Ian Wilmut, the cloner of Dolly the sheep, questions whether "the interpretation of the data offered" by the subsequent cloners of calves is "justified on the basis of the evidence presented." And several letter writers discuss whether "human longevity" can be "predicted by the simple extrapolation of mortality trends."

Quiescence in Nuclear Transfer

When sheep donor cells derived from embryo, fetal, and adult tissues were induced to exit the growth cycle and become quiescent, live lambs were produced after nuclear transfer from such treated cultured cells (*1*). The cells were cultured in reduced serum concentration for several days until there was no evidence of DNA replication as detected by immunofluorescence staining for the S-phase–specific form of proliferating cell nuclear antigen (PCNA) (*2*). However, the majority of cells resumed DNA synthesis when the serum concentration was returned to normal levels for culture. This, together with fluroescence-activated cell sorting (FACS) data, led to the conclusion that the donor cells had diploid DNA content, but were not actively growing.

Cloned calves ACT3, ACT4, and ACT5 at 3 weeks of age.

In a recent report that described the birth of calves after nuclear transfer from fetal fibroblasts (22 May, p. 1256), the authors stated that the donor cells used were not quiescent. The cell-cycle stage of the cells was assessed by FACS analysis and by immunofluorescence for PCNA. FACS analysis measures DNA content, but does not distinguish between actively growing diploid cells (G_1) or nonactively growing diploid cells that have arrested the cell cycle after mitosis and cell division but before DNA replication (G_0). This measurement established that 56% of the cells were diploid, but did not determine how many were in G_0 or G_1.

Because the protocol for PCNA staining used by the authors fixes the soluble complex that is present at all stages of the cell cycle (*3*), the observation provides no information about cell-cycle stage; but because only 82% of the cells were positive for PCNA, it may indicate that some of the cells were dead (*4*).

In short, no evidence is provided either in this or in a previously reported study (*5*) that the small proportion of donor cells whose use resulted in development to viable calves or fetuses was not quiescent. In fact, there is every reason to expect that quiescent cells were present in the cultures, as they had reached 70 to 80% confluence before use as donors, in which condition contact inhibition causes cells to become quiescent (*6*). Additionally, the passage number of the cultures at the time of nuclear transfer is not specified (with time during the culture of primary cell populations an increasing number of cells cease division). Such nongrowing cells, described as senescing, have been reported to enter a presenescent G_0 phase (*7*). In these circumstances, the interpretation of the data offered by the authors is not justified on the basis of the evidence presented.

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References

1. K. H. S. Campbell, J. McWhir, W. A. Ritchie, I. Wilmut, *Nature* **380**, 64 (1996); I. Wilmut, A. Schnieke, J. McWhir, A. Kind, K. H. S. Campbell, *ibid.* **385**, 810 (1997).
2. I. R. Kill, J. M. Bridger, K. H. S Campbell, G. Maldano-Codina, C. J. Hutchison, *J. Cell Sci.* **100**, 869 (1991).
3. R. Bravo and H. Macdonald-Bravo, *J. Cell Biol.* **105**, 1549 (1987).
4. K. Campbell, *Nature Med.* **4**, 557 (1998).
5. W. M. Zawada *et al., ibid.*, p. 569.
6 S. Gustincich and C. Schneider, *Cell Growth Differ.* **9**, 753 (1993).
7 I. R. Kill and S. Shall, *J. Cell Sci.* **97**, 473 (1990); I. R. Kill *et al., ibid.* **107**, 571 (1994).

Response

We consider it highly unlikely that the cloned calves in our study were actually derived from a small population of G_0 cells. First, the cells used to produce the calves had been cultured for approximately 8 to 10 population doublings, far from senescence, which occurs at about 35 population doublings. Second, our cell-cycle analysis indicates that a confluency level of 70 to 80%, as defined in our laboratory, is in the middle of the log phase of growth. Third, contact inhibition of cells does not occur at 70 to 80% confluency and, in fact, does not occur at 100% confluency. Contact inhibition only occurs after physical crowding of cells in the dish. Finally, even if a small population of cells was induced to arrest cell division by the culture conditions, they would be overgrown, very rapidly, by the actively dividing population of cells. Although we believe this indirect evidence is sufficient to warrant the conclusions in our paper, we are now also in the process of testing the question directly.

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The Future of Long Life

In his Research commentary "The future of human longevity: A demographer's perspective" (*Science*'s Compass, 17 Apr., p. 395), John R. Wilmoth states that human longevity could be predicted by the simple extrapolation of mortality trends: "[t]he appeal of extrapolation lies in long-term stability of the historical mortality decline." This decline is supported by his fascinating graph depicting remarkably regular decreases in U.S. mortality rates over the last 97 years.

Unfortunately, the data on age-specific mortality rates published by the Social Security Administration (*1*) are complex and are not consistent with the declared long-term stability of the historical mortality decline. For example, the U.S. death rates for ages 30 to 34 and 35 to 39 have been *increasing* since 1985 both for males and females (*1*). The impression of long-term stability of mortality decline comes from the use of the aggregated death rate calculated by Wilmoth for the mixture of people of different ages (standardized to the U.S. population in mid-1990). The virtual stability of the historical decline of this highly aggregated mortality index is not very informative and might even be misleading (as is the fallacy of one stable mean body temperature for all patients in hospitals).

The future of human longevity is a complex and as yet unresolved multidisciplinary scientific problem. More research is needed on the driving forces of the age-specific mortality rates and their possible biological limits (*2*), not just extrapolation.

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