An Active Chromatin Structure Acquired by Translocated *c-myc* Genes

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We used general sensitivity to DNase I digestion to analyze the chromatin structure of *c-myc* genes in seven murine plasmacytomas. In every case, the 3' portion of *c-myc* juxtaposed with C_α displayed a much more DNase I-sensitive chromatin structure than untranslocated *c-myc* or, in one case analyzed, the reciprocally translocated 5' portion. Our data suggest the presence of regulatory sequences near the C_α gene segment.

Reciprocal translocations juxtapose the *c-myc* oncogene with immunoglobulin loci in murine plasmacytomas and human Burkitt lymphomas (1, 8, 9, 12-14, 17, 21, 26, 37, 40, 42). Frequently, translocation in plasmacytomas removes the normal *c-myc* promoters, and the translocated *c-myc* gene is transcribed from cryptic promoters located in the intervening sequence 5' of the first coding exon (4, 5, 24, 34, 40). Although steady-state levels of transcripts from such translocated genes vary, they are usually relatively high, whereas transcripts from the normal *c-myc* gene on the untranslocated chromosome are undetectable (1, 2, 4, 34, 40). Recent studies (33) demonstrate that truncated *c-myc* mRNA which originates from translocated genes is more stable than normal *c-myc* mRNA. However, this finding does not explain why the cryptic *c-myc* promoters, which initiate transcription of the truncated mRNA, are activated after chromosomal translocation. We wish to understand at the molecular level the basis for the transcriptional activation of translocated *c-myc* genes.

The consistent juxtaposition of immunoglobulin sequences with the *c-myc* gene suggests that perhaps immunoglobulin sequences are responsible for this activation. In addition, somatic cell hybrid studies demonstrate that the cryptic promoters of truncated *c-myc* genes adjacent to immunoglobulin switch, and constant regions are active, in a B-cell background but not in lymphoblastoid or nonlymphoid cell backgrounds (10, 31, 32). This demonstrates a requirement for B-cell-specific factors and suggests that B-cell factors normally involved in immunoglobulin gene transcription may also activate the cryptic *c-myc* promoters translocated adjacent to immunoglobulin. With few exceptions (7, 11, 16, 22, 39), the known immunoglobulin heavy-chain enhancer (3, 19, 29, 30) does not remain on the same chromosome as the translocated *c-myc* gene and thus is not responsible for this activation (18, 24, 29, 35). Since DNase I-sensitive structure is associated with active genes (23, 44-46), we reasoned that an analysis of *c-myc* gene chromatin in plasmacytomas might provide insight into mechanisms responsible for activation of the cryptic promoters. A role for chromatin structure in the activation of translocated *c-myc* genes has been suggested previously (10, 25, 29, 33). We now report that in seven different plasmacytomas the translocated *c-myc* gene shows a strikingly more open chromatin structure, as evidenced by general DNase I sensitivity, than that of the untranslocated allele.

DNase I sensitivity was measured by preparing 1×10^8 to 2×10^8 nuclei by using 0.5% Triton X-100 and a Dounce homogenizer as previously described (41). The nuclei were counted and adjusted to 10^8 nuclei per ml, and during incubation at 37°C, DNase I (Worthington; 5 to 14 μ g/ml) was added. Samples were removed at selected times, and the digestion was stopped by adding 7 volumes of 4 mM EDTA (pH 7.5) and 1% sodium dodecyl sulfate. DNA samples were purified, restricted in standard conditions, and analyzed by

FIG. 1. Restriction maps of *c-myc* and C_α clones in M603. *c-myc* coding regions are represented by dark boxes, and the untranslated 5' *c-myc* exon is indicated by a hatched box. The C_α exons are represented by open boxes, and the switch regions are indicated by S_α/S_μ . The direction of transcription is shown for *c-myc* and C_α by horizontal arrows, and the wavy arrow marks the translocation juncture of murine chromosomes 12 and 15. The asterisks mark the previously identified immunoglobulin heavy-chain enhancer. Subcloned fragments used as hybridization probes are labeled below the maps. The IVS1 *myc* probe is an MSP1 fragment (6), and the 3' C_α probe is from a cDNA (15). The restriction enzyme labels are as follows: H, *Hind*III; B, *Bam*HI; X, *Xba*I; S, *Sst*I. An *Eco*RI site exists in the middle C_α exon at the juncture of the 3' and 5' C_α probes. The *Hind*III sites in the chromosome 12 portion of the translocated 5' *myc* region have not been completely mapped. The parentheses indicate that the presence of V or D segments or both is inferred from blotting but has not been demonstrated by cloning.

