*Int. J. Cancer:* **33,** 477-481 (1984)

# TRIPLICATION OF ONE CHROMOSOME No. 15 WITH AN ALTERED *c-myc* CONTAINING EcoRI FRAGMENT AND ELIMINATION OF THE NORMAL HOMOLOGUE IN A T-CELL LYMPHOMA LINE OF AKR ORIGIN (TIKAUT)

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**An ouabain- and thioguanine-resistant subline (TIKAUT) of spontaneous AKR lymphoma, TKA, was trisomic for chromosome 15 and contained a single 33 kb EcoRI fragment, containing the oncogene *c-myc*. The original TKA lymphoma and derived *in vitro* line contained the same 33 kb fragment, as well as a normal 22 kb fragment. It has been concluded that the original 15-trisomic TKA tumor has duplicated a 15-chromosome that contained the changed fragment, while maintaining the normal fragment as well. Subsequently, in the derived TIKAUT line, the changed chromosome duplicated again, giving rise to three copies, and the normal homologue was eliminated altogether. This confirms our earlier somatic hybrid study showing that the duplicated 15-chromosome of a T-cell leukemia confers an advantage on the cell that favors tumorigenicity, whereas the normal homologue exerts a counteracting influence. Therefore, in the course of tumor progression, the changed chromosome tends to be amplified, whereas its normal homologue tends to be eliminated.**

TIKAUT is an ouabain- and TG-resistant subline of a spontaneous AKR-derived thymic lymphoma, TKA (Spira *et al.*, 1981). Like the majority of murine T-cell lymphomas, irrespective of origin, TIKAUT is trisomic for chromosome 15 (Dofuku *et al.*, 1975; Wiener *et al.*, 1978*a*,*c*).

As part of our analysis concerning the significance of 15 trisomy in murine T-cell leukemia, we have previously fused TIKAUT with normal CBA T6T6 fibroblasts that carry a 14;15 translocation (Spira *et al.*, 1981). This permitted a cytogenetic distinction between the three morphologically unchanged leukemia-derived chromosomes No. 15, on the one hand, and the two normal fibroblast-derived translocation chromosomes, on the other. High- and low-tumorigenic hybrids were compared by chromosome banding. While the number of the other autosomes was close to the expected number in all hybrids, chromosome 15 showed a consistent, tumorigenicity-related numerical difference. The leukemia-derived chromosome No. 15 increased from three to five or six in the high-tumorigenic hybrids, with a concomitant reduction of the fibroblast-derived chromosomes from two to one. In the low-tumorigenic hybrids the ratio remained close to the expected 3:2 (2.6:2 on the average). We have concluded that a changed gene on the leukemia-derived chromosome 15 may require amplification for the expression of the tumorigenic phenotype to overcome some trans-acting regulatory influence that emanates from the homologous chromosome of the normal fibroblast.

Chromosome 15 carries the *c-myc* oncogene. In the majority of murine plasmacytomas, *c-myc* is rearranged, concomitantly with the 12;15 translocation that places it within the immediate neighborhood of the IgH locus on chromosome 12 (Adams *et al.*, 1982; Cory *et al.*, 1983; Harris *et al.*, 1982; Shen-Ong *et al.*, 1982). T-cell lymphomas do not carry this translocation. Experiments with mice that carried reciprocal translocations involving chromosome 15 have shown, however, that the T-lymphoma-associated 15-trisomy affects the *myc*-carrying distal segment of chromosome 15 (Spira *et al.*, 1980; Wiener *et al.*, 1978*b*). It is therefore possible that *c-myc* or regulatory sequences in its neighborhood may be involved in the genesis of T-cell leukemia as well.

Subsequently, Adams *et al.* (1982) have shown that the TIKAUT lymphoma contained a modified *c-myc* containing EcoRI fragment, 33 kb in size, instead of the normal 21.5 kb fragment (Cory *et al.*, 1983*b*). While most of the plasmacytomas contain both a changed and a normal *c-myc* carrying fragment, TIKAUT contained only one modified 33 kb fragment.

