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Genetics

Human c-*myc onc* gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells

(somatic cell hybrids/Southern blotting technique/recombination/cancer)

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ABSTRACT **Human sequences related to the transforming gene (v-*myc*) of avian myelocytomatosis virus (MC29) are represented by at least one gene and several related sequences that may represent pseudogenes. By using a DNA probe that is specific for the complete gene (c-*myc*), different somatic cell hybrids possessing varying numbers of human chromosomes were analyzed by the Southern blotting technique. The results indicate that the human c-*myc* gene is located on chromosome 8. The analysis of hybrids between rodent cells and human Burkitt lymphoma cells, which carry a reciprocal translocation between chromosomes 8 and 14, allowed the mapping of the human c-*myc* gene on region (q24→qter) of chromosome 8. This chromosomal region is translocated to either human chromosome 2, 14, or 22 in Burkitt lymphoma cells.**

Sequences homologous to the transforming gene (v-*myc*) of avian myelocytomatosis virus (MC29) have been shown to be present in the genome of organisms widely separated phylogenetically (1–4). Although the product and the function of the c-*myc* gene are not known, several examples suggest that abnormal regulation of these sequences may be involved in malignant transformation *in vivo*. In approximately 80% of avian leukosis virus-induced B-cell lymphomas in chickens, viral DNA sequences, including the viral transcriptional promoter, integrated in the vicinity of the c-*myc* locus (5–7). Increased c-*myc* transcription was detected in these tumors, implicating activation of this gene as a direct consequence of viral infection in lymphomagenesis (5–7). In human cells the c-*myc* gene is transcribed in a variety of tissues and increased levels of *myc* mRNA have been detected in different tumors (8, 9). The highest levels have been detected in the cell line HL-60 derived from neoplastic cells from a patient with acute promyelocytic leukemia (9). The c-*myc* gene has been shown to be stably amplified in the genome of these cells as well as in primary, uncultured leukemic cells from the same individual (10), suggesting that c-*myc* amplification may have been involved in the leukemic transformation in this case.

These studies suggest that different mechanisms—namely, activation by a viral promoter or gene amplification—may lead to *onc*-gene activation, which, in turn, may cause neoplastic transformation. Another mechanism for abnormal expression of c-*myc* or other cellular *onc* genes might be through chromosomal rearrangements which are a common feature of several types of tumors. In fact, this concept has been formally proposed (11, 12). The identification of the normal chromosomal location of the c-*myc* gene in the human genome might allow a test of this hypothesis by studying the location and regulation of expression of the c-*myc* gene in selected groups of tumors displaying specific cytogenetic abnormalities. Moreover, the study of the mechanism of c-*myc* amplification in human malignant cells may be facilitated by the analysis of the genetic environment from which the amplification event supposedly originated. In the present study we have mapped the human c-*myc* gene within a relatively small region of chromosome 8 which is translocated in the malignant cells of most cases of Burkitt lymphoma.

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MATERIALS AND METHODS

Somatic Cell Hybrids. LM-TK$^-$ mouse fibroblasts (13) or mouse myeloma cells (14) were fused with either human lymphocytes, Burkitt lymphoma cells (15, 16), or HL60 cells (17), and hybrids were selected as described (14–16) and studied for the expression of isozyme markers assigned to each of the different human chromosomes (14). Hybrids between mouse myeloma and Daudi Burkitt lymphoma cells and between LM-TK$^-$ cells and P3 HR1 cells have been described (13, 16). Three of these hybrids have retained the 14q$^+$ chromosome from either Daudi cells (hybrid clone 3F1) or P3-HR1 cells (hybrid clones M44 C1 2S5 and M44 C1 2S9) and have lost the normal 8 and the 8q$^-$ chromosomes. Hybrid clones M44 C1 2S5 and M44 C1 2S9 contained only the 14q$^+$ chromosome and no other human chromosome. Clone 3F1 has lost the human chromosomes 8, 8q$^-$, and 14 but has retained the 14q$^+$ and other human chromosomes. These clones were positive for the expression of human nucleoside phosphorylase (a marker for chromosome 14) and negative for human glutathione reductase (a marker for chromosome 8).

