axis grating coheres with a grating of similar contrast on the same axis and slips when paired with a grating of any relative contrast on a different cardinal axis, there is no one channel, such as the luminance pathway, that supplies the sole input to perception of motion.

Recent suggestions about the analysis of motion give very different roles to the neural system involved in processing luminance variation ('luminance mechanisms') and to that of isoluminant chromatic variation ('chromatic mechanisms'). The former system is assumed to include the magnocellular layers of the LGN and to play a major part in the perception of motion. The latter system includes the parvocellular layers of the LGN and is supposed to be insensitive to motion[7]. Our experiments show that both isoluminant stimuli and luminance stimuli contribute to motion; moreover, they do so through separate motion-analysing mechanisms tuned to individual cardinal axes.

The great difference in the perception of moving plaids modulated along cardinal axes and those modulated along intermediate directions implies that colour space is anisotropic with respect to the perception of motion. The analysis of motion seems to take place within independent mechanisms tuned to the cardinal directions of colour space. Gratings modulated along directions halfway between cardinal axes produce coherent motion because they share vector components along the cardinal axes. The same sort of vector analysis could be applied to other basis sets of vectors besides those on the cardinal axes. For example, the common component vectors might lie along directions halfway between the cardinal axes. There is psychophysical[8] and electrophysiological[9] evidence of mechanisms maximally responsive to stimuli modulated along non-cardinal directions. However, our results show that for the analysis of motion the visual system does not work this way.

Among the conclusions to be drawn from this work is that the apparently seamless variation of colour and brightness that people experience hides the fact that there are three discrete systems that operate quite independently in certain respects. The present results and those on habituation[1] imply mechanisms more narrowly tuned than as yet revealed by physiological investigations[4-6,9]. Furthermore, they are inconsistent with at least one of the two commonly accepted notions that there is only one cortical motion centre, the membrana tympani, and that it is colour-blind. □

Received 15 May; accepted 25 September 1990.

1. Krauskopf, J., Williams, D. R. & Heeley, D. W. *Vision Res.* **22,** 1121-1131 (1982).
2. Adelson, E. H. & Movshon, J. A. *Nature* **300,** 523-525 (1982).
3. MacLeod, D. I. A. & Boynton, R. M. *J. opt. Soc. Am.* **69,** 1183-1186 (1978).
4. Wiesel, T. N. & Hubel, D. H. *J. Neurophysiol.* **29,** 1115-1156 (1966).
5. DeValois, R. L., Abramov, I. & Jacobs, G. H. *J. opt. Soc. Am.* **56,** 966-977 (1966).
6. Derrington, A. M., Krauskopf, J. & Lennie, P. *J. Physiol. Lond.* **357,** 241-265 (1984).
7. Krauskopf, J., Williams, D. R., Mandler, M. B. & Brown, A. M. *Vision Res.* **26,** 23-32 (1986).
8. Livingstone, M. S. & Hubel, D. H. *J. Neurosci.* **7,** 3416-3468 (1987).
9. Lennie, P., Krauskopf, J. & Sclar, G. *J. Neurosci.* **10,** 649-669 (1990).

ACKNOWLEDGEMENTS. This research was supported by the National Eye Institute and the United States Air Force Office of Scientific Research. The advice and comments of J. A. Movshon and Q. Zaidi are gratefully acknowledged.

Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl-2*

Andreas Strasser, Alan W. Harris, Mary L. Bath & Suzanne Cory

The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

THE putative oncogene *bcl-2* is juxtaposed to the immunoglobulin heavy chain (*Igh*) locus[1-3] by the t(14;18) chromosomal translocation typical of human follicular B-cell lymphomas[4]. The *bcl-2* gene product (refs 5, 6) is not altered by the translocation, but its expression is deregulated[6-8], presumably by the *Igh* enhancer Eμ. Constitutive *bcl-2* expression seems to augment cell survival, as infection with a *bcl-2* retrovirus enables certain growth factor-dependent mouse cell lines to maintain viability when deprived of factor[9,10]. Furthermore, high levels of the *bcl-2* product can protect human B and T lymphoblasts under stress[11,12] and thereby confer a growth advantage[12-14]. Mice expressing a *bcl-2* transgene controlled by the *Igh* enhancer accumulate small non-cycling B cells which survive unusually well *in vitro*[15-17] but do not show a propensity for spontaneous tumorigenesis[15,16]. In contrast, an analogous *myc* transgene, designed to mimic the *myc–Igh* translocation product typical of Burkitt's lymphoma and rodent plasmacytoma[18], promotes B lymphoid cell proliferation and predisposes mice to malignancy in pre-B and B lymphoid cells[19-22]. Previous experiments have suggested that *bcl-2* can cooperate with deregulated *myc* to improve *in vitro* growth of pre-B and B cells[9,11]. Here we describe a marked synergy between *bcl-2* and *myc* in doubly transgenic mice. Eμ–*bcl-2*/*myc* mice show hyperproliferation of pre-B and B cells and develop tumours much faster than Eμ–*myc* mice. Surprisingly, the tumours derive from a cell with the hallmarks of a primitive haemopoietic cell, perhaps a lymphoid-committed stem cell.

We have developed several strains of transgenic mice harbouring a *bcl-2* complementary DNA under the control of the SV40 promoter and the 5′ *Igh* enhancer[16]. Mice of the most well-characterized strain, EμSV-*bcl*-2-22, develop large accumulations of B lymphocytes but no tumours, at least within the first 12 months of life (ref. 15, and our unpublished observations). When these mice were crossed with a well characterized Eμ-*myc* strain[21], striking effects of coexpression of the two transgenes were revealed by blood cell analysis of healthy 3-week-old mice (Fig. 1). All the transgenic mice had elevated white cell counts but the levels in the doubly transgenic mice were 50- to 100-fold higher than normal. As in Eμ-*myc* mice[20], the leukocytes in Eμ-*bcl-2*/*myc* mice were all large and therefore presumably proliferating, unlike those in Eμ-*bcl*-2 mice, which were small and non-cycling[15,16]. Immunofluorescence analysis established

TABLE 1 Cells in lymphoid organs of terminal Eμ-*bcl-2*/*myc* mice

Surface phenotype	Thymus	Lymph node	Bone marrow	Spleen
Tumour cells CD45 (B220)$^+$, Ig$^-$, PB76$^-$, Thy-1^{low}, CD4$^+$, Sca-1$^+$	~80%	≥90%	22±11%	16±5%
Pre-B cells CD45 (B220)$^+$, Ig$^-$, PB76$^+$, Thy-1$^-$, CD4$^-$, Sca-1^1	~10%	≤5%	62±11%	28±4%
B cells CD45 (B220)$^+$, Ig$^+$, PB76$^-$ Thy-1$^-$, CD4$^-$, Sca-1$^-$	~10%	≤5%	16±5%	56±5%

B220$^+$ cells comprised >90% of all bone marrow, spleen and lymph node cells and ~80% of thymus cells. The numbers shown are the mean proportions (±s.d.) of CD45R (B220)$^+$ cells from 4 terminally ill mice that stained with fluorochrome-labelled monoclonal antibodies (see legend to Fig. 3) specific for each of the other indicated cell surface molecules. Quantitation was by dual-fluorescence flow cytometry on a FACScan flow cytometer (Becton-Dickinson).

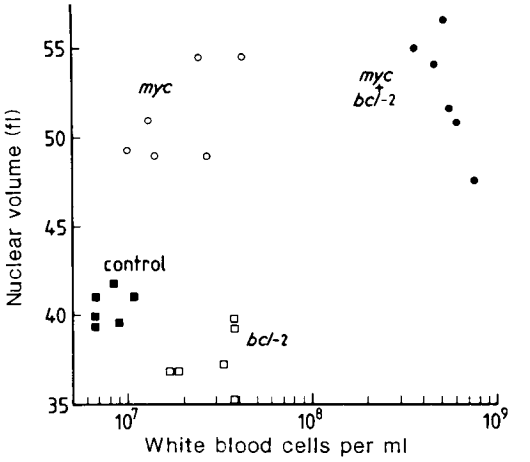

FIG. 1 Distinctive blood profiles induced by *bcl*-2 and *myc* transgenes. Leukocytes from 3-week-old mice were counted and sized in a ZM Coulter counter and channelizer as nuclei which were obtained by lysing diluted blood with Zaponin (Coulter) as described[21].

that the excess white cells in the blood of doubly transgenic mice comprised both pre-B and B cells (30% and 70% respectively).

