References

[1] K. Kita, K. Shiomi, and S. Ōmura, Parasitology in Japan: Advances in drug discovery and biochemical studies, Trends in Parasitol. 23 (2007) 223-229.

[2] A. Kroger, V. Geisler, E. Lemma, F. Theis and R. Lenger, Bacterial fumarate respiration, Arch. Microbiol. 158 (1992) 311–314.

[3] T. Kuramochi, H. Hirawake, S. Kojima, S. Takamiya, R. Furushima T. Aoki, R. Komuniecki, and K. Kita, Sequence comparison between the flavoprotein subunit of the fumarate reductase (complex II)of the anaerobic parasitic nematode, *Ascaris suum* and the succinate dehydrogenase of theaerobic, free-living nematode, *Caenorhabditis elegans*. Mol. Biochem. Parasitol. 68 (1994) 177-87.

[4] F. Saruta, H. Hirawake, S. Takamiya, Y-C. Ma, T. Aoki, K. Sekimizu, S. Kojima and K. Kita, Cloning of a cDNA encoding the small subunit of cytochrome b_{558} (cybS) of mitochondrial fumarate reductase (complex II) from adult *Ascaris suum*, Biochim. Biophys. Acta 1276 (1996) 1–5.

[5] H. Amino, H. Wang, H. Hirawake, F. Saruta, D. Mizuchi, R. Mineki, N. Shindo, K. Murayama, S. Takamiya, T. Aoki, S. Kojima and K. Kita, Stage-specific isoforms of *Ascaris suum* complex II: the fumarate reductase of the parasitic adult and the succinate dehydrogenase of free-living larvae share a common iron-sulfur subunit, Mol. Biochem. Parasitol. 106 (2000), 63–76.

[6] K. Kita, S. Takamiya, Electron-transfer complexes in *Ascaris* mitochondria, Adv Parasitol. 51 (2002) 95-131.

[7] E. Tomitsuka, K. Kita, H. Esumi, Regulation of succinate-ubiquinone reductase and fumarate reductase activities in human complex II by phosphorylation of its flavoprotein subunit, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 85 (2009) 258-65.

[8] E. Tomitsuka, K. Kita H. Esumi, The NADH-fumarate reductase system, a novel mitochondrial energy metabolism, is a new target for anticancer therapy in tumor microenvironments, Ann. N. Y. Acad. Sci. 201 (2011) 44-9.

[9] M.P. Paranagama, K. Sakamoto, H. Amino, M. Awano, H. Miyoshi, K. Kita, Contribution of the FAD and quinone binding sites to the production of reactive oxygen species from *Ascaris suum* mitochondrial complex II, Mitochondrion 10 (2010) 158-65.

[10] R. D. Guzy, B. Sharma, E. Bell, N. S. Chandel, P. T. Schumacker, Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis, Mol Cell Biol. 28 (2008) 718-731.

[11] M.A. Selak, S.M. MacKenzie, H. Boulahbel, D.G. Watson, K.D. Mansfield, Y. Pan, M.C. Simon, C.B. Thompson, E. Gottlieb, Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase, Cancer Cell 7 (2005) 77-85.

[12] R. Komuniecki, B.G. Harris, J. Marr, M. Mueller, (Eds.), Biochemistry and Molecular Biology of Parasites,

Academic Press, London, 1995, pp. 49-66.

[13] A.G.M. Tielens, J. Van Hellemond, The electron transport chain in anaerobically functioning eukaryotes, Biochim. Biophys. Acta 1365 (1998) 71-78.

[14] K. Kita, H. Hirawake, S. Takamiya, Cytochromes in the respiratory chain of helminth mitochondria, Int. J. Parasitol. 27 (1997) 617-630.

[15] J. Matsumoto, K. Sakamoto, N. Shinjyo, Y. Kido, N. Yamamoto, K. Yagi, H. Miyoshi, N. Nonaka, K. Katakura, K. Kita and Y. Oku, Anaerobic NADH-Fumarate Reductase System Is Predominant in the Respiratory Chain of *Echinococcus multilocularis*, Providing a Novel Target for the Chemotherapy of Alveolar Echinococcosis, Antimicrob. Agents. Chemother. 52 (2008) 164-170.

[16] P. Kohler, R. Bachmann, Mechanisms of respiration and phosphorylation in *Ascaris* muscle mitochondria, Mol Biochem Parasitol. 1 (1980) 75-90.

[17] H. Oya, K. Kita, in: E. Bennet, C. Behm, C. Bryant (Eds.), Comparative Biochemistry of Parasitic Helminths, Chapman and Hall, London, 1988, pp. 35-53.

[18] S. Takamiya, R. Furushima, R.H. Oya, Electron transfer complexes of *Ascaris suum* muscle mitochondria: I. Characterization of NADH-cytochrome *c* reductase (complex I-III), with special reference to cytochrome localization, Mol. Biochem. Parasitol. 13 (1984) 121-134.

[19] S. Takamiya, K. Kita, H. Wang, P.P. Weinstein, A. Hiraishi, H. Oya, T. Aoki Developmental changes in the respiratory chain of *Ascaris* mitochondria, Biochim. Biophys. Acta 1141 (1993) 65-74.

[20] S.T. Cole, C. Condon, B.D. Lemire, J.H. Weiner, Molecular biology, biochemistry and bioenergetics of fumarate reductase, a complex membrane-bound iron-sulfur flavoenzyme of *Escherichia coli*, Biochim. Biophys. Acta 811 (1985) 381-403.

[21] A. Hiraishi, Fumarate reduction systems in members of the family *Rhodospirillaceae* with different quinone types, Arch. Microbiol. 150 (1988) 56-60.

[22] F. Iwata, N. Shinjyo, H. Amino, K. Sakamoto, M.K. Islam, N. Tsuji, K. Kita, Change of subunit composition of mitochondrial complex II (succinate-ubiquinone reductase/quinol-fumarate reductase) in *Ascaris suum* during the migration in the experimental host, Parasitol. Int. 57 (2008) 54-61.

