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Chromatin Structure of the Human c-*myc* Oncogene: Definition of Regulatory Regions and Changes in Burkitt's Lymphomas

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A. Introduction

Chromosomal translocations of the *myc* oncogene are a consistent feature of all Burkitt's lymphomas and are also observed in many murine plasmacytomas. These translocations of *myc* occur into the immunoglobulin loci and they result in a general increase in *myc* transcription, but this increase in *myc* is variable [2, 6, 7, 10, 11, 21, 22]. Since *myc* may be regulated during the cell cycle (see [9]), deregulation may mean expression at the inappropriate time, which in turn may result in only a modest overall increase in transcription of *myc* in Burkitt's lymphomas. On the other hand, the true (and unidentified) precursor cell of Burkitt's lymphomas may have a very low level of *myc* transcription and we are as yet unable to assess properly the true increase in transcription as a consequence of translocations.

In any case, another observation points to a loss of the normal control mechanism governing *myc* in Burkitt's lymphomas. The nontranslocated *myc* allele is transcriptionally silent in Burkitt's lymphomas as well as in plasmacytomas [2, 19, 21] and this has led to the prediction that the *myc* gene is under negative control [10, 13]. Thus, to understand how translocations affect *myc* expression it is critical to understand how *myc* is regulated. We therefore identified the presumed regulatory sequences near *myc* by DNAase I hypersensitivity studies [17].

DNAase I hypersensitivity is due to a discrete region on chromatin that is very sensitive to DNAase I [18, 23]. Hypersensitive sites appear near many different DNA sequences which are known to be functionally important for gene expression, as is the case of the immunoglobulin kappa light chain and heavy chain enhancers [14]. In fact, hypersensitive regions may bind regulatory proteins [5].

We will discuss here the location of DNAase I hypersensitivity sites immediately 5′ of *myc* near sequences that we suspect on the basis of other data to be functionally important. We will also dicuss the dramatic difference in chromatin structure between the translocated and the nontranslocated alleles in two Burkitt's lymphomas, BL 31 and BL 22. The nontranslocated allele features one strong hypersensitive site, a probable site for mediating negative transcriptional control of *myc*. The deregulation of the translocated *myc* allele in BL 31 is likely to be the result of the immunoglobulin heavy chain enhancer, juxtaposed with the *myc* gene in that lymphoma.

B. Results and Discussion

In order to study the effect of a translocation on the chromatin structure of *myc*,

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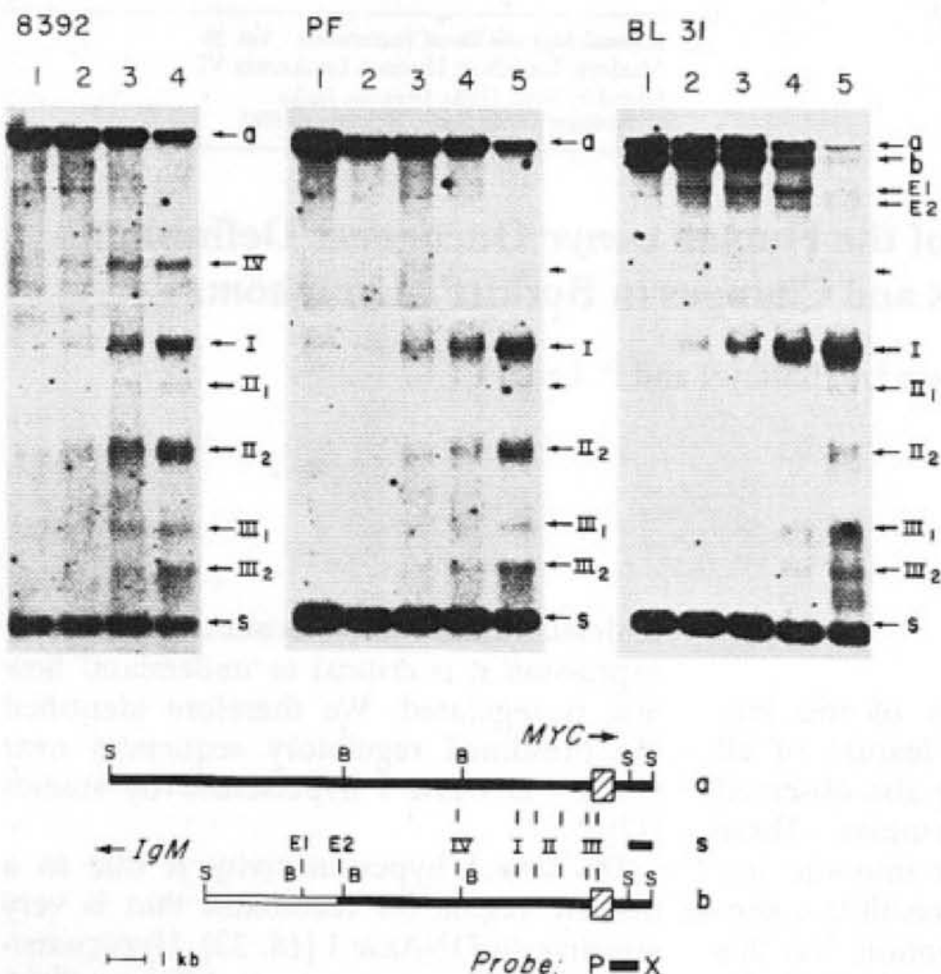