The doubly transgenic animals became terminally ill at 5–6 weeks of age, much faster and more synchronously than littermates bearing only the Eμ-*myc* transgene (Fig. 2). Autopsy revealed enlargement of the spleen, lymph nodes and thymus. Histological sections disclosed masses of lymphoblasts occupying these organs and extensively invading the bone marrow, liver, lungs and kidneys, a picture characteristic of disseminated malignant lymphoma.

Transplantation tests established that the tissues had been invaded by tumour cells. For five of six Eμ-*bcl*-2/*myc* mice tested, every normal histocompatible recipient transplanted with 10^6–10^7 cells from thymus, lymph node, bone marrow or spleen developed a widely disseminated tumour and leukaemia within 26–67 days. The tumours all proved to be of novel cell type (Fig. 3*a*). They expressed the general B lineage surface marker CD45R(B220), but not the pre-B/plasma cell marker PB76 (ref. 23) or the B cell hallmark, membrane-bound immunoglobulin. Intriguingly, they expressed three markers found on haemopoietic spleen colony-forming cells[24,25]: Sca-1, Thy-1 (low levels) and CD4. Although Thy-1 and CD4 are also found on T cells, other T-cell surface antigens such as CD3 and the α/β antigen receptor were not present, and CD8 expression was marginal. There was no sign of the myeloid markers Mac-1, -2 and -3 (data not shown), nor of BP-1/6C3, an antigen found on many early B lymphoid tumour cells, although not on those induced by *myc*[26].

The surface phenotype of the tumour cells differs from those of the earliest murine lymphoid cell lines isolated previously[27,28] and is suggestive of a haematolymphoid stem or progenitor cell. Molecular analysis supports this diagnosis. The transplanted tumours displayed no evidence of rearrangement of the *Igh* or the γ or β T-cell antigen receptor loci (Fig. 3*b*). Nor were transcripts detectable by northern blot analysis for terminal deoxynucleotidyl transferase, the pre-B cell-specific $\lambda 5$ gene[29], or the recently described V(D)J recombination activating gene *RAG*-1 (ref. 30), all of which were readily apparent in control pre-B cell lines (not shown). As expected, the tumour cells expressed both transgenes, the level of Eμ-*bcl*-2 transcripts being comparable to that in the non-tumorigenic spleen cells from Eμ-*bcl*-2 mice and ~10-fold higher than that of the endogenous *bcl*-2 gene in a pre-B line (70Z). One representative tumour has retained its highly characteristic phenotype and genotype through eleven rounds of serial transplantation.

In addition to their tumour, the Eμ-*bcl*-2/*myc* mice had a marked excess of pre-B and B cells (Table 1). Large pre-B cells were particularly prominent in the bone marrow but, as in Eμ-*myc* mice[20], they were also present in the spleen, which is normally devoid of pre-B cells. Most B cells in the spleen and bone marrow were also large, unlike those in Eμ-*bcl*-2 (and normal) mice[15,16]. Thus *myc* effects were dominant. The excess enlarged B-lineage cells were apparent even in 2-day-old doubly transgenic animals.

Despite their conspicuous overproduction, the pre-B and B cells in the Eμ-*bcl*-2/*myc* mice were not malignant. Splenic DNA analysed by Southern blotting revealed no signs of clonal dominance and no pre-B or B lymphomas arose on transplantation of spleen or bone marrow where these cells predominated (Table 1). Furthermore, these cell populations failed to yield rapid outgrowth of cells in culture, in marked contrast to the spontaneous pre-B and B lymphomas of Eμ-*myc* mice, which can readily be grown as cell lines[19]. Cultures of spleen and bone marrow from Eμ-*bcl*-2/*myc* mice initially showed no cell growth and slowly lost viability, but after several weeks clonal pre-B or B cell lines emerged, presumably as a result of acquired mutation(s). We infer from these observations that constitutive *bcl*-2 and *myc* expression does not suffice fully to transform pre-B or B cells, a conclusion consistent with our earlier studies on Eμ-*myc* bone marrow cells infected with a *bcl*-2 retrovirus[9].