[23] T.M. Iverson, C. Luna-Chavez, G. Cecchini, D.C. Rees, Structure of the *Escherichia coli* fumarate reductase respiratory complex, Science 284 (1999) 1961-1966.

[24] C.R. Lancaster, A. Kröger, M. Auer, H. Michel, Structure of fumarate reductase from *Wolinella succinogenes* at 2.2 A resolution, Nature 402 (1999) 377-85.

[25] K. Kita, C. Vibat, S. Meinhardt, J. Guest, R. Gennis, One-step purification from *Escherichia coli* of complex II (succinate: ubiquinone oxidoreductase) associated with succinate-reducible cytochrome b_{556}, J. Biol. Chem. 264 (1989) 2672-2677.

[26] G. Cecchini, I. Schroder, R.P. Gunsalus, E. Maklashina, Succinate dehydrogenase and fumarate reductase from *Escherichia coli*, Biochim. Biophys. Acta. 1553 (2002) 140-157.

[27] C.R.D. Lancaster, Structure and function of succinate: quinone oxidoreductases and the role of quinol: fumarate reductases in fumarate respiration. In: Zannoni, D. (Eds.), Respiration in Archaea and Bacteria: Diversity of Prokaryotic Electron Transport Carriers. Kluwer Academic Publishers, The Netherlands, 2004, pp. 57-85.

[28] J.J. Van Hellmond, A. van der Klei, S.W.H. van Weelden, A.G.M. Tielens, Biochemical and evolutionary aspects of anaerobically functioning bacteria, Phil. Trans. R. Soc. B. 358 (2003) 205-215.

[29] K. Kita, H. Hirawake, H. Miyadera, H. Amino and S. Takeo, Role of complex II in anaerobic respiration of the parasite mitochondria from *Ascaris suum* and *Plasmodium falciparum*, Biochim. Biophys. Acta, 1553 (2002) 123-139

[30] H.P. Indo, M. Davidson, H.C. Yen, S. Suenaga, K. Tomita, T. Nishii, M. Higuchi, Y. Koga, T. Ozawa, H.J. Majima, Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage, Mitochondrion 7, (2007) 106-118.

[31] P. Jezek, L. Hlavata, Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism, Int. J. Biochem. Cell Biol. 37 (2005) 2478-2503.

[32] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417, (2009) 1-13.

[33] J. St-Pierre, J.A. Buckingham, S.J. Roebuck, M.D. Brand, Topology of superoxide production from different sites in the mitochondrial electron transport chain, J. Biol. Chem. 277 (2002) 44784-44790.

[34] I.K. Srivastava, H. Rottenberg, A.B. Vaidya, Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite, J. Biol. Chem. 272 (1997) 3961-3966.

[35] S. Looareesuwan, C. Viravan, H.K. Webster, D.E. Kyle, D.B. Hutchinson, C.J. Canfield, Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand, Am. J. Trop. Med. Hyg. 54 (1996) 62-66.

[36] D. Syafruddin, J.E. Siregar, S. Marzuki, Mutations in the cytochrome *b* gene of *Plasmodium berghei* conferring resistance to atovaquone, Mol. Biochem. Parasitol. 104 (1999) 185-194.

[37] I. K. Srivastava, J. M. Morrisey, E. Darrouzet, F. Daldal, A. B. Vaidya, Resistance mutations reveal the atovaquone-binding domain of cytochrome *b* in malaria parasites, Mol. Microbiol. 33 (1999) 704-711.

[38] P. Kohler, R. Bachmann, The effects of the antiparasitic drugs levamisole, thiabendazole, praziquantel, and chloroquine on mitochondrial electron transport in muscle tissue from *Ascaris suum*, Mol. Biochem. Parasitol. 14 (1978)155-163.

[39] A. Armson, W.B. Grubb, A.H.W. Mendis, The effect of electron transport (ET) inhibitors and thiabendazole on the fumarate reductase (FR) and succinate dehydrogenase (SDH) of *Strongyloides ratti* infective (L3) larvae, Int. J. Parasitol. 25 (1995) 261-263.

[40] S.Omura, H. Miyadera, H. Ui, K. Shiomi, Y. Yamaguchi, R. Masuma, T. Nagamitsu, D. Takano, T. Sunazuka, A. Harder, H. Kölbl, M. Namikoshi, H. Miyoshi, K. Sakamoto and K. Kita, An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 60-62

[41] T. Yamashita, T. Ino, H. Miyoshi, K. Sakamoto, A. Osanai, E. Nakamaru-Ogiso and K. Kita, Rhodoquinone reaction site of mitochondrial complex I, in parasitic helminth, *Ascaris suum*, Biochim. Biophys. Acta 1608 (2004) 97-103

[42] H. Miyadera, K. Shiomi, H. Ui, Y. Yamaguchi, R. Masuma, H. Tomoda, H. Miyoshi, A. Osanai, K. Kita, S. Omura, Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase), Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 473-477.

[43] A. Osanai, S. Harada, K. Sakamoto, H. Shimizu, D.K. Inaoka, K. Kita, Crystallization of mitochondrial rhodoquinol-fumarate reductase from the parasitic nematode *Ascaris suum* with the specific inhibitor flutolanil, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 65 (2009) 941-954.

[44] V. Yankovskaya, R. Horsefield, S. Törnroth, C. Luna-Chavez, H. Miyoshi, C. Léger, B. Byrne, G. Cecchini, S. Iwata, Architecture of succinate dehydrogenase and reactive oxygen species generation, Science 299 (2003) 700-704.

[45] F. Sun, X. Huo, Y. Zhai, A. Wang, J. Xu, D, Su, M, Bartlam, Z, Rao, Crystal structure of mitochondrial respiratory membrane protein complex II, Cell 121 (2005) 1043-1057.

[46] L.S. Huang, G. Sun, D. Cobessi, A.C. Wang, J.T. Shen, E.Y. Tung, V.E. Anderson, E.A. Berry, 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme, J. Biol. Chem. 281 (2006) 5965-5972.

[47] J.P. Bayley, P. Devilee and E.M.P. Taschner, The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency, BMC. Med.Genet. 6 (2005) 39.

