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Hypoxia Inducible Factor pathway proteins in high-altitude mammals

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Abstract

Humans and other mammals inhabit hypoxic high-altitude locales. In many of these species, genes under positive selection include ones in the Hypoxia Inducible Factor (HIF) pathway. One is *PHD2* (*EGLN1*), which encodes for a key oxygen sensor. Another is *HIF2A* (*EPAS1*), which encodes for a PHD2-regulated transcription factor. Recent studies have provided insights into mechanisms for these high-altitude alleles. These studies have (1) shown that selection can occur on nonconserved, unstructured regions of proteins, (2) revealed that high altitude-associated amino acid substitutions can have differential effects on protein:protein interactions, (3) provided evidence for convergent evolution by different molecular mechanisms, and (4) suggested that mutations in different genes can complement one another to produce a set of adaptive phenotypes.

Keywords

High altitude and hypoxia

A salient characteristic of **high altitude** (see Glossary) is **hypobaric** hypoxia. At 12,000 ft (3,700 m), which is the elevation of the cities of La Paz (Andean Altiplano) and Lhasa (Tibetan plateau), the oxygen concentration is only about 63% of that at sea level. Yet humans and other mammals have lived for millennia on these plateaus and other high-altitude locales such as the Ethiopian Simien plateau. Hypoxia presents a major challenge for mammals living at high altitude primarily because oxygen is the terminal electron acceptor for mitochondrial oxidative phosphorylation, the bioenergetic process that efficiently produces ATP in cells. In the absence of oxygen, cells rely on substantially less efficient means of generating ATP, such as glycolysis.

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In multiple high-altitude mammalian species, such as Tibetans, Andeans, high-altitude North American deer mice, Andean horses, and Tibetan dogs [1–6], signals of natural selection have been identified in genes of the **Hypoxia Inducible Factor** (HIF) pathway, the central transcriptional pathway by which metazoans respond to hypoxia [7–13]. A major gap in our knowledge regarding high-altitude adaptation is the functional characterization of **adaptive alleles** and their encoded proteins. While other reviews have discussed the identification of high-altitude genes and physiologic aspects of high-altitude adaptation [7,13], this review will focus on recent and perhaps unexpected observations that have begun to emerge from functional studies of mammalian high-altitude HIF pathway alleles, particularly those of Tibetans, Andeans, and high-altitude North American deer mice and especially those that produce amino acid changes.

The HIF pathway

HIF is a heterodimeric transcription factor that consists of an α subunit, of which HIF-1α and HIF-2α are the major paralogues, and a common β subunit, **Aryl Hydrocarbon Receptor Nuclear Translocator** (ARNT). Each subunit contains structured N-terminal **basic Helix-Loop-Helix** (bHLH) and **Per-Arnt-Sim** (PAS) domains that contact the other subunit and DNA [14]. The remainder of each subunit is largely unstructured. HIF-1α and HIF-2α have overlapping and distinct gene targets [15]. HIF-1α is the key isoform that upregulates genes of glycolysis, thereby promoting the shift from aerobic to anaerobic metabolism. HIF-2α plays a central role in regulating the *ERYTHROPOIETIN* gene (and therefore red cell mass), the *EDN-1* gene (which encodes for the potent vasoconstrictor Endothelin-1 that regulates regional blood pressure in organs such as the lung), and the **hypoxic ventilatory response** (HVR). Humans and mice with gain of function mutations in the *HIF2A* gene display **erythrocytosis** and elevated **pulmonary arterial pressure** (PAP), while inducible knockout of *Hif2a* in mice blunts the erythrocytosis and the increase in HVR following exposure to chronic hypoxia (also known as the **ventilatory acclimatization to chronic hypoxia**, or VAH) [16–20].

In an oxygen-dependent manner, the enzyme **Prolyl Hydroxylase Domain protein 2** (PHD2, also known as EGLN1) prolyl hydroxylates HIF-α in its **oxygen-dependent degradation** (ODD) domain [9] (Figure 1A, left). This modification promotes the binding of the von Hippel Lindau (VHL) protein, which targets HIF-α for degradation [21]. Under hypoxia, this modification is arrested, leading to the stabilization of HIF-α, its dimerization with ARNT, and the activation of target genes (Figure 1A, right). Humans with partial loss of function *PHD2* mutations display erythrocytosis, and mice with acute global ablation of *Phd2* display augmented HVR as well as erythrocytosis [18,20,22,23].

In a parallel pathway, **Factor Inhibiting HIF** (FIH) asparaginyl hydroxylates HIF-α in its **C-terminal transcriptional activation domain** (CTAD) (Figure 1A, left) [24,25]. This modification blocks the binding of the transcriptional coactivator **Creb Binding Protein** (CBP, or its paralogue p300). Under hypoxia, this modification is arrested, leading to the recruitment of CBP/p300 to promote the transcription of HIF target genes [26] (Figure 1A, right). HIF-α also recruits, via its PAS domain, the H3K4 methyltransferase SET1B to promote transcription [27].

This core HIF pathway is integrated with two ancient pathways that enhance the efficiency of prolyl hydroxylation. In the first, PHD2 employs a zinc finger domain located at its N-terminus to bind a Pro-Xaa-Leu-Glu (PXLE) motif in the ribosomal chaperone NACA [28] (Figure 1B, left). NACA, in turn, is positioned at the ribosomal exit tunnel [29], thereby allowing the recruitment of PHD2 to cotranslationally modify HIF-α in a manner enhanced by a translational pause sequence in HIF-α. A knockin mouse mutation in the PXLE motif of Naca that ablates its interaction with Phd2 leads to erythrocytosis [28]. PHD2 also employs this same zinc finger to bind a PXLE motif in the HSP90 cochaperone p23 [30] (Figure 1B, right). The p23 protein is a component of the HSP90 complex that folds proteins, and HIF-α is a client of the HSP90 pathway [31]. Thus, recruitment of PHD2 by p23 promotes posttranslational modification of HIF-α. A knockin mouse with a mutation in the PXLE motif of p23 that ablates its interaction with Phd2 leads to augmented HVR [32]. In summary, these zinc finger-guided interactions with NACA and p23 increase the efficiency by which PHD2 can cotranslationally and then postranslationally prolyl hydroxylate HIF-α, respectively, and they play important roles in the control by PHD2 of red cell mass and respiration, respectively.

As noted above, the phenotype of knockin mice with mutations in the PXLE motif of Naca differs from that of mice with mutations in the PXLE motif of p23 [28,32]. The basis for this is not clear. One possibility is that there is a difference in the relative protein levels of NACA and p23 in the renal erythropoietin (EPO)-producing cells that regulate red cell mass and the Type I cells of the carotid body that regulate respiration, leading to a cell-dependent differential reliance of PHD2 on NACA versus p23. Alternatively, the ability of NACA to facilitate PHD2-induced hydroxylation of HIF-2α is itself dependent on a translational pause site in HIF-2α [28], and the strength of this pause site might differ in cell-type specific manner.