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FIG. 2. DNase I sensitivity studies on M603 chromatin. At the top of each lane is indicated the time of DNase I digestion in minutes; the probes are indicated at the bottom of the blots. Fifteen micrograms of DNA was loaded into each lane. (A) Ethidium bromide-stained 1% agarose gel of DNA purified from DNase I-treated (14 μ g/ml) nuclei. This preparation was used in the restriction digests and blots shown in panels B through F. (B) Blot of a *Hind*III digest hybridized with the IVS1 *myc* probe. The 7.0- and 4.5-kb fragments represent the translocated and normal *c-myc* alleles. (C) Rehybridization of blot in panel B with the 3' C_α probe. The 7.0- and 4.4-kb bands represent the translocated and the active or truncated C_α alleles, respectively. (D) Blot of an *Eco*RI digest hybridized with the 5' C_α probe. The 13.0-, 8.5-, and 5.1-kb fragments represent the C_α adjacent to *c-myc*, the truncated C_α , and multiple copies of the active C_α , respectively, corresponding to clones $\alpha 30$, $\alpha 9$, and $\alpha 6$ in Fig. 1. (E) Blot in panel C, rehybridized with a β -globin cDNA (pCR1 β maj9) (36). The major and minor globin *Hind*III fragments are 8.5 and 1.1 kb, respectively. (F) *Xba*I digest hybridized with the 5' *myc* probe. The 4.2- and 3.5-kb fragments correspond to the 5'-reciprocal and the normal 5' *c-myc* regions, respectively. (G) Ethidium bromide-stained gel showing DNA purified from M603 nuclei treated with less DNase I (4.5 μ g/ml). (H) *Hind*III digest of DNA in panel G, blotted and hybridized with the 3' *myc* probe. The 7.0- and 4.5-kb fragments represent the translocated and normal *c-myc* alleles, respectively.

Southern blotting using the hybridization conditions of Wahl et al. (43).

We first studied murine plasmacytoma M603, in which translocation of the *c-myc* gene to a nonexpressed C_α gene segment removes the normal *c-myc* promoters and 5' noncoding exon and activates 15 cryptic promoters 5' of the first *c-myc* coding exon (34). The restriction maps of the different C_α and *c-myc* genes in M603 are shown in Fig. 1 (6, 9, 34). The top map shows the normal *c-myc* gene on the nontranslocated chromosome 15. The next two maps show the 5' and 3' portions of the *c-myc* gene, which was divided by a reciprocal translocation with the C_α region of chromosome 12. The first noncoding *c-myc* exon is juxtaposed with the heavy-chain enhancer and J_H region. The remaining two *c-myc* coding exons are juxtaposed with C_α . The last two maps show the other C_α alleles in M603. There are two to four copies of the functionally rearranged allele shown in the fourth map. The last map shows an abortive deleted form of the active C_α allele which is also present in M603. Indicated

restriction fragments were used as probes in the following experiments.

Figure 2 shows a DNase I analysis of *c-myc* and C_α genes in M603. In Fig. 2A, M603 DNA from DNase I-digested nuclei is shown in an ethidium bromide-stained agarose gel. Figure 2B shows a Southern blot of a *Hind*III digest of that DNA, hybridized with the IVS1 *myc* probe (Fig. 1), and shows the sensitivity of the normal (4.5-kilobase [kb]) and 3' translocated (7.0-kb) *c-myc* genes. The translocated *c-myc* gene (7.0 kb) was much more sensitive to DNase I than the normal *c-myc* gene (4.5 kb). An experiment with a lower DNase I concentration yielded the same result (Fig. 2G and H).

