skeleton? Surprisingly, however, altering the size of different muscles can produce dramatic alterations in the bones to which they attach[6,7]. Animal — including primate — models of jaw-muscle transposition or removal show that growth patterns of the craniofacial skeleton can be radically altered by changing muscle anatomy[8,9]. From these studies it is clear that, over time, the abrupt reduction in masticatory muscle size and contractile force that would have arisen within the *MYH16*-mutant ancestor could have had a considerable impact on cranial morphology. Those effects might well have included a reduction in stress across the bones of the braincase, allowing it to become larger.

Some serious issues, however, are not fully addressed by such a model. The evolutionary acquisition of reduced jaw-muscle size needs explaining in terms of its adaptive significance, independent of any perceived role in craniofacial morphogenesis. Specifically, given the deleterious nature of the *MYH* mutations implicated in human disease, it is unclear how a similar change would have become 'fixed' in the ancestral hominid population. Several explanations could be advanced to counter this ideological roadblock, such as a contemporaneous shift in diet (say, to an increased reliance on meat eating), or a growing dependence on hands rather than the jaw in food preparation. Stedman *et al.*[1] largely ignore the issue, perhaps because it requires a separate, detailed examination. But whatever the immediate consequence of *MYH16* inactivation was, it is now an indicator that a critical change occurred in the hominid masticatory apparatus around 2.5 million years ago.

What is the significance of these findings, and do they shed any light on human origins? Although there is a rough consensus about the individual features that define fossil species within the genus *Homo*, the sequence in which individual traits were acquired during hominid evolution remains controversial. Furthermore, the definition of which character traits were essential for the appearance of the modern human form is equally contentious. The reasons for this are familiar to anyone who tries to explain morphological transitions over large evolutionary distances based primarily on the fossil record. Such explanations hinge on finding so-called 'transitional forms', where a particular fossil is so indelibly etched with the tell-tale signs of what something was, and what it was going to become, that an inescapable evolutionary theory simply tumbles out of the dirt. Not unsurprisingly, such fossils are very rare indeed, and fossils charting the course of hominid evolution are no exception. Stedman and colleagues' identification[1] of the first molecular difference between human and non-human primates, traceable to an anatomical difference in the fossil record, provides independent evidence and fresh ideas with which to describe the mechanistic basis of hominid evolution.

We can hope that this study[1] represents the vanguard of a new wave of analyses that focus on the genetic basis of human evolution. Any hypothesis about human origins must take into account our understanding of the hominid fossil record. But it is studies such as these that will put meat on the bones of any theory. With the impending completion of the project to sequence the chimpanzee genome, the tantalizing prospect of whole-genome comparisons between humans and our closest living relative is not too far away. It has been suggested that such a comparison could throw up around 40 million nucleotide differences between humans and chimpanzees[10]. Identifying which of these differences encode the essential elements of being human is a daunting task. Sophisticated comparative genomic and expression-profile analyses have nonetheless already been completed, revealing patterns in gene evolution and expression that may guide us to functionally important differences[11–13]. More than any other report before it, however, the study by Stedman *et al.* suggests that the genetic basis of human evolution can and will be defined. ■

Pete Currie is in the Developmental Biology Program, Victor Chang Cardiac Research Institute, Sydney 2010, Australia.
e-mail: p.currie@victorchang.unsw.edu.au

1. Stedman, H. H. *et al. Nature* **428,** 415–418 (2004).
2. Wood, B. *Nature* **355,** 783–790 (1992).
3. Acakpo-Satchivi, L. *et al. J. Cell Biol.* **139,** 1219–1229 (1997).
4. Allen, D. L., Harrison, B. C., Sartorius, C., Byrnes, W. C. & Leinwand, L. A. *Am. J. Physiol. Cell Physiol.* **280,** C637–C645 (2001).
5. Martinsson, T. *et al. Proc. Natl Acad. Sci. USA* **97,** 14614–14619 (2000).
6. McPherron, A. C., Lawler, A. M. & Lee, S. J. *Nature* **387,** 83–90 (1997).
7. Hamrick, M. W., McPherron, A. C., Lovejoy, C. O. & Hudson, J. *Bone* **27,** 343–349 (2000).
8. Hohl, T. H. *J. Maxillofac. Surg.* **11,** 149–156 (1983).
9. Brennan, M. & Antonyshyn, O. *Plast. Reconstr. Surg.* **97,** 13–24 (1996).
10. Olson, M. V. & Varki, A. *Nature Rev. Genet.* **4,** 20–28 (2003).
11. Caceres, M. *et al. Proc. Natl Acad. Sci. USA* **100,** 13030–13035 (2003).
12. Clark, A. G. *et al. Science* **302,** 1960–1963 (2003).
13. Wildman, D. E., Uddin, M., Liu G., Grossman, L. I. & Goodman, M. *Proc. Natl Acad. Sci. USA* **100,** 7181–7188 (2003).

