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Forum Review

Redox Regulation of Lung Development and Perinatal Lung Epithelial Function

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ABSTRACT

Throughout gestation, low oxygen tensions are a dominant feature of the fetal environment and so may be important in sustaining a normal pattern of lung morphogenesis until the moment of birth. As breathing begins, the equilibration of the lung lumen to postnatal P_{O_2} evokes a series of physiologic and morphogenic maturation events that are partially reversible by hypoxia. In this review, we discuss the experimental evidence that fetal and perinatal oxygen tensions differently influence lung morphogenesis through oxygen- and redox-responsive signaling pathways and identify five loci at which this regulation may occur: (I) proliferation of undifferentiated lung mesenchyme as governed by hypoxia-regulated transcription factors (HIF-1α, C/EBPβ); (II) transient production of reactive oxygen species (ROS) and nuclear oxidation of the perinatal lung epithelium; (III) nuclear transport and oxidation of thioredoxin in hand with the acute activation of nuclear factor-κB (NF-κB); (IV) ROS-evoked chronic rise in intracellular glutathione and thioredoxin redox buffering capacity; and (V) NF-κB-dependent increase in transepithelial Na^+ transport and lung lumenal fluid clearance. Although not exhaustive, this analysis leads us to the conclusion that redox events that occur in the lung during gestation, parturition, and the early neonatal period may dramatically influence the expression of genes and physiological events that are crucial to the successful transition from fetal to postnatal lung maturation. *Antioxid. Redox Signal.* 7, 92–107.

INTRODUCTION

LOW OXYGEN TENSIONS are a persistent feature of the gestational environment from conception (P_{O_2}~55 mm Hg), throughout embryonic implantation and early placentation (P_{O_2} 0–13 mm Hg) (for review, see 56). Although the onset of placental gas exchange establishes regulated oxygen delivery to fetal tissues, late gestation P_{O_2} values for umbilical artery, vein, and amniotic fluid are constrained below maternal venous levels (23, 30, and 12 mm Hg, respectively), despite a 12-fold increase in placental gas-exchange surface area from 12 weeks to full-term (50). By comparison, these cord blood P_{O_2} values parallel arterial–venous P_{O_2} measurements from mountaineers breathing without supplementary O_2 at the summit of Everest [8848 m; alveolar P_{O_2} = 35 mm Hg (100)] and illustrate the basis for Barcroft's description of the gestational environment as "Everest *in utero*," a phrase he coined to reflect the shared characteristics between high-altitude adaptation and fetal development at the limit for aerobic function (4).

Despite the low ambient P_{O_2} of the uterine environment, precise roles for oxygen-sensing mechanisms in organ morphogenesis remain poorly defined. However, recent studies of the early stages of placental growth have yielded evidence that such mechanisms may be fundamental to the development of fetal gas exchange (for review, see 27). These studies suggest that low uterine P_{O_2} values at the point of implantation sustain the proliferation of embryo-derived cytotrophoblasts, which differentiate into a motile, nonproliferative phenotype in oxygenated regions located toward the maternal vasculature. This oxygen gradient therefore creates an axis of trophoblast differentiation and chemotaxis that underpins the growth of the chorionic villi and the formation of the gas exchange interface between mother and fetus (discussed further under *Hypoxic Regulation of Cell Differentiation into Branched Pla-*

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cental and Lung Structures). In establishing O_2-sensing and signaling pathways as crucial to the development of fetal respiration, these studies set an important precedent for exploring oxygen-signaling pathways as active participants in the development of pulmonary gas exchange.

Human lung development is initiated in the fifth week of gestation and so precedes the onset of placental gas exchange at 12 weeks. Lung morphogenesis passes through five stereotypic sequences, each of which involves the differentiation of progenic mesenchyme cells into defined airway structures: (I) the formation of primitive lung anlage as projections of endodermal epithelium from the primitive foregut to form the laryngotracheal groove (5 weeks); (II) separation of the laryngotracheal groove from the foregut to form the primitive trachea and bronchial buds (5–6 weeks), (III) asymmetric, bilateral branching of the primary bronchial buds (6 weeks) followed by secondary branching into the splanchnic mesenchyme to form the trilobed (right) and bilobed (left) lung rudiments (7 weeks), (IV) repeated lobar branching to 16 generations (7–16 weeks), and (V) completion of branching to 23 generations (16–24 weeks) followed by alveolarization (28 weeks to 7 years postnatal). The processes of budding and bifurcation (stages I–IV) depend partly on the secretion of fluid into the rudimentary airway spaces that creates a distending pressure necessary for effective three-dimensional lung growth. This persists into the very final stages of gestation where hormonal influences increase the expression, assembly, and membrane insertion of epithelial Na^+ channel (ENaC) subunits that underpin the Na^+-driven reabsorption of lumenal fluid into the vasculature, the subsequent equilibration of airway P_{O_2} to postnatal levels (~100 mm Hg), and postnatal alveolar development. These events are of crucial importance for the survival of the infant into independent life, and are superimposed by a transition from the lowest to the highest naturally encountered systemic P_{O_2} values.

Although the production of reactive oxygen species (ROS) by a wide range of oxidative stressors, including hyperoxia, is well established as causative in a wide range of lung pathologies, relatively little is known about the contribution of these pathways to the natural regulation of lung development and function. The aim of this review is therefore to assess the developmental significance of fetal and postnatal oxygen tensions and lung epithelial redox status in influencing lung morphogenesis and physiology that are required to establish normal pulmonary respiration following birth.

HYPOXIA-INDUCIBLE FACTOR-1α (HIF-1α) AND NUCLEAR FACTOR-κB (NF-κB): DIFFERENTIAL REGULATORS OF GENE EXPRESSION BY OXYGEN

The discovery that local perturbation in extracellular P_{O_2} can be detected and responded to by autonomous cellular oxygen-sensing pathways ranks among the most significant observations to be documented recently in respiratory biology. This homeostatic effect operates at all levels of respiratory organization, and is of fundamental importance in the adaptation of gas-exchange systems to support life in a wide range of aerobic environments (44, 98). Although much controversy surrounds the identity of the cellular "oxygen sensors" involved in transducing change in extracellular P_{O_2} into a physiological signal, much progress had been made in identifying the effector pathways that underpin the subsequent homeostatic response.

HIFα, and NF-κB are two such genomic effectors that are expressed in embryonic tissues from the early preimplantation cleavage stages (7, 14, 68) and that are correspondingly poised to coordinate responses to differences in P_{O_2}. HIFα is a basic helix–loop–helix (bHLH)-Per-ARNT-Sim (PAS) transcription factor that is well documented as a hypoxic inducer of (currently) 28 genes involved in regulating vascular tone, vasculogenesis, dietary iron uptake, blood-cell production, glycolytic ATP synthesis, ATP consumption, and cell proliferation/differentiation (98). The HIF transcriptional complex is made up of a DNA binding α subunit, whose stability is governed by P_{O_2}, and a constitutively expressed β subunit, similar to the aryl hydrocarbon nuclear translocator (ARNT), which functions in nuclear transport and the initiation of transcription. To date, three highly homologous isoforms of the α subunit have been characterized, with HIF-1α being a dominant, widely expressed form, and HIF-2α and HIF-3α isoforms showing a more limited, tissue-specific pattern of expression (61, 90, 101).

Whereas hypoxia (<40 mm Hg) favors HIFαβ dimerization, activation, and DNA binding, the stabilization of the HIFα subunit is the rate-limiting activating event. The search for an O_2-receptive mechanism that could account for this effect revealed the presence of an O_2-dependent degradation domain (ODD) that resides between residues 401 and 603 in the C-terminus of all HIFα isoforms (Fig. 1). This contains a recognition sequence for the von Hippel–Lindau E3 ubiquitin ligase complex (pVHL) that regulates the rate of α-subunit turnover through ubiquitin-mediated proteasomal targeting. This interaction crucially depends on the oxygen-dependent hydroxylation of prolyl residues 402 and 564 contained within the N- and C-terminal ODD by a group of Fe(II) and 2-oxoglutarate-dependent, non-heme dioxygenases, termed proline hydroxylase domain 1–3 (10, 30). An additional hydroxylation event at an asparaginyl residue (Asn803) is regulated by factor inhibiting HIF, which blocks interaction of the HIFα C-terminal activation domain (C-TAD) with the transcriptional coactivator, p300, and so contributes towards a dual mechanism of HIF inactivation in oxygenated tissues (46, 58). In accord with the essential criteria for oxygen sensing, these enzymes possess a high K_M for oxygen, are rapidly inactivated during hypoxic transitions thereby amplifying HIFα abundance, utilize Fe(II) as a cofactor, and are inhibited by Fe(II) chelation or substitution with cobaltous ions. As their activity is both necessary and sufficient for the activity of HIF transcriptional activation, these enzymes are implicated as the rate-limiting oxygen-sensing mechanism that regulates adaptive hypoxic gene expression (10, 11).

