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Gene Duplication and Evolutionary Innovations in Hemoglobin-Oxygen Transport

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Abstract

During vertebrate evolution, duplicated hemoglobin (Hb) genes diverged with respect to functional properties as well as the developmental timing of expression. For example, the subfamilies of genes that encode the different subunit chains of Hb are ontogenetically regulated such that functionally distinct Hb isoforms are expressed during different developmental stages. In some vertebrate taxa, functional differentiation between co-expressed Hb isoforms may also contribute to physiologically important divisions of labor.

Gene Duplication and the Evolution of Novel Protein Functions

Gene duplication is known to play an extremely important role in the evolution of new protein functions. Following the complete duplication of a protein-coding gene, functional redundancy between the two daughter copies will often entail a relaxation of selective constraints that permits the accumulation of degenerative mutations in one or both copies (52, 107). In the majority of cases, one of the two gene duplicates will be rendered functionless by inactivating mutations. However, in a small minority of cases, the fixation of previously forbidden mutations may lead to the acquisition of a novel function and/or expression pattern in one copy or the other. In such cases, both duplicate copies may be selectively retained in the genome, and they can then evolve new functions or divide up ancestral functions.

The diversification of the vertebrate globin gene family provides an excellent example of the role of gene duplication in promoting evolutionary innovation. In this review I highlight several important case studies. First, I describe how the proto hemoglobin (*Hb*) and myoglobin (*Mb*) genes originated via whole-genome duplication in the common ancestor of vertebrates. This duplication event facilitated a physiological division of labor between O_2-binding proteins with distinct roles in respiratory gas transport. I then describe how repeated rounds of gene duplication and divergence promoted the functional diversification of the subfamilies of globin genes that encode the different subunit polypeptides of tetrameric Hb. These globin genes are ontogenetically regulated such that functionally distinct Hb isoforms (isoHbs) are expressed during different stages of prenatal development and postnatal life. I end by discussing the possible functional significance of Hb multiplicity in the definitive red blood cells of different vertebrate groups.

Phylogenetic Insights Into Gene Family Evolution

Phylogenetic reconstructions permit inferences about the branching relationships among homologous members of a multigene family that have diversified via successive rounds of duplication and divergence. In comparisons among different species, phylogenetic reconstructions provide a means of distinguishing different types of homology. Specifically, the congruence or lack of congruence between a species tree and the gene tree contained within it enables us to distinguish “paralogous” genes (which trace their common ancestry to duplication events) and “orthologous” genes (which trace their common ancestry to speciation events; that is, they descend from a common ancestral gene by phylogenetic splitting at the organsimal level) (**FIGURE 1A**).

The Role of Hemoglobin in Blood-Gas Transport

Hb is a red blood cell protein that plays an essential role in sustaining aerobic metabolism by transporting O_2 from the respiratory exchange surfaces (e.g., lungs, gills, or skin) to the cells of respiring tissues. In jawed vertebrates (gnathostomes), Hb is a tetrameric protein composed of two α-chain subunits and two β-chain subunits. Each of these subunit polypeptides contains a heme group: an iron atom at the center of a poryphyrin ring, which reversibly binds a single O_2 molecule in the ferrous state (Fe^{2+}). The related myoglobin (Mb) protein stores O_2 and facilitates intracellular O_2 diffusion from the sarcolemma to the mitochondria of cardiac and skeletal muscle cells (35, 93). In contrast to the tetrameric Hb protein, Mb is a monomer and is therefore structurally similar to a single heme-bearing subunit of Hb. Mb and the individual Hb subunits have similar heme-coordination chemistries, but Mb has a much higher O_2 affinity than Hb. This fulfills an important requirement of an efficient O_2-transporting system, since the storage molecule (Mb) should have a higher O_2 affinity than the carrier molecule (Hb) at the low P_{O_2} that prevails in the cells of aerobically metabolizing tissues.

The evolution of Hb as a specialized O_2-transport protein played a key role in the evolution of aerobic energy metabolism in early vertebrates. Without Hb to augment blood O_2 content, the fluid convection of physically dissolved O_2 in the blood plasma would not be generally sufficient to meet the cellular O_2 demands of relatively large, mobile vertebrates. The one remarkable exception to this rule are the Notothenioid icefish that inhabit the freezing, ice-laden waters surrounding the continental shelf of Antarctica. Notothenioid fish in the family Channichthyidae do not express Hb, and many species do not express Mb either (76).

