Neuroglobin and cytoglobin in search of their role in the vertebrate globin family

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Abstract

Neuroglobin and cytoglobin are two recent additions to the family of heme-containing respiratory proteins of man and other vertebrates. Here, we review the present state of knowledge of the structures, ligand binding kinetics, evolution and expression patterns of these two proteins. These data provide a first glimpse into the possible physiological roles of these globins in the animal’s metabolism. Both, neuroglobin and cytoglobin are structurally similar to myoglobin, although they contain distinct cavities that may be instrumental in ligand binding. Kinetic and structural studies show that neuroglobin and cytoglobin belong to the class of hexa-coordinated globins with a biphasic ligand-binding kinetics. Nevertheless, their oxygen affinities resemble that of myoglobin. While neuroglobin is evolutionarily related to the invertebrate nerve-globins, cytoglobin shares a more recent common ancestry with myoglobin. Neuroglobin expression is confined mainly to brain and a few other tissues, with the highest expression observed in the retina. Present evidence points to an important role of neuroglobin in neuronal oxygen homeostasis and hypoxia protection, though other functions are still conceivable. Cytoglobin is predominantly expressed in fibroblasts and related cell types, but also in distinct nerve cell populations. Much less is known about its function, although in fibroblasts it might be involved in collagen synthesis.

Keywords: Neuroglobin; Cytoglobin

1. Globins: vertebrate novices add complexity to the family

Globins are small globular metalloproteins typically consisting of about 150 amino acids and comprising
eight $\alpha$-helical segments (named A-H) that display a characteristic 3-over-3 $\alpha$-helical sandwich structure. This conserved ‘globin fold’ identifies them as members of the globin protein superfamily [1–3], which also comprises truncated versions whose globin fold consists of only four $\alpha$-helices [4]. Globins contain a heme prosthetic group (Fe-protoporphyrin IX), by which they can reversibly bind gaseous ligands like O$_2$, CO and NO. Most known globins fulfil respiratory functions, supplying the cell with adequate amounts of O$_2$ for aerobic energy production via the respiratory chain [5–7]. They are phylogenetically ancient molecules whose intricate adaptive evolution is demonstrated by their widespread occurrence in bacteria, fungi, plants, invertebrate and vertebrate animals [8]. In man and other vertebrates, the heterotetrameric hemoglobin (Hb) is located in red blood cells and transports O$_2$ from the respiratory organs to the tissues via the circulatory system, and CO$_2$ (at the N-terminal amino groups of the protein chains) in the reverse direction [5]. The monomeric vertebrate myoglobin (Mb) is present in cardiac and striated muscle, where it acts as a local O$_2$ store and probably facilitates intracellular diffusion of O$_2$ to the mitochondria [7,9]. In addition, Mb has an important function as a dioxygenase, converting potentially harmful NO radicals into innocuous nitrate [10]. With exciting data like these, globins continue to be amongst the best-studied cases in terms of structure, function and evolution in the world of protein families.

A few years ago, intrigued by the discovery of unexpected globins in the insect model organism Drosophila [11,12], we set out to systematically search human and mouse genome project sequence databases for the presence of novel, additional globins in vertebrates. First found was neuroglobin (Ngb), a globin predominantly expressed in nervous tissue [13]. Shortly after that, a fourth vertebrate globin type was described independently by three groups [14–16] and is now officially named cytoglobin (Cygb), based on its widespread expression in mammalian tissues. These findings add considerable complexity to our view on O$_2$ metabolism in the vertebrate cell and may have substantial biomedical implications. The review is intended to summarize the structural, gene expressional and functional data on Ngb and Cygb available until November 2004 (Fig. 1), thereby providing perspectives for future research on these molecules.

