giving the axonal cytoskeleton a long-range order. Despite the molecular composition differences between the axon initial segments and distal axons [for example, ankyrin-G and βIV-spectrin are confined in the axon initial segment by an exclusion effect of the distal axon proteins ankyrin-B and βII-spectrin (31)], the cytoskeletal organization is similar between the initial and distal segments of the axons, both adopting a quasi-1D, periodic structure. Interestingly, we found this periodic cytoskeleton structure to be present only in axons, not in dendrites, which instead primarily contained long actin filaments running along the dendritic axis. Although the microscopic interactions between the molecular components of the axon cytoskeleton are probably similar to those between the erythrocyte analogs (9, 10), the overall structure of this quasi-1D, periodic cytoskeleton in axons is distinct from the 2D, pentagonal or hexagonal structure observed for the erythrocyte membrane cytoskeleton (11, 12). In Drosophila motoneuron axons near the neuromuscular junctions, spectrin and ankyrin appear to organize into an erythrocyte-like, pentagonal or hexagonal lattice structure (16), which is distinct from the quasi-1D, periodic, ladder-like structure that we observed in the axons of vertebrate brains. Whether the difference is due to invertebrate versus vertebrate animals or peripheral versus central nervous systems is a topic for future investigations.

The periodic, actin-spectrin–based cytoskeleton observed here may not be involved in myosin-dependent axonal transport. If the analogy to the erythrocyte membrane cytoskeleton holds, the capped short actin filaments in the ringlike actin structures in axons are probably bound by tropomyosin (9, 10), which could potentially prevent the binding of myosins. Myosin-dependent axonal transport could, however, be mediated by the long actin filaments that run along the axon shaft. The quasi-1D, periodic, actin-spectrin cytoskeleton may instead provide elastic and stable mechanical support for the axon membrane, given the flexibility of spectrin. Elastic and stable support is particularly important for axons, because they can be extremely long and thin and have to withstand mechanical strains as animals move (37). Indeed, the loss of β-spectrin in Caenorhabditis elegans leads to spontaneous breaking of axons, which is caused by mechanical strains generated by animal movement and can be prevented by paralyzing the animal (37). The highly periodic submembrane cytoskeleton can also influence the molecular organization of the plasma membrane by organizing important membrane proteins along the axon. We found that sodium channels were distributed periodically along the axon initial segment in a coordinated manner with the underlying actin-spectrin cytoskeleton. An axonal plasma membrane with periodically varying biochemical and mechanical properties may not only influence how an action potential is generated and propagated, but might also affect how axons interact with other cells.

References and Notes
28. Supplementary materials, including materials and methods, are available on Science Online.

Acknowledgments: We thank M. Raszband for providing the βIV-spectrin and sodium channel antibodies and M. Ericson, B. Kasthuri, R. Schaley, G. Hao, and C. Speer for help in the preparation of hippocampal tissue slices. This work is supported in part by the NIH and the Collaborative Innovation Award from the HHMI. K.Z. is a HHMI investigator.

Supplementary Materials
www.sciencemag.org/cgi/content/full/science.1232251/DC1
Materials and Methods
Figs. S1 to S10
References (30–42)
1 November 2012; accepted 5 December 2012
Published online 13 December 2012;
10.1126/science.1232251
ity played a key role in its evolution (3), yet the by-products of oxidative metabolism [such as reactive oxygen species (ROS)] can produce harmful side effects including DNA damage (4). We hypothesize that genetic changes during the evolution of flight in bats likely included adaptations to limit collateral damage caused by by-products of elevated metabolic rate. Another phenomenon that has sparked intense interest in recent years is the discovery that bats maintain and disseminate numerous deadly viruses (5). In this context, we further hypothesize that the long-term coexistence of bats and viruses must have imposed strong selective pressures on the bat genome, and the genes most likely to reflect this are those directly related to the first line of antiviral defense—the innate immune system.