Prolyl/asparaginyl hydroxylases potentially represent important loci for ROS regulation of HIFα activity and possess an expression pattern that limits inappropriate hypoxic gene expression. Several isoforms of this enzyme are known to increase in lung and other tissues during various forms of oxidative stress (*e.g.*, 48, 70, 77), whereas the expression of

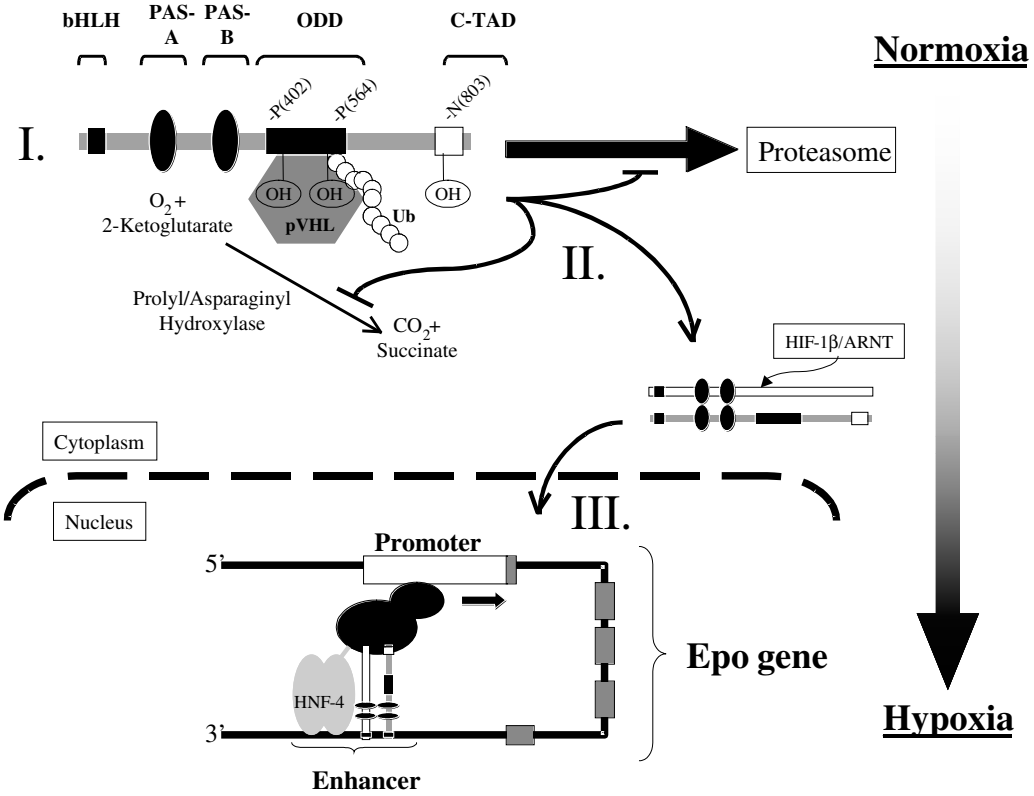

FIG. 1. O_2-dependent regulation of erythropoietin by HIF-1α. Under normoxic conditions (**I**), HIF-1α is maintained inactive through the hydroxylation of proline residues 402 and 564 in the oxygen dependent degradation domain (ODD) by O_2-dependent prolyl hydroxylases. This serves as a binding motif for pVHL ubiquitin ligase, which targets nascent HIF-1α protein for proteasomal degradation. Additionally, asparaginyl hydroxylation of Asn803 within the C-terminal transactivation domain (C-TAD) inhibits interaction with the transcriptional coactivator, p300. As P_{O_2} falls, so prolyl/asparaginyl hydroxylase activity becomes inhibited (**II**) and HIF-1α protein accumulates in the cytosol. Dimerization with the nuclear translocator, HIF-1β/ARNT allows passage to the nucleus (**III**), where the HIF-1αβ binds to a conserved hypoxia response element and forms a viable transcriptional complex through the association of Asn803 with p300. In the case of the erythropoietin (Epo) gene, this association occurs within the 3' enhancer region and also depends on interaction with hepatocyte nuclear factor-4 (HNF-4) and other proteins within the transcriptional initiation complex. ARNT, aryl hydrocarbon nuclear translocator; bHLH, basic helix–loop–helix conserved domain; PAS-A and PAS-B, Period-ARNT-Sim conserved domains A and B; pVHL, von Hippel–Lindau tumor suppressor protein; Ub, ubiquitin. Figure is adapted from references 11 and 13. Dimensions are not to scale.

prolyl hydroxylase-2 increases during chronic hypoxia, leading to a rapid destabilization of HIF-1α on reoxygenation (24). Additionally, prolyl hydroxylase activity is inhibited dose-dependently by nitric oxide (NO) donation under normoxic conditions leading to HIF-1α accumulation in the absence of hypoxia (65, 83).

Despite the suggestion from these studies that hydroxylation events within the HIFα ODD and C-TAD are probably open to redox regulation, models of HIF-1α regulation centered on ROS have yielded little consensus on the direction (*i.e.*, activation or inhibition) or mechanism of activation. The NADPH oxidase model posits that the tonic rate of superoxide anion ($O_2^{\bullet -}$) production from a plasma membrane-bound cytochrome b_{558}-containing NAD(P)H oxidase (NPO) may determine the stability of HIF-1α (1). NPO is a membrane-bound catalytic multimer consisting of $gp91^{phox}$ and $p22^{phox}$ subunits that make up the b_{558} flavocytochrome core, with regulation by interaction with $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$ cytosolic subunits, as well as the GTP-binding proteins Rac2 and Rap1A. The model predicts that decreasing oxygen availability results in substrate limitation of NPO-catalyzed $O_2^{\bullet -}$ production and decreased $O_2^{\bullet -}$ transfer into the cytosol via 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS)–sensitive anion channels. This lowers superoxide dismutase hydrogen peroxide (H_2O_2) formation and favors the overall reduction of the cytosol (31, 39). Although there is evidence that the NPO complex is widely expressed (107) and is inhibited by low P_{O_2}, cobaltous ions, and iron chelation, HIF-1α activity is retained in $gp91^{phox}$ knockout mice and in cells isolated from patients with chronic granulomatous disease, a condition associated with defective NPO activity (2, 99). However, these observations are mitigated by studies showing that a flavoprotein cytochrome P450 NAD(P)H reductase activity, normally involved in the transfer of electrons to microsomal cytochrome p450 and heme oxygenase, can compensate for the removal of $gp91^{phox}$ activity and has been shown to independently regulate hypoxia-evoked gene expression [erythropoietin (Epo), vascular endothelial growth factor (VEGF)] (72).

A second model proposed by Chandel *et al.* (17) suggests that ROS produced by mitochondria during the transition to-

ward hypoxia may stabilize HIFα subunits. Studies using respiratory chain inhibitors have revealed two major sites of ROS production in mitochondria. The oxidation of ubiquinol prior to electron transfer to complex III proceeds through a two-stage electron transfer reaction where the first electron is donated to the Fe_2-S_2 Rieske protein and the second is transferred to cytochrome *b* of complex III. Blockade of the second electron transfer using antimycin A causes the production of $O_2^{\bullet -}$ at complex III, which is inhibited if the initial reduction of Fe_2-S_2 Rieske protein is blocked using using myxothiazol (17, 18). The second source of ROS arises from the accumulation of electrons at the N-1a Fe_2-S_2 redox carrier center of complex I and may be blocked by rotenone (55). In both cases, $O_2^{\bullet -}$ produced in the matrix that is not converted to H_2O_2 by Cu/Zn superoxide dismutase exits to the cytosol through DIDS–sensitive anion channels expressed in the inner and outer mitochondrial membranes. Chandel *et al.* (17, 18) observed that the hypoxic production of ROS could be suppressed by inhibition of complex I, whereas antimycin A blockade at complex III did not attenuate the rate of ROS production. Similarly, HIF-1α stability was abolished in the presence of antioxidants, by blockade of electron transport proximal to complex III, and was absent in cells devoid of a functional respiratory chain (ρ^o). This work implies that an intact respiratory chain is required for HIF-1α stabilization; however, other studies have failed to show that HIF-1α activity is absent from ρ^o cells (87, 93). In addition, other studies show a contradictory requirement for reductants in the induction of HIF-1α activity (41) and inhibition by H_2O_2 (31). Whatever the mechanism, there is a clear role for ROS in regulating HIFα activity; the recent identification of prolyl/asparaginyl hydroxylases as the oxygen sensors that determine HIFα stability should serve to crystallize research efforts in this area.