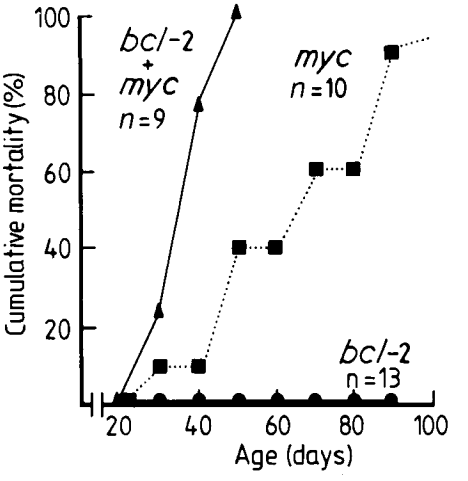

FIG. 2 Cumulative mortality in transgenic mice carrying both a *bcl*-2 and a *myc* transgene compared with mice carrying only one transgene. Data are expressed in terms of the age at which mice became terminally ill and were killed; *n* indicates the number of animals of each transgenic type.
METHODS. Six litters were derived from matings between three EμSV-*bcl*-2-22 (*C57BL/6JWehi* × *SJL/JWehi*)*F2* females and two males of the C57BL/6JWehi-backcrossed (N11) subline of the Eμ-*myc* 292-1 transgenic line[21]. The Eμ-*bcl*-2 line[16] expresses a human *bcl*-2 cDNA (residues 1–939 of the clone isolated by Cleary *et al.*[3]) under the control of the *Igh* enhancer and an SV40 promoter. Doubly transgenic progeny were identified by hybridization of tail DNA to an SV40 probe and also to a pUC12 plasmid probe which detects sequences present in the Eμ-*myc* transgene[19]. Mice were judged as terminally ill when they displayed a persistently hunched posture, slow movements and laboured respiration.

Is the *bcl-2/myc* combination solely responsible for transformation of the more primitive cell type? Tumour cells were not detectable in very young Eμ-*bcl-2/myc* mice, because no tumours arose on transplantation of spleen and thymus cells from two 2-day-old animals. But a 10-day-old Eμ-*bcl-2/myc* mouse yielded the characteristic primitive tumours, even though cells of that phenotype were undetectable by immunofluorescence and flow cytometry (<5% of B220$^+$ cells) in the donor animal. As tumour cells did not become apparent for at least two weeks after the initiation of embryonic haemopoiesis, it could be argued that transformation required a somatic mutation, such as activation of another oncogene. On the other hand, the slow emergence of a malignant clone might reflect the low frequency of the target cell and the low probability that it will activate the *Igh* enhancer and therefore express the transgenes. If so, constitutive *myc* and *bcl-2* expression might be enough for complete transformation of this cell type.

Clearly, *bcl-2/myc* synergy has provided access to an intriguing cell. It is unlikely to be the neoplastic counterpart of a multipotential haemopoietic stem cell as that cell is believed to lack expression of CD4 and B220 (ref. 24). Furthermore, expression of both transgenes is governed by the *Igh* enhancer, which is more likely to be activated in a lymphoid stem or progenitor cell. Intriguingly, our tumour cells share some, but not all, properties with two recently discovered types of primitive lymphoid cell: the Thyl^{lo} B220$^+$ B cell progenitor[31] and the earliest known intrathymic T cell precursor, which has been characterized as Thyl^{lo}, CD4^{lo}, Sca-1$^+$, but B220$^-$, CD8$^-$, CD3$^-$ and α, β T cell receptor$^-$ (L. Wu *et al.*, manuscript submitted). Neither *myc*[32] nor *bcl-2* (refs 9, 10) promotes autonomous cell growth and, despite being readily transplantable, the tumour cells do not proliferate *in vitro*, which is consistent with their retaining a requirement for growth factors. Despite the addition of a range of exogenous growth factors to the medium, including interleukins 1–7, either singly or in combinations, the tumours have remained resistant to cultivation *in vitro*. Thus, the tumour cells may provide an effective assay for new factors controlling the growth and differentiation of early haemopoietic cells.