[48] B.E. Baysal, On the association of succinate dehydrogenase mutations with hereditary paraganglioma, Trends Endocrinol. Metab. 14 (2003) 453-459.

[49] C. Eng, M. Kiuru, M.J. Fernandez and L.A. Aaltonen, A role for hypoxic mitochondrial enzymes in inherited neoplasia and beyond, Nat. Rev. Cancer 3 (2003) 193-202

[50] P.J. Pollard, N.C. Wortham and I.P. Tomlinson, The TCA cycle and tumorigenesis: The examples of fumarate hydratase and succinate dehydrogenase, Ann. Med. 35 (2003) 632-639.

[51] P. Rustin and A. Rotig, Inborn errors of complex II–unusual human mitochondrial diseases, Biochim. Biophys. Acta 1553 (2002) 117-122.

[52] N. Ishii, T. Ishii, P.S. Hartman, The role of the electron transport SDHC gene on lifespan and cancer, Mitochondrion 7 (2007) 24-38.

[53] H.X. Hao, O. Khalimonchuk, M. Schraders, N. Dephoure, J.P. Bayley, H. Kunst, P. Devilee, C.W. Cremers, J.D.

Schiffman, B.G. Bentz, S.P. Gygi, D.R. Winge, H. Kremer, J. Rutter, SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma, Science 325 (2009) 1139-1142.

[54] M.A. Birch-Machin, R.W. Taylor, B. Cochran, B.A. Ackrell, D.M. Turnbull, Late-onset optic atrophy, ataxia, and myopathy associated with a mutation of a complex II gene, Ann. Neurol. 48 (2000) 330-335.

[55] T. Bourgeron, P. Rustin, D. Chretien, M. Birch-Machin, M. Bourgeois, E. Viegas-Pequignot, A. Munnich and A. Rotig, Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency, Nat. Genet. 11 (1995) 144-149.

[56] B. Parfait, D. Chretien, A. Rötig, C. Marsac, A. Munnich, P. Rustin, Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome, Hum. Genet. 106 (2000) 236-243.

[57] R. Van Coster, S. Seneca, J. Smet, R. Van Hecke, E. Gerlo, B. Devreese, J. Van Beeumen, J.G. Leroy, L. De Meirleir, W. Lissens, Homozygous Gly555Glu mutation in the nuclear-encoded 70 kDa flavoprotein gene causes instability of the respiratory chain complex II, Am. J. Med. Genet. A. 120 (2003) 13-18

[58] D. Ghezzi, P. Goffrini, G. Uziel, R.Horvath, T. Klopstock, H. Lochmüller, P. D'Adamo, P. Gasparini, T.M. Strom, H. Prokisch, F. Invernizzi, I. Ferrero, M. Zeviani, SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy, Nature Genetics 41 (2009) 654-656.

[59] E. Tomitsuka, H. Hirawake, Y. Goto, M. Taniwaki, S. Harada, K. Kita, Direct evidence for two distinct forms of the flavoprotein subunit of human mitochondrial complex II (succinate-ubiquinone reductase), J. Biochem. 134 (2003) 191-195

[60] E. Tomitsuka, Y. Goto, M. Taniwaki, K. Kita, Direct evidence for expression of type II flavoprotein subunit in human complex II (succinate-ubiquinone reductase), Biochem Biophys. Res. Commun. 311 (2003) 74-79

[61] M. Salvi, N. Morrice, A. Brunati, A. Toninello, Identification of the flavoprotein of succinate dehydrogenase and aconitase as in vitro mitochondrial substrates of Fgr tyrosine kinase, FEBS Letters, 581 (2007) 5579-5585

[62] H. Esumi H, J. Lu, Y. Kurashima, T. Hanaoka, Antitumor activity of pyrvinium pamoate, 6-(dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl-1H-pyrrol-3-yl)ethenyl]-1-me thyl-quinolinium pamoate salt, showing preferential cytotoxicity during glucose starvation, Cancer Sci. 95 (2004) 685-690.

[63] B. E. Baysal, R. E. Ferrell, J. E. Willett-Brozick, E. C. Lawrence, D. Myssiorek, A. Bosch, A., A. van der Mey, P. E. Taschner, W. S. Rubinstein, E. N. Myers, C. W. Richard, C. W., C. J. Cornelisse, P. Devilee, B. Devlin, Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma, Science 287 (2000) 848-851.

[64] J. M. Weinberg, M. A. Venkatachalam, N. F. Roeser, I. Nissim, Mitochondrial dysfunction during hypoxia/reoxygenation and its correction by anaerobic metabolism of citric acid cycle intermediates, Proc. Natl. Acad. Sci. U. S. A .97 (2000) 2826-2831.

[65] A. Hirayama, K. Kami, M. Sugimoto, M. Sugawara, N. Toki, H. Onozuka, T. Kinoshita, N. Saito, A.

Ochiai, M. Tomita, H. Esumi, and T. Soga, Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry, Cancer Res. 69 (2009) 4918-4925.

Legend to Figures

Figure 1. Complex II is the member of TCA cycle and respiratory chain

Complex II catalyzes the oxidation of succinate to fumarate in the TCA cycle and transports the electron generated by this oxidation to ubiquinone in the respiratory chain. Generally, complex II consists of four subunits. Flavoprotein (Fp) subunit contains a flavin adenine dinucleotide prosthetic group and iron sulfur protein (Ip) subunit contains three iron-sulfur clusters. There are two hydrophobic cytochrome*b* (Cyb L, Cyb S) subunits. The succinate binding site is located in Fp subunit, while the quinone binding site is formed by three subunits, Ip, Cyb L and CybS. Complex II also catalyzes the reduction of fumarate, a reverse-reaction of succinate dehydrogenase, in the respiratory chain of mitochondria from anaerobic animals, such as *Ascaris suum*, as well as anaerobic bacteria.

