Exploring metabolomic changes due to cortisol deficiency in early development using the ferredoxin (fdx1b) null-allele zebrafish

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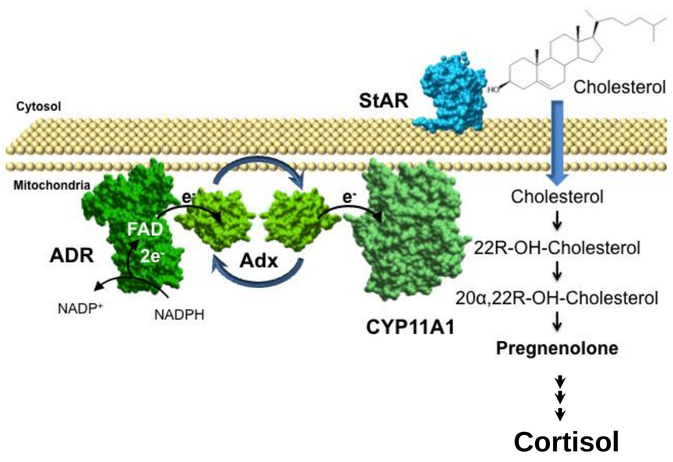
Aim

Significant gaps remain on the understanding of the *in vivo* impact of cortisol deficiency on metabolic pathways during embryonic development. Herein, we present a newly established cortisol deficient zebrafish mutant line in order to investigate into the pathogenic effects of cortisol deficiency *in vivo*.

Introduction

Cortisol production requires electron transfer mediated by ferredoxin (FDX1, Adx)



The zebrafish model for development and endocrine research

- Vertebrate
- Large offspring, small embryos (high-throughput studies, drug screens)
- Transparency of embryos (Life imaging)
- Rapid development
- Easy genetic manipulation (TALENs)
- High conservation of endocrine system to human

Summary

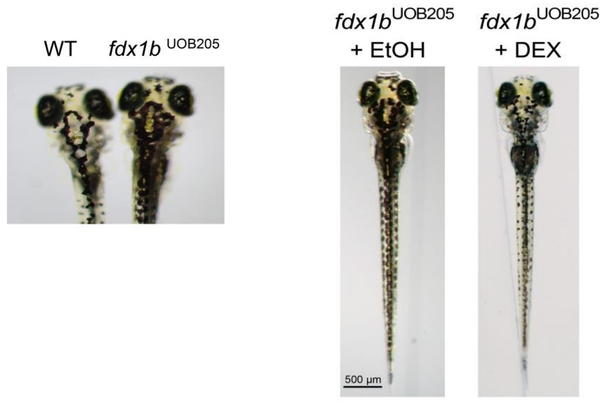
- We have generated a mutant fdx1b (equivalent of human FDX1) null-allele zebrafish line
- fdx1b deficient embryos are darker due to a failure in Visual Background Adaptation (VBA) behaviour
- VBA in the fdx1b mutants is rescued after dexamethasone treatment
- *pomc* expression is significantly increased in fdx1b null-allele larvae
- Cortisol synthesis and signalling are significantly impaired
- fdx1b null-allele larvae have a blunted cortisol response to stress
- Metabolic profiling reveals changes in energy synthesis and biomolecule generation

Conclusion

The fdx1b null-allele zebrafish line is a promising *in vivo* model to explore the pathophysiologic impact of glucocorticoid deficiency on energy metabolism relevant to early development and potentially adult life.

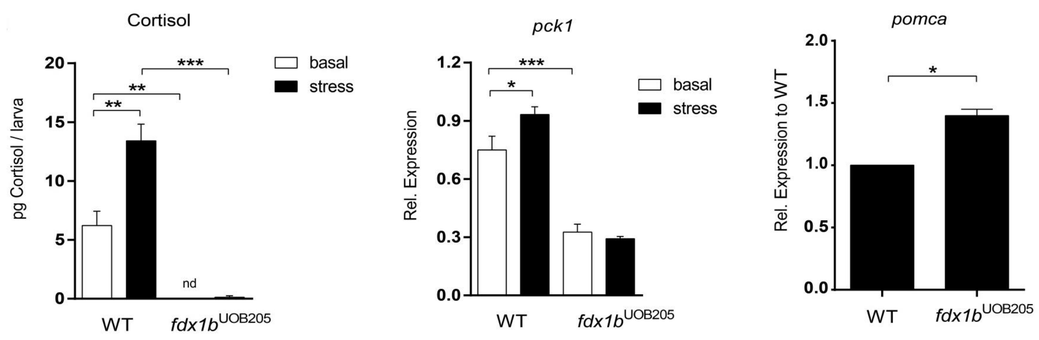
Results

fdx1b null-allele zebrafish larvae reveal a failure in their Visual Background Adaptation (VBA) behaviour



The VBA behavior can be rescued with the synthetic steroid hormone dexamethasone (DEX)

