respiratory chain of the larval mitochondria, where the respiratory system performs aerobic metabolism to fumarate respiration using fumarate as terminal electron acceptor instead of oxygen in the adult worm. In fumarate respiration, the enzyme complex II (fumarate reductase QFR) reduces fumarate in the reverse reaction to yield complex II (succinate dehydrogenase SQR), which catalyzes succinate oxidation in an aerobic environment. The reducing equivalent from nicotinamide adenine dinucleotide hydrogen is transferred to a reduced gradient of rhodoquinone, after which fumarate reductase functions as a terminal oxidase in the respiratory chain. The switches in the life cycle of the two complexes IIs were listed on the cover of *Parasitology Today* (now *Trends in Parasitology*) in 1992.

Expression of these two complexes II is regulated at the genetic level, and the fact that the four subunits are sequentially changed during its migration in the liver, heart, lung, small intestine of its host, aids the enzymatic complex conversion from SQR to QFR. The analysis revealed that there is an *A. suum*-specific pocket at the binding site of rhodoquinone. Genetic study also revealed the evolution of *A. suum* fumarate respiration, including rhodoquinone. SQR was evolved from the anaerobic bacterial fumarate respiratory QFR with the emergence of oxygen on the earth. Interestingly, it was revealed that eukaryotic QFR was a new evolutionary function of energy metabolism that was acquired during the establishment of parasitism in mammals. This research study clearly disproved a consensus that eukaryotic QFR has evolved directly from bacterial QFR and has been internationally recognized as a significant method for the understanding of the evolution of parasites.

Prof. Kita is also conducting research on other parasitic helminthes, from nematodes such as filaria and anisakis to flatworms such as echinococcus, schistosome, and fasciola indicating that

fumarate respiration is a universal system of energy metabolism in these helminthes. More recently, it has been shown that certain types of cancerous cells such as pancreatic cancer cells that grow under conditions of low-nutrition and low-oxygen tension adapt perfectly to the cancer microenvironment as a result of fumarate respiration. Thus, fumarate respiration is an important and universal strategy of adaptation in a hypoxic environment.

3. Energy Metabolism in Protozoan Parasites

The research conducted by Prof. Kita extended not only to the helminthes but also to protozoan parasites, which are single-cell parasites. It is pertinent to note that the studies also covered two other species; the trypanosomes and malaria parasites. Prof. Kita has been in Paraguay for almost 2 years as a team leader in the International Medical Cooperation, where he realized that there is no magic bullet in the endemic Chagas disease in Latin America caused by the parasite *Trypanosoma cruzi.* After returning to Japan, he expanded his study to cover the parasite *Trypanosoma brucei*, a pathogen of African sleeping sickness, and began research into the system of energy metabolism in the trypanosomes. As a result, he found that cyanide-insensitive oxidase, a terminal oxidase of the *T. brucei* mitochondrial respiratory chain, was essential for the growth of the parasite in the host bloodstream. In addition, he discovered a natural product, ascofuranone, which specifically inhibits the enzyme at very low concentrations of nM or less. Although this enzyme was very unstable and had not been analyzed at all, he and his group established an expression system in *Escherichia coli* and were able to clarify the difference that exists between the cytochrome *c* oxidase of mammalian mitochondria and the mechanism of inhibition of ascofuranone by crystal structure analysis. Further studies on the American-type *T. cruzi* also revealed that the mitochondrial complex II, which has four subunits, is a novel enzyme with 12 subunits and Ip subunit is fragmented. Evolutionary analysis revealed that this unique feature originated in Euglenozoa and it progressed with the addition of other accessory subunits. He also clarified the presence of dihydroorotate dehydrogenase localized in the cytoplasm of *T. cruzi*, and found that it is a key enzyme that links energy metabolism and nucleic acid metabolism.

In addition, he and his research colleague are conducting research on malaria parasite, which is currently one of the three majorly known infectious diseases, from the perspective of organelle diversity. In the erythrocytes of mammals, ATP is synthesized by glycolysis, but in the mosquito vector, it has been revealed that ATP levels are maintained by the process of oxidative phosphorylation. He also conducted research on the apicoplasts that share a common ancestor with chloroplasts, and found that Perkinsus apicoplasts, which are related protozoa to the malaria parasite and are a parasite of the oyster, do not contain DNA in the apicoplast. This is a first report on the apicoplast without DNA.

