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

The mechanism of unidirectional flow in alligator lungs is yet to be determined, but our data support Hazelhoff’s model (I), 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 bronchi. 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 countercurrent mechanism has been hypothesized (II), but a countercurrent mechanism cannot be ruled out. Furthermore, the importance of unidirectional airflow for gas exchange efficiency in the alligator lung is yet to be determined, but our data support Hazelhoff’s model (I), in which key features of the bronchial tree give rise to unidirectional airflow (I).

Previous scenarios for the evolution of unidirectional air flow are that it arose in dinosaurs convergent in theropods and pterosaurs (I3, I5), or not at all in dinosaurs because of a hepatic piston mechanism of breathing (I4). 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 (I3, I5). Crocodilians and birds are crown-group Archosauria. Therefore, in contrast to previous views, we suggest that unidirectional flow evolved before the divergence of crocotaursan and dinosaurian archosaurs and was present in the basal archosaurs and their descendants, including phytosaurs, aetosaurs, “rauisuchians,” and crocodylomorphs. The crocotaursan and, somewhat later, the dinosaurs supplanted the synapsids as the dominant members of the Triassic terrestrial vertebrate assemblage, with Triassic mammals existing as duminutive mouselike forms (I6, I7). 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 archosauromorpha, animals such as Euparkeria, appear to have expanded their capacity for vigorous exercise (I8) during a period of relative environmental hypoxia (I9). In bird lungs, unidirectional airflow coupled with a cross-current 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.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5963/338/DC1

Materials and Methods

Fig. S1
References
Movies S1 to S3

G Protein Subunit Go13 Binds to Integrin αIIbβ3 and Mediates Integrin “Outside-In” Signaling

Haixia Gong, Bo Shen, Panagiotis Flevaris, Christina Chow, Stephen C.-T. Lam, Tatyana A. Voyno-Yasenetskaia, 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) Go13 directly bound to the integrin β3 cytoplasmic domain and that Go13-integrin interaction was promoted by ligand binding to the integrin αIIbβ3 and by guanosine triphosphate (GTP) loading of Go13. Interference of Go13 expression or a myristoylated fragment of Go13 that inhibited interaction of αIIbβ3 with Go13 diminished activation of protein kinase c-Src and stimulated the small guanosine triphosphatase (GTPase) RhoA (4–7). Subsequent cleavage of the c-Src binding site in β3 by calpain allows activation of RhoA, which stimulates cell retraction (7, 8). The molecular mechanism coupling ligand-bound αIIbβ3 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). Go13, when activated by GPCRs, interacts with Rho guanine-nucleotide exchange factors and stimulates RhoA activation.

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Platelet lysates were immunoprecipitated with control mouse IgG, antibody to \( \alpha_{\text{IIb}} \beta_{3} \), or antibody to \( G_{\text{13}} \). Immunoprecipitates were immunoblotted with antibody to G\( \text{.src} \) Tyr416. Platelets from \( G_{\text{13}} \)-transfected stem cell recipient mice showed >80% decrease in G\( \text{src} \) expression (Fig. 1B). When platelets were allowed to adhere to immobilized fibrinogen (\( \alpha_{\text{IIb}} \beta_{3} \)) binding to immobilized fibrinogen does not require prior “inside-out” signaling activation. Platelets depleted of \( G_{\text{13}} \) spread poorly as compared with control platelets (Fig. 1A and fig. S2). The inhibitory effect of \( G_{\text{13}} \) deficiency is unlikely to be caused by its effect on GPCR-stimulated \( G_{\text{13}} \) signaling because (i) washed resting platelets were used and no GPCR agonists were added, and (ii) prior treatment with 1 mM aspirin [which abolishes thromboxane\( \text{A}_{2} (\text{TXA}_{2}) \) generation (17)] did not affect platelet spreading on fibrinogen (fig. S2), making unlikely the endogenous TXA\( _2 \)-mediated stimulation of \( G_{\text{13}} \). Furthermore, \( G_{\text{13}} \) siRNA inhibited spreading of Chinese hamster ovary (CHO) cells expressing human \( \alpha_{\text{IIb}} \beta_{3} \) (123 cells) (18), which was rescued by an siRNA-resistant \( G_{\text{13}} \) (fig. S3). Thus, \( G_{\text{13}} \) appears to be important in integrin “outside-in” signaling leading to cell spreading.