In conclusion, the case for *bcl-2* as a *bona fide* oncogene has been considerably strengthened by this demonstration of its tumorigenic co-operativity with *myc* in the whole animal. Although the cell most readily rendered fully malignant was, surprisingly, a primitive lymphoid precursor, the copious and enlarged pre-B and B cells in the doubly transgenic mice attest to a synergistic action of constitutive *bcl-2* and *myc* expression within more differentiated B lineage cells. These could be the counterpart of the rare B lymphoid tumours in man that bear both a *bcl-2* [14; 18] and a *myc* [8; 14] chromosome translocation[33–35], although those high-grade neoplasms may also have acquired other somatic mutations. □

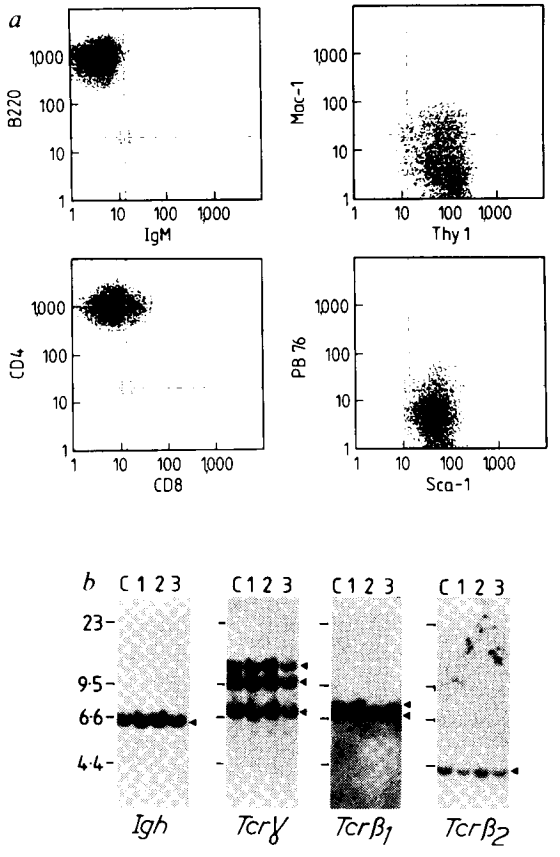

FIG. 3 Characterization of tumour cells in Eμ-*bcl-2/myc* doubly transgenic mice. *a*, Surface phenotype analysis of cells from an enlarged mesenteric lymph node. *b*, Southern blot analysis of *Igh* and *Tcr* gene status in three independent tumours (1–3) and, as a control, liver (C) from a (C57BL/6 × SJL)F_1 mouse. Size markers in kilobases are shown to the left.
METHODS. Immunofluorescence staining and analysis have been described[23]. The cell surface antigens and their identifying monoclonal antibodies were: CD45R (B220) (14.8 and RA3-6B2), IgM (5.1), Mac-1 (M1/70), Thy-1 (J1J and 30-H12), CD4 (GK1.5), CD8 (53-6.72), PB76 (G-5.2; ref. 22) and Sca-1 (E13-161.7; ref. 36). They were conjugated with fluorescein isothiocyanate or phycoerythrin or, alternatively, biotinylated and revealed with phycoerythrin-streptavidin. *Eco*RI-digested and size-fractionated tumour DNA was hybridized with J_{H4} and *Tcr* γ cDNA probes; *Hind*III digests were hybridized with Cβ1 and Jβ2 genomic probes (the germline Cβ1 fragments differ between C57BL/6 and SJL mice). The origin of the probes is detailed elsewhere[37]; they were labelled with ^{32}P by random priming.

Received 4 June; accepted 13 September 1990.

