546. European project AQUA-FAANG: the epigenetic landscape of the Atlantic Salmon; focus on liver tissue

M.-O. Baudement*, G. B. Gillard, T. Podgorniak, L. Grønvold, S. Lien and M. P. Kent

Centre for Integrative Genetics (CIGENE), Faculty of Biosciences, Norwegian University of Life Sciences, 1430 Ås, Norway; marie-odile.baudement@nmbu.no

Abstract

As part of the European project AQUA-FAANG, we produced a body map of the epigenetic and regulatory landscape of Atlantic salmon, spanning different tissues in both sexes and at two stages of maturity. For this purpose, we developed and applied refined protocols for Chromatin ImmunoPrecipitation assays (ChIP-seq) and Transposase-Accessible Chromatin assays (ATAC-seq), complemented with RNA sequencing (RNA-seq). Our design of combining epigenetic and gene expression data sourced from the same individuals provides detailed insights into genome regulatory mechanisms. We showcase results here by comparing regulatory differences between liver of both mature and immature male and female salmon. We identify that differential expression of genes is associated with chromatin histone modifications and/or accessibility dichotomy between sex or maturity.

Introduction

The European project AQUA-FAANG aims to describe genome regulation of the most commercially important fish species in European aquaculture with refined protocols, which in turn will promote a better understanding of the biology of these organisms to improve fish production, welfare and commercial product quality (description of the project available at: https://www.aqua-faang.eu).

As part of this project, we will perform an epigenetic and regulatory analysis across different tissues, sexes and maturity states of the Atlantic salmon. We sampled five tissues (liver, brain, gonad, muscle, gill) from three mature and three immature male and female individuals (12 fish total, 60 samples) to analyse histone modifications by ChIP-seq (H3K4me1, H3K4me3, H3K27ac, and H3K27me3), chromatin accessibility by ATAC-seq and RNA-seq for gene expression. We describe here our results from liver tissue as a proof of concept. We find examples of highly expressed genes (*GYS2*), gender specific expression (*lipocalin*) as well as differentially expressed genes related to maturity (*EVLA*, *nAChRα4*). Together, these results corroborate the high quality of the genomic information obtained through our protocols and analysis, and future ability to describe the epigenetic landscape.

Materials & methods

Dissection of Atlantic salmon. Fish were euthanised by an overdose of tricaine methanesulfonate and sexed. Tissues were dissected, snap-frozen and conserved at -80 °C.

Chromatin ImmunoPrecipitation assays. Tissues were dissociated by dounce homogenization and crosslinked with 1% formaldehyde. Glycine was added and material was washed and recovered by centrifugation. The nuclei were resuspended with sonication buffer containing 1% Sodium Dodecyl Sulfate. Sonicated material was purified by proteinase K treatment and columns, quantified by Qubit and fragmentation was assessed using a Bioanalyzer. Tubes containing fragments with expected size (200-700 base pairs; 350 median) was diluted and used for immunoprecipitation. Detailed protocol available at: NMBU_SOP_ChIP_protocol

R.F. Veerkamp and Y. de Haas (eds)

DOI: 10.3920/978-90-8686-940-4_546, © M.-O. Baudement *et al.* 2022

Transposase-accessible chromatin assays. Tissues were dissociated by dounce homogeneization. Nuclei were recovered, exempt from mitochondria contamination, after slow centrifugation in iodixanol gradient and washing step. 50,000 purified nuclei were resuspended with reaction mix containing transposase (100 nM final). Transposed DNA was then cleaned-up, PCR-amplified and assessed using a Bioanalyzer. Detailed protocol available at: NMBU_SOP_ATAC_protocol

Gene expression assays. PolyA RNA was isolated and purified with RNAeasy kit from pieces of tissues conserved in RNAlater buffer. RNA quality was assessed using a Bioanalyzer and Nanodrop. Detailed protocol available at: NMBU_SOP_RNAextraction_protocol

Library preparation and library sequencing. ChIP libraries were prepared with the Microplex v3 kit from Diagenode. ATAC libraries were prepared with NEBNext Ultra II Q5 Mater mix of Illumina. PolyA RNA libraries were prepared with NEBNext Ultra directional RNA Library Preparation kit for Illumina. All libraries were sequenced with an Illumina NovaSeq 6000 (150 base pair, paired end reads).