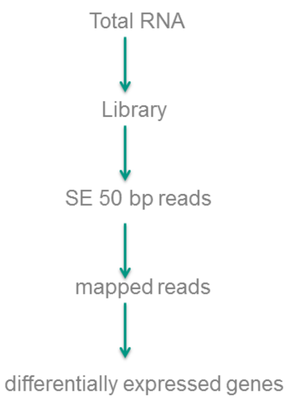
fdx1b null-allele zebrafish larvae are impaired in cortisol synthesis, cortisol regulated gene expression and in their stress response

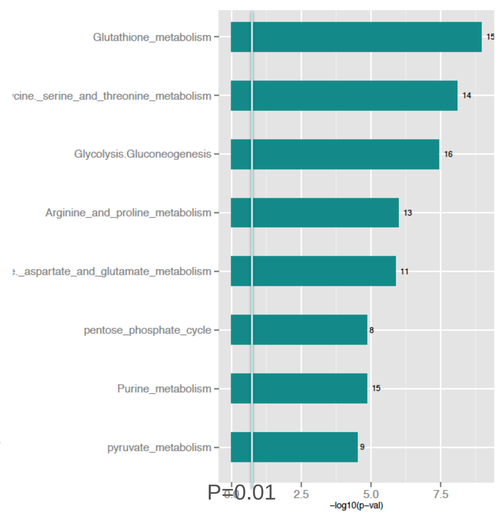


Cortisol deficiency leads to metabolic changes in pathways involved in energy and biomolecule synthesis

Transcriptomics

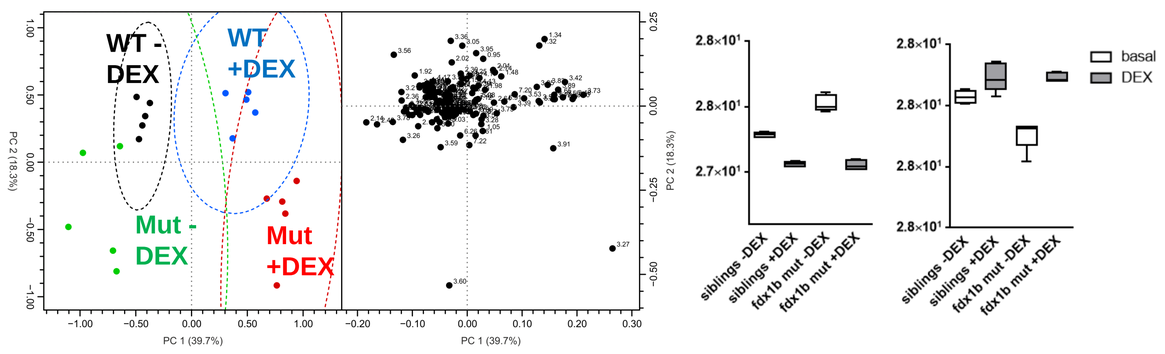
RNA-seq





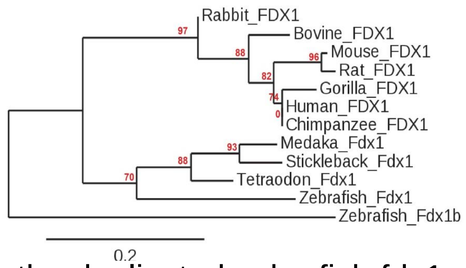
Metabolomics

Nuclear magnetic resonance (NMR) spectroscopy

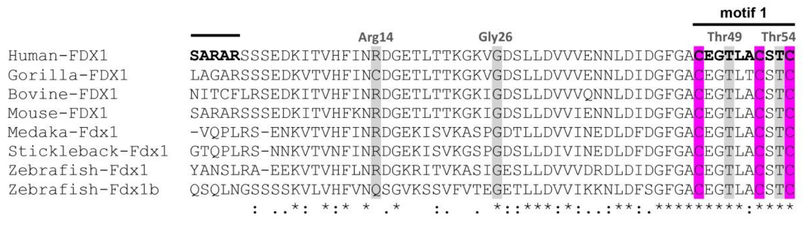


Material and methods

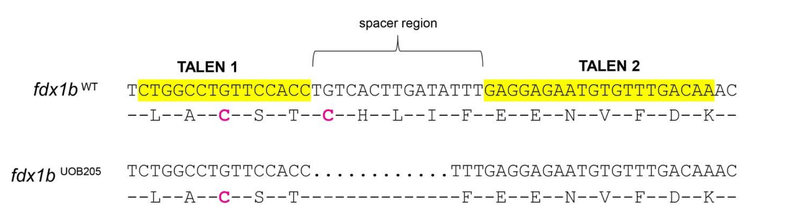
Establishing a fdx1 null-allele zebrafish mutant line using Transcription Activator-like Effector Nucleases (TALENs)



From the duplicated zebrafish fdx1 genes (fdx1, fdx1b), fdx1b is mediating cortisol synthesis



Fdx1b binding TALEN sites target the conserved motif 1 including cysteine residues for Fe/S binding



Generation of an allele (fdx1b^{UOB205}) with a 12 bp in-frame deletion removing a conserved cysteine in motif 1