on days 3, 7, 10 or 14 after SIV infection. Although initiating treatment on days 7, 10 and 14 significantly reduced peak plasma virus levels, treatment from day 3 completely blocked the emergence of detectable viraemia; this was also evidenced by the absence of SIV-specific antibody-based and cellular immune responses in these animals. The authors found no proviral DNA in the animals' peripheral-blood mononuclear cells (which include $CD4^+$ T cells) before treatment initiation on day 3, but proviral DNA was already detectable in their lymph nodes and the mucosal linings of the gastrointestinal tract. This crucial finding suggests that the viral reservoir may be first seeded in the lymphoid and mucosal tissues, a result with important implications for HIV-1 eradication strategies.

Most significantly, the authors observed viral rebound in all animals after ART was stopped. This occurred even when ART that fully suppressed detectable viraemia was initiated at day 3 and continued for 6 months, a treatment period that allows elimination of labile infected cells and thus reveals stable reservoirs. The observed rebound suggests that the viral reservoir is seeded surprisingly early in SIV-infected animals. However, the animals treated from day 3 showed a slightly delayed viral rebound compared with those that started ART at later times. Using a sophisticated model of viral dynamics, the authors show that the time to viral rebound is correlated with total viraemia during the acute phase of infection.

These data indicate that the viral reservoir could be seeded substantially earlier than previously assumed — a sobering finding that poses additional hurdles to HIV-1 eradication efforts. If this evidence from SIV-infected animals reflects what happens early in HIV-1 infection in humans, it would mean that it is nearly impossible to initiate ART before viral reservoirs have seeded, because viraemia is not detectable at this point. In other words, reservoir seeding precedes any clinical evidence of infection. However, although early treatment may not prevent reservoir seeding, it has been consistently shown to reduce the size of the latent reservoir[8–10], and infected individuals who receive early treatment could have a lower barrier to cure in future eradication strategies.

Whitney and colleagues' findings are of particular interest in light of a study last year[16] reporting that a disseminated SIV infection could be cleared by vaccine-induced T-cell-based immune responses. The different outcomes of these two studies may be partly due to the fate of infected cells during acute infection. Early initiation of ART immediately stops subsequent new infection of susceptible cells, but does not affect the fate of cells that are already infected. A small fraction of these infected cells survive and revert back to a resting state, thereby seeding the latent reservoir (Fig. 1). By contrast, vaccinated animals have pre-existing SIV-specific cytotoxic T cells that can clear the infected cells before they go into latency, thus preventing the viral reservoir from becoming established.

It remains to be seen whether clinical studies will confirm Whitney and colleagues' observations, because substantial differences exist between SIV infection in rhesus macaques and HIV-1 infection in humans. As mentioned by the authors, the SIV dose used in their study may be much higher than the typical amount of HIV-1 involved in sexual transmission, perhaps resulting in a higher level of early viral replication. Nevertheless, the striking findings of the early seeding of the viral reservoir in mucosal and lymphoid tissues before viraemia is detected suggest that new approaches, in addition to early treatment, will be necessary to eradicate HIV-1 infection. ■

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1. Finzi, D. *et al. Science* **278,** 1295–1300 (1997).
2. Chun, T.-W. *et al. Proc. Natl Acad. Sci. USA* **94,** 13193–13197 (1997).
3. Finzi, D. *et al. Nature Med.* **5,** 512–517 (1999).
4. Siliciano, J. D. *et al. Nature Med.* **9,** 727–728 (2003).
5. Richman, D. D. *et al. Science* **323,** 1304–1307 (2009).
6. Whitney, J. B. *et al. Nature* **512,** 74–77 (2014).
7. Chun, T.-W. *et al. Proc. Natl Acad. Sci. USA* **95,** 8869–8873 (1998).
8. Chun, T.-W. *et al. J. Infect. Dis.* **195,** 1762–1764 (2007).
9. Hocqueloux, L. *et al. J. Antimicrob. Chemother.* **68,** 1169–1178 (2013).
10. Ananworanich, J. *et al. PLoS ONE* **7,** e33948 (2012).
11. Salgado, M. *et al. Retrovirology* **8,** 97 (2011).
12. Sáez-Cirión, A. *et al. PLoS Pathogens* **9,** e1003211 (2013).
13. Persaud, D. *et al. N. Engl. J. Med.* **369,** 1828–1835 (2013).
14. Ledford, H. *Nature* http://dx.doi.org/10.1038/nature.2014.15535 (2014).
15. Hill, A. L., Rosenbloom, D. I. S., Fu, F., Nowak, M. A. & Siliciano, R. F. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1406663111 (2014).
16. Hansen, S. G. *et al. Nature* **502,** 100–104 (2013).

