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Unidirectional Airflow in the Lungs of Alligators
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Supporting Online Material

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Materials and Methods
Figs. S1 to S13
Tables S1 and S2
References

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Unidirectional Airflow in the Lungs of Alligators

C. G. Farmer[1]* and Kent Sanders[2]

The lungs of birds move air in only one direction during both inspiration and expiration through most of the tubular gas-exchanging bronchi (parabronchi), whereas in the lungs of mammals and presumably other vertebrates, air moves tidally into and out of terminal gas-exchange structures, which are cul-de-sacs. Unidirectional flow purportedly depends on bellowslike ventilation by air sacs and may have evolved to meet the high aerobic demands of sustained flight. Here, we show that air flows unidirectionally through parabronchi in the lungs of the American alligator, an amphibious ectotherm without air sacs, which suggests that this pattern dates back to the basal archosaurs of the Triassic and may have been present in their nondinosaur descendants (phytosaurs, aetosaurs, rauisuchians, crocodylomorphs, and pterosaurs) as well as in dinosaurs.

Airflow in the avian lung is believed to be unique because gases move in the same direction during inhalation and exhalation through small tubes, the parabronchi. Although airflow is caused by volumetric changes in air sacs, the unidirectional pattern is achieved without mechanical valves. Soot-laden air was used to demonstrate unidirectional flow, and this pattern of airflow was attributed to the configuration of the bronchi giving rise to jetting and Venturi effects (in which increases in fluid velocity decrease lateral pressure) (*1*, *2*).

Crocodilian lungs are distinct from bird lungs and are thought to have a large alveolar-arterial blood gas difference, large ventilation-perfusion inhomogeneity, and parenchyma consisting of cubicles (ediculae) (*3*–*5*). However, the topography of the intrapulmonary bronchus and of the first generation of bronchi is similar in birds and crocodilians (*3*, *6*, *7*).

[1]Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA. [2]Department of Radiology, Musculoskeletal Division, 50 North Medical Drive, Room 1A71, University of Utah Health Sciences Center, Salt Lake City, UT 84132, USA.

*To whom correspondence should be addressed. E-mail: cg.frmr@gmail.com

Key features of the avian aerodynamic valve appear to be present in the alligator lung. The green bronchus shown in Fig. 1, the cervical ventral bronchus (CVB), is strikingly similar to the avian ventral bronchus that connects with the cervical air sacs. The small ostium to the CVB opens into a funnel-shaped vestibule acutely angled with the intrapulmonary bronchus, so that the bronchus makes a hairpin turn ventrocranially before coursing cranially to the apex of the lung (Fig. 1A, B). Distal to the CVB ostium, the intrapulmonary bronchus widens and becomes partially enclosed as it curves caudally and laterally and gives rise to a pair of small medial paracardiac bronchi (Fig. 1, red bronchi), a large individual dorsolateral bronchus (Fig. 1, chartreuse bronchus), and three caudal bronchi originating from a common terminal intrabronchial chamber (Fig. 1, blue bronchi). Their orifices are larger than the CVB orifice and better aligned with the intrapulmonary bronchus. Most of these latter bronchial passages spiral dorsolaterally toward the apex of the lung in a manner similar to the avian dorsal bronchi. We have discovered that the dorsal bronchi connect to each other and to the CVB through numerous anastomosing parabronchi, approximately 1 to 1.5 mm in diameter at the orifice to the bronchi (arrowheads, Fig. 1C) [supporting online material (SOM)]. As in birds, small ostia to caudoventral bronchi (arrows, Fig. 1C) occur opposite the ostia of the dorsal bronchi.