4. Mitochondrial Respiratory Chain as a Drug Target

The diversity in the respiratory chain of the electron transport chain involved in the adaptation to conditions of hypoxia from bacteria and parasites to cancer cells as described in the above sections is a promising target for drugs because of variations among host organisms. After his transfer to the Institute of Medical Science, The University of Tokyo in the year 1991, he had discovered inhibitors of fumarate respiration such as nafuredin, atpenin, and flutolanil through joint

research with Prof. Kazuro Shiomi of the Kitasato Institute. Ascofuranone, as described in Section 3, is a compound produced by the filamentous fungi discovered in the year 1972 by a group led by Dr. Gakuzo Tamura of the Faculty of Agriculture, The University of Tokyo as a compound showing anticancer and antiviral activities. Ascofuranone kills trypanosomes in 1 min and has been known to cure infected goats overnight. The target site cyanide-insensitive oxidase was first found in the mitochondria of plants. Collaborative studies with the discoverer, Prof. Tony Moore, University of Sussex, are also elucidating the features of this enzyme in plants. In addition, ascofuranone inhibits complex II of *Echinococcus multilocularis*, which infects nearly 50% of Hokkaido red foxes, without effective drugs for human patient. Recently, the effect was actually proved in animal experiments. These findings demonstrate that several parasitic diseases are zoonotic in nature, and the achievements of Prof. Kita are of great significance from the perspective of One Health that aims to maintain and preserve all living things, including the human species, on earth.

In summary, studies by Prof. Kiyoshi Kita on the mechanism of adaptation to hypoxia by various respiratory chain electron transport systems from bacteria to parasites and cancerous cells were able to clarify the significance of energy metabolism, evolution of organelles, and drug targets and contributed to both basic biology and global health. These excellent academic research findings provide the foundation for the elucidation of the environmental adaptation strategy of living things, maintenance of the natural environment, which is a crucial issue worldwide, and preservation of various species.

List of Main Publications (*: the most significant papers)

Articles

1. Formation of a membrane potential by reconstituted liposomes made with cytochrome b_{562}-*o* complex, a terminal oxidase of *Escherichia coli* K12. Kita, K., Kasahara, M. & Anraku, Y. (1982) ***J. Biol. Chem.*** 257, 7933–7935.
2. Terminal oxidases of *Escherichia coli* aerobic respiratory chain. I. Purification and properties of cytochrome b_{562}-*o* complex from cells in the early exponential phase of aerobic growth. Kita, K., Konishi, K. & Anraku, Y. (1984) ***J. Biol. Chem.*** 259, 3368–3374.
3. *Terminal oxidases of *Escherichia coli* aerobic respiratory chain. II. Purification and properties of cytochrome b_{558}-*d* complex from cells grown with limited oxygen and evidence of branched electron-carrying systems. Kita, K., Konishi, K. & Anraku, Y. (1984) ***J. Biol. Chem.*** 259, 3375–3381.
4. Electron-transfer comlex of *Ascaris suum* muscle mitochondria. III. Composition and fumarate reductase activity of complex II. Kita, K., Takamiya, S., Furushima, R., Ma, Y.-C., Suzuki, H., Ozawa, T. & Oya, H. (1988) ***Biochim. Biophys. Acta (Bioenergetics)*** 935, 130–140.
5. One-step purification from *Escherichia coli* of complex II (succinate:ubiquinone oxidoreductase) associated with succinate-reducible cytochrome b_{556}. Kita, K., Vibat, C.R.T., Meinhardt, S., Guest, J.R. & Gennis, R.B. (1989) ***J. Biol. Chem.*** 264, 2672–2677.
6. Sequence comparison between the flavoprotein subunit of the fumarate reductase (Complex II) of the anaerobic parasitic nematode, *Ascaris suum* and the succinate dehydrogenase of the

aerobic, free-living nematode, *Caenorhabditis elegans*. Kuramochi, T., Hirawake, H., Kojima, S., Takamiya, S., Furushima, R., Aoki, T., Komuniecki, R. & Kita, K. (1994) ***Mol. Biochem. Parasitol.*** 68, 177–187.

