DIEL CHANGES IN THE MOLECULAR PHYSIOLOGY OF RAINBOW TROUT GILL

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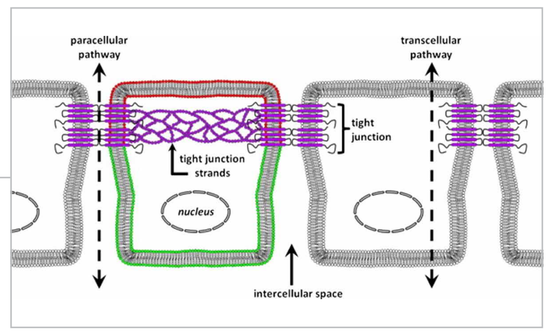

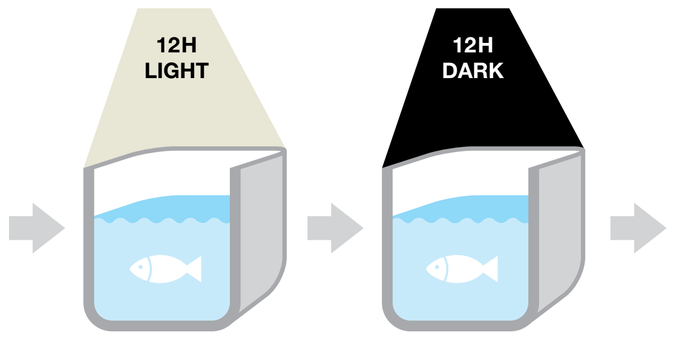

Abstract

This study measured transcript abundance of tight junction (TJ) proteins, core-clock genes and osmoregulatory hormone receptors in rainbow trout (Oncorhynchus mykiss) gills after fish were exposed to 12:12 h Light:Dark cycles. Results from real time qPCR showed that transcript encoding ocln, tric, cldn-6, -28b, -31 mRNA showed statistically significant oscillations while the abundance of cldn-8d, -10c, 32a did not change. Several core-clock gene homologs (clock2, per1 and per2) had diel rhythms that peaked at the dark-to-light transition or mid-light, while cry3 abundance did not show a significant change. Also transcript abundance of gr-1, -2 (glucocorticosteroid receptors) and mr (mineralocorticoid receptor) did not significantly differ over the 24 h period, but prl-r (prolactin receptor) peaked during the middle of the dark cycle. In addition, 11β-hsd and mel-1a (melatonin receptor) peaked at the dark-to-light transition and showed no significant change, respectively. Data suggest that diel changes in select TJ proteins of the rainbow trout gill epithelium, and factors that might influence TJs, occur.

Research Question

Does photoperiod induce diel changes in transcript abundance of ionoregulatory factors extracted from rainbow trout gills?

Results & Conclusion

The proposed hypothesis stating that diel changes in the expression of TJ proteins, core-clock genes and other TJ complex regulatory factors will be observed in the gill was supported by findings in this report. Overall, the abundance of more than half of the gene transcripts examined in this study had statistically significant rhythms. Another main finding was that TJ protein transcript abundance oscillated in gill tissues, highlighting that even though the gill is not directly exposed to light, molecular processes in the gill follow a rhythm that is entrained by light; possibly speaking to the many roles that the gill carries out in order to maintain homeostasis. Interestingly, all transcripts had an increased abundance either in the middle of the dark phase (cldn-28b, -31, and prl-r); at the dark-to-light transition (ocln, tric, clock2, per1, 11b-hsd); or in the middle of the light phase (cldn-6, -31 and per2); but never at the light-to-dark transition. This suggested that increased gene expression occurred in anticipation of light, or in the first part of the light phase. These variations may also reflect the unique roles that TJ proteins have in regulating epithelial permeability. In fact, previous studies in cultured trout gill epithelia helped classify Tric, Ocln, Cldn-28b, and Cldn-31 as 'tight' TJ proteins that reduced paracellular permeability. In mammalian epithelia, Cldn-6 was also considered to be a barrier-forming TJ protein which restricted paracellular Na+ movement. Although Cldn-8d was also associated with gill epithelium tightening, transcript abundance remained constant during the 24 hour cycle.

Another finding was that the period of several gene transcripts was not exactly 24 h. For example, the abundance of cldn-31 had a period of 14h while the abundance of cldn-6 had a period of 30h. This may indicate that while the expression of certain genes is cyclical, it may not be entrained by 12h: 12h light and dark phase, which usually synchronizes rhythms to a 24h period. In sum, the findings in this study tell a more complete story of how the TJ complex changes with time, emphasizing that not only is the molecular physiology of the gill in a dynamic state, but also, that this dynamic state is entrained by light, an abiotic factor.

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Methodology

Fish were acclimated to 12:12 h Light:Dark cycles for 2 weeks and fed randomly after which 10 fish were sampled every 4 h for a total of 24 h cycle. Transcript abundance was measured in whole gill extracts using real time qPCR. Diel rhythms were determined using One Way ANOVA and Cosinor Analysis.