The anatomical similarity with the avian lung led us to hypothesize that airflow might also be unidirectional in crocodilians. Previously, measurements of airflow in alligator lungs did not identify unidirectional flow (*8*). Perry discussed the possibility that crocodilians have unidirectional airflow, but concluded that airflow is tidal in crocodilians and that unidirectional flow evolved in coelurosaurian-grade dinosaurs (*9*–*12*). Furthermore, for avian-style respiration to occur in birds and nonavian dinosaurs, abdominal air sacs have been presumed to be critical (*13*), and the hepatic piston mechanism of ventilation of crocodilians has been presumed incompatible (*14*).

To test the hypothesis that airflow in alligator lungs is unidirectional, we implanted dual thermistor flowmeters in the CVB (green bronchus of Fig. 1) and a dorsal bronchus (a blue bronchus of Fig. 1) of four alligators, artificially ventilated the lungs with both negative and positive pressure inspiration, and observed that air in the CVB moved in a cranial-to-caudal direction and air in the dorsal bronchus moved in a ventrolateral to dorsomedial direction during expiration and during both types of inspiration (Fig. 2).

To determine whether similar patterns of flow occur in vivo, we monitored airflow in the CVB during normal breathing in five alligators with single-bead thermistors and in one alligator with a dual thermistor flow meter. In the former experiments, the flow continued during the transition from inspiration to expiration rather than dropping to zero, as would be the case if the direction of the flow had reversed. In vivo recordings with the dual thermistor flow meter showed that the air moved in a cranial-to-caudal direction during both inspiration and expiration; which is the same pattern of flow observed in excised lungs (Fig. 3). The amplitude of the expiratory flow was greater than the amplitude of the inspiratory flow, as in the excised lungs.

We also visualized flow by filling an excised lung with saline containing fluorescent microspheres. In the CVB, the microspheres flowed in a cranial-to-caudal direction as the fluid was pushed into and pulled out of the lung (movies S1 and S2). In the parabronchi, the spheres also flowed unidirectionally (movie S3). All three methods—in vivo recordings of airflow, recordings of airflow in artificially ventilated excised lungs, and visualization of water flow in artificially ventilated lungs—indicate that fluid flows unidirectionally through the lungs of alligators in a strikingly birdlike pattern.

Our data suggest that airflow in the alligator is extremely birdlike, but it is unknown how it is possible to have unidirectional flow without air sacs and with diaphragmatic breathing. A mechanism for unidirectional flow in bird lungs as a consequence of the topography of the intrapulmonary bronchi (*1*) that does not depend on air sacs or the mechanics of breathing is shown in Fig. 1, H and I. During exhalation, the configuration of the laterobronchial ostia may cause air to move dorsally to enter the ostia of the dorsobronchi, rather than leaving the lung directly by way of the mesobronchus (*1*). During inspiration, rapid flow of air along the intrapulmonary bronchus past the ostia to the ventrobronchi may reduce lateral pressure at this location because of the Venturi effect, and the low pressure may act

