A biologically based computational model for the hypothalamic-pituitary-thyroid (HPT) axis in *X. laevis* larvae

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Supplemental Information SI3a

Thyroid gland morphometric parameters during larval development in *Xenopus laevis*

Histological images of thyroid glands in a model amphibian, *Xenopus laevis*, were generated for a 2009 publication aimed at standardizing histopathological assessments for effects of thyroid disrupting chemicals. Although the images were originally utilized as a baseline for subjective pathology severity scoring, the images contained many useful morphological features that could be evaluated quantitatively to inform computational model parameterization. The images were utilized here to digitally measure the following gland morphological features through a critical developmental period of thyroid-mediated metamorphosis: total gland area at the approximate largest cross-sectional area, follicle number, total follicle area, follicular lumen area, follicular cell area, follicular cell nuclei area, follicular nuclei count. Based on these measurements, estimates of generalized gland morphometry could be made by each focal Nieuwkoop and Faber (NF; 1994) developmental stage. These data are collected to provide physical parameter estimates for computational modeling of amphibian thyroid physiology.

Data description

Although the 41 image files containing the thyroid gland sections and measurement layers are provided, they are not themselves the data nor figures. The raw and summarized data are provided as an Excel workbook in Supplemental Information (Section SI3b) with each of 15 tabs/spreadsheets corresponding to an individual image/replicate with associated raw data and within-replicate averages for each. There is also a “Summary” spreadsheet that conglomerates all the individual replicate data and then

presents the averages per developmental stage (n = 3). Summary graphs for each parameter over the developmental window are embedded within the summary spreadsheet.

Experimental design, materials, and methods

The image file to be analyzed was imported into Image-Pro Premier. A spatial calibration for the image was created using the scale bar that was embedded into each image file. The calibration was verified by using the software to measure the scale bar after spatial calibration, and the new measurement was embedded onto the image next to the scale bar. Using the polygon measurement tool, the gland area was measured, and was designated as "Gland" under the class tag. Next, the follicle, colloid space, and nuclear areas for each follicle was measured and assigned the appropriate class tag. When all features had been measured for every follicle in the gland, the data was exported from Image-Pro Premier into a new sheet in a Microsoft Excel spreadsheet. Then, all measurement drawings were embedded onto the image file along with the scale bar calibration measurement. The new image file with measurement drawings was exported as .jpeg file and inserted onto the Excel sheet with the measurement values.

In the Excel sheet, measurements were grouped for each follicle, follicular lumen, and associated thyrocyte nuclei using an alternating fill coloration of cells for each follicle group. This aided in the visual identification of individual follicle data in an expansive spreadsheet. A summary table was created for each follicle, and area values were either transferred or calculated for follicle, follicular lumen, thyrocyte total area, thyrocyte nuclear area, and thyrocyte cytoplasmic areas, plus thyrocyte nuclei counts. The tables were grouped together, then several calculations were performed for each gland:

1. Calculated averages for follicle area, lumen area, thyrocyte area, nuclear area, and cytoplasmic area.
2. Total values for follicle area, lumen area, thyrocyte area, nuclear area, and cytoplasmic area.
3. Total numbers of follicles and nuclei.

After the data on each image file was reviewed and verified for correctness, a new sheet was created to summarize the measurement data listed above across the 3 gland measurements for each NF stage and included all the NF stages evaluated. The average and standard deviation was calculated for each gland, and line graphs for each measured value vs. NF stage was created to visualize the differences between the NF stages.

There were some noted issues with nuclear count and area measurements. In regions that contained multiple cellular layers, the cellular and nuclear membranes that were apparent and in sharp focus were used for measurements. In regions with nuclei overlap, individual nuclei were identified and measured as determined by nuclear membranes. Where individual nuclei could not be identified, the nuclear measurement included multiple nuclei. The small numbers of thyrocyte nuclei that were actively dividing were included in the analysis. The gland total areas increased through the NF stages analyzed. Total gland area in the 200x magnification images 56-03, 57-01, 57-02, 57-03, 58-02, and 58-03 exceeded image boundaries. Total gland area measurements for these samples were acquired through analysis of the 40x image file of the same bilateral gland sections as the 200x images.

The data quality review included visual verification that confirmed each follicle, colloid space, and associated thyrocyte nuclei were grouped appropriately by alternating the fill coloration of cells in the spreadsheet.

References

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