Chromosome aberration analysis in atomic bomb survivors and Thorotrast patients using two- and three-colour chromosome painting of chromosomal subsets

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(Received 1 September 1995; revision received 15 March 1996; accepted 19 March 1996)

Abstract. Chromosomal translocations in peripheral lymphocytes of three healthy Hiroshima atomic (A)-bomb survivors, as well as three Thorotrast patients and two non-irradiated age-matched control persons from the German Thorotrast study were studied by two- and three-colour fluorescence in situ hybridization (chromosome painting) with various combinations of whole chromosome composite probes, including chromosomes 1, 2, 3, 4, 6, 7, 8, 9 and 12. Translocation frequencies detected by chromosome painting in cells of the A-bomb survivors were compared with results obtained by G-banding. A direct comparison was made, i.e. only those cells with simple translocations or complex aberrations detected by G-banding were taken into consideration which in principle could be detected also with the respective painting combination. The statistical analysis revealed no significant differences from a 1:1 relationship between the frequencies of aberrant cells obtained by both methods. The use of genomic translocation frequencies estimated from subsets of chromosomes for biological dosimetry is discussed in the light of evidence that chromosomes occupy distinct territories and are variably arranged in human lymphocyte nuclei. This territorial organization of interphase chromosomes implies that translocations will be restricted to chromatin located at the periphery of adjacent chromosome territories.

1. Introduction

Chromosome aberrations in PHA-stimulated human peripheral blood lymphocytes have provided a reliable indicator to quantitatively assess human exposure to ionizing radiation (Sasaki and Miyata 1968, Awa et al. 1978, Sasaki et al. 1987). The presence of chromosome aberrations in lymphocytes and bone marrow cells has been demonstrated for at least four decades after exposure to atomic (A)-bomb radiation (Awa et al. 1978, Kamada and Tanaka 1983). The majority of observed aberrations was identified as ‘stable’ chromosome aberrations, such as translocations, while cells with ‘unstable’ aberrations, such as dicentrics, were mostly eliminated due to mitotic failure (Kamada and Tanaka 1983). On the contrary, Thorotrast patients have shown high frequencies of both unstable and stable types of aberrations several decades after a single angiographic injection with radioactive thorium dioxide (ThO$_2$). This difference results from the permanent storage of the radioactive nuclide $^{232}$Th (half-life $1.4\times10^{10}$ years) in the organs of the reticulo-endothelial system. Emission of $\alpha$-particles during radioactive decay (>90% of the total radioactivity) leads to continuous chromosomal damage in these patients (Teixeira-Pinto and Silva 1979, Tanaka et al. 1984, van Kaick et al. 1986, Sasaki et al. 1987, Popp et al. 1990).

For an assessment of long-term biological effects of radiation, the evaluation of stable chromosome aberrations appears to be of particular importance. Stable chromosome aberrations, in particular reciprocal translocations, rarely affect the clonal lifespan of cells and thus may be better suited than dicentrics as a long term or cumulative biological dosimeter. Such a dosimeter will be useful in cases where an irradiation event has occurred a long time ago, in cases where multiple exposures with unknown doses have occurred at unspecified times, as well as in cases with chronic irradiation exposure (Popp et al. 1990). The frequency of stable translocations should increase with the total dose received. While dicentrics can be scored after homogeneous staining of chromosomes,

Previously frequencies of reciprocal translocations for the entire genome were extrapolated from the frequency of reciprocal translocations observed after painting of a chromosomal subset. These estimates were then compared with genomic translocation frequencies obtained by conventional cytogenetic or G-banding techniques (Lucas et al. 1992). The concept that genomic translocation frequencies can be deduced from translocation frequencies obtained for a portion of the genome is based on the assumption that breakpoints are uniformly distributed along the chromosomes and that in a cell population any two breakpoints located on different chromosomes have the same probability to result in a scorible translocation event. The validity of this concept, however, remains uncertain (see §4).