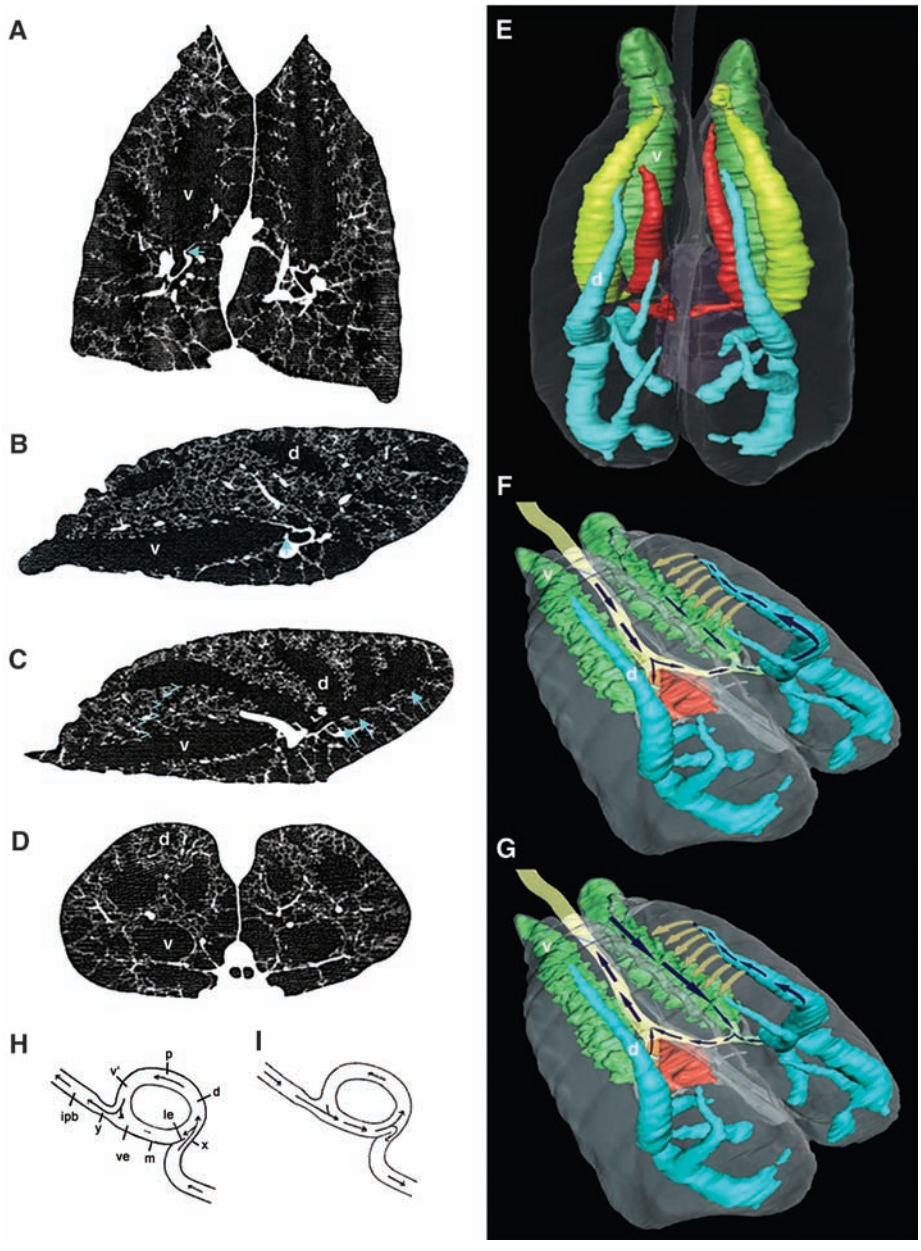

Fig. 1. Airflow in alligator lungs. Computed tomography images (left) show the hairpin turn (blue arrow) into the CVB (v) in the coronal (**A**) and medial sagittal (**B**) views. The lateral sagittal view shows the larger ostia to the dorsal bronchi (**C**), some of the ostia of ventral and lateral bronchi (blue arrows), a parabronchus (blue arrowheads), and the dorsal bronchus in which flow was recorded (d). The axial view (**D**) shows the bifurcation of the primary bronchi. An oblique dorsal view of the major bronchi is shown in (**E**). A simplified view shows airflow during inspiration (**F**) and exhalation (**G**) in the trachea, CVB, dorsal bronchus, and parabronchi. Hazelhoff's model of exhalation (**H**) and inspiration (**I**). ipb, intrapulmonary bronchus; le, guiding dam; ve, vestibulum; v, ventrobronchus; m, mesobronchus; p, parabronchus; d, dorsobronchus; x,y, sites of constriction.

