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業績報告書シリーズ

電離放射線被曝後，長年月を経過した被曝者の転座頻度の迅速分析について§

Rapid Translocation Frequency Analysis in Humans Decades after Exposure to Ionizing Radiation

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要　約

電離放射線被曝者の末梢血に存在する転座のゲノム当たりの頻度を測定するために，全染色体プローブ(個々の染色体全域にわたるプローブ)を用いた蛍光in situハイブリダイゼーション(FISH)法の有効性について分析した結果を報告する。第一に，FISH法によって得られた転座頻度(F_p)から，ゲノム当たりの転座頻度(F_G)を求めるための関係式 $F_p = 2.05f_p(1-f_p)F_G$ を導いた。ここでf_pは全染色体プローブで染色されたゲノムを表す。以下に示す諸事実から，この関係式の正当性が証明された。すなわち(a)この式から予測される転座識別効率は，f_p値が変わっても，実験値に対して一定である。(b)in vitro照射実験から，FISH法による転座頻度に関する線量反応曲線が，通常の染色体分析法によるdicentric頻度の線量反応曲線と良く一致する。また，(c)広島原爆被爆者20例，およびY-12原子炉臨界事故による電離放射線被曝作業者4例の分析から，FISH法により推定されたゲノム当たりの転座頻度と，Gバンドによる転座頻度とはほぼ同じであること，などである。FISH法による転座頻度の線量反応曲線は，広島原爆被爆者と培養開始後第一分裂期のリンパ球に対するin vitro照射実験との間でほぼ同じであることも証明された。被曝と分析の間の時間差とは関係なく，急性放射線被曝の影響を評価する上でFISH法を用いた転座頻度の分析が有用であるとの結論に達した。

§本業績報告書は研究計画書RP 10-89に基づく。本報告にはこの要約以外に訳文はない。本報告はInt. J. Radiat. Biol., Vol. 62, No. 1, 1992, 53–63に掲載され，別刷を出版社から購入した。承認 1992年6月1日。雑誌の別刷りには掲載されていないが，この調査に含まれる20人の原爆被爆者について，放影研の基本名簿番号，培養番号および性別に記載したリストを，別刷りの付録として最終ページにまとめた。

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Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation

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Abstract. This paper presents an analysis of the utility of fluorescence *in situ* hybridization (FISH) with whole-chromosome probes for measurement of the genomic frequency of translocations found in the peripheral blood of individuals exposed to ionizing radiation. First, we derive the equation: $F_p = 2{\cdot}05 f_p(1-f_p)F_G$, relating the translocation frequency, F_p, measured using FISH to the genomic translocation frequency, F_G, where f_p is the fraction of the genome covered by the composite probe. We demonstrate the validity of this equation by showing that: (a) translocation detection efficiency predicted by the equation is consistent with experimental data as f_p is changed; (b) translocation frequency dose-response curves measured *in vitro* using FISH agree well with dicentric frequency dose-response curves measured *in vitro* using conventional cytogenetic procedures; and (c) the genomic translocation frequencies estimated from FISH measurements for 20 Hiroshima A-bomb survivors and four workers exposed to ionizing radiation during the Y-12 criticality accident are approximately the same as the translocation frequencies measured using G-banding. We also show that translocation frequency dose response curves estimated using FISH are similar for Hiroshima A-bomb survivors and for first division lymphocytes irradiated *in vitro*. We conclude with a discussion of the potential utility of translocation frequency analysis for assessment of the level of acute radiation exposure independent of the time between analysis and exposure.

1. Introduction

Accurate estimation of the level of radiation exposure is important immediately after exposure as a guide for medical treatment and at longer times after exposure to assess possible health consequences. Frequency of structurally aberrant chromosomes in the peripheral blood cells of persons exposed to ionizing radiation has been used since the 1960s to estimate dose (Bender and Gooch 1966, Sasaki and Miyata 1968, Littlefield *et al.* 1980, Lloyd *et al.* 1975, 1981). In most studies, this has been accomplished by measuring the frequency of dicentric chromosomes and calibrating this against a standard dose response curve measured for human lymphocytes irradiated *in vitro*. Dicentric chromosomes have been analysed because they can be scored rapidly without banding (Lloyd *et al.* 1975, 1980, Evans *et al.* 1979). However, the use of dicentric chromosome frequencies for biological dosimetry is complicated because the frequency of cells carrying such chromosomes decreases with time after exposure (Awa *et al.* 1978, Bauchinger 1968, Buckton *et al.* 1978). This is especially problematic when dosimetry is required for occupationally or accidentally exposed individuals (e.g. radiation workers or individuals exposed in nuclear accidents such as that at Chernobyl) because the time between exposure and analysis may be considerable or even unknown.

Estimation of dose from the frequency of stable aberrations such as translocations would be preferable for these cases (Bender *et al.* 1988, Buckton 1983, Littlefield and Joiner 1978, Dutrillaux *et al.* 1985). Unfortunately, measurement of the low translocation frequencies usually found in exposed humans has been too difficult and time consuming using conventional staining or banding analysis to be routinely practical. However, recent advances in chromosome staining using fluorescence *in situ* hybridization (FISH) facilitate such measurements. Lucas *et al.* (1989a) and van Dekken and Bauman (1988), for example, showed that FISH probes for chromosome 1*q*12 and 1*p*36 allowed rapid detection of structural aberrations involving chromosome 1*p* (about 4% of the gemome). This approach allowed sufficient aberrations to be scored to permit statistically significant detection of radiation effects produced by exposure to as little as 0·2 Gy γ-irradiation (Lucas *et al.* 1989a). Since those studies, the fraction of detectable translocations has been increased by

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hybridizing with composite, whole-chromosome probes for one or more chromosomes (Pinkel *et al.* 1988, Lucas *et al.* 1989c, Straume *et al.* 1991). Cremer *et al.* (1990) used FISH with whole-chromosome probes to measure radiation dose-response curves for cells irradiated *in vitro*. Most recently, Weier *et al.* (1991) developed composite probes that allow discrimination between symmetrical translocations and dicentric chromosomes. In that approach, FISH with whole-chromosome probes was used to stain selected chromosomes so that they appeared yellow, propidium iodide was used to stain all chromosomes so that they appeared red, and FISH with probes specific to centromeric alpha satellite sequences was used to stain all of the chromosome centromeres so that they appeared blue. Thus, structural aberrations were revealed by their red-yellow staining pattern, and discrimination between translocations and dicentrics was based on centromere number.

We evaluate here the utility of FISH for analysis of translocation frequencies in the peripheral blood of exposed individuals. Specifically, we describe and evaluate the accuracy of an equation relating the frequency of translocations measured using FISH with one or more whole chromosome probes to the genomic translocation frequency. We use this relationship to compare translocation frequencies measured using FISH with translocation frequencies measured using conventional cytogenetic techniques for four workers at the Y-12 plant in Oak Ridge exposed in 1958 and 20 Hiroshima A-bomb survivors exposed in 1945. Finally, we discuss the possibility that translocation frequency analysis can be used to estimate dose many years, even decades, after whole-body radiation exposure.

2. Methods

2.1. In vitro *irradiation*

Blood irradiation *in vitro* was accomplished as previously described (Lucas *et al.* 1989a). Briefly, heparinized whole blood from a healthy male was irradiated with 1·25 MeV γ-rays from a 141 TBq ^{60}Co source at the Lawrence Berkeley Laboratory. Doses were: 0 (unexposed controls), 0·42, 0·91, 1·89 and 3·89 Gy. Dose rate at the exposure position (0·924 m from the source) was 0·9 Gy/min. Dosimetry was done using Victoreen ion chambers and readings were corrected appropriately. In this case, the Gy/R conversion was 0·00966 for 1·25 MeV γ-rays (NBS Handbook 85, 1964).

2.2. *Cell culture and metaphase spread preparation*

Irradiated and control lymphocytes at the Lawrence Livermore National Laboratory (LLNL) were separated from whole blood by centrifugation through a continuous density gradient formed by mixing whole blood with a commercially available separation medium (Sepracell-MN, Sepratech Co., Oklahoma City, OK). Cells were established in suspension cultures in upright T-75 flasks in 50-ml RPMI 1640 medium containing 20% fetal calf serum and stimulated to proliferate with phytohemagglutinin (PHA, 0·15 mg/ml). Colcemid was added to the cultures after 48 h for 4 h, and metaphase spreads were prepared by the method of Evans *et al.* (1979).

Peripheral blood samples from four Y-12 criticality accident victims were obtained during routine medical evaluation in 1983 and 1988. Each blood sample consisted of 10 ml of blood drawn into a heparin treated vacutainer, coded with an ID number. Samples taken in 1983 were cultured at Oak Ridge Associated Universities and metaphase spreads were prepared (Fry *et al.* 1990). Samples taken in 1988 were shipped on ice to LLNL. Lymphocytes were placed into culture immediately upon arrival as described above. Metaphase spreads were prepared as described above.

Peripheral blood from 20 A-bomb survivors in Hiroshima was obtained during routing medical evaluation at the Radiation Effects Research Foundation (RERF). Heparinized whole blood from each donor (2 ml) was cultured for 48 h at RERF using upright 25 cm^2 tissue culture flasks (Corning) in 10-ml RPMI 1640 medium containing 20% fetal calf serum and PHA (0·15 mg/ml). Colchicine was added to the cultures after 46 h of incubation and metaphase spreads were prepared as described previously (Awa *et al.* 1978). Approximately 20 slides were prepared for each culture and shipped to LLNL for analysis by FISH. These slides were coded so that the RERF scoring results were not known before analysis by FISH. Slides from each culture also were prepared and analysed at RERF by G-banding as described below.

2.3. *Conventional cytogenetic staining and G-banding*

Slides prepared for the 1983 samples from the Y-12 victims were stained with Giemsa, metaphases were photographed and aberrations were detected by chromosome grouping (Fry *et al.* 1990). Slides for the A-bomb survivors were coded and then banded.

These were treated with trypsin and stained with Giemsa according to established techniques (Evans *et al.* 1971, Ohtaki *et al.* 1982). All metaphases on the coded slides were photographed and aberrations were checked using 5 × 7 enlarged prints by reading banding patterns of individual chromosomes. Aberrations detected by this procedure were the consensus of three cytogeneticists who analysed the banding patterns independently. Type of aberrations detected by G-banding included translocations, inversions (peri- and paracentric), deletions (terminal and interstitial), insertions, and complex exchanges involving three or more breaks. Only translocations are reported in the present study. Approximately 100 metaphase spreads were scored for each subject.

2.4. *Probe labelling*

Whole-chromosome, composite probes for human chromosomes 1–4 and 15 were prepared from the Bluescribe libraries pBS-1, pBS-2, pBS-3, pBS-4 and pBS-15; respectively (Collins *et al.* 1991). DNA from these libraries was purified and biotin-labelled by nick translation with biotin-dUTP (Bethesda Research Laboratories; Gaitherburg, MD).

A pan-centromeric probe produced by *in vitro* DNA amplification of genomic DNA using the polymerase chain reaction with degenerate alpha satellite primers (Weier *et al.* 1991) was labelled with AAF according to the procedure described by Landegent *et al.* (1984).

2.5. *Hybridization with whole-chromosome probes*

Slides were immersed for 2 min at 70°C in 70% formamide/2 × SSC (SSC is 0·15 M NaCl/0·015 M Na Citrate, pH 7) to denature the target DNA sequences, dehydrated in a 70–85–100% ethanol series, and air dried. A hybridization mix consisting of 50% formamide, 2 × SSC, 10% dextran sulfate, 100 ng/μl sonicated human placental DNA (Sigma), and the required amount of each biotinylated whole-chromosome probe used in a particular hybridization (~4 ng/μl chromosome 1, ~5 ng/μl chromosome 2, ~5 ng/μl chromosome 3, 0·7 ng/μl chromosome 4, and ~5 ng/μl chromosome 15), was heated to 70°C for 5 min to denature the DNA. This hybridization mixture was incubated at 37°C for 1 h to renature preferentially repeated DNA sequences in the probe, thereby preventing them from binding to target DNA during subsequent hybridization. The probe mix (10 μl) was applied to the slide under a 22-mm square coverslip, sealed with rubber cement, and incubated for 16–72 h at 37°C. The coverslip was removed and the slide washed three times in 50% formamide/2 × SSC, pH 7, once in 2 × SSC, and once in PN buffer (mixture of 0·1 M NaH_2PO_4 and 0·1 M Na_2HPO_4 to give pH 8; 0·1% Nonidet P-40 (Sigma)) for 10 min each at 42–45°C. After an additional 10-min wash in PN buffer at room temperature, the bound probe was detected with alternating 20-min incubations (room temperature) in avidin-FITC and biotinylated goat-anti-avidin antibody, both at 5 μg/ml in PNM buffer (PN buffer plus 5% nonfat dry milk, centrifuged to remove solids; 0·02% Na azide). Avidin and anti-avidin incubations were separated by three washes of 3-min each in PN buffer. Two layers of fluoresceinated avidin were applied. (Avidin, DCS grade, and biotinylated goat-anti-avidin were obtained from Vector Laboratories Inc., Burlingame CA.) Slides were counterstained with propidium, iodide, ~0·2 μg/ml in a fluorescence antifade solution (Johnson and Araujo 1981). A Nikon Optiphot microscope with an FITC filter set with a long pass emission filter was used to observe PI and FITC simultaneously. In some cases, a dual-band FITC/Texas red filter set was used to enhance the contrast between red and green fluorescence images.

2.6. *Hybridization with whole-chromosome and centromeric probes*

Hybridization with whole-chromosome and pan-centromere probes was performed as described previously (Weier *et al.* 1991). Briefly, target DNA on slides was prepared as above. A hybridization mix as described above (except that the concentrations of all nucleic acids were doubled) was denatured and incubated at 37°C for 1 h. This was mixed with an equal volume of freshly denatured hybridization mix consisting of 50% formamide, 10% dextran sulfate, 2 × SSC, and 2 ng/μl AAF-labelled alpha satellite pan-centromeric probe, and applied to the slide as above. After washing as above, the slides were incubated for 5 min in PNM. Bound probes were detected by sequential 1-h incubations in: (1) mouse anti-AAF hybridoma supernatant (a gift from Dr R. Baan, TNO, Rijswijk, The Netherlands) containing 5 μg/ml avidin-FITC; (2) 5 μg/ml goat anti-avidin and a 1:75 dilution of AMCA labelled rat anti-mouse antibodies in PNM; and (3) 5 μg/ml avidin-FITC and a 1:75 dilution of AMCA-labelled mouse-anti-rat antibody (AMCA-labelled antibodies from

Jackson ImmunoResearch, West Grove, PA). After each round of incubation, slides were washed twice in PN for 5 min at room temperature and incubated for 5 min in PNM (this was omitted after the last incubation). Slides were counterstained as above. Use of the FITC or dual-wavelength filter set permitted observation of the PI and FITC in the whole-chromosome probes. Use of an ultraviolet excitation filter (~365 nm) and a ~400 nm long pass emission filter allowed observation of the AMCA centromeric staining and PI (PI is excited significantly at 365 nm). When the FITC staining was extremely intense, it also could be seen with 365 nm excitation. In these cases, all three fluorochromes were observed simultaneously.

2.7. *Aberration scoring*

Metaphase spreads from lymphocytes irradiated *in vitro* and from Hiroshima A-bomb survivors were stained yellow with probes for chromosomes 1, 2 and 4, and blue with a pan-centromere probe. All spreads were scored as normal if they showed three pairs of large yellow-stained chromosomes. Structural aberrations were recognized as bi-colour (part red and part yellow) chromosomes. Aberrations were scored as reciprocal translocations if the two derivative chromosomes each showed one blue-stained centromere. They were scored as dicentrics or fragments if the derivative chromosomes showed two or no centromeres, respectively. Metaphase spreads from the Y-12 criticality accident victims were stained using FISH with composite probes for chromosomes 1, 3 and 4. Centromeric staining was not employed in this study. This did not effect the results significantly because of the low frequency of dicentrics in the irradiated individuals. Type of aberrations detected by FISH included translocations, dicentrics, some inversions, some deletions, insertions, and complex exchanges involving three or more breaks. Only reciprocal translocations measured by G-banding and FISH are compared in the present study. All aberrations measured using FISH were recorded on Kodak Ektachrom 400 film.

2.8. *Estimation of genomic translocation frequency*

The relationship between F_p, the frequency of translocations detected by FISH with a few whole chromosome probes, and the total genomic translocation frequency, F_G, was calculated (using the approach suggested previously by Lucas *et al.* 1989b) by assuming that breaks leading to translocations are distributed randomly throughout the genome and that there is no preference for exchanges between particular pairs of chromosomes. In this situation, the probability of a chromosome being involved in a translocation is proportional to its DNA content, C_i (expresed here as a fraction of the total diploid cell DNA content). The probability of an exchange between chromosome i and j (which may be different homologues of the same chromosome type) is then $T(D)C_iC_j$ and the total frequency of translocations is

$$F_G = T(D)\left[\sum_{i=1}^{46}\sum_{j=1}^{46} C_iC_j(i\neq j)\right]$$
$$= T(D)\left[1-\sum_{i=1}^{46} C_i^2\right] = 0{\cdot}976\ T(D) \quad (1)$$

where $T(D)$ is a dose-dependent coefficient and C_i values were obtained using image analysis from an average of 17 male and 17 female donors (Mayall *et al.* 1984). Frequency of bi-colour chromosomes with a single centromere, which are scored as translocations with the FISH technique used here, includes true reciprocal translocations, as well as some complex rearrangements involving three or more chromosomes. The complex rearrangements are rare for doses less than a few Gy and are neglected in this analysis. Fraction of the genome made fluorescent by FISH is defined to be $f_p = \sum C_k$ where C_k's are painted chromosomes. Translocations are detected if the first break is in a region covered by the hybridization probe and the second is in a region not covered, and vice versa. Thus the frequency of translocations detected by painting, F_p, is

$$F_p = T(D)[f_p(1-f_p)+(1-f_p)f_p]$$
$$= T(D)2f_p(1-f_p) = 2{\cdot}05 f_p(1-f_p)F_G. \quad (2)$$

Genomic translocation frequencies F_G determined by FISH in this paper have been converted from the measured frequencies F_p using equation (2).

3. Results

3.1. *Aberration detection*

Figure 1a shows a photomicrograph of FITC plus PI fluorescence from a metaphase spread prepared from the peripheral blood lymphocytes of a Hiroshima A-bomb survivor stained using FISH with

probes for chromosomes 1, 2 and 4. A structural aberration involving one chromosome 2 and one non-target chromosome has resulted in two bi-colour (partly red and partly yellow) derivative chromosomes. Figure 1b shows a photomicrograph of AMCA fluorescence from the same metaphase spread. The aberration was classified as a translocation because the two derivative chromosomes each showed one centromeric hybridization signal. Figures 1c and d show similar photomicrographs for a metaphase carrying an unstable structural aberration involving chromosome 4 and another chromosome type. The aberration was classified as unstable since one derivative chromosome was a dicentric carrying two centromeres and the other was a fragment with no centromere.

3.2. *Distribution of breaks involved in translocations*

The random distribution assumption embodied in equation (2) requires that the frequency of translocations observed for each chromosome be proportional to its DNA content. We checked this by comparing chromosomal DNA content with the frequency of chromosome breaks measured using G-banding for each chromosome type in 3101 metaphase spreads from 52 Hiroshima A-bomb survivors (26 males and 26 females) 35–40 years after exposure. Results are plotted in Figure 2. Linear regression analysis of these data shows that the translocation frequency for the ith chromosome, F_i, increases with chromosomal DNA content, d_i, according to the relation $F_i = 0{\cdot}0058 + 1{\cdot}81 d_i$. Corre-