In an attempt to assess further the validity of chromosome painting as a long-term biological dosimeter, we applied two- and three-colour painting of individual chromosome subsets using several different combinations of directly fluorochrome-labelled whole chromosome composite DNA probes to three Hiroshima A-bomb survivors, as well as three Thorotrast patients and two age-matched controls from the German Thorotrast study (van Kaick et al. 1986). In addition to chromosome painting, DAPI staining was performed to facilitate the scoring of translocations, dicentrics and other aberrations involving the painted chromosomal subsets. Frequencies of cells with translocations observed for painted chromosome sets in cells of the Hiroshima A-bomb survivors were also compared with data from G-banding analysis. A direct comparison was made between the frequencies of cells with translocations detected by painting with each given subset of painted chromosomes, and the frequencies of cells with translocations detected by G-banding, correcting the G-banding frequencies for the same chromosomal subset. In this comparison, only those translocation events detected by G-banding were taken into account which in principle could be detected also by painting with the respective painting combinations. Such a direct comparison has the advantage that its validity does not depend on assumptions regarding the topology of translocation formation in the cell nucleus (see §4). Furthermore, in this way it was possible to include also complex aberrations in the comparison.

2. Materials and methods

2.1. Origin of cells

Three Hiroshima A-bomb survivors, three Thorotrast patients and two non-irradiated control persons were examined. The A-bomb survivors (person 8892: 66-year-old female; person 8971: 74-year-old male; person 8682: 81-year-old male) were in an apparently healthy condition and had no detectable haematological disease. They were exposed at 0·4, 1 and 0·5 km distance from the hypocenter of Hiroshima. Their physical exposure doses were estimated according to the new ABS93D dosimetry system (Hoshi et al. 1994) which was based on the DS86 dosimetry system (Roesch 1987). Two A-bomb survivors (persons 8892 and 8971) had estimated exposure doses of 3·54 and 0·84 Gy respectively. The exposure dose of person 8682 could not be estimated because he was exposed when he was in the basement of a concrete building in a shielded condition.

Thorotrast patients were available through the Thorotrast study of the German Cancer Research Center and were studied 43–45 years after a single injection of Thorotrast. All three Thorotrast patients had developed liver tumours, but had never received radiation therapy. Person 5044, a 61-year-old female had received 26 ml Thorotrast. The present activity measured with a whole-body counter was $7 \times 10^3$ Bq $^{208}$Tl. This corresponds to an estimated dose of $33.8 \times 10^{-2}$ Gy/per year in
the liver, \(104 \times 10^{-2}\) Gy/per year in the spleen, and \(10.4 \times 10^{-2}\) Gy/per year in the red bone marrow. Person 5101, a 66-year-old female, had received 11 ml Thorotrast, present activity \(2.9 \times 10^3\) Bq \(^{208}\)Tl corresponding to \(14.3 \times 10^{-2}\) Gy/per year in the liver, \(44 \times 10^{-2}\) Gy/per year in the spleen, and \(4.4 \times 10^{-2}\) Gy/per year in the red bone marrow. Person 5136, a 59-year-old male received 25 ml Thorotrast, present activity \(6.6 \times 10^3\) Bq \(^{208}\)Tl corresponding to \(32.5 \times 10^{-2}\) Gy/per year in the liver, \(100 \times 10^{-2}\) Gy/per year in the spleen, and \(10.0 \times 10^{-2}\) Gy/per year in the red bone marrow. Control persons were treated in the same hospital at the same time as the Thorotrast patients but were not exposed to Thorotrast (person 7204, a 51-year-old male; person 7223, a 52-year-old female).

2.2. Cell material

Ten ml of peripheral blood were obtained from each person about 45 years after the event (A-bomb explosion, Thorotrast injection) that taken place. Lymphocytes were isolated, stimulated to divide with phytohaemagglutinin (PHA) and cultured for 52 h (A-bomb survivors) or 72 h (Thorotrast patients) using standard techniques (Schwarzacher 1970). Colcemid-arrested metaphase spreads were obtained after hypotonic treatment (0.075 M KCl) and fixation with methanol/acetic acid (3:1 v/v). Chromosome preparations were pretreated with RNase and pepsin as described (Ried et al. 1992).