In contrast to hypoxic gene expression regulated by HIFα, NF-κB regulates gene expression in response to a wide range of oxidative stressors, including raised oxygen tension and the transient production of oxidants released during acute hypoxic perturbations. The NF-κB transcriptional complex is dependent on homo- or heterodimerization with members of the Rel [Rel A (p65), RelB, c-Rel] and NF-κB family (p50 NF-κB1 and p52 NF-κB2) with p65/p50 heterodimer being the dominant associated form (Fig. 2). Whereas HIFα subunits are maintained inactive in normoxia by proteasomal targeting, NF-κB dimers are rendered inactive in the cytosol through their association with an inhibitory factor, I-*k*B. Activation in response to an oxidative stimulus follows the receptor-mediated triggering of a phosphorylation cascade that terminates at the I-*k*B kinase complex (IKK). On activation, this kinase phosphorylates the α subunit of I-*k*B on serine residues 32 and 36 and so targets I-*k*B for ubiquitylation via an E3 ubiquitin ligase and its destruction in the proteasome. Relief of I-*k*B binding to the NF-κB dimer frees the transcription factor for nuclear transport and interaction with DNA consensus binding sequences.

The IKK complex represents a potentially significant locus for the regulation of NF-κB activity by oxidative stressors (hyperoxia, ROS, ionizing radiation). The dominant components of the IKK complex comprise α and β catalytic subunits, together with a γ subunit that binds the complex together. One model proposes that oxidation of Cy179 of both IKKα and β results in a conformational stabilization of the IKK subunits

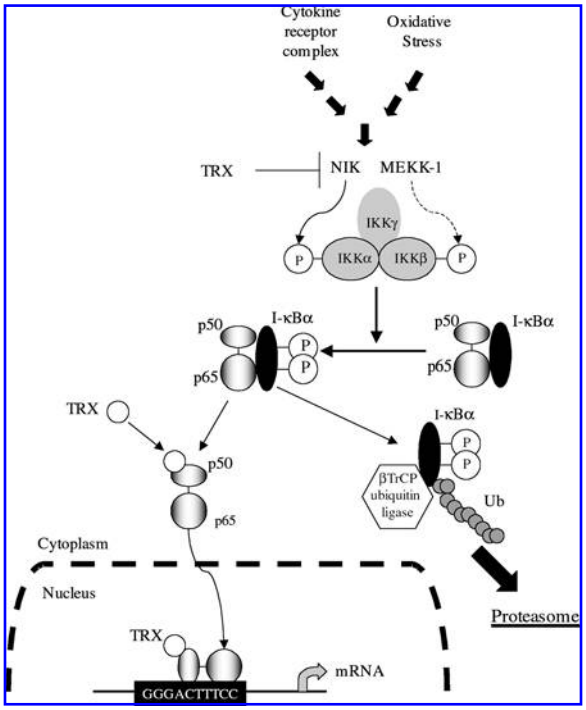

FIG. 2. ROS-dependent activation of NF-κB. NIK and MEKK-1 sit at key convergence points in the activation pathway of NF-κB and may become activated by ROS on oxidation of complexed thioredoxin (TRX). Subsequent activation of the IKK complex leads to the phosphorylation of I-κBα and its degradation *via* the proteasome. Transcriptionally active p50/p65 subunits of NF-kB migrate to the nucleus where TRX-dependent reduction of Cys62 on the p50 subunit facilitates binding to the DNA consensus element and translation. bTrCP, transducin repeat-containing protein E-3 ubiquitin ligase; I-kB, inhibitor of κB; IKK, I-κB kinase; MEKK-1, mitogen-activated protein kinase kinase; NIK, NF-κB inducing kinase; Ub, ubiquitin. "P" denotes phosphorylated residues.

that favors activation (66), whereas another favors the notion of a redox sensor, such as thioredoxin (TRX) (44, 45), which may block the activity of NF-κB activating kinase (NIK) and mitogen-activated protein kinase kinase (MEKK-1), whose phosphorylation targets are IKKα and β, respectively (51, 66, 82, 106). Downstream from IKK, TRX has been shown to reduce Cys62 of the p50 subunit of NF-κB in order to facilitate binding onto its DNA consensus element (45; for review, see 67). However, in contrast to the precise oxygen-sensing role identified for the prolyl/asparaginyl hydroxylases in HIFα activation by hypoxia, there is no distinct oxygen-receptive mechanism identified to date that accounts for the activation of NF-κB under conditions of hyperoxic or oxidative stress.

HYPOXIC REGULATION OF CELL DIFFERENTIATION INTO BRANCHED PLACENTAL AND LUNG STRUCTURES

There is good evidence to suggest that shallow oxygen gradients play a pivotal role in the formation of the branched

gas-exchange structures of the placenta; therefore, this system serves as a useful comparison for probing the role of oxygen in regulating branch morphogenesis of other organs. Placentation begins with an initial phase of embryonic cytotrophoblast proliferation into the endometrial stroma during which cells fuse to form a syncitium (syncytiotrophoblast). A second "differentiation" phase follows during which nonsyncitial cytotrophoblasts condense to form primary villi that invade the maternal decidua, myometrium, and vasculature and which ultimately develop to form the feto-maternal gas-exchange interface. Canigia *et al.* have explored the role played by oxygen in regulating these events using cultured chorionic villial explants and isolated cytotrophoblasts (14, 27). In each case, exposure to fetal Po_2 (23 mm Hg) stimulates cytotrophoblast proliferation and inhibits the expression of a motile, invasive phenotype; conversely, exposure to arterial Po_2 levels causes cell-cycle arrest, increases motility, and leads to phenotypic differentiation into an invasive form that penetrates the maternal spiral arteries and modifies them into low-resistance vessels that supply oxygenated blood to the chorionic membrane (36).

Maintenance of the proliferative undifferentiated phenotype is coupled to low Po_2 tensions through the hypoxic activation of HIF–1α. During placentation, hypoxic activation of the HIF-1α transcriptional complex directly regulates transforming growth factor β3 ($TGF\beta_3$) expression (84) and so maintains cytotrophoblasts in a proliferative nondifferentiated state. Antisense knockdown of HIF-1α in human placental villous explants cultured at fetal Po_2 levels leads to the abrupt suppression of hypoxic $TGF\beta_3$ expression and a switch from a proliferative to a differentiated, motile, and chemotactic trophoblast phenotype (14). This effect may be reproduced at higher Po_2 levels by cobaltous ions and iron chelators, is reversed by administration of free iron chloride, and is blocked by mitochondrial complex I inhibitors (diphenylamine iodinium and rotenone). In this way, regional HIF-1α activation/deactivation as determined by the oxygen gradient from the maternal blood-filled lacunae to the site of implantation determines sites of cytotrophoblast proliferation/differentiation and so regulates for development of the feto-maternal interface.

Unlike that of the placenta, lung morphogenesis is presumed to proceed in a comparatively steady-state low Po_2 environment that likely generates shallow gradients of oxygen from the fetal vasculature into the tissue mass. Whereas several studies have demonstrated that the low fetal Po_2 serves to maintain epithelial lumenal fluid secretion and lung expansion (3, 5, 54, 76), its involvement in maintaining the balance of genes that regulate mesenchymal differentiation and branching morphogenesis is largely unknown. Our studies (57) have shown that gestation day 14 fetal rat lung explants display a 1.5-fold increase in airway surface complexity when cultured for 96 h in serum-free media at fetal (23 mm Hg) versus ambient (142 mm Hg) steady-state Po_2 (Fig. 3). Significantly, the differences in branching morphogenesis we observed were confined to the periphery of the explant, suggesting that the locus of hypoxia dependency is within the region of active mesenchyme differentiation and airway bifurcation. Others have observed that antioxidants [n-acetylcysteine (NAC)] augment airway branching morphogenesis in explants cultured under hyperoxic conditions (ambient Po_2), suggesting that ROS production interferes with the natural process of airway development (32). However, the prooxidant bacterial endotoxin, lipopolysaccharide (LPS), also augments airway bifurcation at permissive concentrations both *in utero* (during chorioamnionitis, for example) and in explants maintained at either fetal or ambient Po_2 (53, 57). As LPS is known to activate the inducible form of nitric oxide synthase (iNOS), it is interesting to note that other studies have reported an increase in airway bifurcation at ambient Po_2 by NO donors at concentrations sufficient to invoke nitrosative/oxidative stress (103). Overall, this body of work serves to highlight the fact that the modulation of redox state in the developing lung bears clear consequences for airway morphogenesis, but fails to offer consensus on the locus, form, or morphogenic outcome of this control.