We performed high-throughput whole-genome sequencing of individual wild-caught specimens of \( P. \) alecto and \( M. \) davidii using the Illumina HiSeq platform (6). More than \( 100 \times \) coverage high-quality reads were obtained for \( P. \) alecto and \( M. \) davidii, which resulted in high-quality assemblies (tables S1 to S3 and fig. S1). The two bat genomes, at \( \sim \)2 Gb, were smaller in size than other mammals (7) (fig. S2), whereas the number of genes we identified was similar to those of other mammals (21,392 and 21,705 in \( P. \) alecto and \( M. \) davidii, respectively) (fig. S3). Both species displayed a high degree of heterozygosity at the whole-genome level (0.45% and 0.28% in \( P. \) alecto and \( M. \) davidii, respectively) (tables S4 and S5), whereas repetitive content accounted for slightly less than one-third of each genome (tables S6 and S7). We identified a novel endogenous viral element derived from *Saimiriine herpesvirus 2* that has expanded to 126 copies in \( P. \) alecto (table S8 and fig. S4). Gene family expansion and contraction analysis (tables S9 to S12) revealed significant expansion (>0.05) of 71 gene families in \( M. \) davidii compared with only 13 in \( P. \) alecto, which may be related to a recent wave of DNA transposon activity (8).

We screened all nuclear-encoded bat genes to identify those for which a single orthologous copy was unambiguously present in both bat species.

![Fig. 1. Comparison of bat biological traits. \( P. \) alecto and \( M. \) davidii represent two distinct Chiropteran suborders and demonstrate diverse evolutionary adaptations. PNG, Papua New Guinea.](image)

![Fig. 2. Phylogenetnic analysis. Maximum-likelihood phylogenetic analysis of 2492 genes from \( M. \) davidii, \( P. \) alecto, and eight mammalian species. Divergence time estimates in blue, gene family expansion events in green, and gene family contraction events in red. MRCA, most recent common ancestor.](image)
as well as in human, rhesus macaque, mouse, rat, dog, cat, cattle, and horse. From this, 2492 genes were used to perform maximum-likelihood and Bayesian phylogenetic analysis (Fig. 2 and figs. S5 to S7). All phylogenetically informative signals, including concatenated nucleotides and amino acids, vigorously supported bats as a member of Pegasoferae (Chiroptera + Perissodactyla + Carnivora) (9), with the bat lineage diverging from the Equus (horse) lineage ~88 million years ago, buttressed by findings at the transcript level (10). However, phylogenetic reconstruction with mitochondrial DNA sequences resulted in bats occupying an outlying position in Laurasiatheria (fig. S8). The incongruence between nuclear and mitochondrial trees likely reflects rapid evolution (fig. S8). To identify mechanisms that facilitated the origin of flight in bats, we surveyed genes involved in detection and repair of genetic damage. A high proportion of genes in the DNA damage pathway were found to be under positive selection in the bat ancestor, including ATM, the catalytic subunit of DNA-dependent protein kinase (DNA-PKc), RAD50, KU80, and MDM2 (Fig. 3A and Table 1). We propose that these changes may be directly related to minimizing and/or repairing the negative effects of ROS generated as a consequence of flight. Additionally in this pathway, TP53 (p53) and BRCAL2 were shown to be under positive selection in M. davidii, whereas LG4 was under positive selection in P. alecto (Table 1). Bat-specific mutations in a nuclear localization signal in p53 and a nuclear export signal in MDM2 (Fig. 3B and fig. S9) may affect subcellular localization and function in both species (11, 12). Other candidate flight-related genes under positive selection in the bat ancestor included COL3A1, involved in skin elasticity, and CACNA2D1, which has a role in muscle contraction (table S13).

