comparable numbers of binding sites (ranging from 1.1×10^6 to 3.8×10^6), the binding affinities (K_a) differed by an order of magnitude, with a value of $2 \times 10^7 M^{-1}$ for the low-affinity cells and the parent cultures and $1.7 \times 10^8 M^{-1}$ for the high-affinity cells.

To assess cell functions, we measured the synthesis of DNA, total protein and collagen, and the production of various collagen types, and we determined the rate of cell growth. The experiments were repeated several times, and representative results are presented as the mean of triplicate cultures (Table 1). The high-affinity cells synthesized about four times as much DNA during a 4-hour treatment with [^{3}H]thymidine as did the parent cultures and about seven times as much as the low-affinity cells (Table 1). Total protein production, assessed after a 20-minute treatment with [^{35}S]methionine, was significantly greater in the high-affinity cells than in the parent cultures and three to four times greater in the high-affinity cells than in the low-affinity cells (Table 1). The proportion of synthesis activity committed to collagen production in the high-affinity cells was about three times that in the parent cultures, with collagen accounting for 40.8 percent and 27.6 percent of total protein in the high-affinity and parent cultures, respectively. The proportion of collagen type I produced by cultures of high-affinity cells and parent cultures was within the range usually seen (*10, 11*) but the production of collagen types III and V was, respectively, about three times and five times greater in the cultures of the high-affinity cells than in the parent cultures (Table 1). In some experiments, production of collagen type V was up to 14 times that in parent cultures.

We have isolated a subset of diploid fibroblasts with unusual phenotypic features. In comparison with the parent cultures from which they were derived, these cells grow and produce proteins at high rates; they commit almost twice as much protein-synthesizing capacity to collagen synthesis, and they produce collagen types III and V in much greater amounts. They possess binding sites for C1q (and therefore presumably for C1) with a significantly higher affinity than that of the parent cultures. All of these features appear to be stable in cultures through at least 12 doublings in our experiment.

Rapid growth and synthesis and the production of large quantities of collagen, especially types III and V, are properties expected of cells in wounds and sites of inflammation. We suggest that factors present at such sites may mitogenically activate one or more subtypes of fibroblast and lead to subtype amplification. Our experiments indicate that C1 may be a key factor in this process and that amplification of a subset of the type we have isolated can occur. As wounds heal and inflammation subsides, and the concentration of C1 decreases, mixtures of subtypes may revert to those characteristic of normal connective tissues.

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Mapping of the Human *Blym*-1 Transforming Gene Activated in Burkitt Lymphomas to Chromosome 1

Abstract. Blym-*1, a transforming gene detected by transfection of NIH 3T3 cells with DNA from Burkitt lymphomas, was mapped to the short arm of chromosome 1 (1p32) by chromosomal in situ hybridization. The* Blym-*1 gene was not physically linked to the cellular* myc *oncogene or to any of the immunoglobulin gene loci implicated in the characteristic chromosomal translocations in Burkitt lymphoma.*

Molecular genetic analyses of several human Burkitt lymphoma cell lines have revealed DNA rearrangements in which there has been a translocation between the cellular oncogene c-*myc* and immunoglobulin gene loci (*1*). The characteristic chromosomal translocations observed in Burkitt lymphomas [t(2;8)(p12;q24), t(8;14)(q24;q32), and t(8;22)(q24;q11)] (*2*) indicated the potential importance of a gene locus on the long arm of chromosome 8 at band q24 (8q24), the band to which the c-*myc* gene was subsequently mapped (*1, 3*). Furthermore, several investigators suggested that the significance of the specific translocations to malignant transformation may have resulted from the alteration of cellular transforming genes by their juxtaposition with specific DNA sequences, that is, immunoglobulin gene sequences (*4*).

Recently a transforming gene has been detected by transfection of NIH 3T3 cells with DNA from six Burkitt lymphomas (*5*). This gene is homologous by molecular hybridization with the *Blym*-1 transforming gene isolated from chicken B-cell lymphoma DNA (*6*), but is not homologous with 12 retroviral transforming genes including v-*myc* and v-*ras*. The involvement of these two distinct genes in B-cell lymphomas of both chickens and humans further supports the hypothesis that c-*myc* and *Blym*-1 may be involved in different stages of progression to neoplasia (*7*).

