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25. The selection for Km^R also prevents us from recovering deletions from the Km^R-proximal IS*903* into *galK* since all of these would lose kanamycin resistance (and none could give two recoverable products whether deletions were reciprocal or not).
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Translocations Among Antibody Genes in Human Cancer

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Geneticists have become increasingly aware of the fact that genes move, that they change position within and between chromosomes, and that they thus alter their relationships to one another with important regulatory and structural consequences. One dramatic example of this occurs as part of the process by which the immunoglobulin (Ig) genes are normally formed by joining subgenomic segments of DNA in various combinations to create the enormous diversity reflected by the immune system (*1*). Another example, by no means normal and with an apparently more sinister outcome, is represented by the somatic translocations that consistently accompany certain human neoplasms (*2*). In recent months, these two phenomena have been physically linked by studies showing that the human cellular oncogene c-*myc* is joined to one of the Ig loci by a translocation that characteristically accompanies a human malignancy called Burkitt lymphoma (*3–7*). Similar results have been obtained in mouse plasmacytomas (*8–13*).

The juxtaposition of these two rather well characterized genetic systems allows us to address two important—if poorly understood—questions: First, what are the molecular genetic consequences of these translocations and how do they alter the normal function and control of these genes? And second, how does their interaction contribute to the process of malignant transformation? While work in a number of laboratories is still in its earliest stages, certain observations already allow us to evaluate the most obvious models and to advance others. Our purpose here will be to consider briefly the normal Ig and *myc* genes before turning to specific molecular aspects of certain Burkitt lymphoma translocations and the early answers they bring to the questions we have posed.

Associations Between Chromosomal Translocations and Neoplasia

The close association between specific chromosomal translocations and certain human neoplasms is well established (*2*). Such translocations are of two types: constitutional, that is, those carried by each of the individual's cells; and somatic, those that arise in a particular cell and are carried by its neoplastic progeny. It is generally held that such constitutional translocations (and other chromosomal abnormalities) predispose an organism to the development of a malignancy, but require a second event, presumably another mutation, to consummate the malignant transformation (*14*). For example, in hereditary renal cell carcinoma there is a constitutional translocation involving chromosomes 3 and 8 [t(3;8)] that is associated with the development of renal cell carcinoma during the fourth decade of life (*15*). Somatic translocations, in contrast, presumably arise in a single somatic cell and contribute to the transformation of that cell and its progeny alone. It is not likely that either translocation is sufficient to accomplish the malignant transformation; it is quite likely that other critical events have occurred or will occur (*16*). Nevertheless, the tight association between particular malignancies and specific translocations [t(9;22) in chronic myelogenous leukemia and t(2;8), t(8;14), and t(8;22) in Burkitt lymphoma] makes a very strong, if circumstantial, argument that these translocations are causally related to the development of the neoplasm.

The discovery of oncogenes, originally noted as transforming genes carried or activated by retroviruses [see review (*16*)], combined with a growing understanding of transcriptional control mechanisms, provided the basis for two molecular models that could explain how a translocation might induce malignancy (*17*). A translocation could disturb the regulation of an oncogene, for example, by providing a new promoter region or some other control element that would activate the oncogene (*18*). Alternatively, it might alter the coding sequence of a gene, changing its protein product from a benign to a malignant form (*19*).

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Summary. The characteristic chromosomal translocations that occur in certain human malignancies offer opportunities to understand how two gene systems can affect one another when they are accidentally juxtaposed. In the case of Burkitt lymphoma, such a translocation joins the cellular oncogene, c-*myc*, to a region encoding one of the immunoglobulin genes. In at least one example, the coding sequence of the rearranged c-*myc* gene is identical to that of the normal gene, implying that the gene must be quantitatively, rather than qualitatively, altered in its expression if it is to play a role in transformation. One might expect to find the rearranged c-*myc* gene in a configuration that would allow it to take advantage of one of the known immunoglobulin promoters or enhancer elements. However, the rearranged c-*myc* gene is often placed so that it can utilize neither of these structures. Since the level of c-*myc* messenger RNA is often elevated in Burkitt cells, the translocation may lead to a deregulation of the c-*myc* gene. Further, since the normal allele in a Burkitt cell is often transcriptionally silent in the presence of a rearranged allele, a model for c-*myc* regulation is suggested that involves a trans-acting negative control element that might use as its target a highly conserved portion of the c-*myc* gene encoding two discrete transcriptional promoters.

