

GENETICS

Paramutable possibilities

Paul D. Soloway

A curious genetic phenomenon allows certain genetic instructions to be passed between generations without the gene variants involved being transmitted. Some spotty mice provide clues to how this might happen.

One of the benefits of sex is that our parents give us two copies of nearly all our genes. So if one copy, or allele, doesn't work, there is a good chance that the other, operating independently, will be sufficient. Exceptions exist, but even in those cases the two alleles usually function independently. In rare cases, however, the alleles interact intimately, even though they reside on separate chromosomes, and whether one allele is expressed depends on directions given by the other. One example of this phenomenon is paramutation, a process in which orders issued by an allele in one generation are remembered in subsequent generations, even if the allele issuing the order is not transmitted. How do chromosomes remember who their partners were in previous generations, and how do those partners continue to exert their control long after they have segregated away? On page 469 of this issue, Minoou Rassoulzadegan *et al.*¹ provide evidence for a mechanism involving small RNA molecules that might underlie paramutation.

In 1956, R. Alexander Brink² coined the term paramutation to describe the unexpected means of regulating purple pigmentation in maize kernels (see ref. 3 for a review). Pigmentation is controlled, in part, by the *R* gene locus. If all the alleles at this locus are recessive alleles (r^r), the kernels are colourless, but when a single dominant allele (R^R) is present, the kernels are typically purple. This is not always the case, however; it depends on what the R^R allele was exposed to in the previous generation. In Brink's experiments, if the R^R allele came from pollen of a plant that also had the R^{st} allele, which caused the kernels to be spotted, then the normally dominant influence of the R^R allele was silenced and kernels showed reduced pigmentation. The untransmitted R^{st} allele exerted its effects in the next generation, overriding the dominance of the R^R allele in violation of Mendel's rules of genetic inheritance. Brink called R^R paramutable and R^{st} paramutagenic, and he showed that the influence of the paramutagenic allele could persist for many generations. Paramutation-like phenomena have been reported in mammals^{1,4-6} and might be of clinical relevance in humans, for example affecting certain forms of diabetes⁴.

The system examined by Rassoulzadegan *et al.* in mice involves an allele of the *Kit* locus called *Kit^{tm1Alf}*. This is a null allele; that is, it makes no functional Kit protein. Heterozygous mice — those with one *Kit^{tm1Alf}* allele and

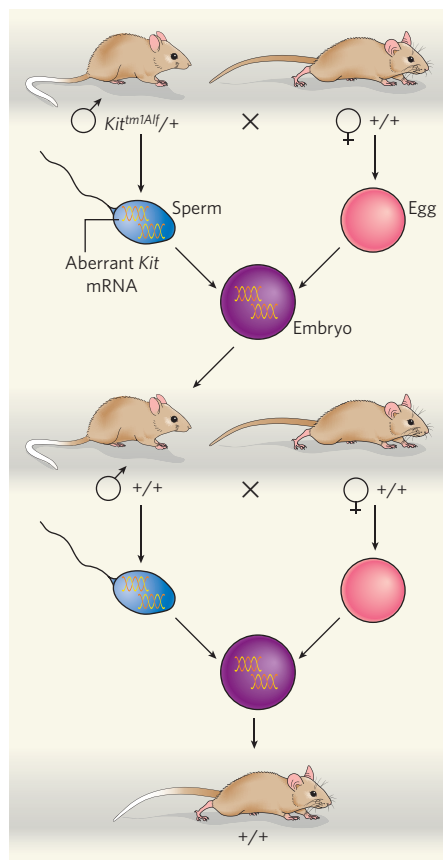


Figure 1 | Model for paramutation at *Kit* as proposed by Rassoulzadegan *et al.*¹. Heterozygous male mice with one *Kit^{tm1Alf}* allele and one normal wild-type (+) allele have a spotted white tail-tip and produce aberrant *Kit* messenger RNAs from the paramutagenic *Kit^{tm1Alf}* allele. These are packaged in sperm and transmitted to the embryo after fertilization of the egg. Progeny carry a wild-type *Kit* allele transmitted by the heterozygous father, but action of the transmitted aberrant RNAs still gives rise to the spotted tail and maintains their production, allowing paramutation of the wild-type *Kit* allele and further transmission of the spotted tail. Loss of aberrant RNAs through attenuated production or dilution over successive generations might lead to gradual loss of paramutation.

one normal 'wild-type' *Kit* allele — had white spotting at their tail tips and showed reduced expression of *Kit* (as measured by production of *Kit* messenger RNA from the gene). When these mice were crossed with wild-type mates, the wild-type progeny showed the same white spotting and reduced *Kit* mRNA levels as their heterozygous parent, even though they were

fully wild type and lacked the null allele that caused spotting in their heterozygous parent. Moreover, these wild-type progeny also had an accumulation of a mixture of smaller RNAs with sequences that matched various parts of the *Kit* mRNA. Notably, sperm precursor cells from male mutants had aberrant *Kit* mRNAs, and their sperm showed an unexpected accumulation of RNA. It is possible that these RNAs are transmitted to the next generation on fertilization, even if the allele from which they arose is not passed on.

