Molecular pathology

Chromosomal translocations in non-Hodgkin's lymphomas (Part I)

F. Vega[1] and L.J. Medeiros

Division of Pathology and Laboratory Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA.

INTRODUCTION

During the process of translocation, a chromosome is disrupted with a segment becoming attached to a different chromosome (1). In many solid tumors, chromosome translocations are typically complex, appear to be random, and therefore are not constant among tumors of the same histological subtype (2). In these tumors, large portions of chromosomes are often deleted during recombination, leading to loss of heterozygosity at the molecular level. In contrast, in leukemia, non-Hodgkin lymphomas (NHLs) and a subset of sarcomas (*e.g.*, Ewing's sarcoma) (3), distinctive chromosomal translocations occur consistently in specific neoplasms, and are thought to be integrally involved in their pathogenesis. Thus, their presence can be used to classify neoplasms, predict response to therapy, and monitor residual disease (4).

Nonrandom chromosomal translocations are a form of gene rearrangement, but unlike antigen receptor gene rearrangements, occur rarely or not at all in normal cells (1). Commonly, chromosome translocations in lymphoid neoplasms are reciprocal; in other words, segments from two chromosomes exchange places. Reciprocal translocations may be further characterized as balanced or unbalanced. In balanced translocations there is no net loss of DNA. Primary chromosomal translocations in NHL are generally balanced translocations, however, secondary chromosomal anomalies tend to be unbalanced. Secondary chromosomal translocations in most types of NHL are poorly characterized at the present time, but may constitute important events in tumor initiation and/or progression (2).

The genes located at the breakpoint sites of a number of chromosomal translocations have been identified. The genes involved are usually oncogenes that encode for several functional classes of proteins, including tyrosine or serine protein kinases, cell surface receptors, growth factors, proteins involved in apoptotic pathways, and transcriptional regulating factors (1). Transcription factors are proteins that are involved in the initiation of gene transcription; they recognize and bind to target sequences located in the regulatory elements of genes,

[1]Dr. Vega is a postdoctoral fellow at the University of Texas M.D. Anderson Cancer Center. He is supported by a grant from "Fundación Pedro Barrie de la Maza", Galicia, Spain.

often functioning in a tissue-specific fashion. Many of these genes were first identified as a result of the molecular characterization of their involvement in translocations in acute and chronic leukemias and NHLs.

In this review article, we provide an overview of chromosomal translocations in NHLs and the utility of molecular genetic methods in their assessment.

TYPES OF CHROMOSOMAL TRANSLOCATIONS IN NON-HODGKIN'S LYMPHOMAS

Chromosomal translocations in hematopoeitic tumors can be subdivided into two types. In the first type, via the translocation an intact gene (or intact coding region) is juxtaposed with another gene, usually one of antigen receptor genes (activation of oncogenes by transposition to an active chromatin domain) (Fig. 1). The oncogene is thus brought under control of active enhancer regions within the antigen receptor gene locus and is overexpressed, resulting in a quantitative increase of qualitatively normal oncoprotein. This type of translocation is most common in NHLs. For example, the t(14;18) in follicular lymphoma results in the *bcl*-2 gene on chromosome 18q21 being juxtaposed with the immunoglobulin (Ig) heavy chain gene locus at 14q32 (3, 5). The *bcl*-2 gene encodes a protein that protects cells from programmed cell death (6).

In the second type of translocation, two genes (usually non-antigen receptor genes) are disrupted and portions of each gene are juxtaposed resulting in a fusion gene, chimeric mRNA, and novel protein (Fig. 2). These fusion proteins are tumor-specific as they do not exist, or rarely exist, in non-malignant cells. The mechanisms involved in tumorigenesis are quite variable from one translocation to another and the specific mechanisms resulting from many translocations remain uncertain (1, 7). This type of chromosomal translocation is most frequently found in acute and chronic myeloid leukemias. For example, the t(15;17) in acute promyelocytic leukemia and the t(9;22) in chronic myeloid leukemia generate the novel fusion genes *pmr-rarα*, and *bcr-abl*, respectively. This type of translocation is less common in NHLs, but currently is known to occur in two NHL types: the t(2;5) in T/null anaplastic large cell lymphoma and the t(11;18) in low-grade B-cell NHL of mucosa-associated lymphoid tissue.

GENERAL MECHANISMS OF CHROMOSOMAL TRANSLOCATIONS IN NON-HODGKIN'S LYMPHOMAS

Common to many translocations in NHLs is the involvement of an antigen receptor gene locus, which provides enhancer or promoter elements that result in

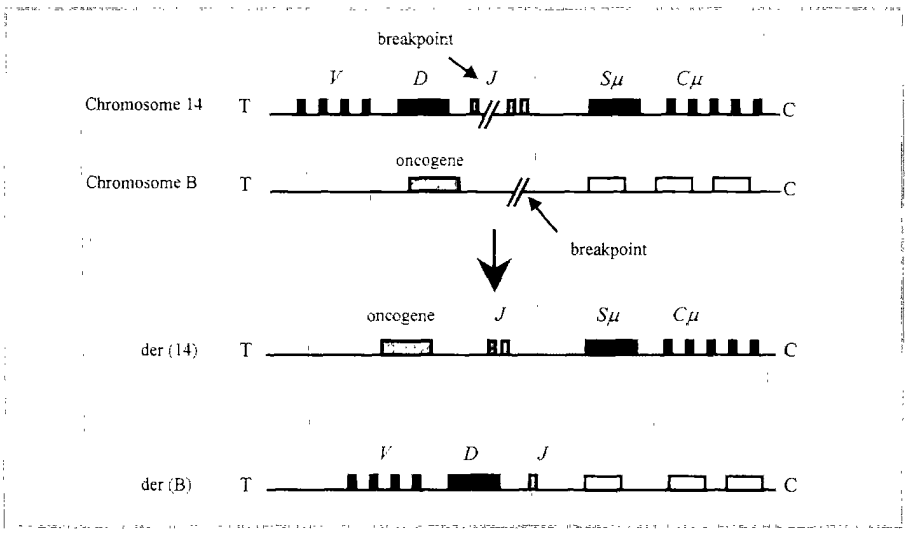


Figure 1. Reciprocal balanced chromosomal translocation in which an oncogene is activated by juxtaposition with the *IgH* gene. The chromosomal translocation breakpoints on chromosome 14 generally occur in the joining region (J) segments. The oncogene lies on the other side of the breakpoint. The oncogene is thus brought under control of active enhancer regions within the antigen receptor gene locus and is overexpressed, resulting in a quantitative increase of qualitatively normal oncoprotein. T = telomere. C = centromere.