Two control experiments showed that the increased DNase I sensitivity of the translocated *c-myc* gene was not due to differences in the target size or sequence of the restriction fragments. First, differential sensitivity was not observed upon DNase I treatment of purified M603 DNA (data not shown). Second, an *Xba*I digest of the DNA in Fig.

FIG. 3. Southern blots of *Hind*III-digested DNA purified from different DNase I-treated plasmacytoma nuclei and hybridized with the 3' *myc* probe. The numbers above each lane indicate time of digestion in minutes. The numbers in parentheses below represent the concentration of DNase I used. Nuclei from tumor or cell lines: TEPC1017 (6.8 μ g/ml), a δ producer; S107 (9.1 μ g/ml), an α producer; MOPC104E (9.1 μ g/ml), a μ producer; J606 (9.1 μ g/ml), a γ 3 producer with an undetermined translocation region; MOPC173 (6.8 μ g/ml), a γ 2a producer; HOPC1 (6.8 μ g/ml), a γ 2a producer.

2A, hybridized with the IVS1 *myc* probe, yielded a normal *c-myc* fragment of 3.5 kb and a translocated fragment of 1.6 kb. Despite its smaller size, the translocated *c-myc* fragment was much more sensitive to the DNase I than the larger normal *c-myc* fragment (data not shown). In addition, the increased sensitivity of the translocated *c-myc* gene cannot be due to hypersensitive sites since no prominent hypersensitive sites were observed. A weak hypersensitive site was occasionally observed on overexposed autoradiograms, but this site was also observed in the normal *c-myc* gene of spleen, liver, and kidney.

To determine the DNase I sensitivity of the C_{α} alleles, the blot in Fig. 2B was washed free of probe and hybridized with the 3' C_{α} probe. The autoradiogram shows a sensitive 7.0-kb fragment containing the C_{α} gene segment adjacent to *c-myc* and a 4.4-kb fragment which, although immediately sensitive to DNase I, persisted longer than the 7.0-kb fragment (Fig. 2C). This is probably because the 4.4-kb fragment contains the abortive C_{α} gene and two to four copies of the active C_{α} gene. To assess the DNase I sensitivity of each C_{α} gene separately, a blot of an *Eco*RI digest was hybridized with the 5' C_{α} probe (Fig. 2D). The 13.0-kb, 8.5-kb, and 5.1-kb *Eco*RI fragments represent the translocated, abortive, and expressed (two to four copies) C_{α} gene segments. All showed sensitivity to DNase I similar to that of the translocated *c-myc* gene. Identical results for the translocated *c-myc* gene and multiple C_{α} gene segments were obtained for plasmacytoma M167 (data not shown).

To study the sensitivity of a known inactive gene, the blot in Fig. 2C was washed and rehybridized with a β -globin probe (Fig. 2E; 36). The sensitivity of the 8.5- and 1.1-kb fragments was the same as that for the normal *c-myc* gene in Fig. 2B. The similar sensitivity of the 8.5- and 1.1-kb

fragments further demonstrated that differences in target size are not critical in this analysis.

Finally, the 5' translocated *c-myc* gene was studied by hybridizing a blot of an *Xba*I digest with the 5' *myc* probe (Fig. 2F). The normal (3.5-kb) and reciprocal 5' (4.2-kb) *c-myc* regions were relatively insensitive to DNase I, like the β -globin gene. This demonstrated that translocation alone does not cause DNase I-sensitive chromatin structure and that the presence of the immunoglobulin enhancer adjacent to the 5' reciprocal fragment of *c-myc* is not sufficient to generate DNase I sensitivity such as that seen for the translocated *c-myc* gene and the C_{α} gene segments.

