of PPARγ adipogenic factors. Because TAZ interacts with both RNX2 and PPARγ, a lingering question is how this interaction is preferentially regulated by matrix stiffness and organization.

The model that emerges from the work of Tang et al. (2013) suggests that it is cell shape that ultimately regulates fate. When cells can round up, they move into chondrogenic and adipogenic lineages. Conversely, when cells can remodel the ECM, including the deposition of new components, they are able to elongate via Rho-driven cellular tension, allowing them to activate β1 integrins, activate FAK, and regulate gene expression via YAP/TAZ. An intriguing finding here is that MT1-MMP proteolysis is necessary for cell elongation under these conditions, and it is likely related to the ability of the cells to deposit and remodel the ECM. An important lingering question is whether the bundling of collagen into thickened fibers when MT1-MMP is lost controls SSC fate. There is probably more at play than we currently understand, and it will be fascinating to see what is uncovered in future studies.

As additional work probes this pathway, it will be of interest to determine whether other substrates of MT1-MMP activity are involved in regulating osteogenic commitment. MT1-MMP cleaves other ECM proteins and cell-surface receptors and releases latent growth factors from the ECM. However, in this story, the important finding that loss of MT1-MMP regulates SSC fate in the presence of a cleavable 3D collagen matrix, and not in 2D culture, suggests that effects are probably attributable to cleavage of collagen rather than other substrates (although it remains possible that 3D culture regulates surface expression of proteins whose cleavage is key to fate specification).

The implications of this work likely reach far beyond SSC fate, as ECM stiffness is emerging as a regulator of multiple cell lineages and MT1-MMP is ubiquitous and necessary for development of the embryo. It will be interesting to discover whether this mechanism is at play in other tissues and for other adult stem cells or, more broadly, at the earliest stages of embryonic stem cell fate determination. Moreover, is this mechanism a key regulator of cancer stem cells or the tumor microenvironment? Recent theories postulate that tumor-associated fibroblasts are derived from the influx of circulating mesenchymal stem cells to the site of the tumor (Karnoub et al., 2007; Mishra et al., 2008), where they deposit an extracellular matrix distinct from the resident fibroblasts. Future studies may determine whether a similar MT1-MMP/β1 integrin/YAP-TAZ signaling axis helps to drive the fate of these cells in the tumor microenvironment.

REFERENCES

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., et al. (2011). Nature *474*, 179–183.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Cell *126*, 677–689.

Huebsch, N., Arany, P.R., Mao, A.S., Shvartsman, D., Ali, O.A., Bencherif, S.A., Rivera-Feliciano, J., and Mooney, D.J. (2010). Nat. Mater. *9*, 518–526.

Karnoub, A.E., Dash, A.B., Vo, A.P., Sullivan, A., Brooks, M.W., Bell, G.W., Richardson, A.L., Polyak, K., Tubo, R., and Weinberg, R.A. (2007). Nature *449*, 557–563.

Mishra, P.J., Mishra, P.J., Humeniuk, R., Medina, D.J., Alexe, G., Mesirov, J.P., Ganesan, S., Glod, J.W., and Banerjee, D. (2008). Cancer Res. *68*, 4331–4339.

Tang, Y., Rowe, R.G., Botvinick, E.L., Kurup, A., Putnam, A.J., Seiki, M., Weaver, V.M., Keller, E.T., Goldstein, S., Dai, J., et al. (2013). Dev. Cell *25*, this issue, 402–416.

Zhong, W., Tian, K., Zheng, X., Li, L., Zhang, W., Wang, S., and Qin, J. (2013). Stem Cells Dev., in press. Published online April 5, 2013.

Zhou, Z., Apte, S.S., Soininen, R., Cao, R., Baaklini, G.Y., Rauser, R.W., Wang, J., Cao, Y., and Tryggvason, K. (2000). Proc. Natl. Acad. Sci. USA *97*, 4052–4057.