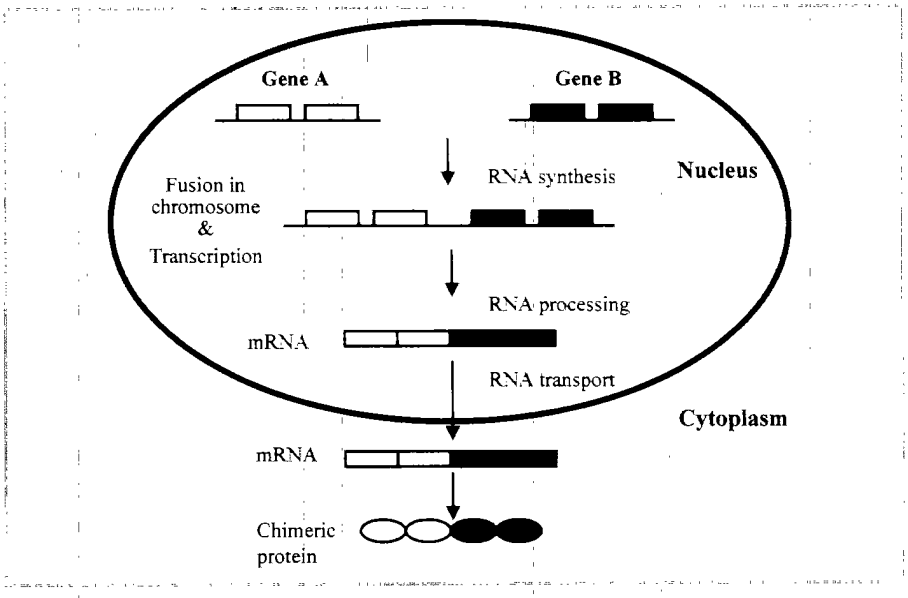


Figure 2. Chromosomal translocation and oncogene activation by gene fusion. In this type of translocation, two genes are disrupted and fused, resulting in chimeric mRNA which is then transported to the cell cytoplasm and translated into a novel chimeric protein.

over-expression of partner genes. The antigen receptor loci of B-cells may be subjected to four types of modification: recombination of the variable (V), diversity (D), and joining (J) regions, somatic hypermutation of the V segments, heavy chain class switching and receptor editing. Occasional failures in the control of these processes appear to play an important role in the generation of chromosomal translocations in B-cell NHLs. The transforming events involved in the pathogenesis of B-cell NHLs occur mainly at two stages of B-cell development: during variable region recombination in B-cell precursors in the bone marrow and during B-cell differentiation in the germinal center microenvironment, as part of T-cell dependent immune responses. Chromosome translocations in T-cell NHLs also may arise from analogous errors of T-cell-receptor gene V(D)J recombination. Somatic hypermutation of V segments is rare and class Ig heavy switching does not occur in normal T-cells.

The development of B-cells in the bone marrow is initiated by a site-specific recombination reaction, V(D)J recombination, of the immunoglobulin heavy and light chain genes (8). Recombination involves the introduction of double-strand breaks at specific recognition signal sequences (RSS) adjacent to the V, D, and J elements (3' to the V regions, 5' and 3' to the D regions, and 5' to the J regions) by the products of the recombination-activating genes, RAG-1 and RAG-2 (9). Defects during the phases in which these physiological breaks are generated, or in which they are joined, can result in chromosomal translocations. In the case of the t(14;18) in follicular lymphoma, the chromosomal breakpoints in the Ig loci are located in the 5' end of J gene segments and frequently occur within or near RSS bordering DH or JH segments (3, 5, 10). In addition, sequences resembling RSS have been described in proximity to *bcl-2* breakpoints at 18q21 (11). The t(7;9) (q34;q32) of precursor T-cell lymphoblastic lymphoma/ leukemia similarly involves breakpoints at RSS flanking D segments of the TCRβ gene on chromosome 7 (12). Also reminiscent of V(D)J coding joints is the frequent finding of non-templated nucleotide insertions at translocation breakpoints (13). These insertions are mediated by terminal deoxynucleotidyl transferase (TdT) during the process of normal V(D)J recombination. Thus, some translocations probably result from mistakes of V(D)J recombination mediated by the RAG-1 and RAG-2 enzymes (14, 15).

Somatic hypermutation is a process by which mutations, mainly single-nucleotide exchanges or small insertions or deletions of DNA, are introduced at a high rate into the Ig V segments in the normal germinal center after exposure to antigen (16-18). These mutations require double-stranded DNA breaks prior to the mutational event and thus increase the likelihood that translo-

cations occur (16). Others (16, 19) recently have suggested that translocations involving the c-*myc* gene in endemic Burkitt's lymphoma probably arise as byproducts of somatic hypermutation, and are less likely to originate from erroneous V(D)J recombination in B-cell precursors, as had been suggested previously. Normal somatic mutation explains the well-known phenomenon that antibody affinity to antigen increases with repeated antigen exposure. Mutations that result in increased affinity are positively selected.

Ig heavy chain switching is a process by which B-cells in the germinal center normally switch their Ig heavy chain constant IgM and IgD regions to IgG, IgA, or IgE (20). This process is mediated by a recombination event that occurs after antigen exposure that results in deletion of DNA between involved switch (S) regions, which are arrays of short tandem repeats, located upstream of each constant-region gene (with the exception of Cδ). This results in a change in the effector functions of the antibody but leaves the V(D)J region unaltered. For several types of B-cell NHL, chromosomal translocations into immunoglobulin heavy chain switch regions have been described, such as c-*myc* translocation in sporadic Burkitt's lymphoma (21-23).

