Strengths and limits of transgenic zebrafish models to study the expression and the perturbation of steroidogenic genes by endocrine disrupting chemicals

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Small fish species such as zebrafish (Danio rerio) are well-recognized models to identify Endocrine Disrupting Chemicals (EDCs), quantify their effects and explore their modes of action. Transgenic zebrafish models can provide reliable and suitable tools to assess EDCs while reducing the costs and the number of animals. The present work intends to review data recently obtained on new transgenic models that we developed to study the expression and the perturbation of several target genes involved in the biosynthesis of hormones and known to be affected by EDCs. These new transgenic lines express either fluorescent (Green or Red Fluorescent Protein) or luminescent (luciferase) proteins under the control of zebrafish promoters of steroidogenic genes coding for Cyp11c1 (11β-hydroxylase), Cyp19a1a (Aromatase A) or Cyp19a1b (Aromatase B). These genes are known to be expressed in various steroidogenic tissues and to play critical roles in the biosynthesis of glucorticoids, androgens and estrogens. Using *in vivo* fluorescence imaging techniques on whole organisms, tissue sections and immunostaining with specific antibodies, transgenes were fully characterized at the cellular and tissue levels demonstrating that the fluorescent reporters perfectly mimic the endogenous protein expressions. At embryo-larval stages, cyp19a1b is expressed in radial glial cells of developing brain while no expression of cyp19a1a and cyp11c1 was found in brain. At these stages, GFP under the control of cyp11c1 promoter was detected in the inter-renal cells. In adults, cyp11c1, cyp19a1a and cyp19a1ab were expressed in gonads of males and females. Exposures of these lines to different compounds revealed a differential regulation depending on the promoter. Given the high auto-fluorescence of some tissues and/or some cells, recoding the reporter fluorescence can be difficult to perform but can be overcome by using luciferase reporter. These newly developed transgenic lines provide relevant models that allowed us to acquire new data on the expression of key steroidogenic genes in zebrafish and their perturbation by endocrine active compounds.

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Improving genome annotation and assembly as a pre-requisite for functional genomics in non conventional models

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The recent advances in functional genomics radically changed the scale and resolution of modern biology, and it holds many promises in the field of comparative endocrinology, often targeted at non conventional models. Functional post-genomic analyses require high quality genome assembly and annotation, and such levels have only been achieved for a few reference genomes. In contrast, most newly completed genome projects produce draft genome assemblies and annotations with (often) hundreds of thousands of fragmented scaffolds and numerous gap, which provide some estimation of the gene repertoire but poorly describe many functional components (location of promoters, gene boundaries...). The quality of these draft genomes may be enough for simple functional genomic analysis.
We used functional genomics to characterize the regulatory network underlying thyroid hormones signaling in the context of Xenopus tropicalis metamorphosis. However, the poor quality of genome assembly and annotation quickly became limiting and prevented accurate data processing and analysis. For example, numerous sequence reads were located outside of genes, thus impacting the quantification of gene expression by RNA-Seq. We thus undertook to re-assemble and re-annotate the genome assembly with paired-end-tags (PETs) sequencing technologies. By using large insert DNA-PET, we reduced the level of genome fragmentation and reduced the number of scaffolds by 60%, correct assembly errors, estimate the real size of assembly gaps and derive a map of structural polymorphisms. We used RNA-PET to capture the 5' and 3' ends of transcripts, precisely demarcate gene boundaries, derive a map of transcription start sites and locate 5' and 3' UTRs. This was completed with conventional paired-end RNA-Seq to better describe the internal structure of genes. These improved references were critical for the processing and biological interpretation of RNA-Seq, ChIP-Seq and ChIA-PET data.

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