Figure 2. Life cycle of *Ascaris suum*

Fertilized eggs grow to be infective L3 under aerobic environment. Infective L3 larvae are ingested by the host, reach the small intestine and hatch there. Afterwards, larvae migrate into host body (liver, heart, lung, pharynx), and finally migrate back to the small intestine and become adults. In the host small intestine, the oxygen concentration is low (pO_2=2.5 ~ 5%) compared with the exogenous environment (pO_2=20%). The metabolic pathway of *A. suum* changes dramatically during its life cycle, to adapt to changes in the environmental oxygen concentration [6].

Figure 3. Glucose metabolism of *A. suum* larval and adult mitochondria

The metabolic pathway of *A. suum* adult has a unique anaerobic electron transport system, NADH-fumarate reductase system. In the phospho*enol*pyruvate carboxykinase (PEPCK)-succinate pathway, phospho*enol*pyruvate (PEP) produced by a glycolytic process is carboxylated to form oxaloacetate and is then reduced to malate. The cytosolic malate is transported into the mitochondria, where it is first reduced to fumarate, and finally to succinate by the rhodoquinol-fumarate reductase activity of complex II. The terminal step is catalyzed by the NADH-fumarate reductase system (Boxed in broken lines) comprised of complex I, rhodoquinone (RQ), and complex II. PEP, phosphoenolpyuvate; PEPCK, phoshoenolpyruvate carboxykinase; OAA, oxaloacete [6].

Figure 4. Chemical structure and redox potentials of the quinones. A. Chemical structures of UQ and RQ. n, numbers of isoprenyl groups in side-chain. B. Redox potentials of quinones and substrates.

Figure 5. NADH-fumarate reductase System of *A. suum* as a target of chemotherapy

The differences in energy metabolisms between host and helminths is an attractive therapeutic targets for

helminthiasis. NADH-fumarate reductase is a part of a unique respiratory system in parasitic helminths and is the terminal step of the phospho*enol*pyruvate carboxykinase-succinate pathway, which is found in many anaerobic organisms. NADH-Fumarate Reductase System is a potential target for chemotherapy. Nafuredin was found to be competitive inhibitor for rhodoquinone binding site of *A. suum* complex II [1].

Figure 6. Schematic representation of *A. suum* complex IIs from larva type and adult type.

The mitochondrial metabolic pathway of the parasitic nematode *A. suum* changes dramatically during its life cycle, to adapt to changes in the environmental oxygen concentration. *A. suum* mitochondria express stage-specific isoforms of complex II. While there is no difference in the isoforms of the Ip and cybL subunits of complex II between L3 larvae and adult A. *suum*, they have different isoforms of complex II subunits Fp (larval, Fp^{L}; adult, Fp^{Λ}) and cybS (larval, $cybS^{L}$; adult, $cybS^{\Lambda}$) in *A. suum* adult respiratory chain, complex II produces high amount ROS [29].

Figure 7. Chemical structure of inhibitors of complex II

A. Nafuresin, a competitive inhibitor for the rhodoquinone binding site of *A. suum* complex II; B. Atpenin A5, a competitive inhibitor for the quinone binding site of complex II of many species; C. Fulutolanil, a competitive inhibitor for the quinone binding site of *A. siuum* complex II.

Figure 8. Fp isoform gene structure

Type I and II Fps differ to each other in six bases in DNA sequences and in two amino acid residues in proteins. Type I Fp gene has an exon-intron structure, while type II Fp gene issuggested to be intron-less. Although type I Fp gene is located on chromosome 5p15, the type II Fp gene is not found in the NCBI database and the location has not been clarified yet [59, 60].

Figure 9. Alignment of Amino acid sequences of Mammalian Fp subunits

Two amino acid desidues in the red box are different in human Fp isoforms. Tyr 586 and Val 614 in type I Fp are changed to Phe 586 and Ile 614 in type II Fp, respectively. Tyr 586 and Val 614 are well conserved among mammals and no animals but human have type II Fp [59].

Figure 10. Positions of Tyr 586 and Val 614 in the structure of porcine complex II

Two amino acid residues different in human isoforms, Y586F and V614I, shown in the cartoon representation of the porcine complex II structure (left) and the close-up view of the region including Y586F and V614I (right). V614I

is surrounded mainly by hydrophobic residues, whereas Y586F by both hydrophilic and hydrophobic residues. Y586 and E598 are in the hydrogen bond distance (3.15 Å) to each other. UQ shows ubiquinone. The numbers of amino acid residues in the box represent the human amino acid sequences and the others are the porcine amino acid sequences.

Table 1 mRNA expression of Fp isoforms in human cultured cells and tissues.

The expression ratio of the two Fp isoforms was analyzed by RT-PCR-RFLP (restriction fragment length polymorphism with AvaII).Total RNAs were obtained from NIPPON GENE (Japan) for normal liver, heart, skeletal muscle, brain, kidney and breast tumor, colon tumor, stomach tumor and uterus tumor. Wako (Japan) for normal pancreas and fetal tissues. Invitrogen (USA) for normal testes and breast tumor, liver tumor, kidney tumor, colon tumor, pancreas tumor, cervix tumor, ovary tumor, prostate tumor. Cells ; Fibroblast and Myoblast : kind gift from Dr. Yu-ichi Goto (National Institute of Neuroscience, Japan) A549, DLD-1 and MCF-7 : kind gift from Mr. Yasuyuki Yamazaki (Taiho pharma_ceutical, Japan) Panc-1 : kind gift from Dr. Yasuhiro Esumi (National Cancer Institute, Japan) Raji : kind gift from Dr. Kazurou Shiomi (Kitasato university, Japan) HT-29, HU-VEC-C, MDA-M-231, BT-20 and T-47D : ATCC (USA). Pancreatic epithelial and stromal cells : DS pharma (Japan).

