The paradox of model organisms

The use of model organisms in research will continue despite their shortcomings

During the 1940s and 1950s, in the early days of molecular biology, biologists tackled the enormous problem of explaining how cells work at the molecular level by applying the tried and tested tools of reductionism. They reduced the complexity of the task in two ways: they focused on a few central molecular mechanisms—replication, transcription, protein synthesis and the control of gene activity—and they chose to use the simplest organisms—bacteria and bacteriophages—in which to study these phenomena. Over time and with more knowledge to hand, biological research expanded to the study of more complex systems, which required the increasing use of higher organisms, including *Caenorhabditis elegans*, *Drosophila*, *Arabidopsis*, zebrafish and rodents.

These model organisms became the irreplaceable tools of fundamental biological and clinical research, and helped scientists to amass an enormous amount of knowledge. However, several high-profile clinical trials in which the use of model organisms failed to predict the serious side effects of some drugs, coupled with the prospect of using human stem-cell lines in trials and the growing sophistication of *in silico* methods, have all cast doubt on the future use of model organisms. This is the case at least for research into human diseases, which, after all, drives much of the research in molecular biology.

…the fate of *E. coli* suggests that model organisms can become even more valuable for studying cellular processes once their biology is well understood

Animal rights activists have seized on this argument, but show little interest in appreciating the huge contribution that model organisms have made to molecular biology. Indeed, it is not an exaggeration to say that research on animals has taught us nearly all we know about cell biology—be it transcriptional control, RNA quality control or the structure of chromatin.

Of course, some organisms have fallen from grace over time. The rat, for example, was often the animal of choice two or three decades ago, but is now less used simply because its genome cannot tolerate the insertion of foreign DNA to anywhere near the extent of the mouse genome.

The question is whether all model organisms will go the way of the rat. There might come a time when the use of whole organisms in fundamental research will decline but, according to Stan Fields, Professor of Genome Sciences and Medicine at Washington State University (Seattle, WA, USA), that is probably a generation away (Fields & Johnston, 2005). Fields believes that all the fundamental pathways, structures and mechanisms of the lower model organisms will be solved in around 20–30 years. This will in no sense represent the end of research using these organisms, but it could bring about a decline in activity, as happened to *Escherichia coli* during the 1980s after some of the basic structural features were resolved.

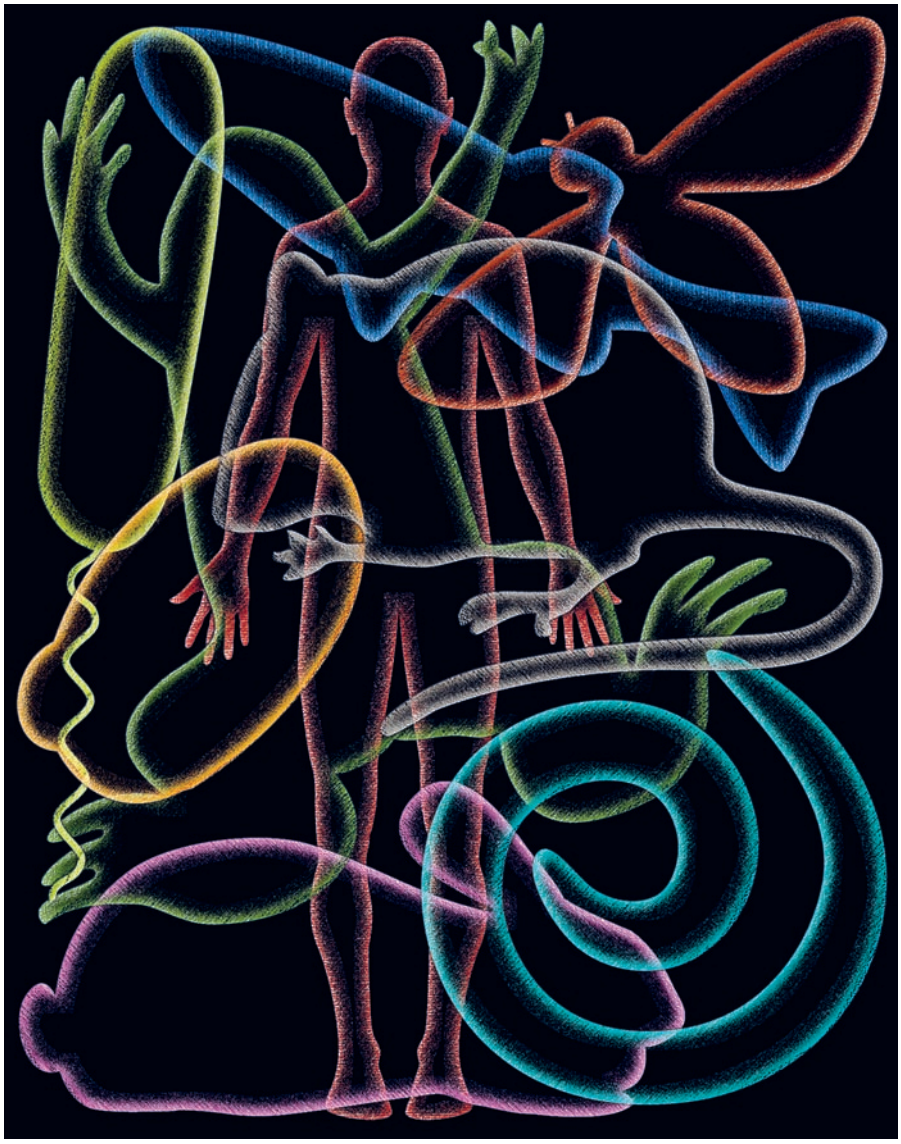
Yet, *E. coli* remains the organism of choice for many scientists, as advances in microscopy and sequencing have allowed an even more detailed probing of its structures and function. It continues to serve as a crucial model to study diseases of the gut and sepsis, and its fate suggests that model organisms can become even more valuable for studying cellular processes once their biology is well understood. Fields suggested that current model organisms, with several others, will therefore continue to populate laboratories, even if the list of their applications changes.