2. Neuroglobin: the distantly related cousin on our nerves

2.1. Protein structure and ligand binding

Ngb is a substantially divergent member of the globin family, displaying only 20–25% amino acid sequence identity to Mbs and Hbs [13, Fig. 2]. Ngb represents a typical Mb-type monomeric globin, which
can bind $O_2$ reversibly [13,17,18]. In spite of its se-
quence differences, Ngb features the conserved globin
fold consisting of the eight $\alpha$-helices A-H, albeit with
some peculiarities which reflect a pronounced adaptive
potential of this basic globin structure (Fig. 3). The
crystal structure of human NGB [19] has recently been
solved, revealing the presence of unusual protein cavi-
ties which are not found as such in Hb and Mb and
which may influence ligand storage and diffusion paths
inside the molecule. The most peculiar structural char-
acteristic of Ngb is the so-called $/C_{212}$ hexa-coordinated
$/C_{213}$ binding scheme of the heme Fe atom in the ferrous
($Fe^{2+}$) deoxy and in the ferric ($Fe^{3+}$) states (Fig. 3).
The crystallographic data have ultimately confirmed
several types of spectroscopic analyses [13,17,20–24],
showing that in the absence of external ligands, the his-
tidine at position 7 of the E helix (HisE7) binds the
heme iron at its sixth, distal position. Thereby, any
external gaseous ligand such as $O_2$ or CO has to com-
pete with the internal His(E7) ligand for Fe binding.
This produces a biphasic ligand-binding kinetics for
Ngb: the displacement of the His(E7) is the rate-limit-
ing, slow step, while the inherent affinity of the Fe
atom after His(E7) displacement is high and makes
the gaseous ligand-binding step fast [17,25]. Heme
hexa-coordination has previously been reported in
plant, bacteria and invertebrate globins [26], and
although this widespread occurrence may suggest a
conserved function, its physiological significance is
not yet understood. Recent kinetic studies show that
even slight variations in pH may cause pronounced
changes in the association rates of exogenous ligands
in Ngb [27]. On the other hand, hexa-coordination in
Ngb and other globins may render the process of exter-
nal ligand binding relatively independent of temperature variations [unpublished data], which might be physiologically relevant under conditions of fluctuating body temperatures, e.g., in poikilothermic animals. Another effect of hexa-coordination may be a protection of the Fe$^{3+}$ iron atom against various oxidizing molecules, which seems to suppress the formation of cytotoxic ferryl (Fe$^{4+}$)-heme [28].

Despite the complex ligand-binding scheme, the overall O$_2$-affinity of mammalian and of fish Ngb is in the range of 1 Torr [13,17,25,29], which is similar to the O$_2$ affinity of Mb. Recently, it was found that human NGB is able to form an internal disulfide bridge at cysteines CD7 and D5 in vitro (see Fig. 2) [18], which may break up under reducing conditions in the cell, e.g., when NADH$^+$ reduction equivalents accumulate under hypoxia. Reduction of the disulfide bond in turn lowers the O$_2$ affinity of Ngb by a factor of 10, which would lead to a release of O$_2$ and thus, possibly, an attenuation of hypoxic stress. It is not yet clear if this mechanism is acting in vivo: while fish Ngsbs possess two cysteines at roughly equivalent positions, rodent Ngbs lack the CD7 cysteine (cf. Fig. 2).

With regard to globin function (see below), it is essential to investigate the possibility of Ngb binding to ligands other than O$_2$, namely the noxious reactive oxygen and nitrogen species, which accumulate in the cell, e.g., after ischemic insult and subsequent reperfusion of the tissue [30]. EPR (electron paramagnetic resonance) and kinetic studies revealed that the binding affinity of the reactive nitric oxide (NO) to Ngb-Fe$^{2+}$ is relatively low compared to penta-coordinate Hbs and Mbs, which is due to protection of the Fe$^{2+}$ by the internal His(E7) ligand [31]. Under an excess of NO, however, Ngb-Fe$^{2+}$-NO may readily form and scavenge peroxynitrite, a harmful oxidizing molecule generated under ischemic conditions [28].