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EVOLUTION

Tooth structure re-engineered

Mice deficient in the EDA protein lack normal tooth features. Restoring EDA in embryonic teeth at increasing doses has now been found to recover these dental features in a stepwise pattern that mimics evolution. SEE ARTICLE P.44

ZHE-XI LUO

A fundamental connection between developmental changes and evolution has long been established[1]. This link is gaining renewed emphasis[2] as molecular studies shed new light on evolution by revealing many genetic modifications that alter developmental processes, in turn changing an organism's shape and structure. On page 44 of this issue, Harjunmaa *et al.*[3] report that, by simply tinkering with the genes and signalling pathways that control the shape of developing teeth, they have remade several different tooth structures *in vitro*. These structures draw a striking parallel with how teeth evolved from those of distant mammalian ancestors to the teeth of modern-day rodents.

Some lineages of therians (marsupial and placental mammals and their kin) that lived in the Mesozoic era, 252 million to 66 million years ago, had 'tribosphenic' molars[4]. The taller front end of the tribosphenic lower molar, called the trigonid, had three cusps — the raised points on the crown of the tooth — for shearing food. The lower back end, the talonid, had a basin-like surface for grinding food[5,6]. The trigonid and talonid of Mesozoic mammals are still recognizable in modern-day rodents, albeit in a highly modified form. As rodents arose from ancestral mammals and diversified into many lineages, cusps that were separate in the Palaeocene epoch 66 million to 56 million years ago[7] became progressively connected by crests — which are more effective for chewing plant food — in a 'cusp-to-crest' dental evolution that occurred in many rodent groups[8,9].

The *ectodysplasin A* (*Eda*) gene encodes a vertebrate signalling protein that is involved in the development of a wide array of structures, from hair to sweat glands[10]. In the embryonic tooth, the EDA protein is active in enamel knots, which are signalling centres and the precursors of adult tooth structures. EDA regulates the position and size of future tooth cusps and their connecting crests[8]. Mice that do not express *Eda* lack normal cusps and crests, and instead have only basic, generalized teeth[10].

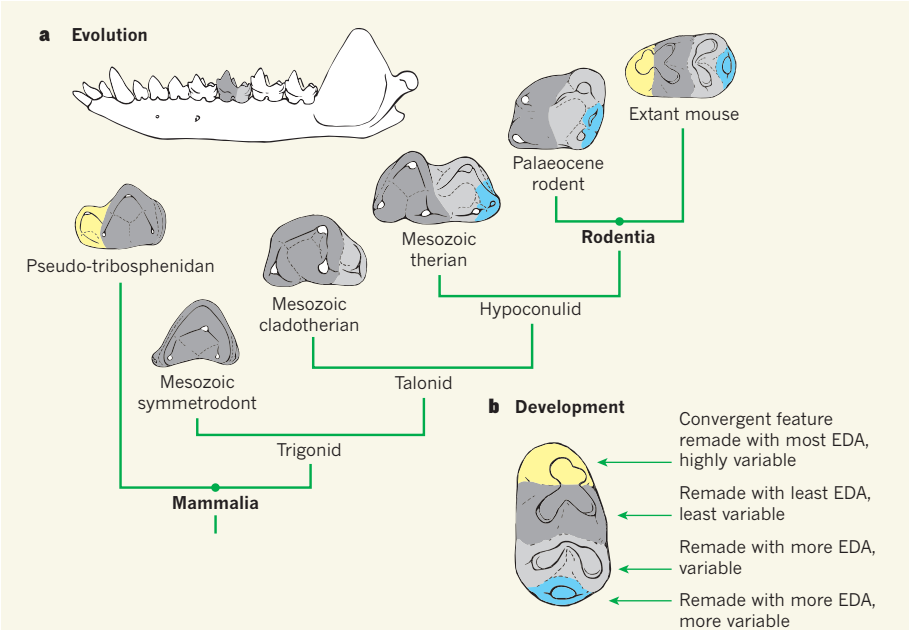

Figure 1 | Reconstructing tooth evolution *in vitro*. **a**, As mammals evolved, their molars (one indicated in the jaw) became ever more complex, because extra tooth features evolved over time. Structures called trigonids (dark grey) evolved first, in early mammals such as the Mesozoic symmetrodonts, followed by talonids (light grey) in a group of Mesozoic therians called cladotherians. Hypoconulids (blue) evolved in Mesozoic therians, and anteroconids (yellow) in advanced rodents. The anteroconids are similar in structure to pseudo-talonids, which evolved separately (convergently) in pseudo-tribosphenic teeth in an early-divergent clade of mammals (the pseudo-talonid is also indicated in yellow). **b**, Deletion of the *Eda* gene in mouse embryos results in the loss of all of these tooth features. Harjunmaa *et al.*[3] show *in vitro* that addition of the EDA protein to embryonic teeth from *Eda*-deficient mice in increasing doses can replay the steps of evolution. Furthermore, features that evolved longer ago respond in a less variable manner than features that evolved more recently.