TIKAUT carries three chromosomes No. 15 with normal morphology (Spira *et al.*, 1981). The presence of a single modified *c-myc* hybridizing EcoRI fragment suggested that a single, changed chromosome 15 may have triplicated. This would involve several consecutive cytogenetic changes. As the first step, one chromosome 15 would duplicate by non-disjunction, giving rise to trisomy. The second and third steps would involve an additional duplication of the same chromosome 15 and the elimination of the non-duplicated homologous normal chromosome.

The possible tumorigenic and/or progression-promoting role of *c-myc* activation by DNA rearrangements in hemopoietic tumors is currently of great interest. In contrast to the plasmacytomas, the *c-myc*-containing region is only modified in a minority of the T-cell lymphomas studied (Adams *et al.*, 1983). TIKAUT is particularly interesting since it was recently shown (Cory *et al.*, 1983*b*, in press) that the modification is due to retroviral insertion upstream from *c-myc*, leading on an activation of *c-myc* transcription.

In the present study, we wished to determine whether the three postulated cytogenetic events that have led to the concurrent presence of 15-trisomy and a single modified *myc*-carrying fragment have already occured in the original TKA tumor, or only during the subsequent history of the ouabain and TG-resistant double mutant. We have compared that original TKA tumor, a spontaneous leukemia of AKR origin, the derived tissue culture line, and the double mutant TIKAUT at the molecular and the cytogenetic levels.

Received: November 25, 1983 and in revised form January 26, 1984.