Given its role as a hypoxia-responsive genomic effector, the HIF complex represents a possible site for redox regulation of lung morphogenesis. Although morphogenic roles for this transcription factor are less clearly defined in lung as compared with the placenta, there is evidence that related bHLH PAS proteins have evolved in close functional association with airway tubule development. The tracheal system of *Drosophila,* a model of branch morphogenesis, has revealed a successional expression of genes that regulate primary invagination of tissue from a progenitor epithelial placode [branchless (bnl), breathless (btl), and stump], secondary branching (pointed, rhomboid, sprouty, and hypersprouty), and the formation of terminal branches (pruned, trimmed, cropped, and misguided). Initiation of tubulogenesis from the embryonic midline placodes depends on the expression of two bHLH PAS transcription factors, trachealess and singleminded (trh and sim), which dimerize with the tango (tgo) gene product to direct the expression of genes that govern tracheal invagination. These *Drosophila* genes share high sequence and functional homology with members of the mammalian HIF-1α-ARNT family (22), where both trh and sim act as transcriptional initiators homologous to HIF-1α that depend on an ARNT homologue, tgo, for nuclear transport and the formation of the transcriptional complex. Whereas experimental knockout of trh results in the complete failure of the tracheal system to develop in *Drosophila*, the null mutation of HIF-1α in mice has no effect until embryonic day 9, a period coinciding with the initiation of lung development and vasculogenesis that is associated with increased HIF-1α expression. Consequently, HIF-1α null mutants display aberrantly enlarged vascular structures, fail to fully initiate lung morphogenesis, and become morbid by embryonic day 10.5 (49, 52, 81). Notably, these events are associated with a regulated loss of mesenchyme tissue pointing to a role for HIF-1α in regulating the survival and differentiation of progenic cells into their ultimate structural form (52). We have also found that HIF-1α is constitutively active in phenotypically differentiated fetal distal lung epithelial cells maintained at fetal Po_2 (23 mm Hg) and that this is abrogated if cells are exposed to early postnatal (76 mm Hg) or ambient (142 mm Hg) Po_2. Notably, this activity is reciprocated by a rapid increase in NF-κB activity over these oxygen tensions (42), suggesting that the activity state of these transcription factors is differentially responsive to Po_2 and may serve as an oxygen-responsive switching mechanism involved in the initiation of pathways crucial for postnatal lung maturation.

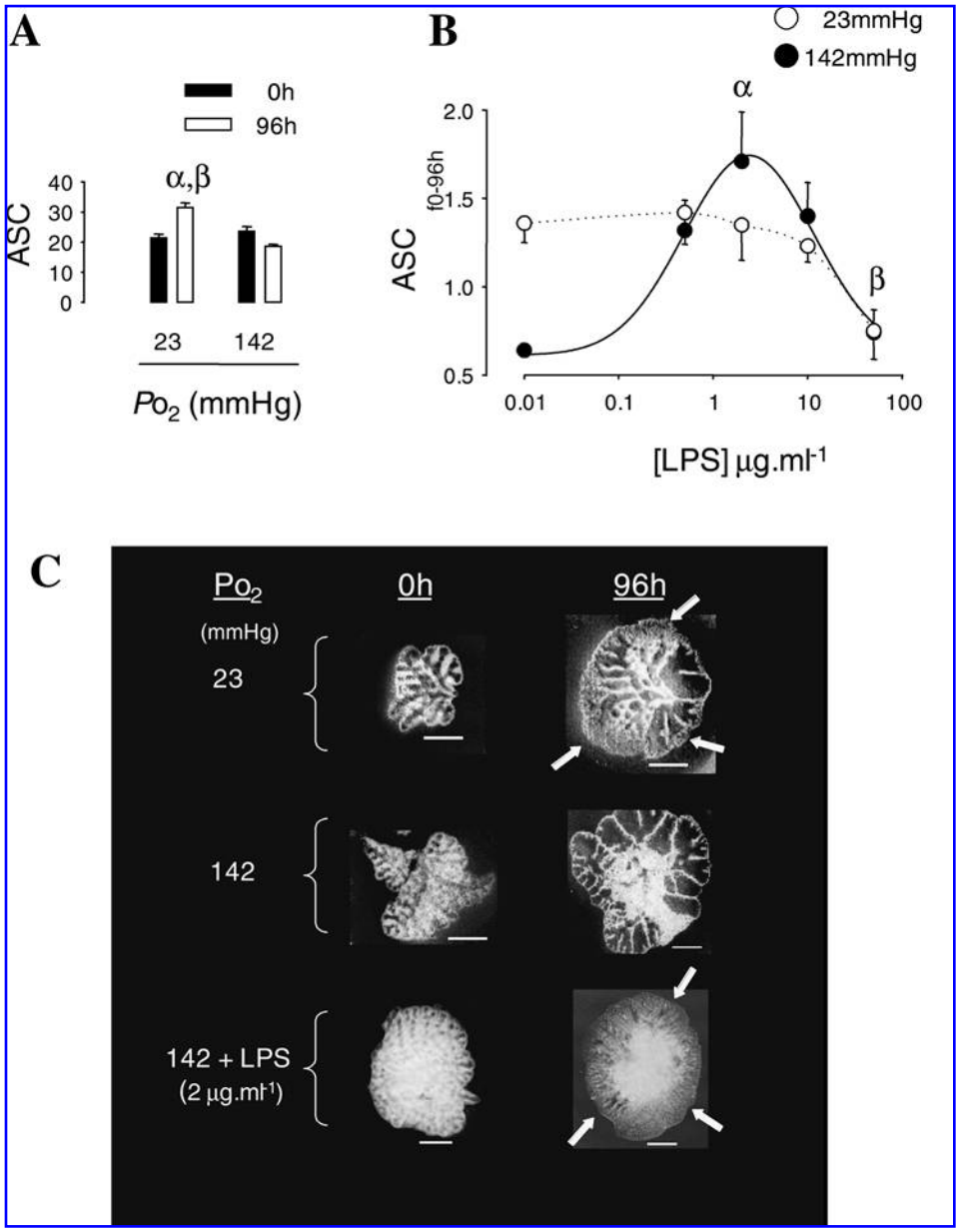

FIG. 3. Influence of oxygen and the prooxidant, lipopolysaccharide (LPS), on airway morphogenesis in fetal rat lung explants. (**A**) Airway surface complexity [ASC; airway perimeter (mm) · airway area (mm^2)] increases 1.5-fold in explants maintained in culture for 96 h at fetal (23 mm Hg) compared with ambient PO_2 (142 mm Hg). Values are means ± SEM. $^{\alpha}p < 0.05$ versus 0-h control; $^{\beta}p < 0.01$ versus 142 mm Hg at 96 h. (**B**) The proinflammatory/prooxidant bacterial endotoxin, LPS, dramatically increases ASC irrespective of culture Po_2 over a permissive concentration range (maximum response at 2 μg/ml). Higher dosages evoke widespread apoptosis and necrosis. Values are means ± SEM. $^{\alpha}p < 0.05$ versus 142 mm Hg control for 96 h; $^{\beta}p < 0.01$ versus 23 mm Hg control. (**C**) Representative images of gestation day 14 fetal rat lung explants maintained at fetal Po_2, ambient Po_2, or ambient Po_2 in the presence of 2 μg/ml LPS for 96 h. Arrows denote regions of airway bifurcation; bar = 1 mm. All figures are based on data from Land and Darakhshan (57).

As with placental cytotrophoblasts, there is some evidence to suggest that hypoxia limits the rate of lung mesenchyme differentiation through the expression of TGF family genes (TGFβ 1–3) and their receptors (TGFβR I–III) [see also O'Reilly (71), this issue]. Although the developing lung probably lacks the O_2 gradients that control the functional expression of this pathway in placenta, the hypoxia dependency of TGFβ genes is readily illustrated by experimental exposure of neonatal lungs to hypoxia. In neonatal rats, ventilation with a 9.5% O_2 atmosphere results in a dramatic increase in TGFβ signaling and TGFβR-I expression that diminishes alveolarization, inhibits the formation of branched airway structures, and blocks epithelial and endothelial differentiation (88, 94). Other studies also demonstrate that the HIF-1α target gene, $TGF\beta_3$ (84), is a potent autocrine inducer of $TGF\beta_1$ (104) and that overexpression of $TGF\beta_1$ *in utero* causes mesenchymal thickening

in hand with lowered epithelial/endothelial differentiation, airway branching morphogenesis, and vasculogenesis (105). Although firm roles for HIF-1α-regulated $TGF\beta_3$ signaling during prenatal lung morphogenesis are yet to be identified, it seems reasonable to hypothesize that this pathway may be crucial in regulating mesenchyme cell mass during lung growth, so ensuring a balance between the rate of stem cell proliferation and terminal differentiation into airway and vascular tissues. In an intriguing addendum to this, it is notable that $TGF\beta_1$ is an effective inhibitor of stress-evoked (iNOS) activity (75) and that iNOS expression and activity increase dramatically in the lung within the final trimester of pregnancy (85). $TGF\beta_1$ expression may therefore negatively regulate the NO-dependent increase in branching morphogenesis observed by Young *et al.* (103) in a manner that is responsive to changes in lung P_{O_2} and redox state.

A number of other important regulators of lung morphogenesis are similarly poised to respond to O_2-evoked signals in the lung. Members of the hepatocyte nuclear factor family (HNF, a homologue of *Drosophila* forkhead) represent additional redox-responsive effectors involved in regulating aspects of lung morphogenesis. These proteins determine epithelial cell lineage fates by acting as dimerization partners for crucial developmental transcription factors, such as Nkx2.1, and are also DNA binding partners for several HIF-1α activated genes (35), through interaction between a TKQE motif at the HNF-4 C-terminus and HIF-1α (92). Similarly, specific protein (SP)-1 family transcription factors regulate the expression of surfactant genes, are activated by hypoxia, and are dimerization partners for HNF-3 (9, 28, 62). Although few studies have addressed the relationship between the HNF transcriptional regulation and lung development during redox stress, by virtue of their widespread association with a number of hypoxia-activated transcription factors, HNF family transcription factors represent key points of association between oxygen availability and lung development.