2.3. DNA probes and chromosome painting

Plasmid libraries from sorted human chromosomes 1, 4 and 8 were kindly provided by Dr Joe Gray (University of California, San Francisco, CA, USA) (Collins et al. 1991). Amplification of these libraries and isolation of plasmid DNA were carried out according to standard protocols (Sambrook et al. 1989). Chromosome-specific library DNA probes directly labelled with fluoro-chromes, termed whole chromosome painting (WCP)-probes, were a generous gift from Imagenetics (now Vysis; Naperville, IL, USA). WCP-probes for chromosomes 1, 2, 4, 6, 7, 8, 9, 12 were conjugated to a fluoresceiniothiocyanate (FITC) derivative (spectrum green; green fluorescence) while WCP-probes for chromosomes 1, 2, 3, 4, 6, 7, 9, 12 were conjugated to a rhodamine derivative (spectrum orange; red fluorescence). In addition, plasmid library DNAs from sorted human chromosomes 1, 4 and 8 were nick-translated with coumarin-4-dU TP (fluoro blue, a generous gift from Amersham, UK; blue fluorescence) (Lengauer et al. 1993). In one experiment, plasmid libraries from human chromosomes 4 and 8 provided as a kind gift by Dr J. Gray (University of California, San Francisco) were nicktranslated with digoxigenin-11-dUTP and biotin-16-dUTP respectively (Boehringer Mannheim, Germany). Chromosome painting was carried out as described by Lichter and Cremer (1992) with minor modifications. Probes for various chromosomes were combined at an amount of 200 ng to 1 \(\mu\)g each and precipitated with sodium acetate (300 mM) in the presence of 100–200 \(\mu\)g cot-I DNA (Life Technologies, Eggenstein, Germany) and 100 \(\mu\)g sonicated salmon sperm DNA (Sigma, Munich, Germany). Following precipitation, the probe DNA was resuspended in the hybridization mixture provided with the Imagenetics WCP-probe kit and mounted with a 15 \(\times\) 15 mm coverslip. Following probe and chromosome denaturation, and hybridization for 65 h at 37 °C, slides with biotin and digoxigenin-labelled probes were washed and probe detection was carried out as described (Lichter and Cremer 1992). Briefly, biotinylated sequences were visualized by avidin conjugated with fluoresceiniothiocyanate (FITC) (Vector Laboratories), whereas digoxigenin-labelled sequences were detected by indirect immunofluorescence using mouse anti-digoxigenin (Boehringer Mannheim) and tetramethylrhodamin (TRITC) conjugated rabbit antimese antibodies (Sigma). In case of probes directly conjugated to fluorescent dyes, slides were washed three times in 2 \(\times\) SSC, followed by a short wash under tap water. All slides were mounted in 0-1% \(p\)-phenylene diamindihydrochloride in glycerol (Johnson and Nogueira 1981). Slides with green and red fluorescent probes were counterstained before with 0.2 mg/ml DAPI. In case of chromosome painting with an additional blue fluorescent probe set, DAPI staining was only carried out after evaluation of translocations involving the blue painted chromosomes.

2.4. Microscopy

Metaphase spreads and corresponding cell nuclei were investigated with a Zeiss Axiophot equipped with a Plan-Neofluar 63 \(\times\)/1.25 oil objective. The following filters were used: filter set 10
(BP 450-490, FT 510, LP 515-565) for spectrum green; filter set 01 (BP 365, FT 395, LP 397) for fluoro blue and DAPI and the double band pass filter set 23 for simultaneous spectrum green and spectrum orange detection (DBP 485/20, 546/12; FT 500/560; DBP 515-530, 580-630) (Carl Zeiss, Oberkochen, Germany). For simultaneous visualization of DAPI, FITC and TRITC the triple band pass filter set F61-002 was applied (AHF Analysentechnik, Tübingen, Germany). Microphotographs were taken on Ektachrome 400 ASA colour slide films by double or triple exposures and are shown without any (digital) processing.

2.5. Molecular and conventional cytogenetic analyses

Painted chromosomes were first identified using the appropriate filters for spectrum green, spectrum orange and fluoro blue. Rearranged chromosomes involving segments from two or more chromosomes were identified due to a change of colour along the chromosome contour. A DAPI specific filter was then used to view painted chromosomes after DAPI staining in order to facilitate the classification of painted chromosomes (Table 2), in particular to judge also whether painted chromosomes were monocentric or dicentric. Another set of metaphase spreads from the three A-bomb survivors was also analysed after routine G-banding. All abnormal cells were karyotyped and identified according to the International System of Chromosome Nomenclature (ISCN 1991).