Receptor editing is a process by which an expressed antibody polypeptide chain, usually κ or λ, is replaced by another one. The process of Ig light-chain loci receptor editing is mediated by secondary rearrangements of the variable-region gene, usually involving upstream V segments and downstream J segments (24). This process also involves DNA-strand breaks and may play a role in the generation of chromosomal translocations.

It also has been suggested that translocations can occur through elements such as χ sequences or alternating purine-pyrimidine tracts, which promote recombination. These elements form negative supercoiled regions (Z-DNA) making DNA more accessible to the enzymatic machinery (25). *Alu* sequences also have been implicated in recombinations and translocations, such as in the t(9;22), where both *abl* and *bcr* introns contain *Alu* sequences in opposite orientation (26).

METHODS FOR DETECTION OF CHROMOSOMAL TRANSLOCATIONS

Nonrandom chromosomal abnormalities are a central feature of the pathogenesis of many types of leukemia and NHL and their detection is presently the standard of care for the diagnosis of certain tumors. The standard technique for detecting chromosomal translocations has been conventional cytogenetic analysis. Chromosome translocations are detected cytogenetically as a fusion of different chromosomes or of non-contiguous segments of a single chromosome, the latter occurring after structural rearrangement, for example, inversion. These studies represent the first step, often localizing the abnormality to a relatively small chromosome locus, where research efforts can be focused to identify the exact genes involved. Once the genes are recognized, the translocations are cloned, and their breakpoints sequenced. Molecular genetics approaches such as Southern blot hybridization, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH) can then be applied to the study of specific chromosomal translocations in NHLs in greater detail. For diagnostic purposes, the best method to assess for a specific chromosomal translocation depends on a number of factors, most importantly the structure of the involved genes, but also the type of sample available for study and the convenience of various methods.

Restriction fragment length analysis (Southern Blot Hybridization)

Restriction fragment length analysis can be used to detect chromosomal translocations, although it is not usually the method of choice. Translocations are suited to detection by this method because the presence of a translocation alters the germline configuration and corresponding restriction enzyme sites of the involved genes (27). To prove the presence of a chromosomal translocation, the cells must be analyzed with probes specific for each chromosome involved. The detection of a non-germline fragment that hybridizes with both probes is evidence of the translocation. For example, to detect the t(14:18) probes specific for the *IgH* on 14q32 and the *bcl*-2 gene on 18q21 are used. A single probe also can be used to assess for gene translocations (28). However, this approach only demonstrates the presence of a breakpoint within the gene, and does not allow detection of the partner chromosome. For example, if analysis with a *bcl*-6 probe demonstrates rearrangement, then an alteration in 3q27 is present. However, no information regarding the partner chromosome is generated.

There are at least two disadvantages of using restriction fragment length analysis to detect chromosomal translocations. These methods are only sensitive to the level of approximately 1-5%, significantly less then PCR, and therefore are not sufficiently sensitive to detect minimal residual disease following therapy. Also, these methods are relatively time-consuming, labor intensive and are limited by the need for large amounts of high-quality DNA.

Polymerase chain reaction

The polymerase chain reaction (PCR) is a technique that allows massive amplification of specific nucleic acid fragments, and can performed in a single test tube (Figs. 3-5). PCR methods are far more convenient than restriction fragment length analysis for detecting chromosomal translocations if the partner chromosomes are constant and the breakpoints are tightly clustered (29). PCR is advantageous because this technique is rapid compared with more laborious methods, such as restriction fragment length analysis, and DNA extracted from archival specimens can be used (30).

There are disadvantages to using PCR to assess for some types of chromosomal translocations. Standard PCR assays can efficiently amplify only small fragments of DNA of less than 1 kb. Thus, PCR is not well suited to assess translocations with widely scattered breakpoints. Furthermore, translocations that involve one constant chromosome with many different partner chromosomes require primers for each chromosome potentially involved. Chromosomal translocations involving the c-*myc* gene in Burkitt's lymphoma exemplify the problems of both widely scattered breakpoints and multiple-partner chromosomes. In Burkitt's lymphoma, translocations involving c-*myc* at 8q24 can be identified in virtually all cases (31). However breakpoints on chromosome 8 are variable and widely scattered (31). Furthermore, c-*myc* may be translocated to one of three chromosome loci, 14q32 *(IgH)*, 2p12 *(Ig)*κ, or 22q11 *(Igλ)*. Standard diagnostic PCR assays thus become impractical because numerous primers sets are needed to amplify all the possible variations of t(8;14) and the variant translocations involving Igκ and Igλ.