Fig. 1. DNAase-I-hypersensitive sites near *myc* in PF, 8392, and BL 31 cells. Nuclei were digested with increasing amounts of DNAase I (*from left to right*) (for details see [17]) and the isolated genomic DNA was restricted with *Sst* I, electrophoresed, blotted onto nitrocellulose, and hybridized with the PX probe. The hypersensitive sites are labeled I, II_1, II_2, III_1, III_2, IV, E1, and E2. E1 is the location of the immunoglobulin enhancer. *a* represents the germline *myc* fragment and *b* is the translocated *myc* allele, both of which are detected by the probe. *S* is an internal size marker genomic *Sst* I fragment which the probe overlaps. *Solid bar* indicates *myc*-derived sequences, while *open bar* indicates Ig- derived sequences. *The box* represents the untranslated first *myc* exon. *S, Sst* I; *B, Bgl*II; *P, Pvu*II; *X, Xba* I

we initially chose a Burkitt's lymphoma in which the translocation point occurs at a considerable distance from the *myc* oncogene. This less common situation occurs in BL 31. Here the *myc* gene is translocated into the IgM locus, with the crossover point occurring about 6 kb upstream of the first and untranslated *myc* exon. Also unusual, though not unique, is that *myc* is now juxtaposed with the immunoglobulin heavy chain enhancer.

DNAase-I-hypersensitive sites in this Burkitt's cell and in the nonmalignant B-cell lines PF and 8392 (EBV-transformed lymphoblastoid lines) were determined essentially as described by Wu [23] (for details see [17]). In this method, nuclei are digested with increasing amounts of DNAase I. Upon isolating and restricting the DNA, the DNAase-I-cutting sites (hypersensitive sites) can be visualized as subbands on genomic Southern blots, in addition to the original genomic restriction fragment. As is shown in Fig. 1 for the lymphoblastoid lines PF and 8392 and the Burkitt's line BL 31, these subbands appear with increasing amounts of DNAase I, from left to right. PF and 8392 cells contain two germline *myc* alleles (fragment a in Fig. 1), whereas BL 31 cells have one translocated (b) and one germline (a) *myc* band. Clearly several DNAase-I-hypersensitive sites emerge and their positions are indicated on the map in Fig. 1 and summarized in Fig. 2.