3. Results

3.1. Two-colour painting of metaphase and interphase chromosomes

The reliability of translocation scoring of painted chromosomes strongly depends on the quality of the whole chromosome paint probes to visualize entire chromosomes from pter to qter. Figure 1 shows photomicrographs recorded on colour slide films without digital processing as examples for two two-colour painting of chromosomes 4 and 8 in normal lymphocyte metaphase spreads and corresponding interphase nuclei. These chromosomes were uniformly painted from pter to qter (with the exception of centromeric heterochromatin which is often faintly labelled due to the suppression with cot1 DNA; see §2). The same quality of chromosome painting was achieved for both indirectly and directly labelled probes used in the present study. Under these conditions translocation events between differently coloured chromosomes could be easily detected except for very small insertions or cryptic translocations involving the chromosome ends. Another important feature demonstrated in Figure 1 is the territorial organization of interphase chromosomes and their variable arrangements in interphase nuclei. Accordingly, chromosome territories, which were part of a given painted chromosome subset, were immediate neighbours in some nuclei, while they were located at remote places in others. Light optical serial sections of chromosome territories, which were fixed and painted under conditions that preserved the 3-D architecture of cell nuclei as much as possible (Cremer et al., 1993), revealed that interdigitation of chromatin from neighbouring territories was limited to the periphery of adjacent territories (our unpublished data). We conclude from these findings that the formation of translocations is necessarily restricted to a peripheral chromatin layer of each chromosome territory. This fact has important implications with regard to the frequency with which translocations between two pairs of chromosomes may occur (see §4).

3.2. Two- and three-colour painting of chromosomal subsets in A-bomb survivors and Thorotrast patients

Two- and three-colour chromosome painting was applied with three combinations of differently coloured WCP-probe sets A-G (Table 1) to peripheral lymphocyte metaphase spreads from three Hiroshima A-bomb survivors, three Thorotrast patients and two age-matched control persons from the German Thorotrast study. For an estimate of the percentage of the genome covered by each probe set A-G, Table 1 gives their DNA content relative to a male diploid chromosome set (Morton 1991). For example, the DNA fraction hybridized by probe set A was calculated...
as \((183\, \text{Mbp} \times 2 + 171\, \text{Mbp} \times 2 + 145\, \text{Mbp} \times 2) / (6349\, \text{Mbp}) \times 100\% = 15.7\%\). In WCP probe combination I (38.4\% genome coverage), WCP probes for chromosomes 6, 7, 9 (probe set A) labelled with spectrum green, were combined with probes for chromosomes 1, 2, 4 (probe set B) labelled with spectrum orange (red). In WCP probe combination II (54.5\% genome coverage) probes for chromosomes 1, 4, 8, 12 (probe set C) labelled with spectrum green were combined with probes for chromosomes 2, 3, 6, 7, 9 (probe set D) labelled with spectrum orange. WCP probe combination III targeted the same chromosomes as combination II, but in three colours, i.e. probes for chromosomes 2, 7 and 9 (probe set E) were labelled with spectrum green, probes for chromosomes 3, 6 and 12 (probe set F) were labelled with spectrum orange and probes for chromosomes 1, 4 and 8 (probe set G) with fluoro blue. In all experiments metaphase spreads were counterstained with DAPI for additional aberration assessment comprising painted chromosome material. In this way any translocation event occurring between green-, red-, and blue-painted, plus DAPI stained chromosomes could be easily detected. Cells with aberrations were classified as cells with simple and cells with complex translocations, and cells with dicentrics, deletions, fragments, inversions, insertions, and rings. Most of the detected chromosome aberrations were translocation events. For example, Figure 2 shows two metaphase spreads from A-bomb survivor A 8892. The metaphase spread in Figure 2a and b was subjected to the two-colour paint probe combination I. Several green/red and green/blue coloured translocation chromosomes can be noted. The metaphase spread shown in Figure 2c and d shows the quality of three-colour chromosome painting achieved with WCP-probe combination III and includes a green-painted deleted chromosome.