Library alignment, quality assessment, peak-calling, differential analysis, and repository. Sequencing data was processed through relevant nf-core pipelines (available at: https://nf-co.re); chipseq, atacseq or rnaseq, to produce results for data quality, read alignments, peak annotations, and gene expression analysis. Libraries of satisfactory quality were submitted to ENA (project PRJEB47410). The latest Ensembl rapid release genome of the Atlantic Salmon (v3) was used for alignment and gene annotations (available at: Salmo_salar_GCA_905237065.2)

Results

Assessment of epigenetic data generated. In this project we have generated and sequenced more than 300 libraries for Atlantic salmon (ChIP, ATAC and RNA). We showcase here a subset of results obtained for liver. To assess if our adaptation of ChIP and ATAC methods for this fish species was reliable (Table 1). We evaluated the similarity of the different ChIP libraries (H3K4me3 and H3K27ac peaks) generated by calculating the Jaccard index (Figure 1A). We saw as expected a progressive reduction of correlation from replicates with the highest correlation to histone modification showing independency of signals, with intermediate correlation by sex or maturity. We saw a large proportion of ChIP peaks overlapping with genomic features as expected (Figure 1B), with a concentration surrounding gene promoters and the transcription start sites (TSS). We saw relatively less concentration of ATAC peaks within promoters.

Table 1. Metrics about ChIP-seq data generated for liver tissue.

Type of library	Number of libraries[1]	Average number of read pairs (millions)		Number of peaks detected (thousand)[2]
		sequenced	aligned	
ChIP-H3K4me3	8	57 (8 SD)	31 (5 SD)	29 (3 SD)
ChIP-H3K27ac	8	56 (7 SD)	30 (5 SD)	27 (10 SD)
ATAC	12	49 (8 SD)	24 (4 SD)	146 (25 SD)
RNA	12	32 (2 SD)	30 (2 SD)	-

[1] Two sexes × two maturity stages × two replicates for ChIP, or three replicates for ATAC and RNA.

[2] MACS2 narrow peak detection for H3K4me3 and H3K27ac, broad peak for ATAC.

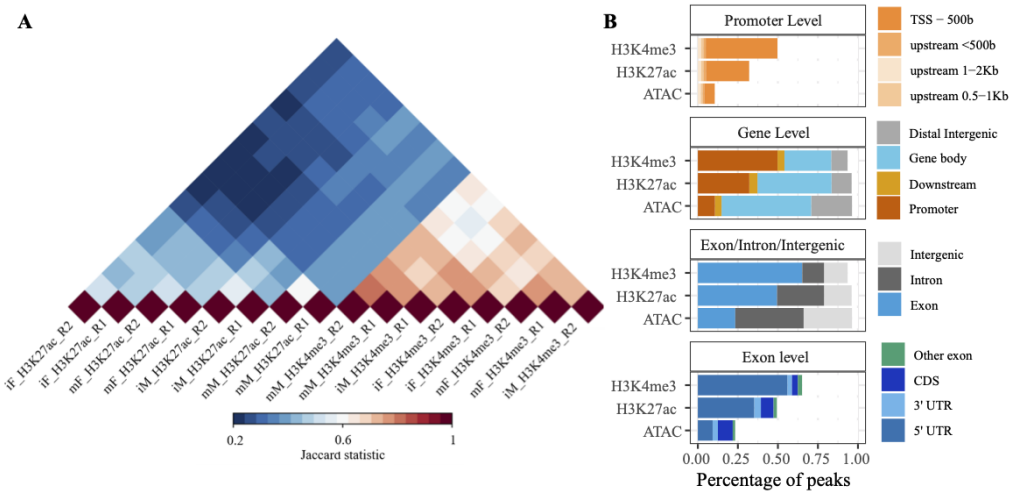

Figure 1. Assessment of peaks detected by ChIP and ATAC in liver. Similarity of peak datasets based on Jaccard statistics for two ChIP marks, H3K27ac and H3K4me3, across different liver libraries: immature female (iF), mature female (mF), immature male (iM), mature male (mM), and replicates (R1 and R2). B. Percentages of ChIP and ATAC peaks overlapping different genomic features.

Sex and maturity specific gene expression relate with chromatin environment. Differentially expressed genes (DEGs) between sex and/or maturity were identified (Figure 2). The chromatin state surrounding these DEGs was assessed by viewing H3K4me3, H3K27ac and ATAC peaks as tracks in a genome browser, alongside RNA expression levels. The gene *GYS2*, coding for a glycogen synthase, was found highly expressed in the liver of all individuals, in agreement with earlier studies in Masu salmon [Furukawa *et al.*, 2018] A gene coding for a lipocalin-like protein, suspected to be involved in sex(male) specificity in Tilapia [Shirak *et al.*, 2008] was found to be specifically expressed in males in our data. The gene *EVLA*, coding for Ena/Vasp protein is reported here has being expressed only in mature liver (independent of gender). EVLA has already been described to be involved in cytoskeleton remodelling (Ena) [Sechi and Wehland, 2004] and gluconeogenesis (vasodilator-stimulated phosphoprotein Vasp) (Tateya *et al.*, 2019), but a possible maturity-specific expression of this gene has not been reported previously. Lastly, a gene coding for a cholinergic receptor, nAChRα4, is presented here as being expressed specifically in immature individuals. Together, this first data exploration shows the high potential of better characterisation and new discoveries of genes potentially important for aquaculture.