We next examined genes of the innate immune system (Table 1). Positively selected genes in the bat ancestor included c-REL, a member of the nuclear factor kB (NF-kB) family of transcription factors, which also contained amino acid changes potentially affecting inhibitor of NF-kB (IkB) binding (fig. S10). In addition to diverse roles in innate and adaptive immunity (13), c-REL plays a role in the DNA damage response by activating ATM (14) and CLSPN (15), whereas ATM is also an upstream regulator of NF-kB (16). The DNA damage response plays an important role in host defense and is a known target for virus interaction (17), which raises the possibility that changes in DNA damage response mechanisms during selection for flight could have influenced the bat immune system.

It is intriguing that both P. alecto and M. davidii have lost the entire locus containing the PYHIN gene family, including AIM2 and IFI16, both of which are involved in sensing microbial DNA and the formation of inflammasomes (fig. S11). The association between PYHIN genes and cell cycle regulation in other species (18) hints that loss of the PYHIN family in bats may be connected to changes in the DNA damage pathway, because at least one PYHIN gene is present in all other major groups of eutherian mammals (19). NLRP3, triggered by both viral infection and ROS in other mammals (20), plays an analogous role to AIM2 in inflammasome assembly and was also under positive selection in the bat ancestor (Table 1).

Natural killer (NK) cells provide a first line of defense against viruses and tumors and include two families of NK cell receptors; killer-cell immunoglobulin-like receptors (KIRs), encoded by genes in the leukocyte receptor complex (LRC), and killer cell lectin-like receptors (KLRs, also known as Ly49 receptors), encoded within the natural killer gene complex (NKC). KLRs and KIRs were entirely absent in P. alecto and reduced to a single Ly49 pseudogene in M. davidii (table S14). KIR-like receptors identified in other species (21) were also absent from both P. alecto and M. davidii genomes, which was supported by transcript analysis in P. alecto (10). This likely indicates that bat NK cells use a novel class of receptors to recognize classical major histocompatibility complex class I molecules. Furthermore, additional LRC members of the immunoglobulin superfamily [including sialic acid–binding immunoglobulin-like lectins (SIGLECs), leukocyte...
immunoglobulin-like receptors (LILRs), carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), and leukocyte-associated immunoglobulin-like receptors (LAIRs) have undergone considerable gene duplication in M. davidii and other mammals yet have almost completely failed to expand in P. alecto (fig. S12). As the genes encoded within the LRC bind a variety of ligands and play multiple roles in immune regulation, these observations have diverse implications for differences in immune function between P. alecto and M. davidii and between bats and other mammals.

We identified seven complete and two partial copies of the digestive enzyme RNASE4 in M. davidii (table S15), whereas P. alecto RNASE4 has acquired a frameshift mutation resulting in loss of catalytic residues (fig. S13). We also identified critical amino acid changes in M. davidii RNASE4 genes (relative to the mammalian consensus) that suggest diversification of substrate specificities (fig. S13). With a proven role in host virus resistance but may also reflect the insectivorous diet of M. davidii, which consumes predominantly fruit, nectar, and other mammals (fig. S13).

M. davidii also differs from P. alecto in aspects including hibernation and echolocation (Fig. 1). Bile salt–stimulated lipase (BSSL), capable of hydrolyzing triglycerides into monoglycerides and subsequently releasing digestible free fatty acids, has been specifically expanded in M. davidii compared with P. alecto and other mammals (fig. S14). In addition, we observed six candidate genes related to hibernation, which showed positive selection in M. davidii and three other hibernating species relative to nonhibernators (table S16). Seven echolocation-related genes, including new candidates WNT8A and FOS (a subunit of the AP–1 transcription factor), had significantly higher ratio of nonsynonymous to synonymous substitutions (dN/dS) in the echolocating M. davidii branch relative to non-echolocating branches (table S17). Of note, the third exon in M. davidii FOXP2 had even greater variation from the mammalian consensus than two previously identified orthologous sites (fig. S15), which suggests a specific transcript variant is involved in echolocation (23).

