Molecular and Genomic Data Identify the Closest Living Relative of Primates
Jan E. Janecka, *et al.*
Science **318**, 792 (2007);
DOI: 10.1126/science.1147555

14. M. S. Grinolds, V. A. Lobastov, J. Weissenrieder, A. H. Zewail, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 18427 (2006).
15. V. A. Lobastov, J. Weissenrieder, J. Tang, A. H. Zewail, *Nano Lett.* **7**, 2552 (2007).
16. H. S. Park, J. S. Baskin, O.-H. Kwon, A. H. Zewail, *Nano Lett.* **7**, 2545 (2007).
17. K. J. Caspersen, E. A. Carter, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6738 (2005).
18. D. R. Trinkle *et al.*, *Phys. Rev. Lett.* **91**, 025701 (2003).
19. F. J. Morin, *Phys. Rev. Lett.* **3**, 34 (1959).
20. C. N. Berglund, H. J. Guggenheim, *Phys. Rev.* **185**, 1022 (1969).
21. K. D. Rogers, *Powder Diffr.* **8**, 240 (1993).
22. A. Cavalleri *et al.*, *Phys. Rev. Lett.* **87**, 237401 (2001).
23. G. I. Petrov, V. V. Yakovlev, J. A. Squier, *Opt. Lett.* **27**, 655 (2002).
24. A. Cavalleri *et al.*, *Phys. Rev. Lett.* **95**, 067405 (2005).
25. A. Cavalleri, M. Rini, R. W. Schoenlein, *J. Phys. Soc. Jpn.* **75**, 011004 (2006), and references therein.
26. H.-T. Kim *et al.*, *Phys. Rev. Lett.* **97**, 266401 (2006).
27. P. Baum, A. H. Zewail, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16105 (2006).
28. D.-S. Yang, N. Gedik, A. H. Zewail, *J. Phys. Chem. C* **111**, 4889 (2007).
29. V. A. Lobastov *et al.*, in *Ultrafast Optics IV*, F. Krausz, G. Korn, P. Corkum, I. A. Walmsley, Eds., vol. 95 of *Springer Series in Optical Sciences* (Springer, New York, 2004), pp. 419–435.
30. J. B. Goodenough, *Phys. Rev.* **120**, 67 (1960).
31. T. C. Koethe *et al.*, *Phys. Rev. Lett.* **97**, 116402 (2006).
32. Given the x-ray wavelength (1.54 Å) and diffraction angle (13.9°) reported (*22*), the Bragg peak (*22*) should be indexed as (011) of the monoclinic phase. It was assigned as (110) of the monoclinic phase, which in fact becomes (110) of the rutile phase upon transformation.
33. D. Maurer, A. Leue, R. Heichele, V. Müller, *Phys. Rev. B* **60**, 13249 (1999).
34. D. Kucharczyk, T. Niklewski, *J. Appl. Crystallogr.* **12**, 370 (1979).
35. J. M. Thomas, *Chem. Br.* **6**, 60 (1970), and references therein.
36. Excess electrons (carriers), which are not bound by strong correlation with the lattice, may redistribute within the excited metallic region but are impeded by the insulating surrounding. Electron diffusion from the probed layer (~10 nm) to the metallic region (~100 nm) occurs in ~100 ps, as calculated from the electron mobility of 1 to 10 $cm^2/(V{\cdot}s)$ for metallic vanadium dioxide (*20*). The diffusion of such electrons into the deeper regions may contribute to generation of shear.
37. Shear motion leads to a change in principal axes (*34*). Because not all Bragg spots are equally well in phase with the Ewald sphere at the same time (*28*), shear motion may enhance or suppress the Bragg intensities to values above or below the initial intensity, as observed.
38. From the reflectivity of 0.28 and the absorption depth of 100 nm at 800 nm (*20*), the threshold fluence corresponds to 450 mJ/mm^3 at the surface. With the unit cell volume of 118 $Å^3$ (*21*), which contains four vanadium atoms, this energy density gives ~0.05 photon per vanadium.
39. G. Ertl, *Adv. Catal.* **45**, 1 (2000).
40. In order to evaluate the maximum range of the intensity decay, we also considered a convoluted step function instead of a decay process. This distinction becomes significant depending on the physics of the process involved. For a step function, we obtained Δt_1 = 760 fs. The overall fit of the transient was repeated 1000 times to estimate the error in the Δt_1 range, which was found to be ±80 fs. We note that changes in intensity occur in a step of 250 fs. For the ps component, the range Δt_2 of 15 ps is evident from the figure.