### 2.2. Phylogeny

Ngb sequences are now known for a broad variety of mammalian and fish species (Fig. 2 and unpublished data) [13,29,32,33]. Our recent finding of putative Ngb orthologs in frogs and chicken strongly indicates its conserved presence in all vertebrate taxa (unpublished data). Phylogenetic reconstructions show that Ngb resembles nerve globins that have been found in some invertebrate species [13]. Recent results have shown the existence of a fifth globin type in lower vertebrates (unpublished data). Together with Ngb, invertebrate nerve and other intracellular globins, and the Ciona intestinalis globins [34], this novel globin of presently unknown function defines a distinct branch of the globin phylogenetic tree. Thus, Ngb is representative of an

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![Fig. 3](image-url). (a) 3-D protein structure of human MB (PDB accession number: 2MM1), NGB (1OJ6) and Cygb (1UMO). In MB, the eight alpha-helices are designated A through H. Note that the globin fold is conserved in all three proteins. (b) Schematic scheme of globin hexa-coordination. The equilibrium of the hexa- and penta-coordinated form is the rate limiting step in ligand binding for Ngb and Cygb. Colors: red, heme group; green, interacting histidines; blue, oxygen ligand.
old globin lineage, which already existed before the separation of Protostomia and Deuterostomia more than 600 million years ago (Fig. 1). Ngb sequence conservation during mammalian evolution has been especially high, pointing to a strongly selected, important function. Evolutionary rate calculations show that mammalian Ngb evolves approximately 3-fold slower than Mb and Hb [35].

2.3. Gene expression patterns

Since the initial discovery of Ngb mRNA in the mammalian brain [13], several studies have confirmed the widespread expression of both, Ngb mRNA and protein, in nerve cells of the central and peripheral nervous system in mammals [32,36-39]. Ngb is exclusively expressed in the cytoplasm of neurons, but not in glia, which may be explained by the presence of candidate neuron-restrictive silencer elements in the Ngb gene region [38,40]. In some studies [36,39], virtually all neurons appear to be Ngb-positive, albeit at regionally substantially different expression intensities. These varying expression levels reconcile these data with other studies that have reported a more focal expression pattern of Ngb [37,38]. Recent mRNA in situ hybridization studies in the zebrafish (Danio rerio) revealed a global pattern of Ngb expression in the fish brain [29].

In addition to the central nervous system (CNS) and the peripheral nervous system (PNS), mammalian Ngb is expressed in endocrine tissues such as the adenohypophysis, adrenal gland, testes [36] and the pancreatic islets of Langerhans [38]. Like neurons, these cell-types are known to be metabolically highly active, which appears to be a general feature of Ngb expression sites.

While the average Ngb protein content in total mouse brain has been estimated to be rather low (ca. 1 μM; [13]), we found that the retina of the mammalian eye is a major site of Ngb expression [41]. Local retinal Ngb concentrations in mouse amount to an estimated 100 μM, which is almost equivalent to Mb content in muscle cells. Ngb is found in the plexiform cell layers and in the inner segments of the photoreceptors, which again coincides with regions of high metabolism and O₂ demand. Retinal Ngb expression has recently been confirmed in the zebrafish [29]. In Danio, we noticed an additional Ngb expression site, namely the chloride cells of the gills, which are known to sustain high metabolic rates during regulation of osmolarity.

2.4. Gene regulation and medical implications

Given that Ngb may be instrumental in sustaining cellular O₂ levels, it is mandatory to study its regulation under conditions of hypoxic and ischemic stress. In cultured rat cortical neurons, Ngb mRNA and protein were shown to be upregulated maximally 2.5-fold after 16 h of anoxia [42]. Admittedly, cell culture hypoxia experiments should be interpreted critically, since the ‘hypoxic’ conditions achieved in the cell incubator may in reality merely reflect physiological O₂ tensions normally present in tissues like the brain [43]. Other authors [37] did not find any upregulation of Ngb mRNA in brains in vivo after prolonged (up to 14 days) exposure of mice to moderate hypoxia (10% O₂). Our preliminary hypoxia experiments appear to confirm a lack of pronounced upregulation [unpublished data]. Clearly, the results on Ngb regulation by hypoxic stress need to be further substantiated. At this moment, the data can at best be taken to indicate a mild short-term response of Ngb to hypoxic stress. Interestingly, bioinformatical sequence evaluations [35] reveal that the Ngb genes in mammals lack conserved hypoxia-responsive sequence elements, which also argues against a strong transcriptional hypoxia response of Ngb mediated by the hypoxia-inducible ‘master’ transcription factor HIF-1 (hypoxia-inducible factor-1). However, the moderate hypoxia response of Ngb in cell culture was reported to be dependent on the mitogen-activated protein kinase (MAPK) signal transduction pathway [44], which is known to interact with the HIF-pathway via the recruitment of p300/CREB transcriptional co-activator. In addition to hypoxia, Ngb seems to be moderately upregulated in cell cultures by the addition of hemin, the ferric chloride salt of heme, which is already known to transcriptionally activate Mb and Hb [44]. This response of Ngb seems to proceed via the soluble guanylate cyclase-protein kinase G (sGC-PKG) signal transduction pathway.