Mammalian high-altitude phenotypes

The three most extensively studied human high-altitude populations are the Tibetans, Andeans, and Ethiopians [33–38]. In addition to humans, a multitude of mammalian species have adapted to high altitude (Table 1) [13,39,40]. Phenotypes are available for a number of these species. For example, Tibetans at altitude display (1) normal hemoglobin levels, in contrast to the elevated hemoglobin levels that are seen in lowlanders who ascend to and live at high altitude; (2) persistently augmented HVR, in contrast to the attenuated HVR that is seen in lowlanders who have lived long term at high altitude; and (3) lower right ventricular pressures and lower incidence of pulmonary hypertension than lowlanders who ascend to and live at high altitude [7,41]. High-altitude North American deer mice (*Peromyscus maniculatus*) display different phenotypes, including (1) a variant hemoglobin that has increased affinity for oxygen and (2) blunted VAH [42,43].

Signatures of natural selection on the mammalian *HIF2A* gene

The gene under strongest selection in Tibetans is *HIF2A* (also known as *EPAS1*) [1,2,44,45]. This high-altitude *HIF2A* allele does not encode for any amino acid changes. Rather, it encompasses a haplotype that is introgressed from Denisovans, an archaic human species,

and consists of intronic sequence changes [46]. This haplotype is also present in high-altitude Sherpas who reside in the eastern Himalayas [47]. Available evidence suggests that Tibetan *HIF2A* allele is a partial loss of function allele (reviewed in [48]). It is possible that this allele may result in decreased *HIF2A* transcription, although there might be other potential effects, such as ones on splicing [49].

In Andean Colla and Quechua populations, signatures of natural selection have also been observed in the *HIF2A* gene [48,50,51]. In this case, there is enrichment for a *HIF2A* allele that encodes for a H194R substitution in the PAS domain of HIF-2α [51]. This amino acid substitution impairs heterodimerization with ARNT, leading to decreased transcriptional activity [48]. In a mouse model with a knockin H194R *Hif2a* mutation, it was observed to result in blunting of hypoxia-induced increases in right ventricular pressure [48]. Together, these observations indicate that the Andean *HIF2A* is a partial loss of function allele.

Exome sequencing of North American high- and low-altitude deer mice revealed evidence for altitude-dependent natural selection acting on the *Hif2a* locus [4]. One of the single nucleotide polymorphisms (SNPs) under strongest selection encodes for a T755M substitution. Thr-755 resides between the ODD and CTAD (Figure 2A). In vitro, the T755M mutation impairs binding of Hif-2α to the transcriptional coactivator CBP but not p300 [52]. Because the HIF target genes affected by loss of CBP versus p300 are not identical [53], this would be predicted to impair transcription of some but not all HIF target genes. In vivo, the high-altitude *Hif2a* haplotype is associated with increased heart rate under hypoxia and impaired VAH [4,54]. Given that impaired VAH is observed in mice with inducible *Hif2a* knockout [18], these findings are consistent with high altitude North American deer mouse *Hif2a* being a partial loss of function allele.

Whole genome sequencing or exome sequencing has been performed on a whole range of additional high-altitude mammals, including Tibetan dogs, cashmere goats, horses, pigs, and Pikas, as well as on North American cattle, the latter being a species susceptible to high altitude-associated pulmonary hypertension [5,6,55–62]. In many of these species, there is evidence of natural selection on the *Hif2a* gene. In a number of species, coding sequence changes in the gene have been reported. While functional characterization of the high-altitude allele has been performed in a few cases [58–60], in many cases the functional consequences are still unknown. These studies are summarized in Table 1, and select amino acid changes in the HIF-2α protein identified in these mammals are highlighted in Figure 2A.

Signatures of natural selection on the human *ARNT2* gene

The *ARNT2* gene has been nominated as a candidate gene under positive selection in high-altitude Ethiopians [63]. This gene encodes for a paralogue of ARNT that has the capacity to heterodimerize with HIF-2α. It is not known whether the high-altitude allele encodes for any amino acid changes. The *ARNT* gene itself has not yet been reported to be under positive selection in this or other high-altitude mammalian populations. Perhaps the dual role of ARNT in both the hypoxic response (via heterodimerization with HIF-α) and the xenobiotic response (via heterodimerization with Aryl Hydrocarbon Receptor) [64]

might constrain the ability of this gene to participate in evolutionary adaptations to high altitude.

Signatures of natural selection on the human *PHD2* gene

In Tibetans, there is also natural selection acting on the *PHD2* gene locus [3,65]. The Tibetan *PHD2* allele consists of a D4E/C127S double amino acid substitution [66]. Several studies have reported an association between the high-altitude *PHD2* allele and lower hemoglobin levels [3,67,68]; in some of these studies, the association was seen in males but not in females [67,68]. In other studies, however, no significant association was seen [69–72]. These latter studies include the one with the largest Tibetan sample size (2,849) among them [70]. That study examined SNP-based haplotypes. Another study that specifically examined the combination of the two amino acid substitutions did not observe an association between D4E/C127S *PHD2* haplotype and hemoglobin levels when examined in highlanders [71]. Additionally, Tibetan *PHD2* is not associated with PAP [68], and Tibetan *Phd2* in mice is not associated with either hemoglobin level or **right ventricular systolic pressure** (RVSP) [32]. In contrast, Tibetan *HIF2A* is associated with decreased PAP [68]. These studies provide evidence that the phenotypes of Tibetan *PHD2* and Tibetan *HIF2A* are not identical.

In parallel, the functional effect of the D4E/C127S substitution has been investigated. It has been reported, using in vitro assays employing recombinant proteins, that Tibetan *PHD2* exhibits increased affinity for oxygen and therefore is a gain of function allele [66]. A gain of function could lead to an association of the Tibetan *PHD2* allele with lower hemoglobin level, which as noted above, has been observed in some, but not all, studies of the Tibetan *PHD2* allele.

A very different, though not mutually exclusive, model for Tibetan *PHD2* has also been reported [73]. The amino acids affected in PHD2, Asp-4 and Cys-127, flank the N-terminal zinc finger of PHD2 (Figure 2B), which promotes cotranslational and posttranslational hydroxylation of HIF-α (Figure 1B) [28,73]. The D4E/C127S substitutions impair interaction of PHD2 with p23 [73]. Since p23 recruits PHD2 to the HSP90 pathway to facilitate HIF-α prolyl hydroxylation, this would be predicted to lead to a partial loss of function. This is supported by a knockin mouse model bearing the D4E/C127S substitution [32]. These mice display increased HVR, which is also observed in mice with *Phd2* haploinsufficiency [74]. Moreover, the increased HVR is phenocopied in mice bearing *p23* knockin mutations that ablate p23 interaction with Phd2. These findings, in turn, are consistent with the observation that Tibetans display augmented HVR. Importantly and in contrast to its interaction with p23, Tibetan PHD2 maintains its binding to NACA, the ribosomal chaperone important for PHD2 control over red cell mass [28]. In accord with this, the Tibetan *Phd2* knockin mice display hemoglobin levels that are similar to wild type mice under either normoxia or hypoxia [32]. This in turn would be consistent with a lack of an association of the Tibetan *PHD2* allele with hemoglobin levels, which as discussed above has been observed in a number of the studies examining the *PHD2* allele.