The O_2-transport Hbs of ancestral vertebrates likely existed in a monomer-oligomer equilibrium as in modern-day lampreys and hagfish (see below), where cooperative O_2-binding stemmed from association-dissociation dynamics. In modern gnathostomes, by contrast, the efficiency of Hb as a specialized O_2-carrier molecule is chiefly attributable to its multisubunit quaternary structure. The interaction between unlike subunits gives rise to the cooperativity of Hb-O_2 binding, whereby O_2 binding of a given heme iron facilitates the binding of subsequent O_2 molecules at the remaining unliganded hemes, and, conversely, O_2 liberated by a heme iron facilitates the unloading of O_2 molecules from the remaining liganded hemes. Thus Hb has a high O_2 affinity at the sites of respiratory gas exchange (the alveoli of the lungs in humans and other mammals) where the P_{O_2} is high, and a reduced affinity at the sites of O_2 delivery in the tissue capillaries where the P_{O_2} is substantially lower. The physiological significance of cooperativity is that it permits efficient O_2 unloading over a relative narrow range of blood O_2 tensions. In addition to cooperativity, which results from interactions between subunits, the O_2 affinity of Hb is also modulated by the binding of allosteric cofactors at sites remote from the heme iron. These cofactors include H^+, Cl^-, CO_2, and a variety of organic phosphates, all of which preferentially bind and stabilize deoxy-Hb, thereby shifting the allosteric equilibrium in favor of the low-affinity “tense-state” quaternary structure (92, 93).

In addition to Hb's familiar role as an O_2 carrier, recent discoveries have revealed that Hb also plays a role in regulating blood flow in the arterial microcirculation. In this process, which may be important for

matching tissue perfusion to local O_2 demand, Hb functions as an O_2 sensor and O_2-responsive nitric oxide (NO) signal transducer, thereby contributing to red cell-dependent hypoxic vasodilation (47). This may be accomplished via enzymatic reduction of nitrite to NO by deoxy-Hb (18, 19, 31) and/or release of bioactive NO from *S*-nitrosylated Hb (2, 77, 108). Both of these proposed mechanisms of vasoregulation are governed by oxygenation-linked allosteric transitions in Hb quaternary structure. These findings suggest that vertebrate Hb has evolved physiologically important interactions with NO in addition to the more familiar interactions with CO, CO_2, and O_2.

Gene Duplication, Genome Duplication, and the Origin of Hemoglobin as an O_2 Carrier

Two rounds of whole-genome duplication in the stem lineage of vertebrates played an important role in promoting the diversification of the globin gene superfamily (37, 38, 42, 60, 80, 81). The progenitors of the *Hb* and *Mb* gene lineages originated as products of one such genome duplication event (42). The retention of the proto *Hb* and *Mb* genes in the ancestor of gnathostomes set the stage for a physiological division of labor between O_2-carrier and O_2-storage functions. In the ancestor of gnathostomes, subsequent duplication of the proto *Hb* gene gave rise to the progenitors of the α- and β-type globins (**FIGURE 1B**). This duplication event occurred ~450 million years ago, before the divergence between the ancestor of cartilaginous fish and the common ancestor of ray-finned fish and tetrapods (32, 42, 80, 81). Functional divergence of the proto α- and β-globin genes permitted the formation of multimeric Hbs composed of unlike subunits ($\alpha_2\beta_2$). The evolution of this heteromeric quaternary structure was central to the emergence of Hb as a specialized O_2-transport protein because it provided a mechanism for cooperative O_2-binding and allosteric regulatory control. Both of these features require a coupling between the effects of ligand binding at individual subunits and the interactions between subunits in the quaternary structure (64).

The ancestral linkage arrangement of the proto α- and β-globin genes is still retained in the genomes of some modern-day amphibians and teleost fish (27, 56). In amniote vertebrates, by contrast, the α- and β-type globin genes are located on different chromosomes (34, 42, 44). In the human genome, the α-globin gene cluster is located on *chromosome 16*, and the β-globin gene cluster is located on *chromosome 11* (**FIGURE 2A**). This reflects the fact that the ancestral β-globin gene was transposed to a new chromosomal location in the lineage leading to modern amniotes (34). Intriguingly, an "orphaned" β-type globin gene (ω-globin) is still found in association with the tandemly linked α-type globin genes in the genomes of monotremes and marsupials (41, 59, 105).

