Hemoglobin Control at Low Oxygen Levels in Cod Explained

The national effort leading to the complete sequencing of the cod genome will undoubtedly result in many new ways of understanding this important fish species. For Øivind Andersen at Nofima Marine at Ås and his recent PhD student Ola Frang Wetten, now at Hedmark University College, participation in the cod genome project gave them the opportunity to find answers to questions tracing back 50 years.

Atlantic Cod. Photo: Hans-Petter Fjeld (from Wikipedia.)

It was in 1961 that Knud Sick, working at the University of Copenhagen, reported in Nature on polymorphic hemoglobins (Hb) in Atlantic Cod, using electrophoresis to detect different Hb forms. In this and later reports, Sick documented Hb differences in populations of cod harvested from different regions in the North Sea and others areas in the North Atlantic. Sick designated two different alleles in his study; one named HbI1 which had an allele frequency corresponding to 61% in the western Baltic Sea, for example, compared to only 3% in the eastern Baltic Sea where the other allele named HbI2 predominated.

In discussing his work, Sick cited several unanswered questions involving his findings; for example, what component of Hb (α versus β subunit) is responsible for the polymorphism, were the electrophoresis methods used sufficient to distinguish different Hb polypeptides, do the different Hbs have different adaptive advantages, are the differences in populations solely caused by genetic differences or does temperature, for example, affect gene expression, as well.

Research following up on Sick's pioneering studies, including key publications involving both Andersen and Wetten, has provided answers to these questions. As far as the question of whether it is the α or β subunit of Hb that is responsible for the observed polymorphism, a publication by Andersen et al. (2009) used PCR to clone and sequence both Hb α or β sequences from reverse-transcribed erythrocyte mRNA. Two different α chains (Hb-α1 and Hb-α2) and four different β chains (Hb-β 1, Hb-β 2, Hb-β 3 and Hb-β4) were found. Based on their coding sequences, amino acid differences were identified that justify the conclusion that it is the Hb-β1 that accounts for the variability observed by Sick with a Met55–Lys62 variant accounting for the HbI-1 allele and a Val55–Ala62 variant accounting for the HbI-2 allele. Cod homozygous for the Met55–Lys62 allele are HbI-1/1 whereas those homozygous for the Val55–Ala62 allele are HbI-2/2. Cod with both alleles are heterozygous HbI-1/2.

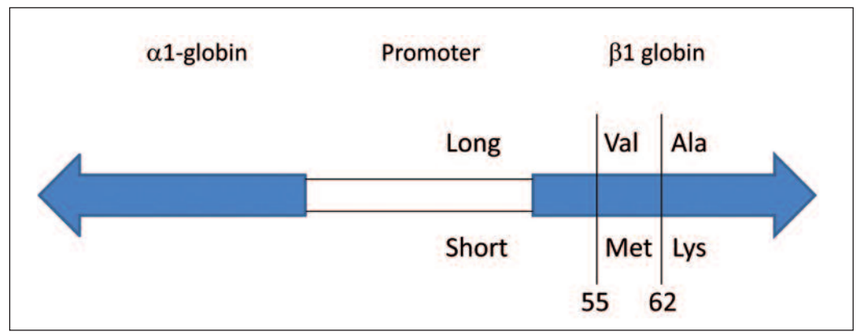

Figure 1. Schematic of the head-to-head orientation of α1-globin and β1-globin genes together with the intergenic promoter region. Two promoter types exist; one corresponding to the long promoter-Val-Ala variant and the other to the short promoter-Met-Lys variant. Diagram based on Star et al. (2011).

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Another question addressed by the Andersen et al. (2009) publication was the adaptive advantages of the HbI-1 allele versus the HbI-2 allele. To deal with this question, the authors used amino sequence information to model the 3D structures of the corresponding proteins in tetrameric Hb complexes. This analysis led to the idea that the Hb coded by the HbI-2 allele, corresponding to the Val55-Ala62 variant would have a higher affinity for oxygen compared to the Hb coded by the HbI-1 allele. This hypothesis fits nicely with the observed distribution of the HbI-2 allele in nature inasmuch as it is associated with low-oxygen waters such as the eastern Baltic Sea and colder Arctic waters of the Atlantic Ocean.

Of course, the completed sequence of cod confirmed Sick's earlier presumption that bands seen after electrophoresis really do represent unique Hb polypeptides. The sequence has also provided interesting information to fill out knowledge on how Hb genes are arranged on the genome and how they respond to temperature/oxygen variations. As far as the crucial Hb-β1 gene is concerned, it was found to be situated adjacent to the Hb-α1 gene in a head-to-head orientation and separated by an intergenic promoter region of about 1.7 kb that varies, depending on genotype. HbI-1 homozygotes with the Val55-Ala62 allele have a so-called 'long promoter' while HbI-2 homozygotes with the Met55-Lys62 allele have a so-called 'short promoter'. When Andersen and Wetten tested the strengths of these alternative promoters, using a luciferase gene expression system in salmon kidney cells, they found that the long promoter gave higher luminescence compared to the short promoter at both 15 C and 20 C. Given that the Hb coded by the Val55-Ala62 allele has lower oxygen affinity compared to Hb coded by the Met55-Lys62 allele, it makes sense that its promoter can compensate for this by elevating relative expression.

Ola Frang Wetten.

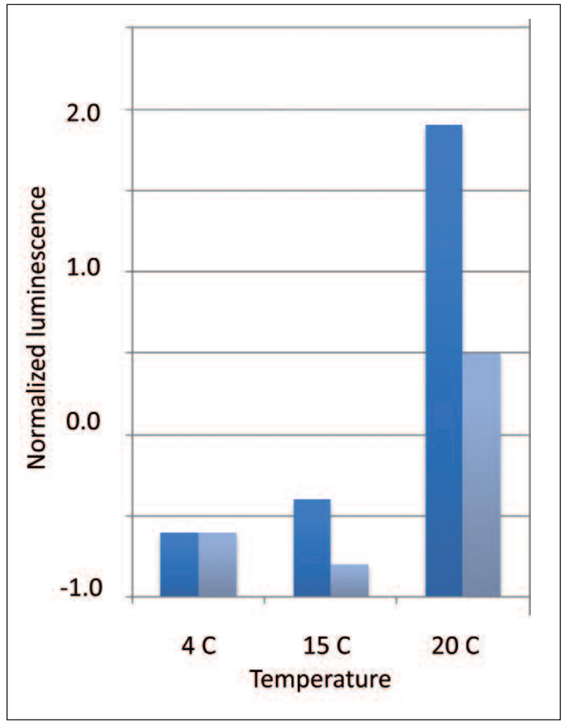

Figure 2. Normalized luciferase luminescence ratios in salmon kidney cells expressing luciferase based on either the long promoter (dark blue bars) or the short promoter (light blue bars) of the β1-globin gene. Data based on Star et al. (2011).

As the authors say (Star et al., 2011), this result "provides a compelling example of the coevolution of structural and regulatory adaptation, and highlights the relationship between temperature and functional molecular variation in the hemoglobin system".

References

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