Turtle Origins: Picking Up Speed

Scott F. Gilbert[1,2,*] and Ian Corfe[1,*]
[1]Institute of Biotechnology, University of Helsinki, 00014 Helsinki, Finland
[2]Department of Biology, Swarthmore College, Swarthmore, PA 19081, USA
*Correspondence: sgilber1@swarthmore.edu (S.F.G.), ian.corfe@helsinki.fi (I.C.)
http://dx.doi.org/10.1016/j.devcel.2013.05.011

Genomes for three species of turtles were recently reported in *Nature Genetics* and *Genome Biology*. The findings of Wang et al. (2013) and Abramyan et al. (2013) place the turtles as a sister group to birds and crocodiles and offer clues to the origins of this group's remarkable physiological traits.

Turtles are bones of contention. Their body plan is unique, appears abruptly in the fossil record, and has resisted attempts to form a consensus as to which group of organisms gave rise to turtles. Rather, there are three extant phylogenies, each modeling a different origin of Testudines (Lyson et al., 2012). Most morphologists tend to favor separating turtles from the crown group of Reptilia, putting them into a distinct and otherwise wholly extinct parareptilian group on the basis of the turtles' characteristic anapsid skull anatomy. Molecular biologists, however, tend to view turtles as normal reptiles whose nuclear and mitochondrial genes demonstrate their affinities to Archosauria (birds and

dinosaurs + crocodilians). A third proposal, using both morphological (ankle) and molecular (microRNA) homologies, suggests that turtles are a sister group to Lepidosauria (lizards, snakes, and the tuatara).

Turtle physiology and anatomy are likewise unique. Turtles are incredibly long-lived (with members of some species routinely living over a century), have temperature-dependent sex determination as their ancestral state, and can survive severely cold, hypoxic, and hypocaloric conditions for years and perhaps decades. Unlike other vertebrates, turtles also have their scapula inside of their ribs, which do not form a rib cage but extend laterally, into the dermis, where they induce and become part of the dorsal shell, the carapace. The ventral exoskeleton of the turtle, the plastron, may be the result of trunk neural crest cells reacquiring the ability to form bone (Gilbert et al., 2007). In short, turtles are highly derived animals.

A turtle genome has thus been anxiously awaited. Now, two recent papers in *Nature Genetics* and *Genome Biology* report the genomes of three representative turtle species. Wang and colleagues (2013) have sequenced the genomes of the sea turtle *Chelonia* and the softshell turtle *Pelodiscus*, whereas Shaffer and colleagues (Abramyan et al., 2013) report the genome of the western painted turtle, *Chrysemys*. The bottom line: examining around 1,000 and 2,000 genes, respectively, these two papers find that turtles are the sister group to archosaurs, splitting off from the ancestors of dinosaurs, birds, and crocodiles around 250 million years ago. These studies thus confirm the placement of turtles suggested in recent, large-scale but nongenomic, comparisons (Chiari et al., 2012; Crawford et al., 2012).

So are these papers the twin meteorites that signal the demise of alternative hypotheses for turtle origins? There are some issues that may allow the continued survival of nonarchosaurian turtle hypotheses. The grouping of turtles and crocodiles, with birds as the sister group, occurs in a subset of the analyses of Wang and colleagues (2013). This may be due to mutation saturation and long-branch attraction, as the crocodile-turtle grouping only appears when analyzing data before the exclusion of the rapidly saturated third codon position. The extremely low nucleotide substitution rate for *Chrysemys* (Abramyan et al., 2013) could, however, lessen the saturation effect. Previous studies have reported the same crocodile-turtle grouping in some or all analyses and additionally blamed the process of reconstructing species trees from gene trees, despite (or perhaps because of) the large number of genes used (Chiari et al., 2012).

Moreover, both studies used only a single analytical method: Wang and colleagues (2013) employ maximum likelihood analyses, while Shaffer and colleagues (Abramyan et al., 2013) employ Bayesian methods. The use of both methods for each data set, and perhaps other methods such as parsimony analysis, might produce increased confidence in the robustness of the sisterhood of turtles and archosaurs. The turtle/archosaur hypothesis can be further tested by the addition of other important species' genomes that would help break up long branches. The tuatara, for instance, would add a deep split in the lepidosaur lineage (Hedges, 2012), and including the genome for a side-necked turtle would add a deeper split within the Testudines. These additions might also increase the dating accuracy of internal turtle relationships; the 95% confidence interval given by Wang and colleagues (2013) for the split date of their two turtle species spans almost 180 million years.