Phylogenetic evidence indicates that erythroid-specific, O_2-transport Hbs evolved independently from different ancestral precursor proteins in the two deepest branches of the vertebrate family tree: gnathostomes and jawless fishes (cyclostomes, represented by lampreys and hagfish) (39, 75). The independent evolution of O_2-transport Hbs in these two anciently diverged vertebrate lineages involved the convergent co-option of distinct globin precursors to perform similar respiratory functions in circulating red blood cells. In the Hbs of both gnathostomes and cyclostomes, multisubunit quaternary structures provide the basis for cooperative O_2 binding and allosteric regulation, but differences in numerous structural details belie their independent origins. In the tertrameric Hbs of gnathostomes, cooperativity stems from an oxygenation-linked transition in quaternary structure between high- and low-affinity conformations (64). In the Hbs of cyclostomes, by contrast, cooperativity stems from an oxygenation-linked dissociation of low-affinity homo- and/or heterodimers into high-affinity monomers (11, 12, 22, 23, 66). Thus the O_2-transport Hbs of gnathostomes and cyclostomes represent superficially similar but structurally distinct design solutions to the challenge of maintaining cellular O_2 supply in support of aerobic metabolism (39).

Gene Duplication and the Developmental Regulation of Hemoglobin Synthesis

In mammals, the arrangements of tandemly linked genes in the α- and β-globin gene clusters are co-linear with the temporal order of expression during development (25, 73). For example, the human α-globin gene cluster is arranged: 5′-ζ (embryonic)-α_2 (fetal and adult)-α_1 (fetal and adult)-3′, and the human β-globin gene cluster is arranged: 5′-ε (embryonic)-$^{G}\gamma$ (fetal)-$^{A}\gamma$ (fetal)-δ (minor adult)-β (major adult)-3′ (**FIGURE 2A**). This same general arrangement also is seen in the α- and β-globin gene clusters of other amniotes, although the individual identities of early and late-expressed genes vary among taxa due to lineage-specific gene duplications and deletions (41, 44, 57, 58, 78).

Evolutionary changes in the developmental timing of isoHb expression are typically associated with changes in oxygenation properties, since the different isoHbs are adapted to perform distinct O_2-scavenging/O_2-transport tasks during different stages of development (7, 92, 106). Evolved changes in functional properties of differentially expressed isoHbs are attributable to amino acid substitutions in paralogous genes that encode the different α- and/or β-type subunits.

During human embryogenesis, O_2 diffusion is sufficient to meet the metabolic demands of the developing embryo until *day 15* postconception (7, 24). At that stage of development, the embryonic α- and β-type globin genes (ζ- and ε-globin, respectively) are transcriptionally activated to produce Hb Gower I ($\zeta_2\varepsilon_2$), which serves as an O_2 carrier (106). After 4 wk of gestation, the heart of the developing embryo becomes septated, the venous and arterial circulations are established, and the placenta begins to develop. During this phase, two additional embryonic isoHbs are synthesized: Hb Gower II ($\alpha_2\varepsilon_2$) and Hb Portland ($\zeta_2\gamma_2$). During the next 6 wk, the placental circulation is established, the yolk sac gradually disappears, and the liver becomes the major site for hematopoeisis, producing definitive, enucleated erythrocytes containing a mix of fetal Hb (HbF; $\alpha_2\gamma_2$) and adult Hb (HbA; $\alpha_2\beta_2$). After ∼20 wk of gestation, the bone marrow becomes established as a secondary site for hematopoesis, producing only HbA (106). At birth, the neonatal circulation consists of erythrocytes containing ∼70% HbF and ∼30% HbA. In 5-mo-old infants, the fraction of HbF in the blood falls to ∼3%, and by 2 years of age, circulating erythrocytes derived from the bone marrow contain ∼97% HbA and ∼3% HBA2 ($\alpha_2\delta_2$) (**FIGURE 2B**).