The Burkitt Lymphoma Model

The matter of how a translocation might affect an oncogene was put in perspective by Ohno *et al.* (*20*), who noticed that there was a correlation between the chromosomes involved in the translocations in human Burkitt lymphomas and murine plasmacytomas and the chromosomes that carry the antibody heavy and light chain genes. Since these translocations occurred in antibody producing cells, Klein (*21*) suggested that such translocations might introduce an oncogene into a position close to one of the Ig transcriptional promoters. The association between Ig genes and the observed translocations was strengthened by mapping data that placed the Ig heavy chain genes on chromosome band 14q32, the κ light chain gene on chromosome band 2p11, and the λ light chain gene on chromosome band 22q11 (*22*), precisely the chromosomal bands representing the breakpoints seen in the Burkitt translocations (albeit that these "bands" extend over 10^7 base pairs of chromosomal DNA). Furthermore, when one of the light chain chromosomes (2 or 22) was involved in the translocation, the affected chromosome usually carried a light chain gene of the type expressed in that particular cell line (*23*). For example, Burkitt cells that contain translocations involving chromosome 2 usually make κ rather than λ light chains. This further suggested that the translocations might involve an actively transcribed locus.

It is also important to recognize that the translocations noted in the Burkitt lymphoma are reciprocal, that is, they represent grossly conservative exchanges of large chromosomal segments (Fig. 1). Moreover, the chromosome common to each of the translocations is chromosome 8 which, according to the model (*21*), should encode the critical oncogene.

The Combinatorial Nature of Immunoglobulin Loci

The Ig loci involved in these translocations have a very special property: they undergo site- or region-specific recombination that is critical for the development of antibody diversity (*1*). As shown in Fig. 2, one such recombination joins widely separated segments of DNA called V, D, and J regions to form active variable (V) regions in heavy (H) and light (L) chain loci. The other, switch (S) recombination, operates only in the heavy chain locus and moves or "switches" the finished heavy chain V-region gene from one heavy chain constant region to another. Both of these types of recombination are a required part of immunocyte development. The physical maps of all three human loci are shown in Fig. 2, together with a diagrammatic representation of the recombinational events.

The signal sequences thought to be involved in VDJ and VJ recombination, pairs of hepta- and nonanucleotides, occur on the edges of the V, D, and J segments that are joined to form the intact V-region sequence. The switch region signals, on the other hand, consist of 1- to 2-kilobase segments of DNA largely made up of recurring pentanucleotide sequences (*24*). One such segment is encoded on the 5′ side of each of the heavy chain constant region genes (except the delta gene). During the switch reaction, the completed V region, initially adjacent to the μ constant region gene, is joined to one of the other switch signals transferring the V region with its encoded specificity and transcriptional control signals to one of the successively encoded heavy chain genes. In this way, each of the γ, ε, and α heavy chains can be expressed in the descendents of a precursor cell that originally expressed only the μ heavy chain. Delta gene expression and the choice between membrane and secreted Ig's seem to depend on alternative messenger RNA processing pathways (*25*).

The Regulatory Consequences of Immunoglobulin Gene Recombination

Both recombinational events occurring during immunocyte maturation have important implications with regard to the regulated expression of the Ig genes. The heavy chain V region is formed by selecting one of several hundred V-region subgenes and joining it to one of several dozen D segments (D segments occur in the heavy chain locus only) and then to one of five or six J segments (see Fig. 2). Since each of the V-region subgenes has a promoter region encoded close to its 5′ end, VDJ recombination brings this promoter close to the constant region sequence, thereby forming a complete Ig coding sequence with its appropriate transcriptional initiation site.

The paradox of why the many potential promoter regions are inactive in their germ line (unrearranged) configuration (*26*) and active when drawn close to the constant region subgene was partially resolved by the recent discovery that a small segment of DNA between the J and switch regions in the heavy chain and between the J and constant regions in the κ chain gene is actually a B-cell specific enhancer sequence (*27*). The enhancer is apparently necessary for the activation of the V-region associated promoters in B cells. (The enhancer sequences are indicated diagrammatically in the heavy chain and κ loci shown in Fig. 2.) Enhancer sequences have yet to be identified in the λ locus or in species other than the mouse, but since sequences homologous to known mouse enhancer sequences are conserved in man (*28*), it is reasonable to postulate that they will be found in other species as well. Thus, at least two elements occur within the Ig loci that could influence gene expression in a B-cell specific manner, the V-region associated promoters and the constant region enhancer sequences.

The Normal Human *myc* Gene

While little is known about the actual function of the c-*myc* protein, the structure of its gene is known in detail (*29*). The gene is encoded in three discontinuous exons separated by two large intervening sequences as shown diagrammatically in Fig. 3A. The first exon has several interesting features (Figs. 3A and 4), including the fact that it forms the major portion of a segment of DNA that is tightly conserved between man and mouse (*30*). Given that this tight evolutionary conservation suggests functional selection, it was surprising to discover that the 550-base-long segment contained no translational initiation codons (eight would have been expected in a random sequence of this length). Furthermore, the sequence contains multiple termination codons in all three reading frames and is, therefore, an untranslated messenger RNA (mRNA) leader sequence. In addition, this region encodes at least two active promoters with transcription initiation sites located about 150 base pairs from one another within the leader sequence (*30*). Curiously, the 5′ ends of transcripts from each of the promoters can be drawn as short stem and loop structures, although many alternative configurations are also possible (*30*).