The DNAase-I-hypersensitive sites I through III are consistently observed,

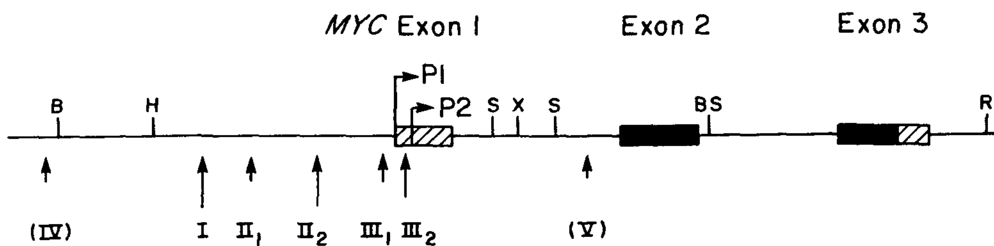

Fig. 2. Location of DNAase-I-hypersensitive sites. The sizes of the *arrows* reflect the approximate relative intensities of the hypersensitive sites in the lymphoblastoid lines. *H, Hind*III; *R, Eco*RI; *P1* and *P2* are the two *myc* promoters; for further description see Fig. 1 and text

whereas sites IV and V are not (site IV is only seen in 8392 cells and site V is very weak in these cells, but much more intense in peripheral T cells; U. Siebenlist, unpublished observation). E1 is located at the immunoglobulin enhancer and E2 lies close to or at the crossover point.

Hypersensitive sites I through III lie within a 2-kb region immediately 5′ of the *myc* gene, a region we thus presume to contain regulatory sequences. Indeed all of these hypersensitive sites coincide with positions that we suspect on the basis of other data to be functionally important. This strengthens our notion that the DNAase-I-hypersensitive sites reflect regions critical to *myc* regulation.

To begin with, the very strong hypersensitive site I is located about 2 kb upstream of the P2 promoter start site, within a sequence region that is well conserved between mouse and man, as seen in a cross-species heteroduplex [1]. Such conservation is usually indicative of functional importance, and, as we will discuss below, this region possibly mediates negative control of *myc.*

The hypersensitive sites III-1 and III-2 are located directly upstream of the two *myc* promoters P1 and P2, respectively. III-1 maps about 100 basepairs 5′ of the P1 'TATA' box in a cystosine-rich stretch of DNA that is very homologous to the – 100 region described by Dierks [4], a region of functional significance for several genes. This sequence may therefore bind a more general transcription factor.

The relatively weak hypersensitive site II-1 lies just 5′ of a sequence which is recognized by a protein from nuclear extracts in vitro (see [17]). Interestingly, site II-2 lies next to a similar sequence. We speculate that the in vitro binding also occurs in vivo, resulting in a hypersensitive site II-1 and possibly also II-2. By comparing these sequences with other competing binding sites next to the human immunoglobulin *mu* gene (L. Henninghausen, unpublished observation) or in the long terminal repeat of adenovirus [17], a conserved sequence emerges (TGGCN_5 GCCAA). The binding site on adenovirus is in fact also recognized by nuclear factor 1, a nuclear protein which has been shown to be necessary for adenovirus replication in vitro [12]. Since purified nuclear factor 1 also binds to the immunoglobulin and *myc* sites (L. Henninghausen, unpublished observation), it is likely to be the protein detected in our nuclear extracts. Although this protein has an identified role in replication of adenovirus, its function at the *myc* locus is yet to be defined.

Is the fact that only the translocated allele in Burkitt's lymphomas is transcribed reflected in the chromatin structures of the two *myc* alleles within the same cell? In BL 31 the two *myc* alleles can be differentiated by employing a probe which hybridizes only to the nontranslocated (germline) *myc* (a), but not to the translocated *myc,* as seen in Fig. 3. The germline *myc* allele in BL 31 has only one hypersensitive site, I, and it is very intense when compared with the contribution from both chromosomes in PF; sites II and III are undetectable. A similar situation exists in BL 22, where the breakpoint on the translocated allele occurs between hypersensitive sites II_1 and II_2 (U. Siebenlist, unpublished observation and [1]). We therefore hypothesize that site I mediates the nega-