41. We are grateful to J. Weissenrieder for helpful discussions, L. H. Tjeng for generously providing some crystals, G. R. Rossman for the crystal-cutting equipment, and L. M. Henling for help with the x-ray measurements. This work was supported by the NSF, by the Air Force Office of Scientific Research, and by the Gordon and Betty Moore Center for Physical Biology at Caltech. P.B. was partially supported by the Alexander von Humboldt Foundation.

12 July 2007; accepted 19 September 2007
10.1126/science.1147724

Molecular and Genomic Data Identify the Closest Living Relative of Primates

Jan E. Janečka,[1] Webb Miller,[2] Thomas H. Pringle,[3] Frank Wiens,[4] Annette Zitzmann,[5] Kristofer M. Helgen,[6] Mark S. Springer,[7] William J. Murphy[1]*

A full understanding of primate morphological and genomic evolution requires the identification of their closest living relative. In order to resolve the ancestral relationships among primates and their closest relatives, we searched multispecies genome alignments for phylogenetically informative rare genomic changes within the superordinal group Euarchonta, which includes the orders Primates, Dermoptera (colugos), and Scandentia (treeshrews). We also constructed phylogenetic trees from 14 kilobases of nuclear genes for representatives from most major primate lineages, both extant colugos, and multiple treeshrews, including the pentail treeshrew, *Ptilocercus lowii*, the only living member of the family Ptilocercidae. A relaxed molecular clock analysis including *Ptilocercus* suggests that treeshrews arose approximately 63 million years ago. Our data show that colugos are the closest living relatives of primates and indicate that their divergence occurred in the Cretaceous.

The origins of modern primates and their fossil relatives remain a topic of intense debate (*1*–*3*), as there has been an increased focus on identifying adaptive evolutionary changes within primates and the dynamics of genome evolution within the primate lineage (*4*, *5*). Resolving higher primate relationships has been challenging, making it difficult to identify character transformations in early primate evolution. An essential part of this challenge is to determine the closest living relative to primates, which would provide a broader context for understanding primate evolution.

DNA sequence and morphological studies, and analyses of rare genomic changes, support the monophyly of treeshrews, colugos (flying lemurs), and primates in the clade Euarchonta, with a sister-group relationship to Glires [which includes rodents and lagomorphs (*3*, *6*–*8*)]. In contrast, the relationships within Euarchonta are not well resolved, most likely because of the rapid evolution of these groups and inadequate sampling within Scandentia and Dermoptera. Three hypotheses have been proposed: (i) a sister-group relationship between treeshrews and primates (*9*–*11*), (ii) a sister-group relationship between colugos and primates [Primatomorpha (*12*)], and (iii) both colugos and treeshrews as sister to the primates [Sundatheria (*2*, *13*)]. Molecular and morphological studies have favored Sundatheria (*3*, *6*, *14*), although support for this hypothesis was lower than for other mammalian interordinal clades (*15*). Primatomorpha, proposed on morphological grounds (*12*), has also been indicated by some molecular studies (*16*, *17*). Other studies have failed to reject alternative hypotheses, and analyses of different character subsets support contradictory topologies (*18*–*20*).

To improve our understanding of early euarchontan evolution and determine the closest living relative of primates, we used two independent molecular approaches. We first screened a nonredundant set of 197,522 protein-coding exons from the human University of California Santa Cruz Known Genes track to identify rare genomic changes (exonic indels) that would potentially support the three a priori hypotheses. We also assembled and analyzed a 14-kb nuclear gene data set of 19 gene fragments in order to estimate a phylogeny and time scale for extant euarchontans. To mitigate against the possible effects of long-branch attraction (LBA), which can incorrectly place rapidly evolving clades

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA. [2]Center for Comparative Genomics and Bioinformatics, Pennsylvania State University, University Park, PA 16802, USA. [3]Sperling Foundation, Eugene, OR 97405, USA. [4]Department of Animal Physiology, University of Bayreuth, D-95440 Bayreuth, Germany. [5]Zoological Institute, Johann Wolfgang Goethe-University, D-60054 Frankfurt/Main, Germany. [6]National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA. [7]Department of Biology, University of California, Riverside, CA 92521, USA.