The remaining portion of the c-*myc* gene contains two coding exons that direct the synthesis of a protein 439 amino acids in length that is particularly rich in amino acids carrying acidic, basic, and hydroxyl groups (*29*). The function of the normal protein is not known. However, when the avian c-*myc* gene is taken up by a retrovirus, it forms a fusion protein with the viral gag (group specific antigen) gene product and, in this form, participates in the transformation of several avian cell types [for a review see (*31*)]. The product of the fused gene is a nuclear protein that binds without apparent specificity to double-stranded DNA (*32*).

Structure of c-*myc* Rearrangements in Burkitt Lymphoma Cell Lines

The search for the crossover point of the translocation that joins chromosomes 8 and 14 in Burkitt lymphoma was facilitated by studies in the mouse that had detected a series of aberrantly rearranged fragments of DNA that were joined to the α Ig switch signal in a large number of murine plasmacytomas (*33*). By taking advantage of the fact that the avian leukosis virus frequently integrates near and activates a c-*myc* gene in avian B-cell lymphoma, we and others were able to show that the murine c-*myc* and the Ig α constant region genes were physically linked in a single fragment of genomic DNA (*8–13*). By using appropriate probes it was further shown that the c-*myc* and the human Ig μ genes were joined in several Burkitt lymphoma cell lines and that c-*myc* had undergone some form of rearrangement in the majority of Burkitt cell lines (*3–7*). An example of such an experiment is shown in Fig. 3B, in which a probe corresponding to the midportion of the human c-*myc* gene detects c-*myc* encoding Eco RI fragments of DNA derived from several Burkitt cell lines. As shown in the figure, the presence of rearranged and nonrearranged c-*myc* fragments suggests that only one of the two c-*myc* alleles has undergone rearrangement in these cell lines and that this rearrangement has occurred within a few thousand bases of the c-*myc* gene. Note, however, that the cell line SER displays only a germ line band implying that the translocation has occurred at a distance greater than can be assessed by using Eco RI restriction sites.

The exact nature of several of these c-*myc* rearrangements has been determined by cloning the relevant genes and determining their structure by direct sequence analysis (*30, 34*). Two examples of rearranged c-*myc* genes derived from Burkitt cell lines are diagramed in Fig. 5. In both cases the c-*myc* gene is joined to a switch region sequence located 5′ to the μ heavy chain constant region gene, the region normally involved in switch-type recombination. In both cases the orientation of the c-*myc* and Ig μ genes are in opposite transcriptional directions. In the case of cell line A (Fig. 5A), the c-*myc* promoter or leader sequence has been retained, but in line B (Fig. 5B), this exon has been lost as a consequence of recombination. Since the observed translocations are reciprocal, the reciprocal product should also be present within the Burkitt cells (*35*). Though these reciprocal products have not been characterized in detail, their expected structure is shown diagrammatically in

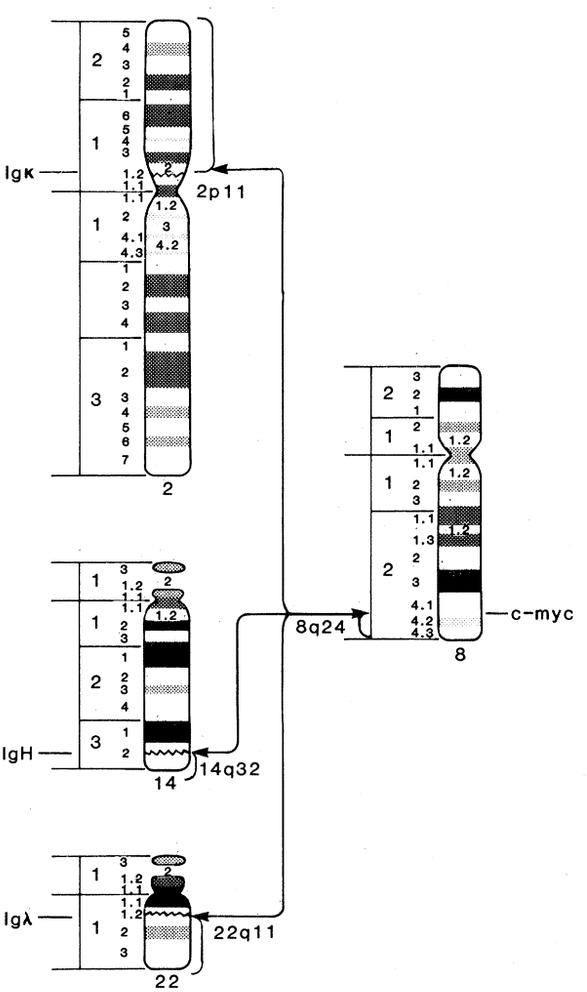


Fig. 1. Diagrammatic representation of the human chromosomes involved in the specific translocations of Burkitt lymphoma. Chromosomes 2, 14, 22, and 8 are shown with their characteristic Giemsa-banding patterns. The positions of the Igκ, IgH, and Igλ (22) chains are indicated, as is the position of the c-*myc* gene. The arrows point to breakpoints at which chromosomes 2, 14, or 22 reciprocally exchange chromosomal segments with chromosome 8. Approximately 75 percent of the translocations involve chromosomes 8 and 14 (*44*).