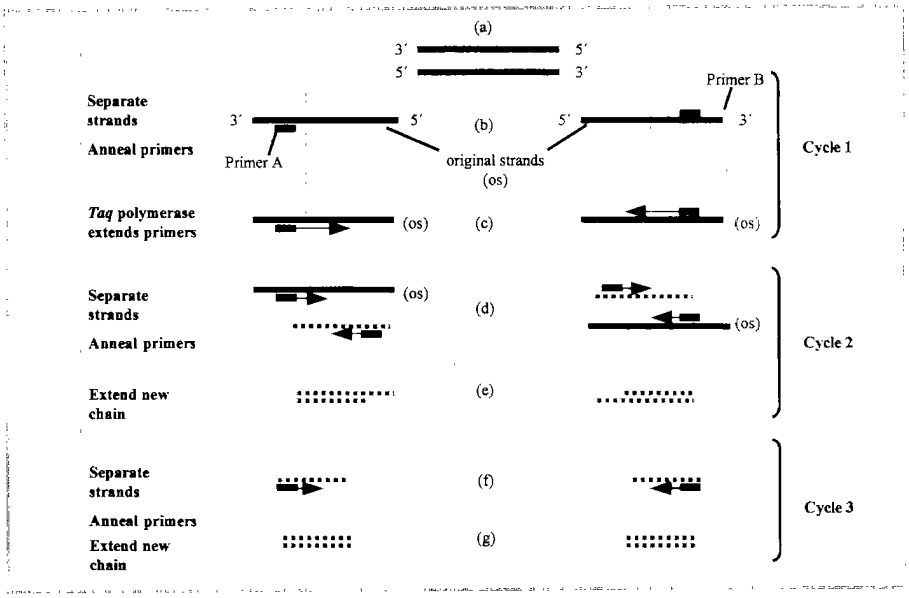


Figure 3. Polymerase chain reaction leads to amplification of the target sequences. The starting material is double-stranded DNA molecule [a]. The strands are denatured (separated) by heating the reaction mixture at a high temperature and then cooling allowing the primers to anneal to DNA flanking the target region, one on each strand [b]. *Taq* polymerase synthesizes new complementary strands of DNA, including the target sequence [c]. The reaction mixture is heated again; the original and newly synthesized DNA strands separate. Four binding sites are now available to the primers, one on each of the two original strands and the two new strands [d] (subsequent events involving the original strands (os) are omitted). *Taq* polymerase synthesizes new complementary strands but the extension of these chains is limited to the target sequence [e]. Primers anneal to the newly synthesized strands [f]. The first of the completely synthesized double-stranded DNA target fragments is shown in [g].

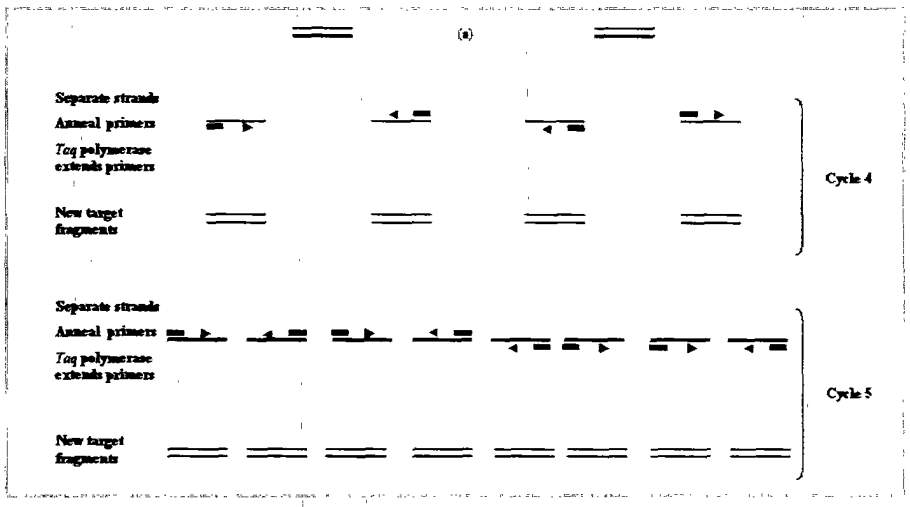


Figure 4. This figure illustrates geometric doubling of target DNA beginning after the third cycle of the PCR reaction. The first of the target fragments completely synthesized after three PCR cycles is shown in [a].

Long-range PCR

Long-range PCR is an alternative approach for the detection of chromosomal translocations that have widely scattered breakpoints. In this technique, primers and conditions are designed to optimize the efficiency of PCR, allowing amplification of relatively long segments of DNA (32).

Recently, two chromosomal translocations with widely scattered breakpoints have been studied by long-range PCR with promising results. Basso *et al.* (33) have shown that PCR can detect the t(8;14) in approximately 80% of pediatric sporadic Burkitt's lymphoma using a multiplex long-range PCR method. A nested long-range PCR technique also has been applied for the detection of

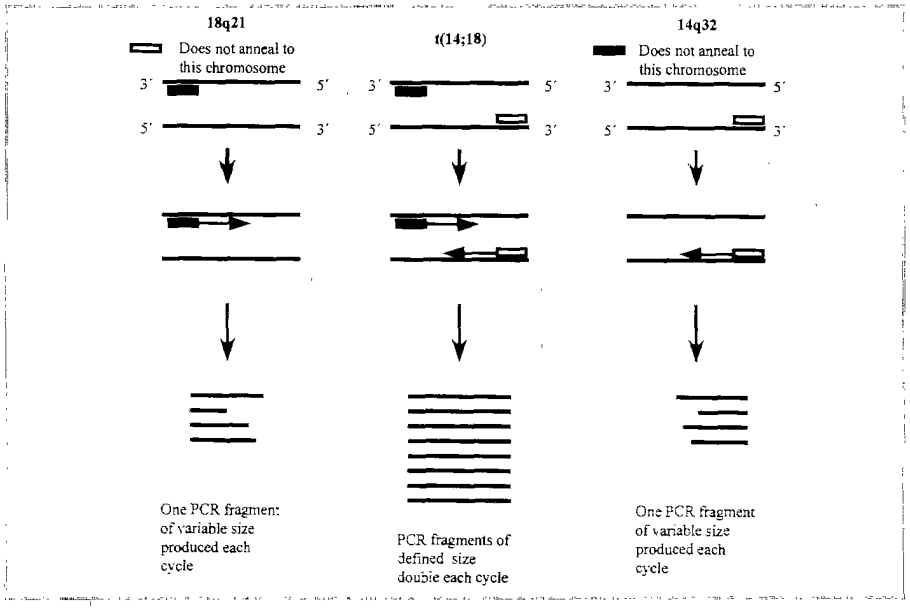


Figure 5. This figure illustrates PCR amplification of the t(14;18)(q32;q21) using one set of primers. If the translocation is not present, the primers are located on different chromosomes. Although these primers will initiate synthesis of new DNA strands on the normal chromosome 14 and chromosome 18, respectively, these strands accumulate at a linear rate because they can be synthesized using only the starting DNA as a template and they are of variable length because their 3' ends are not defined. In contrast, the t(14;18) brings the two primers close together on the same double-stranded DNA molecule. This defined fragment doubles in quantity with each cycle.

the t(2;5) in T/null anaplastic large cell lymphoma in our laboratory (34). Similar to standard PCR, the unique breakpoints yield differently sized amplification products that allow one to exclude contamination of a series of reaction when it occurs (34, 35). Disadvantages to long-range PCR are that this technique requires high quality DNA, which is usually not obtainable from archival specimens, and is more technically demanding than standard PCR.