This same basic pattern of ontogenetic gene switching is observed in all other tetrapod vertebrates that have been examined to date (1, 78, 97, 104). In the α-globin gene cluster, the physiological division of labor between early and late-expressed genes was established in the common ancestor of tetrapod vertebrates, and it appears to have been retained in nearly all descendant lineages. The ancestral arrangement of the tetrapod α-globin gene cluster is 5′-α^E-α^D-α^A-3′ (43, 44), where α^E is orthologous to the embryonic ζ-globin gene in humans and α^A is orthologous to the adult α-globin in humans. In the tetrapod common ancestor, the α^A-globin gene and the (presumably embryonic) progenitor of the α^E/α^D genes originated via tandem duplication of an ancestral proto α-globin gene; the α^E- and α^D-globins originated via a subsequent tandem duplication (43). In modern tetrapods, the α^E-globin gene appears to be expressed exclusively in larval/embryonic erythroid cells, and the α^A-globin gene is expressed in definitive erythroid cells during later stages of prenatal development and postnatal life. In mammals, products of the α^D-globin gene (annotated as "μ-globin" in the human genome assembly) do not appear to be incorporated into functional Hb tetramers. However, the α^D-globin gene is expressed in both primitive and definitive erythroid cells of birds and non-archosaurian reptiles (1, 78).

In contrast to the ancient functional diversification of α-type globin genes (**FIGURE 3A**), the developmental regulation of gene expression in the β-globin gene cluster evolved independently in several different tetrapod lineages (44). For example, in mammals and birds, the β-type globin genes that are expressed during the earliest stages of embryogenesis were independently derived from lineage-specific duplications of the same proto-β-globin gene, that is, the embryonic β-globins of mammals and birds are not "1:1 orthologs" (**FIGURE 3B**). Even within mammals, embryonic β-type globin genes appear to have originated independently as the products of lineage-specific duplication events in monotremes (egg-laying

mammals) and in the common ancestor of marsupials and eutherian mammals (59). Likewise, fetally expressed β-type globin genes originated independently in simian primates (New World monkeys, Old World monkeys, apes, and humans) and in bovid artiodactyls (cattle, antelope, and goats). In most eutherian mammals, the γ-globin gene encodes the β-chain subunit of embryonic isoHbs, but in simian primates, duplicated copies of γ-globin ($^{G}\gamma$ and $^{A}\gamma$) have been co-opted for fetal expression (49, 50). In New World monkeys, $^{G}\gamma$-globin is expressed in nucleated erythroid cells derived from the embryonic yolk-sac (the ancestral condition), but $^{A}\gamma$-globin is expressed in enucleated erythroid cells derived from the fetal liver. In catarrhine primates (Old World monkeys, apes, and humans), both $^{G}\gamma$- and $^{A}\gamma$-globin are fetally expressed. This developmental switch was accompanied by a delay in the fetal expression of the β-globin gene, which is predominantly expressed during postnatal life in mammals. Goodman et al. (32) suggested that the acquisition of fetally expressed Hb may have played an important role in the life history evolution of simian primates because it facilitated an extended duration of fetal development.

Whereas embryonic γ-globin genes were co-opted for fetal expression in simian primates, duplicate copies of the adult β-globin gene were co-opted for fetal expression in bovids (17, 74, 86). Thus the stage-specific expression of fetal isoHbs evolved twice independently from different ancestral states. In simian primates and bovids, the co-option of γ- or β-globin genes for fetal expression was likely facilitated by the fact that redundant or semi-redundant copies of other early or late-expressed β-type globin genes continued to perform their ancestral functions. The acquisition of fetally expressed isoHbs would not have been possible if the ancestor of simian primates had possessed only a single embryonic gene or if the ancestor of bovid artiodactyls had possessed only a single adult-expressed gene, as in contemporary monotremes and marsupials (58, 59).

In humans, the fetally expressed isoHb, HbF ($\alpha_2\gamma_2$), exhibits a slightly lower intrinsic O_2 affinity relative to adult Hb, HbA ($\alpha_2\beta_2$). However, in the presence of physiological concentrations of allosteric cofactors that are present in the red blood cell, HbF exhibits a higher O_2 affinity than HbA due to its reduced sensitivity to the organic phosphate 2,3-diphosphoglycerate (DPG), a metabolite of red cell glycolysis (85) (**FIGURE 4**). During pregnancy, the resultant O_2-affinity difference between HbF in the fetal circulation and HbA in the maternal circulation facilitates O_2 transfer across the placental barrier (7). Since HbF and HbA have identical α-type subunits, the different functional properties must be attributable to substitutions between the γ- and β-globin genes. The reduced DPG sensitivity of HbF relative to HbA appears to be mainly attributable to the amino acid substitution γ143His → Ser, which eliminates two DPG binding sites per tetramer (26), in combination with γ43Glu → Asp, which indirectly affects DPG binding by perturbing the allosteric $\alpha_1\beta_2$ interface (15).