*To whom correspondence should be addressed at the Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Veterinary Medical Administration Building, Room 107, Texas A&M University, College Station, TX 77843–4458, USA. E-mail: wmurphy@cvm.tamu.edu

together, we included nuclear DNA sequences from both living colugos and the second tree shrew family, Ptilocercidae (*21*, *22*).

We identified 300 candidate indels in coding gene exons within Euarchonta. Of these, 104 were excluded because they lacked flanking sequences that were long or conserved enough for primer design, were determined to be anomalous misalignments, or were computationally determined to be paralogous gene alignments (*23*). The lack of a colugo genome sequence required polymerase chain reaction (PCR) amplification of candidate indel–containing exons in the colugo and comparison to the treeshrew genome sequence (*23*). PCR primers were designed for the remaining 196 candidates, of which 75% produced a single band in colugo, distributed in the following categories: 32 indels that were initially primate-specific (shared by anthropoids and strepsirrhines, potentially informative for Primatomorpha); 13 indels shared by primates and treeshrews (potentially informative for colugos being in a basal position or alternatively for euarchontan monophyly); and 102 indels that were treeshrew-specific (potentially informative for Sundatheria).

After excluding noninformative and hypervariable indels (*23*) and the evaluation of additional eutherian genomes (table S1), three indels supported the monophyly of Euarchonta [*N4BP2*, *ZNF12*, and *CDCA5* (figs. S1 to S3)] and corroborate the emerging phylogenetic consensus that primates, colugos, and treeshrews are a monophyletic group (*3*, *8*, *15*, *19*, *20*). No indels placed treeshrews with rodents and lagomorphs (*17*) or treeshrews as basal within Euarchontoglires (*24*). We identified seven indels that supported colugos as the closest living relative of primates [Primatomorpha: *SPBC25*, *SMPD3*, *MTUS1*, *SH3RF2*, *NCOA4*, *TEX2*, and *SSH2* (Figs. 1 and 2 and figs. S4 to S10)]. By contrast, no indels supported Sundatheria, despite a larger number of potentially informative candidates for this hypothesis having been screened. One indel (*ADD2*) supported a sister-group relationship between treeshrews and primates (fig. S11). Taken together, an analysis of these last eight indels by means of a statistical framework (*7*) provides significant support for Primatomorpha [$P < 0.025$ (*23*)].

The monophyly of Primatomorpha was independently confirmed by phylogenetic reconstruction from a 14-kb data set consisting of 19 nuclear gene segments with maximum likelihood [(ML) 90% bootstrap support] and Bayesian (1.00 posterior probability) algorithms (Fig. 2 and fig. S12). Previously, the hierarchical order at the base of the Euarchonta was difficult to resolve with confidence because of contempo-

TEX2 exon 4	Primatomorpha
human	SEEKPPAE---GSEDPKKPPRPQEGTR
chimp	SEEKPPAE---GSEDPKKPPRPQEGTR
orangutan	SEEKPPAE---GSEDPKKPPRPQEGTR
macaque	SEEKPPAE---GSEDPKKPPRPQEGTR
marmoset	SEEKPSAE---GSEDPKKPPRPQEGTR
tarsier	SEEKPPPE---GSEDPQKPPPPQEGTR
bushbaby	LEEKLPAE---GSEDPKKPPHPQEGTR
mouse lemur	LEEKLPVE---GSEDPKKPPHPQEGAR
Phil. flying lemur	VEEKLPAE---GSEDPKKPPVPQEGTR
northern treeshrew	SEDKPPAERELGSEDPKKPPHSQEGTR
pentail treeshrew	SEEKPPAEREPGSEDPKKPPHSQEG-R
mouse	TEEKPPPEKELPSEDLKKPPQPQEGTK
guinea pig	SEEKPPAEKELGSEDPKKPSHPQEGTR
rabbit	SEEKPPAERELASEDPKKPPQPQEGTR
dog	SEEKPPAERELGGEDPKKPPHPQEGTR
horse	SEEKPPTEKEQGVEDPKKPSPPQEGTR
brown bat	SEEKPPAERDLGVEDPKKPPHPQEGTR
cow	CEEKPPAERELGGEDPKKPPHPQEGTR
shrew	SEEKLPAEKELGAEDPKKPAHPQEGTR
armadillo	SEEKPSAERELGSEDSKKPPHSQEGTR
elephant	SEEKPPAERELAGEDPKKPP--LEGTR