Finally, there is evidence to suggest that balanced expression of fibroblast growth factor (FGF) family members may depend on a low P_{O_2} environment to effectively regulate mesenchyme–epithelial differentiation. In the pseudoglandular stage lung (stages III–IV), FGF-9 mRNA is expressed in the pleura and transiently in the bronchial epithelium and stimulates the proliferation and expansion of the mesenchyme via FGF receptor 1c (FGFR-1c) and/or FGFR2c (21). FGF-10 is expressed in the mesenchyme adjacent to the distal termini of the developing airways and becomes diffusely expressed in the mesenchyme toward the end of this developmental stage (6). Whereas FGF-10 may initiate airway branching through proliferation and expansion of mesenchyme, FGF-9 regulates the density of this branching through proliferation and expansion of mesenchyme (20, 21); therefore, the ratio of FGF-9:FGF-10 represents an index of airway branching potential. We found that the mesenchymal expression of FGF-9 protein was greater in explants maintained at fetal as opposed to ambient P_{O_2} and that the mass specific expression of FGF-10 protein did not change with this treatment (57). Morphologically, this correlated with a loss of the surface area fraction of mesenchyme, an increase in airway space, and a significant reduction in airway surface complexity at the higher P_{O_2} (Fig. 4).

Although little is known about the genomic regulation of FGF-9, the 5' flanking region of this gene contains a putative binding site for the transcription factor, CCAAT enhancer binding protein β (C/EBPβ) (20). As demonstrated in other lung tissues (89), culture of explants at fetal P_{O_2} resulted in the nuclear translocation and DNA binding of this protein in the mesenchyme compartment, which mirrored the increase in expression of FGF-9 that occurred during treatments that augmented mesenchyme proliferation. Similarly, deactivation of C/EBPβ occurred with exposure of explants to ambient P_{O_2} and also resulted in a decrease in FGF-9 expression. C/EBP isoforms have been postulated as significant morphogens in the developing lung due to their capacity to regulate cellular turnover and differentiation (for review, see 15). Genomic knockout of the α and β isoforms results in breathing difficulties from birth, central cyanosis, high postnatal mortality, and, in the case of the α isoform, hyperproliferation of alveolar type II cells (23, 33, 95). Both α and δ isoforms regulate the expression of Clara cell secretory protein, an important antiinflammatory and cytoprotective agent in the bronchial airway (16). Precise developmental roles for C/EBPβ in lung are poorly defined; however, in hepatic and colonic tissue, C/EBPβ is a key component of the proliferative response to oxidative injury and is known to mediate mesenchyme proliferation during tissue repair by p53-independent induction of $p21^{WAF1/CIP1}$, an antiapoptotic cell-cycle regulator (12, 19, 71). Based on this information, we have derived a working hypothesis that the activity of this transcription factor in mesenchyme tissue at fetal P_{O_2} is important for maintaining an adequate balance of undifferentiated mesenchyme tissue, via FGF-9 expression, in regions of active airway differentiation.

REDOX EVENTS IN THE PERINATAL PULMONARY EPITHELIUM

Birth and the transition from placental to pulmonary respiration herald a major increase in systemic oxygen tensions that are at their greatest in the lung lumen as P_{O_2} equilibrates from fetal (23 mm Hg) to postnatal values (~100 mm Hg). We have explored the possibility that this change in P_{O_2} is sufficient to alter the redox state of the epithelial gas-exchange surface that is important for signaling postnatal lung maturation events. Polarized, bioelectrically resistant rat fetal distal lung epithelial cells (FDLE) can be used as a physiologically representative culture model of the epithelium that lines the lower reaches of the fetal lung (76, 79, 80). Culture of these cells at fetal P_{O_2} followed by exposure to a rise in P_{O_2} equivalent to that which occurs in the alveolar region as the infant takes its first breaths (~76–100 mm Hg) evokes a three-fold rise in H_2O_2 production that increases to six-fold in cultures exposed to hyperoxic P_{O_2} levels (Fig. 5A); in each case, iron chelation using desferrioxamine or respiratory chain inhibition substantially depleted the production of H_2O_2 and its derivative ROS (43). Similarly, A549 alveolar epithelial cells that are devoid of mitochondrial DNA (ρ^o A549), and so lack a functional respiratory chain, fail to produce H_2O_2 (as fluorescence arising from dichlorofluorescin diacetate oxidation) when exposed to similar shifts in P_{O_2} (Fig. 5B). Taken together, these studies highlight the change in P_{O_2} in the perinatal lung as a stimulus for mitochondrial ROS production that initiates an important sequence of events necessary for postnatal lung function.

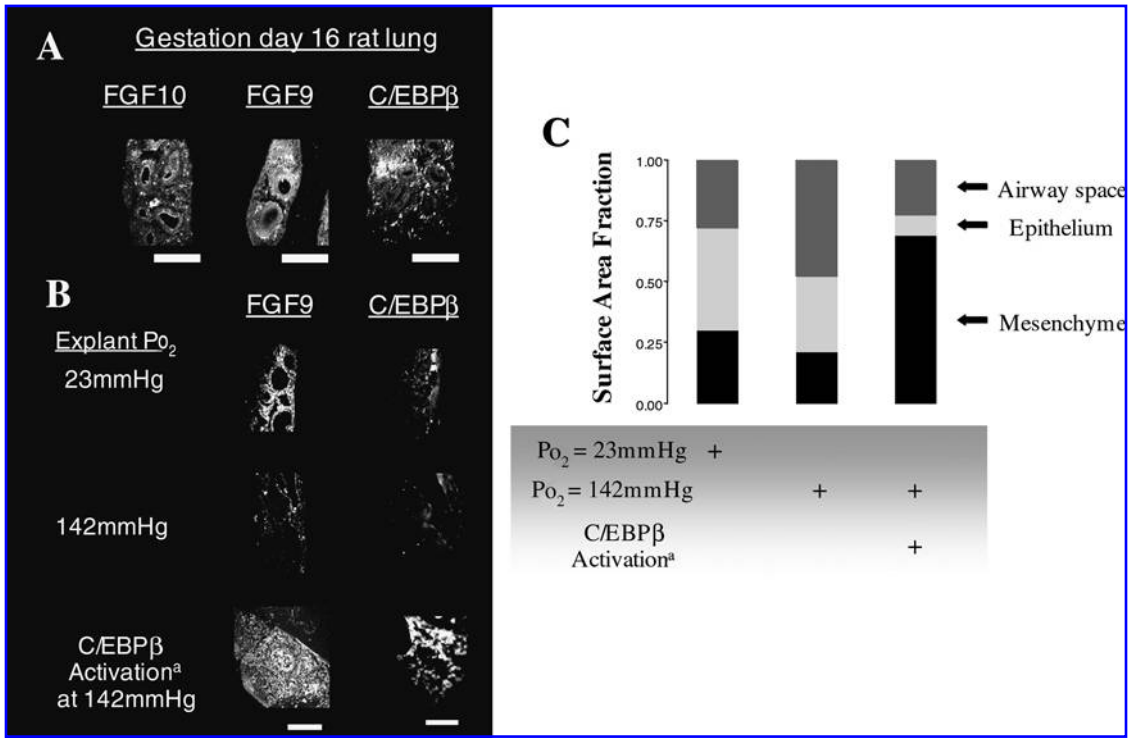

FIG. 4. Culture Po_2 modulates the expression and distribution of growth factors that regulate mesenchyme differentiation. (**A**) Immunohistochemical detection of FGF-10, FGF-9, and C/EBPβ in native gestation day 16 rat lung. Whereas FGF-10 is largely associated with the airways, FGF-9 and C/EBPβ expression is predominantly in the undifferentiated mesenchyme compartment (FGF-9 and 10, fluorescein isothiocyanate; C/EBPβ tetramethylrodamine β isothiocyanate; bar = 345 μm). (**B**) Expression of FGF-9 and its putative transcriptional regulator, C/EBPβ, in gestation day 14 fetal rat lung explants cultured for 96 h at fetal Po_2 (23 mm Hg), ambient Po_2 (142 mm Hg), or ambient Po_2 in the presence of the thymic hormone, thymulin (1 μg/ml), a potent activator of C/EBPβ (57). Culture of explants at ambient Po_2 dramatically lowers FGF-9 protein expression and C/EBPβ nuclear immunoreactivity and is potently reversed by thymulin treatment. Bar = 345 μm (FGF-9) and 50 μm (C/EBPβ). (**C**) Calculation of the surface area fraction occupied by airway space, differentiated epithelium, and mesenchyme reveals that culture of explants at ambient Po_2 is associated with a statistically significant ($p < 0.05$ relative to fetal Po_2) loss of mesenchyme area. In hand with its putative regulation of FGF-9, C/EBPβ activation dramatically reverses this effect by evoking a widespread proliferation of mesenchyme. Figures are based on data from Land and Darakhshan (57).