		Race	Gender	Age	I (%) / II (%)
Tissue (Normal)	Liver*	Caucasoid	Female	15	70 / 30
	Heart*	Caucasoid	Pool of 7 donors		61 / 39
	Skeletal muscle*	—	Male	23	80 / 20
	Brain*	Caucasoid	Male	50	84 / 16
	Kidney*	Caucasoid	Pool of 8 donors		62 / 38
	Pancreas	—	Male	44	30 / 70
	Testes	Caucasoid	Male	19	100 / 0
Cell (Normal)	Fibroblast*	Mongoloid	—	—	94 / 6
	Myoblast*	Mongoloid	—	—	87 / 13
	HUV-EC-C*	—	—	—	88 / 12
	Pancreatic epithelial	—	—	—	100 / 0
	Pancreatic stromal	—	—	—	100 / 0
Tissue (Fetal)	Brain	—	Female	22 weeks	100 / 0
	Brain	—	Male	41 weeks	38 / 62
	Skeletal muscle	—	Male	22 weeks	0 / 100
	Skeletal muscle	—	Female	19 weeks	100 / 0
Tissue (Cancer)	Breast	—	Female	55	100 / 0
	Breast	Mongoloid	Female	Pool of 6 donors	0 / 100
	Liver	Caucasoid	Male	60	0 / 100
	Kidney	Caucasioid	Female	54	23 / 77
	Colon	Caucasoid	Male	75	100 / 0
	Colon	—	—	—	100 / 0
	Pancreas	Mongoloid	Male	32	100 / 0
	Stomach	—	—	—	100 / 0
	Uterus	—	Female	—	100 / 0
	Cervix	Caucasoid	Female	59	23 / 77
	Ovary	Caucasoid	Female	32	100 / 0
	Prostate	—	Male	—	100 / 0
Cell (Cancer)	HT-29*	Caucasoid	Female	44	92 / 8
	A549*	Caucasoid	Male	58	96 / 4
	DLD-1*	—	Male	—	25 / 75
	MCF-7*	Caucasoid	Female	69	23 / 77
	Raji*	Neglod	Male	11	17 / 83
	Panc-1	Caucasoid	Male	56	12 / 88
	MDA-M-231	Caucasoid	Female	51	100 / 0
	BT-20	Caucasoid	Female	78	78 / 22
	T-47D	Caucasoid	Female	54	53 / 47

* Tomitsuka, E., *et al.*, 2003

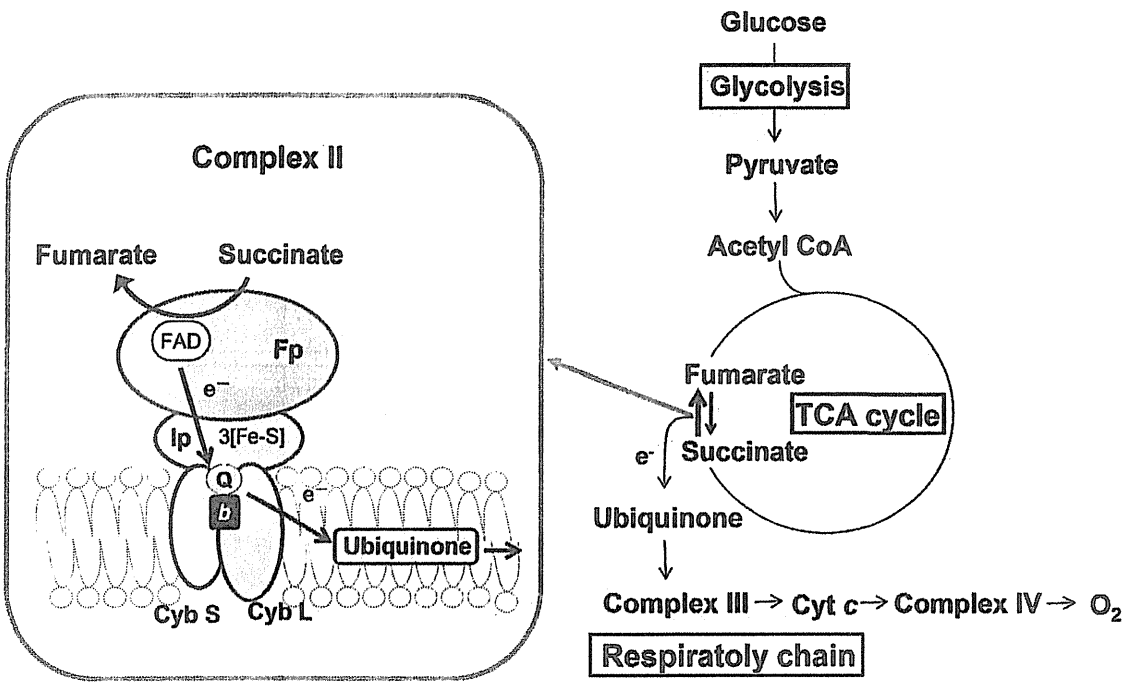

Complex II
Fumarate
Succinate
FAD
Fp
e⁻
Ip
3[Fe-S]
Q
b
e⁻
Ubiquinone
Cyb S
Cyb L
Glucose
Glycolysis
Pyruvate
Acetyl CoA
Fumarate
TCA cycle
e⁻
Succinate
Ubiquinone
Complex III → Cyt c → Complex IV → O2
Respiratoly chain