Chromosome aberrations detected with chromosome paint combinations I–III and DAPI staining in lymphocyte metaphase spreads from A-bomb survivors (A), Thorotrast patients (T), and control persons (C) are listed in Table 2. For each experiment, 63–250 (average 130) metaphase spreads were scored. A cell containing two painted chromosomes with a change in colour (corresponding to a reciprocal exchange of material) was counted as a cell with a simple translocation event. A metaphase spread containing aberrations in three or more painted chromosomes (e.g. two simple translocation events, or one simple translocation event plus a deletion, etc.) was counted as a cell with a complex aberration. In Table 2, for each WCP-probe combination and each case, the number of cells is given in which a specified type of aberration (e.g. simple translocation; complex aberration) was observed. In parentheses, the percentage of abnormal metaphase spreads as well as the total percentage of cells with translocations (simple translocation events plus complex aberrations) is indicated.

Using WCP-probe combinations I–III, several cells with complex aberrations were observed in the cells of the A-bomb survivors A 8892 and A 8682, in addition to cells with simple translocation events. The combined experiments yielded a total percentage of cells with translocations (simple translocation events plus complex aberrations) of 14·7\% \(((26 + 15 + 7)/(176 + 86 + 65) \times 100\%\) for person A 8892 and of 3·3\% for person A 8682 \(((3 + 5 + 2)/(149 + 87 + 63) \times 100\%\).

Application of WCP-probe combinations I and II to cells of persons A 8892, A 8971 and A 8682

<table>
<thead>
<tr>
<th>Whole chromosome painting (WCP) probe combination</th>
<th>Painted fraction of the genome (%)</th>
<th>Green painted chromosomes in probe set (percentage of the genome)</th>
<th>Red painted chromosomes in probe set (percentage of the genome)</th>
<th>Blue painted chromosomes in probe set (percentage of the genome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38·4</td>
<td>set A: 6, 7, 9 (15·7)</td>
<td>set B: 1, 2, 4 (22·7)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>54·5</td>
<td>set C: 1, 4, 8, 12 (24·0)</td>
<td>set D: 2, 3, 6, 7, 9 (30·5)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>54·5</td>
<td>set E: 2, 7, 9 (18·0)</td>
<td>set F: 3, 6, 12 (17·0)</td>
<td>set G: 1, 4, 8 (19·5)</td>
</tr>
</tbody>
</table>

Table 1. Design of whole chromosome painting experiments
Figure 2. Examples of two aberrant lymphocyte metaphase spreads from Hiroshima A-bomb survivor 8892. Conventional microphotographs recorded on colour slide films are shown. (a) Metaphase spread after two-colour painting of chromosomes 6, 7, 9 labelled with spectrum green and of chromosomes 1, 2, 4 labelled with spectrum orange (WCP-probe combination I) shows a cell with complex aberrations, including two red/green painted translocation chromosomes resulting from a reciprocal translocation event t(2;9), and three additional translocation chromosomes with a green painted segment and a blue DAPI-stained segment (compare b). Arrows point to the breakpoint regions. (b) The same metaphase spread after DAPI staining. (c) Metaphase spread after three-colour painting of chromosomes 2, 7, 9 labelled with spectrum green, chromosomes 3, 6, 12 labelled with spectrum orange and chromosomes 1, 4, 8 labelled with fluoro blue (probe combination III). A green-painted (acentric?) fragment can be seen (arrowhead). (d) The same metaphase spread after DAPI staining.
yields total percentages of cells with translocations of 15.6% \((26 + 15)/(176 + 86) \times 100\%)\) for A8892; 1.8% \((2 + 2)/(127 + 99) \times 100\%)\) for A8971; and 3.4% \((3 + 5)/(149 + 87) \times 100\%)\) for A8682.

Since WCP-probe combinations I and II were applied to all three A-bomb survivors, the resulting total percentages of cells with translocations were used to obtain a first rough estimate of the dose which the shielded victim A8682 received. From these figures, one may estimate that the shielded victim A8682 may have received an intermediate dose between that for the other two victims. Cells with aberrations other than translocations were rare in atomic bomb survivors. In particular, in 852 metaphase spreads analysed form the three A-bomb survivors, no dicentric was observed among the painted chromosomes (upper limit of the 0.95% confidence interval: 0.43%).