To confirm the observations made in plasmacytoma M603, we analyzed the DNase I sensitivity of the translocated *c-myc* genes in six other plasmacytoma tumor and cell lines (Fig. 3). All of these plasmacytomas, except J606, have *c-myc* genes translocated to the C_{α} locus, although some of these tumors express isotypes other than alpha. M104E (immunoglobulin M) is unusual in that it has a *c-myc* gene translocated just 3' of the C_{α} gene (24). In all cases, the translocated *c-myc* gene was significantly more sensitive to DNase I than the normal *c-myc* gene.

These results show that *c-myc* genes translocated to the C_{α} region acquire a different chromatin structure from their normal counterparts; their chromatin structure is very similar to that of expressed C genes. Two models for how the translocated *c-myc* genes acquired a sensitive chromatin structure are: (i) translocation into an active chromatin domain caused a change in *c-myc* chromatin which in turn activated the cryptic promoters, or (ii) juxtaposition with some *cis*-acting element near C_{α} activated the cryptic *c-myc* promoters, subsequently causing an open chromatin structure. In either case, our data show that translocation alone

did not cause the altered chromatin structure since the reciprocally translocated 5' *c-myc* region did not acquire the same structure. Furthermore, since activation of translocated *c-myc* genes requires a mature B-cell environment (10, 31, 32), adjacent C_{α} sequences are causally implicated in this altered chromatin structure.

It has been suggested that additional enhancerlike regulatory elements may be located 3' of C_{α} (10, 38). This possibility is consistent with the fact that translocations in plasmacytomas and Burkitt lymphomas nearly always retain the region 3' of C gene segments adjacent to *c-myc*. We have searched for another enhancer in a 5.5-kb region 3' to the translocation breakpoint and have not discovered any significant enhancing activity (20, 28, 29). However, since the endogenous heavy-chain enhancer appears to activate tandem promoters located at least 17.5 kb away (43a), there may be a *cis*-acting element 3' of the region we have analyzed which could activate the cryptic *c-myc* promoters.

