observed fragmentation of mitochondrial reticulum, oxidative phosphorylation disruption, permeability transition induction, that can lead to kidney cell loss. We also explored the defensive mechanisms such as GSK-3 inhibition, hypoxic preconditioning, mitochondria-targeted antioxidant use which prevented mitochondria damaging and increased cell survival. Partially these effects involve action of nonpathologic quantities of ROS and NO. According to our data during ARF mitochondria are the source, sensors and targets for ROS and NO. Delicate regulation of ROS- and NO-production and utilization is of critical importance to determine the cell fate, which can lead to kidney malfunctioning.

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S10.6 The NADPH binding on phagocyte NADPH oxidase

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Neutrophils play an essential role in the body's innate defence against pathogens and are one of the primary mediators of the inflammatory response. Neutrophils use a wide range of microbicidal products, such as oxidants, microbicidal peptides and lytic enzymes to kill invading pathogens. The generation of microbicidal oxidants results from the activation of a multiprotein enzyme complex known as the NADPH oxidase, which catalyses the formation of superoxide anion $O_2^{\cdot-}$. The complex is composed of 4 cytosolic subunits (p40phox, p47phox, p67phox, Rac) and two membrane proteins (gp91phox, p22phox). The aim of this work is to clarify the binding of the primary electron donor NADPH on this complex. Two hypotheses have been proposed, either a binding of the nucleotide on a cytosolic component called p67phox or on the membrane flavocytochrome b558. The availability as a recombinant protein of p67phox has allowed a thorough study on this latest. Several methods have been used such as tryptophan quenching, inhibitor analogs and centrifugal sedimentation. None of these techniques has revealed binding of NADPH on p67phox, in opposite to the gp91phox. In conclusion, as expected, the nucleotide binds on the flavocytochrome b558 close to FAD for an efficient electron transfer, the role of p67phox would be to change the affinity of NADPH to regulate the activity of the complex NADPH oxidase.

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S10.7 The phagocytic NAD(P)H-oxidase: Heterologous expression of membrane flavohemoprotein

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The nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase is an essential multiprotein complex present in phagocytic cells of the innate immune system having for function, in response to a bacterial infection, to generate superoxide from a single electron reduction of molecular oxygen using as electron donor NADPH. The catalytic component of this complex is a membrane bound flavocytochrome *b*, referred to as flavocytochrome *b558* (cyt *b558*) which consists of a heterodimer of two non-covalently linked glycosylated proteins $p22^{phox}$ and $gp91^{phox}$. The biochemical studies indicate that in addition to the two hemes, cyt*b558* contains a functional FAD prosthetic group. There are, at the moment, neither direct structural data of cyt*b558*, nor on the way it binds its redox components. Various studies allowed to propose structural models but which remain still hypothetical. The limiting factor for functional and structural studies is the lack of sufficient amounts of the cyt*b558* in stable, pure and homogenous form. Therefore, we are focusing our efforts in producing the membrane heterodimer by designing an engineered efficient membrane protein expression tool. Thus, we have made attempts to express the cyt*b558* in an *Escherichia coli* system but the eukaryotic *Pichia pastoris* expression system leads to more successful results.

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S10.8 Remodelling of mitochondrial function by the p13 protein of HTLV-1: Effects on reactive oxygen species and cell death

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In previous studies we showed that the p13 protein coded by human T-cell Leukemia/Lymphoma virus type-1 (HTLV-1) is targeted to mitochondria, affects cell turnover in vitro and exerts antitumor effects in experimental transformation models. The present study was aimed at understanding the mechanism of p13 function. Assays employing full-length synthetic p13 and isolated mitochondria showed that p13 triggers an inward K^+ current, resulting in depolarization, increased respiratory chain activity and accumulation of reactive oxygen species (ROS). Similar effects were induced by the K^+ ionophore valinomycin, while the protomophore FCCP reduced ROS production, suggesting that depolarization induced by K^+ vs. H^+ currents has opposite effects on ROS. We next studied the effects of p13 expression on reactive oxygen species production in HeLa and Jurkat T-cells. Results demonstrated that p13-expressing cells exhibit increased ROS levels and cell death. Interestingly, these effects ensued when cells were subjected to glucose deprivation, and were abrogated by treatment with ROS scavengers. Taken together, these findings indicate that by remodelling mitochondrial function, p13 may control mitochondrial ROS production and cell turnover under conditions of metabolic stress.

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S10.9 Flavohemoproteins as potential targets of antibiotics

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The compound miconazole is the most widely used antimycotics. Imidazoles such as miconazole, econazole, ketoconazole and clotrimazole have been recently proposed to coordinate selectively bacterial flavohemoproteins thus inhibiting their oxyde nitric dioxygenases (NOD) activity. This enzymatic conversion thus protects bacteria from the toxic NO and from other damaging NO-derived