The main contention, therefore, lies over the continuing role of model organisms in studying human diseases and developing cures against them (Festing & Wilkinson, 2007; Rollin, 2007), especially with the growing maturity of *in silico* and stem-cell-based techniques. These doubts about animal models are supported by some clinical trials in which they completely failed to predict serious side effects, as was the case of the immunomodulator TGN1412, used to treat autoimmune rheumatoid arthritis.

TGN1412 was withdrawn from development after a catastrophic trial in the UK left six men fighting for their lives in March 2006. The drug was a monoclonal antibody designed to trigger the production of T cells by binding to the T-cell receptor CD28. The crucial part, however, was the supposed accompanying expression of anti-inflammatory cytokines, which, it was hoped, would alleviate rheumatoid arthritis and perhaps other autoimmune conditions. The drug passed various animal trials that yielded *in vivo* and *in vitro* evidence that although the drug stimulated T-cell production as a whole, it led to the preferential production of regulatory T cells and a downregulation of active T cells.

The main contention, therefore, lies over the continuing role of model organisms in studying human diseases and developing cures against them...

However, once it was tested in human volunteers, the drug elicited an unexpected immune response and systemic organ dysfunction, which had not occurred in previous rounds of animal testing, including those in primates (Rosenthal, 2006). All of the men in the trial suffered a severe allergic reaction, leading to the swelling of their skin and mucous membranes. The exact mechanism involved was unclear at first, but one important clue was the fact that the men's white blood cells, including T cells, had vanished almost completely several hours after taking the drug. Federica Marelli-Berg from Imperial College, London, UK, and colleagues eventually concluded that the drug efficiently redirected T cells from the blood stream into organs where they caused tissue damage (Mirenda *et al*, 2007). This effect



occurred in humans because the drug activated memory T cells that normally respond to antigens to which the immune system had been previously exposed, such as infectious agents or vaccines. A similar response did not occur in the laboratory animals because they had been raised in a relatively sterile environment with insufficient exposure to pathogenic antigens to elicit memory T cells. Once TGN1412 was administered to non-laboratory mammals, they also suffered tissue damage.