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LITERATURE CITED

- Adams, J., S. Gerondakis, E. Webb, L. Corcoran, and S. Cory. 1983. Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in Burkitt lymphomas. *Proc. Natl. Acad. Sci. USA* 80:1982-1986.
- Adams, J., S. Gerondakis, E. Webb, J. Mitchell, O. Bernard, and S. Cory. 1982. Transcriptionally active DNA region that rearranges frequently in murine lymphoid tumors. *Proc. Natl. Acad. Sci. USA* 79:6966-6970.
- Banerji, J., L. Olson, and W. Schaffner. 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* 33:729-740.
- Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J. Adams. 1983. Sequence of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B lymphoid tumours. *EMBO J.* 2:2375-2383.
- Calabi, F., and M. Neuberger. 1985. Chromosome translocation activates heterogeneously initiated, bipolar transcription of a mouse *c-myc* gene. *EMBO J.* 4:667-674.
- Calame, K., S. Kim, P. Lalley, R. Hill, M. Davis, and L. Hood. 1982. Molecular cloning of translocations involving chromosome 15 and the immunoglobulin C_{α} gene from chromosome 12 in two murine plasmacytomas. *Proc. Natl. Acad. Sci. USA* 79:6994-6998.
- Corcoran, L., S. Cory, and J. Adams. 1985. Transposition of the immunoglobulin heavy chain enhancer to the *myc* oncogene in a murine plasmacytoma. *Cell* 40:71-79.
- Cory, S., S. Gerondakis, and J. Adams. 1983. Interchromosomal recombination of the cellular oncogene *c-myc* with the immunoglobulin heavy chain locus in murine plasmacytomas is a reciprocal exchange. *EMBO J.* 2:697-703.
- Crews, S., R. Barth, L. Hood, J. Prehn, and K. Calame. 1982. Mouse *c-myc* oncogene is located on chromosome 15 and translocated to chromosome 12 in plasmacytomas. *Science* 218:1319-1321.
- Croce, C., J. Erikson, A. ar-Rushdi, D. Aden, and K. Nishikura. 1984. Translocated *c-myc* oncogene of Burkitt lymphoma is transcribed in plasma cells and repressed in lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA* 81:3170-3174.
- Croce, C., J. Erikson, K. Huebner, and K. Nishikura. 1985. Coexpression of translocated and normal *c-myc* oncogenes in hybrids between Daudi and lymphoblastoid cells. *Science* 227:1235-1238.
- Dalla-Favera, R., M. Bregni, J. Erikson, D. Patterson, R. Gallo, and C. Croce. 1982. Human *c-myc* onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl. Acad. Sci. USA* 79:7824-7827.
- Dalla-Favera, R., S. Martinotti, R. Gallo, J. Erikson, and C. Croce. 1983. Translocation and rearrangements of the *c-myc* oncogene locus in human undifferentiated B-cell lymphomas. *Science* 219:963-967.
- Dunnick, W., B. Shell, and C. Dery. 1983. DNA sequences near the site of reciprocal recombination between a *c-myc* oncogene and an immunoglobulin switch region. *Proc. Natl. Acad. Sci. USA* 80:7269-7273.
- Early, P., M. Davis, D. Kaback, N. Davidson, and L. Hood. 1979. Immunoglobulin heavy chain gene organization in mice: analysis of a myeloma genomic clone containing variable and α constant regions. *Proc. Natl. Acad. Sci. USA* 76:857-861.
- Fahrlander, P., J. Sumegi, J. Yang, F. Wiener, K. Marcu, and G. Klein. 1985. Activation of the *c-myc* oncogene by the immunoglobulin heavy-chain enhancer after multiple switch region-mediated chromosome rearrangements in a murine plasmacytoma. *Proc. Natl. Acad. Sci. USA* 82:3746-3750.
- Gelmann, E., M. Psallidopoulos, T. Papas, and R. Dalla-Favera. 1984. Identification of reciprocal translocation sites within the *c-myc* oncogene and immunoglobulin μ locus in a Burkitt lymphoma. *Nature (London)* 306:799-803.
- Gerondakis, S., S. Cory, and J. Adams. 1984. Translocation of the *myc* cellular oncogene to the immunoglobulin heavy chain locus in murine plasmacytomas is an imprecise reciprocal exchange. *Cell* 36:973-982.
- Gillies, S., S. Morrison, V. Oi, and S. Tonegawa. 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* 33:717-728.
- Gorman, C., L. Moffat, and B. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044-1051.
- Hamlyn, P., and T. Rabbitts. 1983. Translocation joins *c-myc* and immunoglobulin genes in a Burkitt lymphoma revealing a third exon in the *c-myc* oncogene. *Nature (London)* 304:135-139.
- Hayday, A., S. Gillies, H. Saito, C. Wood, K. Wiman, W. Hayward, and S. Tonegawa. 1984. Activation of a translocated human *c-myc* gene by an enhancer in the immunoglobulin heavy chain locus. *Nature (London)* 307:334-340.
- Igo-Kemenes, T., W. Horz, and H. Zachau. 1981. Chromatin. *Annu. Rev. Biochem.* 51:89-121.
- Keath, E., A. Kelekar, and M. Cole. 1984. Transcriptional activation of the translocated *c-myc* oncogene in mouse plasmacytomas: similar RNA levels in tumor and proliferating normal cells. *Cell* 37:521-528.
- Klein, G., and E. Klein. 1985. Evolution of tumours and the impact of molecular oncology. *Nature (London)* 315:190-195.
- Marcu, K., L. Harris, L. Stanton, J. Erikson, R. Watt, and C. Croce. 1983. Transcriptionally active *c-myc* oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia. *Proc. Natl. Acad. Sci. USA* 80:519-523.
- Mercola, M., J. Gorman, C. Mirell, and K. Calame. 1985. Immunoglobulin heavy-chain enhancer requires one or more tissue-specific factors. *Science* 227:266-270.
- Mercola, M., E. Kakkis, J. Prehn, X. Wang, and K. Calame. 1984. Cis-acting effects on transcription of *c-myc* and Vh genes within the immunoglobulin heavy chain locus. *UCLA Symp. Mol. Cell. Biol.* 18:479-489.
- Mercola, M., X. Wang, J. Olsen, and K. Calame. 1983. Transcriptional enhancer elements in the mouse immunoglobulin