In the mouse immortalized hippocampal cell line HN33, antisense-mediated downregulation of Ngb leads to decreased levels of cell survival under hypoxia, while overexpression of Ngb in the same cells improves cell survival. This finding suggests that Ngb may exert some protective effect under hypoxic stress in the nervous system [42]. It is not clear, however, if neuroprotection is due to an O₂ supply function of Ngb or some other function, like the binding of noxious reactive oxygen species (see below). Recently, it was reported that Ngb is also able to promote neurogenic survival in vivo [45]: in rat, intracerebral administration of an Ngb antisense oligodeoxynucleotide increases infarct size by 2-fold and worsens neurological outcome after an induced focal ischemia. In turn, an adeno-associated-virus (AAV)-mediated Ngb overexpression ameliorates ischemic pathology [45]. These studies indicate that Ngb is a candidate target for diagnosis and, possibly, therapy of stroke and of neurodegenerative disorders, which are known to be associated with hypoxia or increased levels of reactive oxygen species.
3. Cytoglobin: myoglobin’s elusive brother

3.1. Protein structure and ligand binding

Cytoglobin (Cygb) shares about 30% amino acid sequence identity with myoglobin (Mb), pointing at a shared evolutionary ancestry [14–16]. Compared to Mb, mammalian Cygb is unusually long, containing 190 amino acids owing to extensions of about 20 amino acids at both, the N- and the C-terminus (Fig. 2). Part of the N-terminal extension may be explained by sequence motif duplication, while the C-terminal extension partly derives from a small additional exon, which has been recruited during mammalian evolution and is lacking in fish Cygb sequences (Fig. 2). Irrespective of these functionally elusive terminal extensions, which are also observed in some invertebrate globins, Cygb features the sequence hallmarks of a standard globin, e.g., the key residues Phe(CD1), His(E7) and His(F8). The crystal structure [46] ultimately proves that the Cygb core folds as a classic globin. Unfortunately, however, no interpretable electron density data were obtained for the extended Cygb termini. In agreement with spectroscopic data [16,47], the crystal structure proves that Cygb is also a hexa-coordinated globin (Fig. 3). Moreover, Cygb displays an unprecedented large apolar protein matrix cavity next to the heme, which is connected to the exterior and may provide a special ‘ligand tunnelling’ pathway [46,48].

Ligand-binding kinetics of Cygb are – as in the case of Ngb – determined by the comparatively slow phase of displacing the internal His(E7) ligand, before an external gaseous ligand can then rapidly bind to the iron atom [25,26]. The resultant O2 affinity of Cygb is also in the range of 1 Torr [16,18]. As shown for Ngb, Cygb is able to form an internal disulfide bridge (albeit at different positions, between Cys(B2) and Cys(E9); cf. Fig. 2). Reduction of this bond lowers O2 affinity of Cygb only moderately, by about 2-fold [18]. While there is no evidence for intermolecular disulfide bonds, biochemical and crystallographic data suggest that full-length Cygb might act as a homodimeric protein, while the truncated form is monomeric [18,46].