The nature of aberrant RNAs is unknown, but they have attracted attention for a variety of reasons. First, paramutation is one of several heritable 'epigenetic' phenomena, in which inherited trait differences arise by covalent modifications to DNA and DNA-bound histone proteins rather than by changes to the DNA sequence of the genes themselves. These modifications can be regulated by small interfering RNAs (siRNAs) generated by the RNA interference (RNAi) pathway, and can arise from transcribed DNA repeats⁷⁻⁹, which are structures found at some loci undergoing paramutation^{4,6,10}. Second, siRNAs and the related microRNAs (miRNAs) can cause the degradation of mRNAs whose sequences they share, blocking production of the encoded proteins¹¹. Finally, RNAs have been proposed to play a role in another non-mendelian system of inheritance¹². In combination with Rassoulzadegan and colleagues' data, these observations raise the possibility that the aberrant RNAs arising from the *Kit^{tm1Alf}* allele include siRNAs, miRNAs or other regulatory RNAs that are packaged in sperm and cause paramutation on transmission to the next generation.

To test this possibility, Rassoulzadegan *et al.* injected total RNA from tissues containing the aberrant *Kit* RNAs into fertilized mouse eggs. This led to spotting in many of the progeny that came to term — and in their progeny. Injected miRNAs designed to degrade *Kit* mRNA had the same effect. Control miRNAs with no similarity to *Kit* unexpectedly also caused spotting, but at a lower frequency, and the spotted animals only rarely transmitted the spotted phenotype to their progeny. It is not certain that spotting caused by the *Kit^{tm1Alf}* allele arises by the same mechanism as spotting induced by injected miRNAs. However, what might be occurring in this system, and possibly in other paramutation models, is that small RNAs produced from a paramutagenic allele are acting on the corresponding paramutable allele or on its transcribed mRNA, effectively silencing it. Because RNAi-mediated degradation of mRNAs produces more siRNAs, the silencing might be propagated if these small RNAs are packaged into germ cells and carried into the next generation. This could allow successive generations to display a certain characteristic, even if the paramutagenic allele that caused it was not transmitted (Fig. 1). Rassoulzadegan and colleagues' proposal that RNAs are involved

in paramutation is strongly supported by work from Vicki Chandler's group showing that paramutation requires an RNA metabolizing enzyme that is involved in other epigenetic phenomena¹³.

Rassoulzadegan and colleagues' model has yet to be validated, and several points need to be clarified. Notably, the mechanism by which aberrant RNAs from *Kit^{tm1Alf}* heterozygotes induce spotting is not known. Does it involve bona fide siRNAs or miRNAs emanating from the mutant allele? Also, are aberrant RNAs causing mRNA degradation or epigenetic modifications at the wild-type allele? Are these effects mediated by RNAi or by other pathways influenced by small RNAs? If siRNAs or miRNAs do emanate from the paramutagenic *Kit^{tm1Alf}* allele, how do they arise, and do similar RNAs arise in other paramutation models? A particularly intriguing possibility is that such RNAs regulate other non-genetic modes of inheritance, such as metabolic or behavioural imprinting. These have far greater consequences for human development than for

spotty mice and maize, but we may learn about such mysterious processes by studying those mouse tales. ■

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1. Rassoulzadegan, M. *et al. Nature* **431**, 469–474 (2006).
2. Brink, R. A. *Genetics* **41**, 872–889 (1956).
3. Chandler, V. L. & Stam, M. *Nature Rev. Genet.* **5**, 532–544 (2004).
4. Bennett, S. T. *et al. Nature Genet.* **17**, 350–352 (1997).
5. Rassoulzadegan, M., Magliano, M. & Cuzin, F. *EMBO J.* **21**, 440–450 (2002).
6. Herman, H. *et al. Nature Genet.* **34**, 199–202 (2003).
7. Aufsatz, W., Mette, M. F., van der Winden, J., Matzke, A. J. & Matzke, M. *Proc. Natl Acad. Sci. USA* **99**, 16499–16506 (2002).
8. Hamilton, A., Voinnet, O., Chappell, L. & Baulcombe, D. *EMBO J.* **21**, 4671–4679 (2002).
9. Volpe, T. A. *et al. Science* **297**, 1833–1837 (2002).
10. Stam, M., Belele, C., Dorweiler, J. E. & Chandler, V. L. *Genes Dev.* **16**, 1906–1918 (2002).
11. Fire, A. *et al. Nature* **391**, 806–811 (1998).
12. Lolle, S. J., Victor, J. L., Young, J. M. & Pruitt, R. E. *Nature* **434**, 505–509 (2005).
13. Alleman, M. *et al. Nature* (in the press).

