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Resolving contradictory reports on cell aging

To the editor:

In a recent commentary on cell aging in this journal (*Nat. Biotechnol.* **16:**396, May 1998), I pointed out that human fibroblasts leave the division cycle irreversibly at an increasing rate with each passage in culture[1] and concluded that the Hayflick limit represents the number of divisions of the longest surviving clone.

I was recently informed of a paper published a decade earlier that demonstrated that monoclonality developed in mass cultures of lymphocytes from all humans tested, with no difference between the replicative lifespan of mass cultures from birth to old age[2]. It also showed, however, a significant inverse relation between donor age and replicative lifespan among randomly isolated individual lymphocyte clones. The replicative lifespan of the lymphocyte clones is much shorter than that of the mass cultures.

The implication is that rare clones that have a prolonged replicative lifespan in mass culture define the Hayflick limit equally for young and old donors, but mask the evidence for differences in accumulated damage between the great majority of lymphocytes from different age groups.

This analysis provides a plausible explanation for the recent failure to find an age-related difference in replicative lifespan between fibroblasts from young and old healthy donors in the largest study ever done,[3] in contrast to the weak relationship reported in the classic study on the subject[4]. The latter was based mainly on cells from refrigerated cadavers or autopsies[3] in which there was likely to be postmortem death of cells that would tend to eliminate the rare, long-lived clones and leave the more representative ones to determine lifespan.

The present analysis would also account for the failure of other investigators to find an age relationship for cell divisions in fibroblasts from healthy donors while finding it in diabetic and prediabetic patients[5]. The clonal results presumably reflect the accumulation of genetic damage in cells with age,[6,7] but give no reason[8,9] to believe there is a fixed limit to cell division in vivo, much less[10] a mechanism such as telomere length to count divisions.

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1. Rabinovitch, P.S. 1983. *Proc. Natl. Acad. Sci. USA* **80:**2951–2955.
2. McCarron, M. et al. 1987. *Mech. Age. & Dev.* **41:**211–218.
3. Cristofalo, V.J. et al. 1998. *Proc. Natl. Acad. Sci. USA* **95:**10614–10619.
4. Martin, G.M., Sprague, C.A. and Epstein, C.J. 1970. *Lab. Invest.* **23:**86–92.
5. Goldstein, S. et al. 1978. *Science* **199:**781–782.
6. Ramsey, M.J. et al. 1996. *Mutation Res.* **338:**95–106.
7. Vijg, J. et al. 1997. *Mech. Ageing and Dev.* **99:**257–271.
8. Cameron, I.L. 1972. *J. Gerontol.* **27:**157–161.
9. Potten, C.S. and Loeffler, M. 1990. *Development* **110:**1001–1020.
10. Frenck, R.W. et al. 1998. *Proc. Natl. Acad. Sci. USA* **95:**5607–5610.

Mycoplasmas as gene therapy vectors?

To the editor:

Gene therapy is based on the delivery of therapeutic genes into target cells. Strategies for gene transfer has been limited so far to two approaches using either viral vector or non viral vector methods, but recent data published in *Nature Biotechnology* suggest a new approach based on bacteria-mediated gene delivery[1]. Traditional strategies are based on the assumption that the introduction of a therapeutic gene *into* target cells is a prerequisite step to any successful gene therapy. The concept of gene therapy can be reformulated however, if we consider the delivery of the therapeutic gene at the cell surface as an alternative to its penetration into cells. Such targeting at the cell surface could be achieved by the use of mycoplasma[2].

Mycoplasmas are the smallest self-replicating living organisms, with a genome in some cases less than 600 kilobases. They are extracellular parasites intimately associated with the surface of the cells they parasitize[3]. Thus, the delivery and the expression of a therapeutic gene at the cell surface, rather than inside, via mycoplasmas could present an alternative to the current concepts and vectors used in gene therapy to produce secreted drugs or proteins.

An interesting feature of mycoplasmas is that they can behave as commensal organisms. Thus, they usually cause only mild symptoms with a tendency to latent infection[4]. This protection against host defense is probably related to the close contact of mycoplasmas to host cell membrane, their ability to adsorb host antigens at their surface, and their antiphagocytic surface properties[4].

In addition, many of the endotoxic substances and antigens found on the cell wall of Gram negative bacteria are absent in mycoplasmas, which are naturally wall-less organisms. Another potential advantage of this approach would be the reduced risk of recombination between DNA constructs and the genome of host cells, since therapeutic DNA remains at the cell surface. Therefore, mycoplasma-mediated gene therapy could well represent an attractive alternative for the production of either cell-permeant drugs or secreted proteins such as a growth factors or hormones.

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1. Grillot-Courvalin, C. et al. 1998. *Nat. Biotechnol.* **16:**862–866.
2. Berger, F. et al. 1999. *Med. Hypotheses*. In press.
3. Howard, C.J. and Gourlay, R.N. 1978. *Sci. Prog. Oxf.* **65:**313–329.
4. Bredt, W. 1976. *Infection* **4:**9–12.

Errata

The December editorial, "Taking stock of spin science," incorrectly referred to a "fetal tissue" research ban, which was in fact lifted by the Clinton administration in 1992. This should have been "embryonic tissue" research ban, which remains in effect. The editors regret the error.