3.2. Phylogeny

Reconstructions of globin phylogeny confirmed that Cygb is distantly related to vertebrate Mbs (Fig. 1), with which it may have shared a common ancestor before the split of jawless and jawed vertebrates about 450 million years ago [15]. Independent evidence for this proposed ‘relationship by gene duplication’ comes from human genome data, showing that Cygb on chromosome 17q25 and Mb on chromosome 22q12 are both parts of paralogous gene groups which have been formed by an ancient large-scale duplication event [49]. Cygb sequences are known from various vertebrates including man, mouse, rat and zebrafish [14–16,50]. We have recently identified Cygb homologues in chicken, in several fish species like Medaka and pufferfishes and in amphibians [unpublished data], which substantiates our evolutionary scenario. In some taxa, we have obtained evidence for independently duplicated Cygb genes, making the evolution of this globin type rather complex. Cygb is very slowly evolving in mammals, and is even slightly more conservative than Ngb [35], which indirectly points towards a strongly selected function.

3.3. Gene expression patterns

Cytoglobin mRNA was originally detected by Northern blot hybridization in essentially all tissues of the mammalian body, indicating a very widespread expression pattern [15,16]. At the same time, the rat ortholog of Cygb was independently isolated by a proteomics approach from the fibroblast-related stellate cells of the liver (and it was therefore dubbed ‘stellate cell activation-associated protein’ STAP) [14,50]. Subsequently, it was reported that Cygb protein is localized exclusively within the cell nuclei in a wide variety of tissues [38]. Two recent studies have re-investigated the expression pattern of Cygb [51,52]. Using independently derived antibodies, both report that Cygb is cytoplastically expressed in fibroblasts and fibroblast-related cell types in a broad variety of splanchnic organs like liver, heart, muscle, gut, kidney, lung and pancreas. The earlier publications on the presence of Cygb (synonym STAP) in the fibroblast-like hepatic stellate cells were therefore confirmed. In addition, Cygb expression was also detected in bone osteoblasts and in tracheal chondroblasts, but not in mature osteocytes and chondrocytes [51]. In summary, the data by the majority support the specific expression of Cygb in the cytoplasm of cells that are actively engaged in the production of extracellular matrix components in visceral organs. Adding complexity to the Cygb expression pattern, our results suggest that Cygb is also expressed in specific, but ill-defined neuronal cell populations in the brain, as well as in peripheral nerve cells [51]. Here, Cygb immunostaining yielded signals in both, the cytoplasm and the nucleus, possibly pointing at a specific role of Cygb in nervous tissues.

3.4. Gene regulation and medical implications

Cygb was originally described as a protein that is upregulated in activated, fibroblast-like hepatic stellate cells during liver fibrosis, and recent data demonstrate Cygb expression in stellate cells of fibrotic pancreas tissue during pancreatitis and in fibroblast-like cells from necrotic regions in the kidney after diet-induced chronic nephropathy in rats [14,52]. In primary cultures of rat hepatic stellate cells, Cygb expression is slightly aug-
mented by addition of recombinant transforming growth factor β (TGFβ) and platelet-derived growth factor-B (PDGF-B), serum factors, which accelerate stellate cell activation [52]. Addition of protein kinase inhibitors suggests that Cygb may be regulated via a protein kinase C (PKC)-dependent signal transduction pathway.

When NIH 3T3 fibroblast cells were transfected with a Cygb expression construct and, subsequently, collagen α1 (I) synthesis was induced by TGFβ, a substantial enhancement of collagen production was observed in the Cygb-transfected cells as compared to non-Cygb-expressing wild-type 3T3 cells [52]. This result suggests a stimulatory, yet undefined role of Cygb in collagen expression, a finding that is corroborated by the shutdown of Cygb expression during osteoblast and chondroblast maturation [51]. By exposing mice to hypoxic conditions, we have shown that Cygb is upregulated 2- to 3-fold in heart and liver [51], which is in good agreement with the presence of conserved hypoxia-responsive sequence elements in the Cygb gene region [35]. Acute tissue hypoxia is a stimulatory signal in processes like osteogenesis, chondrogenesis and wound healing [53,54], in which collagens are massively produced, thereby possibly creating a link between the above observations on Cygb regulation. In summary, Cygb may have substantial biomedical impact due to its involvement in organ fibrosis and in the production of extracellular matrix collagens during normal tissue development and fibrotic pathogenesis.