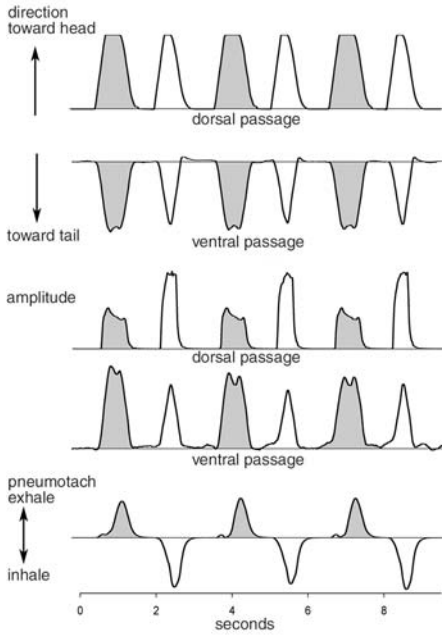

Fig. 2. Airflow in excised lungs. The top two traces show the direction of intrapulmonary airflow recorded from the dorsal bronchus (d in Fig. 1) and CVB (v in Fig. 1) with dual thermistor flow meters. The middle two traces show the relative amplitude of these flows. The bottom trace shows flow into and out of the trachea, recorded with a pneumotach.

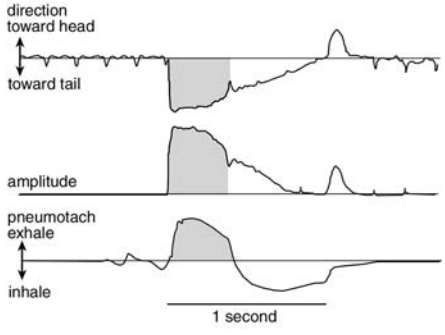

Fig. 3. Airflow observed in vivo. The top trace shows the direction of intrapulmonary airflow recorded in the CVB (v of Fig. 1) with a dual thermistor flow meter. The small pulse of air moving toward the head after airflow in the trachea has ceased occurs when the glottis is closed and the muscles of the trunk relax (*21*). The middle trace shows the amplitude of the flow. The bottom trace shows flow into and out of the trachea, recorded with a pneumotach.

as a suction pump to draw air past the mouths of the ventrobronchi into the mesobronchus (*1*). A glass model demonstrated how this geometry gives rise to unidirectional airflow (*1*).

The mechanism of unidirectional flow in alligator lungs is yet to be determined, but our data support Hazelhoff's model (*1*), in which key features of the bronchial tree give rise to unidirectional flow. During inspiration, air may jet past the obliquely oriented vestibule of the CVB to enter the larger dorsal bronchial openings and reduce lateral pressure at the CVB orifice to draw air from the CVB into the intrapulmonary bronchus. During exhalation, air in the caudoventral bronchi may jet dorsally (blue arrows in Fig. 1C) to enter the ostia of the dorsobronchi. In this way, a simple arrangement of the bronchi by themselves might give rise to unidirectional airflow. Also, the mechanism of gas exchange in crocodilians is not known; a crosscurrent mechanism has been hypothesized (*11*), but a countercurrent mechanism cannot be ruled out. Furthermore, the importance of unidirectional airflow for gas exchange efficiency in the alligator lung is not known and cannot be determined from our data, which consist of measurements of airflow.

Previous scenarios for the evolution of unidirectional airflow are that it arose in dinosaurs of coelurosaurian grade (*12*), convergently in theropods and pterosaurs (*13*, *15*), or not at all in dinosaurs because of a hepatic piston mechanism of breathing (*14*). Our findings contrast with these previous views in several ways. They demonstrate that the hepatic piston mechanism of breathing, which crocodilians have but birds lack, does not preclude the evolution of unidirectional flow and that pneumaticity, which crocodilians lack, cannot be used to diagnose unidirectional airflow in fossil taxa, as previously suggested (*13*, *15*). Crocodilians and birds are crown-group Archosauria. Therefore, in contrast to previous views, we suggest that unidirectional flow evolved before the divergence of crurotarsan and dinosaurian archosaurs and was present in the basal archosaurs and their descendants, including phytosaurs, aetosaurs, "rauisuchians," and crocodylomorphs. The crurotarsans and, somewhat later, the dinosaurs supplanted the synapsids as the dominant members of the Triassic terrestrial vertebrate assemblage, with Triassic mammals existing as diminutive mouselike forms (*16*, *17*). The roles of contingency and competition in the faunal turnover that occurred in the aftermath of the End Permian mass extinction are controversial. The basal archosaurs and archosauromorphs, animals such as *Euparkaria*, appear to have expanded their capacity for vigorous exercise (*18*) during a period of relative environmental hypoxia (*19*). In bird lungs, unidirectional airflow coupled with a crosscurrent mechanism of gas exchange facilitates the extraction of oxygen under conditions of hypoxia (*20*). If such a lung was present at the base of the archosaur radiation, this clade may have been better able than the synapsids to compete for niches that required a capacity for vigorous exercise.