Fig. 1. An example of a coding sequence indel supporting the Primatomorpha hypothesis. A three–amino acid deletion in exon 4 of the *TEX2* gene is present in all major primate lineages and both colugo genera (shaded gray) but is absent in all treeshrew lineages and eutherian outgroup representatives. See figs. S1 to S10 for full alignments and descriptions of additional supporting indels for Euarchonta and Primatomorpha.

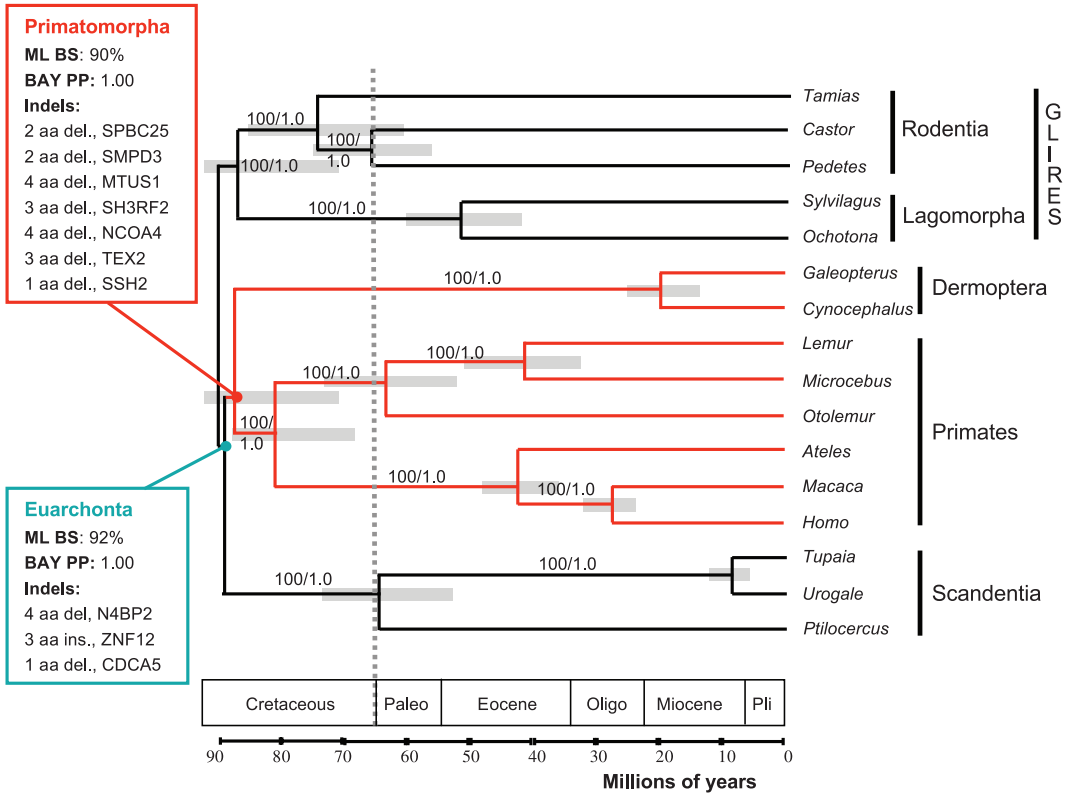

Fig. 2. A maximum-likelihood phylogeny of the superorder Euarchonta, with rodent and lagomorph lineages as outgroups. Branch lengths were estimated under an F84 model of sequence evolution and the relaxed molecular clock approach, implemented in the program MULTIDIVTIME (*23*). Bootstrap (BS) values and Bayesian posterior probabilities (BPPs) are shown on branches for which these values are 100% and 1.0, respectively. Amino acid (aa) indels (ins, insertion; del, deletion) supporting the monophyly of Euarchonta and Primatomorpha are listed in boxes to the left, along with respective BS and BPP values. A molecular time scale is presented below the tree (*23*). The 95% credibility intervals (CIs) are shown as gray bars spanning each node. The point estimates and 95% CIs for all nodes are presented in table S4.

raneous divergence of ancestral lineages during the Cretaceous, LBA, and limited taxon and gene sampling (*25*). The results from our expanded data set (table S2) contrast with previous studies supporting Sundatheria. When *Ptilocercus lowii* and both colugo genera are included, the ML and Bayesian trees become consistent with rare genomic changes. The importance of *P. lowii* was evident when it was removed from the data set; ML trees lacked significant bootstrap support for the divergence between primates, treeshrews, and colugos (fig. S13).