4. Two globins in search of their role in the family (and in the cell)

Theoretically, and partly in analogy to other globins, we can consider several possible cellular functions for Ngb and Cygb (Fig. 4) and discuss them in light of the currently available data:

(a) As with Mb and many other Mb-type molecules, both novel globins could either store O2 or assist in the diffusion of O2 within the cell towards the mitochondria [7].

(b) Both globins could function as oxygen sensor proteins, which have been well-studied in bacteria [55]. Alternatively, they could be involved in other intracellular signalling pathways.

(c) Ngb and Cygb might act as terminal oxidases, regenerating NAD⁺ to support glycolysis and sustain ATP production under hypoxic conditions, as proposed for maize hemoglobin [56].

(d) Both globins could be instrumental as scavengers of reactive oxygen or nitrogen species, which are produced, e.g., after reperfusion/re-oxygenation following ischemia.

(e) As proven for Mb in mammalian muscle cells [10], they could possess dioxygenase activity, converting harmful excess NO into innocuous nitrate.

(f) Several cytoplasmatic enzymes use molecular O2 for chemical reactions, and globins like Ngb or Cygb could supply these other enzymes with adequate amounts of O2.

In the case of Ngb, much indirect evidence favours scenario (a). As initially pointed out [13], it certainly makes sense for highly O2-demanding and metabolically active cells like neurons to possess a specialized respiratory protein, which helps to sustain aerobic metabolism, possibly by acting as a ‘salvage’ O2 store on the encounter of acute hypoxia. The correlation between Ngb expression rates and metabolic activity is intriguing, most notably in the case of the vertebrate retina, where Ngb localization perfectly matches the zones of strongest O2 consumption and is even found strictly associated with mitochondria-rich segments of the photoreceptors [41]. Adding to this argument, the nerve globin present in the bivalve mollusc Tellina has been proven to sustain neuronal activity under hypoxic stress [57]. While the overall amount of Ngb protein in the mammalian brain may be low, there are pronounced differences in expression levels. For certain highly active brain regions, and of course the most strongly Ngb-expressing retina, Ngb levels appear to be sufficient for an Mb-like O2 supply function. Moreover, some structural and physiological features of Ngb are essentially Mb-style. It must be clearly pointed out, however, that Ngb rapidly autoxidizes to metNgb(Fe³⁺) [17]. To function as an O2 supply, we therefore have to postulate a yet unidentified Ngb-reducing enzymatic activity, in analogy to Mb reductase in muscle. The neuroprotective effects of Ngb (over)expression [42,45] are certainly in line with an O2 supply function. Also, a short-term storage role will not necessarily require a pronounced upregulation of globins under hypoxia, as shown for Mb in hypoxic muscle during athletic training [58]. One may even ask whether any gross physiological hypoxia response on the globin level can be expected in those mammalian species which are usually not adapted to hypoxic conditions.

We regard the current evidence for Ngb as a signalling protein (scenario b) as rather weak. Globin-coupled O2 sensors [55] have until now only been found in Archaea and bacteria and usually require signal-transducing protein domains in addition to the globin part. It has been proposed based on in vitro studies using surface plasmon resonance that oxidized Ngb(Fe³⁺) is involved in intracellular signalling under oxidative stress by inhibiting release of GDP from Gα proteins and triggering release of the Gβγ complex, thereby enhancing cell survival [59]. The interaction of Ngb with G proteins was postulated on the basis of a proposed sequence similarity be-
between Ngb and regulators of G protein signalling (RGS) and RGS domains of G protein-coupled receptor kinases. The evidence given for this similarity, however, is very weak and it will require further studies to substantiate this proposed Ngb involvement in signal transduction in vivo. Recently, the β2 subunit of the Na,K-ATPase ion pump was also reported to interact with Ngb in a yeast two-hybrid assay and in immunoprecipitation experiments [60]. This protein, however, is membrane-bound with a pronounced expression in astroglial cells [61], while Ngb is clearly cytoplasmically expressed only in neurons. These methods of identifying potential Ngb partner molecules should therefore be used with caution. Finally, it should be noted that a role of both Ngb and Cygb in O2 sensing appears unlikely, because at least in vitro their observed ligand affinities [13,17] are much higher than those of functional sensors like the HIF prolyl-4-hydroxylases that are known to work under natural cellular O2 concentrations [62].