In addition, we have studied a hybrid clone between a Chinese hamster ade$^-$E gly$^-$B mutant, which also carries two additional markers, asn$^-$ (asparagine requiring) and HPRT$^-$ (deficient in hypoxanthine phosphoribosyltransferase) (18), and human peripheral lymphocytes. The Chinese hamster mutant parent was derived from the CHO-K1 cell line (18). This hybrid, 706B6-40 C1 17, a subclone of hybrid 706B6 (18), has retained only human chromosome 8 (Fig. 2). The parental hybrid 706B6 contained only human chromosomes 8 and 14 (18).

Isozyme Analysis. Hybrid cells were studied for the expression of isozyme markers that were assigned to each of the different human chromosomes by starch gel or cellulose acetate gel electrophoresis: 1, enolase 1 (EC 4.2.1.11); 2, isocitrate dehydrogenase (EC 1.1.1.42); 3, β-galactosidase (EC 3.2.1.23); 4, phosphoglucomutase 2 (EC 2.7.5.1); 5, hexosaminidase B (EC 3.2.1.30); 6, glyoxalase 1 (EC 4.4.1.5) and phosphoglucomutase 3 (EC 2.7.5.1); 7, β-glucuronidase (EC 3.2.1.31); 8, glutathione

Abbreviation: kb, kilobase.

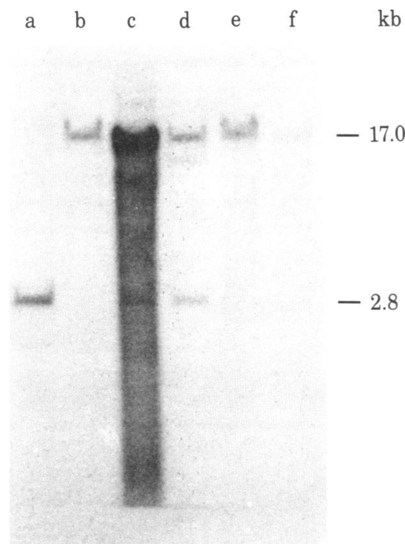

FIG. 1. Segregation of the human c-*myc* homologue in mouse–human hybrids. Lanes: a, normal human DNA; f, normal mouse DNA; b–e, DNAs derived from four mouse–human hybrids.

reductase (EC 1.6.4.2); 9, aconitase (EC 4.2.1.3); 10, glutamate oxaloacetate transaminase (EC 2.6.1.1); 11, lactate dehydrogenase A (EC 1.1.1.27); 12, lactate dehydrogenase B (EC 1.1.1.27); 13, esterase D (EC 3.1.1.1); 14, nucleoside phosphorylase (EC 2.4.2.1); 15, mannosephosphate isomerase (EC 5.3.1.8); 16, adenine phosphoribosyltransferase (EC 2.4.2.7); 17, galactokinase (EC 2.7.1.6); 18, peptidase A (EC 3.4.11.-); 19, glucosephosphate isomerase (EC 5.3.1.9); 20, adenosine deaminase (EC 3.5.4.4); 21, superoxide dismutase 1 (EC 1.15.1.1); 22, arylsulfatase (EC 3.1.6.1); X chromosome, glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

DNA Extraction and Southern Blot Analysis. DNA from various mouse, human, and hybrid cell lines was prepared by cell lysis, proteinase K digestion, extraction with phenol, and precipitation with ethanol as described (19). Thirty micrograms of DNA was digested with 60 units of the appropriate restriction endonuclease in standard conditions recommended by the supplier (New England BioLabs). Fragments were separated by electrophoresis on a 0.8% agarose gel. DNA was denatured and transferred to nitrocellulose as described by Southern (20). Hybridization and autoradiography were performed according to Wahl *et al.* (21), except that filter washing was performed in 0.45 M NaCl/0.045 M sodium citrate/0.5% sodium dodecyl sulfate, pH 7, at 60°C for 2 hr.

Probe for Hybridization. A recombinant pBR322 plasmid (clone pMC41RC) that contains the entire 3′ c-*myc* exon was used for this study. This plasmid, the preparation of which has been described (10), was derived from a recombinant phage (λ-LMC41) containing the entire human c-*myc* gene (4). pMC41RC DNA was used as a probe after labeling with [^{32}P]dCTP and [^{32}P]dATP by nick-translation (22).