NANOMATERIALS

Display of flexibility

László Forró

Treated the right way, carbon nanotubes can be moulded into large, flexible electron-emitting sheets. The material is one half of what's needed for an electronic display you could fold up and slip in your pocket.

As far as their electrical properties are concerned, the long, cylindrical carbon molecules known as carbon nanotubes are notoriously Janus-faced: picking out a nanotube from a newly fabricated batch, one cannot know whether it will be a conductor or an insulator. In principle, both types are useful, and the basic transport, mechanical and optical properties of each kind are more or less known. But not knowing how to tune the synthesis to yield just one type is a considerable handicap to the use of nanotubes as, for example, components in integrated circuits. Because of this, attention has concentrated on applications for which this uncertainty is irrelevant: nanoswitches¹, motors², actuators³ and yarns for composite materials⁴, to name but a few.

One particularly promising application of carbon nanotubes is the flat-panel display. Such displays exploit the fact that, owing to quantum-mechanical tunnelling effects, nanotubes are very efficient emitters of electrons when placed under electric fields. They thus act as a source of electrons that is similar to the cathode-ray tube used in conventional monitors, but one that is just a few millimetres thick and operates at a fraction of the power. The Samsung Advanced Institute of Technology in South Korea has recently reported the

fabrication of a 30-inch flat television screen, based on field emission from carbon nanotubes, that is close to commercialization⁵. Now, writing in *Nano Letters*, Yung Joon Jung and colleagues⁶ report the assembly of the field-emission part of a screen using carbon-nanotube electrodes embedded in a polymer matrix. Their simple idea could be a big step towards the implementation of large-area displays that are not only flat, but flexible too.

The authors' method consists of patterning islands of catalytic particles on a surface of silicon dioxide. Using the technique of chemical vapour deposition, they synthesize vertically aligned carbon-nanotube pillars 500 micrometres in diameter and 100 micrometres high. This large area of nanotube pillars is impregnated with dimethylsiloxane, a polymer precursor. Heating the resulting matrix to 100 °C polymerizes it, and the nanotube-polymer composite can be peeled off (Fig. 1). These embedded nanotube pillars show excellent emission characteristics, with a field-enhancement factor (defined as the ratio of the electric field at the tip of the pillar to the applied electric field) of 10,000.

The originality of the work lies in the polymer precursor, which perfectly wets the individual nanotubes within the pillars. Without



Figure 1 | Flexible friend. Jung and colleagues⁶ nanotube-polymer composite, with its evenly spaced carbon pillars clearly visible.

this wetting, surface tension would have shrunk the pillars, and the mutual screening of the electric field at tips of neighbouring nanotubes would have damped down the exceptional field-enhancement factor. Crucially, the nanotubes preserve their emission properties even if the matrix is severely bent.

Although this highly flexible nanotube-polymer composite could prove to be a central part of a future foldable flat-screen display, there is still a long way to go. A second, essential component will be a flexible screen that, as in a conventional display, fluoresces when struck by electrons sent out by the field-emitting part. This screen must be kept at a constant separation from the field-emitting composite, even when the display is bent. Constructing such a component will be difficult, but is not an insurmountable task.

The potential applications of flexible displays are many. One could conceive of a flexible electronic newspaper, the pages of which are reloaded using wireless network technology, and which you could bend or roll up after reading. More whimsically, projecting a car's surroundings onto its body with such a display would make it invisible — allowing James Bond to die another day. Perhaps one could even envisage a time when the opera diva changes her dress between scenes by simple reprogramming.

Composites of carbon nanotubes and polymers are not the only candidates to inspire such unbridled imagination. Organic light-emitting diodes are another, but these have their own problems, suffering from short lifetimes owing to air sensitivity, fatigue and the like. Thus, contributions such as that of Jung and colleagues⁶ play an important part in ensuring the emergence of the flexible display from the realm of science fiction to that of science fact. ■

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