We were interested in determining the chromosomal location of the human *Blym*-1 transforming gene and its possible relation to c-*myc* or the immunoglobulin gene loci, loci known to be active in cells from Burkitt lymphomas. By using chromosomal in situ hybridization we were able to assign the human *Blym*-1 gene to the short arm of chromosome 1 (1p32) (Fig. 1), a locus unlinked to either c-*myc* or immunoglobulin gene sequences.

The probe we used for the chromosome hybridization was a plasmid containing the ^{3}H-labeled 0.95-kb biologically active Eco RI fragment of the human

Blym-1 gene (*5*). Under stringent conditions of washing as previously described (*5*), the 0.95-kb Eco RI fragment hybridized to a single Hind III fragment of approximately 5 kb in cellular DNA from normal human embryonic fibroblasts, Burkitt lymphomas, and NIH 3T3 cells transformed by Burkitt lymphoma DNA, but not to DNA of nontransformed NIH 3T3 cells. For in situ hybridization procedure and analysis we used a modification of the technique of Harper and Saunders (*8*), which has been described previously (*9*).

Analyses of 150 metaphase spreads from normal human male lymphocytes revealed that approximately 6 percent of all the silver grains were located on or directly beside chromosome 1 at band p32 (Fig. 2), and approximately 25 percent of all cells scored showed hybridization at 1p32. The percentage of all grains located at 8q24.1 (c-*myc*), 2p12 (immunoglobulin κ light chain), 14q32 (heavy chain), and 22q11 (λ light chain) was 0.6, 0.6, 1, and 0, respectively. Secondary peaks of grain accumulation were noted at 1q32 (1.6 percent), 2q13 (1.8 percent), 3p21 (2.3 percent), and 14q24 (1.8 percent). Statistical analysis of these data by the Poisson distribution with the number of grains per chromosome band adjusted by the relative size of the band in a 400-band stage chromosome idiogram (*10*) revealed a highly significant distribution at 1p32 ($P < 10^{-70}$) and at 2q13 ($P < 10^{-24}$) and a much less significant distribution at 14q24 ($P < 10^{-3}$). Other peaks were not significant at a P value of < 0.001. Because the chicken *Blym*-1 probe hybridizes to a small family of related human sequences under low stringency conditions (*6*), the finding of a highly significant grain accumulation at a secondary site by chromosomal in situ hybridization despite a single band of hybridization in a Southern blot may be a result of the different stringencies of the two hybridization techniques. We believe that the blotting studies with human *Blym*-1 were more stringent than the in situ analyses, although such data may not strictly be comparable. Thus the homology to sequences at 2q13 could possibly have been a sampling artifact.

