tubular cells, which results in mitochondrial uncoupling and increased O_2 consumption. This mechanism may be protective against diabetes-induced oxidative stress, but will increase O_2 usage. The subsequently reduced O_2 availability may contribute to diabetes-induced progressive kidney damage.

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S12.34 The alternative oxidase as a tool to study mitochondrial function and to correct mitochondrial pathologies

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Alterations in mitochondrial function are related with different diseases (e.g. Parkinson or diabetes). It is not clear if such alterations are caused by the increase in mitochondrial Reactive Oxygen Production (mtROS) generation, deficits in ATP synthesis or both. This lack of knowledge make difficult to produce more efficient treatments. In order to clarify the mechanism involved in different kind of mitochondrial pathologies we have introduced the alternative oxidase (AOX) gene of *Ciona intestinalis* in the genome of Drosophila melanogaster. AOX expression in Drosophila decreases mtROS generation and partially by-pass the blockage of respiration elicited by inhibitors of both complexes III (antymicin A) and IV (KCN). Thus, AOX flies have new physiological properties as for example resistance in vivo to respiratory inhibitors and increase survival at low temperatures (4 °C). Moreover, AOX is able to correct mitochondrial alterations related with increases in oxidative stress. Mutations in DJ-1 gene provoke a Parkinson-like phenotype in Drosophila (e.g. alterations in locomotive function). Thus, DJ-1 mutant flies produce more mtROS without major alteration in the mitochondrial oxygen consumption. AOX expression in DJ-1 mutants flies either decreases mtROS generation to normal levels or rescue the alteration in locomotive function. Our results indicate that AOX is a potent model to study the molecular mechanism of mitochondrial pathologies.

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S12.35 Mitochondria as regulators of apoptosis through the redox state of cytochrome *c*

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When in cytosol cytochrome *c* triggers caspase activation by apoptosome, and oxidized cytochrome *c* is more effective in this process than reduced form of the enzyme. We investigated which cellular activities can reduce cytochrome *c* and by doing so may regulate apoptosis. When added to the cytosols from control or staurosporine-treated, apoptotic cells cytochrome *c* was gradually reduced whereas in homogenates of apoptotic cells it was rapidly oxidised by mitochondrial cytochrome oxidase (COX). The cytochrome *c* reducing activity of cell homogenates (but not cytosols) was enhanced in the presence of NADH. NADH-dependent reduction of cytochrome *c* in homogenates was significantly inhibited by DIDS or by removal of mitochondria indicating that this activity may be related to the mitochondrial porin/VDAC. Isolated heart mitochondria exhibited high rates of DIDS-inhibitable NADH-cytochrome *c* reductase activity. In liver mitochondria, DIDS only partially inhibited NADH-cytochrome *c*-reductase suggesting that more than one enzyme may be responsible for this activity. To test whether inhibition of COX or enhancement of cytosolic NADH can increase reduction of cytochrome *c* in cells and rescue them from apoptosis we incubated cells with staurosporine in the presence of azide and lactate. Such treatment decreased the rate of staurosporine-induced caspase activation by 40%, indicating that increasing the level of NADH may inhibit or delay caspase activation by mechanisms that may involve reduction of cytochrome *c* in the cytosol. Altogether our data suggest that mitochondria can regulate caspase activation by reducing or oxidizing cytochrome *c* released into cytosol after induction of apoptosis.

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S12.36 Oxidative stress in hypercholesterolemic LDL receptor knockout mice: Role of mitochondrial NADP-linked substrates and intracellular calcium levels

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Recently, we demonstrated that hypercholesterolemic LDL receptor knockout (LDLr k/o) mice present increased mitochondrial and cellular ROS production and a lower antioxidant capacity probably due to a large consumption of reducing equivalents from NADPH to sustain high rates of lipogenesis. Here we show that when k/o mice were treated with citrate containing drinking water during one week, the rates of oxygen consumption supported by endogenous NAD(P)-linked substrates, ROS production and NADPH oxidation by liver mitochondria were partially restored. We also observed that spleen mononuclear cells isolated from the k/o mice present cytosolic free Ca^{2+} concentrations and ROS production 2–3 times higher than the controls. To ascertain the role of Ca^{2+} in the k/o mice lymphocyte ROS production, we treated the k/o mice with verapamil, an L-type Ca^{2+} channel antagonist. The increase of ROS generation and Ca^{2+} concentration were partially inhibited in spleen mononuclear cells, but no effect was verified in liver mitochondrial ROS production and NADPH oxidation rates. These data demonstrate that the oxidative stress in spleen and liver of LDLr k/o mice results from distinct mechanisms. While liver mitochondria are deficient in NADPH-linked substrates, spleen lymphocytes are activated by high intracellular Ca^{2+} concentrations.

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S12.37 The inhibitory protein $1F_1$ regulates cellular sensitivity to staurosporine-induced cell death

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IF_1 inhibits the reverse activity of the ATP synthase, limiting mitochondrial ATP consumption during pathological states (e.g. ischaemia). IF_1 expression has also been reported to be upregulated in neoplastic cell lines. Thus, IF_1 may play a fundamental role in the