order to indicate that the enhancer sequence normally associated with the heavy chain μ region would not be retained by the chromosome that carries the rearranged c-*myc* gene (compare to Fig. 2). Thus, these rearranged c-*myc* genes can neither use a promoter region normally used by Ig heavy chain genes, nor can they be influenced by its known enhancer. In addition, the complete sequence of the amino acid coding exons of the translocated c-*myc* gene shown in Fig. 5A (BL22) has been determined and is identical to that of the normal gene, suggesting that their protein products are also qualitatively identical (*30*).

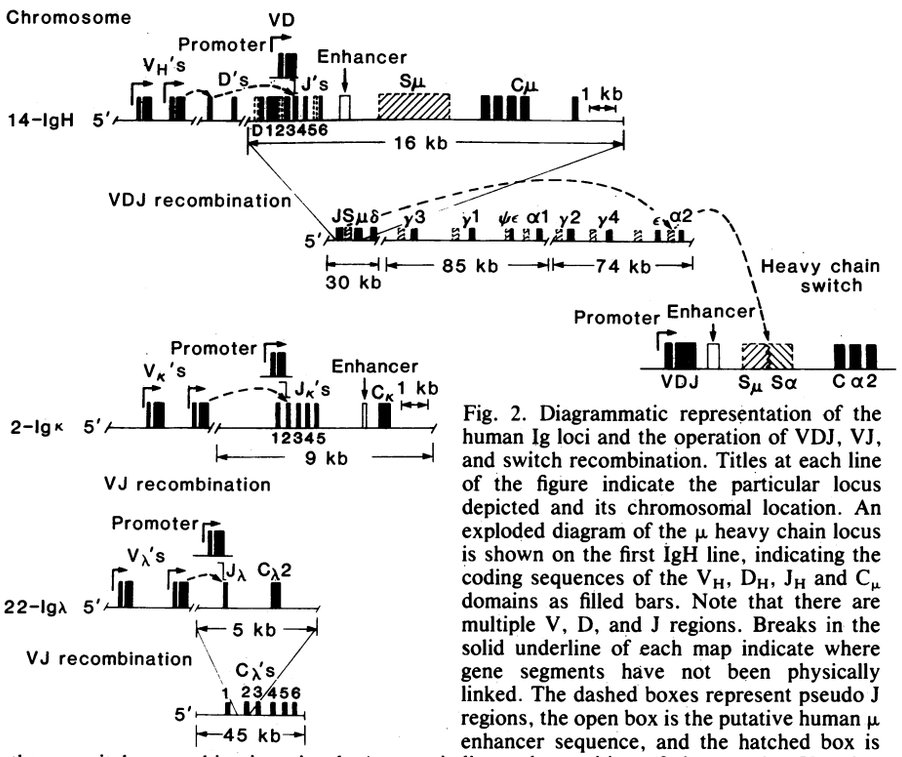


Fig. 2. Diagrammatic representation of the human Ig loci and the operation of VDJ, VJ, and switch recombination. Titles at each line of the figure indicate the particular locus depicted and its chromosomal location. An exploded diagram of the μ heavy chain locus is shown on the first IgH line, indicating the coding sequences of the V_H, D_H, J_H and $C_μ$ domains as filled bars. Note that there are multiple V, D, and J regions. Breaks in the solid underline of each map indicate where gene segments have not been physically linked. The dashed boxes represent pseudo J regions, the open box is the putative human μ enhancer sequence, and the hatched box is the μ switch recombination signal. Arrows indicate the position of the putative V-region promoters and above the line is a recombined VD sequence at the position where it will join a J region to form a complete and active VDJ sequence (VDJ recombination). The second line shows the physical map of the entire heavy chain locus. The dashed arrows indicate segments joined by a sample switch reaction which draws a complete VDJ sequence from its initial position adjacent a μ constant region domain to a switch signal near the α2 heavy chain gene. An exploded diagram of the "switched" gene is shown in the last line with the retained enhancer indicated. The second diagram (Igκ) similarly shows the human κ locus with a dashed arrow indicating the path of VJ recombination. The putative enhancer region is shown. The first line of the third diagram (Igλ) shows an exploded diagram of VJ recombination occurring in one of the six human λ constant region genes. Note there are multiple V regions, but presumably only one J region associated with each of the six λ constant region sequences (lower line).