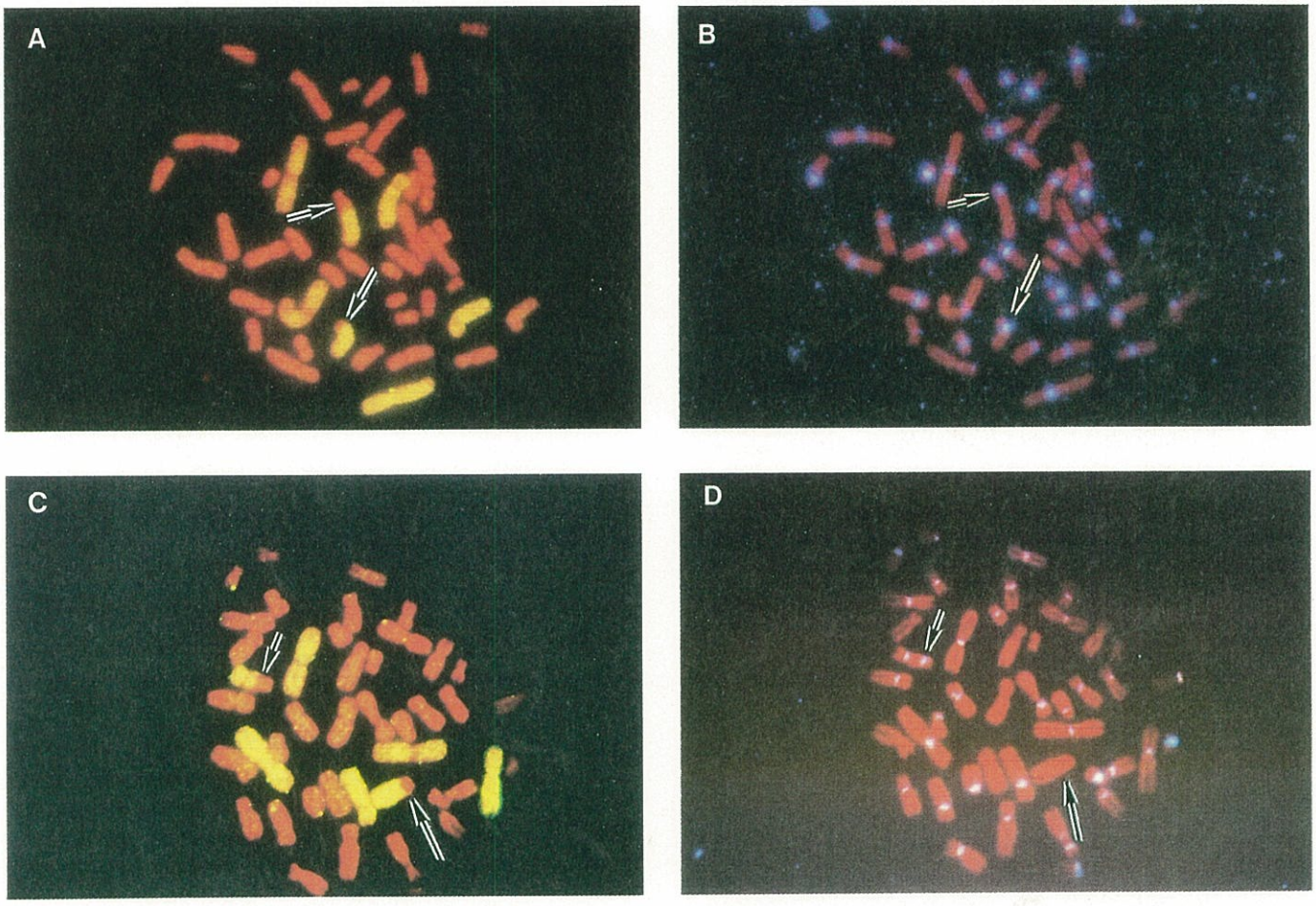

Figure 1. Photomicrographs showing FISH with whole-chromsome probes for chromosomes 1, 2 and 4 and a pan-centromere probe to human metaphase spreads. Whole chromosome-probe binding sites appear yellow, unstained DNA appears red, and the binding sites of the pan-centromere probes appear blue. (a) Yellow (FITC) and red (PI) fluorescence from a metaphase spread carrying a translocation between chromosome 2 and a non-target chromosome. (b) Blue (AMCA) fluorescence from the same spread. Two derivative chromosomes show only one centromere each. (c) Yellow (FITC) and red (PI) fluorescence from a metaphase spread carrying a dicentric chromosome. (d) Blue (AMCA) fluorescence from the same spread. One derivative chromosome shows two centromeres and the other shows none.

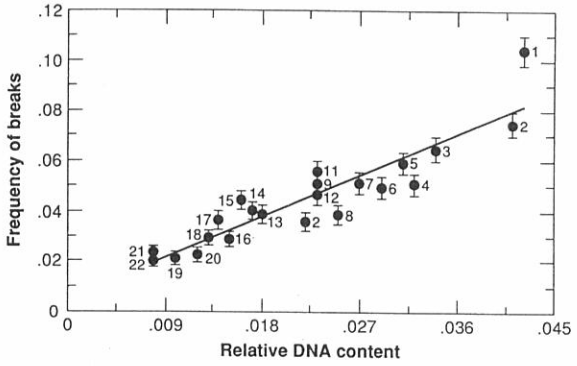

Figure 2. Chromosome translocation break frequencies (breaks/cell) *versus* chromosome DNA content for all human chromosomes. These data were determined from G-banding analysis of 3101 metaphase spreads prepared from peripheral blood lymphocytes of 52 Hiroshima A-bomb survivors. Error bars show the uncertainty in the counting statistics (one standard deviation).

lation coefficient for the regression is 0·915. Only the translocation frequency from chromosome 1 is significantly off the regression line ($p = 0{\cdot}03$).

3.3. *Dependence of detection efficiency on f_p*

Equation (2) predicts that the efficiency with which translocations are detected using FISH changes in a parabolic manner as a function of f_p, the fraction of the genome covered by the composite probe. We tested this relation experimentally by measuring the frequencies of translocations detected after FISH with probes for chromosomes 4, 1, 1+4, 1+3+4 and 1+2+3+4+15 to metaphase spreads from the Y-12 worker exposed to the highest dose of radiation. These probes covered 0·062, 0·080, 0·142, 0·207, and 0·318 of the total genome; respectively. Figure 3 shows a fit of equation (2) to the experimental measurements. Data and equation agree well over the range tested.

3.4. In vitro *translocation frequencies*

The validity of equation (2) also was tested by comparing the estimated genomic translocation frequency measured by FISH and dicentric frequencies reported in the literature (summarized by Bender *et al.* 1988) for first division lymphocytes after *in vitro* irradiation. Frequencies of these two types of aberrations are expected to be equal at the first division after irradiation (in this study the induction of translocations and dicentrics at various doses for ^{60}Co-γ-irradiated lymphocytes were 72 translocations and 72 dicentrics at 4·0 Gy, and 20 translocations and 18 dicentrics at 2·0 Gy). The comparison is shown in Figure 4. Results of three dicentric dose-response curves for ^{60}Co-γ-irradiated human lymphocytes are compared with two genomic translocation frequency curves generated using FISH. One analysis based on FISH shows genomic translocation

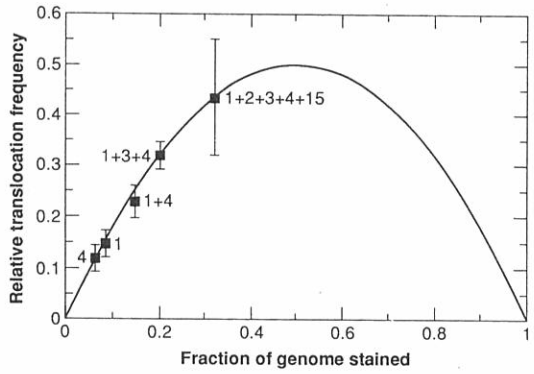

Figure 3. Fraction of translocations detected *versus* fraction of genome stained using FISH. Solid line represents equation (2). Data points are fractions of transloctions detected after staining chromosomes 4, 1, 1+4 and 1+3+4, and 1+2+3+4+15 in metaphase spreads of a Y-12 worker exposed to ionizing radiation. Error bars show the uncertainty in the counting statistics (one standard deviation).

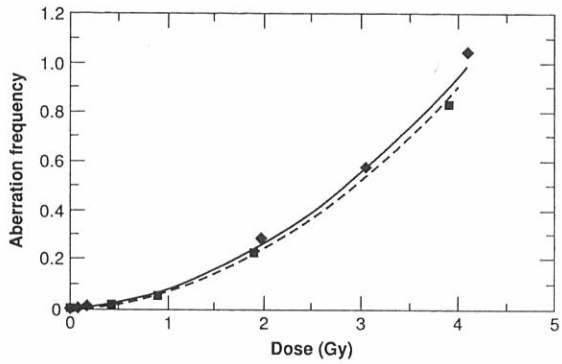

Figure 4. Genomic structural aberation frequencies (events/cell) estimated using equation (2) from analyses of ^{60}Co gamma-ray irradiated human lymphocytes at the first division after irradiation. Two studies are shown: (a) translocation frequencies estimated using FISH with probes for 1, 2 and 4 (solid squares) and (b) translocation plus discentric frequencies (divided by two) estimated using FISH with probes flanking 1p (◆). Dashed line shows an average dicentric frequency dose-response curve from studies reported by Bauchinger *et al.*, Lloyd *et al.*, and Littlefield (summarized in Bender *et al.* 1988). Solid line shows the fit of a second-order polynomial to both sets of dose-response data obtained using FISH. Equation for the fit was $F_G = 0{\cdot}0069 + 0{\cdot}0023D + 0{\cdot}053D^2$, where F_G is the genomic translocation frequency and D is the radiation dose in Gy.

frequencies estimated following hybridization with probes for chromosomes 1, 2 and 4 ($f_p = 0{\cdot}22$). The other shows the sum of genomic dicentric and translocation frequencies (divided by two) estimated using FISH with probes flanking chromosome 1*p* (Lucas *et al.* 1989a, $f_p = 0{\cdot}04$). Translocation and dicentric frequencies were summed and divided by two for this comparison because discrimination between these types of aberrations was sometimes difficult using probes flanking chromosome 1*p*. In general, the five dose response curves are in good agreement, showing that genomic translocation frequencies estimated from analysis of a few chromosomes or from a fraction of one chromosome seem to be acurate.

3.5. *Comparison between conventional cytogenetics and FISH*

The validity of equation (2) was checked further by comparing the genomic translocation frequencies estimated using equation (2) from FISH measurements with translocation frequencies measured using G-banding or conventional staining for 20 Hiroshima A-bomb survivors and four workers irradiated during a criticality accident at the Y-12 plant in Oak Ridge. FISH with probes for chromosomes 1, 2 and 4 in conjunction with the pan-centromere probe was used in the A-bomb survivor study and FISH with probes for chromosomes 1, 3 and 4 was used in the Y-12 study. Comparisons of FISH and G-banding results for the A-bomb survivors were made using slides prepared from the same blood cultures. However, the Y-12 study compared results obtained using conventional staining made from samples taken in 1983 with those obtained using FISH from samples taken in 1988. The translocations measured by conventional staining were multiplied by 1·33 to account for the reduced scoring efficiency using this method (Ohtaki *et al.* 1982, Fry *et al.* 1990). Tables 1a and b and Figure 5 compare the translocation frequencies measured by the various techniques. Slope of the linear regression between the G-banding and FISH measurements for the Hiroshima A-bomb survivors is 0·75 and correlation coefficient is 0·98. However, probability that the slope of the regression line is different from 1 is only 0·7 and thus is not statistically significant.

3.6. *Comparison between* in vitro *and* in vivo *measurements*

Figure 6 compares the average *in vitro* translocation frequency dose-response curve shown in Figure 3 for lymphocytes at the first division post-irradiation with the *in vivo* translocation frequency dose-response data for A-bomb survivors measured 45 years after exposure. DS86 dose estimates ($\pm 30\%$ uncertainty) to the bone marrow of each A-bomb survivor were used for this comparison. The uncertainty estimate is from RERF (1987). A neutron radiobiological effectiveness (RBE) of 10 was assumed for this analysis. In general, the A-bomb survivor dose-response data are similar in shape and magnitude to the *in vitro* dose response curves. For a more detailed comparison of Hiroshima data and *in vitro* results see Straume *et al.* (1992).

4. Discussion

The desirability of employing presumably stable chromosomal aberrations, such as reciprocal translocations, for biological dosimetry has long been recognized. While some measurements of translocation frequencies have been made using conventional staining or chromosome banding, the effort required has prevented wide-scale exploration and application of this approach. The recent development of FISH for chromosome staining has significantly altered the situation. The main advantage of FISH-based translocation analysis is that the aberrations are made distinct so that they can be scored rapidly. In addition, discrimination between translocations and dicentrics can be done accurately using centromeric staining with a pan-centromere probe as shown in Figure 1. This is particularly important when the translocation and dicentric frequencies are of similar magnitude (e.g. in analysis of cells at first division after *in vitro* exposure or within a few months of *in vivo* exposure). Average scoring rates for the Y-12 and A-bomb studies in this paper were ~ 1000 metaphases per week per analyst. The majority of that time was spent searching for metaphase spreads. Automated metaphase finding should increase this rate by 10 fold so that analysis of 10,000 spreads per exposed individual becomes possible. This should allow detection of the increase in translocation frequency resulting from exposure to 0·1–0·2 Gy of acute X-irradiation assuming: (a) that the translocation frequency before exposure is known and equal to the average background genomic frequency of $0{\cdot}008 \pm 0{\cdot}002$ (SE) translocations per cell that we measured in eight unexposed individuals (see below); (b) that the *in vivo* dose-response curve follows the *in vitro* curve of Figure 4; and (c) that f_p is selected so that the translocation detection efficiency, F_p/F_G, is 35%. Background frequency was measured in six persons who were in the control

Table 1a. Translocation frequencies in A-bomb survivors measured by FISH for chromosomes 1, 2 and 4 and by G-banding.

		FISH			G-banding		
Subjects	Dose[a] (Sv)	Cells	Translocations total (#1, #2, #4)	Trans/cell (genomic	Cells	Translocations	Trans/cell
[redacted]	2·22	490	31 (10, 7, 14)	0·179	100	10	0·10
	0	2027	5 (4, 0, 1)	0·007	100	0	0·00
	1·63	824	49 (20, 14, 15)	0·168	100	16	0·16
	0·62	455	16 (8, 2, 6)	0·099	100	5	0·05
	1·06	284	19 (4, 6, 9)	0·189	100	15	0·15
	2·61	178	19 (4, 8, 7)	0·302	100	37	0·37
	0·66	687	15 (6, 4, 5)	0·062	100	6	0·06
	1·32	343	17 (7, 6, 4)	0·140	97	15	0·15
	2·31	141	29 (11, 8, 10)	0·582	100	72	0·72
	1·94	743	16 (6, 7, 3)	0·061	100	6	0·06
	4·82	216	23 (8, 10, 5)	0·301	100	44	0·44
	1·46	375	16 (4, 9, 3)	0·121	100	7	0·07
	1·86	146	39 (14, 11, 14)	0·756	97	90	0·93
	0	813	4 (2, 1, 1)	0·014	100	1	0·01
	2·22	178	26 (6, 12, 8)	0·413	100	46	0·46
	1·84	205	18 (9, 4, 5)	0·248	100	29	0·29
	0·96	416	16 (9, 1, 6)	0·109	100	17	0·17
	1·42	378	33 (10, 13, 10)	0·247	100	41	0·41
	0·86	530	14 (8, 4, 2)	0·075	100	14	0·14
	1·30	105	16 (10, 3, 3)	0·431	100	44	0·44

[a]Dose to bone marrow assuming DS86 dosimetry and a neutron RBE of 10.

Table 1b. Translocation frequencies in Y-12 criticality accident victims measured by FISH for chromosomes 1, 3 and 4, and by conventional staining.

		FISH			CONVENTIONAL		
Subjects	Dose[b] (Sv)	Cells	Translocations total (#1, #3, #4)	Trans/cell (genomic	Cells	Translocations	Trans/cell[c] × 1·33
[redacted]	4·61	2400	146 (60, 39, 47)	0·185	298	36	0·161
	3·41	1674	58 (29, 14, 15)	0·106	200	11	0·073
	2·98	2449	42 (16, 9, 17)	0·051	294	12	0·054
	0·29	3001	13 (4, 3, 6)	0·013	200	2	0·013

[b]Dose to blood based on sodium-24 activation (Patton *et al.* 1958) and assuming an RBE of 2 for the fast neutron dose.
[c]Frequency multiplied by 1·33 to account for the lower scoring efficiency using this method (see text).

group of the Hiroshima study and two unexposed laboratory workers. Sensitivity of the approach might be increased further by increasing the translocation detection efficiency. The highest efficiency that can be achieved using single colour FISH is $\sim 50\%$ when $f_p = 0{\cdot}5$: Figure 3. This may be increased by using multi-colour FISH to stain differentially larger portions of the genome. However, the increased efficiency may come at the cost of increased analytical complexity and analysis time per spread. Additional work will be needed to determine the optimal strategy.

Equation (2) was derived to allow intercomparison of FISH analysis using different sets of whole chromosome probes and comparison of FISH results with translocation frequencies determined using conventional chromosomal staining or banding. The equation is based on the assumption that the translocation breakpoints are distributed uniformly throughout the genome so that the same information can be obtained from sampling any portion of it. Measurement of breakpoint distributions and several quantitative tests support the adequacy of equation (2) for the present studies.

Figure 2 shows that the frequency of translocation breakpoints in a chromosome is related linearly to chromosomal DNA content in 52 Hiroshima survivors ($r = 0{\cdot}915$), up to 45 years after exposure. The small non-zero intercept at $d_i = 0$ and the deviation of the translocation frequency for chromosome 1

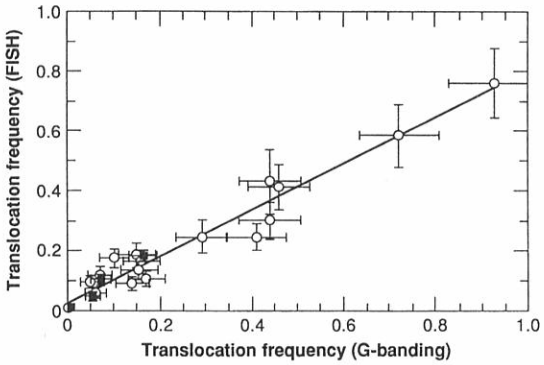

Figure 5. Comparison of genomic translocation frequencies estimated using FISH and conventional cytogenetic techniques. ○, translocation frequencies measured for Hiroshima A-bomb survivors using FISH for chromosomes 1, 2 and 4 were converted to genome equivalents using equation (2) and plotted against translocation frequencies measured by G-banding for all chromosomes for the same individuals. ■, translocation frequencies measured for irradiated Y-12 workers using FISH for chromosomes 1, 3 and 4 were converted to genome equivalents using equation (2) and plotted against genomic translocation frequencies measured for all chromosomes for the same workers by analysis of conventionally stained metaphase spreads and multiplying the translocations measured by 1·33 to account for the lower scoring efficiency using this method (see text). All error bars show the uncertainty in the counting statistics (one standard deviation).