In Andeans, signatures of positive selection have also been identified in the *PHD2* gene by SNP genotyping [50,65]. The Andean *PHD2* allele, which does not contain any coding sequence substitutions, is associated with increased VO$_2$max, a measure of aerobic capacity [75]. Certain SNPs in this high-altitude Andean allele (rs479200, rs480902) are also enriched in high-altitude Southeast Asian Indian populations [76]. The precise mechanism by which these or other SNPs might act remains to be determined.

Convergent evolution

The possibility of convergent evolution is raised by the observation of natural selection acting on the *HIF2A* locus in multiple species, including humans (Tibetans and Andeans), North American deer mice, Andean horses, and Tibetan dogs, goats, horses, and pigs (Table 1). Likewise, there is evidence for natural selection acting on the *PHD2* locus in humans (Tibetans and Andeans) and possibly Tibetan cattle.

Importantly, there is evidence that convergent evolution can act by different molecular mechanisms (Figure 3). For example, high-altitude North American deer mouse T755M Hif-2α shows a defective interaction with Cbp in vitro (Figure 3B) [52], and deer mice with the *Hif2a* allele shows impaired VAH [54], consistent with a partial loss of function. Andean H194R HIF-2α displays weakened heterodimerization with ARNT in vitro (Figure 3C), and mice with a knockin Andean *Hif2a* mutation (H194R) display resistance to hypoxia-induced pulmonary hypertension; both observations are consistent with a partial loss of function [48]. Tibetan *HIF2A* likewise displays phenotypic properties consistent with a partial loss of function (reviewed in [48]). However, in contrast to the situation with high-altitude deer mouse or Andean HIF-2α, there are no coding sequence mutations in Tibetan HIF-2α, and current evidence is consistent with it being due to decreased transcription (or possibly splicing) of the *HIF2A* gene (Figure 3D).

It should be noted that core HIF pathway genes are not invariably targets of natural selection in high-altitude mammals. For example, high-altitude Ethiopian gelada monkeys, like Tibetans, do not exhibit elevated hemoglobin concentrations [77]. However, in contrast to Tibetans, they do not display evidence of natural selection acting on the *Hif2a* gene.

Selection on nonconserved, unstructured regions of proteins

Natural selection often targets the coding sequences of conserved, structured regions of proteins, resulting in functional changes that can either be readily understood or rationalized based on known three-dimensional structures of the proteins in question. An unexpected finding that has emerged from the recent research on high-altitude HIF pathway alleles is that in some cases, selection has occurred on coding sequences of nonconserved, unstructured regions of proteins. One example of this is Tibetan PHD2. One component of the allele, C127S [66], affects an amino acid that is not conserved (Figure 2B) and is likely to reside in an unstructured region of PHD2 between the N-terminal zinc finger and the C-terminal prolyl hydroxylase domain. The other component of the Tibetan allele, D4E [66,67], affects a conserved amino acid that is just N-terminal to the zinc finger (Figure 2B).

It is not known whether Asp-4 resides in a disordered region or alternatively, an ordered part of the protein, perhaps in association with the zinc finger.

A second example is high-altitude North American deer mouse Hif-2α [4]. The T755M mutation affects an amino acid that is not conserved (Figure 2A) and is likely to reside in an unstructured region of Hif-2α between the ODD domain and the CTAD. Interestingly, both the Tibetan PHD2 C127S and the North American deer mouse Hif-2α T755M mutation revert the amino acids to ones present in related species, raising the possibility that the result is reversion to the ancestral amino acid. In the case of PHD2, Ser is present in the position corresponding to Cys-127 in PHD2s from primates such as chimpanzee, orangutan, gorilla, and marmoset (labeled Pt, Pa, Gg, and Cj, respectively, in Figure 2B). Likewise, in the case of Hif-2α, Met is present in the corresponding position in rodent species such as house mouse, rat, and golden hamster (labeled Mm, Rn, and Ma, respectively, in Figure 2A). The basis for this potential reversion to the ancestral amino acid as opposed to some other amino acid is not known. Even more puzzling is how these substitutions can mechanistically produce functional defects since they are predicted to reside in unstructured regions of the protein that are distant from sites of known protein:protein interactions in these proteins. It is noteworthy that the functional effect of specific substitutions can be context dependent. For example, interchanges between Met and Thr at position 755 in Hif-2α have functional effects on Cbp binding in the context of deer mouse Hif-2α but not house mouse Hif-2α [52].

Differential effects of coding sequence mutations on protein:protein interactions

Another notable finding that has emerged from the recent research is that in some cases, the functional result is a differential effect on protein:protein interactions. One example of this is Tibetan PHD2. The D4E/C127S substitution impairs interaction with the HSP90 cochaperone p23 but not the ribosomal chaperone NACA (Figure 4A) [28,73]. The impaired interaction with p23 could account for the augmented HVR seen in Tibetans, since p23 plays an important role in this arm of the HIF pathway in mice [32]. On the other hand, the preserved interaction with NACA, which plays an important role in PHD2 control of red cell mass, could account for the observation that Tibetans are not predisposed to erythrocytosis. This preserved interaction is further supported by the observation that mice with Tibetan *Phd2* display normal hemoglobin levels and that, after crossing with mice with a knockin *Naca* mutation that ablates Phd2 interaction, the resulting mice display erythrocytosis [28,32].

A second example of a differential effect on protein:protein interactions is high-altitude North American deer mouse Hif-2α. The T755M substitution impairs interaction with the transcriptional coactivator Cbp but allows maintenance of interaction with its closely related paralogue, p300 (Figure 4B) [52]. While very similar in overall structure, Cbp and p300 serve genetically non-redundant roles in the activation of Hif target genes [53]. It is plausible that this partial loss of function may account for selective effects of the high-altitude North

American deer mouse *Hif2a* allele. For example, this allele is associated with reduced VAH but not hemoglobin levels in this high-altitude species [4,54].

These findings suggest that these alleles with differential protein:protein interaction effects might have been selected for during evolution in order to avoid widespread pleiotropic effects that might otherwise accompany strong loss or gain of function mutations. The observation that they are partial as opposed to complete loss of function is consistent with the notion that these alleles modulate, as opposed to completely ablate or activate, the hypoxic response in adaptive ways.

Complementary effects of apparently antagonistic alleles

Tibetans show evidence of natural selection acting on two key genes of the HIF pathway, *PHD2* and *HIF2A*, with studies supporting both being partial loss of function alleles [1,2,32,68,70,73,78]. The partial loss of function of the zinc finger of Tibetan PHD2 would be predicted to lead to augmented HIF activity. Decreased transcription of the *HIF2A* allele, in contrast, would be predicted to lead to attenuated HIF-2α activity. On the surface, this is puzzling because these are antagonistic activities.