Conclusions

We showcased here a first look at linking gene expression with the epigenetic landscape. Deeper exploration of this dataset will help to establish a better understanding of gene regulation of the salmonid genome as well as the discovery of new traits crucial for the sustainability of the fish industry for the coming years.

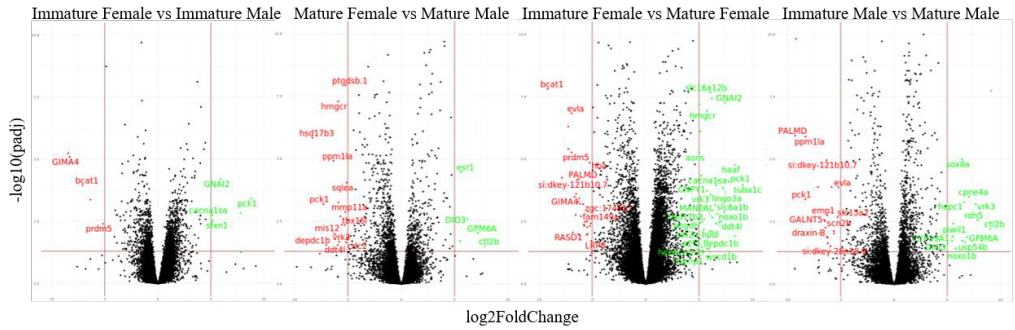

Figure 2. Differentially expressed genes in liver. Volcano plot highlighting genes highly differentially expressed between maturities or sexes in liver. Down-regulated (in red) and up-regulated genes (in green) between conditions are highlighted (FDR adjusted *P*-value<0.5 and absolute log2 fold change >5). For an overview, we display results within x and y limits up to an absolute value of 10.

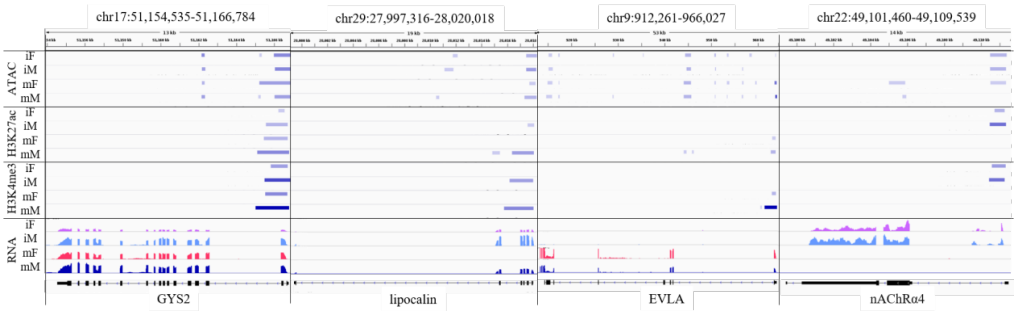

Figure 3. Epigenetic patterns of differentially expressed genes. Peaks from ChIP (H3K4me3, H3K27ac) and ATAC data are observed alongside RNA expression levels for select genes expression specificity: *GYS2* (no specificity), *lipocalin* (male-specific), *EVLA* (mature-specific) and *nAChRα4* (immature-specific).

References

Buenrostro J., Wu B., Chang H., and Greenleaf W. (2016) Curr Protoc Mol Biol 109:21.29.1–21.29.9. https://doi.org/10.1002/0471142727.mb2129s109

Chawla A., Nagy C., and Turecki G. (2021) IJMS 22(14) 7612. https://doi.org/10.3390/ijms22147612

Furukawa F., Irachi S., Koyama M., Baba O., *et al.* (2018) CBPA 225:74–82. https://doi.org/10.1016/j.cbpa.2018.07.003

Ma S. and Zhang Y. (2020) Mol Biomedicine 1(1) 9. https://doi.org/10.1186/s43556-020-00009-w

Sechi A. S. and Wehland J. (2004) Frontiers in Bioscience-Landmark 9(2) 1294-1310. https://doi.org/10.2741/1324

Shirak A., Golik M., Lee B-Y., Howe A. E., *et al.* (2008) Heredity 101:405–415. https://doi.org/10.1038/hdy.2008.68

Tateya S., Rizzo-De Leon N., Cheng A. M., Dick B. P., *et al.* (2019) PloS ONE 14(4):e0215601. https://doi.org/10.1371/journal.pone.0215601