RESULTS

Mapping of the Human c-*myc* Gene. In the genomes of both humans and rodents, c-*myc* sequences are represented by at least one gene and a complex pattern of incompletely related sequences which may represent pseudogenes (4). The complete human c-*myc* gene was previously cloned and characterized (4), but to determine its chromosomal location it was necessary to use as a probe a subclone that does not detect any of the putative pseudogenes either in human or in rodent DNA. Clone pMC41RC, which represents the 3′ exon of the human c-*myc* gene, detects a single copy sequence in both genomes and the *Sst* I human c-*myc* fragment [2.8 kilobases (kb); Fig. 1] can be distinguished from the corresponding sequences present in mouse DNA (17 kb; Fig. 1). Fig. 2 shows the results of the analysis of a panel of hybrid clones that were studied for the expression of isozyme markers assigned to each of the human chromosomes and for the presence of the human c-*myc* homologue. Because only chromosome 8 segregates concordantly with the presence of the human c-*myc* gene, we assign this gene to human chromosome 8. To confirm this finding we have studied a somatic cell hybrid between Chinese hamster and human cells that has retained only human chromosome 8 and no other human chromosome (Figs. 2 and 3). As shown in Fig. 4, this hybrid clone contains the human c-*myc* homologue.

	Human Chromosomes																							
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Human c-myc
DSK1B2A5 Cl2			■				■	■						■		■	■					■		+
DSK1B2A5 Cl20														■	■		■	■			■	■	■	−
Nu 9						■	■																	−
PAFxBalbIV Cl5		■			■			■				■							■					+
GMxLM Cl3				■											■		■			■				−
GMxLM Cl4				■				■							■		■			■		■		+
GMxLM Cl5				■				■				■			■					■		■		+
GMxLM Cl6				■							■	■				■				■		■		−
706B6-40 Cl17								■																+
77-B10 Cl5	■				■			■	■	■			■	■		■				■			■	+
77-B10 Cl8	■		■		■			■	■				■	■				■		■			■	+
77-B10 Cl25	■		■		■				■	■			■	■						■			■	−
77-B10 Cl26	■		■		■			■	■	■				■				■		■			■	+
77-B10 Cl28	■		■		■									■				■					■	−
77-B10 Cl30			■		■					■			■	■						■			■	−
77-B10 Cl31	■		■		■			■	■	■			■	■				■		■			■	+

FIG. 2. Presence of the human c-*myc* homologue in a panel of rodent–human hybrid clones. A black square indicates that the hybrid clone named in the left column contains the chromosome named in the upper row. An empty square indicates that the hybrid clone has lost the human chromosome indicated in the upper row.

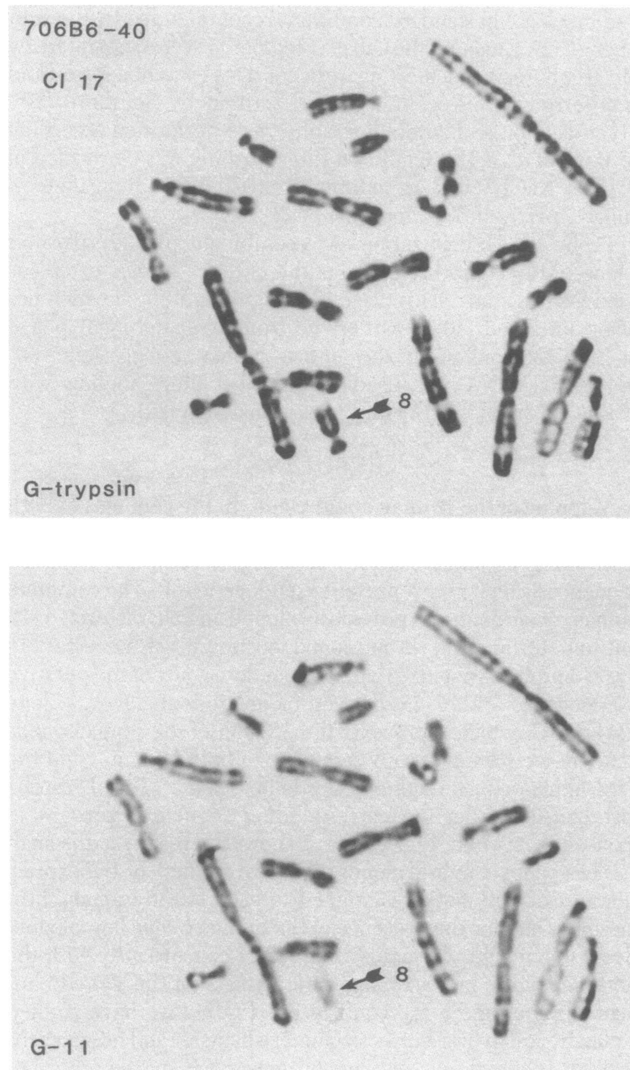

FIG. 3. Metaphase of Chinese hamster–human hybrid 706B6-40 C1 17, which has retained human chromosome 8 and no other human chromosome, stained by the trypsin/Giemsa method (*Upper*) and by the G-11 method (*Lower*).