In other eutherian mammals, the requisite Po_2 difference between the maternal and fetal circulations is accomplished via changes in red cell DPG concentrations that differentially modulate the O_2 affinities of structurally identical Hbs. Viviparous vertebrates employ an astounding diversity of mechanisms for maintaining the Po_2 differential between maternal and fetal circulations, only some of which involve genetically based differences in the oxygenation properties of isoHbs with stage-specific expression (17, 45, 90, 92, 98). One consistent pattern across all vertebrates is that, within a given species, isoHbs that are expressed during early embryogenesis have higher O_2 affinities and lower cooperativities than isoHbs expressed later in prenatal development or in postnatal life (13, 45, 92, 97, 104). In humans, for example, the embryonic isoHbs (Hb Gower I, Hb Gower II, and Hb Portland) have uniformly higher O_2 affinities and lower cooperativities than the later expressed HbF and HbA (7, 10).

Functional Differentiation of Co-Expressed Hb Isoforms

Most eutherian mammals possess multiple copies of α- and β-type globin genes that are co-expressed during postnatal life (30, 40, 41, 53, 58, 59, 61, 70, 82). Adult-expressed genes of the same subunit type

typically have highly similar coding sequences and therefore encode identical or nearly identical polypeptides. Thus, in definitive red blood cells, isoHbs that incorporate the different α- and β-type subunits typically have very similar functional properties (14, 46, 51, 71, 83).

The situation is quite different in other tetrapods. The majority of birds, reptiles, and amphibians co-express multiple structurally and functionally distinct Hb isoforms during adult life (20, 33, 57, 78, 79). Crocodilians are a notable exception, since all species that have been examined to date express a single adult Hb (94, 100, 101). Birds typically express two main isoHbs in definitive red blood cells: HbA (the major isoHb, with α-chain subunits encoded by the α^A-globin gene) and HbD (the minor isoHb, with α-chain subunits encoded by the α^D-globin gene). Both isoHbs incorporate the same β-chain subunits. In all bird species that have been examined to date, the minor HbD exhibits a substantially higher O_2 affinity than the major HbA in the presence of physiological concentrations of allosteric cofactors (16, 29, 33, 54, 57, 65). Turtles, lizards, and snakes also express homologous HbA and HbD isoHbs in definititive erythrocytes. [The α-type globin genes are orthologous to those in birds, but the β-type globins are not necessarily 1:1 orthologs (44, 78).] However, the reptilian pattern of isoHb differentiation is a mirror image of the avian pattern: In the few non-archosaurian reptiles that have been investigated, HbD is the major isoHb, and (at least in turtles and snakes) it has a lower O_2 affinity than other isoHbs that incorporate products of the α^A-globin gene (20, 78, 79).

Since the HbA and HbD isoHbs exhibit appreciable differences in O_2-binding properties, regulatory changes in the HbA-to-HbD ratio could conceivably provide an effective mechanism for reversibly modulating blood-O_2 affinity in response to changes in environmental O_2 availability or changes in internal metabolic demands (33, 36, 95). Among sauropsid vertebrates, however, there is no evidence to suggest that isoHb switching plays an important role in acclimatization to environmental hypoxia. Birds that are native to different elevations in the Andes exhibit consistent differences in Hb-O_2 affinity due to genetically based increases in the O_2 affinities of HbA and HbD in highland taxa, but there are no detectable elevational differences in HbA-to-HbD ratios (16, 29, 54, 65). Likewise, in turtles, the HbA-to-HbD ratio does not change during acclimation to hypoxia (20).

The Root Effect and IsoHb Differentiation In Teleost Fish

To assess the possible physiological significance of Hb multiplicity, teleost fishes are an ideal group to study. First, teleosts exhibit the highest levels of functional isoHb diversity among vertebrates (28, 45, 48, 88, 91, 103). The extensive repertoire of α- and β-type globin genes in this group is partly attributable to a teleost-specific whole-genome duplication event (56). Second, teleosts inhabit aquatic environments that span an extraordinarily broad range of variation in O_2 availability, salinity, ionic composition, pH, and temperature. In principle, the expression of multiple isoHbs with graded O_2 affinities and allosteric regulatory capacities could broaden the permissible range of O_2 tensions for efficient tissue O_2 delivery (88, 89). The isoHb differentiation in some groups may be adaptive in this regard. Most notably, a number of taxa, including eels, catfish, and salmonids, express two electrophoretically distinct isoHb classes (designated as “anodic” and “cathodic”) that exhibit pronounced differences in intrinsic O_2 affinity and buffer capacity, and sensitivity to pH, temperature, and organic phosphates (6, 48, 88, 89, 91, 99, 102, 103).