Reverse-transcriptase PCR

An alternative approach to the problem of widely scattered breakpoints is to synthesize complementary DNA (cDNA) from mRNA by using reverse transcriptase (36). In the process of normal transcription, introns are spliced out of mRNA and exons are brought into close proximity (Fig. 6). PCR primers specific for these exons therefore anneal in close proximity cDNA, allowing standard PCR methods to be used. However, reverse transcriptase PCR (RT-PCR) has its drawbacks. Working with RNA is technically demanding and requires ribonuclease-free conditions. In addition, RT-PCR methods generate amplicons of the same size and thus, contamination of a series of RT-PCR reactions cannot be excluded easily.

5'→3' exonuclease-based real-time PCR

Recently, we developed real-time PCR assays based on the 5'→3' exonuclease activity of *Taq* polymerase to detect the t(14;18) (37) and t(11;14) (37-39). These methods permit amplicon-detection without post-PCR processing. The theory and methods of real-time PCR assays have been described in detail (39-41). Briefly, a nonextendable oligonucleotide probe internal to the primer set, labeled with a 5' reporter fluorescent dye and a 3' quencher fluorescent dye, is included in each PCR assay. When the probe is intact, the reporter dye emission is quenched owing to the physical proximity of the reporter and quencher dyes (41). However, with DNA synthesis the 5' exonuclease activity of *Taq* polymerase hydrolyzes the probe during the extension phase of PCR, releasing the reporter dye from the probe and 3' quenching dye (Fig. 7). Consequently, fluorescence increases in proportion to the concentration of the DNA templates, which rises geometrically in the presence of DNA amplification, but only linearly in its absence (Fig. 8). Therefore, real-time PCR assays allow accurate quantification of the amplification products, and there is emerging evidence to suggest that amplicon quantity, in addition to its presence or absence, may convey useful clinical information.

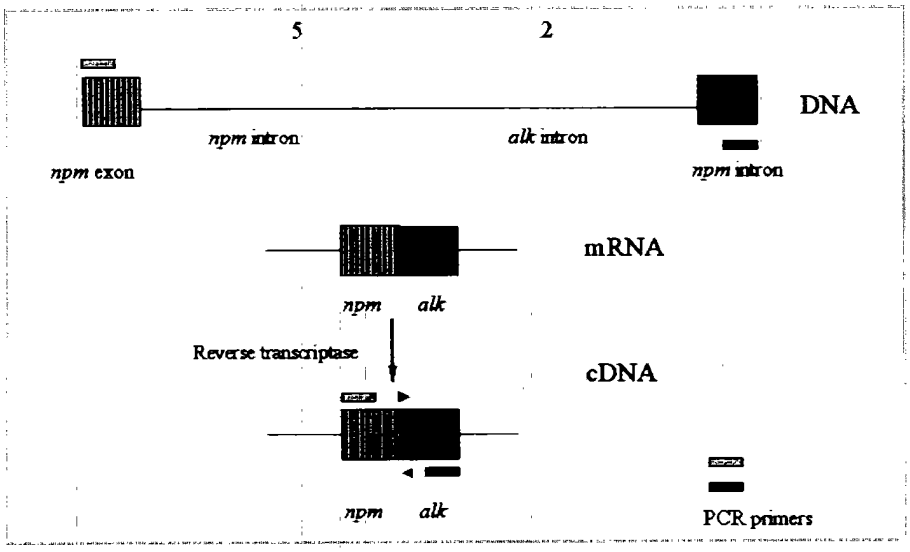


Figure 6. Schematic illustration of reverse-transcriptase PCR for the t(2;5)(p23;q35). The translocation occurs within introns and the breakpoints are widely scattered (not shown here), precluding amplification of genomic DNA using standard PCR methods. The exons are constant, allowing the use of a set of exon-specific primers, but the distance between the exons is too great to perform standard PCR. However, if messenger RNA is transcribed into complementary DNA by reverse transcriptase, exon-specific primers are brought in close proximity allowing PCR amplification using standard methods.

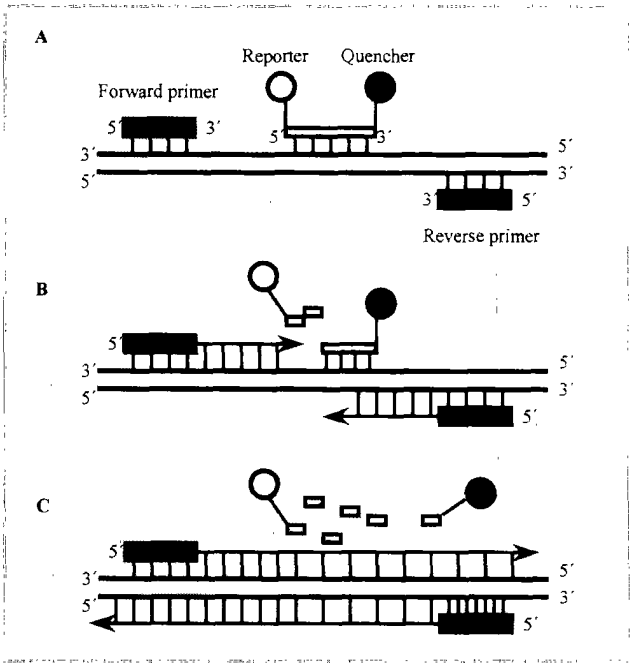


Figure 7. Schematic illustration of sequence-specific annealing and 5'3' exonuclease cleavage of the fluorescent dye-labeled probe. (A) Annealing of the primers (black boxes) and probe (gray box) to the target sequence. (B) Extension of the primer and the initiation of cleavage of the probe at its 5' end by *Taq* polymerase. (C) Release of the probe from the target strand. The separation of the reporter dye from the quencher dye abrogates the quencher effect and results in an increase in the fluorescence signal of the reporter dye.

