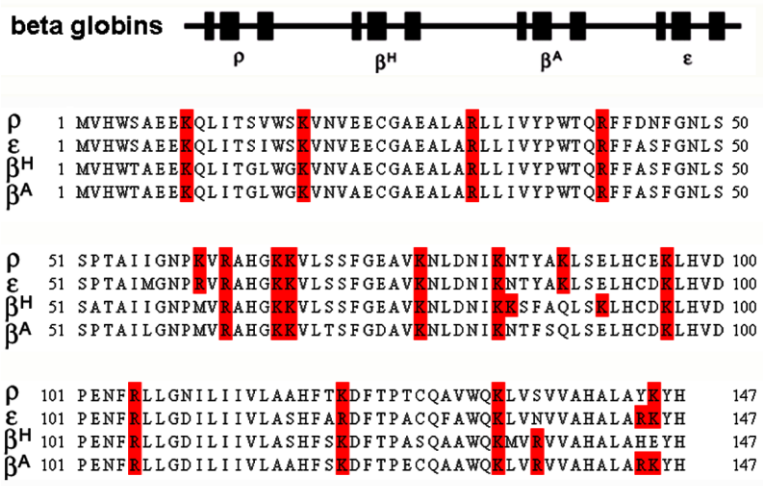
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Zeroing in on beta globins in the early embryo

May 5, 2008 – Our red blood cells, or erythrocytes, act as vehicle for conveying oxygen from the respiratory system to the cells of the body, a feat they achieve thanks to the ornate structure of hemoglobin, a molecule comprising alpha and beta globin subunits and an iron-based heme. There are multiple types of both alpha and beta globin, and different combinations of these types result in differing degrees of affinity for oxygen, which has implications in both embryonic development and adult physiology. In the chicken, the three subtypes of alpha globin (π, αD and αA) and the four beta globins (ρ, βH, βA and ε) show different spatiotemporal patterns of expression during the formation of the red blood system during embryogenesis, a process that plays out in two waves – an initial phase of primitive extraembryonic erythropoiesis followed by a second wave that takes place within the embryo. This transition is characterized by a shift from the embryonic beta globins (ρ and ε) to βA, the major adult beta globin. But due to technical limitations, attempts to study the details of this sequence of events have yielded somewhat conflicting results.



Each of the four beta globins has unique tryptic digestion pattern that can be used for mass-spectrometry based semi-quantitative assessment of its relative abundance.

Now, in a study published in *Developmental Dynamics*, Cantas Alev and colleagues in the Laboratory for Early Embryogenesis (Guojun Sheng; Team Leader) and other CDB labs have shown through protein analyses that βA is expressed even in the early stage of primitive erythropoiesis in the chicken.

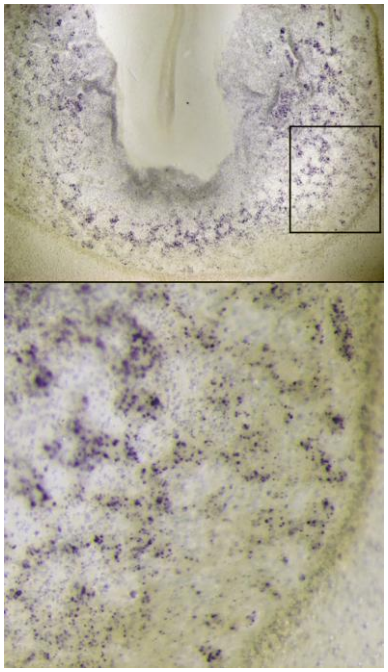
Previous reports using classical embryology and molecular biology had suggested that there was a clear separation between primitive (embryonic) and definitive (adult) beta globins, and that the transition between these two stages occurred as early as embryonic day 4 (E4) or as late as day 13. Analysis of mRNA transcripts, in particular, had indicated a switching on and rapid increase of βA expression in the period between days 5 and 7 of embryogenesis.

Alev et al., however, took a new approach to the question, first using mass spectrometry to sort out and quantify each of the globins from samples of embryonic blood. In contrast to previous reports indicating that βA expression is first detectable at around E2 (stage HH13 in the Hamburger-Hamilton system for evaluating chicken development), the team found that it is present as early as two days earlier, coincident with the primitive wave of blood development. Looking even earlier, they found signs of βA expression as early as HH8, with significant transcription by HH9. Interestingly, even at this early stage of development, the level of the purportedly "adult" beta globin βA was seen concurrent with that of the "embryonic" ρ subtype (although at a much lower

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

level). In situ hybridization further showed that, by HH10~11, βA beta globin is expressed in the extraembryonic mesoderm as well.

The findings from this multi-technique strategy are in general agreement with the scheme in which the transition from primitive (ρ and ε) to definitive (βA) beta globins occurs in the period of E5~7, but pushes back the start point for βA expression to the earliest stage of blood formation, suggesting that the distribution of the various subtypes may be a function of relative abundances rather than strict transcriptional control. "We still don't know what βA is doing in the primitive stage of hematopoiesis," admits Alev. "But it's interesting that the other adult beta globin, βH, is completely undetectable at that stage, so we'd like to try to work out how to account for these difference in the regulation of transcription."



Expression of betaA in primitive erythrocytes revealed by intron-specific in situ hybridization.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs