cipitation are of great significance for food production and water resource management, even more so than changes in temperature, which have received much attention in recent years. The trends in zonally averaged precipitation over the last 30 to 40 years are similar to changes predicted by GCM experiments with doubled CO_2 levels. All models simulate overall increases in midlatitude precipitation, and most models suggest precipitation decreases in tropical and subtropical regions, in agreement with observed conditions since 1950. Temperatures over the Northern Hemisphere as a whole have also increased during this interval, although temperatures over land areas declined from the early 1940s to the early 1960s, followed by a warming in the last 20 years (*2*). It would be unwise to link increases in greenhouse gases to trends in the precipitation of specific areas merely because these trends are consistent with theoretical experiments. The magnitude of the observed trends, their geographical distribution, and differences between seasons need to be examined in more detail for both GCM results and observational data. Nevertheless, important fluctuations in precipitation have occurred over large regions, and precipitation trends may provide an additional indicator (together with temperature) in the evaluation of CO_2-induced climatic change.

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24. It is unlikely that this upward trend is due to abrupt data inhomogeneities as data from many different countries are combined; about one-third of gridded points in this zone are from the Soviet Union, one-third from Europe, and one-third from North America (Fig. 2).
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Detection of Minimal Residual Cells Carrying the t(14;18) by DNA Sequence Amplification

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By means of the polymerase chain reaction (PCR) technique, DNA sequences were amplified that flank the crossover sites of a characteristic chromosomal translocation for follicular lymphomas, t(14;18)(q32;q21). This technique permitted the detection of cells carrying the t(14;18) hybrid DNA sequences at a dilution of 1:100,000. The remission marrow and blood samples of a patient with follicular lymphoma and the t(14;18) failed to show any abnormality by morphological examination and conventional Southern blot analysis. However, the t(14;18) hybrid DNA sequences were detected by the PCR technique. Thus, this technique is a highly sensitive tool to detect minimal residual cells carrying the t(14;18) and has the potential to identify a subpopulation of patients with subclinical disease.

HIGH FREQUENCY OF RECURRENCE is one of the major problems in cancer treatment. Relapse from clinically undetectable residual disease is the most likely mechanism. Detection of minimal residual disease is extremely difficult since tumor-specific markers are not readily available. Molecular technology has provided a means to demonstrate residual disease by identifying clonal rearrangement patterns that are present in malignant hematopoietic cells (*1*). Southern blot hybridization detects neoplastic cells at levels as low as 1% of the total number of cells (*2*). However, one of

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the major drawbacks is that it is difficult to be certain that faint nongermline bands indeed represent clonal rearrangements. Furthermore, no rearranged bands can be detected in cases in which the concentration of neoplastic cells is below 1%. Theoretically, this low a concentration of neoplastic cells occurs frequently while patients are in remission.

Saiki *et al.* have recently utilized a new technique, sequence amplification by polymerase chain reaction (PCR), to diagnose sickle cell anemia prenatally (*3*). This technique is highly sensitive. It requires small amounts of DNA (less than 1 μg) and can amplify copies of target DNA sequences exponentially. We were interested in using the PCR technique to preferentially amplify DNA sequences flanking the crossover sites of a chromosomal translocation that is characteristic of a tumor. Since cells carrying the translocation are unique to the malignant hematopoietic cells, detection and amplification of such sequences can be used as evidence for minimal residual disease.

The karyotypic abnormality t(14;18) (q32;q21) has been observed in approximately 90% of human follicular lymphomas (*4–6*). This translocation frequently results in rearrangement of a putative oncogene "*bcl*-2" which resides at chromosome 18 (band q21) (*7*). In the majority of cases with the t(14;18), the molecular breakpoints on chromosome 18q21 cluster within a 4.3-kb Hind III restriction fragment or more specifically a 2.8-kb Eco RI–Hind III restriction fragment that has recently been designated the t(14;18) major breakpoint cluster region (mbr) (*8–12*). DNA sequencing of the crossover sites in seven representative cases revealed that breakpoints on chromosome 18q21 were located within 450 bp of each other and breakpoints on chromosome 14 were located close to the 5′ end of one of the joining (J) segments (J1 to J6) of the immunoglobulin heavy chain locus (J_H) (*12–14*).

Taking advantage of these characteristics, we made two synthetic oligonucleotides as primers for the PCR. These two primers were expected to flank the crossover sites of the t(14;18) in the majority of cases. Primer 18q21(+), 5′-TTTGACCTTTAG-3′, is complementary to the sequences of the (−)-strand of chromosome 18q21 (*12–14*); primer $J_H(-)$, 3′-CAGAGGAGTCCA-5′, is complementary to the sequences at the 3′ end of each (+)-strand J segment (*15*). Therefore, this primer always flanks the breakpoint on the J_H region even though the breakpoint varies from J1 to J6 from case to case. For t(14;18), copies of the (+)-strand hybrid 18q21–J_H DNA sequences were synthesized from primer 18q21(+) and copies of the (−)-strand hybrid 18q21–J_H DNA sequences were synthesized from primer $J_H(-)$. New copies of the (+)-strand and the (−)-strand hybrid 18q21–J_H DNA sequences in turn became templates of primer $J_H(-)$ and primer 18q21(+), respectively. Therefore, DNA sequences flanking the crossover sites of the t(14;18) were amplified exponentially as PCR was carried out (Fig. 1A). In contrast, such amplification could not be generated in cases without the t(14;18) because no new templates for the primers could be synthesized (Fig. 1B). These two primers could also be used as probes to detect newly synthesized hybrid 18q21–J_H DNA sequences that were generated in case of the t(14;18), but not the new copies of 18q21(+) or $J_H(-)$ DNA sequences that were synthesized in cases without the t(14;18). Another oligonucleotide, 18q21(+)II (5′-CACAGACCCACCCAGAGCCC-3′), deduced from the mbr region [27 bases 3′ to the primer 18q21(+)], was used as an "internal" probe to further confirm that the PCR-amplified DNA segments contained the hybrid 18q21–J_H sequences (*12–14*). Since 18q21(+)II is derived from sequences of the (+)-strand

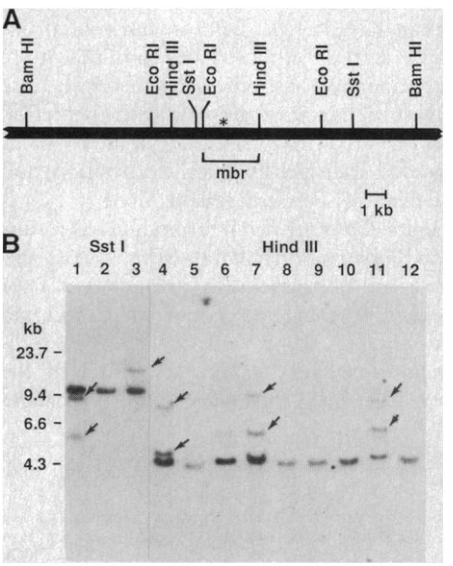

Fig. 2. (**A**) Partial restriction enzyme map surrounding the mbr region of chromosome 18 (band q21). The solid bar represents the germline DNA structure of chromosome 18 (band q21) (*8, 12*). The horizontal line labeled mbr indicates the t(14;18) major breakpoint cluster region and the probe used for Southern blot hybridization to map the chromosomal breakpoints of the t(14;18) on chromosome 18q21. The asterisk indicates the t(14;18) breakpoint hot spot where primer 18q21(+) was deduced (*12, 14*). (**B**) Southern blot hybridization with mbr probe. DNAs were digested with Sst I or enzyme Hind III and separated on a 0.8% agarose gel, transferred to a nylon filter, and hybridized with a radioactively labeled mbr probe. The rearranged bands are indicated by arrows. Lanes 1 to 3 were Sst I–digested samples A, B, and C, respectively. Lanes 4 to 6 were Hind III–digested samples A, B, and C, respectively. Lanes 7 to 10 were DNA samples obtained from patient 1 and were digested with Hind III (lane 7: pretreatment bone marrow sample obtained in October 1985; lane 8: remission blood sample obtained in June 1986; lane 9: remission marrow sample obtained in June 1986; lane 10: remission marrow sample obtained in September 1986). Lanes 11 and 12 were DNA samples obtained from patient 2 and were digested with Hind III (lane 11: pretreatment lymph node sample obtained in January 1986; lane 12: remission marrow sample obtained in October 1986).

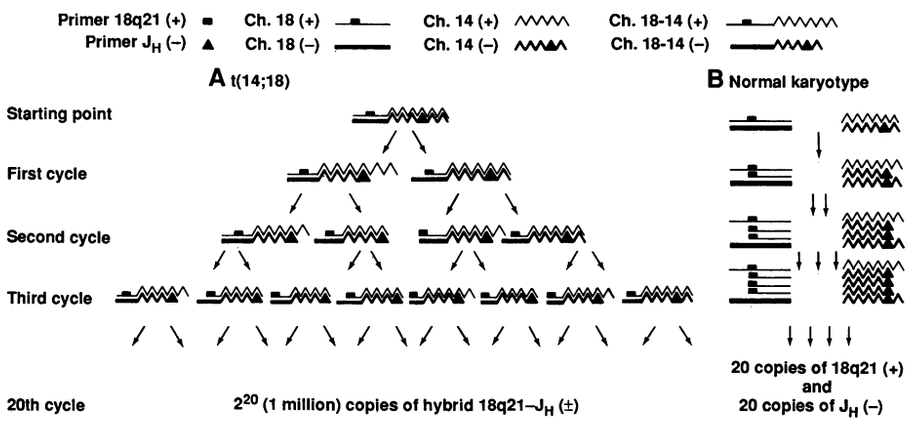

Fig. 1. Schematic illustration of the mechanism by which PCR preferentially amplifies the hybrid 18q21–J_H DNA sequences, but not the normal DNA sequences. (**A**) In case of the t(14;18), the hybrid 18q21–$J_H(+)$ and 18q21–$J_H(-)$ DNA sequences were synthesized from primer 18q21(+) and primer $J_H(-)$, respectively. The primers are also complementary to the newly synthesized hybrid 18q21–$J_H(\pm)$ DNA sequences, which, in turn, become templates for the primers. Therefore, exponential amplification of the hybrid 18q21–$J_H(\pm)$ DNA sequences are generated, that is, $Y = (1 + E)^N$, where Y is the extent of yield, E is the mean efficiency per PCR cycle, and N is the number of PCR cycles carried out. If $E = 100\%$ and $N = 20$, the final yield is 2^{20} copies of hybrid 18q21–$J_H(\pm)$ DNA sequences. (**B**) In case of a normal karyotype, the newly synthesized 18q21(+) and $J_H(-)$ DNA sequences cannot be templates for the primers. Therefore, the final yield is calculated as the following formula: $y = 2n \times e$, where y is the extent of yield, n is the number of PCR cycles, and e is the mean efficiency per cycle.

chromosome 18q21, it cannot hybridize with sequences extended from primer 18q21(+). In contrast, it will hybridize with sequences extended from primer $J_H(-)$ in case of the t(14;18). Therefore, signals detected by the radioactively labeled 18q21(+)II probe strongly indicate presence of the hybrid 18q21–J_H sequences.

To study the feasibility of using PCR to amplify the DNA sequences flanking the crossover sites of the t(14;18), we selected three representative DNA samples for our initial experiment. Sample A was from a lymph node of follicular lymphoma with the t(14;18). Sample B was from a lymph node of reactive lymphadenitis with normal karyotype. Sample C was from a lymph node of follicular lymphoma with the t(14;18). These samples were analyzed by Southern blot hybridization with a human genomic DNA fragment specific for the mbr region of chromosome 18q21 (Fig. 2A) (*8, 12*). As shown in Fig. 2B, lanes 1 and 4, two rearranged bands were detected in sample A, indicating the breakpoint on chromosome 18q21 occurring within mbr. In sample C, only one rearranged band was detected by Sst I and no rearrangement was detected with Hind III (Fig. 2B, lanes 3 and 6). Therefore, the breakpoint occurred 3′ to mbr (within the Hind III–Sst I restriction fragment). These rearranged bands also comigrated with the immunoglobulin J_H gene, which confirmed that breakpoints on chromosome 14 are located in the J_H region.

These three samples were subjected to PCR. The hybrid 18q21–J_H DNA sequences in sample A were amplified and the signals were detected after the 15th cycle by radioactively labeled 18q21 and J_H primers (Fig. 3A). The amplified DNA segments also hybridized with radioactively labeled 18q21(+)II, which further confirmed the presence of the hybrid 18q21–J_H sequences (Fig. 4, lanes 1 through 3). Sample B was not amplifiable because there were no hybrid 18q21–J_H DNA sequences present. Sample C could not be amplified because primer 18q21(+) was too far upstream (>1 kb) from the breakpoint. Theoretically, samples with breakpoints occurring 5′ to primer 18q21(+) could not be amplified either. Even though two universal primers for PCR are used, it may not be possible to amplify the hybrid 18q21–J_H DNA sequences for each case with the t(14;18). However, this approach is justifiable since it is not practical to make different primers for each individual case on the basis of DNA sequencing. Since approximately 60% of follicular lymphoma samples were mapped to have the t(14;18) breakpoint occurring within the mbr region (*8, 10*), we estimate that close to 60% of follicular lymphomas will be amplifiable by our PCR technique.

Another experiment was performed to determine whether PCR could preferentially amplify the hybrid 18q21–J_H DNA sequences, but not the normal DNA sequences. Dilutions of sample A in sample B were subjected to PCR. At 1:100 and 1:5000 dilutions, the hybrid 18q21–J_H DNA sequences were amplified and signals were detected after the 20th cycle of PCR as shown in Fig. 3B and Fig. 4, lane 4. At a 1:100,000 dilution, a strong and convincing signal was detected at the 30th cycle by using a mixture of radioactively labeled primers 18q21(+) and $J_H(-)$ as a probe (Fig. 3C, lane 3). The presence of the hybrid 18q21–J_H sequence was also further confirmed by the radioactively labeled "internal" probe.

We also performed sequential follow-up studies in two patients with follicular lymphoma and the t(14;18). The pretreatment tumor samples were shown to have chromosomal breakpoints occurring within mbr (Fig. 2B, lanes 7 and 11). The remission marrow and blood samples obtained from these two patients were first analyzed by morphological examination and conventional Southern blot hybridization with a radioactively labeled mbr probe. All the samples

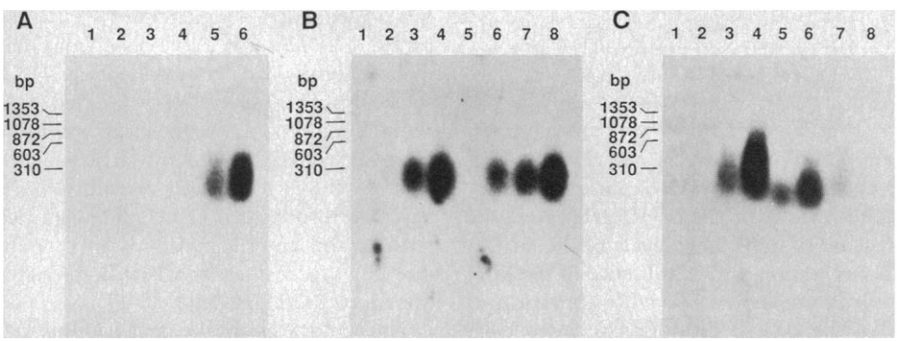

Fig. 3. Southern blot analysis of PCR-amplified genomic DNA with radioactively labeled primer 18q21(+) and primer $J_H(-)$. Samples (1 μg) of genomic DNA were dispensed in microcentrifuge tubes, then denatured at 100°C for 5 minutes, centrifuged for 10 seconds to remove the condensation, and adjusted to 100 μl in a buffer containing 10 m*M* tris, *p*H 7.5, 50 m*M* NaCl, 10 m*M* $MgCl_2$, 1.5 m*M* deoxynucleotide triphosphate (each of four was used), 1 μ*M* primer 18q21(+), and 1 μ*M* primer $J_H(-)$. The samples were then transferred to a 30°C heat block for 2 minutes to allow annealing, followed by the addition of 1 μl of the Klenow fragment of *Escherichia coli* DNA polymerase I (1 unit/μl) and 1 μl of 0.1*M* dithiothreitol (DTT), extension of DNA sequences was allowed to continue for 5 minutes. The cycle—denaturation, reannealing, and extension—was repeated for 19 to 29 more times, except that subsequent denaturations were performed at 95°C for 2 minutes. The amplified DNAs were loaded on a 2% alkaline agarose minigel, fractionated by electrophoresis (50 V) for 2 hours, and then neutralized and transferred to a nylon filter. Prehybridization, hybridization, and washing of filters were as described (*3*). The mixture of primer 18q21(+) and $J_H(-)$ was radioactively labeled to a specific activity of >3 μCi/pmol oligonucleotides with [γ-^{32}P]adenosine triphosphate and used as a probe. Autoradiography with a single intensification screen was at −70°C for 48 hours. (**A**) Hind III–digested sample A (1 μg; lane 1) was compared with PCR-amplified sample A (25 ng) at cycles 3, 7, 10, 15, and 20 (lanes 2 to 6, respectively). (**B**) PCR amplification of sample dilutions of sample A in sample B (25 ng per lane): (Lanes 1 to 4) 1:100 dilution amplified for 10, 15, 20, and 24 cycles, respectively; (lanes 5 to 8) 1:5000 dilution amplified for 15, 20, 25, and 30 cycles, respectively. (**C**) (Lane 1) Sample B at 30 cycles; (lanes 2 and 3) mixture of samples A and B in 1:100,000 ratio amplified for 20 and 30 cycles of PCR, respectively; (lanes 4 to 6) remission marrow and blood samples obtained from patient 1 and subjected to PCR for 27 cycles, corresponding to samples shown in Fig. 2, lanes 8 to 10, respectively; (lane 7) pretreatment tumor sample obtained from patient 2 and amplified for 20 cycles of PCR; (lane 8) remission marrow sample obtained from patient 2 and subjected to PCR for 27 cycles. Size markers, Hae III–digested ϕ×174.

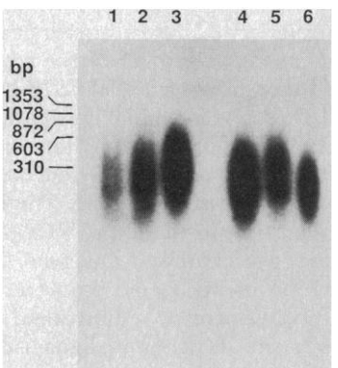

Fig. 4. Southern blot analysis of PCR amplified genomic DNA with radioactively labeled oligonucleotide 18q21(+)II. The PCR, radioactive labeling, hybridization, washing, and autoradiography were carried out as described in the legend to Fig. 3 except that the radioactively labeled oligonucleotide 18q21(+)II with specific activity of >3 μCi/pmol was used as a probe and autoradiography was carried out for 20 hours. (Lanes 1, 2, and 3) 25 ng of sample As were amplified for 20, 25, and 30 cycles, respectively; (lane 4) 25 ng of mixture of a 1:5,000 dilution of sample A in sample B were subjected to PCR for 40 cycles; (lanes 5 and 6) 25 ng of remission marrow samples obtained from patient 1 in June and September of 1986, respectively, were amplified for 40 cycles. Size markers, Hae III–digested ϕ×174.

appeared normal since none of them demonstrated any morphologic abnormality or rearranged bands (Fig. 2B, lanes 8, 9, 10, and 12). However, hybrid DNA sequences were markedly amplified and thus clearly detected by the PCR technique in samples obtained from patient 1 (Fig. 3C, lanes 4 through 6 and Fig. 4, lanes 5 and 6), indicating the presence of residual neoplastic cells carrying the t(14;18). Even though the hybrid 18q21–J_H DNA sequences were amplifiable in the pretreatment tumor sample obtained from patient 2 (Fig. 3C, lane 7), no hybrid DNA sequences were detected in the remission marrow sample by the PCR technique (Fig. 3C, lane 8). These findings indicate that the concentration of neoplastic cells carrying the t(14;18) was too low to be detected by our current techniques or the patient was completely free of tumor and there were no t(14;18) target DNA sequences present for amplification.

We have shown the feasibility of using the PCR technique to detect minimal numbers of neoplastic cells carrying a chromosomal translocation. Detection of small numbers of circulating monoclonal B cells by flow cytometry or clonal immunoglobulin gene rearrangement in patients with follicular lymphoma in remission has been reported (*1, 16*). The sensitivity of our approach far exceeds the sensitivity limit achieved by conventional Southern blot analysis or the flow cytometric method. Detection of minimal neoplastic cells by means of chromosomal translocation and PCR will make it possible to address the following important biological and clinical questions that could not be answered before. Do patients in long-term remission have quiescent tumor cells with proliferative potential? Can detection of minimal residual tumor cells predict early relapse? Do patients with persistent minimal residual disease after prolonged treatment require non–cross-resistant therapy to prevent relapse? The answers will help in understanding tumor biology and designing strategies for cancer treatment.

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*erb*B-2 Is a Potent Oncogene When Overexpressed in NIH/3T3 Cells

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A wide variety of human tumors contain an amplified or overexpressed *erb*B-2 gene, which encodes a growth factor receptor–like protein. When *erb*B-2 complementary DNA was expressed in NIH/3T3 cells under the control of the SV40 promoter, the gene lacked transforming activity despite expression of detectable levels of the *erb*B-2 protein. A further five- to tenfold increase in its expression under influence of the long terminal repeat of Moloney murine leukemia virus was associated with activation of *erb*B-2 as a potent oncogene. The high levels of the *erb*B-2 product associated with malignant transformation of NIH/3T3 cells were observed in human mammary tumor cells that overexpressed this gene. These findings demonstrate a new mechanism for acquisition of oncogenic properties by genes encoding growth factor receptor–like proteins and provide a functional basis for the role of their overexpression in the development of human malignancies.

EVIDENCE THAT RETROVIRAL ONCOgenes can encode proteins that are homologous to either growth factors or growth factor receptors has shed new light on the mechanisms by which cells become malignant. For example, v-*sis* is derived from the gene encoding one chain of the platelet-derived growth factor (*1*). In addition, v-*erb*B and v-*fms* are derived from the genes encoding the epidermal growth factor (EGF) (*2*) and colony-stimulating factor–1 (CSF-1) (*3*) receptors, respectively. A further linkage between growth control and oncogenes is the common tyrosine kinase activity of many growth factor receptors (*4*) and several viral oncogene products (*5*). These connections have motivated a search in nonvirally induced neoplasia for abnormalities of genes involved in the pathways by which growth factors stimulate normal cell growth.

Shih *et al.* identified a transforming gene in ethylnitrosourea (ENU)-induced rat neuroblastomas by means of the NIH/3T3 transfection assay (*6*). This oncogene, termed *neu*, was found to encode a product immunologically related to the *erb*B/EGF receptor (*7*) and to exhibit a cell surface location. Independently, we cloned and partially sequenced an *erb*B-related gene that was detected because of its amplification in a human mammary carcinoma (*8*). This same human sequence, designated *erb*B-2 (*9, 10*) or HER-2 (*11*), was also cloned from normal genomic and complementary DNA (cDNA) libraries, respectively. Sequence analysis has shown that the structure of the predicted *erb*B-2/HER-2 product has significant homology to the EGF receptor (*9, 11*). Several lines of evidence, including sequence analysis and chromosomal mapping, indicate that *neu* is an activated rat homolog of human *erb*B-2/HER-2 (*9, 11–14*).

Thus far, the established mechanisms that activate genes encoding growth factor receptors to become oncogenes appear to involve structural alterations of their coding sequences. These include truncation or substitutions (*15*) as well as mutational alterations (*14*). While growth factor receptor–like genes with altered coding sequences have been readily detected as transforming genes by DNA transfection analysis, human tumors containing such amplified or overexpressed genes have not yielded transforming genes detectable by this approach (*8, 10*). These findings have suggested that overexpression alone may convert the gene for a normal growth factor receptor into an oncogene. In the present studies, we have investigated the effects of overexpression of the normal coding sequence for the growth

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