A Bayesian relaxed molecular clock approach with eight fossil constraints estimated the origin of Euarchontoglires at 88.8 million years ago (My), Euarchonta at 87.9 My, and Primatomorpha at 86.2 My (see Fig. 2 and table S4 for 95% credibility intervals). Our divergence dates for Hominoidea/Cercopithicoidea (26.8 My), Anthropoidea (41.7 My), *Lemur*/*Microcebus* (40.4 My), Strepsirhini (62.1 My), and Primates (79.6 My) were very similar to those estimated from an independent 59.7-kb alignment of the *CFTR* gene region (*26*) (table S4). The rapid divergence across the basal euarchontan nodes explains why, despite the seven indels and high bootstrap and Bayesian support for Primatomorpha, we were not able to reject the Sundatheria hypothesis on the basis of sequence data alone (Shimodaira-Hasegawa test, $P = 0.065$) (*23*). We did reject an alliance of treeshrews and primates ($P = 0.047$), despite the single discrepant indel supporting primates + tree shrews. This observation is similar to other findings of incomplete lineage sorting in the common ancestor of rapidly diversifying eutherian clades (*27*, *28*).

The inclusion of nuclear gene sequences from ptilocercid treeshrews allowed us to date the origin of extant treeshrews (Scandentia) to ~63.4 My (Fig. 2 and table S4), near the Cretaceous-Tertiary boundary, concomitant with divergence estimates of many eutherian orders and consistent with the long-fuse model of eutherian diversification (*25*). This deep divergence between *Ptilocercus* and other scandentians complements profound anatomical and behavioral distinctions that have been documented between these groups (*2*, *13*, *21*, *29*) and vindicates recent classifications that have separated *Ptilocercus* in a unique family, Ptilocercidae (*21*, *22*). As the sole living representative of a eutherian lineage that diverged in the early Tertiary along with many modern mammalian orders, we suggest that the phylogenetic uniqueness of *Ptilocercus*, combined with its restriction to lowland forest habitats within a relatively limited global range, should render it an important conservation priority in global context.

Because our conclusions imply that colugos, rather than treeshrews, are the most appropriate outgroup for Primates in studying the evolution of adaptive traits, these results may affect the placement of euarchontan fossils and our understanding of primate genomic evolution (*3*–*5*). For example, a recent morphological analysis supporting Sundatheria placed extinct plesiadapiforms in a monophyletic clade with Primates (*3*), in contrast to Beard (*12*), who identified plesiadapiforms as members of Dermoptera, within Primatomorpha. Our reanalysis of the data set from (*3*) that constrains the monophyly of Euprimates and Dermoptera agrees with the placement of plesiadapiforms as the sister group to Euprimates, though this result is only weakly supported (*3*) (fig. S14). Finally, our results indicate that a draft genome sequence from a colugo is a necessary prerequisite to accurately reconstruct the ancestral primate genome (*5*).