An important functional specialization of the anodic isoHbs involves an extreme form of pH sensitivity known as the Root effect, whereby the low-affinity “T-state” conformation of deoxyHb is strongly stabilized at low pH (3, 4, 8, 9, 62, 63). In tissues such as the retina and the gas gland of the swim bladder, reductions of blood pH in dense, counter-current capillary networks (*rete*) trigger the release of Hb-bound O_2 via the Root effect, thereby promoting O_2 secretion at high P_{O_2}. The evolution of the Root effect represents a key physiological innovation in teleosts, since O_2 secretion in the ocular *choroid rete* increases the O_2 diffusion gradient to highly aerobic cells in the avascular retina (which enhances high-acuity

vision), and O_2 secretion into the swim bladder provides a mechanism of buoyancy regulation (which facilitated the colonization of deep sea habitats). In addition to these well known functional specializations, recent in vitro and in vivo studies have demonstrated that Root effect Hbs, in conjunction with mechanisms for maintaining an arterial-venous pH difference, also play a significant role in general tissue O_2 delivery (67-69).

The differentiation between (anodic) Root effect Hbs and cathodic isoHbs that have low to normal pH sensitivities may represent a physiologically significant division of labor for tissue O_2 delivery, especially under hypoxic and/or hypercapnic stress (21, 48, 84, 88, 89, 103). Since the cathodic isoHbs typically have higher O_2 affinities than the Root effect Hbs, they may help secure arterial O_2 loading under conditions of severe hypoxia where the Root effect Hbs would not be fully saturated. Likewise, since the cathodic isoHbs typically have far lower pH sensitivities, they may help secure tissue O_2 delivery during stress-induced acidosis if the red cell β-adrenergic response is not sufficient to safeguard intraerythrocytic pH.

Marine and freshwater fishes must often contend with extreme vicissitudes of O_2 availability on a daily or seasonal basis. Given that adult-expressed isoHbs of teleost fish often exhibit physiologically significant differences in oxygenation properties, it seems plausible that regulatory adjustments in red cell isoHb composition could represent an important mechanism of phenotypic plasticity in blood-O_2 transport. Experiments involving the African cichlid *Haplochromis ishmaeli* revealed that exposure to chronic hypoxia during postnatal development induced changes in the relative expression of functionally distinct isoHbs that increased blood-O_2 affinity (72). However, as a mechanism of physiological plasticity during adulthood, there is not much evidence to suggest that regulatory changes in red cell isoHb composition make significant contributions to the acclimatization response to hypoxia (45, 103). In fishes, reversible changes in red cell pH and concentrations of allosteric effectors appear to represent far more important mechanisms for modulating blood-O_2 affinity in response to changes in O_2 availability (5, 48, 55, 87, 88, 91, 96, 103).

Conclusion

The duplication and functional divergence of globin genes has promoted a number of key physiological innovations in respiratory gas transport during vertebrate evolution. The physiological division of labor among developmentally regulated isoHbs has clear adaptive significance in viviparous and oviviparous vertebrates alike. Aside from isoHbs with unique specializations of function such as the Root effect Hbs of teleost fishes, the adaptive significance of Hb multiplicity in the definitive erythrocytes of vertebrates is generally unclear. It is also possible that Hb multiplicity confers physiological benefits that are not directly related to inherent oxygenation properties of the proteins. For example, Hb multiplicity may increase Hb solubility in the red blood cell, thereby increasing blood-O_2 carrying capacity by raising the upper limit of intracellular Hb concentration (45, 89). An important line of future research is to elucidate the physiological significance and evolutionary origins of less well understood functions of Hb.