Figure 1

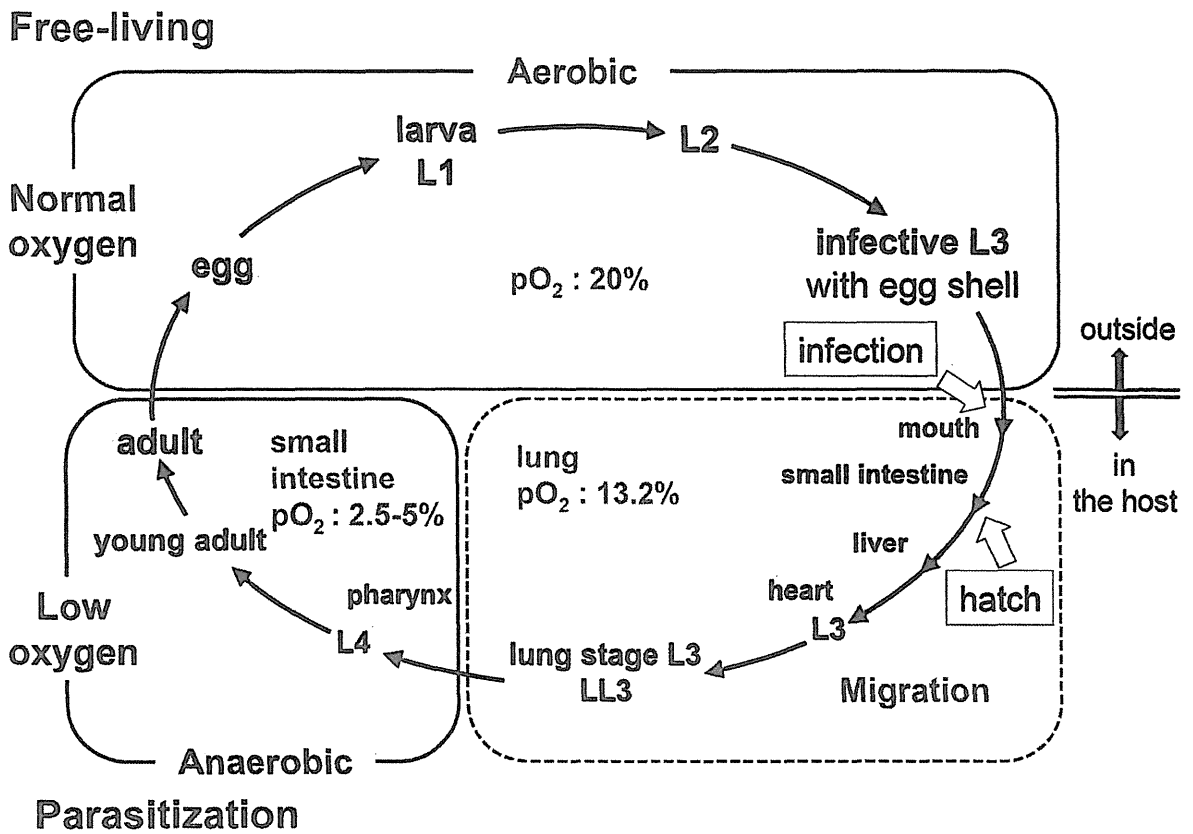

Free-living
Aerobic
larva
L1
L2
Normal
oxygen
egg
pO2 : 20%
infective L3
with egg shell
infection
outside
in
the host
mouth
small intestine
liver
heart
hatch
L3
lung
pO2 : 13.2%
lung stage L3
LL3
Migration
adult
small
intestine
pO2 : 2.5-5%
young adult
pharynx
L4
Low
oxygen
Anaerobic
Parasitization