Consequences of c-*myc* Translocation at the Level of Expression

The activity of the c-*myc* gene as measured by the steady-state level of c-*myc* mRNA is elevated in a number of human non-Burkitt tumors (*36*). The situation in Burkitt lymphoma is less clear and the analysis is made difficult because little is known about the expression of c-*myc* in normal tissue (*5, 7, 37*). Nevertheless, our own experience indicates that the level of c-*myc* transcript in Burkitt lines is usually, but not always, elevated when compared to EBV-immortalized, nonmalignant lymphoblastoid cells (*38*). Hence, there is variation in c-*myc* expression among Burkitt lines. Similarly, certain mouse plasmacytomas seem to have increased levels of c-*myc* transcript while others do not (*10, 11, 13, 39*).

In the absence of consistent findings, we have turned to a more revealing analysis of the c-*myc* mRNA transcript in Burkitt cells. By using S1-nuclease analysis, we are able to detect and quantitate the steady-state levels of the mRNA transcripts derived from each of c-*myc*'s two promoters. As shown in Fig. 6, the normal gene in the lymphoblastoid cell line, IARC 100, uses both promoter regions with the steady-state level of transcripts favoring the promoter producing the shorter transcript, P2. However, in the two Burkitt lines the longer transcript increases relative to the shorter.

Obviously, this relation between c-*myc* promoters will not prevail in every Burkitt cell line (see, for example, the c-*myc* gene illustrated in Fig. 5B which has lost its promoter or leader exon entirely). Indeed, most of the c-*myc* genes analyzed in murine plasmacytomas have lost their promoter-leader exons (*8–13*). Nevertheless, the conserved nature of the sequence, the presence of dual promot-

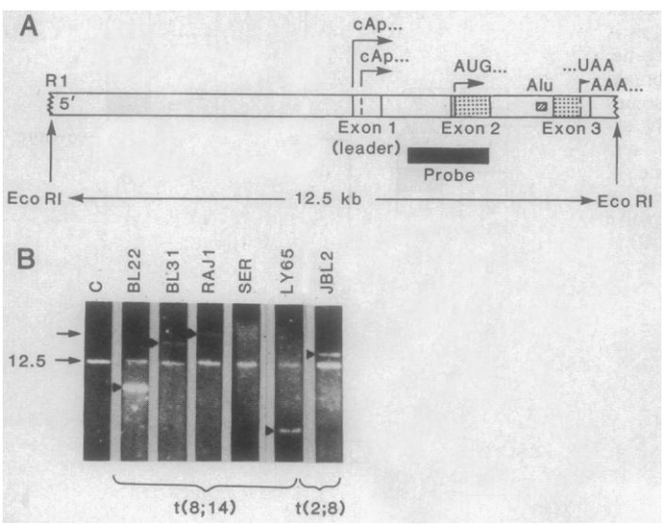


Fig. 3. Physical map of the human c-*myc* gene and demonstration of rearranged c-*myc*–containing fragments in Burkitt lymphoma cells. (A) The diagram shows a 12.5-kb Eco R1 fragment encoding the c-*myc* gene. The boxes within the segment represent the three c-*myc* exons. The first exon encodes two transcription initiation points, indicated as arrows with the "cAp. . ." symbols. The position of the translational initiation and termination codons are shown, as is the polyadenylate addition site (AAA. . .). A small hatched box in the second intervening sequence indicates the position of a highly repeated "Alu" sequence. (B) Each lane represents an in situ hybridization analysis (Southern blot) of DNA derived from normal and Burkitt lymphoma cell lines that have been digested with Eco R1 and probed with the c-*myc* DNA fragment [indicated by the solid bar in (A)]. The designation of each Burkitt cell line is indicated above each lane and the translocation found in each line is indicated below each lane. These rearrangements have been described previously (*3*). Lane C, the control, contains DNA derived from normal human white blood cells. The normal 12.5-kb Eco R1 fragment is indicated by an arrow and the rearranged c-*myc* fragments are indicated by triangles.

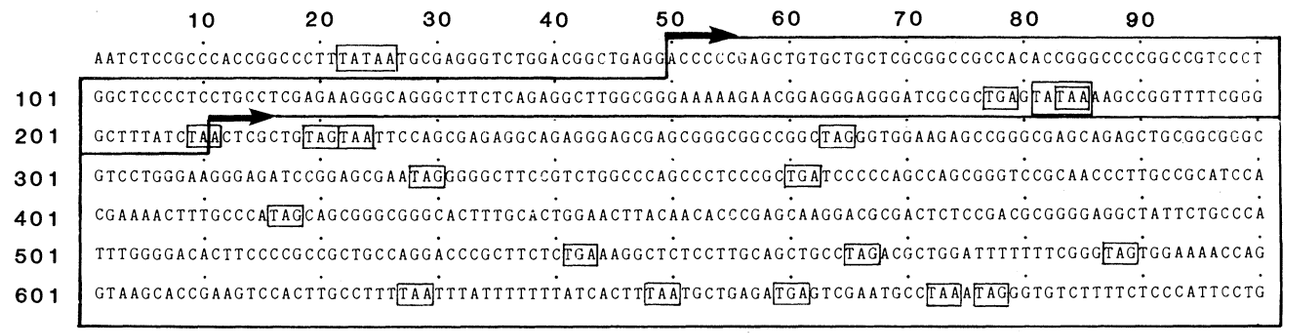


Fig. 4. Sequence of the untranslatable leader exon of the c-*myc* gene. The sequence of the leader exon (*29*) is shown with small boxes around its two TATAA boxes and each of the termination codons that occur within its transcribed sequence. The transcribed sequence is also boxed with heavy arrows indicating the positions of the two transcription initiation points.