1. Tsujimoto, Y., Finger, L. R., Yunis, J., Nowell, P. C. & Croce, C. M. *Science* **226,** 1097–1099 (1984).
2. Bakhshi, A. *et al. Cell* **41,** 889–906 (1985).
3. Cleary, M. L., Smith, S. D. & Sklar, J. *Cell* **47,** 19–28 (1986).
4. Fukuhara, S., Rowley, J. D., Variakojis, D. & Golomb, H. M. *Cancer Res.* **39,** 3119–3128 (1979).
5. Tsujimoto, Y. N., Ikegaki, N. & Croce, C. M. *Oncogene* **2,** 3–7 (1987).
6. Chen-Levy, Z., Nourse, J. & Cleary, M. L. *Molec. cell. Biol.* **9,** 701–710 (1989).
7. Reed, J. C., Tsujimoto, Y., Alpers, J. D., Croce, C. M. & Howell, P. C. *Science* **236,** 1295–1299 (1987).
8. Graninger, W. B., Seto, M., Boutain, B., Goldman, P. & Korsmeyer, S. J. *J. clin. Invest.* **80,** 1512–1515 (1987).
9. Vaux, D. L., Cory, S. & Adams, J. M. *Nature* **335,** 440–442 (1988).
10. Nunez, G. *et al. J. Immun.* **144,** 3602–3610 (1990).
11. Tsujimoto, Y. *Oncogene* **4,** 1331–1336 (1989).
12. Reed, J. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 3660–3664 (1990).
13. Reed, J. C., Haldar, S., Cuddy, M. P., Croce, C. M. & Makover, D. *Oncogene* **4,** 1123–1127 (1989).
14. Nunez, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 4589–4593 (1989).
15. McDonnell, T. J. *et al. Cell* **57,** 79–88 (1989).
16. Strasser, A. *et al. Curr. Topics Microbiol. Immun.* (in the press).
17. McDonnell, T. J. *et al. Molec. cell. Biol.* **10,** 1901–1907 (1990).
18. Cory, S. *Adv. Cancer Res.* **47,** 189–234 (1986).
19. Adams, J. M. *et al. Nature* **318,** 533–538 (1985).
20. Langdon, W. Y., Harris, A. W., Cory, S. & Adams, J. M. *Cell* **47,** 11–18 (1986).
21. Harris, A. W. *et al. J. exp. Med.* **167,** 353–371 (1988).
22. Schmidt, E. V., Pattengale, P. K., Weir, L. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **85,** 6047–6051 (1988).
23. Strasser, A. *Eur. J. Immun.* **18,** 1803–1810 (1988).
24. Spangrude, G. J., Heimfeld, S. & Weissman, I. L. *Science* **241,** 58–62 (1988).
25. Fredrickson, G. G. & Basch, R. S. *J. exp. Med.* **169,** 1473–1478 (1989).
26. Morse, H. C. III *et al. J. exp. Med.* **165,** 920–925 (1987).
27. Palacios, R. & Steinmetz, M. *Cell* **41,** 727–734 (1985).
28. Alessandrini, A., Pierce, J. H., Baltimore, D. & Desiderio, S. V. *Proc. natn. Acad. Sci. U.S.A.* **84,** 1799–1803 (1987).
29. Sakaguchi, N. & Melchers, F. *Nature* **324,** 579–582 (1986).
30. Schatz, D. G., Oettinger, M. A. & Baltimore, D. *Cell* **59,** 1035–1048 (1989).
31. Tidmarsh, G. F., Heimfeld, S., Whitlock, C. A., Weissman, I. L. & Müller-Sieburg, C. E. *Molec. cell. Biol.* **9,** 2665–2671 (1989).
32. Langdon, W. L., Harris, A. W. & Cory, S. *Oncogene Res.* **3,** 271–279 (1988).
33. Pegoraro, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 7166–7170 (1984).
34. Gauwerky, C. E., Haluska, F. G., Tsujimoto, Y., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 8548–8552 (1988).
35. Gauwerky, C. E., Huebner, K., Isobe, M., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **86,** 8867–8871 (1989).
36. Aihara, Y., Bühring, H-J., Aihara, M. & Klein, J. *Eur. J. Immun.* **16,** 1391–1399 (1986).
37. Hariharan, I. K. *et al. Molec. cell. Biol.* **9,** 2798–2805 (1989).

ACKNOWLEDGEMENTS. We thank J. M. Adams for discussion and comments on the manuscript, D. Schatz and M. Oettinger for the *RAG-1* cDNA, F. Melchers for the λ5 probe, K. Patane for animal husbandry, S. Mihajlovic for histological sections, M. Stanley for technical assistance and I. Collins for typing this manuscript. A.S. is the recipient of a fellowship from the Swiss National Science Foundation. This work was supported by the National Health and Medical Research Council of Australia and the US National Cancer Institute.