Figure 2

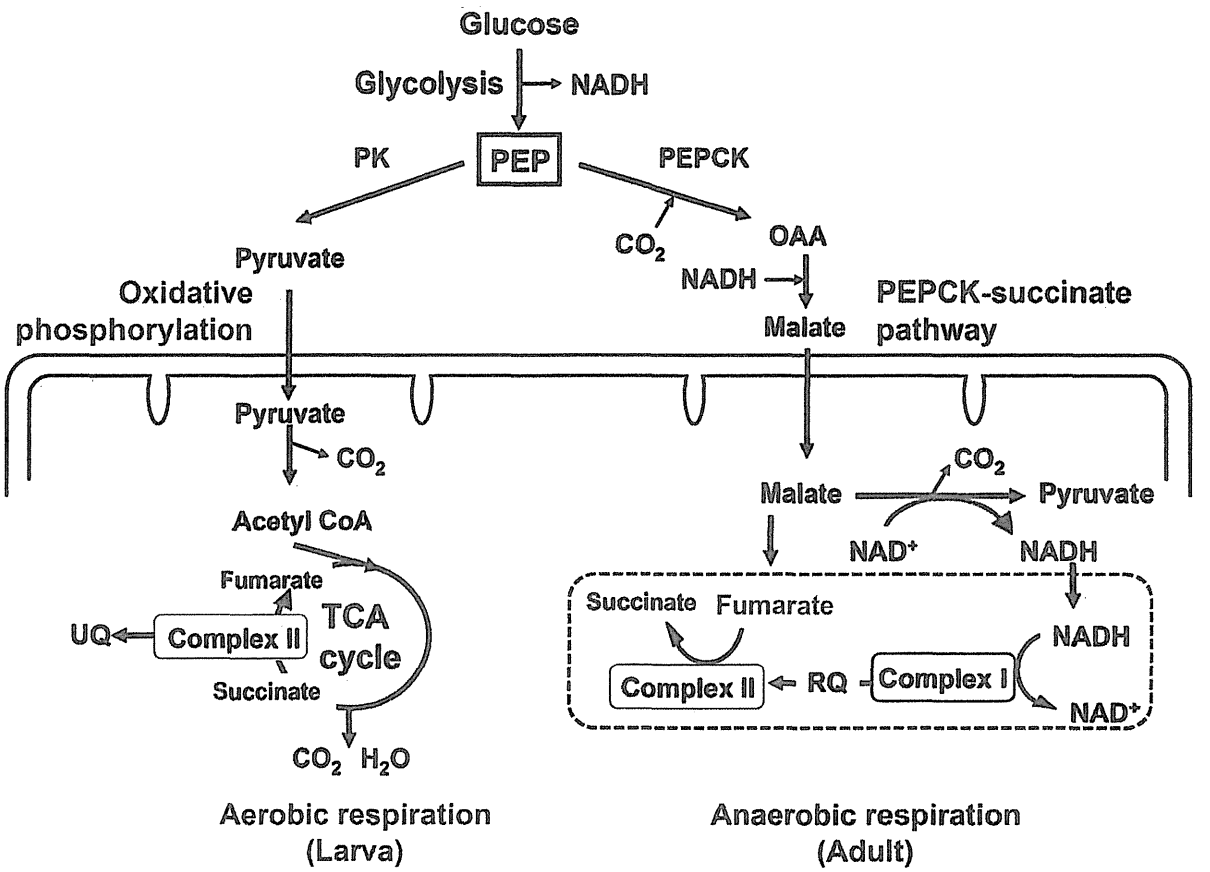
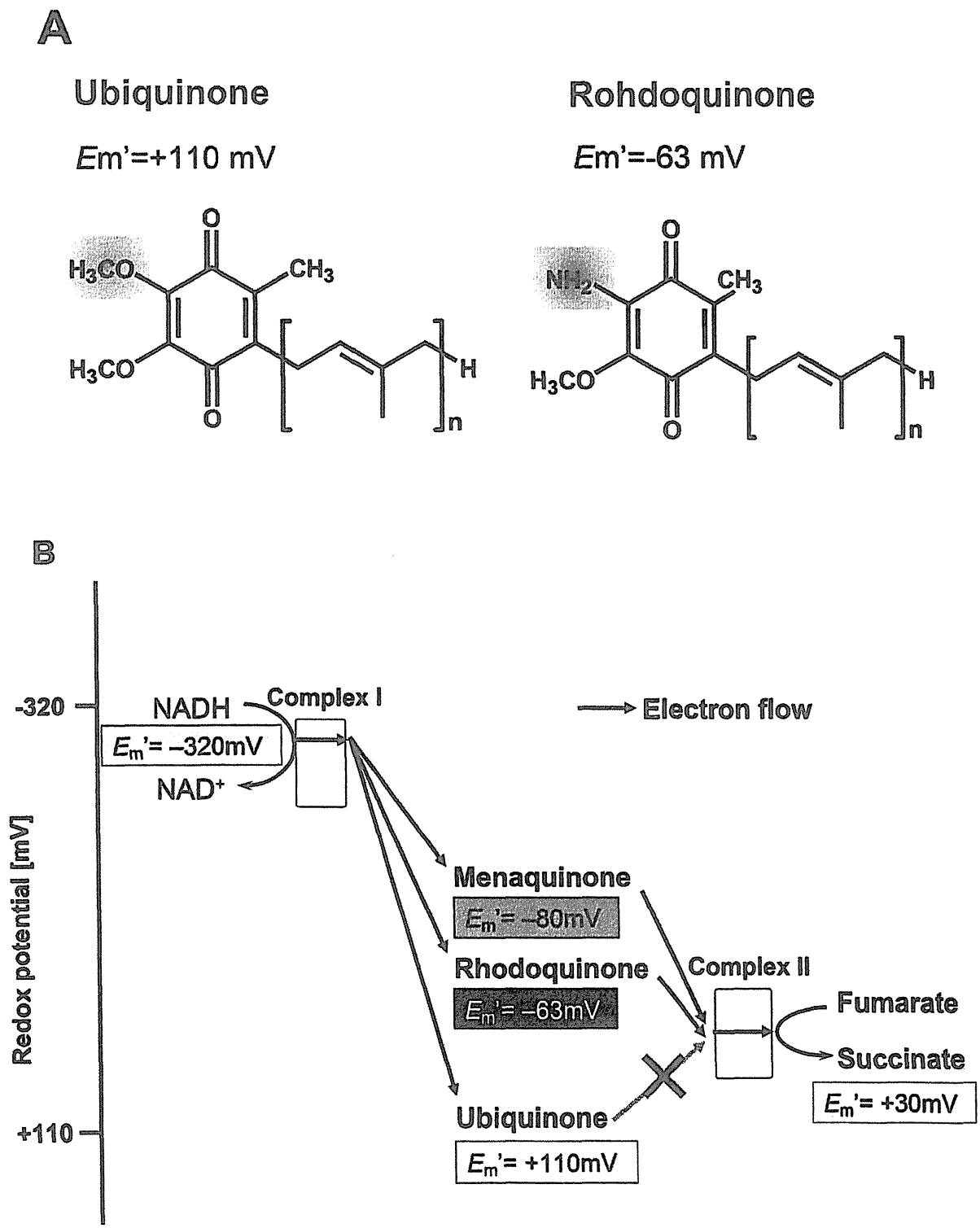

Glucose
Glycolysis
NADH
PK
PEP
PEPCK
CO_2
OAA
NADH
Malate
Pyruvate
Oxidative phosphorylation
PEPCK-succinate pathway
Pyruvate
CO_2
Acetyl CoA
Fumarate
UQ
Complex II
TCA cycle
Succinate
CO_2 H_2O
Malate
CO_2
Pyruvate
NAD^+
NADH
Succinate
Fumarate
NADH
Complex II
RQ
Complex I
NAD^+
Aerobic respiration (Larva)
Anaerobic respiration (Adult)

Figure 3

Figure 4


A
Ubiquinone
Em'=+110 mV
Rohdoquinone
Em'=-63 mV
B
-320
+110
Redox potential [mV]
NADH
Em'= –320mV
NAD+
Complex I
Electron flow
Menaquinone
Em'= –80mV
Rhodoquinone
Em'= –63mV
Ubiquinone
Em'= +110mV
Complex II
Fumarate
Succinate
Em'= +30mV

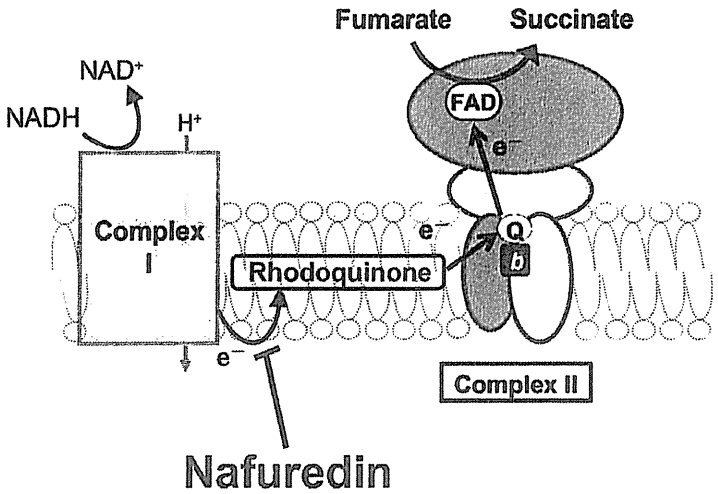

Fumarate
Succinate
NAD+
NADH
H+
FAD
e−
Complex
I
e−
Q
b
Rhodoquinone
e−
Complex II
Nafuredin