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Supporting Online Material

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Fig. S1
References
Movies S1 to S3

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G Protein Subunit $G\alpha_{13}$ Binds to Integrin $\alpha_{IIb}\beta_3$ and Mediates Integrin "Outside-In" Signaling

Haixia Gong, Bo Shen, Panagiotis Flevaris, Christina Chow, Stephen C.-T. Lam, Tatyana A. Voyno-Yasenetskaya, Tohru Kozasa, Xiaoping Du*

Integrins mediate cell adhesion to the extracellular matrix and transmit signals within the cell that stimulate cell spreading, retraction, migration, and proliferation. The mechanism of integrin outside-in signaling has been unclear. We found that the heterotrimeric guanine nucleotide–binding protein (G protein) $G\alpha_{13}$ directly bound to the integrin β_3 cytoplasmic domain and that $G\alpha_{13}$-integrin interaction was promoted by ligand binding to the integrin $\alpha_{IIb}\beta_3$ and by guanosine triphosphate (GTP) loading of $G\alpha_{13}$. Interference of $G\alpha_{13}$ expression or a myristoylated fragment of $G\alpha_{13}$ that inhibited interaction of $\alpha_{IIb}\beta_3$ with $G\alpha_{13}$ diminished activation of protein kinase c-Src and stimulated the small guanosine triphosphatase RhoA, consequently inhibiting cell spreading and accelerating cell retraction. We conclude that integrins are noncanonical $G\alpha_{13}$-coupled receptors that provide a mechanism for dynamic regulation of RhoA.

Integrins mediate cell adhesion and transmit signals within the cell that lead to cell spreading, retraction, migration, and proliferation (*1*). Thus, integrins have pivotal roles in biological processes such as development, immunity, cancer, wound healing, hemostasis, and thrombosis. The platelet integrin $\alpha_{IIb}\beta_3$ typically displays bidirectional signaling function (*2*, *3*). Signals from within the cell activate binding of $\alpha_{IIb}\beta_3$ to extracellular ligands, which in turn triggers signaling within the cell initiated by the occupied receptor (so-called "outside-in" signaling). A major early consequence of integrin "outside-in" signaling is cell spreading, which requires activation of the protein kinase c-Src and c-Src–mediated inhibition of the small guanosine triphosphatase (GTPase) RhoA (*4*–*7*). Subsequent cleavage of the c-Src binding site in β_3 by calpain allows activation of RhoA, which stimulates cell retraction (*7*, *8*). The molecular mechanism coupling ligand-bound $\alpha_{IIb}\beta_3$ to these signaling events has been unclear.

Heterotrimeric guanine nucleotide–binding proteins (G proteins) consist of Gα, Gβ, and Gγ subunits (*9*). G proteins bind to the intracellular side of G protein–coupled receptors (GPCRs) and transmit signals that are important in many intracellular events (*9*–*11*). $G\alpha_{13}$, when activated by GPCRs, interacts with Rho guanine-nucleotide exchange

Department of Pharmacology, University of Illinois at Chicago, 835 South Wolcott Avenue, Room E403, Chicago, IL 60612, USA.

*To whom correspondence should be addressed. E-mail: xdu@uic.edu