One resolution to this apparent paradox lies in the differential effect of the Tibetan PHD2 mutation on protein:protein interactions. As noted above, Tibetan PHD2 is defective in its interaction with p23 [73] (Figure 4A). Since the PHD2:p23 interaction is critical for maintenance of HVR (Figure 5A, left), this could account for the augmented HVR observed in Tibetans (Figure 5B, left). At the same time, Tibetan PHD2 maintains its interaction with NACA (Figure 5B, right) [28]. Since the PHD2:NACA interaction is critical for appropriate control of red cell mass (Figure 5A, right), this explains why a *PHD2* allele that otherwise shows a defect in p23 interaction (which leads to increased HVR) does not predispose to erythrocytosis. Thus, Tibetan *PHD2* behaves very differently than complete loss of function of *PHD2*. For example, inducible global loss of function of *Phd2* in mice is accompanied by both increased HVR and erythrocytosis [18,22,23]. In the setting of a Tibetan *PHD2* allele with this selective defect, a hypomorphic *HIF2A* allele could then be the main factor accounting for the protection against erythrocyosis and pulmonary hypertension that has been observed in Tibetans (Figure 5B, right). Thus, complementary effects of apparently antagonistic alleles can produce a set of phenotypes that neither allele could produce by itself.

Naturally, this now leads to the question of why the presumed loss of function Tibetan *HIF2A* allele does not depress HVR and act in the opposite direction of the loss of function Tibetan *PHD2* allele in the control of ventilation. The Tibetan *HIF2A* haplotype consists of multiple SNPs which, when tested in vitro using reporter genes and select cell lines, decrease transcription [79]. It is conceivable that these SNPs may have different effects in the EPO-producing interstitial cells of the renal cortex as compared to the Type I cells of the carotid body that play a critical role in oxygen sensing and maintenance of the HVR. In other words, it is plausible that the Tibetan *HIF2A* allele may exert its effects in a cell type-specific manner.

Concluding remarks

Functional correlations for high-altitude HIF pathway alleles are just beginning to emerge, and further investigations will reveal the extent to which the trends highlighted here extend to other species. Such studies may address other intriguing issues as well, such as whether these alleles select for other phenotypes (see Outstanding questions). The availability of in vitro and in vivo experimental methods to accurately model coding and noncoding nucleotide changes is critical. There are certainly challenges. It may be difficult to predict a priori which nucleotide changes will be functionally important. With coding sequence changes, for example, we now know that they sometimes occur in nonconserved, unstructured regions (Figure 2). Amino acid changes may also have differing effects. Their effects may only be manifest in specific amino acid contexts (e.g., high-altitude deer mouse Hif-2α) [52] or only within specific cells. That being said, a more complete understanding of human high-altitude adaptation may provide the basis for pharmacologically mimicking it. Such an approach could facilitate high altitude adaptation in low altitude residents who sojourn to high altitude, or alternatively ameliorate chronic mountain sickness (Monge's disease) that can afflict long term high-altitude residents. More broadly, such an approach may also have an impact on more commonplace ischemic cardiovascular diseases, such as heart attack or stroke.

Acknowledgments

Glossary

Adaptive allele
a gene variant that confers a survival or fitness benefit in an environment

Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT)
a transcription factor, also known as HIF-β, that form heterodimeric complexes with a number of other transcription factors, including HIF-α and Aryl Hydrocarbon Receptor

Basic Helix-Loop-Helix (bHLH)
a protein domain that binds to DNA which structurally consists of a basic region followed by two α helices separated by a loop

C-terminal transcriptional activation domain (CTAD)
a segment of HIF-α that binds to Cbp/p300 in manner that is regulated by site-specific asparaginyl hydroxylation. It is likely largely unstructured

Creb binding protein (Cbp)
a transcriptional coactivator with histone acetyl transferase activity that binds to a number of transcription factors, including HIF-α, to facilitate transcription at target genes

Erythrocytosis
excessive red blood cells, leading to increased circulating red cell mass

Factor Inhibiting HIF (FIH)

an oxygen-dependent asparaginyl hydroxylase that hydroxylates and thereby downregulates the activity of the HIF-α CTAD

High altitude

typically defined as altitudes >8,000 ft (>2,500 m), an altitude at which oxygen saturation of hemoglobin in the blood begins to fall

Hypobaric

low atmospheric pressure, associated with high altitude

Hypoxia Inducible Factor (HIF)

a heterodimeric transcription factor consisting of α and β subunits that is the main mediator of the transcriptional response to hypoxia in animals

Hypoxic ventilatory response (HVR)

the increase in breathing that occurs upon acute (seconds to minutes) exposure to hypoxia

Oxygen-dependent degradation (ODD) domain

a segment of the HIF-α protein that is site-specifically prolyl hydroxylated by PHD2, thereby marking HIF-α for degradation. It is likely largely unstructured

Per-Arnt-Sim (PAS)

a globular protein domain originally identified in the Period, Arnt, and Sim proteins

Prolyl Hydroxylase Domain protein 2 (PHD2)

an oxygen-dependent prolyl hydroxylase that is the central oxygen sensor in the HIF pathway

Pulmonary artery pressure (PAP)

the pressure in the pulmonary arteries that supply the lungs, elevations of which indicate pulmonary hypertension

Right ventricular systolic pressure (RVSP)

the pressure in the right ventricle during systole. Since the right ventricle pumps blood through the pulmonary arteries, elevations in RVSP can be a reflection of pulmonary hypertension

Ventilatory acclimatization to chronic hypoxia (VAH)

the augmentation of HVR that occurs upon chronic (hours to weeks) exposure to hypoxia

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Outstanding questions

- Are all high-altitude *HIF2a* alleles loss of function alleles?

- Can cell-type specific effects of high-altitude HIF pathway alleles be demonstrated?

- Why are phenotypic outputs of high-altitude HIF pathway alleles not always identical across species? For example, a presumed loss of function *HIF2A* allele is associated with low Hb in Tibetans but not in high-altitude deer mice. Conversely, this allele is associated with blunted VAH in high-altitude deer mice but not in Tibetans.

- What other phenotypes are selected for by high-altitude HIF pathway alleles?

- Can noncoding sequence changes in high-altitude alleles be modeled in mice or other experimental animals?

- Can high-altitude alleles such as the hypomorphic *PHD2* and *HIF2A* alleles be mimicked pharmacologically to treat conditions such as chronic mountain sickness?

Highlights

- Many high-altitude mammals, including humans, display signatures of selection on genes of the HIF pathway, particularly *HIF2A* and *PHD2*.

- *HIF2A*, encoding the transcription factor HIF-2α, is a recurrent target of selection. Mechanisms of convergent evolution include impaired heterodimerization, defective coactivator recruitment, and possibly decreased transcription of the *HIF2A* gene.