Regional Mapping of the Human c-*myc* Gene. Specific chromosome rearrangements involving human chromosome 8 are a common finding in Burkitt lymphoma (23, 24). In this disease a reciprocal chromosome translocation between chromosome 8 and either chromosome 14, 2, or 22 has been observed (23–26) and the breakpoint on chromosome 8 is at band q24. We have previously characterized somatic cell hybrids between mouse cells and either Daudi (15, 16) or P3-HR1 (13) Burkitt lymphoma cells and have obtained hybrids that contain the $14q^+$ chromosome characteristic of Burkitt lymphoma cells (13, 15, 16). Hybrids M44 C1 2S5 and M44 C1 2S9 retained the human $14q^+$ chromosome from the P3-HR1 parent and no other human chromosome (unpublished data). Both of these hybrid clones

Table 1. Presence of the human c-*myc* homologue in hybrids with the Burkitt lymphoma $14q^+$ chromosome

Hybrids	Isozymes		Chromosomes				Human
	NP	GSR	8	$8q^-$	14	$14q^+$	c-*myc*
M44 C1 2S5	+	−	−	−	−	+	+
M44 C1 2S9	+	−	−	−	−	+	+
3F1	+	−	−	−	−	+	+

NP, nucleoside phosphorylase; GSR, glutathione reductase.

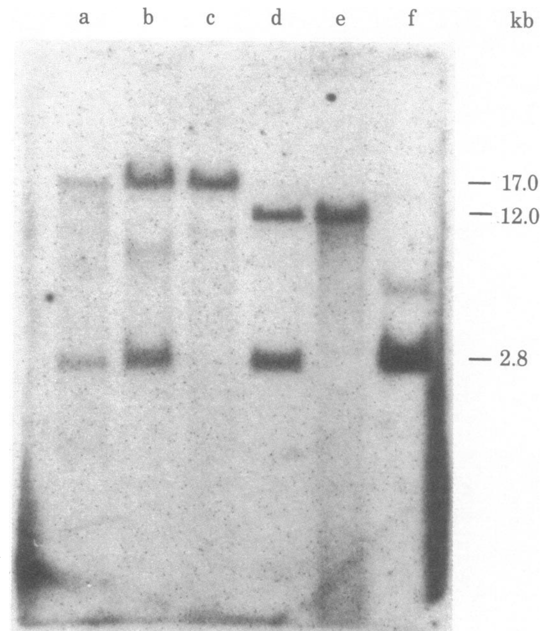

FIG. 4. Presence of the human c-*myc* homologue in rodent–human cell hybrids. Lanes: a, hybrid 3F1 DNA; b and c, hybrids DSK1B1A5 C1 2 and DSK1B2A5 C1 2 DNAs, respectively; d, hybrid 706B6-40 C1 17 DNA; e, CHO-K1 Chinese hamster DNA; f, normal human DNA.

have retained the human c-*myc* gene (Table 1). Hybrid 3F1, which has retained the $14q^+$ chromosome from the Daudi parent and has lost the normal 8 and the $8q^-$, contains the human c-*myc* gene as well (Fig. 4, lane a; Table 1). Therefore, we conclude that the human c-*myc* gene is located on region q24→qter of human chromosome 8 (Fig. 5). Because the human c-*myc* gene is present in the $14q^+$ chromosome of both Daudi and P3-HR1 cells we also conclude that the human c-*myc* gene is distal to the breakpoint on human chromosome 8 in these independent Burkitt lymphoma cell lines. No obvious rearrangement of the human c-*myc* DNA segment was detected in the $14q^+$ chromosome of two Burkitt lymphoma cell lines (Fig. 4, lane a).