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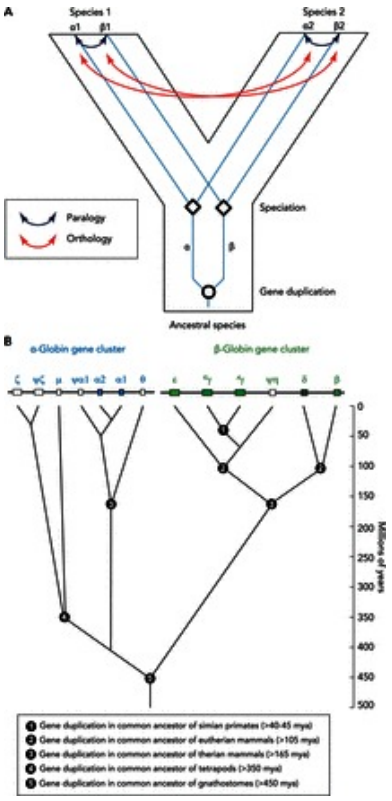
Figures and Tables

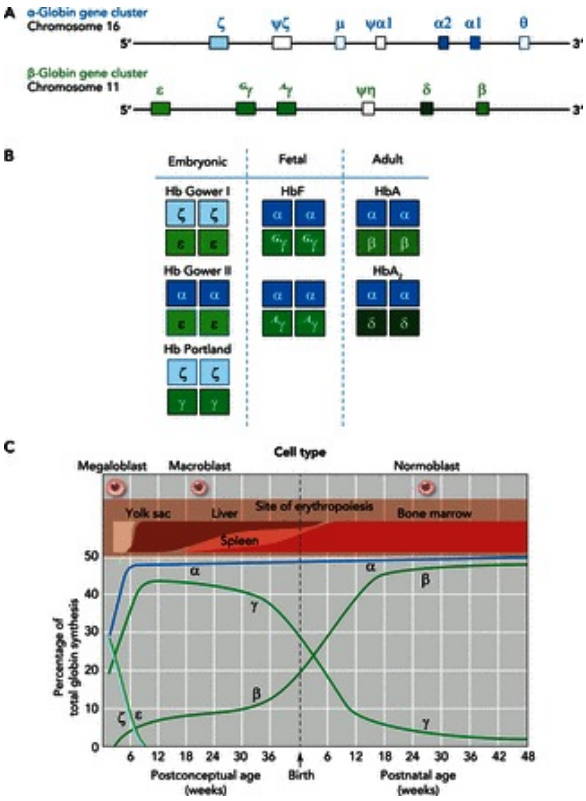
FIGURE 1.



Phylogenetic reconstructions reveal the branching relationships among members of a multigene family that have diversified via successive rounds of duplication and divergence

A: paralogous genes trace their common ancestry to duplication events, whereas orthologous genes trace their common ancestry to speciation events. *B*: phylogenetic diversification of the α- and β-globin gene subfamilies. The human α- and β-globin gene clusters are shown at *top*. Pseudogenes are denoted by the Ψ symbol. In the human α-globin gene cluster, for example, Ψζ denotes an inactivated copy of the embryonic ζ-globin gene. The tree depicts phylogenetic relationships among the paralogous gene duplicates. The inferred timing of duplication events is indicated on the vertical axis. Note that the human μ-globin gene is orthologous to the α^D-globin gene of other tetrapods, as discussed in the text.

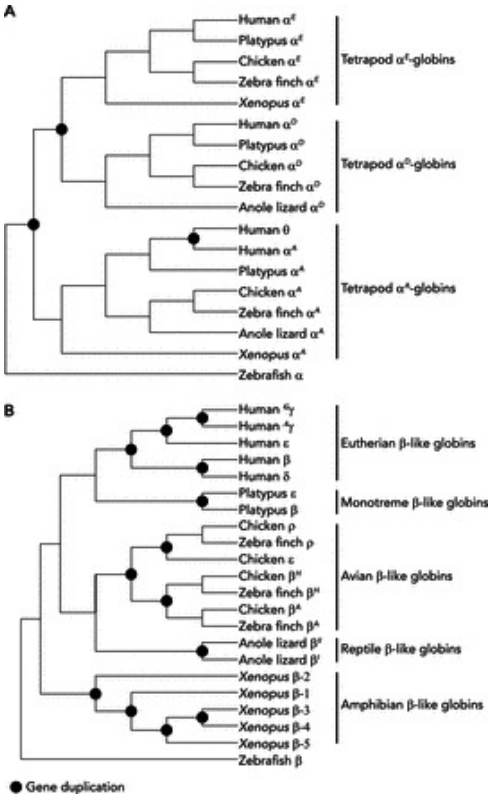
FIGURE 2.



