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The *myc* Oncogene and Lymphoid Neoplasia: From Translocations to Transgenic Mice

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A. Introduction

The c-*myc* proto-oncogene encodes a nuclear phosphoprotein which probably plays a crucial role in growth control [4]. The protein has DNA-binding activity in vitro, but its function remains unknown. While avian retroviruses carrying the closely related v-*myc* sequence rapidly transform myeloid cells, the cellular *myc* gene has been strongly implicated in several types of lymphoid neoplasia. The fundamental mechanism releasing the oncogenic potential of c-*myc* is believed to be *deregulation* of its expression. Most chicken bursal lymphomas resulting from infection with avian leukosis virus, which does not itself bear an oncogene, carry a provirus near or within the c-*myc* gene [6]. About a quarter of T lymphomas with a retroviral aetiology also bear a c-*myc*-associated provirus [3]. Expression of the c-*myc* gene in these tumours is governed by the promotor and/or the enhancer in the viral long terminal repeat (LTR) [6, 13, 3]. In most plasmacytomas of the mouse and Burkitt lymphomas of man, a chromosome translocation couples the c-*myc* gene to the IgH constant region locus, presumably bringing c-*myc* under the control of factors that regulate heavy-chain expression [11, 8, 4].

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B. Induction of B-Cell Neoplasia by a c-*myc* Gene Coupled to Immunoglobulin Enhancers

The evidence connecting deregulated *myc* expression with neoplasia was persuasive but remained circumstantial. Transgenic mice provided the means of testing the hypothesis directly. The results were dramatic: Transgenic mice carrying an essentially normal c-*myc* gene remained healthy, as did those with a *myc* gene devoid of its putative regulatory sequences [1]. However, mice bearing a cellular *myc* gene linked to the regulatory region within the LTR of the murine mammary tumour virus were found to have an increased susceptibility to mammary carcinomas [16]. Linkage to the SV40 promoter/enhancer also provoked tumours, but the incidence was relatively low [1]. In marked contrast, 13 or 14 of 15 primary transgenic animals bearing c-*myc* coupled to the heavy-chain enhancer (Eμ) developed lymphomas, as did six of 17 with c-*myc* linked to the kappa enhancer [1]. Thus c-*myc* is innocuous as a transgene in its "native state", or even after removal of certain regulatory sequences. Once under the control of a strong exogenous regulatory element, however, it becomes a potent tumourigenic agent.

We have now made a detailed study of the disease induced by the Eμ-*myc* transgene (Harris et al., in preparation). Similar pathology was observed in several independent lines bred from different primary transgenic

mice, so the chromosomal location of the transgene does not play a major role. One line was followed for five generations over 12 months, and 96% of the mice bearing the Eμ-*myc* locus succumbed to tumours before 6 months of age. In most cases, the disease pattern was a disseminated lymphoma involving most of the lymph nodes and often (but not always) the thymus. The lymphoma was usually accompanied by leukaemia. Some animals developed only a thymoma, or succumbed to a bowel obstruction probably caused by proliferation of tumour cells within the intestinal wall. The tumours grew rapidly, and most animals had to be killed within a month of exhibiting palpable inguinal lymph nodes. Transplantation tests established that the proliferating lymphoid cells were truly malignant. Indeed, injection of only 100 cells from one of the donors was sufficient to induce tumours in syngeneic recipients.

As expected, all the tumours exhibited relatively abundant transcription of the Eμ-*myc* transgene. Significantly, however, *no* normal c-*myc* transcripts could be detected [1], even after sensitive S1 analysis. Thus, the normal c-*myc* alleles had apparently been suppressed as a result of constitutive expression of the transgene. This result exactly parallels the situation found earlier in Burkitt's lymphomas and murine plasmacytomas, where expression was shown to be restricted to the translocated c-*myc* allele, the normal allele being essentially silent [2, 12]. The data favour the hypothesis [11, 14] that normal c-*myc* regulation operates via a negative feedback loop, possibly involving a repressor. The normal allele is presumed to be silenced via the protein produced by the rearranged allele, which is itself refractory to repression.

To identify which cell types had undergone transformation, about 50 different primary tumours were dissected from 20 mice and established in culture. All proved to be B lymphoid in origin, including those derived from thymic lymphomas. Thus, none displayed the T cell marker Thy 1, but all exhibited rearrangement of the J_H and/or Jκ loci and all expressed B-lineage-specific markers. While expecting to find B-cell neoplasia, we were somewhat surprised not to find any examples of T-cell or even myeloid tumours, because the IgH enhancer is thought to be active in at least some T and myeloid cells [7, 5].