In the three Thorotrast patients (estimated accumulated total doses in the red bone marrow: T5101, 1.9 Gy; T5136, 4.4 Gy; T5044, 4.6 Gy), but not in the two age-matched control persons, WCP-probe combination I yielded both cells with simple translocation events and cells with complex aberrations. The total percentages of cells with simple translocation events plus complex aberrations was 8.7, 9.0, and 6.3% respectively for the three patients and ≤1% for the two control persons. In the case of the Thorotrast patient T5101, chromosome painting was performed with the two WCP-probe combinations I and II, yielding similar results. In contrast to the A-bomb survivors, in 494 cells analysed from Thorotrast patients, eight cells with dicentrics were found (95% confidence interval: 0.7–3.2%), whereas in the 602 control cells, again no dicentrics were detected (upper limit of the 95% confidence range: 0.61%). The statistically significant difference in the yield of dicentrics observed in Thorotrast patients and in A-bomb survivors is compatible with the biological difference between the chronic exposure with \(^{232}\text{Th}\) and the single acute irradiation by the Hiroshima bomb. PHA stimulated lymphocyte cultures evaluated from Thorotrast patients were terminated after 72 h. A shorter culture time, as it was applied for A-bomb survivors (52 h), might have resulted even in a higher rate of dicentrics.

### 3.3. Comparison of frequencies of cells with translocations detected by chromosome painting with frequencies detected by G-banding

G-banding provides the gold standard for the counting of translocation events. In this report, the frequencies of cells with translocations obtained by G-banding were compared with the frequencies of cells obtained by painting. Here, instead of calculating total genomic translocation frequencies from the painting data and comparing these genomic estimates with the total translocation frequencies obtained by G-banding analysis, a more direct comparison was made which avoids assumptions how to calculate genomic translocation frequencies. Furthermore, by making the comparison between the frequencies of cells with translocations, it was possible to...
include also complex aberration events. For comparison with the painting data, the chromosome aberrations (all chromosomes) after G-banding were analysed in a total of 328 metaphase spreads of the three A-bomb survivors A8892 (112 cells), A8971 (110 cells), and A8682 (106 cells). With very few exceptions, translocation events only were found. In a few cells, it was not possible to clearly identify an aberrant chromosome; these cells were excluded from further consideration.

A direct comparison of G-banding data with data from painted chromosomes has to take into account that translocation events between chromosomes painted with the same colour cannot be detected. To account for this, the following procedure was used for the further analysis of G-banded metaphase spreads:

1. For every cell with aberrations, the aberrations (mostly translocation events) were listed for all chromosomes.

2. For each individual WCP-probe combination used, it was noted whether the G-banded aberration would have been detected also in the corresponding painting experiment, e.g. by forming a bicoloured chromosome; the G-banded cell containing such a ‘detectable’ aberration was classified as an aberrant cell detectable by the corresponding WCP-probe combination. Thus, depending on the WCP combination, a cell with G-banded aberrations could be classified, e.g. as a ‘cell with a simple translocation’ by WCP-probe combination I and as a ‘cell with complex aberrations’ by WCP-probe combination II. To obtain frequencies, the number of the cells classified in this way were divided by the total number of metaphase spreads analysed by G-banding.

For example, the G-banding analysis of 112 metaphase spreads of A-bomb victim A8892 yielded a total of 29 cells with aberrations; for all chromosome regions of the aberrant chromosomes, the colour was noted by which this region would have been painted using probe combination I; three cells were excluded from further consideration because one or more regions of aberrant chromosomes could not be unequivocally identified; 14 of the remaining 26 cells were classified as containing simple translocations or complex aberrations detectable with this probe combination (I). Thus, the frequency of these cells (called G-banding-cells) was 14/112 = 0.125, and the percentage of G-banding cells was 12.5%. If the classification was restricted to cells which would have been classified as cells with simple translocations only, the frequency of these latter cells was 12/112 = 0.107 for probe combination I.

Assuming probe combination II, 17 cells of the 112 analysed (15.2%) were classified as containing simple or complex translocations detectable with this probe combination. If the classification was restricted to cells which would have been classified as cells with simple translocations, the frequency of these latter cells would have been 13/112 = 0.116.
The percentages of G-banding-cells obtained in this way for A 8892, A 8971, A 8682, and the probe combinations I–III, were compared with the results (Table 2) obtained experimentally by chromosome painting. For example, the percentage of G-banding-cells obtained for probe combination I and cells from person A 8892 (12.5%) was compared with the percentage of cells (FISH-cells) with simple and complex translocations (14.8%) detected by the corresponding painting experiment. The outcome for the eight pairs (percentage of G-banding cells; FISH-cells) is shown in Figure 3.