Fluorescence *in situ* hybridization

One of the recent advances in cytogenetic analysis that has greatly facilitated our ability to detect chromosomal abnormalities is fluorescence *in situ* hybridization (FISH). FISH allows the analysis of both dividing and non-dividing cells (27). FISH can be performed on peripheral blood and bone marrow aspirates smears, cytospin cell preparations, colony assays, nuclei extracted from imprints made from fresh tissue or frozen tissue, and nuclei obtained from formalin-fixed paraffin-embedded tissue sections (42-44).

For chromosomal translocations with widely dispersed breakpoints, FISH methods are advantageous because large probes can be used that span large regions of DNA (>100 kb), thereby detecting widely dispersed breakpoints. Recently, the feasibility and validity of two-color FISH for the detection of chromosomal translocations has been well illustrated (45). DNA probes for the fusion genes involved in many of the chromosomal translocations involved in leukemia and NHLs are now commercially available.

For detection of translocations by FISH, three approaches can be used. The simplest one is a colocalization assay, using two probes (one from each of the fusion

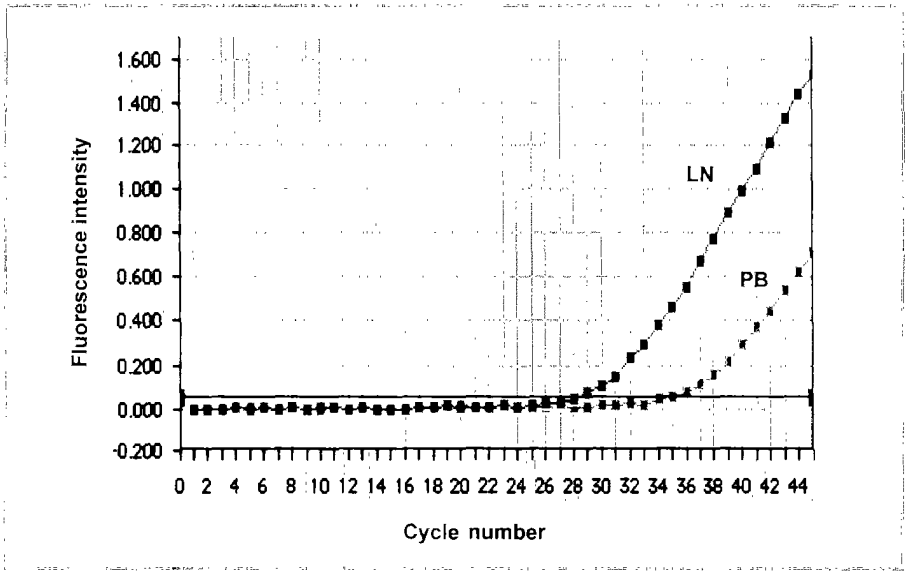


Figure 8. t(11;14)(q13;q32). Linear amplification plots of real-time PCR assays for detecting *bcl*-1 MTC/JH in lymph node (LN) and peripheral blood (PB) samples from a patient with mantle cell lymphoma. The fluorogenic probe is hydrolized emitting fluorescence only if the *bcl*-1 MTC/JH DNA fusion sequences are present. Note that a lower fluorescent signal was observed in the PB compared with the LN specimen. When these specimens were analyzed for ß-actin, superimposed amplification plots were observed, indicating that the difference in *bcl*-1 MTC/JH DNA fusion sequence fluorescence signal between the two specimens was a true reflection of the number of tumor cells and not due to variation in the initial quantity of DNA in the two reactions.

genes), differentially labeled and detected with two different-colored fluorochromes (red and green, respectively). An interphase cell positive for the translocation will exhibit a red-green (yellow) fusion signal representing the translocation, and single red and green signals correspond with the normal chromosome homologs. However, the false-positive rate using this approach is relatively high, approximately 5% (46). A second approach is a segregation assay, with one probe telomeric and one centromeric to the breakpoint region also labeled in two different colors. The presence of the translocation separates these signals. The third approach is three-color FISH, combining segregation and colocalization detection assays.

The ability to combine interphase FISH analysis with immunological staining for cell surface antigens, such as the fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasms (FICTION) technique, (47) provides a powerful method to combine cell by cell analysis with morphology and immunophenotype.

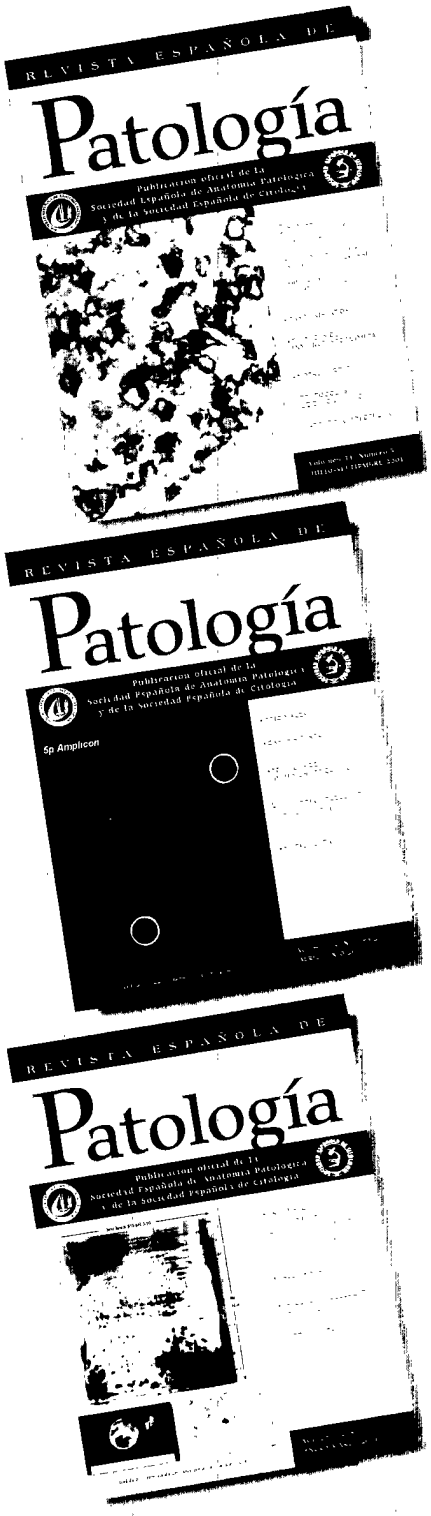
The term DNA fiber-FISH is used to describe a collection of methods for performing FISH on DNA stretched out as fibers on a glass slide. These methods are powerful tools for the analysis of chromosomal translocations with widely scattered breakpoints (48, 49). Individual DNA fibers can be released from the nucleus and fixed on a slide. Because of the linearity of the template, adjacent probes generate adjacent linear signals without overlap. Thus, hybridization with multiple, contiguous differently colored probes generates a linear, easily recognizable signal referred to as "color barcode". In cases with a translocation, breaks within this barcode are easily detected and due to the linear relationship between physical and genomic distance in DNA fibers, each breakpoint can be accurately mapped relative to well defined markers in a range of 1 to 500 kb (50). Co-localization of the translocated sequences on single DNA fibers can be visualized by using color barcodes for both loci. This method has great potential for mapping and detecting chromosomal breakpoints within large genomic regions of several hundreds of kilobases. The usefulness of this technique has been demonstrated by the mapping of 11q13 breakpoints in mantle cell lymphoma (50).