ers, the inversion of transcript ratios, and the frequent loss of the promoter-leader sequence in oncogenic translocations point to the importance of this region in regulating the expression of the c-*myc* gene. Furthermore, a number of studies in the mouse system have indicated that expression of the normal (unrearranged) allele is often profoundly reduced in plasmacytomas carrying a rearranged c-*myc* allele (*6, 13*). Indeed, in each case in which we have been able to distinguish the products of the normal and translocated alleles in Burkitt cell lines, we have found that the transcript of the normal allele is either undetectable or profoundly reduced by comparison to that of the product of the translocated allele (*38*). Very recent experiments involving the expression of normal and translocated Burkitt chromosomes in mouse myeloma–human Burkitt lymphoma cell lines are also consistent with this conclusion (*40*).

The Deregulation of c-*myc*: A Model

In view of the variety of crossover points that occur in c-*myc* translocations, we are likely to find that there are a number of mechanisms by which the regulation of the c-*myc* can be altered. Indeed, the collection of translocations represented in the Burkitt lymphomas provides an array of mutations that will help us to understand the controls that operate upon both the c-*myc* and Ig genes. To this end, any regulatory scheme should allow us to explain at least three observations: the fact that the normal c-*myc* allele is often inactive or less active in the presence of the rearranged allele, the significance of the conserved dual promoter-leader exon of the c-*myc* gene and its P1/P2 transcript ratio, and the fact that the Ig loci are so consistently involved in these B-cell translocations.

Granting that little is known, let us nevertheless consider the regulatory model shown diagrammatically in Fig. 7. The model holds that that c-*myc* is normally regulated by a trans-acting repressor. More complex models, including those that involve positive control elements, are possible; but negative regulation requires fewer assumptions. It is also attractive to suppose that this repression is medicated directly or indirectly by the cellular level of the c-*myc* protein, although repression unrelated to the c-*myc* protein cannot be ruled out. Thus, an elevated level of c-*myc* protein produced from the deregulated, translocated allele in a Burkitt cell could bring about the repression of the normal c-*myc* allele. Let us further assume that the target of this trans regulation is the dual promoter-leader segment conserved at the 5′ region of the c-*myc* gene. Since some of the translocations we observe damage this region (*38*), they can be thought of as operator-promoter constitutive mutations that defeat this control. This could be accomplished by deleting the first exon or subtly altering its structure. Such alterations occur even at a great distance from the site of chromosomal recombination (*39*). Alternatively, deregulation could also occur by substituting an overriding positive control element for a flanking region of c-*myc* DNA or by changing the structure of the c-*myc* or succeeding regulatory proteins (a repressor constitutive mutation).

The control function could also operate either to influence the level of mRNA, the promoter used, or both. The fact that the two promoter regions generate mRNA's that differ in their untranslated leader sequences and are differentially expressed in lymphoblastoid and Burkitt cells allows us to suggest that deregulation, in certain cases, shifts transcription to the first promoter region generating mRNA's with longer leader sequences which, in turn, might affect their ability to direct the synthesis of c-*myc* protein. It is also possible that the regular control element acts as a destabilizer of c-*myc* mRNA rather than a con-

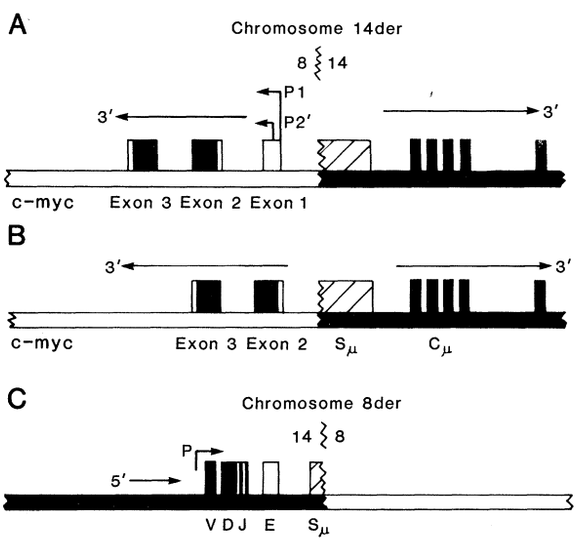
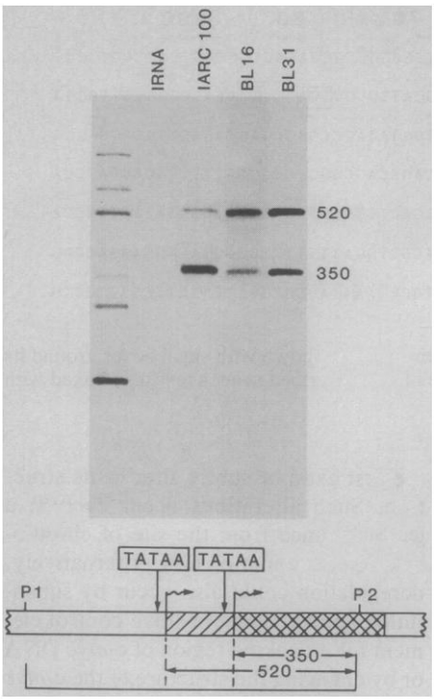