- It has been observed that high altitude-associated amino acid changes in PHD2 and HIF-2α occur in nonconserved, unstructured regions of these proteins, and these changes can selectively alter interactions with other proteins, producing partial as opposed to complete loss of function.

- In Tibetans, pairing of a partial loss of function *PHD2* allele with a partial loss of function *HIF2A* allele could produce a panel of adaptive phenotypes that neither allele alone could produce, raising the possibility that this could be mimicked pharmacologically.
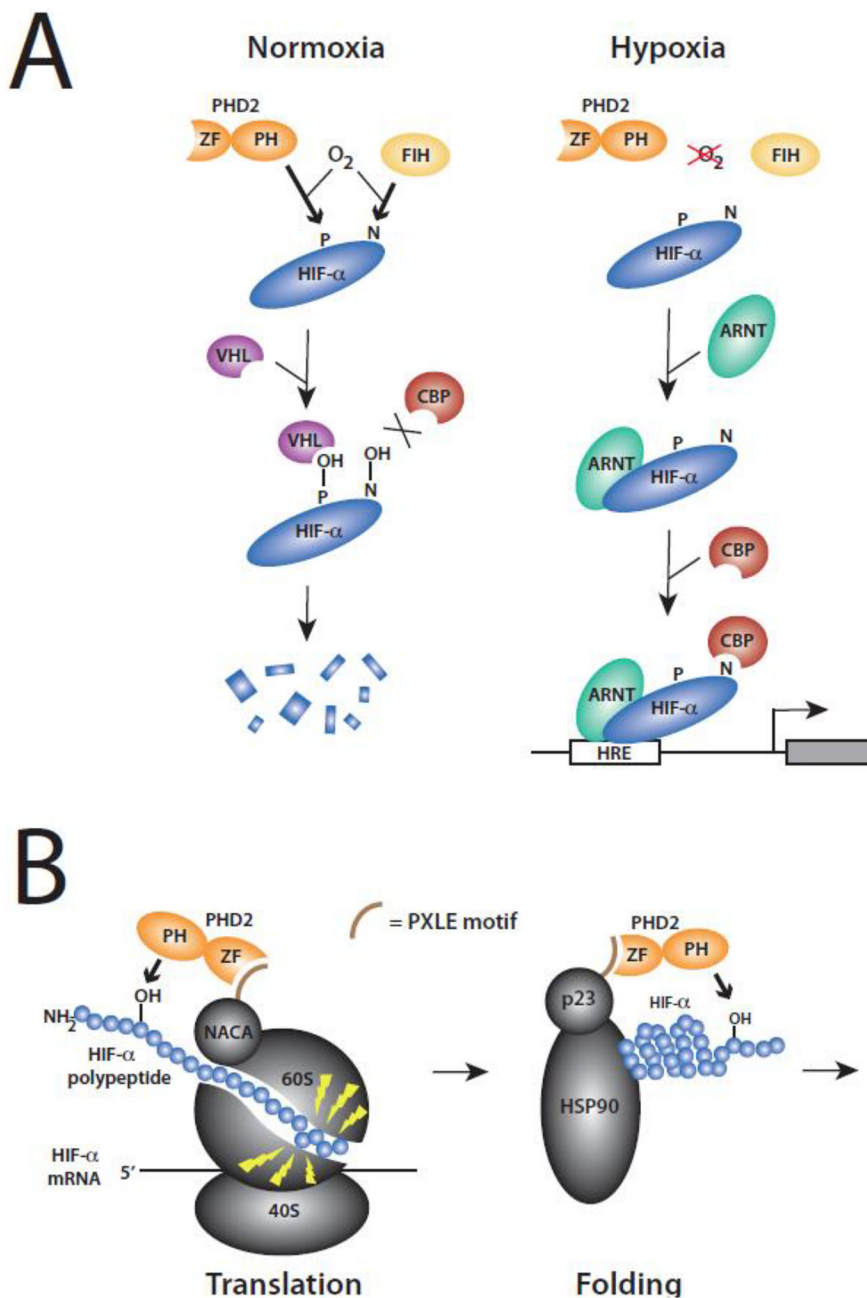
Figure 1. The HIF pathway.

(A) Core components. Under normoxia, PHD2 [which contains zinc finger (ZF) and prolyl hydroxylase (PH) domains] and FIH site-specifically hydroxylate prolyl (P) and asparaginyl (N) residues, respectively, on HIF-α in an oxygen-dependent manner. In human HIF-1α, these residues are Pro-564 and Asn-803, respectively. In human HIF-2α, they are Pro-531 and Asn-847, respectively. Prolyl hydroxylated HIF-α is recognized by VHL, promoting degradation of the former. Asparaginyl hydroxylation blocks binding of CBP/p300 (only CBP shown). Under hypoxia, both hydroxylation reactions are inhibited, allowing HIF-α heterodimerization with ARNT, recruitment of CBP/p300, and activation of HIF target

genes. (B) Coupling of HIF prolyl hydroxylation to translation and protein folding. Left, PHD2 employs its zinc finger to allow binding to NACA and recruitment to ribosome, where it cotranslationally hydroxylates HIF-α in a manner enhanced by translational pausing (indicated by lightning bolts). For further details, see [28]. 60S and 40S refer to ribosomal subunits. Right, PHD2 employs its zinc finger to allow binding to p23 and recruitment to the HSP90 complex, where it postranslationally hydroxylates HIF-α, which is a HSP90 client.
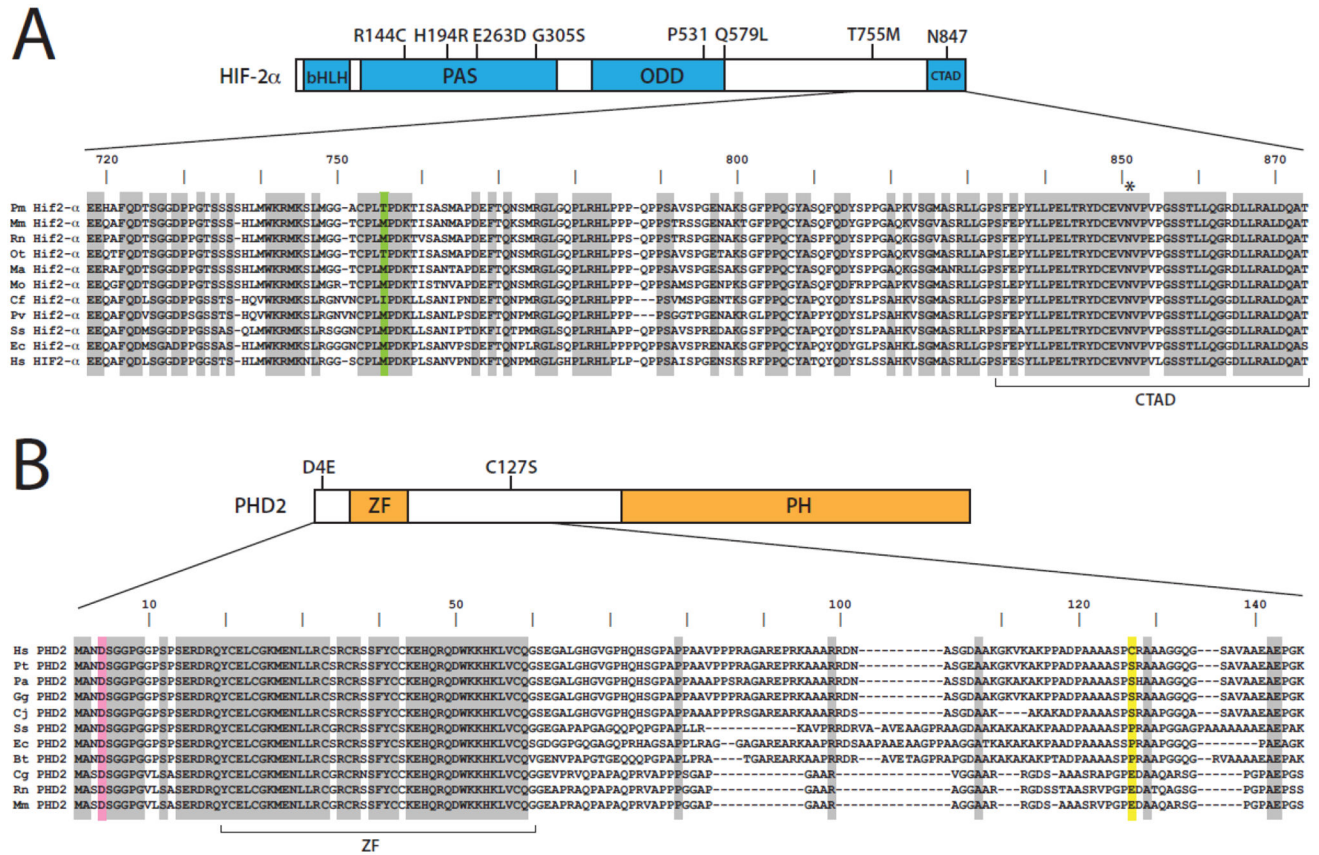
Figure 2. HIF-2α and PHD2.