Perinatal ROS production is buffered through a broad spectrum increase in the expression of several antioxidant enzymes. Among the most important secreted antioxidants in the lung at this time is the extracellular isoform of superoxide dismutase (EC-SOD), which is highly expressed and secreted by alveolar type II cells, upper airway epithelial cells, and vascular endothelium. The secretion of EC-SOD into the extracellular space parallels the increase in breathing at birth (69) and, by rapidly scavenging $O_2^{\bullet -}$. ($K_D \approx 1 \times 10^9$; 63), secondarily extends the activity half-life of NO (74) synthesized in epithelial and vascular tissues during the early perinatal period and limits the generation of peroxynitrite ($ONOO^-$). By implication, EC-SOD activity may be important in conserving pulmonary vasodilation as well as NO-dependent aspects of airway morphogenesis in the postnatal period. The expression of EC-SOD occurs in direct response to the perinatal rise in lung lumenal Po_2 and is chronically suppressed in neonates previously exposed to hypoxia *in utero* (37). Although the basis for this dependency for raised oxygen is not yet known, EC-SOD expression is potently raised by NF-κB-dependent induction of interleukin-1α, tumor necrosis factorα, and interferon-γ (8, 64). Outside the lung, there is also some evidence that EC-SOD expression is increased by NO synthesis (34), suggesting that a positive feedback loop may exist involving EC-SOD expression and maintenance of NO activity.

Glutathione biosynthesis also parallels the increase in perinatal lung ROS production (Fig. 5C and D). The synthesis of the reduced form of glutathione (GSH) is transiently increased in rat lung over the first 6 h following birth and is matched by a two-fold fall in the overall content of the oxidized form (GSSG). Activities of glutamate cysteine ligase (GCL), the rate-limiting enzyme in glutathione biosynthesis, and glutathione synthase are both increased 1.5-fold within 3 h of birth and account for a three-fold postnatal increase in total glutathione concentrations. This effect is reproduced in FDLE cultures when shifted from fetal to postnatal Po_2 where a 2.5-fold increase in cellular [GSH] is accompanied by an increase in GCL activity of similar magnitude (40). GCL is composed of a catalytic 70-kDa subunit (GCLC) and a 30-kDa modifier subunit (GCLM) that function to lower the K_M of the GCL heterodimer for glutamate from 18.2 m*M* to a physiological concentration of 1.2 m*M* while increasing the K_I for GSH. Both subunits are expressed widely in fetal tissues and show no developmental change in either distribution or expression

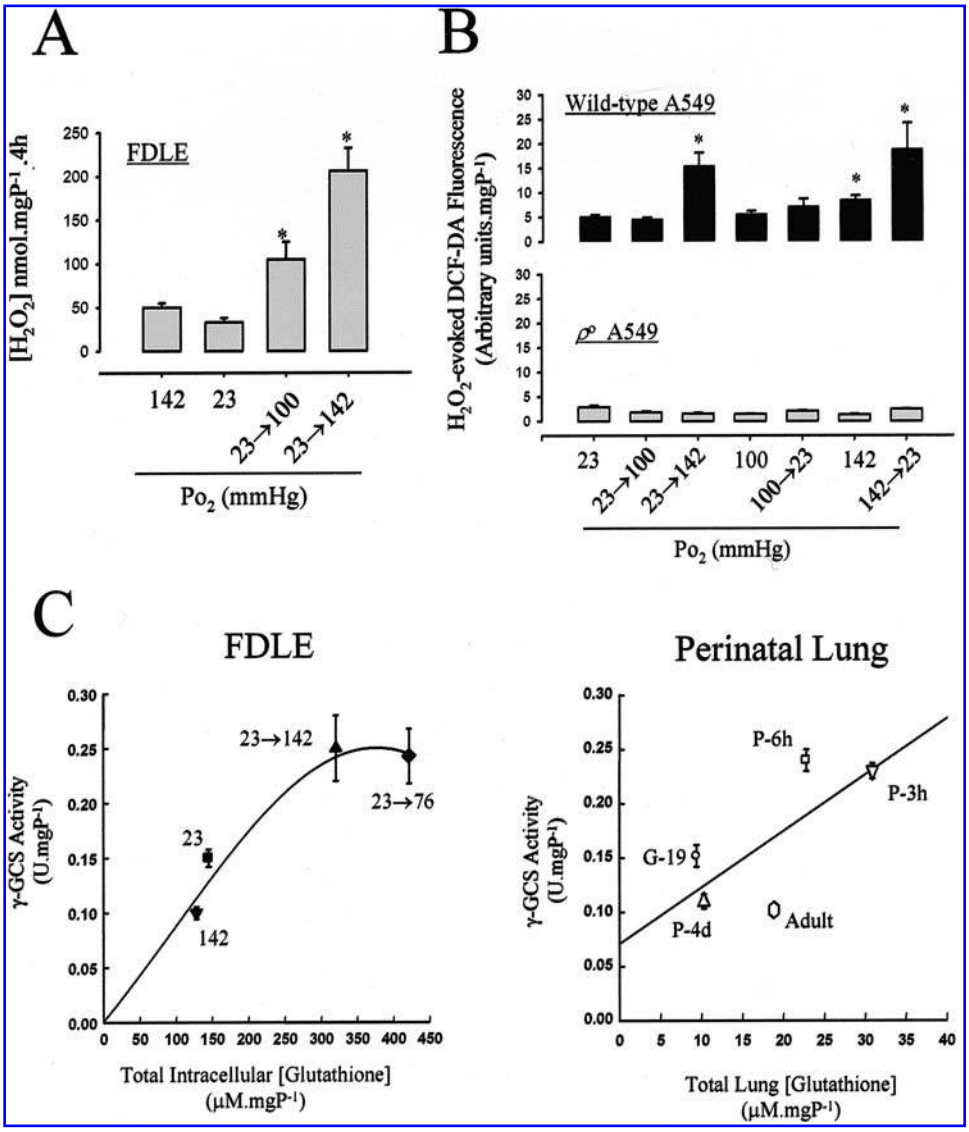

FIG. 5. The perinatal rise in Po_2 is sufficient to evoke ROS production and glutathione biosynthesis in lung epithelial tissue. (**A**) H_2O_2 production over 4 h in fetal distal lung epithelial cell cultures maintained in the steady state at ambient (142 mm Hg) or fetal (23 mm Hg) Po_2 or removed from the fetal oxygen tension for 4 h to postnatal (100 mm Hg) or ambient Po_2 (*$p < 0.05$ versus 23 mm Hg). Data are from Haddad and Land (40). (**B**) H_2O_2-evoked dichlorofluorescin diacetate (DCF-DA) oxidation and DCF fluorescence in human alveolar A549 cells cultured under steady-state conditions at fetal, postnatal, or ambient Po_2, or exposed to deoxygenating or oxygenating shifts in Po_2 for 4 h (upper graph). An identical treatment series was applied to ρ° A549 cells lacking mitochondrial DNA (lower graph). Incubation at 142 mm Hg, de-oxygenation (142 → 23 mm Hg), and oxygenation (23 → 142 mm Hg) evoked significant increases in DCF fluorescence ($p < 0.05$ versus 23 mm Hg steady state, ANOVA post hoc Tukey's HSD, $n = 6$); however, no statistical differences were found among treatments applied to in ρ° A549 cultures ($n = 6$). ρ° A549 were generated by exposure to 25 ng/ml ethidium bromide over several passages in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 1 m*M* pyruvate, and 1 m*M* uridine. Mitochondrial DNA depletion was confirmed by the gradual loss of ability to amplify a 450-bp fragment of the cytochrome oxidase II gene that is encoded exclusively on the mitochondrial genome. Po_2 manipulations were conducted using a MACS VA500 microaerophilic workstation. (**C**) Exposure of FDLE cells to early postnatal Po_2 (76 mm Hg) or ambient Po_2 for 6 h evokes a rise in glutamyl cysteine ligase [GCL; also γ-glutamyl cysteine synthetase (γ-GCS)] activity and total cellular glutathione content that is mirrored in the perinatal rat lung. Figures are modified from Haddad and Land (40).

levels (59). However, both subunits are transcriptionally upregulated in response to a wide range of oxidative stressors, including quinone-evoked mitochondrial H_2O_2 production (60, 86, 91, 102). The 5' promoter region of GCLC contains several putative transcription factor binding sites, including several antioxidant responsive elements (ARE) and others for activator protein (AP)-1, AP-2, metal response element, SP-1, and NF-κB (see 60 and references therein); however, recent studies with bleomycin-induced ROS production reveal particular roles for factors binding to both NF-κB and ARE pro-

moter elements (26). The GCLM promoter contains a similar complement of transcription factor binding sites, but in contrast to GCLC, lacks a consensus site for NF-κB and is dependent on *denovo* protein synthesis prior to transcriptional initiation. In the perinatal lung epithelium, the rise in [GSH] matches the time frame for the perinatal increase in ROS production and NF-κB activity; hence, the increase in GCL activity that accompanies this response likely arises from ROS-evoked expression of both subunits. As such, an initial transient oxidation of the perinatal lung represents a potentially important signal for triggering aspects of postnatal lung maturation.