There also disadvantages to using FISH methods. As compared with PCR, FISH methods are less sensitive for following minimal residual disease and are relatively time consuming. Unlike conventional cytogenetics, which allows assessment of the entire genome, FISH is highly focused and therefore allows assessment of only a limited portion of DNA.

REFERENCES

1. Rabbits TH. *Chromosomal translocations in human cancer.* Nature 1994; 372: 143-149.
2. Johansson B, Mertens F, Mitelman F. *Primary vs secondary neoplasia-associated chromosomal abnormalities-balanced rearrangements vs. genomic imbalances?* Genes Chromosomes Cancer 1996; 16: 155-163.
3. Le Beau MM, Rowley JD. *Chromosomal abnormalities in leukemia and lymphoma: Clinical and biological significance.* Adv Hum Genet 1986; 15: 1-54.
4. Bakhshi A, Jensen JP, Goldman P et al. *Cloning the chromosomal breakpoint of t(14;18) human lymphomas: Clustering around JH on chromosome 14 and near a transcriptional unit on 18.* Cell 1985; 41: 899-906.
5. Cleary ML, Sklar J. *Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18.* Proc Natl Acad Sci USA 1985; 82: 7439-7443.
6. Korsmeyer SJ. *Programmed cell death: Bcl-2.* Important Adv Oncol 1993; 19-28.
7. Look AT. *Fusion genes and their hybrid proteins in human leukemias and lymphomas.* Proc Assoc Am Physicians 1995; 107: 175-180.
8. Tonegawa S. *Somatic generation of antibody diversity.* Nature 1983; 302: 575-581.
9. Oettinger MA, Schatz DG, Gorka C, Baltimore D. *RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination.* Science 1990; 248: 1517-1523.
10. Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC, Croce CM. *Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation.* Science 1984; 224: 1403-1406.
11. Showe LC, Croce CM. *The role of chromosomal translocations in B- and T- cell neoplasia.* Annu Rev Immunol 1987; 5: 253-277.
12. Tycko B, Reynolds TC, Smith SD, Sklar J. *Consistent breakage between consensus recombinase heptamers of chromosome 9 DNA in a recurrent chromosomal translocation of human T cell leukemia.* J Exp Med 1989; 169: 369-377.
13. Tycko B, Sklar J. *Chromosomal translocations in lymphoid neoplasia: A reappraisal of the recombinase model.* Cancer Cells 1990; 2: 1-8.
14. Hiom K, Melek M, Gellert M. *DNA transposition by RAG1 and RAG2 proteins: A possible source of oncogenic translocations.* Cell 1998; 94: 463-470.
15. Agrawal A, Eastman QM, Schatz DG. *Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system.* Nature 1998; 394: 744-751.
16. Goossens T, Klein U, Kuppers R. *Frequent occurrence of deletions and duplications during somatic hypermutations: Implications for oncogene translocations and heavy chain disease.* Proc Natl Acad Sci USA 1998; 95: 2463-2468.