(A) Top, HIF-2α. Select high-altitude mutations shown, including R144C (Tibetan horse), H194R (human Andeans), E263D (Tibetan horse), G305S (Tibetan dogs), Q579L (Tibetan goat), and T755M (high-altitude deer mice). bHLH = basic helix-loop-helix, PAS = Per-Arnt-Sim domain, ODD = oxygen-dependent degradation, CTAD = C-terminal activation domain. Bottom, sequence of residues 718–874 of deer mouse (*Peromyscus maniculatus*, XP_015860532) Hif-2α, along with corresponding ones from house mouse (*Mus musculus*, NP_034267), rat (*Rattus norvegicus*, Q9JHS1), Southern grasshopper mouse (*Onychomys torridus*, XP_036026399), golden hamster (*Mesocricetus auratus*, XP_040593547), creeping vole (*Microtus oregoni*, XP_041486961), dog (*Canis lupus familiaris*, XP_005626137), harbor seal (*Phoca vitulina*, XP_032266944), pig (*Sus scrofa*, NP_001090889), horse (*Equus caballus*, XP_005600062), and human (*Homo sapiens*, NP_001421). Green = Thr-755 in deer mouse Hif-2α and the corresponding residues in orthologues. Asterisk, Asn-851. (B) Top, PHD2. Positions of the Tibetan D4E and C127S substitutions shown. ZF = zinc finger, PH = prolyl hydroxylase domain. Bottom, sequence of residues 1–146 of human (*Homo sapiens*, Q9GZT9) PHD2 is shown, along with corresponding ones from chimpanzee (*Pan troglodytes*, JAA34701), orangutan (*Pongo abelii*, XP_002809347), gorilla (*Gorilla gorilla gorilla*, XP_004028630), marmoset (*Callithrix jacchus*, XP_003735339), pig (*Sus scrofa*, XP_020929239), horse (*Equus caballus*, XP_014588968), cow (*Bos taurus*, NP_001192975), Chinese hamster (*Cricetulus griseus*, XP_007651724), rat (*Rattus norvegicus*, NP_848017), and mouse (*Mus musculus*, Q91YE3). Pink = Asp-4 and yellow

