



Fig. 3 *a*, Characterization of NEO^R colonies and cell morphology. Upper photograph, NEO^R granulocyte-macrophage colony at a magnification of 250 \times ; the lower photograph, Geimsa staining of NEO^R cells; granulocytes (neutrophil cells are small with characteristic doughnut shaped nuclei) and macrophages (large irregular shaped cells) at a magnification of 400 \times . *b*, Analysis of *neo* RNA in NEO^R bone marrow colony cells.

Methods: *a*, In two experiments all colonies from several plates (30 colonies in total) were picked, and analysed for cell morphology. Colonies were spread onto slides and the cells were stained with Geimsa. Erythroid content was determined by benzidine staining. *b*, NEO^R colony cells from six methylcellulose plates or the same number of uninfected NEO^S colonies grown in the absence of G418 were washed from the methylcellulose and put into short-term liquid culture containing spleen conditioned medium in microtitre wells. G418 was added to the NEO^R cell cultures at 650 $\mu\text{g ml}^{-1}$. After 12 days of further growth the cells had reached a total of approximately 2.5×10^5 cells. The cells were collected and used in a whole cell blot technique according to Manzari *et al.*¹². In brief, cell suspensions at a concentration of 5×10^5 cells ml^{-1} were serially diluted (1:2) in phosphate-buffered saline, and 200 μl samples of each dilution were applied to nitrocellulose soaked in $10 \times \text{SSC}$ using a 96-well filtration manifold (Schleicher and Schuell). Wells were washed three times in $2 \times \text{SSC}$, 0.1% SDS followed by three washes in $2 \times \text{SSC}$. After air drying, the filters were baked at 80 $^\circ\text{C}$ in a vacuum oven. The filters were prehybridized overnight at 42 $^\circ\text{C}$ in $5 \times \text{SSC}$, 50 mM sodium phosphate (pH 6.5), $5 \times \text{Denhardt's}$ solution, 250 $\mu\text{g ml}^{-1}$ sonicated salmon testes DNA (preheated for 5 min at 100 $^\circ\text{C}$) and 50% deionized formamide, and hybridized for 20 h at 42 $^\circ\text{C}$ in the same solution containing 10% dextran sulphate and ^{32}P -labelled *HindIII/BamHI neo* fragment derived from pBRneo¹⁰ (2×10^6 c.p.m. ml^{-1}) essentially as described by Wahl *et al.*¹⁴. After hybridization, filters were washed three times for 5 min each at room temperature and twice at 50 $^\circ\text{C}$ for 1 h each with $2 \times \text{SSC}$ and 0.1% SDS, followed by two washes for 1 h each at 50 $^\circ\text{C}$ in $0.1 \times \text{SSC}$ and 0.1% SDS. Lane 1, NEO virus infected Rat-2 cells (Go-1b); lane 2, Rat-2 cells; lane 3, NEO^R bone marrow colony cells; lane 4, uninfected bone marrow colony cells. The top dots contain 10^5 cells and each successively lower dot contains a twofold serial cell dilution down to 3×10^3 cells per dot.

provide a simple, reproducible method for the stable insertion of DNA sequences into haematopoietic progenitors.

Retrovirus vectors, while providing the unique advantages described earlier over DNA-mediated gene transfer techniques, may have a number of drawbacks including: (1) virus spread; (2) constraints on the size of the transferred genome; (3) gene sequences which inhibit viral replication and; (4) transformation of certain cell types. However, there are possible solutions for all of these problems. First, Mann *et al.*¹³ have developed techniques which eliminate virus spread by producing defective viruses, such as MLV-NEO.I, in the absence of helper virus.

Second, only 2 kb of the viral genome is required for virus replication in the presence of helper virus proteins leaving up to 8 kb for insertion of DNA into retrovirus vectors. Since retrovirus vectors can be used to both express and transduce genes lacking their homologous promoters⁸, small cDNA clones should provide an alternative to large genomic clones. Third, although polyadenylation signals may interfere with retrovirus infectivity, these inhibitory sequences can be deleted⁹ or genes can be inserted into viral vectors in the opposite orientation of transcription to the viral LTR⁸. Finally, although the genomes of some retroviruses include viral oncogenes, Moloney MLV and MLV-NEO.I do not contain an oncogene and we have no evidence of cell transformation in the NEO^R marrow colonies.

The ability to transduce normal bone marrow cells provides the opportunity to address a number of questions central to fundamental and clinical aspects of haematopoiesis. Experiments are currently in progress to determine whether other committed progenitor cells and pluripotent haematopoietic stem cells can also be transduced to NEO^R ; whether non-selectable genes can be transferred into mouse bone marrow cells via retrovirus vectors; and finally, whether the expression of these genes is regulated during the differentiation along different haematopoietic cell lineages.

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Errata

IN the letter 'Basaltic systems and a corresponding states equation for crystal growth rates by R. Dearnley, *Nature* **304**, 151-152 (1983), in line 20 from the bottom of page 152 the value for r.m.s.d. should read 0.331.

IN the letter 'Discovery of an IR echo from a supernova dust cloud' by J. R. Graham *et al.*, *Nature* **304**, 709-710 (1983), equation (3) should read:

$$v = (2.2 \pm 0.2) \times 10^5 \left(\frac{a}{0.1 \mu\text{m}} \right)^{-1/2} \left(\frac{L_{\text{SN}}}{10^{43} \text{ erg s}^{-1}} \right)^{1/2} \text{ km s}^{-1} \quad (3)$$

Corrigendum

IN the review article 'Lunar magnetism, polar displacements and primeval satellites in the Earth-Moon system' by S. K. Runcorn, *Nature* **304**, 589-596 (1983), the legend to Fig. 4 should now read: Palaeoequators and multiring basins of Imbrian and Upper Nectarian age in northern hemisphere of near side and southern hemisphere of far side excepting Hertzprung (on the present equator), and of Lower Nectarian age in southern hemisphere of near side and northern hemisphere of far side excepting Korolev (near the present equator). The directions (or possible alternative directions) from which impacting bodies came are shown by arrows.