Figure 5

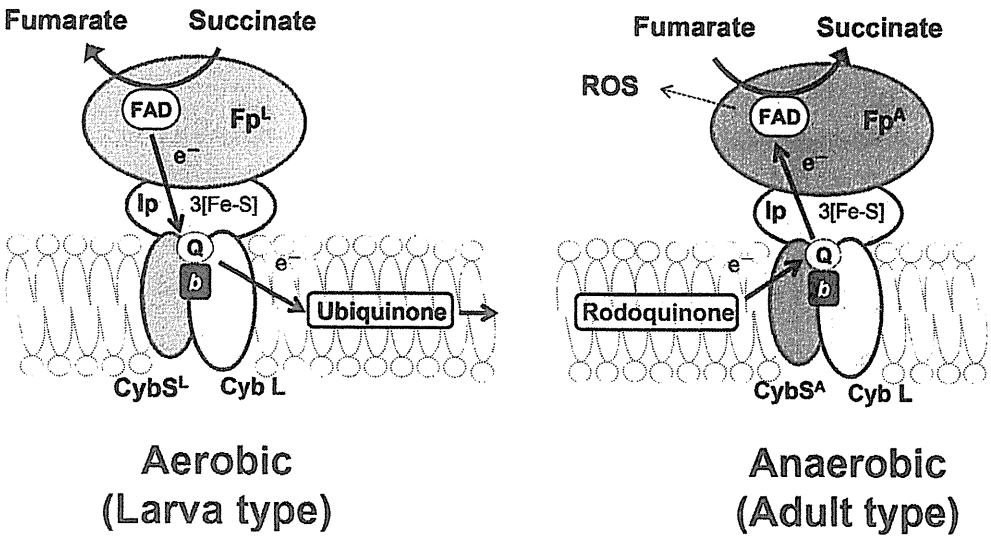
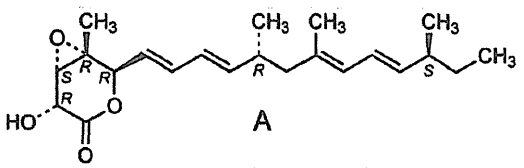
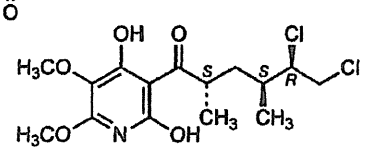

Fumarate
Succinate
FAD
Fp^L
e⁻
Ip
3[Fe-S]
Q
b
e⁻
Ubiquinone
CybS^L
Cyb L
Aerobic
(Larva type)
Fumarate
Succinate
ROS
FAD
Fp^A
e⁻
Ip
3[Fe-S]
Q
b
e⁻
Rodoquinone
CybS^A
Cyb L
Anaerobic
(Adult type)

Figure 6


CH3
CH3
CH3
CH3
CH3
O
S
R
R
R
R
S
HO
O
O
A


OH
O
Cl
H3CO
S
S
R
Cl
CH3
CH3
H3CO
N
OH
B

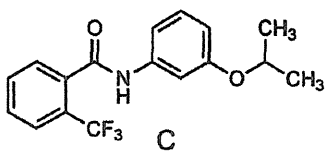

O
CH3
N
H
O
CH3
CF3
C

Figure 7

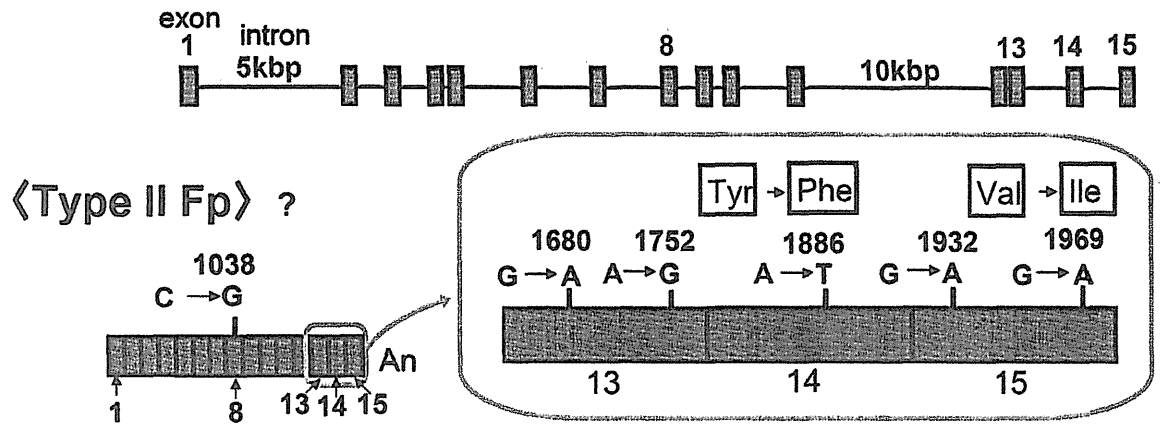

〈Type I Fp〉 Chromosome 5p15
exon
1
intron
5kbp
8
10kbp
13
14
15
〈Type II Fp〉 ?
1038
C →G
An
1
8
13 14 15
Tyr
Phe
Val
Ile
1680
1752
1886
1932
1969
G →A
A →G
A →T
G →A
G →A
13
14
15

Figure 8


Human type I Fp 578 HWRKHTLSYVDVGTGKVTLEYRPVI DKTLNEADCATVPPAI RSY
Human type II Fp 578 HWRKHTLSFVDVGTGKVTLEYRPVI DKTLNEADCATIPPAI RSY
Rat Fp 570 HWRKHTLSYVDTKTGKVTLDYRPVI DKTLNEADCATVPPAI RSY
Mouse Fp 578 HWRKHTLSYVDIKTGKVTLEYRPVI DKTLNEADCATVPPAI RSY
Bovine Fp 582 HWRKHTLSYVDIKTGKVTLEYRPVI DRTLNETDCATVPPAI GSY
Y586F
V614I

Figure 9