The expression of α- and β-type globin genes is developmentally regulated, resulting in the synthesis of functionally distinct isoHbs

A: structure of the human α- and β-globin gene clusters. *B*: the set of structurally distinct embryonic, fetal, and adult Hb isoHbs, with subunits encoded by each of the pre- and postnatally expressed α- and β-type genes. *C*: developmental timeline for changes in the expression levels of the various α- and β-type genes from the earliest stages of embryogenesis to the end of the first year of life. *C* was adapted from Ref. 106 with permission from *British Medical Bulletin*.

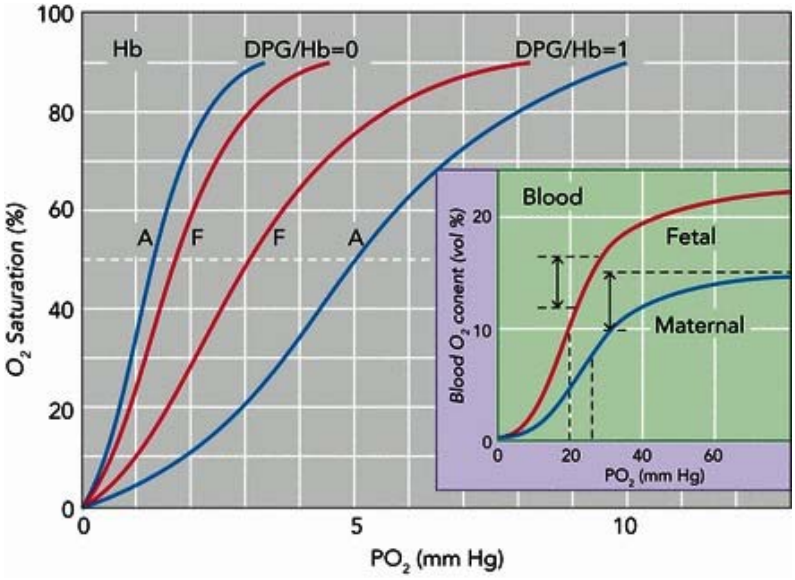
FIGURE 3.



Diagrammatic phylogenies depicting the inferred relationships among members of the α- and β-globin gene subfamilies in tetrapods

In each tree, nodes depicted as filled symbols represent gene duplication events. The remaining nodes represent speciation events (phylogenetic splitting at the organismal level). *A*: phylogeny of α-type globin genes in representative tetrapod lineages. Note that the three paralogs (α^E-, α^D-, and α^A-globin) are reciprocally monophyletic relative to one another. As discussed in the text, the α^E- and α^D-globin genes are products of a duplication event that occurred in the stem lineage of tetrapods. Orthologs of the embryonic α^E-globin gene are known as α^L-globin in amphibians, π-globin in birds, and ζ-globin in mammals. The human ortholog of the α^D-globin gene is known as μ-globin. *B*: phylogeny of β-type globin genes in representative tetrapod lineages. Note that eutherian mammals, monotremes, birds, nonavian reptiles, and amphibians each inherited an ortholog of the same proto β-type gene, which then underwent one or more rounds of duplication and divergence to produce distinct repertoires of β-type globins in each descendent lineage. The depicted phylogenies are based on data reported in Refs. 43, 44, 59.

FIGURE 4.



O_2-equilibrium curves of human adult and fetal isoHbs

O_2-equilibrium curves of human adult and fetal isoHbs (*A* and *F*, respectively). Data are shown for "stripped" Hbs (purified Hbs that are stripped of organic phosphates and other allosteric cofactors) in the absence and presence of equimolar concentrations of 2,3-diphosphoglycerate (DPG:Hb = 0 and DPG:Hb = 1, respectively) at 20°C and pH 7.2 (the approximate intraerythrocytic pH value). *Inset*: O_2-equilibrium curves for maternal and fetal blood (solid and dashed lines, respectively) at 37°C and extracelluar pH 7.4 (corresponding to an intracellular pH of 7.2), illustrating the difference in arteriovenous O_2 content (double-headed arrows), as well as the higher O_2 affinity and higher O_2-carrying capacity of fetal blood. Adapted, with permission, from Refs. 85, 90 and used with permissions from the *Journal of Biological Chemistry* and the *Israel Journal of Zoology*.