As the reciprocal regulation of HIF-1α and NF-κB activities by perinatal shifts in Po_2 occurs within the approximate time frame of change in epithelial [GSH], we assessed the potential for this redox buffer to alter the O_2 responsiveness of these transcription factors. We found that experimental augmentation of [GSH] using NAC stabilized and activated HIF-1α, and deactivated NF-κB, during hyperoxic shifts in Po_2, an event that would appear to implicate a state of reduction as representative of the hypoxic fetal lung. However, augmentation of [GSSG] using pyrrolidine dithiocarbamate and depletion of total cellular glutathione using the GCL inhibitor, l-buthionine [*S,R*]-sulfoximine, blocked the activation of both transcription factors irrespective of Po_2 (41). Moreover, we have observed that the timeline of HIF-1α activation and deactivation coincides invariably with a transient rise in [GSSG]:2[GSH] as cells equilibrate to a new hypoxic or normoxic steady state, suggesting that oxidation may be an important component of the regulation of this transcription factor (Land, unpublished observations). Although these results suggest that altered total cellular glutathione buffering can modulate the O_2 induction pattern of transcription factors, it has to be acknowledged that these treatments do not reflect physiological conditions and that ROS buffering by GSH occurs as a secondary generalized response to the production of an oxidizing stimulus. We therefore examined the potential for localized regulation of transcription factor activation by protein thiols in response to changes in Po_2.

TRX, a widely expressed, potent protein oxidoreductant, is known to exert redox control over the activation of transcription factors by dithiol–disulfate redox exchange to modulate their binding to DNA [see review by Nakamura *et al.* (67), this issue]. TRX reductase (TR) catalyzes the transfer of reducing equivalents from NADPH, via its own disulfide bond, to the substrate (TRX) disulfide and so is the rate-limiting determinant of TRX redox status. The expression of mRNAs encoding TRX and TR remains constant throughout development and shows no sign of change until the early neonatal period. Significantly, this increase is potentiated *in vivo* by exposure of perinatal lungs to hyperoxic Po_2 levels and is also mirrored in lung explants cultured at raised oxygen tensions, suggesting that the change in lung lumenal Po_2 is a dominant signal for TRX/TR expression in the perinatal lung (25). TRX has been identified as a dimerization partner for a number of transcription factors, including HIF-1α and NF-kB, and so offers a means for transducing local differences in redox potential to modulate transcriptional regulation (29, 44, 45, 97). We found that HIF-1α readily coimmunoprecipitates with TRX using an anti-TRX antibody from nuclear extracts obtained from lung epithelial cells during equilibration to fetal Po_2. Notably, slow equilibration of cultures from ambient to fetal Po_2 levels (142–23 mm Hg) revealed that the stabilization and nuclear transfer of this transcription factor to the nucleus were associated with an oxidation and homodimerization of nuclear TRX that is not recovered until steady-state conditions are reached. Similarly, deactivation/destabilization of HIF-1α during a return to ambient Po_2 coincides with a transient nuclear TRX oxidation event that is accompanied by significant H_2O_2 production (Figs. 5 and 6). Although our own studies are yet to extend to NF-κB, others have demonstrated that the inflammatory enactivation of this transcription factor in the lung is similarly dependent on TRX binding and cotranslocation to the nucleus and that subsequent DNA binding and gene expression are inhibited by supraphysiological elevation of [GSH] by NAC (41). As the oxidized TRX dimer is not a substrate for TR and so does not participate further in redox signaling, these studies raise a number of important considerations, namely, that, (I) the TRX transport to the nucleus occurs at changes in oxygen tension that are physiologically representative of fetal and postnatal Po_2 levels, (II) the biological effects of TRX are confined to the early stages of equilibration to a new steady-state Po_2; and (III) the nuclear environment is sufficiently oxidative to evoke the dimerization and inactivation of TRX during the latter stages of equilibration to either fetal or ambient Po_2 levels. These data accord with recent evidence that indicates that oxidative stress can alter nuclear histone acetylation and DNA unwinding and so facilitate access for transcription factor DNA binding and enhanced gene expression in various lung cells (38). Transient oxidation of the nuclear compartment, irrespective of increased cellular GSH buffering, may therefore be requisite for the completion of proper transcriptional initiation during transitions to or from hypoxia; importantly, these effects are apparent in lung epithelial cells shifted between physiologically pertinent oxygen tensions.

PERINATAL Po_2 AND LUNG FLUID HOMEOSTASIS

Throughout gestation, the secretion of Cl^- against an electrochemical gradient creates an osmotic driving force that drives fluid into the lung lumen and so creates a distending pressure that is necessary for three-dimensional airway morphogenesis. Immediately before birth, the secretion of liquid slows as increased Na^+ absorption from the lung lumen drives fluid into the vasculature and so triggers airway clearance in preparation for gas exchange. Although the rise in maternal adrenaline titer during labor is crucial for initiating the lung's Na^+-absorbing phenotype, other factors must contribute to the maintenance of this phenotype as the lungs' capacity to absorb Na^+ from the lumen is normally retained throughout adult life despite a rapid, postnatal fall in circulating adrenaline levels. The first indication that the rise in lung lumenal Po_2 at birth might influence pulmonary ion transport came from studies of lung tissue explanted from fetal rats (5). These studies showed that increased Po_2 caused a fall in wet to dry weight ratio of explants derived from late-gestation fetuses, and established that this was accompanied by a reduction in the number and

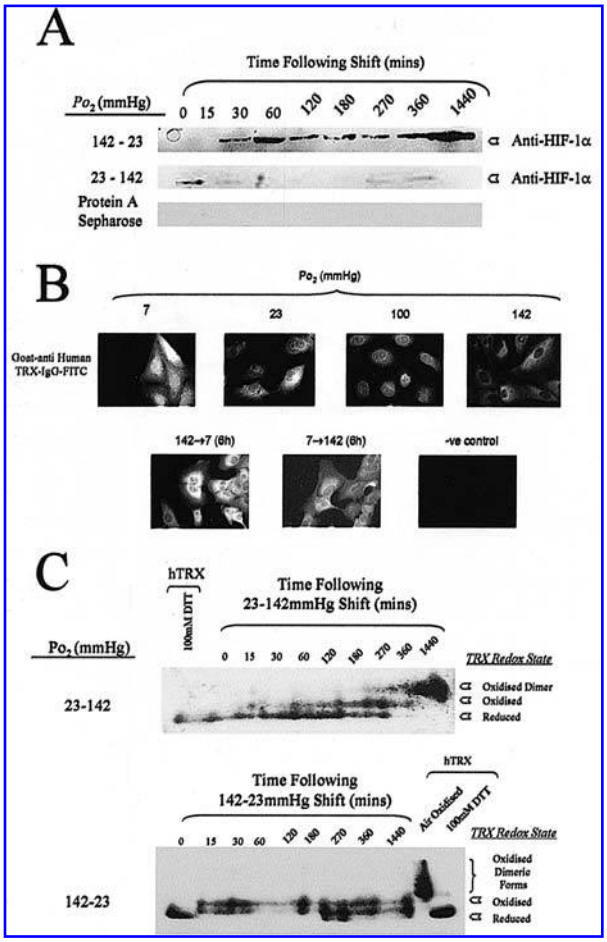

FIG. 6. TRX nuclear activity and redox state are altered by PO_2. (**A**) HIF-1α displays a reversible association with TRX in A549 cells during equilibration from ambient to fetal P_{O_2}. Nuclei were obtained and lysed at times shown during the equilibration period as described (43). Proteins eluting with protein A-Sepharose-conjugated goat anti-human TRX IgG (America Diagnostica) were fractionated by western blotting, and HIF-1α was resolved by horseradish peroxidase (HRP) chemiluminescence using rabbit anti-human HIF-1α IgG (Novus Biologicals) as described (57). Blots are representative of four experiments. (**B**) TRX is associated with the nucleus at steady-state oxygen tensions equal to, or below, fetal P_{O_2} and shows strong perinuclear localization during transient oxygenating or deoxygenating events. A549 cells were cultured under the steady-state or transient P_{O_2} regimen shown. Cells were fixed in 4% paraformaldehyde, permeabilized with phosphate-buffered saline (PBS) containing 0.1% Triton X-100, blocked in PBS containing 1% serum albumin (30 min each step), and incubated overnight with goat anti-human TRX IgG (1:200 dilution). Fluorescein isothiocyanate (FITC)-conjugated anti-goat IgG (1:500 dilution) was used to visualize the distribution of fluorescence evinced using a Zeiss Axioskop fluorescent microscope. Each image is representative of four experiments. (**C**) TRX is oxidized during equilibration of A549 cells toward or away from fetal P_{O_2}. Cell protein was lysed at the times shown and carboxymethylated as described by Das *et al.* (25) taking care to avoid further perturbation in P_{O_2}. Carboxymethylated proteins were fractionated on a 15% native polyacrylamide gel and western blotted, and TRX was resolved by HRP chemiluminescence using anti-human TRX IgG. Control lanes reveal location of dithiothreitol (DTT)-reduced or air-oxidized TRX. Blots are representative of three experiments for each P_{O_2} regimen.

size of the fluid-filled cysts present in these cultures. However, increased P_{O_2} had no discernible effect on lung tissue from early-gestation fetuses (5), although precocious sensitivity to O_2 could be induced by exposing the cells to T_3/glucocorticoids. These data thus suggest that a rise in ambient P_{O_2} may directly influence pulmonary fluid balance, and later studies of FDLE cells showed that such increases in P_{O_2} can stimulate amiloride-sensitive Na^+ transport and raise the abundance of α-, β- and γ-ENaC mRNA (76). Pulmonary Na^+ transport is thus inherently sensitive to P_{O_2}, implying that the rise in alveolar P_{O_2} occurring at birth may provide a drive for maintained Na^+ transport in the postnatal period despite rapidly falling adrenaline levels.