References and Notes

1. S. Tavaré, C. R. Marshall, O. Will, C. Soligo, R. D. Martin, *Nature* **416**, 726 (2002).
2. E. J. Sargis, *Science* **298**, 1564 (2002).
3. J. I. Bloch, M. T. Silcox, D. M. Boyer, E. J. Sargis, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1159 (2007).
4. M. Goodman, L. I. Grossman, D. E. Wildman, *Trends Genet.* **21**, 511 (2005).
5. E. Pennisi, *Science* **316**, 218 (2007).
6. W. J. Murphy *et al.*, *Science* **294**, 2348 (2001).
7. P. J. Waddell, H. Kishino, R. Ota, *Genome Inform.* **12**, 141 (2001).
8. J. O. Kriegs, G. Churakov, J. Jurka, J. Brosius, J. Schmitz, *Trends Genet.* **23**, 158 (2007).
9. R. D. Martin, *Primate Origins and Evolution* (Princeton Univ. Press, Princeton, NJ, 1990).
10. M. J. Novacek, *Nature* **356**, 121 (1992).
11. J. Shoshani, M. C. McKenna, *Mol. Phylogenet. Evol.* **9**, 572 (1998).
12. K. C. Beard, in *Mammal Phylogeny: Placentals*, F. S. Szalay, M. J. Novacek, M. C. McKenna, Eds. (Springer, New York, 1993), pp. 129–150.
13. E. J. Sargis, *Evol. Anthropol.* **13**, 56 (2004).
14. F.-G. R. Liu *et al.*, *Science* **291**, 1786 (2001).
15. M. S. Springer, M. J. Stanhope, O. Madsen, W. W. de Jong, *Trends Ecol. Evol.* **19**, 430 (2004).
16. R. M. Adkins, R. L. Honeycutt, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10317 (1991).
17. P. J. Waddell, S. Shelley, *Mol. Phylogenet. Evol.* **28**, 197 (2003).
18. J. Schmitz, M. Ohme, B. Suryobroto, H. Zischler, *Mol. Biol. Evol.* **19**, 2308 (2002).
19. O. Madsen *et al.*, *Nature* **409**, 610 (2001).
20. W. J. Murphy *et al.*, *Nature* **409**, 614 (2001).
21. M. C. McKenna, S. K. Bell, *Classification of Mammals Above the Species Level* (Columbia Univ. Press, New York, 1997).
22. K. M. Helgen, in *Mammal Species of the World*, D. E. Wilson, D. M. Reeder, Eds. (John Hopkins Univ. Press, Baltimore, MD, 2005), pp. 104–109.
23. See supporting material on *Science* Online.
24. D. Huchon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7495 (2007).
25. M. S. Springer, W. J. Murphy, E. Eizirik, S. J. O'Brien, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1056 (2003).
26. M. E. Steiper, N. M. Young, *Mol. Phylogenet. Evol.* **41**, 384 (2006).
27. H. Nishihara, M. Hasegawa, N. Okada, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9929 (2006).
28. W. J. Murphy, T. H. Pringle, T. Crider, M. S. Springer, W. Miller, *Genome Res.* **17**, 413 (2007).
29. L. H. Emmons, *Tupai: A Field Study of Bornean Tree Shrews* (Univ. of California Press, Berkeley, CA, 2000).

30. This work was supported in part by NSF (grants EF0629849 to W.J.M. and EF0629860 to M.S.S.) and the National Institutes of Health (grant HG02238 to W.M.). We thank A. Jambhekar, T. Crider, A. Wilkerson, V. David, K. Durkin, D. Wilson, L. Grassman Jr., and A. Wilting for technical advice and support and the Broad Institute/Massachusetts Institute of Technology, Baylor College of Medicine–Human Genome Sequencing Center, and Washington University Genome Sequencing Center for access to unpublished sequence data. Sequences from this study have been deposited in GenBank with accession numbers EU142140-EU142251 and EU213052-EU213059.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5851/792/DC1
Materials and Methods
Figs. S1 to S14
Tables S1 to S5
References

9 July 2007; accepted 1 October 2007
10.1126/science.1147555

A Gene Regulatory Network Subcircuit Drives a Dynamic Pattern of Gene Expression

Joel Smith, Christina Theodoris, Eric H. Davidson*

Early specification of endomesodermal territories in the sea urchin embryo depends on a moving torus of regulatory gene expression. We show how this dynamic patterning function is encoded in a gene regulatory network (GRN) subcircuit that includes the *otx*, *wnt8*, and *blimp1* genes, the cis-regulatory control systems of which have all been experimentally defined. A cis-regulatory reconstruction experiment revealed that *blimp1* autorepression accounts for progressive extinction of expression in the center of the torus, whereas its outward expansion follows reception of the Wnt8 ligand by adjacent cells. GRN circuitry thus controls not only static spatial assignment in development but also dynamic regulatory patterning.

The genomic regulatory code that controls the specification of the future skeletogenic, gut endoderm, and nonskeletogenic mesodermal components of the sea urchin embryo is embodied in a gene regulatory network (GRN). The GRN states the interactions of about 50 genes encoding transcription factors, as determined in an extensive perturbation analysis along with other data (*1*, *2*). The subcircuits of this network control the establishment of tran-