Perhaps the best chance of recovering a phylogeny markedly different from those of Wang, Abramyan, and colleagues would be through enlarging the data sets to include fossils and neontological nonmolecular data. Recent methodological advances have allowed such "phenomic" data sets to approach the size of moderate molecular data sets and so equally influence phylogenetic relationships in combined analyses (O'Leary et al., 2013) or have enabled truly simultaneous analysis of fossil and molecular data in dating phylogenies (Ronquist et al., 2012). Combining these methods with the wealth of gene data provided by the turtle genomes would allow relationships to be tested in the presence of relevant fossils, which have been shown to frequently produce significant effects on the phylogenetic relationships and dates of splits recovered (Cobbett et al., 2007; Ronquist et al., 2012). While the new data provide a very convincing argument for turtles splitting off from early archosaurs, there are still further tests that can be made.

Beyond examining turtle origins, the papers provide complementary insights into turtle development and physiology. Wang and colleagues (2013) focus on evo devo aspects of turtles and find that turtles conform to the pattern of other vertebrates in having an hourglass-shaped pattern of orthologous gene expression. The expression of turtle genes is most similar to that of chick gene expression during the vertebrate phylotypic stage. This agrees with earlier developmental studies showing that turtles develop like most vertebrates before going their turtle-specific way. Abramyan and colleagues (Abramyan et al., 2013) focused their attention on the remarkable turtle physiology. Turtles are among the most anoxia-tolerant animals known, and *Chrysemys* may be the reigning champion. The authors find that anoxic conditions cause a 128-fold increase in the expression of the apolipoprotein-encoding gene *APOLD1* in the brain (and 19-fold in the heart ventricle.) They also find that their turtle has the slowest nucleotide substitution rate of any known vertebrate. It seems the only thing turtles did abruptly was entering the fossil record.

Both groups also find that turtles seem to have lost some genes along the way. *Chelydra* and *Pelodiscus* lack the *ghrelin* gene (which regulates hunger stimulation and homeostasis) and *CXCL10* (which, among other properties, is involved in regulating insulin secretion). *Chrysemys* lacks *ATP50* (whose downregulation increases longevity in *Caenorhabditis elegans*). The absence of these genes could possibly be involved in the strange metabolism and longevity of turtles. It will be important to compare these two databases to see whether the same genes are missing from both sets.

There are also some genes that seem to have evolved rapidly in the turtle. These include the gene encoding microsomal glutathione transferase-3, whose protein product has been associated with longevity and resistance to oxidative stress. Also, the microRNA *miR-29b*, involved in regulating glucose transport, is different than in other organisms. Interestingly, but not unexpectedly, the genes whose quantitative expressions

are elevated most after the phylotypic period, compared to other vertebrates, are those genes whose products are involved in bone formation and responsiveness to vitamin D.

Indeed, much of what is reported is a normal vertebrate genome. The genes for vertebrate sex determination are all present and accounted for, but we haven't been given clues as to how they become regulated by temperature. And the genes for bone formation are there, but we are given no instruction manual as to how the turtle uses them to make its shell. Turtles seem to accomplish their remarkable anatomical and physiological feats using the same basic set of genes as their amniote relatives. Indeed, the Wang et al. (2013) paper shows that *WNT5a*, usually involved in limb formation, seems to be reutilized in the formation of the turtle carapacial ridge. Evolution generates its novelties by tinkering with existing genes, rarely creating something from scratch. Genomes are inventories, the descriptive first step in determining how organisms evolve their specific traits. In addition to quality and quantity, pattern and context are critically important. Having these genomes will make possible the study of the *cis*-regulatory structure of genes and how they may be integrated in new ways to make the unique anatomical and physiological properties of the Testudines. Turtle progress is slow, but rarely steady. These papers may be the great sprint forward allowing us to understand how vertebrate embryos were modified to produce such morphological and physiological wonders as turtles.

REFERENCES

Abramyan, J., Badenhorst, D., Biggar, K.K., Borchert, G.M., Botka, C.W., Bowden, R.M., Braun, E.L., Bronikowski, A.M., Bruneau, B.G., Buck, L.T., et al. (2013). Genome Biol. *14*, R28.

Chiari, Y., Cahais, V., Galtier, N., and Delsuc, F. (2012). BMC Biol. *10*, 65.