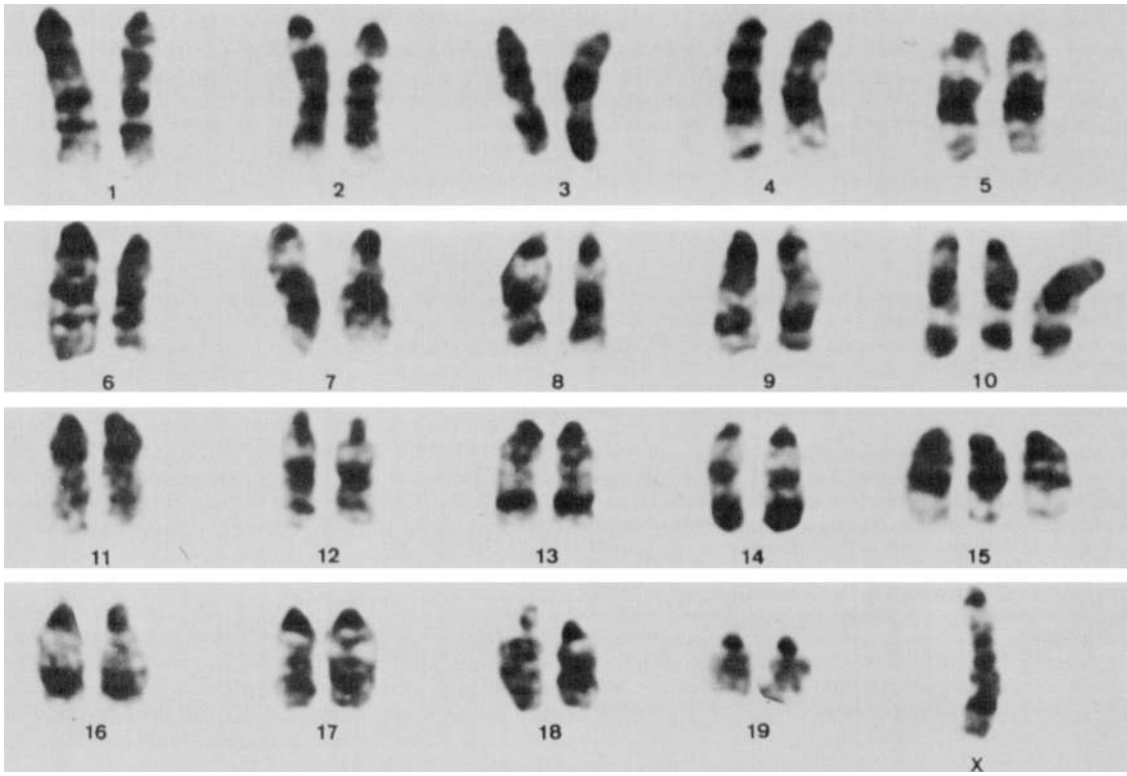


FIGURE 1 – G-banded metaphase plate of the ouabain- and thioguanine-resistant TIKAUT cell line, serially propagated *in vitro* for more than 2 years. Note the presence of trisomy 15. This long-established culture line also has some secondary chromosomal changes, such as trisomy 10 and the loss of one X chromosome.

## MATERIAL AND METHODS

### *Cells*

The TIKAUT line was derived from a spontaneous thymic lymphoma in an AKR mouse. It was adapted to tissue culture, mutagenized with EMS (ethyl methane sulfonate, Sigma, P.O. Box 14508, St. Louis, Mo 63178, USA) 100 μg/ml and made resistant to 50 μg/ml TG (thioguanine) and 3 mM ouabain by stepwise selections. Karyotyping shows a stemline containing 41 chromosomes with trisomy of chromosome 15 (Spira *et al.*, 1981). TIKAUT grows progressively after subcutaneous transplantation of small-cell doses in 100% of syngeneic AKR recipients. TIKAUT was used in our earlier somatic hybrid studies, concerned with the relationship between chromosome 15 and tumoriginicity (Spira *et al.*, 1981). Since that study in 1981, the line was mostly kept in frozen storage. It was thawed and propagated on RPMI medium, supplemented with 10% fetal calf serum (FCS), prior to the present study.

For comparison with TIKAUT, we have also thawed the frozen ampoule of the original, unselected TKA tumor, from the eight *in vivo* passage. The *in vivo* tumor was propagated by subcutaneous passage in syngeneic AKR mice.

A new, unselected tissue culture line was derived from the ninth passage of the *in vivo* TKA tumor. It is designated as the "TKA *in vitro* line" and propagated in tissue culture in the same way as TIKAUT. At the time of the present study, it has been in culture for 6 months.

### *Chromosomal examination*

Metaphase spreads were prepared by harvesting tissue-cultured cells 24 h after splitting. After hypotonic treatment (0.075 M KCl for 10 min), the pellet was fixed in four changes of acetic acid: methanol (1:3), spread on wet slides, air-dried and stained with alkaline Giemsa solution (pH 8).

Banded methaphase plates were prepared according to a slight modification of Wang and Fedoroff's method (Wang and Fedoroff, 1971). Metaphase plates with a high banding quality were used to identify the relevant trisomic chromosomes under the microscope. Whole karyotypes were also analyzed from magnified photographs. Chromosome identification followed the nomenclature of the Committee on Standardized Genetic Nomenclature for Mice (1972).

*Southern blot hybridization for* c-myc

High-molecular-weight DNA was digested to completion with EcoRI as recommended by the manufacturer (Boehringer, Mannheim, Fed. Rep. Germany). DNA fragments were electrophoresed through 0.8% agarose gel and transferred to nitrocellulose filter BA85, (Schleicher and Schuell GmbH., D-3354 Dassel, W. Germany). DNA hybridization was carried out with the nick-translated EcoRI-Cla I fragment of the cloned human *c-myc* gene (Dalla-Favera *et al.*, 1982), as described by Sümegi *et al.* (1983).