In summary, comparative analysis of P. alecto and M. davidii genomes has provided insight into the phylogenetic placement of bats and has revealed evidence of genetic changes that may have contributed to their evolution. Gene duplication events played a particularly prominent role in the evolution of M. davidii bats and may have helped contribute to their speciation. Concentration of positively selected genes in the DNA damage checkpoint pathway in bats may indicate an important step in the evolution of flight, whereas evidence of change in components shared by the DNA damage pathway and the innate immune system raises the interesting possibility that flight-induced adaptations have had inadvertent effects on bat immune function and possibly also life expectancy (24). The data generated by this study will help to address major gaps in our understanding of bat biology and to provide new directions for future research.

References and Notes
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6. Materials and methods are available as supplementary materials on Science Online.

Acknowledgments: We thank H. Field, C. Smith, and M. Yu for helping source genome DNA; K. Iwahana and J. J. Boomsma for constructive discussion; and M. Cowled for graphics assistance. We acknowledge financial support from the China National Genbank at Shenzhen, CSIRO (Office of the Chief Executive Science Leaders Award, Julius Award), The Australian
Tunable Signal Processing Through Modular Control of Transcription Factor Translocation

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Signaling pathways can induce different dynamics of transcription factor (TF) activation. We explored how TFs process signaling inputs to generate diverse dynamic responses. The budding yeast general stress-responsive TF Msn2 acted as a tunable signal processor that could track, filter, or integrate upstream signals in an input-dependent manner. This tunable signal processing appears to originate from dual regulation of both nuclear import and export by phosphorylation, as mutants with one form of regulation sustained only one signal-processing function. Versatile signal processing by Msn2 is crucial for generating distinct dynamic responses to different natural stresses. Our findings reveal how complex signal-processing functions are integrated into a single molecule and provide a guide for the design of TFs with "programmable" signal-processing functions.

Many transcription factors (TFs) display diverse activation dynamics in response to various external stimuli (1–4). To investigate how TFs process upstream signals, we studied the Saccharomyces cerevisiae general stress–responsive TF Msn2 (5). In the absence of stress, Msn2 is phosphorylated by protein kinase A (PKA) and localized to the cytoplasm; in response to stress, Msn2 is dephosphorylated and translocates to the nucleus, where it induces gene expression (5).

Natural stresses elicit highly variable dynamics of Msn2 nuclear translocation (Fig. 1A) (6, 7), which are thought to result from oscillatory signaling inputs (presumably PKA activity) (8). To study how Msn2 processes oscillatory PKA inputs, we used an engineered yeast strain (6) carrying mutations in all three PKA isoforms that are crucial for generating distinct dynamic responses to different natural stresses. Our findings reveal how versatile signal processing by Msn2 is crucial for generating distinct dynamic responses to different natural stresses.

Fig. 1. Tunable signal-processing behaviors of Msn2. (A) Illustration of the distinct single-cell dynamic responses of Msn2 to various stresses. (B) Steady-state abundance of Msn2 in the nucleus in response to various concentrations of 1-NM-PP1. In response to each concentration of 1-NM-PP1, Msn2 exhibited uniform and stable nuclear localization in single cells and did not exhibit stochastic fluctuations as observed in natural stress responses. Open circles: responses to different concentrations of 1-NM-PP1; closed circles: responses to 3 μM and 0.2 μM 1-NM-PP1, which are used as high- and low-amplitude inputs, respectively, for the following analyses. AU, arbitrary unit. (C) Averaged single-cell time traces of Msn2 nuclear localization (bottom: n = 50 cells; error bar: single-cell variances) in response to oscillatory inputs with high and low amplitudes (top). (Left) High-amplitude input produced by 3 μM 1-NM-PP1; (right) low-amplitude input produced by 0.2 μM 1-NM-PP1. Pulse duration of 3 min; pulse interval of 2 min. To emphasize the fact that 3 μM 1-NM-PP1 elicited a steady-state response that is about twice the response elicited by 0.2 μM 1-NM-PP1, the top y axes are not presented on a linear scale.