7. *Stage-specific isoforms of Complex II (succinate-ubiquinone oxidoreductase) in mitochondria from the parasitic nematode, *Ascaris suum*. Saruta, F., Kuramochi, T., Nakamura, K., Takamiya, S., Yu, Y., Aoki, T., Sekimizu, K., Kojima, S. & Kita, K. (1995) ***J. Biol. Chem.*** 270, 928–932.
8. *Oral and intraperitoneal treatment of *Trypanosoma brucei brucei* with a combination of ascofuranone and glycerol in mice. Yabu, Y., Minagawa, N., Kita, K., Nagai, K., Honma, M., Sakajo, S., Koide, T., Ohta, N. & Yoshimoto, A. (1998) ***Parasitol. Int.*** 47, 131–137.
9. *An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Ōmura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Harder, A., Kölbl, H., Namikoshi, M., Miyoshi, H., Sakamoto, K. & Kita, K. (2001) ***Proc. Natl. Acad. Sci. USA*** 98, 60–62.
10. Trypanosome alternative oxidase as a target of chemotherapy. Nihei, C., Fukai, Y. & Kita, K. (2002) ***Biochim. Biophys. Acta*** 1587, 234–239.
11. *Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). Miyadera, H., Shiomi, K., Ui, H., Yamaguchi, Y., Masuma, R., Tomoda, H., Miyoshi, H., Osanai, A., Kita, K. & Ōmura, S. (2003) ***Proc. Natl. Acad. Sci. USA*** 100, 473–477.
12. Rhodoquinone reaction site of mitochondrial complex I, in parasitic helminth, *Ascaris suum.* Yamashita, T., Ino, T., Miyoshi, H., Sakamoto K., Osanai, A., Nakamaru-Ogiso, E. & Kita, K. (2004) ***Biochim. Biophys. Acta (Bioenergetics)*** 1608, 97–103
13. Mitochondria and apicoplast of *Plasmodium falciparum*: Behaviour on subcellular fractionation and the implication. Kobayashi, T., Sato, S., Takamiya, S., Komaki-Yasuda, K., Yano, K., Hirata, A., Onitsuka, I., Hata, M., Mi-ichi, F., Tanaka, T., Hase, T., Miyajima, A., Kawazu, S., Watanabe, Y. & Kita, K. (2007) ***Mitochondrion*** 7, 125–132.
14. *Anaerobic NADH-fumarate reductase system is predominant in the respiratory chain of *Echinococcus multilocularis*, providing a novel target for the chemotherapy of alveolar echinococcosis. Matsumoto, J., Sakamoto, K., Shinjyo, N., Kido, Y., Yamamoto, N., Yagi, K., Miyoshi, H., Nonaka, N., Katakura, K., Kita, K. & Oku, Y. (2008) ***Antimicrob. Agents Chemother.*** 52, 164–170.
15. A cryptic algal group unveiled: A plastid biosynthesis pathway in the oyster parasite *Perkinsus marinus*. Matsuzaki, M., Kuroiwa, H., Kuroiwa, T., Kita, K. & Nozaki, H. (2008) ***Mol. Biol. Evol.*** 25, 1167–1179.
16. Structures of *Trypanosoma cruzi* dihydroorotate dehydrogenase complexed with substrates and products: Atomic resolution insights into mechanisms of dihydroorotate oxidation and fumarate reduction. Inaoka, D.K., Sakamoto, K., Shimizu, H., Shiba, T., Kurisu, G., Nara, T., Aoki, T., Kita, K. & Harada, S. (2008) ***Biochemistry*** 47, 10881–10891.
17. *Novel mitochondrial Complex II isolated from *Trypanosoma cruzi* is composed of 12 peptides including a heterodimeric Ip subunit. Morales, J., Mogi, T., Mineki, S., Takashima, E., Mineki, R., Hirawake, H., Sakamoto, K., Ōmura, S. & Kita, K. (2009) ***J. Biol. Chem.*** 284, 7255–7263.

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20. Cloning and characterization of hypoxia-inducible factor-1 subunits from _Ascaris suum_ — A parasitic nematode highly adapted to changes of oxygen conditions during its life cycle. Goto, M., Amino, H., Nakajima, M., Tsuji, N., Sakamoto, K. & Kita, K. (2013) ***Gene*** 516, 39–47.
21. *Structure of the trypanosome cyanide-insensitive alternative oxidase. Shiba, T., Kido, Y., Sakamoto, K., Inaoka, D.K., Tsuge, C., Tatsumi, R., Takahashi, G., Balogun, E.O., Nara, T., Aoki, T., Honma, T., Tanaka, A., Inoue, M., Matsuoka, S., Saimoto, H., Moore, A.L., Harada, S. & Kita, K. (2013) ***Proc. Natl. Acad. Sci. USA*** 110, 4580–4585.
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Reviews

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2. Electron-transfer complexes in *Ascaris* mitochondria. Kita, K. & Takamiya, S. (2002) ***Adv. Parasitol.*** 51, 95–131.
3. *Parasitology in Japan: Advances in drug discovery and biochemical studies. Kita, K., Shiomi, K. & Ōmura, S. (2007) ***Trends Parasitol.*** 23, 223–229.
4. Spread and evolution of *Plasmodium falciparum* drug resistance. Mita, T., Tanabe, K. & Kita, K. (2009) ***Parasitol. Int.*** 58, 201–209.
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