

A12.14 FUNCTIONAL CHARACTERIZATION OF HEMOGLOBIN ISOFORMS FROM HIGH AND LOW ALTITUDE GEESE SPECIES

WEDNESDAY 5 JULY, 2017 14:50

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Each year bar-headed goose (*Anser indicus*) migrate over the Himalaya to overwinter in India. Energetically demanding flap flight at altitudes where the oxygen is reduced to approximately 30% of sea level is an astonishing achievement in part due to an adaptive increase in the oxygen affinity of bar-headed goose hemoglobin (Hb), compared to that of lowland greylag goose (*Anser anser*). Although avian red blood cells contain two Hb isoforms, HbA and HbD, the oxygen affinities of Hb isoforms of bar-headed and greylag goose have not been characterized separately yet. In the present study, we used equilibrium methods to characterize in detail oxygen-binding and allosteric properties of purified HbA and HbD from bar-headed and greylag goose, in presence and absence of the allosteric effector inositol hexaphosphate (IHP), an analogue of the avian red blood cell organic phosphate. Both bar-headed goose Hb isoforms displayed higher intrinsic oxygen affinity than the respective greylag goose Hbs. This difference in Hb oxygen affinity between the species was even more marked with added IHP, which reduced oxygen affinity at least 9-fold. Curve fitting analyses according to the two-state MWC model indicated that the effect of IHP was primarily due to stabilisation of highly labile tense state molecules. Moreover, IHP stabilised low-affinity tense and high-affinity relaxed Hb tetramers which otherwise tended to dissociate to dimers. The present study provides a novel detailed analysis on the allosteric regulation properties of geese isoHbs explaining the high oxygen affinity of bar-headed goose hemoglobins at a molecular level.

A12.19 IONS BEFORE OXYGEN: LARVAL LAMPREYS AND PROTOCHORDATE REPRESENTATIVES QUESTION THE ORIGINS OF CHORDATE GILL FUNCTION

WEDNESDAY 5 JULY, 2017 15:45

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The gill is believed to have played a key role in the adaptive radiation of early chordates by supplanting the skin as the dominant site for oxygen uptake. In this untested hypothesis, shifting oxygen uptake to the gill relaxed constraints associated with oxygen uptake at the skin, thus allowing chordates to increase body size, dermal

thickness and activity. Interestingly, larval teleosts undergo similar increases to body size, dermal thickness and activity during development. However, the larval teleost gill first supplants the skin as the dominant site for ion flux well before oxygen uptake. Here, we want to determine if the gill also became critical for ion flux before oxygen during early chordate evolution. We use larval lampreys as a representative system to test how increasing organism size might have affected early chordate gill function. We directly measure the flux of oxygen, carbon dioxide, ammonia and sodium simultaneously at the gill and skin of whole larvae in vivo across a range of body sizes (0.02-2.00 g). We find that sodium flux is dominated by the gill at much smaller body sizes than gas flux, even when challenged by hypoxia and temperature. Complimentary data collected in more ancestral protochordate representatives (amphioxus and acorn worms) also indicate a dominant role for the gill in ion flux before gas. Our results suggest that ion regulation rather than gas exchange might have been the primary function of the early chordate gill, questioning long-standing views of chordate gill origins and the constraints shaping early chordate evolution.

A12.20 INTERACTIVE EFFECTS OF SALINITY AND AMMONIA STRESS ON MARINE FISH: INSIGHTS FROM GENOME-WIDE TRANSCRIPTIONAL ANALYSIS

WEDNESDAY 5 JULY, 2017 16:00

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The objective of the present study was to elucidate genome-wide transcriptional responses underlying reduced seawater salinity challenge and ammonia toxicity as single and combined factor in European seabass (*Dicentrarchus labrax*). Fish were progressively acclimated to normal seawater (32 ppt) and reduced seawater salinity (10 ppt). Following acclimation to these salinities for two weeks, fish were exposed to high environmental ammonia (HEA, 1.18 mM representing 50% of 96 h LC₅₀) for 12h, 84h and 180h. RNA sequencing (Illumina HiSeq platform) of brain tissue samples from the experimental conditions yielded a total of 12,490 contigs, out of which 2,128 were significantly modulated in any of the applied treatments (salinity, HEA or combination). Low salinity resulted in the up-regulation of 370 and down-regulation of 832 contigs, whereas in HEA exposure 122 contigs were up-regulated and 270 contigs were down-regulated. While looking for the combinatorial effects, we found that 74 contigs were commonly up-regulated and 94 were down-regulated in both these treatments. Gene enrichment analysis revealed that genes differentially expressed in response to salt stress were highly enriched for Na⁺ transporter, cell adhesion and communication, transmembrane signalling receptor, Na⁺/K⁺-ATPase and oxidative stress. Genes which were differentially expressed to HEA showed enrichment in amino acid biosynthetic/metabolic process, anti-oxidant system, neurotransmitter and ion regulation. In combination treatment, ion transporters, oxidative and anti-oxidant related genes were highly represented. Overall, our data highlights the complexity of transcriptional responses which are triggered when fish face individual and/or combinatorial environmental challenges.