In one sense, this study muddied the waters because it suggested that the side effects could have been detected in an animal model and that the fault lay in unnatural laboratory conditions. In practice, however, it is impossible, almost by definition, to conduct clinical studies on populations of non-laboratory animals, which in any case would fan the flames of anti-vivisection arguments. Moreover, the unanticipated immune response in the TGN1412 trial did not depend on the individual phenotype of the volunteers, which further bolsters the argument for using human tissue derived from stem-cell lines—in preference to animal models—to screen for drug reactions from genetic subgroups. "Some drugs are toxic only in some genetic backgrounds," commented Stephen Minger, head of the Stem Cell Laboratory at King's College, London, UK, which is one of two departments in the UK granted a licence to conduct research into hybrid embryos created by injecting a human nucleus into enucleated bovine oocytes. He argues that stem cells could be used to generate multiple cell lines geared to the discovery and screening of candidate drugs with much greater predictive powers than animal models.

In practice [...] it is impossible, almost by definition, to conduct clinical studies on populations of non-laboratory animals, which in any case would fan the flames of anti-vivisection arguments

Conversely, stem cells and human tissue are not sufficient to model more complex organ systems, such as the immune system, and would not have picked up the impact of TGN1412 on the transport of memory T cells. Indeed as Fields pointed out, "even for human disease research, recapitulating disease phenotypes in human stem cell lines does not by necessity mean that we will fully understand the disease process from these experiments. For fundamental processes—like gene regulation—we will have to make sense of them first in the simplest models—yeast, worms, flies, etc—then in the mouse, and only then will we know enough to fully decipher them in the human case."

Animal models have certainly been extremely helpful in identifying specific genes or pathways implicated in particular human diseases. "A large number of human disease-relevant genes and pathways have only been identified in the past two decades through intense research on experimentally tractable model organisms," noted Erich Brunner, a scientist at the Proteomics and Technology Development Center for Model Organism Proteomes at the Institute of Molecular Biology at the University of Zurich in Switzerland. "About 60% of the human disease genes that have been identified thus far have counterparts in the fly and worm and these organisms are used all over the world to study and characterize the molecular pathways these genes are involved in."

Brunner cited a 1999 review article (Edwards, 1999), which describes how

several crucial pathways for human cancer have been discovered in animal models. These include the famous pathway known as Hedgehog involved in growth and development, which is conserved in nearly all animals from fruit flies to humans. Its discovery in *Drosophila* earned Edward B. Lewis, Christiane Nüsslein-Volhard and Eric F. Wieschaus the Nobel Prize for Medicine and Physiology in 1995, even before the role of the Hedgehog pathway in human cancer came to light (Altaba, 1999).

In a few cases, animal models have blown the lid off previously unknown human disease pathways. For example, we owe our current understanding of the mechanism behind haemochromatosis, or iron overload, to the zebrafish (Donovan *et al*, 2000). A team that included Len Zon, who is now at the Children's Hospital of the Howard Hughes Medical Institute (Boston, MA, USA), used positional cloning to identify the gene, the mutation of which leads to excessive iron uptake during digestion in the zebrafish. "This novel gene has a human orthologue—derived from a common ancestor—involved in how the human genome takes up iron," Zon said. "That was the first time a zebra fish mutant predicted human disease."

In a few cases, animal models have blown the lid off previously unknown human disease pathways

Zon adopted the zebrafish as a model organism largely because fertilization occurs exogenously, which makes it much easier to study blood development during early growth in the external embryo. "I wanted a period of time when there weren't blood cells and a period of time when these arose, so that I could study that process," Zon commented. He argued that the zebrafish is as good as any other vertebrate model, including humans, for studying the fundamental mechanisms of blood development: "I now think all vertebrates are equally complex as far as the blood system is concerned."

Zon conceded that this observation does not apply to complex neuronal function; but that even here, animal models have helped to elucidate many of the pathways underpinning the more complex behavioural functions of higher mammals. Jerry Yin, Professor of Genetics and Psychiatry at the University of Wisconsin-Madison (Madison, WI, USA), chose to use *Drosophila* to study the molecular mechanisms of learning and memory formation. The fly has the obvious advantage of short generation times and there are a suite of molecular tools for generating knockouts to create mutants. In Yin's words, the organism is, "complex enough to have interesting behaviours in terms of learning, memory formation, addiction, aggression, and social activities, but is simple enough to allow researchers to 'see' and 'discover' some of the basic logic." He added: "mammals are too complex, most of the time, to 'see' the simpler, underlying logic. The basic 'biological logic' for almost all problems is first worked out in simpler organisms, then the answers are 'searched for' in mammals."

