Evaluation of ROS production in OXPHOS-deficient models of *C. elegans* as future models to evaluate therapeutic potential of metallothioneins

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Introduction: During their lifetime cells can experience various kinds of stress. The stress can either be exogenous or endogenous and this can make alterations to the wild type physiological process. A group of these stressors, reactive oxygen species (ROS), are mainly generated via mitochondria as a by-product of the oxidative phosphorylation system (OXPHOS). Overproduction of ROS may lead to oxidative damage to lipids, proteins and DNA, which has a causative effect in the development of aging and other diseases, particularly deficiencies in the OXPHOS system (mitochondrial disease, MD). Several antioxidants have been investigated *in vitro* for their therapeutic potential in limiting ROS damage, including endogenously induced metallothioneins (MTs). *In vivo* models of MD where the involvement of ROS in the pathology could be confirmed, have been lacking - thus limiting the evaluation of MTs in scavenging ROS. We have selected *C. elegans* as a model for future studies and compared various strains with OXPHOS deficiencies as potential candidates for future studies on MT's scavenging potential (1,2 &3).

Methodology: A literature review was done to identify *C. elegans* strains with mutations in the various OXPHOS genes that could potentially result in excessive ROS production. After acquiring and setting up maintenance of these worms, genetic confirmation of the mutations was done and the deficiencies confirmed by high-resolution respirometry (Oroboros). Production of ROS were measured by fluorescence microscopy using the peroxide-sensitive fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFDA).

Results: The strains that were identified are shown in Table 1. The genetic mutations of these strains were confirmed with DNA sequencing. TK22 and GA184 showed a decrease of 18,9% and 27,7%, respectively, in oxygen consumption compared to the wild type (N2). Surprisingly, CW152 showed a 10,2% increase in oxygen consumption. ROS analyses revealed a 10,4% increase in the TK22 strain when compared to the wild type. The other strains still need to be further investigated.

Discussion: From the selected *C.elegans* strains that were studied, only a few strains seems suitable for investigating OXPHOS deficiencies and ROS production. Thus-far there are at least one strain, TK22, that produces excessive ROS related to an OXPHOS deficiency. Although experimentation is still progressing the initial results show that at least a selection of these strains may be suitable as models to further investigate MT scavenging against ROS in OXPHOS deficient strains; despite further experimentation.

Table 1: *C.elegans* strains selected for the evaluation as potential models to study the ROS-scavenging effect of MT's.

Genotype	Strain name	Deficiency	Mutation	Phenotypic characteristics
mev-1(kn1)	TK22	CII	c.213G > A Missense mutation	Shortened lifespan, reduced brood size
sod-2(gk257)	GA184	Superoxide dismutase	159 Base-pair deletion at 1254	Slow growing, reduced brood size
nuo-1(ua1)	LB25	CI (NADH Ubiquinone Oxidoreductase)	1190bp deletion at 352	Low brood size, short life span
gas-1(fc21)	CW152	NDUFS2	c.501C > T Missense mutation	Reduced lifespan, low brood size
isp-1(qm150)	MQ887	CIII	c.673C > T Point mutation	Slow development, reduced brood sizes
nuo-6(qm200)	MQ1333	NDUFB4	c.116G > A Missense mutations	Slow development

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