Fig. 5. Diagrammatic representation of DNA fragments formed by translocations between chromosomes 8 and 14. (A) Map of the c-*myc* (open bar) and Ig μ (filled bar) genes as they are rearranged in the Burkitt line BL22 (*30*). The coding sequences are indicated by boxes on the chromosomal DNA segment. The filled boxes have amino acid–coding function. The hatched box is the μ switch signal. The transcriptional direction of each segment is given by the arrow above it. (B) Map of the genes as they rearranged in the Burkitt line Ly 65 (*46*). Symbols are as in (A). (C) Hypothetical representation of the reciprocal product that one would expect in the translocation shown in (A) (BL22), assuming it were entirely conservative and assuming the Ig gene had undergone VDJ recombination. The symbols have the same value as above, but the VDJ segment refers to completed IgH V-region gene (see Fig. 2) and the E segment refers to the position of the suspected human IgH enhancer sequence (*26*).



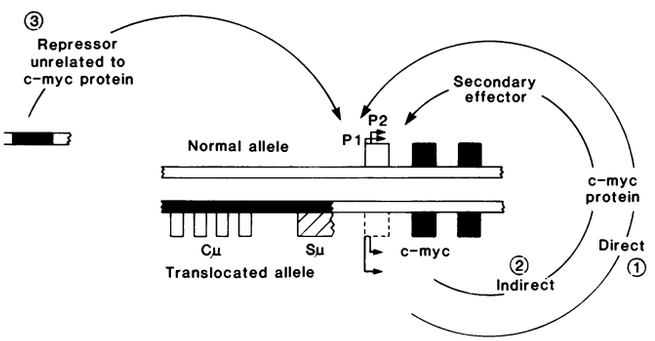


Fig. 6 (left). Analysis of the transcripts derived from normal and Burkitt lymphoma cell lines. IARC 100 is a lymphoblastoid control cell line, whereas BL16 and BL31 are Burkitt cell lines that carry the 8;14 translocation. The mRNA was analyzed by the technique of Berk and Sharp (*45*) as modified (*30*, *46*) to obtain uniformly labeled, single-stranded probes that were synthesized by cloning the Pst (P) fragment indicated in the figure in the MP9 vector system. The first lane represents size control standards (an Eco R1/Hind III digest of the plasmid pBR322) of the following lengths: 1630 bp, 1000 bp, 630 bp, 507 bp, 396 bp, 344 bp, 298 bp, 220 bp, and 150 bp. The bottom diagram shows the map of the probe derived from the first c-*myc* exon. The position of each of the two TATAA boxes is shown as are the expected lengths of the protected fragments that would arise from the two c-*myc* promoters. Fig. 7 (right). A model for the trans negative control of normal c-*myc* expression. The diagram of the normal (upper) and translocated c-*myc* (lower) genes are as described in Fig. 4. The two c-*myc* promoters are indicated by arrows and the symbols P1 (5′ most) and P2. The arrows indicate the direction of transcription. The model is described in the text but indicates three potential models of trans-mediated repression of the normal c-*myc* gene: (i) direct autorepression by the c-*myc* protein, (ii) indirect autoregulation by a repressor that responds to the level of c-*myc* protein, or (iii) regulation by a repressor unrelated to the c-*myc* protein. The target of this repression is suggested to be the conserved dual promoter-leader sequence of the c-*myc* gene. In some Burkitt translocations this target is damaged or lost, thus releasing the translocated c-*myc* gene from this trans control, altering its expression or promoter choice (P1 or P2) in the Burkitt cell (see Fig. 5B). The negative control element is referred to as a repressor but could also serve to destabilize c-*myc* mRNA.

ventional repressor that would prevent its transcription.

One virtue of this trans-negative control model is that it is consistent with the behavior of the normal c-*myc* allele in Burkitt cells and it allows us to focus on an evolutionarily conserved segment of the c-*myc* gene as a potential cis-acting control site. The other virtue, in view of the availability of DNA-mediated B-cell transforming protocols (*19*) and the relevant cloned genes, is that it is not difficult to design tests for the model. Obviously, it would be useful to have an assay for the level of the c-*myc* protein in control and transformed cells. Progress in this regard has been reported recently in the form of an antibody directed against the avian c-*myc* protein (*41*). It is also possible to link the putative c-*myc* control region to a more easily assayed protein and view its function in cells producing greater and lesser amounts of the c-*myc* transcript. In particular, the possibility that c-*myc* is subject to tight temporal regulation, especially with respect to the cell cycle, deserves consideration.

