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Identification of a duplicated P450 side-chain cleavage enzyme (Cyp11a2) defines initiation and maintenance of interrenal steroidogenesis in zebrafish

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Background

Cytochrome P450 side-chain cleavage enzyme (CYP11A1) catalysis the first and rate-limiting step in steroidogenesis, the conversion of cholesterol into pregnenolone. Current data suggests that zebrafish Cyp11a1 is the human ortholog facilitating steroidogenesis in the zebrafish interrenal gland (counterpart of the mammalian adrenal gland), gonad and brain. By database mining we have identified a duplicated zebrafish *cyp11a* gene designated as *cyp11a2*, sharing an 80% protein identity with Cyp11a1.

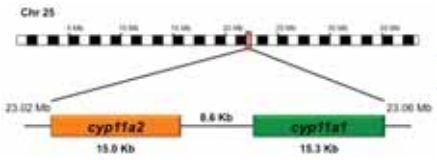
Aim: To characterise the spatio-temporal expression and enzymatic properties of the two zebrafish *cyp11a* orthologs using *in vitro* and *in vivo* studies.

Conclusions

- **Cyp11a1 is essential during early zebrafish development.**
  cyp11a1 is expressed in early zebrafish embryos and showed a significantly reduced, although essential, *in vitro* enzymatic activity compared to human CYP11A1.
- **Cyp11a2 is the functional ortholog of human CYP11A1.**
  cyp11a2 expression is only detected after the interrenal gland is formed.
  In vitro Cyp11a2 activity is similar to human CYP11A1.
  Cyp11a2 deficiency is associated with cortisol insufficiency and metabolic abnormalities.
- **Overall, this study proves the value of zebrafish as a comprehensive *in vivo* model in translational research of adrenal disease.**