One of Yin's most important discoveries in *Drosophila*, he said, was that the CREB (cAMP-responsive-element-binding protein), which regulates the expression of various genes in many cells, including neurons, had a crucial role in enhancing long-term memory (Yin *et al*, 1995). CREB is highly conserved and appears to be vital for long-term memory in almost all animals, according to Yin, even if the actual encoding is carried to much greater levels of sophistication in the higher mammals, especially primates and humans.

Model plant organisms, notably *Arabidopsis*, corn and maize, have also yielded valuable insights into fundamental processes. There are some such processes, of course, which are unique to plants and of potential value for agriculture—one being the phenomenon of apomixes, whereby a flowering plant produces seed asexually from the mother without requiring fertilization from pollen. If this could be engineered into food crops, it would be possible to replicate desirable varieties and potentially to provide farmers with the means to perpetuate their crops without having to pay for new seed every year. Although this might not please seed producers, there are groups working to identify the genes and their alleles underlying apomixes in the relatively few species that exhibit the phenomenon in the wild. "There are about 400 species that produce seeds genetically identical to the mother plant," said Ueli Grossniklaus, who specializes in plant reproduction at the Institute of Plant Biology at the University of Zurich in Switzerland.

Grossniklaus is also studying the phenomenon of genomic imprinting, which occurs both in seed-producing plants and in placental mammals. In both cases, females contribute virtually all of the nutrients needed for the early development of the offspring. It is in the interests of fathers to pass on alleles that stimulate growth of their progeny, whereas mothers would seem to be better off passing on silent copies of their alleles in order to spread resources more equitably across their offspring. As Grossniklaus pointed out, genomic imprinting must almost certainly have evolved independently in plants and animals, as their last common ancestor was unicellular, thus offering little scope for competition between parents. But both plants and mammals seem to have recruited common tools for imprinting that must either have existed in their common ancestors, or converged subsequently.

...the need for [model organisms] will only diminish once most of the fundamental mechanisms of biology have been solved...

Grossniklaus found that plants rely on a particular protein complex called Polycomb to identify which parent particular alleles come from so that they can be silenced if they come from the mother (Köhler *et al*, 2005). Almost the same complex had already been discovered in mice. With genomic imprinting being implicated in several human disorders, it seems that model organisms will continue to have an important role over the next decade or more.

The paradox of model organisms seems to be that the need for them will only diminish once most of the fundamental mechanisms of biology have been solved to allow the greater use of both human tissue cultures and *in silico* methods for drug discovery. To reach that point, however, requires the extensive use of model organisms. Given the enormous number of unresolved questions that remain in biology, even if the use of model organisms changes over time, they will remain an integral research tool for molecular biologists.

REFERENCES

Altaba AR (1999) Gli proteins and hedgehog signaling in development and cancer. *Trends Genet* **15:** 418–425

Donovan A *et al* (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* **403:** 776–780
Edwards PAW (1999) The impact of developmental biology on cancer research: an overview. *Cancer Metastasis Rev* **2:** 175–180
Festing S, Wilkinson R (2007) The ethics of animal research. Talking point on the use of animals in scientific research. *EMBO Rep* **8:** 526–530
Fields S, Johnston M (2005) Whither model organism research? *Science* **307:** 1885–1886
Köhler C, Page DR, Gagliardini V, Grossniklaus U (2005) The *Arabidopsis thaliana* MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. *Nat Genet* **37:** 28–30
Mirenda V, Jarmin SJ, David R, Dyson J, Scott D, Gu Y, Lechler RI, Okkenhaug K, Marelli-Berg FM (2007) Physiologic and aberrant regulation of memory T-cell trafficking by the costimulatory molecule CD28. *Blood* **109:** 2968–2977
Rollin B (2007) Animal research: a moral science. Talking point on the use of animals in scientific research. *EMBO Rep* **8:** 521–525
Rosenthal E (2006) British Rethinking Test Rules After Drug Trial Nearly Kills 6. *The New York Times*, April 8
Yin JC, Del Vecchio M, Zhou H, Tully T (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell* **81:** 107–115

Philip Hunter

doi:10.1038/embor.2008.142