- heavy chain locus. *Science* **221**:663–665.
30. **Neuberger, M.** 1983. Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells. *EMBO J.* **2**:1373–1378.
 31. **Nishikura, K., A. ar-Rushdi, J. Erikson, E. DeJesus, D. Dugan, and C. Croce.** 1984. Repression of rearranged μ gene and translocated *c-myc* in mouse 3t3 cells $\times$ Burkitt lymphoma cell hybrids. *Science* **224**:399–402.
 32. **Nishikura, K., A. ar-Rushdi, J. Erikson, R. Watt, G. Rovera, and C. Croce.** 1983. Differential expression of the normal and of the translocated human *c-myc* oncogenes in B cells. *Proc. Natl. Acad. Sci. USA* **80**:4822–4826.
 33. **Piechaczyk, M., J. Yang, J. Blanchard, P. Jeanteur, and K. Marcu.** 1985. Posttranscriptional mechanisms are responsible for accumulation of truncated *c-myc* RNAs in murine plasma cell tumors. *Cell* **42**:589–597.
 34. **Prehn, J., M. Mercola, and K. Calame.** 1984. Translocation affects normal *c-myc* promoter usage and activates fifteen cryptic *c-myc* transcription starts in plasmacytoma M603. *Nucleic Acids Res.* **12**:8987–9007.
 35. **Rabbitts, T., A. Forster, R. Baer, and P. Hamlyn.** 1983. Transcription enhancer identified near the human $C\mu$ immunoglobulin heavy chain gene is unavailable to the translocated *c-myc* gene in a Burkitt lymphoma. *Nature (London)* **306**:806–809.
 36. **Rougeon, F., and B. Mach.** 1977. Cloning and amplification of α and β mouse globin gene sequences synthesized in vitro. *Gene* **1**:229–239.
 37. **Shen-Ong, G., E. Keath, S. Piccoli, and M. Cole.** 1982. Novel *myc* oncogene RNA from abortive immunoglobulin gene recombination in mouse plasmacytomas. *Cell* **31**:443–452.
 38. **Showe, L., M. Ballantine, K. Nishikura, J. Erikson, H. Kaji, and C. Croce.** 1985. Cloning and sequencing of a *c-myc* oncogene in a Burkitt's lymphoma cell line that is translocated to a germ line alpha switch region. *Mol. Cell. Biol.* **5**:501–509.
 39. **Siebenlist, U., L. Hennighausen, J. Battey, and P. Leder.** 1984. Chromatin structure and protein binding in the putative regulatory region of the *c-myc* gene in Burkitt lymphoma. *Cell* **37**:381–391.
 40. **Stanton, L., R. Watt, and K. Marcu.** 1983. Translocation, breakage and truncated transcripts of *c-myc* oncogene in murine plasmacytomas. *Nature (London)* **303**:401–406.
 41. **Storb, U., R. Wilson, E. Selsing, and A. Walfied.** 1981. Rearranged and germline immunoglobulin κ genes: different states of DNase I sensitivity of constant κ genes in immunocompetent and nonimmune cells. *Biochemistry* **20**:990–996.
 42. **Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder.** 1982. Translocation of the *c-myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl. Acad. Sci. USA* **79**:7837–7841.
 43. **Wahl, G., M. Stern, and G. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzoyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. USA* **76**:3683–3687.
 - 43a. **Wang, X., and K. Calame.** 1985. The endogenous immunoglobulin heavy chain enhancer can activate tandem V_H promoters separated by a large distance. *Cell* **43**:659–665.
 44. **Weintraub, H.** 1984. Histone-H1-dependent chromatin superstructures and the suppression of gene activity. *Cell* **38**:17–27.
 45. **Weintraub, H., and M. Groudine.** 1976. Chromosomal subunits in active genes have an altered conformation. *Science* **193**:848–856.
 46. **Weisbrod, S.** 1982. Active chromatin. *Nature (London)* **297**:289–295.