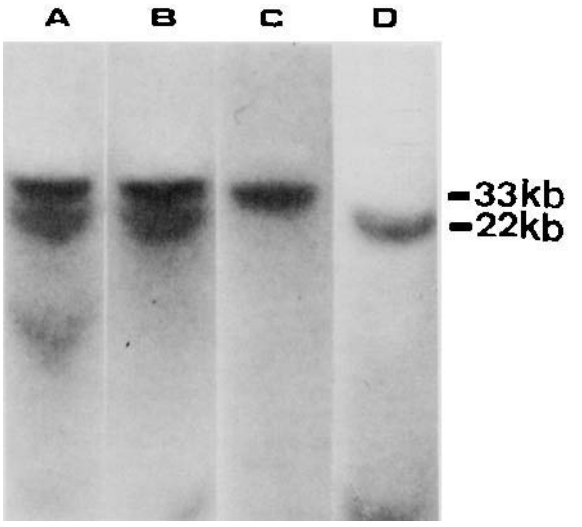


FIGURE 2 – Restriction enzyme analysis of the *c-myc* locus in genomic DNA obtained from (*a*) TKA, 8th *in vivo* passage; (*b*) TKA, *in vitro* line; (*c*) TIKAUT double mutant; (*d*) Moloney-virus-induced lymphoma 4-Rb 1;15 × C57B1-F (Spira *et al.*, 1979). Hybridization was carried with the nick-translated (Rigby *et al.*, 1977) EcoRI-ClaI fragment of the cloned human *c-myc* locus (Dalla-Favera *et al.*, 1982) as described by Wahl *et al.* (1979). Filters were washed in 0.2 × SSC, 0.02% SDS at 65° C for 2 × 30 min.

## RESULTS

*Chromosomal analysis*

The original TKA lymphoma, the derived tissue culture line and the TIKAUT double mutant were analyzed for chromosomal constitution by chromosome counts and G-banding, as summarized in Table I. The modal chromosome number was already 41 in the primary explant culture. Banding analysis showed that the extra chromosome was a No. 15. This corresponds to the most frequent chromosomal change in murine T-cell lymphomas. No other chromosomal changes were identified (Fig. 1).

The ouabain- and TG-resistant double mutant showed the same chromosomal constitution, with a 41 mode and an extra chromosome No. 15.

*Molecular studies*

In agreement with the previous findings of Cory *et al.* (1983*b*) and Adams *et al.* (1982), TIKAUT was found to have a single 33-kb-long *c-myc*-specific EcoRI fragment on Southern blot analysis, using a subclone of the human *c-myc* locus as the hybridization probe, as shown in Figure 2.

To examine whether the 33 kb EcoRI fragment was already present in the original *in vivo* TKA tumor, from which the TIKAUT line was derived by *in vitro* establishment and selection for ouabain and TG resistance, we thawed the cells from the eighth passage of TKA. As shown in Figure 2, it had both the changed 33 kb fragment and a 22 kb *c-myc* fragment, corresponding to the normal sequence. This suggested that the eighth passage of the tumor already known to be trisomic for chromosome 15 contained both types of sequences. This conclusion was only tentative, however, since *in vivo* tumors always contain infiltrating host cells and the normal sequences may have been derived from them. To examine this possibility, we have established a new *in vitro* line from the 9th TKA passage generation and examined it by restriction enzyme analysis after 6 months in culture. As shown in Figure 2, the TKA *in vitro* line carried both the changed and the normal sequences. This shows that the findings on the *in vivo* tumor genuinely reflect the status of the *c-myc* sequences in the lymphoma cells themselves. The most reasonable explanation is that three independent cytogenetic events have affected chromosome 15 in the TIKAUT line. First, one 15 hromosome that already contained the changed *c-myc*-chromosome 15 that carried the normal sequence was maintained as well, as indicated in the densitometry scanning of the relevant autoradiograms (Figs. 2 and 3). The subsequent development must have involved at least two different cytogenetic changes, in unknown order: an