To evaluate whether there is a linear relationship between the two measurements, we performed a special regression analysis proposed by Passing and Bablok (1983, 1984) and Bablok et al. (1988), since standard regression technique is not adequate in this situation. If both percentages of G-banding cells and of FISH-cells measured the same percentage of cells with simple translocations and complex aberrations, the values should vary symmetrically around the diagonal with zero mean difference. No significant ($p > 0.05$) deviation of the Passing-Bablok regression line from the diagonal was detected. In addition, the frequency of cells with simple translocation events obtained by G-banding and counted for the different WCP-probe combinations as outlined above, was compared with the frequencies of cells with simple translocations detected by painting (Table 2). Again, no significant deviation was observed from a 1:1 relationship.

4. Discussion

In this study, painting of a panel of chromosomal subsets was applied to quantitatively assess radiation-induced chromosomal translocations in Hiroshima survivors and in Thorotrast patients. These two types of persons reflect two typical situations of long term biological dosimetry (see §1). The Hiroshima survivors received a single acute radiation dose a few years before the chronic radiation exposure started in the Thorotrast patients more than four decades ago. To assess further the usefulness of multicolour painting of chromosomal subsets for the scoring of translocation events, seven probe sets were hybridized in various combinations, painting up to nine pairs of chromosomes simultaneously (genome coverage 54-5%) in two and three colours respectively. Translocations occurring between differently painted chromosomes were easily detected by a change in colour noted along the chromosome contour. In addition, DAPI-fluorescence was used to facilitate the classification of painted chromosome material.

For the three A-bomb survivors, the average percentages of cells with translocation chromosomes obtained with WCP-probe combinations I and II ranged from 1.8 to 15.6% reflecting a range of estimated physical exposure doses from 0.84 to 3.54 Gy known for two of the survivors (A 8971 and A 8892). For the third victim who survived close (0.5 km) to the hypocenter under shielded conditions, a first rough estimate of the received dose may be calculated to be in the order of 1-6 Gy from the present data, neglecting the constant and the linear term in the linear-quadratic dose–response relationship (cf. Lucas et al., 1992). In the case of the three Thorotrast patients, the percentages of cells with translocation events (simple and complex) obtained with WCP-probe combination I varied from 6.3% (T 5044), 8.7% (T 5101) to 9.0% (T 5136) reflecting an accumulated dose range of red bone marrow of several Gy. For the two Thorotrast-control persons, the percentages of cells with translocation events (simple and complex) estimated from the painting experiments were many times lower. Cells with dicentrics were observed for the three Thorotrast patients (chronic radiation exposure), but neither for the two age-matched control persons of the Thorotrast study nor for the three A-bomb survivors (single acute radiation exposure).

While the lower limit of the confidence limits of the percentage of dicentrics observed in cells from Thorotrast patients was 0.7%, the upper confidence limit of the percentage of dicentrics in cells of A-bomb victims was 0.43%. The dicentric frequencies may be compared with results obtained more than two decades earlier by Sasaki and Miyata (1968). For cells from survivors in Hiroshima corresponding to a similar distance as for A 8892 and A 8971, a percentage of dicentrics of $14/1573 \approx 0.9\%$ (0.5 km distance) and of $12/(1370+679) = 0.6\%$ (1 km distance) was observed. Considering that the present study has been performed more than two decades later, our results are compatible with a progressing elimination of dicentrics after the single acute A-bomb event whereas dicentrics were continually reproduced by the continuous internal radiation the Thorotrast patients were exposed to.

In the present study, dicentrics in the painted chromosomal subsets were scored using additional DAPI staining. Notably, FISH of centromeric and
telomeric segments of chromosomes will facilitate the automated detection of dicentrics (Rutowitz 1992).

In comparison with attempts to automate the detection of translocations in banded metaphase spreads, the automated analysis of painted chromosomes is greatly facilitated (Popp et al. 1990, Cremer et al. 1992, Fantes et al. 1995). In the first case, complex patterns (hundreds of bands per cell) in a huge variety of combinations have to be analysed, while in the latter case simple colour changes along the segmented chromosome contours are counted. This approach should become particularly useful to monitor acute single or chronic exposures with low radiation doses or in a case of catastrophes. It is expected that chromosome painting in combination with automated analysis will become the method of choice for translocation detection in biological dosimetry.