HOW DOES O_2 CONTROL Na^+ TRANSPORT?

Despite its potential importance, the mechanism that allows a physiologically relevant change in P_{O_2} to stimulate pulmonary Na^+ transport is not well understood. O'Brodovich and colleagues first showed that increases in P_{O_2} can raise the cellular abundance of mRNA encoding α-, β- and γ-ENaC and thus suggested that the increased Na^+ transport may be due to increased expression of this gene family, which would be expected to cause a rise in Na^+ conductance (G_{Na^+}) and thus facilitate Na^+ transport. Support for this model came from the fact that the promoter region of the α-ENaC gene contains a binding site for NF-κB and that this transcription factor displays a pattern of activation that temporally matches the rise in P_{O_2} and ROS production in the distal lung (73). It was thus suggested that increases in P_{O_2} might stimulate Na^+ transport by evoking NF-κB-mediated ENaC expression (42, 78), and the formulation of this testable hypothesis prompted an examination of the temporal relationship between the different components of this proposed pathway in O_2-stimulated rat FDLE cells. These experiments clearly confirmed that increases in P_{O_2} did activate NF-κB and established that this response was well established 5–10 minutes after P_{O_2} was raised. The stimulation of Na^+ transport, on the other hand, was a slower response that developed over the following 24 h. However, although these experiments also provided the first direct demonstration of O_2-evoked activation of the α-ENaC promoter, they showed this response was slow, occurring only after P_{O_2} had been raised for 24–48 h. This surprising result prompted functional studies in which G_{Na^+} was measured directly in O_2-stimulated cells. It thus became clear that no overt rise in G_{Na^+} occurred until P_{O_2} had been raised for 24–48 h, and so this effect, in common with the increased activity of the α-ENaC promoter, is delayed. Physiologically relevant increases in P_{O_2} evoke all of the key responses identified in O'Brodovich's model, but examination of the temporal relationship between the various components of the response shows clearly that NF-κB-mediated ENaC expression cannot be involved in the genesis of O_2-evoked Na^+ transport. Indeed, more recent work has supported this conclusion by showing that, at least under certain conditions, O_2-evoked increases in G_{Na^+} can occur with no change in ENaC mRNA abundance (80). Although increases in P_{O_2} can activate the α-ENaC promoter, the available data suggest that the resultant increase in ENaC expression may

occur as a physiological adaptation to the increased rate of transepithelial Na^+ transport rather than being its cause. It is interesting, however, that pharmacological blockade of NF-κB activation does attenuate the effects of O_2 upon G_{Na^+}, and so this transcription factor may well play a central role in this response (42).

Earlier studies of FDLE cells showed that physiologically relevant increases in Po_2 also increase the Na^+ extrusion capacity of the basolateral Na^+ pump, and this response (79), in contrast to the increased ENaC expression, precedes the increase in short circuit current (I_{SC}), by 3–4 h. As this transport protein is ultimately responsible for providing the driving force for transepithelial Na^+ transport, it is tempting to attribute the O_2-evoked stimulation of Na^+ transport to the increased capacity of this pump. However, significant problems must be addressed before this model can be accepted. Although O_2-evoked activation of the Na^+ pump is now well documented, the physiological basis of this response in the perinatal lung is completely unknown. Some authors have noted Po_2-evoked increases in mRNA encoding the pump's component subunits, suggesting that increased gene expression may be involved, but not all available data are consistent with this model. Moreover, it is abundantly clear from classical studies of Na^+-absorbing epithelia that rate of transepithelial Na^+ transport is limited by the rate at which Na^+ can diffuse down the inwardly directed electrochemical gradient across the apical membrane. Simply increasing the amount of Na^+ extruded by the Na^+ pump capacity cannot lead to increased transepithelial Na^+ transport without a corresponding rise in G_{Na^+} and yet studies of O_2-stimulated pneumocytes have shown that no overt change in G_{Na^+} occurs until the O_2-evoked increase in I_{SC} is fully established. However, it is worth noting that these studies used a method that measures the apical membrane's "constitutive" conductance at fixed ionic conditions, and would therefore fail to reveal changes in G_{Na^+} that were mediated by small, highly mobile messengers. It is interesting, in this context, that cytoplasmic [Na^+] and [Cl^-] have been shown to mediate inhibitory control over G_{Na^+} in other Na^+-absorbing cells. This control over G_{Na^+} appears to form part of a rapid, feedback mechanism that may well allow increases in Na^+ pump capacity to be transduced into changes in the overall rate of Na^+ transport. It is therefore important to determine the extent to which such feedback control of G_{Na^+} occurs in lung epithelia.

CONCLUSIONS

In this review, we have identified a number of loci at which fetal Po_2 and the transition to postnatal Po_2 may potentially sustain or actively regulate lung development and maturation. Although not exhaustive, these are: (I) the potential for hypoxia to sustain the transcriptional activity of HIF-1α, C/EBPβ, HNF, and SP family transcription factors coupled with the expression of TGFβ and FGF-9 genes involved in mesenchyme proliferation and differentiation; (II) the transient production of ROS and nuclear oxidation of the perinatal lung epithelium; (III) nuclear transport and oxidation of TRX in hand with the acute activation of NF-κB; (IV) ROS-evoked chronic rise in intracellular glutathione and TRX redox buffering capacity; and (V) NF-κB-dependent increase in transepithelial Na^+ transport and lung lumenal fluid clearance. The implication of this set of observations is that modulation of the natural redox events that occur in the perinatal lung (by, for example, oxygen therapy) stand to widely affect normal lung developmental processes, a fact that is readily observed in the progression of perinatal lung disorders such as bronchopulmonary dysplasia and cystic fibrosis. In a recent review of the genetic mechanisms currently understood to be involved in regulating lung morphogenesis, Warburton *et al.* (96) acknowledge the fact that we are only presently emerging from the identification phase of candidate morphogenic genes to a point where their mechanistic interactions can be addressed. We highlight development at fetal Po_2 and the physiological change from fetal to perinatal Po_2 levels as significant mechanistic factors that superimpose the developmental expression of these genes.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-1, activator protein-1; ARE, antioxidant response element; ARNT, aryl hydrocarbon nuclear translocator; bHLH, basic helix–loop–helix; C/EBP, CCAAT-enhancer binding protein; C-TAD, C-terminal activation domain; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; EC-SOD, extracellular superoxide dismutase; EnaC, epithelial Na^+ channel; Epo, erythropoietin; FDLE, fetal distal lung epithelial cells; FGF, fibroblast growth factor; FGFR, FGF receptor; FITC, fluorescein isothiocyanate; G_{Na^+}, Na^+ conductance; GCL, glutamate cysteine ligase (γ-glutamyl cysteine synthetase); GCLC, catalytic subunit of GCL; GCLM, modifier subunit of GCL; $gp91^{phox}$, glycosylated 91-kDa subunit of NADPH oxidase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; HIF, hypoxia-inducible factor; HNF, hepatocyte nuclear factor; H_2O_2, hydrogen peroxide; I-κB, inhibitor of κB; IKK, inhibitor of κB kinase; iNOS, inducible isoform of nitric oxide synthase; I_{SC}, short circuit current; LPS, lipopolysaccharide; MEKK-1, mitogen-activated protein kinase kinase; NAC, *N*-acetylcysteine; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; NO, nitric oxide; NPO, NADPH oxidase; $O_2^{\bullet -}$, superoxide anion; ODD, oxygen-dependent degradation domain; PAS, *Drosophila* "period," ARNT and *Drosophila* "singleminded" proteins; pVHL, von Hippel–Lindau protein; ROS, reactive oxygen species; SP, specific protein; TGF, transforming growth factor; TGFR, TGF receptor; TR, TRX reductase; TRX, thioredoxin.

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