= Cys-127 in human PHD2, and the corresponding residues in orthologues. In (A) and (B), alignment produced using the Clustal W method (Lasergene MegAlign v15.3.0), and shading indicates residues conserved in all species.
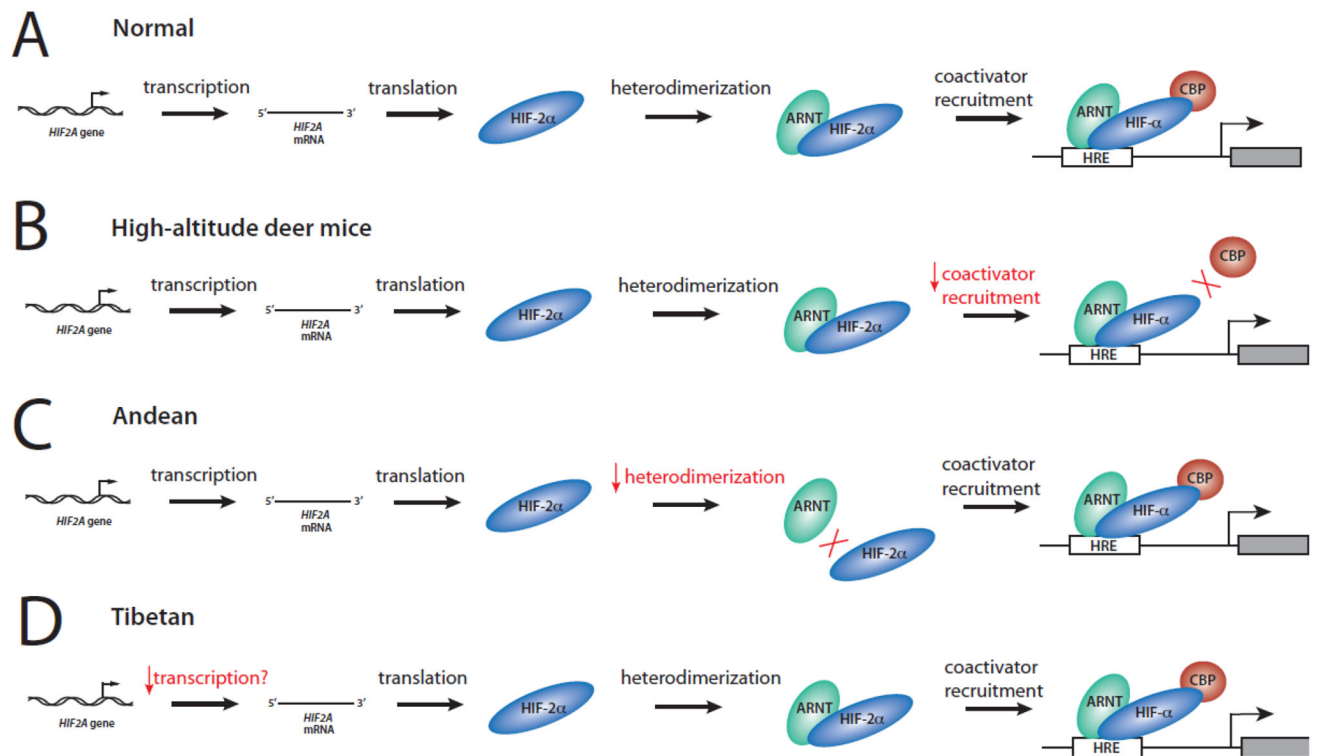
Figure 3. Convergent evolution acting on the *HIF2A* gene occurring by different mechanisms. (A) Normal transcription of the *HIF2A* gene followed by translation, heterodimerization with ARNT, binding to Hypoxia Response Elements (HRE), and recruitment of the coactivator CBP. (B) In high-altitude North American deer mice, there is impaired binding of HIF-2α to the transcriptional coactivator CBP. (C) In Andeans, there is impaired heterodimerization of HIF-2α with ARNT. (D) In Tibetans, there is possibly decreased transcription of the *HIF2A* gene.
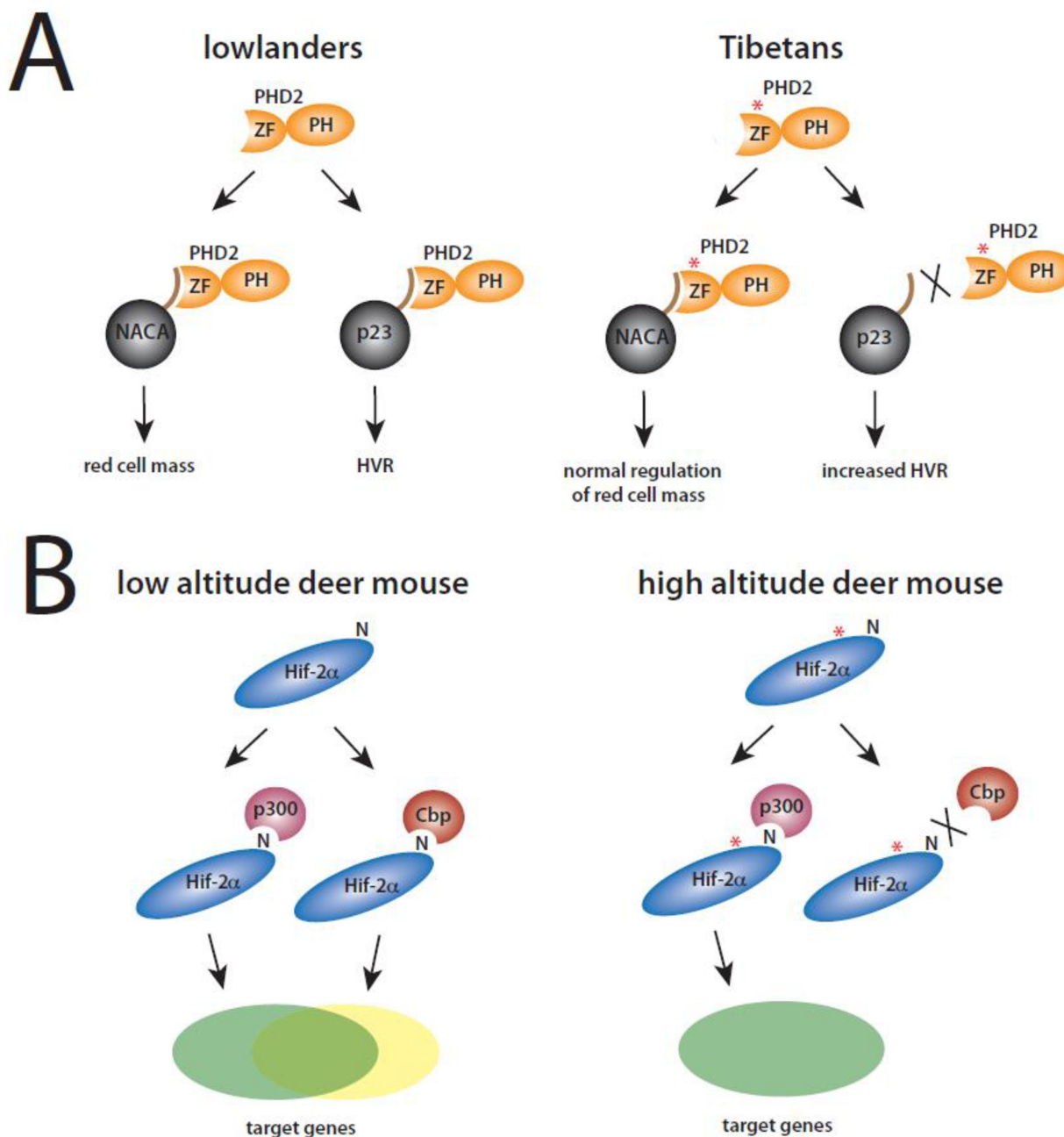
Figure 4. High-altitude mutations in the HIF pathway can differentially affect protein:protein interactions.

(A) Left, the zinc finger of wild type PHD2 of lowlanders binds to NACA and p23 and therefore maintains appropriate control of red cell mass and HVR, respectively. Right, the zinc finger of Tibetan PHD2 has a defect in interaction with p23 but not with NACA. Hence, there is wild type (normal) control of red cell mass but increased HVR. ZF = zinc finger, PH = prolyl hydroxylase domain, asterisk = D4E/C127S double amino acid substitution. (B) Left, wild type low-altitude deer mouse Hif-2α can interact with both Cbp and p300, leading to activation of both Cbp- and p300-dependent genes (yellow and green, respectively). Right, high-altitude deer mouse Hif-2α has a defect in interaction with Cbp