**TABLE I** – SUMMARY OF CHROMOSOMAL ANALYSIS ON THE PRIMARY EXPLANT OF TKA, THE DERIVED TKA *IN VITRO* LINE, AND THE TIKAUT DOUBLE MUTANT

| Cell line | Time in culture | Chromosome analysis | Chromosome No. | | G-banding |
|---|---|---|---|---|---|
| | | | Range | Mode | |
| TKA | 5 days | Primary culture | 40-41 | — | |
| TKA | 10 days | Primary culture | 40-41 | 41 | |
| TKA | 1 month | Established primary culture | 41 | 41 | |
| TKA | 18 months | Established *in vitro* line | 40-41 | 41 | Trisomy 15 |
| TKA | 2 years | EMS-treated *in vitro* line | 31-41 | — | |
| TIKAUT | Derived from TKA | TG- and ouabain-resistant mutant line | 40-41 | 41 | Trisomy 15 |

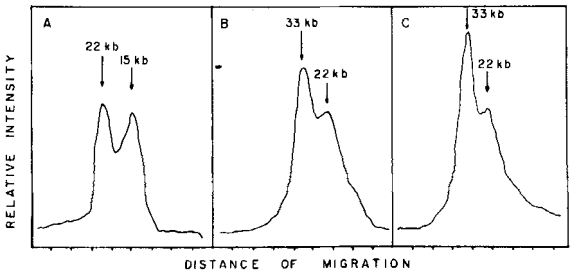


FIGURE 3 – Densitometry scanning of the autoradiograms shown in Figure 2. (*a*) Germ-line and rearranged EcoRI *c-myc* specific fragments obtained from a diploid MCF-virus induced tumor. (*b*) Germ-line and rearranged EcoRI *c-myc* specific fragments obtained from TKA 8th *in vivo* passage (Fig. 2, lane *a*). (*c*) Germ-line and rearranged EcoRI *c-myc* specific fragments obtained from TKA *in vitro* line (Fig. 2, lane *b*).

additional duplication of one chromosome 15, carrying the changed sequence, and elimination of the normal homologue.

## DISCUSSION

In mouse plasmacytoma (Adams *et al.*, 1982, 1983; Calame *et al.*, 1982; Cory *et al.*, 1983*b*; Harris *et al.*, 1982; Shen-Ong *et al.*, 1982) and human Burkitt's lymphoma (Erikson *et al.*, 1983; Taub *et al.*, 1982) specific chromosome translocations often lead to rearrangement of the *c-myc* gene, with subsequent activation of *myc*-transcription.

Opinions differ on the relative significance of the constitutive switch-on of the *c-myc* oncogene, as contrasted to the possible abnormalization of its protein product (for review see Klein, 1983). Neither one of the two alternatives can serve as a general explanation. Changed responsiveness of the rearranged gene to growth and differentiation stimuli is more likely to emerge as the salient feature.

In the chromosome 15-trisomic T-cell lymphomas, the situation is even less clear. It is noteworthy, however, that the duplication of the *c-myc* carrying distal segment of the chromosome is particularly important, as shown by studies on lymphomas that carried reciprocal translocations involving chromosome 15 (Spira *et al.*, 1980; Wiener *et al.*, 1978*c*). The significance of 15 trisomy for the genesis of T-cell leukemias has been particularly reinforced by our studies on Robertsonian translocation-carrying mice (Spira *et al.*, 1979), where the entire Robertsonian chromosome duplicated, if 15 was involved, but not otherwise (Spira *et al.*, 1983), and by the occurrence of cryptic 15-trisomies (Spira *et al.*, 1980).