Results

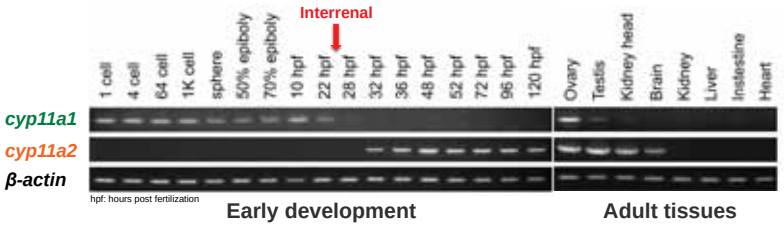
Zebrafish *cyp11a* paralog genes



Cyp11a1 *vs* Cyp11a2: 80% protein identity

Cyp11a *vs* hCYP11A1: 45% protein identity

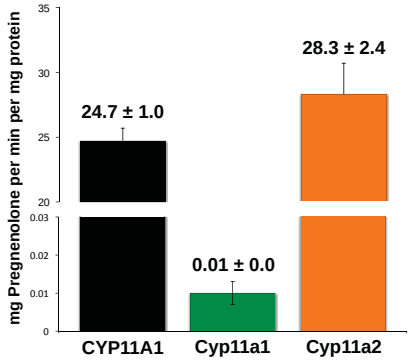
Spatio-temporal *cyp11a* expression

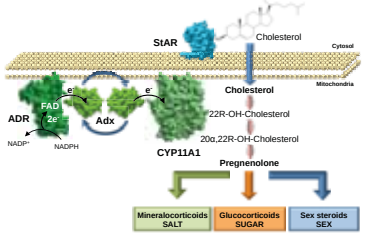


***cyp11a1* is expressed during early zebrafish development and in adult gonads**

***cyp11a2* is expressed after the interrenal gland develops and in adult steroidogenic tissues**

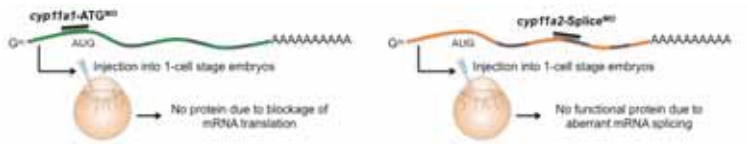
Cyp11a *in vitro* enzyme activity



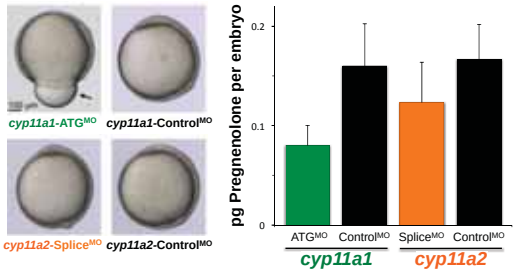


Cyp11a1 enzyme activity is significantly lower than human CYP11A1 and Cyp11a2 activities

In vivo cyp11a knockdown studies

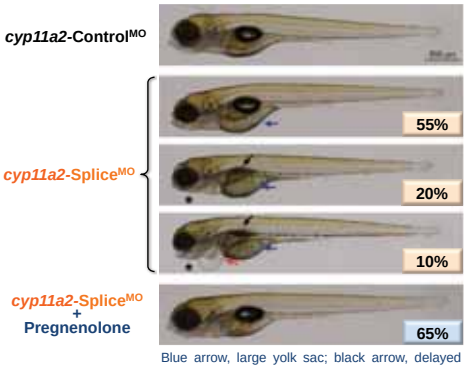


At 10 hours post-fertilisation



***cyp11a1* knockdown** results in **epiboly abnormalities** and **decreased pregnenolone** synthesis during early zebrafish development

At 120 hours post-fertilisation

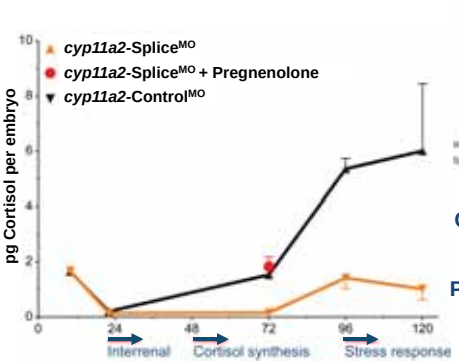


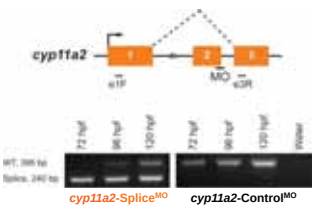
Blue arrow, large yolk sac; black arrow, delayed inflation of swim bladder; red arrow, pericardiac oedema; asterisk, craniofacial malformations.

Cyp11a2 deficiency is associated with **late-onset metabolic abnormalities** in zebrafish larvae

Pregnenolone rescues the phenotype in Cyp11a2 deficient larvae

Cortisol synthesis in *cyp11a2* morphants





Cyp11a2 deficiency impairs *de novo* cortisol synthesis

Pregnenolone supplementation rescues cortisol synthesis in *cyp11a2* morphants

Methods

cyp11a expression was determined by RT-PCR. Functional assays were performed using COS7 cells transiently co-overexpressing zebrafish *cyp11a* or human *CYP11A1* and adrenodoxin cDNAs. CYP11A1 enzyme activity was assessed by the conversion of 22R-hydroxycholesterol into pregnenolone. Pregnenolone was measured by LC/MS/MS. *In vivo cyp11a* knockdown studies were performed by injecting specific antisense morpholinos in 1-cell stage embryos. Rescue experiments on *cyp11a2* morphants were performed by supplementing fish media with 50 nM pregnenolone from 10 hours post-fertilisation. Untreated or pregnenolone supplemented *cyp11a2* morphants and mismatch controls were collected at different developmental stages for steroid extraction. Collected embryos were homogenized and steroids were extracted in dichloromethane. Cortisol was measured in controls and morphants by LC/MS/MS.

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