Crystallization

How come you look so good?

Roger J. Davey

Crystallized brown sugar is quite miraculous: it takes just a few 'seeds' in the crystallization process to trigger the formation of many times more crystals. Now we are starting to understand why.

The generation of a solid from a solution through crystallization might sound such a simple, familiar process that one could be forgiven for thinking that it is fully understood. But despite the work of Wilhelm Ostwald[1] on crystal nucleation, and the development of classical nucleation theory[2], this is not so. Take an everyday example such as brown sugar, millions of tonnes of which are crystallized annually, to be dissolved in tea and coffee. The crystals are all of uniform size — no really big ones, no really small ones — and all are nicely faceted (Fig. 1). This is no accident: the effect is achieved by a process known as 'seeding', in which small crystals of pulverized sugar are introduced in the solution to act as seeds on which crystal growth can start. This seeding, however, does not follow the rules of the garden, with one new plant (or in this case, crystal) per seed. In fact, the amount of seed needed to catalyse the whole crystallization may be less than 1% of the mass of the final product. How do so few seeds give rise to so many crystal nuclei?

We do not really know for sure, and so I am intrigued by the work of Cacciuto and colleagues[3], reported on page 404 of this issue. Through computer simulations, Cacciuto *et al.* have investigated just how a seeding process might work, choosing the case where the seed is a foreign substance rather than a crystal of the solute (as it might be in, for instance, the seeding of clouds to induce rain).

For simplicity and generality, however, Cacciuto *et al.* have focused not on specific molecules but on crystallization in a colloidal suspension of hard, nanometre-size spheres. Using clever simulation methodologies, they have explored how the curvature and size of seeds, both spherical and cylindrical, affect nucleation. When the seeds are hollow spherical sections, varying in radius from 10 to 150 times the diameter of the crystallizing colloidal particles, it seems that nucleation is easier on the convex side of a seed's surface than on its concave side. For cylindrical seeds, it is easier still. This 'experiment' reveals what looks like a 'nucleus factory', centred on a seed particle (see Fig. 4 on page 405), which allows the final number of crystals to be larger than the number of seeds.

Experimentally, the question and application of seeding may go back to Louis Pasteur's discovery in the mid-nineteenth century of optical activity in crystals of sodium ammonium tartrate. The compound's optical activity is due to its chirality, the fact

R.J. DAVEY

Figure 1 **Hey, brown sugar: Cacciuto *et al.*[3] offer new insight into the seeding of crystallization.**

that it can exist in two forms, or enantiomers, that are non-superimposable mirror images of each other. In 1866, Desiré Gernez wrote to his former colleague Pasteur, describing the result of an interesting experiment[4]. Following on from Pasteur's work, Gernez had discovered that the addition of seed crystals of pure enantiomer to a racemic solution of the tartrate — one containing equal amounts of the two enantiomers — yielded, not a racemic solid, but crystals of the same chirality as the seed. Separations based on this observation have become known as 'resolutions by entrainment'[4] and are part of the armoury of the modern-day chemical-process developer.

Not surprisingly, it was chemical engineers, interested in designing continuous crystallization processes, for whom seeding (or secondary nucleation, as they termed it) became a central issue. In 1934, Ting and McCabe[5] showed that solutions of magnesium sulphate could be nucleated more reproducibly at moderate supersaturations in the presence of seeds. Today, commercial crystallization processes operate at suspension densities of perhaps 20%, ensuring that seeding levels are always high.

Some clever experiments[6] in the 1970s on the seeded nucleation of enantiomers of sodium chlorate revealed that, as long as the supersaturations were not too high, all the crystals were enantiomerically identical to the seed. The experiments also showed that the new crystals originated from the seeds through their contact with the crystallizing vessel. We now know that secondary nucleation, and hence seeding, can often be more effective because of mechanical and liquid-shear damage at the seed surface[7]. Such damage would remove potential nuclei from the seed, allowing them to become free-growing crystals. This seems to be a tantalizing reflection of what Cacciuto *et al.*[3] have now shown.

Time, I think, for some new experiments.