While the distal segment of chromosome 15 thus carries some gene(s) that are involved in the genesis of T-cell leukemias, it is much less clear whether activation and/or abnormalization of *c-myc* is involved. In an extensive study, the Australian group (Adams *et al.*, 1982) has examined 18 T-cell lymphomas. Three, including TIKAUT, contained a changed *c-myc*-carrying fragment. TIKAUT was the only one among them that had no corresponding normal fragment. Subsequently, we have examined 19 Moloney-virus- or MCF-virus-induced T-cell lymphomas, of which 4 were trisomic and 15 diploid (Wirschubsky Z., unpublished observations). In this material, 18 lymphomas contained only normal *c-myc* sequences, whereas one contained both a changed and a normal sequence. A change in the *c-myc* carrying fragment, as detected by the restriction enzyme-cutting – Southern blot approach is thus certainly not a general feature of murine T-cell leukemias. This does not mean that it may not play a significant role in the lymphomas where it occurs[1]. It is of great interest that Cory *et al.* (1983*b*) have recently found retroviral sequences inserted immediately 5′ of *c-myc* in all three T-cell lymphomas that contained a changed *c-myc* fragment. One of them, TIKAUT, was found to carry an intact retroviral genome of the MCF type. These authors have also shown that *c-myc* is highly transcribed in the TIKAUT lymphoma.

Our earlier study dealt with the relationship between chromosome 15 and tumorigenicity in a series of somatic hybrids derived from the fusion of TIKAUT with normal diploid CBAT6T6 fibroblasts. Since T6 mice carry a reciprocal 14;15 translocation, we could readily distinguish the three morphologically normal 15 chromosomes contributed by the TIKAUT lymphoma and the two 14;15 translocation chromosomes, contributed by the normal fibroblast parent.

In the highly tumorigenic hybrids, the lymphoma-derived No. 15 chromosomes increased in number from three to five or six, while the number of the No. 15 chromosomes derived from the normal fibroblast parent decreased from two to one. Low-tumorigenic hybrids contained the same chromosomes in the expected 3:2 ratio. This suggested that chromosome 15 of the normal cell exerted some trans-acting control that prevented the expression of tumorigenicity, whereas the amplification of a changed gene on chromosome 15 of the leukemic cell favored the malignant phenotype.

This interpretation may also throw some light on the significance of 15-trisomy during the leukemogenic process itself. It may be presumed that the first event is the activation of a cellular oncogene, perhaps by the insertion of a retrovirus in its neighborhood, a process that is actually supported for the TIKAUT lymphoma itself by the recent findings of Cory *et al.* (1983*b*). Unlike the plasmacytoma and Burkitt-lymphoma-associated translocations where *c-myc* constitutively is activated by its transposition into the immunoglobulin region of the B-cell, the oncogene remains in its natural position in TIKAUT. It may find itself under the competing influence of the retroviral promoter, inserted in the more or less close neighborhood of its 5′ end, and of the normal control signals that regulate *c-myc* expression. The latter may partly or wholly emanate from the unchanged homologous chromosome. The limitations imposed by this competition on the expression of the tumorigenic phenotype may then be most easily overcome by a duplication of the changed chromosome by non-disjunction. A second change in the homologous chromosome, due to mutation, deletion, or a second retroviral insertion, would be much less likely to occur.

This interpretation implies that the 15-chromosome complement of the trisomic T-cell lymphoma is func-

[1] Furthermore, it cannot be excluded that changes may have occurred outside the restriction enzyme sites (*e.g.* EcoRI) containing germ-line *c-myc* sequences.

tionally heterogeneous. Two copies would contain a structurally or regulatively changed oncogene, while the third would carry the normal homologue.

The present findings on the TIKAUT line and its TKA ancestor are directly relevant in this context. The presence of both an altered and a normal *c-myc* containing fragment in TKA is in line with the postulated functional heterogeneity of the 15-trisomic lymphoma. Band intensities on the Southern blot are in line with the expected 2:1 ratio (Fig. 3). The subsequent events in the TIKAUT double mutant are strongly reminiscent of our earlier findings on somatic hybrids (Spira *et al.*, 1981). They also provide an interesting example of tumor progression. In TIKAUT, the chromosome No. 15, that carries the changed *c-myc*-containing fragment has duplicated once again, to a total of three copies, wheres the normal homologue has been eliminated altogether.