Cobbett, A., Wilkinson, M., and Wills, M.A. (2007). Syst. Biol. *56*, 753–766.

Crawford, N.G., Faircloth, B.C., McCormack, J.E., Brumfield, R.T., Winker, K., and Glenn, T.C. (2012). Biol. Lett. *8*, 783–786.

Gilbert, S.F., Cebra-Thomas, J.A., and Burke, A.C. (2007). In Biology of Turtles, J. Wyneken, M.H. Godfrey, and V. Bels, eds. (Boca Raton, FL: CRC Press), pp. 1–16.

Hedges, S.B. (2012). BMC Biol. *10*, 64.

Lyson, T.R., Sperling, E.A., Heimberg, A.M., Gauthier, J.A., King, B.L., and Peterson, K.J. (2012). Biol. Lett. *8*, 104–107.

O'Leary, M.A., Bloch, J.I., Flynn, J.J., Gaudin, T.J., Giallombardo, A., Giannini, N.P., Goldberg, S.L., Kraatz, B.P., Luo, Z.X., Meng, J., et al. (2013). Science *339*, 662–667.

Ronquist, F., Klopfstein, S., Vilhelmsen, L., Schulmeister, S., Murray, D.L., and Rasnitsyn, A.P. (2012). Syst. Biol. *61*, 973–999.

Wang, Z., Pascual-Anaya, J., Zadissa, A., Li, W., Niimura, Y., Huang, Z., Li, C., White, S., Xiong, Z., Fang, D., et al. (2013). Nat. Genet.

Filopodia: The Cellular Quills of Hedgehog Signaling?

Andrew P. McMahon[1,*] and Sean M. Hasso[1]
[1]Department of Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, Keck School of Medicine, University of Southern California, Los Angeles, CA 90089, USA
*Correspondence: amcmahon@med.usc.edu
http://dx.doi.org/10.1016/j.devcel.2013.05.008

Reporting in *Nature*, Sanders et al. (2013) implicate filopodial projections in Sonic hedgehog (Shh) patterning of the limb. Actin-based filopodia transport Shh from producing cells, while filopodia of responding cells bear Cdon and Boc: coreceptors in the Shh pathway. These findings suggest a new mechanism of ligand movement and transmission.

Among the many signaling factors that coordinate cell interactions during development, the Hedgehog family continues to intrigue. Vertebrate Hedgehog signals operate in a wide variety of tissue interactions, but the Sonic hedgehog (Shh) morphogen has garnered the most attention. In the two best-studied systems, limb and neural tube patterning, Shh moves from discrete organizing centers—the zone of polarizing activity (ZPA) and notochord, respectively—forming a concentration gradient within each target field (Lewis et al., 2001). Concentration and duration of signaling are integrated by receiving cells to generate distinct neural progenitor subtypes in the developing nervous system and digit pattern in the limbs (Dessaud et al., 2007; Yang et al., 1997).

Whereas morphogens like Nodal undergo simple processing and diffuse through their target field, the production, release, and movement of Shh is more complex. Processing of Hedgehog family members generates a dual-lipid modified membrane-associated protein. The lipid moieties, palmitylation at the N terminus and cholesterol at the C terminus of the secreted Shh protein, govern multimerization, release, activity, and range of action of the signal. Through an elegant use of genetic cell labeling and live imaging in the chick limb, reported in *Nature*, Sanders et al. (2013) now suggest a new means of Shh signal

Update

Developmental Cell
Volume 25, Issue 6, 24 June 2013, Page 655

DOI: https://doi.org/10.1016/j.devcel.2013.06.001

Developmental Cell
Erratum

Turtle Origins: Picking Up Speed

Scott F. Gilbert* and Ian Corfe*
*Correspondence: sgilber1@swarthmore.edu (S.F.G.), ian.corfe@helsinki.fi (I.C.)
http://dx.doi.org/10.1016/j.devcel.2013.06.001

(Developmental Cell *25*, 326–328; May 28, 2013)
The original version of the Preview text incorrectly stated the location of the scapula in turtles and the genus of the green sea turtle sequenced in the recent paper discussed in the Preview. The turtle scapula is inside the ribcage, and the green sea turtle genus is *Chelonia*. The Preview has been updated online with these corrections.