Dorian Gray mice

Robin A. Weiss

NEWLY generated transgenic mice appear to be able to grow indefinitely without ageing, yet with programmed death. This dramatic development is the work of several groups, and has been accomplished in three stages. First, a gene encoding tithonin was introduced from the carp (*Cyprinus carpa*), a freshwater fish that has the capacity for perpetual growth; the *tith* gene confers longevity on mice despite many symptoms of ageing[1]. Second, by selecting *tith* mutants with more stable function at 37 °C, the mice show no signs of senescence[2,3]. Third, by crossing these animals with others expressing mutant *p53* and an inducible *bcl*-2 gene, the mice can be triggered to die within 48 hours by apoptosis[4].

Carp

These new findings bring to fruition ideas pioneered by Sigmund Obispo in the Stoyte Institute of Life Sciences in California. As reported in these columns[5], Obispo started by inquiring why some long-lived poikilotherm vertebrates such as the carp, and the giant Galápagos tortoise, do not cease growing at sexual maturity, and show little evidence of senescence at the cellular and tissue level. After many a summer investigating various blind alleys, Obispo's laboratory isolated a bacterium, *Campylobacter limnia*, from the jejunum of carp which releases a substance promoting indefinite growth. This growth factor was identified as a small (6K) protein named longevin[6]. Following cloning and expression of the gene for the longevin precursor (preprolongevin), recombinant protein was inoculated into mice. Surprisingly, the treated animals lost their whiskers and developed scales all over the body resembling those on the tails of normal mice[7]. It eventually became apparent that bacterial longevin exerts its effect in fish by forming complexes with a host protein, tithonin, produced by neurosecretory chromaffin cells in the gut[8]. When the tithonin–longevin dimer is administered to adult mice, their life-span is extended unless they develop antibodies to the protein; treatment of young mice delayed the fusion of epiphyses of the long bones.

Tithonin derives its name from Tithonus, the hero upon whom Zeus granted eternal life without eternal youth. In a thought-provoking review[9], Obispo suggests that by extending normal life-span, he has reversed neoteny, the evolutionary mechanism expounded by Huxley to explain the maintenance of embryonic or fetal features in adults.

Although the *tith* gene is widely conserved among bony fish and reptiles, homologous sequences have not been detected in mice or humans, suggesting that mammals represent natural knockout species[10]. To study the phenomenon further, Maunciple *et al.*[1] generated transgenic mice expressing the carp *tith* gene under the control of the pancreatic amylase promoter. Tithonin again inhibited epiphyseal fusion, allowing the mice rapidly to attain a weight of about 300 g before they died of cardiac arrest, at a mean age of 146 days.

Maunciple *et al.*[1] considered that the uncoupling of signal transduction resulting in unlimited growth together with early senescence was due to the lack of longevin in the secreted complexes. But attempts to make longevin transgenics proved to be non-viable, with or without the *tith* gene. It now appears that ageing is due to the extremely short half-life of tithonin in warm-blooded animals.

Two groups have provided evidence that a genetically modified tithonin gene overcomes senescence. Mond *et al.*[2] used site-specific mutagenesis to achieve a more stable protein. Their transgenic mice grow indefinitely without developing scales, though they become photophobic. Kawaguchi *et al.*[3] followed a more elegant route by cloning *tith* from Koi carp that live in the thermal springs on Mount Asama. These fish are so valuable and venerable that it was necessary to employ PCR amplification of *tith* from a single scale to obtain the warm-adapted sequence. The 'K-tith' mice grow to only 1.5 times normal body weight and appear to be healthy in every other respect. It is too early to ascertain their life-span, but none of the heterozygotes has died after more than 600 days. A great deal needs to be done to elucidate the mechanism of *tith*-dependent growth in mammals. Maunciple is looking for a partner protein analogous to longevin. She also has preliminary evidence (personal communication) for activation of c-*myc* in the *tith* mice.

The ramifications of these discoveries are exciting, though potentially disturbing. For example, there is speculation that tithonin might reverse the symptoms of Zachary's progeria (ZP). This is a rare autosomal recessive syndrome with incomplete penetrance, possibly triggered by *Campylobacter* infection[11]; the disease combines achondroplasia and premature senescence. Although there is no homologue to *tith* in humans, tithonin would be expected to promote bone growth and perhaps delay the ageing process. Retroviral vectors carrying *tith* complementary DNA are being tested in mice, but research is hampered by the lack of a close animal model for ZP. Another application under investigation is the development of *tith* transgenic cattle. Viandegene Inc. in Chicago are already making progress in this field[12], and the Kobe Soma Corp. are supporting the Japanese team.

One environmental issue is the problem of releasing *tith*-transgenic animals that might never die except by accident or in the abattoir. This is on its way to being solved by linking *tith* expression with apoptosis genes. Ariellos *et al.*[4] crossed *tith* transgenes with mice constitutively expressing mutant *p53* and conditionally expressing *bcl*-2. Both *bcl*-2 and *tith* are linked to a promoter with a response element for the γ-opiate receptor. So long as a minute amount (0.6 ng l^{-1}) of morphine is added to the drinking water, both proteins are synthesized and the animals remain in fine health. On removing the narcotic, nuclei in striated muscle and liver undergo immediate apoptosis. Cell death becomes irreversible within six hours, the body temperature drops and the mice die in 40–48 hours.

Cattle

It comes as no surprise that the generation of ageless, self-destructing higher organisms is viewed with concern. Whereas some welcome the brave new world that has such creatures in it, others predict dire consequences of pursuing this line of research. Alarm was expressed at the recent Genethics Conference[13], after Bokanovsky suggested that apoptotic death in transgenic cattle would be both humane and would provide tenderized meat. A breeding moratorium was mooted, reminiscent of the Asilomar Conference on DNA cloning 18 years ago. □

Robin A. Weiss is at the Chester Beatty Laboratories of the Institute of Cancer Research, Fulham Road, London SW3 6JB, UK.

1. Maunciple, V. A., Boone, P. S. & Obispo, S. *Gerontol. Res.* **26**, 958–967 (1989).
2. Mond, M., Marx, B. F. & Watson, H. *J. devl Biol.* **34**, 1044–1056 (1992).
3. Kawaguchi, Y. *et al. Jap. J. expl Endocrinol.* **13**, 1418–1427 (1993).
4. Ariellos, C., Prosperou, K. K., Kaliban, D. W., Mirander, B. P. & Kawaguchi, Y. *Proc. natn. Acad. Sci.* (in the press).
5. Correspondent, A. A. *Nature* **296**, 392–393 (1982).
6. Boone, P. S. *et al. Gerontol. Res.* **8**, 267–271 (1982).
7. Gonister, J. E., Boone, P. S., Maunciple, V. A. & Obispo, S. *J. molec. Dermatol.* **4**, 809–822 (1985).
8. Tennison, A. L. *et al. Growth Form* **III**, 780–788 (1987).
9. Obispo, S. *Adv. Gerontol.* **6**, 1–39 (1990).
10. Maunciple, V. A. *et al. Am. J. Nutr.* **79**, 2332–2344 (1988).
11. Pfitzner, H. O. *et al. Arch. hum. Genet.* **82**, 1413 (1991).
12. Bokanovsky, B. S. & Bokanovsky, S. B. Illinois patent file, no. Z010493A (1992).
13. Cassandra, S. in *Genethics and Transgenics* (ed. Orestes, A. G.) 895–987 (Academia, 1993).