Tumor progression is a never-ending exercise in the selection of new variants that are less responsive to *in vivo* or *in vitro* growth control than the original type (Foulds, 1958; Klein and Klein, 1957). The history of TIKAUT clearly exemplifies this in relation to a known oncogene-containing region and the simplest mechanism of chromosomal variation, non-disjunction. The findings strongly support the concept that the relative balance between chromosomes that carry a changed locus and its normal counterpart has a decisive influence on the expression of the tumorigenic phenotype.

ACKNOWLEDGEMENTS

This investigation was supported by PHS grant No. 2 R01 CA 14054-10, awarded by the National Cancer Institute, DHHS and by the Swedish Cancer Society. The help of Dr. Eva Maria Fenyö in the establishment of the TIKAUT line is gratefully acknowledged.

REFERENCES

ADAMS, J.M. GERONDAKIS, S., WEBB, E., CORCORAN, L.M., and CORY, S., Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. *Proc. nat. Acad. Sci. (Wash.),* **80,** 1982-1986 (1983).

ADAMS, J.M., GERONDAKIS, S., WEBB, E., MITCHELL, J., BERNARD, O., and CORY, S., Transcriptionally active DNA region that rearranges frequently in murine lymphoid tumors. *Proc. nat. Acad. Sci. (Wash.),* **79,** 6966-6970 (1982).

CALAME, K., KIM, S., LALLEY, P., HILL, R., DAVIS, M., and HOOD, L., Molecular cloning of translocations involving chromosome 15 and the immunoglobulin C $\alpha$ gene from two murine plasmacytomas. *Proc. nat. Acad. Sci. (Wash.),* **79,** 6994-6998 (1982).

COMMITTEE ON STANDARDIZED GENETIC NOMENCLATURE FOR MICE, Standard karyotype of the mouse, *Mus musculus. J. Hered.,* **63,** 69-71 (1972).

CORY, S., ADAMS, J.M., STEVEN, D., GERONDAKIS, S.D., MILLER, J.F.A.P., GAMBLE, J., WIENER, F., SPIRA, J., and FRANCKE, U., Fusion of DNA region to murine immunoglobulin heavy chain locus corresponds to plasmacytoma-associated chromosome translocation. *Europ. mol. Biol. Org. J.,* **2,** 213-216 (1983*a*).

CORY, S., BERNARD, O., GERONDAKIS, S., WEBB, E., MITCHELL, J., CORCORAN, L.M., and ADAMS, J.M., Interchromosomal translocation of the *c-myc* oncogene in B-lymphoid tumors. Structural and functional consequences. *Proc. International Congress of Immunology,* Kyoto, Adademic Press (1983*b*). In press.

DALLA-FAVERA, R., GELMANN, E.P., MARTINOTTI, S. FRANCHINI, G., PAPAS, T.S., GALLO, R.C., and WONG-STAAL, F., Cloning and characterization of different human sequences related to the onc gene (*v-myc*) of avian myelocytomatosis virus (MC 29). *Proc. nat. Acad. Sci. (Wash.),* **79,** 6497-6501 (1982).

DOFUKU, R., BIEDLER, J.L., SPENGLER, B.A., and OLD, L.J., Trisomy of chromosome 15 in spontaneous leukemia of AKR mice. *Proc. nat. Acad. Sci. (Wash.),* **72,** 1515-1517 (1975).

ERIKSON, J., ABBAS AR-RUSHDI, DRWINGA, H.L., NOWELL, P.C., and CROCE, C.M., Transcriptional activation of the translocated *c-myc* oncogene in Burkitt lymphomas. *Proc. nat. Acad. Sci. (Wash.),* **80,** 820-824 (1983).

FOULDS, I.M.A., The natural history of cancer. *J. chron. Dis.,* **8,** 2-37 (1958).

HARRIS, L.J., LANG, R.B., and MARCU, K.B., Non-immunoglobulin-associated DNA rearrangements in mouse plasmacytomas. *Proc. nat. Acad. Sci. (Wash.),* **79,** 4175-4179 (1982).