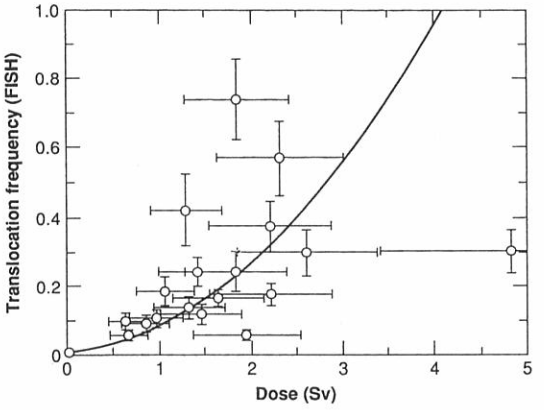

Figure 6. Comparison of the *in vitro* genomic translocation frequency dose-response curve estimated using FISH with the translocation frequency dose-response data measured using FISH for 20 Hiroshima A-bomb survivors. Line shows the second order-polynomial fit from Figure 4 to the *in vitro* translocation frequency dose response data. A-bomb translocation frequencies are plotted against DS86 estimates of dose to the bone marrow assuming a neutron RBE of 10 (○). Error bars on the translocation frequencies are the uncertainties in the Poisson counting statistics (one standard deviation) and the error bars on the doses are the ±30% uncertainty estimated for the DS86 doses (RERF, 1987).

from the regression line are not consistent with assumptions associated with equation (2). However, they do not seem sufficiently large to invalidate the equation.

Three studies based on FISH also were used to evaluate the accuracy of equation (2). First, Figure 3 shows that the dependence of the measured translocation frequency, F_p, on the fraction of the genome stained is correctly described by equation (2). Second, Figure 4 shows that the dose dependence of the genomic translocation frequencies, F_G, determined at first division after *in vitro* exposure using chromosome painting with f_p values of 0·04 and 0·22 are in close agreement with each other. These translocation dose response curves also are very close to published dicentric frequency dose response curves. Third, Figure 5 and Tables 1a and b compare the genomic translocation frequencies, F_G, determined by FISH with the frequencies determined by G-banding (A-bomb survivors) or conventional staining (Y-12). Measurements for the Hiroshima survivors were on different samples of the same blood cultures, while the analyses of the Y-12 victims were separated in time by 5 years. Linear regression between the two methods of measurement for the A-bomb survivors shows a slope of 0·75, which is not different statistically from 1 in this data set. However, additional studies are needed to define more accurately the slope of the regression line and to examine the reasons for the observed individual deviations between the two measurements.

Use of translocation frequency analysis for *in vivo* biological dosimetry requires that the relationship between dose and translocation frequency be known with some accuracy. One possibility is to use *in vitro* calibration curves such as that in Figure 4 for this purpose. This presumes that the translocation frequency measured for peripheral lymphocytes immediately after irradiation is the same as that measured in the peripheral blood years after exposure. The study of Buckton *et al.* (1978) of ankylosing spondylitic patients, suggests that the translocation frequencies measured in peripheral blood do appear to be generally stable with time after exposure. Also, the agreement in Figure 6 between the translocation frequency dose-response curve measured *in vitro* and the translocation frequency dose-response data for the Hiroshima A-bomb survivors measured here, suggests that, with few exceptions, translocation frequencies of individuals do not appear to change substantially with time after exposure. In fact, when all known uncertainties are considered, only four of 20 A-bomb survivors have translocation frequencies that are significantly different from the *in vitro* curve (Straume *et al.* 1992). By contrast, dicentrics mea-

sured on the same slides for both Hiroshima and Y-12 individuals had decayed to background; six dicentrics were measured in 6000 full genome-equivalent cells for Hiroshima and two dicentrics in 4000 full genome-equivalent cells for Y-12.

Variation of the individual translocation frequencies for the Hiroshima survivors about the *in vitro* dose response curve, and the Y-12 dose-response data (not plotted in Figure 6) which lie substantially below the *in vitro* curve, may at least in part be explained by errors in dose estimation (e.g. Figure 6, and we note that literature reports of the physiological responses of the irradiated Y-12 workers were less than expected from the estimated doses). It is also possible that the dynamics of the hemopoietic system may affect the correspondence between the *in vitro* dose-response curve and individual translocation frequencies years after exposure. For example, the lymphocytes that contribute to the *in vitro* dose-response curve were fully differentiated when irradiated while the cells measured in the peripheral blood years after exposure were less mature memory cells or bone marrow stem cells when irradiated. Radiosensitivity and repair characteristics of these populations may differ between individuals and thus lead to an uncoupling between the *in vitro* curve and the *in vivo* data. In addition, transient clonal expansion of specific lymphocyte subsets as a result of immunological challenge could, at least in principle, cause fluctuations in the translocation frequencies measured for irradiated individuals, thereby complicating estimation of individual dose. However, clonal expansion does not appear to be a frequent occurrence in irradiated populations. For example, in the 20 Hiroshima survivors studied here, only two individuals appeared to have clones of cells with stable aberrations and in these survivors the clonal cells contributed less than 5% of the cells with translocations. Thus, clones did not contribute significantly to the translocation frequencies measured here. Translocation analysis using FISH with whole-chromosome probes is sufficiently rapid and precise to allow further investigation of these issues.

Translocation frequency analysis for estimation of dose should be particularly useful for large populations such as radiation workers, A-bomb survivors, and the citizens in the communities surrounding Chernobyl, where analysis immediately after exposure is inconvenient or logistically impossible. Translocation frequency analysis also may be useful in studies of chronically exposed populations because stable aberrations such as translocations are expected to accumulate during exposure.

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Appendix
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MF # = Master File number : 基本名簿番号　Sex = 性　M = 男性
F = 女性　Culture # = 培養番号