Cell proliferation

Paths to immortality and back

from T.B.L. Kirkwood

RECENT exciting developments in the characterization of cellular and viral oncogenes show that the immortalization of mammalian cells is an important feature of their progression from normality to malignancy[1,2]. This finding makes natural sense; populations of normal cells exhibit only finite division potential when grown in culture, and it is commonly held that there is an intrinsic limit to cell proliferation that must be overcome *in vivo* before a cancer can be formed. (But immortality is not absolutely required; if the potential for cell division is great enough a lethal tumour can be found without it.) At the same time, it is widely believed that the limited growth of cells such as fibroblasts is a contributing factor in the process of ageing[3].

If both these ideas are true, then cancer and ageing are intimately connected at the cellular level, and if we could but understand the mechanism by which one process occurs, we should be better placed to understand the other. Behind the simple dichotomy between finite and infinite cell population growth, however, lie complex issues.

First, there are two quite different types of theories to explain why cells stop proliferating. One postulates a rigourously programmed series of events specifically aimed at terminating cell division; the other, that cells cease growing because they accumulate random defects, particularly among macromolecules. The two mechanisms call for quite different explanations of how cells can escape the ageing process and become immortalized[4], although testing for this difference is by no means easy.

Second, while the growth behaviour of a population of cells is necessarily determined by the replicative properties of the individual cells within it, the relationship is not always obvious. If immortal cells give rise to mortal cells which undergo only a finite number of divisions before they die, a culture consisting initially of immortal cells may nevertheless have only a finite lifespan[5]. This surprising outcome arises because the immortal cells are 'diluted' by their mortal progeny and, in appropriate circumstances, may become so scarce that they are lost altogether.

Two recent studies, both by groups with long-standing interests in the problems of cellular ageing, suggest that the transformation of normal cells to immortality may not be as irreversible as is generally thought, and throw light on some of the difficulties in interpreting the phenomenon of immortalization.

Pereira-Smith and Smith[6] used fusions between different combinations of mortal and immortal somatic cells to see whether cellular immortality is dominant or recessive. When normal cells were hybridized with three simian virus 40 (SV40)-transformed cell lines and with a variety of cell lines (including HeLa) derived from malignant tumours, in all cases the phenotype of immortality was recessive, although variant immortal cells did arise at low frequency (around 1 in 10^5) within some of the non-proliferating hybrid populations. These results confirm and significantly extend earlier reports of the dominance of mortality over immortality in human cell hybrids[7-9].

More strikingly, Pereira-Smith and Smith have also found that in fusions between some pairs of immortal cell lines, growth is finite but that in others, all hybrids cells can divide indefinitely. This surprising result is interpreted as support for the hypothesis of programmed cessation of cell division, the argument being that immortality can arise from various distinct dysfunctions in the programme and that, among hybrids between cell lines immortalized in different ways, complementation can restore the operation of the programme for mortality. I will, however, explain that this finding does not militate against the alternative hypothesis of random damage as strongly as Pereira-Smith and Smith have claimed; in any case, for a trait as complex as immortality, the classical concepts of dominance and recessiveness may be too narrow.

The second study, by Huschtscha and Holliday[10], details the events surrounding transformation by SV40 of the human diploid cell strain MRC-5, comparing two permanent cell lines obtained therefrom. The lines differ in several respects, including morphology, modal chromosome number, glucose requirement and, in particular, stability of the immortal phenotype.

One of the lines (called MRC-5V1) is unusually unstable in that the growth of some sub-lines slows over a period of many population doublings, the cultures dying out in a manner closely resembling the behaviour of normal cells. By recovering cells frozen in liquid nitrogen, the line was kept growing through 750 population doublings, raising the question whether the immortal phenotype is carried only by a small sub-population of cells which may on occasion have been lost. Regrettably, Huschtscha and Holliday were unable to test this hypothesis directly by growing individual clones of MRC-5V1 cells, but independent studies with HeLa and other cell lines show that immortal cell populations, even after many generations of selection *in vitro*, may contain a sizeable fraction (up to 40 per cent) of cells whose potential for division is limited[6,11]. If a similar, or even more extreme, situation should pertain *in vivo*, which seems quite plausible because selection for immortality *per se* will not begin until normal clonal lifespan is exhausted, it could explain why it is not always easy to establish permanent cell lines from tumour biopsies and why tumorigenesis itself sometimes seems a hit-or-miss phenomenon.

So what of the mechanism limiting normal cell division? If it contributes to the ageing of the organism as a whole, it must be understood in an evolutionary context as much as in any other[4]. The popular view of ageing as an adaptive mechanism to prevent overcrowding and promote species' adaptability, which lends direct support to the hypothesis of programmed cell death, is regarded by most evolutionary biologists as untenable[12]. On the other hand, there is quite good support for the view that ageing is the consequence of the energy-saving strategy of not repairing cellular damage too well[4,13]. This 'disposable soma' hypothesis suggests that somatic cells are switched to a repair level which is optimally efficient from the organism's point of view even if less than optimal for the cell. If this is the case, transformation of a cell to immortality can be understood as a product of clonal selection acting on rare mutations or epigenetic changes which enhance the capacity of a cell to cope with damage, either by repairing it more effectively or by diluting it with more rapid cell division. It is possible that such a change might be 'recessive' and, therefore, consistent with the data of Pereira-Smith and Smith[6].

The advances made in recent months in understanding cell immortalization suggest that a new path to unravelling the mechanisms of carcinogenesis and cell ageing may have been opened up. In working our way down this path, it will be helpful to keep the competing hypotheses of both these fields firmly in mind. Looking back nearly a hundred years, it is amusing to note the sharp prejudice of Vines[14], who wrote of Weismann's theory of cell ageing that it is "absurd to say that an immortal substance can be converted into a mortal substance". Poor Professor Vines has been proved wrong. The conversion appears to work both ways, and with any luck we may soon know why. □

T.B.L. Kirkwood is at the National Institute for Medical Research, Mill Hill, London NW7 1AA.

1. Cairns, J. & Logan, J. *Nature* **304**, 582 (1983).
2. Land, H., Parada, L.F. & Weinberg, R.A. *Science* **222**, 771 (1983).
3. Hayflick, L. *Scient. Am.* **242**, 42 (1980).
4. Kirkwood, T.B.L. & Cremer, T. *Hum. Genet.* **60**, 101 (1982).
5. Kirkwood, T.B.L. & Holliday, R. *J. theor. Biol.* **53**, 481 (1975).
6. Pereira-Smith, O.M. & Smith, J. R. *Science* **221**, 964 (1983).
7. Bunn, C.L. & Tarrant, G.M. *Expl Cell Res.* **127**, 385 (1980).
8. Muggleton-Harris, A.L. & De Simone, D.W. *Somat. Cell Genet.* **6**, 689 (1980).
9. Pereira-Smith, O.M. & Smith, J.R. *Somat. Cell Genet.* **7**, 411 (1981).
10. Huschtscha, L.T. & Holliday, R. *J. Cell Sci.* **63**, 77 (1983).
11. Martinez, A.O. *et al. In Vitro* **14**, 996 (1978).
12. Medawar, P.B. in *The Uniqueness of the Individual* 2nd edn, 28 (Dover, New York, 1981).
13. Kirkwood, T.B.L. *Nature* **270**, 301 (1977).
14. Vines, S.H. *Nature* **40**, 621 (1889).