17. Wilson PC, de Bouteiller O, Liu YJ et al. *Somatic hypermutation introduces insertions and deletions into immunoglobulin V genes.* J Exp Med 1998; 187: 59-70.
18. Jacob J, Kelsoe G, Rajewsky K, Weiss U. *Intraclonal generation of antibody mutants in the germinal centers.* Nature 1991; 354: 389-392.
19. Hoang AT, Lutterbach B, Lewis BC et al. *A link between increased transforming activity of lymphoma-derived MYC mutant alleles, their defective regulation by p107, and altered phosphorylation of the c-myc transactivation domain.* Mol Cell Biol 1995; 15: 4031-4042.
20. Liu YJ, Arpin C, de Bouteiller O et al. *Sequential triggering of apoptosis, somatic mutation, and isotype switch during germinal center development.* Semin Immunol 1996; 8: 169-177.
21. Magrath I. *The pathogenesis of Burkitt's lymphoma.* Adv Cancer Res 1990; 55: 133-270.
22. Ye BH, Chaganti S, Chang CC et al. *Chromosomal translocations cause deregulated BCL-6 expression by promoter substitution in B cell lymphoma.* EMBO J 1995; 14: 6209-6217.
23. Chesi M, Nardini E, Brents LA et al. *Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3.* Nat Genet 1997; 16: 260-264.
24. Harada K, Yamagishi H. *Lack of feedback inhibition of V kappa gene rearrangement by productively rearranged alleles.* J Exp Med 1991; 173: 409-415.
24. Boehm T, Mengle-Gaw L, Kees UR et al. *Alternating purine-pyrimidine tracts may promote chromosomal translocations seen in a variety of human lymphoid tumours.* EMBO J 1989; 8: 2621-2631.
25. Denny CT, Shah N, Odgen S et al. *Localization of preferential sites of rearrangement within BCR gene in Philadelphia chromosome positive acute lymphoblastic leukemia.* Proc Natl Acad Sci USA 1989; 86: 4254-4258.
26. Cossman J, Uppenkamp M, Sundeen J, Coupland R, Raffeld M. *Molecular genetics and the diagnosis of lymphoma.* Arch Pathol Lab Med 1988; 112: 117-127.
27. Dalla-Favera R, Ye BH, Cattoretti G et al. *BCL-6 in diffuse large-cell lymphomas.* Important Adv Oncol 1996; 9: 139-148.
28. Baumforth KRN, Nelson PN, Digby JE, O'Neil JD, Murray PG. *The polymerase chain reaction.* J Clin Pathol Mol Pathol 1999; 52: 1-10.
29. Pan LX, Diss TC, Isaacson PG. *The polymerase chain reaction in histopathology.* Histopathology 1995; 26: 201-217.
30. Pelicci PG, Knowles DM, Magrath I, Dalla-Favera R. *Chromosomal breakpoints and structural alterations of the c-myc locus differ in endemic and sporadic forms of Burkitt lymphoma.* Proc Natl Acad Sci USA 1986; 83: 2984-2988.
31. Cheng S, Chang SY, Gravit P, Respess R. *Long PCR.* Nature 1994; 369: 684-685.
32. Basso K, Frascella E, Zanesco L, Rosolen A. *Improved long distance polymerase chain reaction for the detection of t(8;14)(q24;q32) in Burkitt's lymphomas.* Am J Pathol 1999; 155: 1479-1485.
33. Sarris AH, Luthra R, Papadimitracopoulou V et al. *Amplification of genomic DNA demonstrates the presence of the t(2;5)(p23;q35) in anaplastic large cell lymphoma, but not in other non-Hodgkin's lymphomas, Hodgkin's disease, or lymphomatoid papulosis.* Blood 1996; 88: 1771-1779.
34. Luthra R, Pugh WC, Waasdorp M et al. *Mapping of genomic t(2;5)(p23;q35) breakpoints in patients with anaplastic large cell lymphoma by sequencing long-range PCR products.* Hematopathol Mol Hematol 1998; 11: 173-183.
35. Kawasaki ES, Wang AM. *Detection of gene expression.* In: H.A. Erlich (Ed.). PCR Technology. Stockton Press, New York 1989; 89-97.
36. Luthra R, McBride A, Cabanillas F, Sarris A. *Novel 5' exonuclease-based real-time PCR assay for the detection of t(14;18) (q32;q21) in patients with follicular lymphoma.* Am J Pathol 1998; 153: 63-68.
37. Luthra R, Sarris A, Hai S et al. *Real-time 5' 3' exonuclease-based PCR assay for detection of the t(11;14)(q13;32).* Am J Clin Pathol 1999; 112: 524-530.
38. Luthra R, Medeiros LJ. *5'3' exonuclease-based real-time PCR methods for detecting the t(14;18) and t(11;14) in non-Hodgkin's lymphomas.* J Clin Assay 2000; 23: 6-14.
39. Higuchi R, Dollinger G, Walsh P, Griffith R. *Simultaneous amplification and detection of specific DNA sequences.* Biotechnology 1992; 10: 413-417.
40. Livak KJ, Flood SJA, Marmaro J, Giusti W, Deetz K. *Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.* PCR Methods Appl 1995; 4: 357-362.
41. Li JY, Gaillard F, Moreau A et al. *Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization.* Am J Pathol 1999; 154: 1449-1452.
42. Bentz M, Schroder M, Herz M, Stilgenbauer S, Lichter P, Dohner H. *Detection of trisomy 8 on blood smears using fluorescence in situ hybridization.* Leukemia 1993; 7: 752-757.
43. Poddighe PJ, Van Der Lely N, Voojis P, De Witte T, Ramaekers FCS. *Interphase cytogenetics on agar cultures: A novel approach to determine chromosomal aberrations in hematopoietic progenitor cells.* Exp Hematol 1993; 21: 859-863.
44. Seiber R, Matthiesen P, Harder S et al. *Application of interphase fluorescence in situ hybridization of the detection of the Burkitt translocation t(8;14)(q24;q32) in B-cell lymphomas.* Blood 1998; 91: 984-990.
45. Kearney L. *The impact of the new FISH technologies of the cytogenetics of haematological malignancies.* Br J Haematol 1999; 104: 648-658.
46. Weber-Matthiesen K, Winkemann M, Muller-Hermelink A, Schelberger B, Grote W. *Simultaneous fluorescence immunophenotyping and interphase cytogenetics: A contribution to the characterization of tumour cells.* J Histochem Cytochem 1992; 40: 171-175.
47. Wiegant J, Kalle W, Mullenders L et al. *High-resolution in situ hybridization using DNA halo preparations.* Hum Mol Genet 1992; 1: 587-591.
48. Parra I, Windle B. *High-resolution visual mapping of stretched DNA by fluorescent hybridization.* Nat Genet 1993; 5: 17-21.
49. Vaandrager JW, Schuuring E, Zwikstra E et al. *Direct visualization of dispersed 11q13 chromosomal translocations in mantle cell lymphoma by multicolor DNA fiber fluorescence in situ hybridization.* Blood 1996; 88: 1177-1182.



A partir del 1 de enero de 2002
el Comité de Redacción
de la Revista Española de Patología
se traslada a la sede central
de la SEAP.

Los trabajos destinados
a ser publicados
deben remitirse a esta dirección:
Sociedad Española
de Anatomía Patológica

C/ Áncora 3, 2º B - 28045 Madrid
Tfno./Fax: 915 398 628
e-mail: seap@seap.es

Hasta esa fecha se mantiene
la dirección actual,
tanto para la recepción de los trabajos
como para cualquier otra consulta.