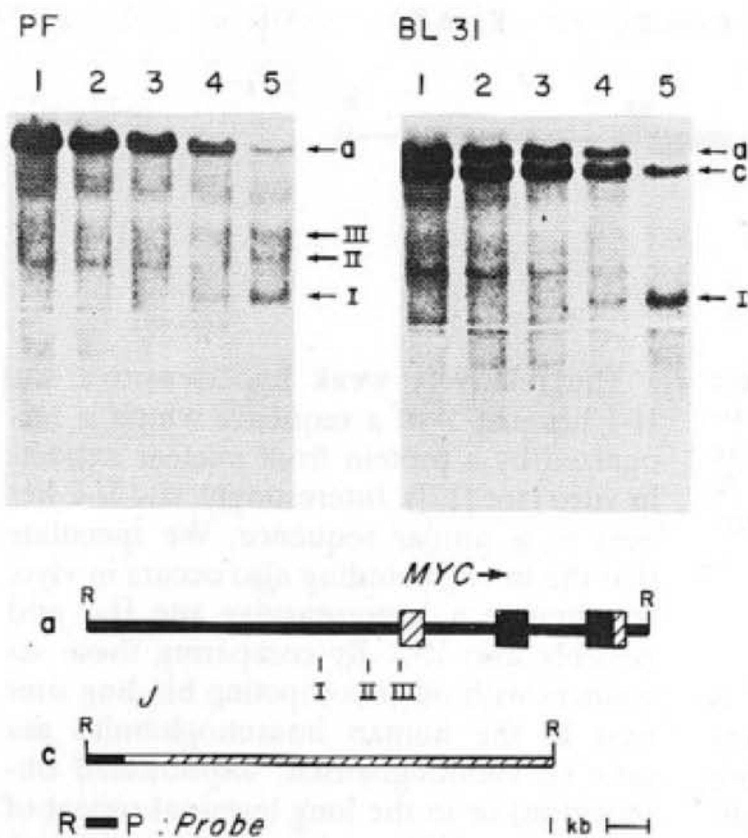

Fig. 3. The nontranslocated *myc* allele in BL 31 has only one very intense DNAase-I-hypersensitive site. The analysis was similar to the one described in Fig. 1, except that a different probe was used (*R-P*), which hybridizes to the germline *myc* allele (*Eco*RI: fragment *a*) and the reciprocal product of the translocation process (*Eco*RI: fragment *c*). *J*, immunoglobulin J region; *hatched bar* indicates an immunoglobulin rearrangement; *R*, *Eco*RI; *P*, *Pst*I; for further description see Fig. 1

tive transcriptional control that appears to operate on the germline *myc* gene in all Burkitt's cells and plasmacytomas where this could be analyzed (see "Introduction"). In one of several possible scenarios then, we imagine that the abnormally high production of *myc* from the translocated allele precipitates increased activity in a *trans*-acting repressor which functions through site I on the germline *myc* allele. This, in turn, represses transcription of *myc*, possibly by preventing transcriptional factors from binding at site III.

Elimination of site I by the translocation process may explain deregulation of *myc* in BL 22, but how does the translocated *myc* allele escape repression in BL 31, where the chromosomal breakpoint does not cut the regulatory region apart? Sites III_1 and III_2 are very intense on the translocated allele, suggesting that the translocation interferes with the function of the hypothetical *trans*-acting repressor proposed above. In BL 31, this may be due to the immunoglobulin heavy chain enhancer, which is presumably functional, since it is itself associated with a hypersensitive site (see Fig. 1). Interestingly, insertion of an ALV LTR 5′ of the chicken *myc* gene changes the chromatin structure of that gene as well [16]. Here, the enhancer may directly activate the promoters, possibly by allowing transcriptional factors to bind near sites III-1 and III-2. Of course other not yet identified elements either removed or introduced by the translocation could also contribute the chromatin changes observed and thus lead to the deregulation of *myc*.

The presented data lead to a new interpretation of how translocations in general may deregulate the *myc* gene. We would like to suggest that the structural alteration or elimination of site I (like in BL 22) might account for the observed loss of the normal control mechanisms governing this gene. Many translocations interrupt or eliminate this site and the untranslated first exon [2, 3, 8, 15, 20, 21]. In addition, it is possible that this region is mutated as a consequence of a translocation [15, 21]. Of course, other mutational changes of elements may further affect the deregulation of the *myc* gene. In BL 31, site I is retained and most likely not mutated and here the strong dominant effect of the immunoglobulin enhancer may cause deregulation. Experiments testing these hypotheses are in progress.

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