Roger J. Davey is at the Molecular Materials Centre, Department of Chemical Engineering, UMIST, Manchester M60 1QD, UK.
e-mail: roger.davey@umist.ac.uk

1. Ostwald, F. W. *Z. Phys. Chem.* **22**, 289–302 (1897).
2. Garside, J. & Davey, R. J. *From Molecules to Crystallizers — An Introduction to Crystallization* Ch. 3 (Oxford Univ. Press, 2000).
3. Cacciuto, A., Auer, A. & Frenkel, D. *Nature* **428**, 404–406 (2004).
4. Jacques, J., Collet, A. & Wilen, S. H. *Enantiomers, Racemates, and Resolutions* 223 (Wiley, Chichester, 1994).
5. Ting, H. H. & McCabe,W. L. *Ind. Eng. Chem.* **26**, 1201–1207 (1934).
6. Denk, E. G. & Botsaris, G. D. *J. Cryst. Growth* **13/14**, 493–499 (1972).
7. Mullin, J. W. *Crystallization* 3rd edn, Chs 5, 6 (Butterworth–Heinemann, Burlington, MA, 1992).

RNA interference

Human genes hit the big screen

Andrew Fraser

Genetic screens are powerful tools for identifying the genes involved in specific biological processes. At last, RNA interference makes large-scale screens possible in mammalian cells.

One of the most intuitive ways to learn how a complicated machine works is to take it apart piece by piece — a directed 'learning by breaking'. For biologists, teasing apart the machinery underlying the form and function of an organism can be done, most simply, by removing genes one at a time and looking at the effect. One experimental method for turning genes off is known as RNA interference (RNAi; Box 1, overleaf); this has shot to prominence because it allows almost any gene of known sequence to be shut down with apparently magical ease[1].

In two of the biologist's favourite model organisms, nematode worms and fruitflies, RNAi has been used to turn off almost every one of their genes[2,3]. Such genome-wide RNAi surveys of gene function have remained out of reach in mammals — until now, that is, for on pages 427 and 431 of this issue Paddison *et al.*[4] and Berns *et al.*[5] report the generation of tools to allow RNAi mass-screening of mammalian genes. This at last makes it possible to carry out genetic screens in mammalian cells in culture.

There is a range of effective strategies for RNAi in mammalian cells (reviewed in ref. 6), and they differ principally in the method for getting the double-stranded RNA (dsRNA) that specifically interferes with the target gene into the cells. In one method, rather than synthesize the dsRNA chemically before introducing it into the cells, the interfering dsRNA is made directly by the cells themselves. A vector directing the transcription of precise short hairpin RNAs — shRNAs — by RNA polymerase III is introduced into the cells; these transcribed shRNAs are processed by the cell to give the small interfering dsRNAs (siRNAs) that turn off the target gene. shRNA-expressing vectors allow for sustained RNAi in a wide range of cell lines (including embryonic stem cells, the subject of much current research). A complete library of shRNA-expressing vectors designed to target each and every gene in a mammalian genome would thus allow genome-wide RNAi-based genetic screens in cells in culture. Put simply, for any process that we are interested in (cell division, response to DNA damage and so on), with such an shRNA library we could screen every gene in the human genome and ask if it is involved.

Both groups[4,5] have converged on the same basic shRNA library approach, each generating a retrovirus-based library capable of targeting around a third of human genes; the genes were chosen for their potential roles in disease. Different shRNAs often interfere to differing extents with a target gene, so at least three shRNAs have been cloned for most genes. This multiple coverage not only provides an internal control, but may also allow comparison of both strong and weak 'knock-downs' of a specific gene in an analogous way to a classical genetic approach[7].

Berns *et al.*[5] used their library to search for genes that affect the function of *p53*, a tumour-suppressor gene that kills or 'arrests' cells with damaged DNA. They screened around 8,000 human genes to find those required for a *p53*-dependent arrest of cell proliferation and identified six genes, including *p53* itself. Further assays confirm that these genes — which include a histone acetyl transferase and a histone deacetylase, two key regulators of gene expression — do indeed play a role in *p53*-induced cell-cycle arrest and senescence. This ability to survey the gene functions of a full third of the human genome so rapidly is breathtaking, and the success of the subsequent assays underscores the quality of this approach.

The retrovirus-based vectors used by both groups are excellent for many cell-based screens. But they cannot be used for the stable expression of shRNAs in all cell types — this requires moving the shRNA-encoding inserts to different vectors. The shRNA library described by Paddison *et al.*[4] incorporates an elegant system for shuttling the inserts into any destination vector simply using bacterial mating. Their sequence-verified shRNA library targets almost 10,000 human genes; the shRNAs have also been chosen to allow targeting of the mouse orthologues (equivalents) of those human genes, if possible, and over 5,000 mouse