but not with p300. Hence, there is impaired activation of Cbp- but not p300-dependent genes. N = Asn-851 (equivalent to Asn-847 in human), the site of asparaginyl hydroxylation in Hif-2α. Asterisk = T755M amino acid substitution.
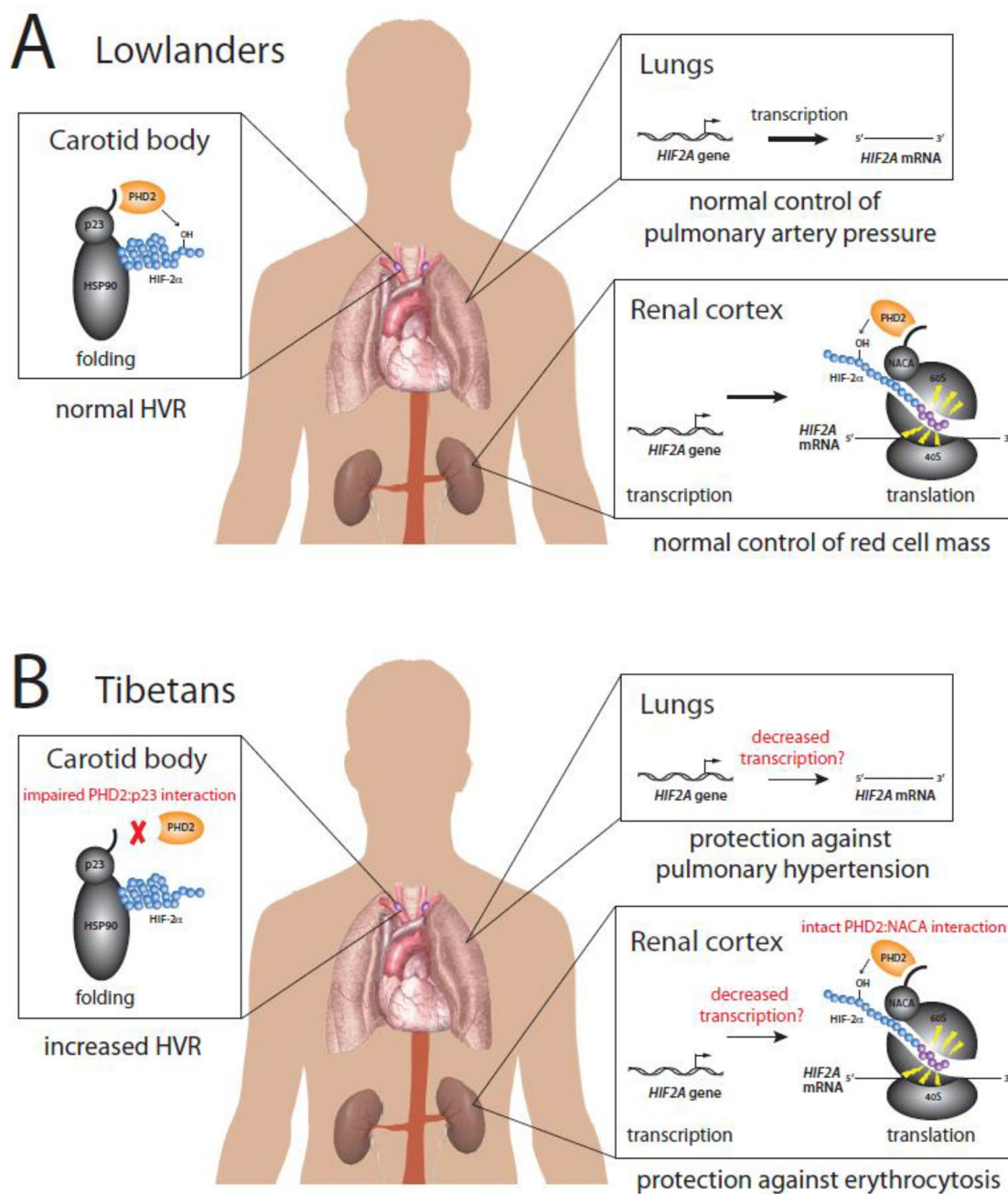
Figure 5. Model for HIF pathway alleles in Tibetan adaptation to high altitude.
(A) Transcription, translation, and protein folding of HIF-2α in tissues of lowlanders. For simplicity, only select processes are shown in each tissue. Appropriate control of HVR, pulmonary artery pressure, and red cell mass is maintained. (B) In Tibetans at high altitude, impaired interaction of PHD2 with p23 in the carotid body leads to increased HVR (left). In the renal cortex, PHD2 interaction with NACA is preserved in the EPO-producing cells; however, HIF-2α activity is decreased, possibly due to decreased transcription of the *HIF2A*

gene, leading to protection against erythrocytosis (right). Decreased transcription in the lungs may also lead to protection against pulmonary hypertension (right).

Table 1.

Mammalian high-altitude HIF pathway alleles

Species[a]	Gene	Amino acid changes	Evidence of selection	Associations	Functional characterization	References
H. sapiens (T)	HIF2A	ND[b]	yes	↓Hb, ↓PAP, ↑expression of HIF2A mRNA	↓expression of reporter gene	[1,2,44–46,68,70,78–80]
H. sapiens (A)	HIF2A	H194R	yes	↑FeNO	↓heterodimerization with ARNT, ↓expression of reporter gene, Attenuated hypoxia-induced PH in mice	[48,51]
P. maniculatus (N)	Hif2a	T755M	yes	↑HR under hypoxia, ↓VAH	↓binding to Cbp in vitro	[4,52,54]
C. familiaris (T)	Hif2a	G305S, D494E, V500M, P750S	yes	↓blood flow resistance		[6,55,56]
B. taurus (N)	Hif2a	E270Q, P362L, A606T, G610S, A671G, L701F	no	↑PAP		[61,62]
C. hircus (T)	Hif2a	Q579L	yes	↑MCHC		[57]
E. caballus (T)	Hif2a	R144C, E263D	yes	↓Hb	↑stability of Hif-2α protein	[58]
E. caballus (A)	Hif2a	NR	yes			[5]
S. scrofa (T)	Hif2a	ND	yes		↓expression of reporter gene	[59]
O. curzoniae (T)	Hif2a	24 aa insertion	NR		↑stability of Hif-2α protein	[60]
H. sapiens (T)	PHD2	D4E/C127S	yes	*	↓binding to p23 in vitro, Preserved binding to NACA in vitro, ↑HVR and VAH in mice, ↑affinity for oxygen in vitro	[3,32,44,45,65–67,71,73,80]
H. sapiens (A)	PHD2	ND	yes	↑VO$_2$max[c]		[65,75]
H. sapiens (I)	PHD2	NR	NR	↑pulmonary edema[d], ↑expression of PHD2 mRNA[d]		[76]
B. taurus (T)	Phd2	NR	NR	↓Hb, ↓rbc		[81,82]
P. uncia (T)	Phd2	K228M[e]	NR			[83]
H. sapiens (E)	ARNT2	NR	yes			[63]

[a]The locales are indicated in parentheses and are as follows: T, Tibet; A, Andes; N, North America; I, India; E, Ethiopia

[b]Abbreviations are as follows: ND, not detected; NR, not reported; Hb, hemoglobin concentration; PAP, pulmonary arterial pressure; FeNO, fractional concentration of exhaled nitric oxide; PH, pulmonary hypertension; VAH, ventilatory acclimatization to chronic hypoxia; MHCH, mean corpuscular hemoglobin concentration; VO2max, maximal oxygen consumption; rbc, red blood cell concentration.

[c] TT genotype of rs1769793

[d] TT genotype of rs479200

[e] Annotation according to full length protein (XP_049500971.1)

[*] One study looking specifically at the D4E/C127S haplotype did not report any association with Hb in highlanders [71]. Another study examining D4E or C127S individually have reported associations with decreased Hb [67]. Additional studies examining other SNPs have reported either no association or an association with decreased Hb [3,68–70].