The role of Ngb as a terminal oxidase for sustaining glycolysis under hypoxia (scenario c) has not yet been investigated. According to the ‘lactate shuttle’ hypothesis, it is currently believed that in normoxia and even under functional activation of a brain region, glycolysis occurs predominantly in the astroglia, which produces substantial amounts of lactate [63]. This lactate is taken up by neurons, which appear to have a preference to oxidize imported lactate instead of producing lactate/pyruvate by their own glycolysis. Under this scenario, Ngb as a purely neuronal protein should not play a substantial role in glycolytic energy production. Energy depletion under hypoxia, however, may stimulate enhanced glucose oxidation in neurons, compensating for a reduction in lactate supply by astroglia.

A function of Ngb as a scavenger of reactive oxygen species (ROS) and nitrogen species (scenario d) would be consistent with the neuroprotective effect of Ngb after ischemia and reperfusion of brain tissue [42,45], when such harmful molecules are known to form. A possible chemistry showing how Ngb(Fe2+) might react first with NO and then with peroxynitrite has recently been published [28]. It remains to be shown whether this chemistry works in vivo at physiological NO concentrations. At present, a classic MbO2-like NO dioxygenase activity (scenario e) may be regarded improbable for Ngb. To our knowledge, this issue has been studied, without any publication of positive results so far. Ngb and NO synthase do not seem to strictly co-localize in neurons [36]. The NO donor sodium nitroprusside did not increase Ngb expression in cultured cells [42], although an upregulation might not strictly be required for the dioxygenase reaction.

Less data are currently available for Cygb and several possible cellular roles can still be hypothesized for this protein. On the one hand, Cygb shows a globin fold and an O2 affinity reminiscent of Mb, to which it is phylogenetically related. On the other hand, Cygb features peculiarities like its N- and C-terminal extensions, which might be mediating special protein–protein interactions, plus the heme hexa-coordination, a redox-dependent O2 affinity and special cavities for ligand diffusion. Based on the expression pattern, which is not as global as suggested by its name, we envisage that Cygb will perform distinct functions in the cytoplasm of fibroblast-like cells, and in the nuclear and cytoplasmic compartments of yet to be defined nerve cell populations [51]. Cygb certainly has its most prominent role in cells of the fibroblast lineage. Any proposed function here must take...
into account the following: (i) fibroblast-like cells are not known to be metabolically active in general, but engage in the massive production of extracellular matrix proteins like collagen; (ii) collagen synthesis consumes molecular O$_2$ directly to the collagen prolyl-hydroxylases (scenario production. One formal possibility is that Cygb provides size that Cygb could be involved in collagen proteins like collagen; (ii) collagen synthesis consumes not known to be metabolically active in general, but enhance collagen expression [52]. We therefore hypothesize that Cygb could be involved in collagen production. One formal possibility is that Cygb provides O$_2$ directly to the collagen prolyl-hydroxylase (scenario in Fig. 4), although the lower O$_2$ affinity of the collagen prolyl-hydroxylase [62] appears to be at odds with this mechanism. Alternatively, Cygb might participate in some unknown signalling pathway, ultimately augmenting collagen synthesis.

Although vertebrate Ngb and Cygb were discovered only recently, many labs have since contributed significantly to our knowledge on the biochemistry, structure, comparative physiology and molecular genetics of these proteins. Future studies should focus on ultimately understanding the function(s) of intracellular globins in the metabolism of eukaryotic cells and on their biomedical impact.

Note added in proof


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