The Role of the Immunoglobulin Locus

We have alluded to a possible function of the Ig locus in affecting the expression of the c-*myc* gene, by contributing either a powerful positive control element or a region-wide configuration of chromatin that is more conducive to c-*myc* expression in B cells. It is still tempting to consider the role that the recombination systems of the Ig genes might have played in generating these translocations. While it is clear that not all the translocated c-*myc* genes will break into an Ig locus at a switch signal, most of the t(8;14) translocations do involve this region. Thus, although it is now established that the c-*myc* gene does not encode an extensive switchlike sequence (*42*), a convincing evaluation of this possibility awaits the analysis of variant translocations (*23*) in which the c-*myc* gene translocates to one of the light chain loci that do not employ switching functions.

The Role of the c-*myc* Gene in Transformation

The specific role that the c-*myc* protein plays in normal cells is still obscure. Its presence in the nucleus (*41*) and the ability of c-*myc*-related proteins to bind to double-stranded DNA (*32*) lead one to consider any of the cardinal reactions of mitosis, DNA replication, and transcription that might profoundly affect cell growth and division. Once again, answers to this question must await the development of techniques that allow the isolation of the c-*myc* protein and its further biochemical analysis. Specific antisera should greatly facilitate this process, as should the availability of artificial mutations that alter the gene's ability to transform normal cells. In the accompanying article by Weinberg (*16*), it is shown that in certain cell systems c-*myc* cannot transform cells by itself but requires a complementary oncogene. Indeed, an additional transforming gene, B*lym*, has been isolated from Burkitt cells (*43*). Complementation assays, coupled with detailed knowledge of the c-*myc* gene and the Ig region into which it falls, provide an experimental framework in which to seek answers to this important question.

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Cellular Oncogenes and Multistep Carcinogenesis

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Summary. Two dozen cellular proto-oncogenes have been discovered to date through the study of retroviruses and the use of gene transfer. They form a structurally and functionally heterogeneous group. At least five distinct mechanisms are responsible for their conversion to active oncogenes. Recent work provides experimental strategies by which many of these oncogenes, as well as oncogenes of DNA tumor viruses, may be placed into functional categories. These procedures may lead to definition of a small number of common pathways through which the various oncogenes act to transform cells.

Two independent lines of work, each pursuing cellular oncogenes, have converged over the last several years. Initially, the two research areas confronted problems that were ostensibly unconnected. The first focused on the mechanisms by which a variety of animal retroviruses are able to transform infected cells and induce tumors in their own host species. The other, using procedures of gene transfer, investigated the molecular mechanisms responsible for tumors of nonviral origin, such as those human tumors traceable to chemical causes. We now realize that common molecular determinants may be responsible for tumors of both classes. These determinants, the cellular oncogenes, constitute a functionally heterogeneous group of genes, members of which may cooperate with one another in order to achieve the transformation of cells.

Retrovirus-Associated Oncogenes

An initial insight into cellular oncogenes came from study of Rous sarcoma virus (RSV). Retroviruses such as RSV have been studied intensively for the past decade, in part because of their unusual molecular biology involving reverse transcription and the high-efficiency integration of their genomes into the cellular chromosome. Another of their traits, still poorly understood, opened up study of cellular oncogenes: retroviruses are able to pick up and transduce cellular genetic information.

Upon dissecting the genome of RSV, Stehelin, Varmus, Bishop, and Vogt found two distinct portions (*1*). The first portion includes the genes responsible for viral replication, which involves the complex processes of reverse transcription, integration, and progeny virus particle formation. The other portion contains the *src* gene, which enables the virus to induce sarcomas in vivo and to transform chicken fibroblasts in monolayer culture. This *src* gene is now known to encode the structure of the tyrosine kinase termed pp60*src* (*2*). As these workers showed (*1*), the *src* oncogene is not a bona fide viral gene at all, but rather stems from a closely related gene residing in the genome of the chicken. This antecedent gene, sometimes termed a proto-oncogene, is a normal cellular gene and an integral part of the chicken genome (*3*).

This work proved that the cellular genome contains a gene that can exhibit strong transforming properties when properly activated. RSV served as a paradigm for more than 30 other animal retroviruses, each of which was also shown to have acquired a cellular oncogene during its brief evolution. Retroviruses thus represent useful devices to scan the cellular genome for the presence of proto-oncogenes. It seems that these hybrid transforming retroviruses usually exist ephemerally, picking up and activating a host proto-oncogene, inducing a tumor, and dying together with the afflicted host. Timely isolation of the virus from a tumor-bearing host

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