To determine whether \( G_{\text{13}} \) serves as an early signaling mechanism that mediates integrin-induced
activation of c-Src, we measured phosphorylation of c-Src at Tyr416 (which indicates activation of c-Src) in control and fibrinogen-bound cells. Depletion of Go13 in mouse platelets or 123 cells abolished phosphorylation of c-Src Tyr416 (Fig. 1C and fig. S3), indicating that Go13 may link integrin αIIbβ3 and c-Src activation. Because c-Src inhibits RhoA (7, 19), we also tested the role of Go13 in regulating activation of RhoA. RhoA activity was suppressed to baseline 15 min after platelet adhesion and became activated at 30 min (Fig. 1C), which is consistent with transient inhibition of RhoA by c-Src (7). The integrin-dependent delayed activation of RhoA was not inhibited by depletion of Go13, indicating its independence of the GPCR-Go13-RhoGEF pathway (Fig. 1C). In contrast, depletion of Go13 accelerated RhoA activation (Fig. 1C). Thus, Go13 appears to mediate inhibition of RhoA. The inhibitory effect of Go13 depletion on platelet spreading was reversed by Rho-kinase inhibitor Y27632 (Fig. 1A), which suggests that Go13-mediated inhibition of RhoA is important in stimulating platelet spreading. These data are consistent with Go13 mediating integrin “outside-in” signaling inducing c-Src activation, RhoA inhibition, and cell spreading.

The integrin αIIbβ3 was coimmunoprecipitated by antibody to Go13, but not control immunoglobulin G (IgG), from platelet lysates (Fig. 2A). Conversely, an antibody to β3 immunoprecipitated Go13 with β3 (Fig. 2B). Coimmunoprecipitation of β3 with Go13 was enhanced by guanosine triphosphate γS (GTP-γS) or AlF4 (Fig. 2A and fig. S4). Thus, β3 is present in a complex with Go13, preferentially the active GTP-bound Go13. To determine whether Go13 directly binds to the integrin cytoplasmic domain, we incubated purified recombinant Go13 with β3 integrin cytoplasmic domain fused with GST (GST-β3CD). Purified Go13 bound to GST-β3CD, but not to GST (Fig. 2C). Purified Go13 also bound to the β3 integrin cytoplasmic domain fused with GST (GST-β3CD) (Fig. 2D). The binding of Go13 to GST-β3CD and GST-β3CD was detected with GDP-loaded Go13.

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**Fig. 3.** Effects of mSRI on integrin-induced c-Src phosphorylation, RhoA activity, and platelet spreading. (A) Washed human platelets pretreated with DMSO, mSRI, or scrambled control peptide were allowed to adhere to fibrinogen and then solubilized at indicated time points. Proteins from lysates were immunoblotted with antibodies to c-Src phosphorylated at Tyr416, c-Src, or RhoA. GTP-bound RhoA was measured by association with GST–Rhotekin rho-binding domain (GST-RBD) beads (26). See fig. S4 for quantitative data. (B) Spreading of platelets treated with 0.1% DMSO, scrambled control peptide, or mSRI, in the absence or presence of C3 toxin, Y27632, or 0.01 U/ml thrombin. Platelets were stained with Alexa Fluor 546–conjugated phalloidin.

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**Fig. 4.** The role of Go13 in clot retraction and dynamic RhoA regulation. (A) Effect of 250 μM mSRI peptide on clot retraction of human platelet-rich plasma compared with DMSO and scrambled peptide. Clot sizes were quantified using Image J (mean ± SD, n = 3, t test). (B) Comparison of clot retraction (mean ± SD, n = 3, t test) mediated by control siRNA and Go13-depleted platelets. (C to F) Platelets were stimulated with thrombin with or without 2 mM RGDS and monitored for turbidity changes of platelet suspension caused by shape change and aggregation (C). The platelets were then solubilized at indicated time points and analyzed for amount of β3 coimmunoprecipitated with Go13 (D) and amount of GTP-RhoA bound to GST-RBD beads (E) by immunoblot. (F) Quantitative data (mean ± SD) from three experiments. (G) A schematic illustrating Go13-dependent dynamic regulation of RhoA and crosstalk between GPCR and integrin signaling.
but enhanced by GTP-γS and AlF₄⁻ (Fig. 2, C and D), indicating that the cytoplasmic domains of β₃ and β₁ can directly interact with G(13) and that GTP enhances the interaction. The G(13)β₃ interaction was enhanced in platelets adherent to fibrinogen, and by thrombin, which stimulates GTP binding to G(13) via GPCR (Fig. 2E). Hence, the interaction is regulated by both integrin occupancy and GPCR signaling.