KLEIN, G., Specific chromosomal translocation and the genesis of B-cell-derived tumors in mice and men. Minirev. *Cell,* **32,** 311-315 (1983).

KLEIN, G., and KLEIN, E., The evolution of independence from specific growth stimulation and inhibition in mammalian tumour cell populations. *Symp. Soc. Biol.,* **11,** 305-328 (1957).

RIGHBY, P.W.J., DIECKMANN, M., RHODES, C., and BERG, P.J., Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. *J. mol. Biol.,* **113,** 237-251 (1977).

SHEN-ONG, G.L., KEATH, E.J., PICCOLI, S.P., and COLE, M.D., Novel *myc* oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. *Cell,* **31,** 443-452 (1982).

SPIRA, J., BABONITS, M., WIENER, F., OHNO, S., WIRSCHUBSKY, Z., HARAN-GHERA, N., and KLEIN, G., Non random chromosomal changes in Thy-1 positive and Thy-1-negative lymphomas induced by 7,12-dimethylbenzanthracene in SJL mice. *Cancer Res.,* **40,** 2609-2616 (1980).

SPIRA, J., WIENER, F., BABONITS, M., GAMBLE, J., MILLER, J., and KLEIN, G., The role of chromosome 15 in murine leukemogenesis. I. Contrasting behavior of the tumor vs. normal parent-derived chromosomes No. 15 in somatic hybrids of varying tumorigenicity. *Int. J. Cancer,* **28,** 785-798 (1981).

SPIRA, J., WIENER, F., and KLEIN, G., Robertsonian translocation studies on the significance of trisomy-15 murine T-cell leukemia. *Cancer Genet. Cytogenet.,* **9,** 45-50 (1983).

SPIRA, J., WIENER, F., OHNO, S., and KLEIN, G., Is trisomy cause or consequence of murine T-cell leukemia development? Studies on Robertsonian translocation in mice. *Proc. nat. Acad. Sci. (Wash.),* **76,** 6619-6621 (1979).

SÜMEGI, J., SPIRA, J., BAZIN, H., SZPIRER, J., LEVAN, G., and KLEIN, G., Rat *c-myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t (6:7) chromosomal translocations. *Nature (Lond.),* **306,** 497-498 (1983).

TAUB, R., KIRSCH, I., MORTON, C., LENOIR, G., SWAN, D., TRONICK, S., AARONSON, S., and LEDER, P., Translocation of the *c-myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. nat. Acad. Sci. (Wash.),* **79,** 7837-7841 (1982).

WAHL, G.M., STERN, M., and STARK, G.R., Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl paper and rapid hybridization by using dextran sulfate. *Proc. nat. Acad. Sci. (Wash.),* **76,** 3683-3687 (1979).

WANG, H.C., and FEDOROFF, S., Banding of human chromosomes treated with trypsin. *Nature (Lond.),* **235,** 52-54 (1971).

WIENER, F., OHNO, S., SPIRA, J., HARAN-GHERA, N., and KLEIN, G., Chromosome changes (trisomies 15 and 17) associated with tumor progression in leukemias induced by radiation leukemic virus. *J. nat. Cancer Inst.,* **61,** 227-238 (1978*a*).

WIENER, F., OHNO, S., SPIRA, J., HARAN-GHERA, N., and KLEIN, G., Cytogenetic mapping of the trisomic segment of chromosomes 15 in murine T-cell leukemia. *Nature (Lond.),* **275,** 658-660 (1978*b*).

WIENER, F., SPIRA, J., OHNO, S., HARAN-GHERA, N., and KLEIN, G., Chromosome changes (trisomy 15) in murine T-cell leukemia induced by 7,12-dimethylbenz(*a*)anthracene (DMBA). *Int. J. Cancer,* **22,** 447-453 (1978*c*).