Harjunmaa and colleagues grew *Eda*-deficient teeth *in vitro*, and found that cusps and crests could be restored by adding EDA. They demonstrated, with the aid of computer models, that different doses of EDA alter tooth morphogenesis (the process by which structures are shaped as they develop), akin to the dental transformations that occurred as rodents evolved from their Mesozoic mammalian ancestors (Fig. 1). For example, the trigonid — the first part of the tribosphenic molar to have evolved — is regenerated with only a small dose of EDA. However, a higher dose of EDA is required to restore the talonid, which evolved more recently[5].

The cusp-to-crest morphogenesis of mouse molars is controlled by a gene network that includes genes encoding the signalling proteins fibroblast growth factor 3 (*Fgf3*; ref. 9) and sonic hedgehog (*Shh*)[11]. An increase or decrease in *Fgf3* causes over- or under-development of tooth features, respectively[9]. Harjunmaa and co-workers found that reducing the concentration of SHH in *Eda*-deficient teeth regenerated the ancestral features of Palaeocene rodents, reversing the cusp-to-crest transformation of modern-day rodents. Thus, molecular manipulations that alter tooth morphogenesis *in vitro* can replay evolution, either forward, to mimic the fossil record, or in reverse.

Perhaps the most exciting insight from Harjunmaa and colleagues' work is that ancestral structures show a more uniform response to the addition or removal of EDA or SHH than those that evolved more recently or independently in different lineages (convergent evolution). For example, addition of a low dose of EDA reliably restored the trigonid, as expected of ancestral features, which are typically evolutionarily well conserved owing to their long history. By contrast, higher doses restored the talonid in many, but not all, teeth — development of this feature was more variable in response to EDA. This is consistent with the theory[6] that the talonid basin evolved convergently in different mammal lineages, but has reduced in size in some carnivoran or insectivoran mammals.

The hypoconulid is a talonid cusp in some mammals, but is enlarged and forms a separate lobe in mice. The authors found that full development of this structure requires a higher dose of EDA than does the rest of the talonid, and shows even wider variation in its response to EDA. Finally, the anteroconid in mice — another feature that arose late in rodent evolution — requires the highest EDA dose to regenerate, and shows the broadest variation when regenerated. Its position on the tooth corresponds to the 'pseudo-talonid' that arose in some early-divergent mammals that died out before the end of the Mesozoic. Harjunmaa and co-workers' experiment therefore demonstrates that modern-day mice still have the developmental potential to replicate evolutionary events that occurred long ago, in the now-extinct mammals of the Mesozoic[12].

The level of EDA required to give rise to individual molar characteristics therefore seems to provide information about how robust their development is. When studying how morphogenesis drives evolution[13], it will be crucial to bear in mind that the sensitivity of a particular tooth feature to EDA activity may indicate the likelihood of an evolutionary transformation producing that feature. For example, as mentioned above, talonid-like features evolved twice — in the basal diversification of modern mammals and in early-divergent groups of the Mesozoic. Variable sensitivities to gene-expression dosage and signalling strength can serve as a measure of the evolutionary variability of each tooth feature, and may underpin the many convergences and reversals of tooth evolution observed in the mammalian fossil record.

Eda and *Shh* have varying effects on many vertebrate structures, so it can be hard to tease apart which evolutionary feature is controlled by which part of the gene network. Harjunmaa *et al.* have cleared this hurdle in a welcome development that brings us closer to being able to test how changes in morphogenesis affect the final shape of evolving teeth as seen in the fossil record. Genetic engineering of developmental processes *in vitro* is a fruitful way to decipher how the shape of organs or other biological structures is modified by evolution. ■

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1. Gould, S. J. *Ontogeny and Phylogeny* (Belknap, 1985).
2. Abzhanov, A. *Trends Genet.* **29,** 712–722 (2013).
3. Harjunmaa, E. *et al. Nature* **512,** 44–48 (2014).
4. Kielan-Jaworowska, Z., Cifelli, R. L. & Luo, Z.-X. *Mammals from the Age of Dinosaurs: Origins, Evolution, and Structure* (Columbia Univ. Press, 2004).
5. Crompton, A. W. *Zool. J. Linn. Soc.* **50** (Suppl. 1), 65–87 (1971).
6. Luo, Z.-X., Cifelli, R. L. & Kielan-Jaworowska, Z. *Nature* **409,** 53–57 (2001).
7. Meng, J. & Wyss, A. R. *J. Mamm. Evol.* **8,** 1–71 (2007).
8. Gomes-Rodrigues, H. *et al. Nature Commun.* **4,** 2504 (2013).
9. Charles, C. *et al. Proc. Natl Acad. Sci. USA* **106,** 22364–22368 (2009).
10. Mikkola, M. J. & Thesleff, I. *Cytokine Growth Factor Rev.* **14,** 211–224 (2003).
11. Cho, S.-W. *et al. Development* **138,** 1807–1816 (2011).
12. Luo, Z.-X., Ji, Q. & Yuan, C.-X. *Nature* **450,** 93–97 (2007).
13. Carroll, S. B., Grenier, J. K. & Weatherbee, S. D. *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design* 2nd edn (Blackwell, 2005).

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