DISCUSSION

Nonrandom chromosome translocations and consistent chromosome changes have been observed in numerous human malignancies (27, 28). We have hypothesized that a knowledge of the chromosomal location of human homologues of known ret-

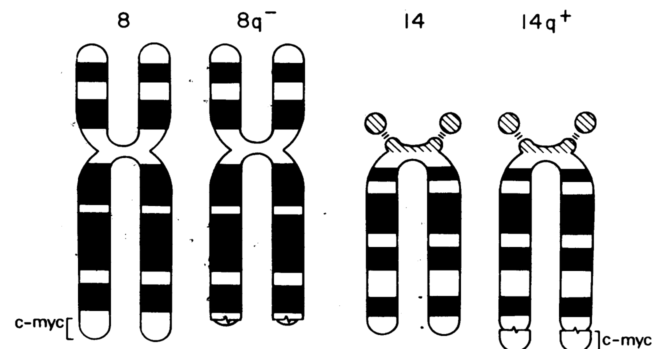

FIG. 5. Diagram of the t(8;14) chromosome translocation observed in Burkitt lymphoma. The reciprocal translocation results in the formation of two marker chromosomes: $8q^-$ and $14q^+$. The results of the experiments described in Table 1 indicate that the human c-*myc* homologue is distal to the breakpoint on human chromosome 8 in both Burkitt lymphoma cell lines that were analyzed.

roviral oncogenes might provide important information concerning their possible involvement in the expression of neoplasia (29, 30). Hypothetically, the juxtaposition of an *onc* gene to an active promoter due to chromosome translocation might result in its abnormal expression and in malignant transformation.

The human c-*myc* homologue is located on human chromosome 8, as shown by the analysis of rodent–human somatic cell hybrids. A consistent chromosomal change involving chromosome 8 is the cytogenetic hallmark of human Burkitt lymphomas. In this malignancy a reciprocal translocation involving a portion of chromosome 8 and chromosome 14 or, less frequently, chromosomes 2 and 22 has been described repeatedly. Interestingly, the same portion of chromosome 8 is consistently involved and chromosomes 14, 2, and 22 carry immunoglobulin genes (14, 31–33). In the case of chromosome 14, it has actually been shown that the breakpoint occurs within the region carrying human heavy chain, variable region genes (16). In this study we show that the c-*myc* gene is located on the same region of chromosome 8 that is translocated in Burkitt lymphoma malignant B lymphocytes. This observation, together with the fact that c-*myc* activation causes a B-cell malignancy in chickens (5, 7), points toward possible involvement of the c-*myc onc* gene in Burkitt lymphoma.

In a study of a limited number of Burkitt lymphoma cell lines *myc*-related RNA levels were not significantly above the levels found in other human normal or neoplastic tissues (9). These findings argue against the simple model of increased c-*myc* expression after translocation in Burkitt lymphoma. In this respect, the c-*myc* location on region q24→qter of chromosome 8 may be coincidental and may not be involved in the process of malignant transformation. However, it should be noted that although *myc* mRNA levels are not elevated in Burkitt lymphoma, the malignant cells represent a population of B lymphocytes for which normal controls at the same stage of differentiation have not been investigated. Thus, *myc* mRNA levels detected in Burkitt lymphoma cells may be different than those in the normal counterpart populations. Alternatively, a failure in gene regulation rather than abnormal activation may be critical. The genomic relocation of the c-*myc* gene may have caused a failure in mechanisms that control repression of this gene. This possibility is suggested indirectly by the evidence that c-*myc* expression is switched off during *in vitro* differentiation of the human myeloid cell line HL-60 (9). Constitutive expression of c-*myc* resulting from a chromosomal translocation in Burkitt cells or from gene amplification in HL-60 cells (10) may prevent normal cellular differentiation by maintaining the cells in an immature proliferative state.

Although these alternatives have not yet been examined, the results presented here suggest that a more detailed study of c-*myc* expression is needed in human normal and transformed B lymphocytes as well as in the frequent cases of acute nonlymphocytic leukemias with chromosome 8 trisomy (34).

Note Added in Proof. At the time this manuscript was submitted for review, we have found rearrangements at the 5′ region of the c-*myc* gene in 6 of 11 Burkitt lymphoma cell lines (8, 14) with the chromosome translocation. In some cases, for example, in the cell lines CA46 and JD, the rearrangement brings the c-*myc* gene in close proximity of the $C\mu$ immunoglobulin gene. On the other hand, no rearrangement was detected within 20 kb 5′ of the c-*myc* gene in the Daudi cell line (16), indicating heterogeneity in the breakpoint on both chromosomes 8 and 14 in Burkitt lymphoma. Similar data have been reported by Taub *et al.* (35).

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