The tumours represented several stages within the B differentiation lineage. About 40% were surface Ig-positive B cells, while the rest were pre-B cells of varying maturity having different combinations of IgH and κ rearrangements. While some were apparently stable, others continued to differentiate, either in vivo or in tissue culture. Thus, Eμ-*myc*-induced tumourigenesis does not totally prevent further differentiation.

C. Tumourigenicity Requires More than Deregulation of c-*myc*

Cancer has long been regarded as a multistep process. More recently, this concept has been represented in molecular terms as the need for collaboration between two (or more) oncogenes [9, 15]. It might be argued from the nearly invariant development of tumours by Eμ-*myc* mice that deregulation of c-*myc* is itself sufficient to cause cancer. However, this does not appear to be the case. Firstly, Eμ-*myc* tumours are clonal [1], even though all B-lineage cells express Eμ-*myc*. Secondly, the onset of tumours is highly variable and can occur as early as at 3 weeks of age and as late as at 6 months or more. Both these features argue that an additional change gives one cell a proliferative advantage over its fellows. Thirdly, and most compelling, in contrast to the tumour cells, the lymphoid cells from young animals which have not yet developed enlarged lymph nodes fail to induce tumours when injected in large numbers into syngeneic recipients [10]. Thus, even though the Eμ enhancer is turned on early in B-cell ontogeny, Eμ-*myc* mice exhibit a true pre-neoplastic phase.

D. Eμ-*myc* Promotes a Benign Polyclonal Expansion of Early B-Lineage Cells

Pre-lymphomatous Eμ-*myc* mice exhibit profoundly disturbed B-cell differentiation. We have analyzed this condition in some detail [10], because it provides a unique opportunity to discover the consequences of con-

stitutive *myc* expression in "normal" cells. Cell surface marker analysis of foetal livers and the various lymphoid organs of young mice revealed a remarkable expansion of early B-lineage cells at the expense of mature B cells. The increase in pre-B cells is evident as early as at 18 days of gestation, and by 7 days after birth approximately half the cells in the bone marrow are pre-B cells, mostly the early Ly-5(B220)$^+$ ThB$^-$ type. In young adults, the expansion includes late pre-B cells and involves the spleen as well as the bone marrow. Overall, these animals exhibit a 4- to 5-fold increase in pre-B cell numbers and about a 30% reduction in sIg$^+$ B cells. Analysis of bone marrow DNA for J_H rearrangement established that the expansion is polyclonal and probably also includes a considerable number of pre-B cells which have not yet commenced J_H rearrangement.

The B-lineage cells in Eμ-*myc* mice differ remarkably in their size profile from those in normal mice. The small resting B cell and its immediate precursor, the small B220$^+$ ThB$^+$ pre-B cell, are absent from Eμ-*myc* mice, and all the B-lineage cells are large. Moreover, analysis of cellular DNA content suggests that at least one third of the pre-B and B cells are in cycle. We conclude that constitutive *myc* expression promotes and maintains B cells in cycle and may indeed preclude a G_0 state.

A notable consequence of Eμ-*myc* expression is the acquisition of the Ia surface antigen by many pre-B cells. Ia is normally found only on sIg$^+$ B cells, increasing after activation by mitogen or antigen plus growth factors. The significance of premature Ia expression is not clear, but it may indicate that enforced *myc* expression partially replaces the need for certain growth factors.

Clearly, Eμ-*myc* expression has affected both mitogenesis and differentiation within the B-cell lineage. To account for these results, we have proposed [10] that the level of c-*myc* expression is an important factor in setting the probability of self-renewal versus maturation during differentiation, with increased *myc* expression favouring self-renewal, as shown in Fig. 1.

In summary, constitutive *myc* expression strongly predisposes to malignancy. Its consequence for B-cell differentiation is to favour self-renewal over maturation, and this results in a significant expansion of early cells. The increased proliferative potential presumably increases the probability of one cell within the population undergoing further change and becoming a fully malignant clone. It may be significant that the expanded population primarily comprises pre-B cells which actively undergo DNA rearrangement and which therefore may be more susceptible to genetic accident.

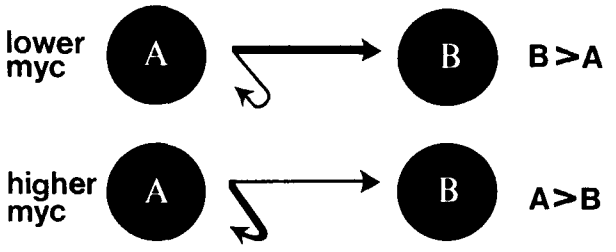

Fig. 1. A model for the role of c-*myc* in differentiation. The balance between self-renewal and maturation is set by the level of c-*myc* expression, with higher levels favouring self-renewal

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