To map the β₃ binding site in G(13), we incubated cell lysates containing Flag-tagged wild type or truncation mutants of G(13) (Fig. S5) with GST-β₃CD beads. GST-β₃CD associated with wild-type G(13) and the G(13) 1 to 212 fragment containing α-helical region and switch region I (SRI), but not with the G(13) fragment containing residues 1 to 196 lacking SRI (Fig. 2F). Thus, SRI appears to be critical for β₃ binding. To further determine the importance of SRI, G(13)β₃ binding was assessed in the presence of a myristoylated synthetic peptide, Myr-LALRPTKGHIY (mSRI), corresponding to the SRI sequence of G(13) (197 to 209) (21, 22). The mSRI peptide, but not a myristoylated scrambled peptide, inhibited G(13) binding to β₃ (Fig. 2G), indicating that mSRI is an effective inhibitor of β₃-G(13) interaction. Therefore, we further examined whether mSRI might inhibit integrin signaling. Treatment of platelets with mSRI inhibited integrin-dependent phosphorylation of c-Src Tyr526 and accelerated RhoA activation (Fig. 3A). The effect of mSRI is unlikely to result from its inhibitory effect on the binding of RhoGEFs to G(13) SRI because G(13) binding to RhoGEFs stimulates RhoA activation, which should be inhibited rather than promoted by mSRI (22). Thus, these data suggest that β₃-G(13) interaction mediates activation of c-Src and inhibition of RhoA. Furthermore, mSRI inhibited integrin-mediated platelet spreading (Fig. 3B), and this inhibitory effect was reversed by C3 toxin (which catalyzes the ADP ribosylation of RhoA) or Y27632, confirming the importance of G(13)β₃-dependent inhibition of RhoA in platelet spreading. Thrombin promotes platelet spreading, which requires cdc42/Rac pathways (23). However, thrombin-promoted platelet spreading was also abolished by mSRI (Fig. 3B), indicating the importance of G(13)β₃ interaction. Thus, G(13)-integrin interaction appears to be a mechanism that mediates integrin signaling to c-Src and RhoA, thus regulating cell spreading.

To further determine whether G(13) mediates inhibition of integrin-induced RhoA-dependent contractile signaling, we investigated the effects of mSRI and depletion of G(13) on platelet-dependent clot retraction (shrinking and consolidation of a blood clot requires integrin-dependent retraction of platelets from within) (7, 8). Clot retraction was accelerated by mSRI and depletion of G(13) (Fig. 4, A and B, and fig. S6), indicating that G(13) negatively regulates RhoA-dependent platelet retraction and coordinates cell spreading and retraction. The coordinated cell spreading-retraction process is also important in wound healing, cell migration, and proliferation (24).

The function of G(13) in mediating the integrin-dependent inhibition of RhoA contrasts with the traditional role of G(13), which is to mediate GPCR-induced activation of RhoA. However, GPCR-mediated activation of RhoA is transient, peaking at 1 min after exposure of platelets to thrombin, indicating the presence of a negative regulatory signal (Fig. 4, D and F). Furthermore, thrombin-stimulated activation of RhoA occurs during platelet shape change before substantial ligand binding to integrins (Fig. 4, C, D, and F). In contrast, after thrombin stimulation, β₃ binding to G(13) was diminished at 1 min when G(13)-dependent activation of RhoA occurs, but increased after the occurrence of integrin-dependent platelet aggregation (Fig. 4, E and F). Thrombin-stimulated binding of G(13) to α(IIb)β₃ and simultaneous RhoA inhibition both require ligand occupancy of α(IIb)β₃ and are inhibited by the integrin inhibitor Arg-Gly-Asp-Ser (RGDS) (Fig. 4, D to F). Thus, our study does not support only a function of integrin α(IIb)β₃ as a noncanonical G(13)-coupled receptor but also a new concept of G(13)-dependent dynamic regulation of RhoA, in which G(13) mediates initial GPCR-induced RhoA activation and subsequent integrin-dependent RhoA inhibition (Fig. 4G). These findings are important for our understanding of how cells spread, retract, migrate, and proliferate, which is fundamental to development, cancer, immunity, wound healing, hemostasis, and thrombosis.

References and Notes

15. V. Senyak et al., Cancer Res. 69, 262 (2009).
21. Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Functional and Evolutionary Insights from the Genomes of Three Parasitoid Nasonia Species

The Nasonia Genome Working Group*†

We report here genome sequences and comparative analyses of three closely related parasitoid wasps: *Nasonia vitripennis*, *N. giraulti*, and *N. longicornis*. Parasitoids are important regulators of arthropod populations, including major agricultural pests and disease vectors, and *Nasonia* is an emerging genetic model, particularly for evolutionary and developmental genetics. Key findings include the identification of a functional DNA methylation tool kit; hymenopteran-specific genes including diverse venoms; lateral gene transfers among Vox viruses, *Wolbachia*, and *Nasonia*; and the rapid evolution of genes involved in nuclear-mitochondrial interactions that are implicated in speciation. Newly developed genome resources advance *Nasonia* for genetic research, accelerate mapping and cloning of quantitative trait loci, and will ultimately provide tools and knowledge for further increasing the utility of parasitoids as pest insect-control agents.

Parasitoid wasps are insects whose larvae parasitize various life stages of other arthropods (for example, insects, ticks, and mites). Female wasps sting, inject venom, and lay eggs on or in the host, where the developing offspring consume and eventually kill it. Parasitoids...