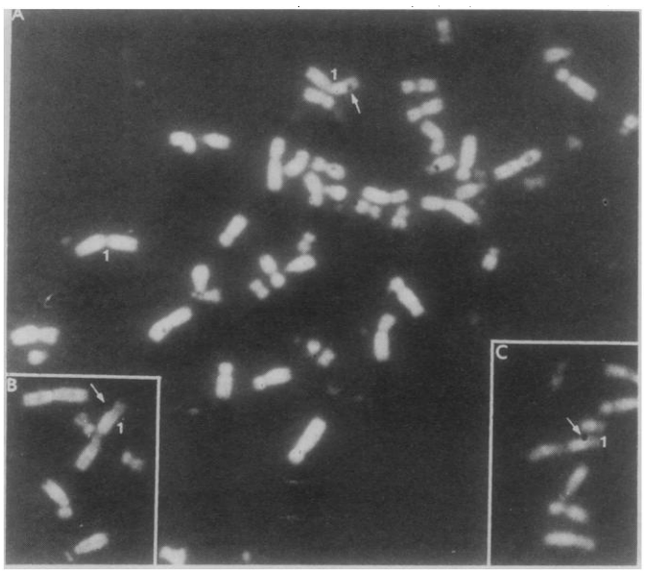

Fig. 1. Photographs of partial metaphase spreads from normal male lymphocytes. The chromosomes were hybridized overnight at 37°C with the biologically active 0.95-kb Eco RI fragment of the *Blym*-1 gene cloned in pBR322 and labeled with tritium to a specific activity of 2.5×10^7 counts per minute per microgram. Chromosomes are stained with quinacrine mustard dihydrochloride and photographed with a combination of incident ultraviolet and transmitted visible light. A silver grain can be seen at 1p32.

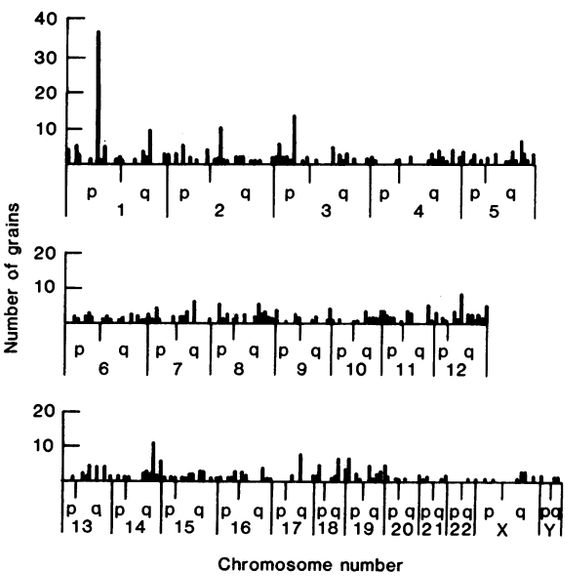

Fig. 2. Histogram showing the distribution of silver grains from 150 metaphase spreads from normal male lymphocytes. A highly significant grain deposition can be seen at 1p32, the site to which we have assigned the *Blym*-1 probe, as well as a secondary peak at 2q13 ($P < 10^{-70}$ and $P < 10^{-24}$, respectively) as determined from an analysis of the data by the Poisson distribution, $P\ (r) = \mu^r\ e^{-\mu}/r!$ where μ is the total number of grains counted divided by the measured length of the total karyotype in centimeters at the 400 band stage from a standardized chromosome idiogram (*10*), r is the total number of grains counted at the chromosome band of interest (that is, 1p32, 2q13) divided by the measured length of the chromosome band of interest in centimeters from the standardized idiogram, and $e = 2.71828$.

The relation, if any, between the location of *Blym*-1 on chromosome 1 at band p32 and the cytogenetic anomalies characteristic of Burkitt lymphoma is unclear. Although a few reports have suggested a nonrandom involvement of chromosome 1 in Burkitt lymphoma in addition to the characteristic 2;8, 8;14, and 8;22 translocations (*14*), the rearrangements or numerical alterations of this chromosome do not involve band p32. However, various abnormalities of chromosome 1 have been found to occur occasionally in many, if not all, tumor types (*15*). In a survey of 218 human neoplasms, chromosomal breakpoints in chromosome 1 were found to cluster

This study of the location of human *Blym*-1 and the previous analysis of this probe (*5*) indicate that there is no homology with the c-*myc* gene. Furthermore, chromosomal in situ hybridization indicates that these two loci are unlinked and reside on different human chromosomes. Therefore, the activation of both *Blym*-1 and c-*myc* in Burkitt lymphomas is not related to the physical proximity of the genes. In addition, the chromosomal location of *Blym*-1 is distinct from that of other transforming genes analyzed to date, including n-*ras*, which is located between the centromere of chromosome 1 and band p21 by chromosomal in situ hybridization (*11*). Although nucleotide sequence analysis of chicken *Blym*-1 indicates that it encodes a small protein with significant homology to the amino terminal region of transferrin proteins, it is of interest that *Blym*-1 is not syntenic to the transferrin receptor gene. The transferrin receptor gene has been regionally mapped to 3q23→qter (*12*), with which the melanoma-associated antigen p97 has been found to cosegregate in somatic cell hybrids (*13*).

within or adjacent to the centromeric heterochromatin (49.9 percent), in bands p22–p32 (14.6 percent), q31–32 (14.3 percent), and p36 (8.2 percent) (*16*). Whether this preponderance of chromosome 1 abnormalities is the result of clonal evolution of malignant cells, perhaps related to an unidentified proliferative advantage for the cell, or is part of the initial event in neoplastic transformation remains to be determined. Furthermore, the relation of these chromosomal abnormalities to the oncogenes located on chromosome 1 is unknown. The large physical distance between the mapped oncogenes and the common chromosome 1 lesions, especially 1q31–32, suggest that if these abnormalities play a role in the malignant transformation of the tumors in which they are found, additional oncogenes may also be mapped to this chromosome.

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Gene for T-Cell Growth Factor: Location on Human Chromosome 4q and Feline Chromosome B1

Abstract. *T-cell growth factor (TCGF) or interleukin-2 (IL-2), an immunoregulatory lymphokine, is produced by lectin- or antigen-activated mature T lymphocytes and in a constitutive manner by certain T-cell lymphoma cell lines. By means of a molecular clone of human TCGF and DNA extracted from a panel of somatic cell hybrids (rodent cells × normal human lymphocytes), the TCGF structural gene was identified on human chromosome 4. In situ hybridization of the TCGF clone to human chromosomes resulted in significant labeling of the midportion of the long arm of chromosome 4, indicating that the TCGF gene was located at band q26–28. Genomic DNA from a panel of hybrids prepared with HUT-102 B2 cells was examined with the same molecular clone. In this clone of cells, which produces human T-cell leukemia virus, the TCGF gene was also located on chromosome 4 and was apparently not rearranged. The homologous TCGF locus in the domestic cat was assigned to chromosome B1 by using a somatic cell hybrid panel that segregates cat chromosomes. Linkage studies as well as high-resolution G-trypsin banding indicate that this feline chromosome is partially homologous to human chromosome 4.*

T-cell growth factor (TCGF) was originally discovered as a component of conditioned medium from lectin-stimulated primary cultures of human lymphoid cells that permitted long-term proliferation in vitro (*1, 2*). Exploitation of this discovery led to the cloning of T-cell populations of defined function and specificity (*3*) and the use of TCGF to establish cell lines from certain mature T-cell tumors (*4*). These cells respond to TCGF without prior activation with lectin or antigen, apparently because of constitutive expression of the TCGF receptor (*5*). From these cell lines the retrovirus human T-cell leukemia virus (HTLV) was isolated (*6*). TCGF-dependent lymphocyte cultures of normal or leukemic individuals are invariably mature T cells as determined by the expression of an array of T-cell markers and the lack of any B cell or immature lymphoid determinants (*1*). Although normal lymphocyte cultures require the continuous presence of TCGF for growth, many of the HTLV-infected cell lines gradually become independent of exogenous TCGF (*2*), suggesting that they either produce their own TCGF or by-pass the normal TCGF–TCGF receptor system required for proliferation.

Homogeneously purified human TCGF is a single polypeptide with a molecular weight of 15,000 (*7*). Recently, almost identical molecular clones of the structural gene for human TCGF were derived from complementary DNA (cDNA) libraries of a leukemic T-cell line (Jurkat) (*8*) and from normal peripheral blood lymphocytes (*9*). Use of cloned TCGF sequences as probes suggested that human TCGF is encoded by a single cellular gene containing introns. We have used a molecular TCGF clone to map the gene in the normal human genome and in the genome of the HUT-102 B2 lymphoma cell line from which the original isolate of HTLV was obtained. HUT-102 B2 has become TCGF-independent and produces low levels of TCGF messenger RNA (mRNA) and biologically active TCGF (*2, 9*).

TCGF was mapped in normal cells with use of a panel of 43 somatic cell hybrids (rodent cells × normal human lymphocytes) (*10*). The human chromo-