In this study, a direct comparison was made between the frequency of cells with translocations observed for a given subset of painted chromosomes and the frequency of cells with translocations obtained by G-banding for the same subset of chromosomes, deducing those translocations which would not have been detectable by painting using a given WCP-probe combination. No statistically significant deviation from a 1:1 relationship was observed.

Using single-colour FISH, Lucas et al. (1992) scored reciprocal translocation frequencies in atomic bomb survivors for single painted autosomes 1 or 4, as well as for painted chromosomal subsets 1, 4, 1 + 4, 1 + 3 + 4, and 1 + 2 + 3 + 4 + 15. Genomic translocation frequencies were extrapolated from these data and compared with translocation frequencies obtained by conventional cytogenetic staining techniques. A fit statistically not different from a 1:1 ratio was obtained.

In this report, we used different subsets of autosomes (chromosomes 1, 2, 4, 6, 7, 9 and 1, 2, 3, 4, 6, 7, 8, 9, 12 respectively) in two- and three-colour FISH assays. Furthermore, in contrast with Lucas et al., a direct comparison was made between G-banding data and painting data, without calculating total genomic translocation frequencies. This was possible by counting in the G-banding analysis those aberrant chromosomes only which would have been detected also with the corresponding WCP-probe combination.

In one mode of evaluation, the frequency of cells with simple (i.e. reciprocal) translocations detected by painting was compared with the corresponding frequency of cells with simple translocations detected by G-banding; no significant deviation was found from a 1:1 ratio. This way of comparison is closest to the comparison of reciprocal translocation frequencies performed by Lucas et al. (1992).

In a second mode of evaluation, a first attempt was made to directly compare the percentages of cells with both simple translocations and complex aberrations detected by G-banding and painting, respectively. This allowed a full, direct comparison between painting and G-banding also for higher doses where an increased number of cells with complex aberrations is induced. Again, no significant deviation from a 1:1 ratio between G-banding and painting data was detected.

Both modes of evaluation indicate the validity of two- and three-colour chromosome painting for scoring of cells with simple translocations as well as for scoring of cells with simple translocations or with complex aberrations. This also confirms the validity of automatic evaluation procedures for biological dosimetry where the scoring is based on the classification of cells containing two or more bicoloured chromosomes (Popp et al. 1990, Cremer et al. 1992).

As noted above ($§1$) a valid extrapolation of genomic translocations frequencies from frequencies counted for specific chromosomal subsets implies the assumption that the translocation breakpoints are distributed uniformly throughout the genome. Although the data of Lucas et al. (1992) suggest that this assumption may be justifiable for practical purposes and as a first approximation, we have refrained from such an extrapolation for several reasons. Significant deviations from a simple correlation of the number of chromosome interchanges from a uniform distribution of breaks along the chromosomes have been observed for several chromosomes in a previous study of lymphocytes of atomic bomb survivors (Tanaka et al. 1983). The reasons for such deviations are not clear at present. Within the framework of an initially uniform distribution of translocation breakpoints, differential repair and cellular selection provides a possible explanation of the non-randomness of breakpoints involved in interchanges. Another explanation which has been rarely considered so far concerns the fact that interphase chromosomes occupy discrete territories in the cell nucleus with limited interdigitation of chromatin at territory surfaces (Figure 1; Cremer et al., 1995, Eils et al. 1995, 1996, our unpublished 3D-reconstructions of neighbouring chromosome territories visualized in two-colour painting.
experiments of paraformaldehyde fixed, three-dimensionally intact human amniotic fluid cell nuclei).

Accordingly, translocation formation should be restricted to chromatin located at or close to the surface of adjacent chromosome territories (Savage and Papworth 1973, Savage 1990, Cremer et al. 1995b). The detailed three-dimensional organization of chromosome territories is not known at present. A random walk/giant loop model (Sachs et al. 1995) assumes that chromosome territories may consist of flexible chromatin loops averaging 3 million base pairs with a random walk backbone. If so, one can expect that any point of the chromatin fiber forming a given giant loop may have a similar probability to be exposed at the chromosome territory periphery. Accordingly, any segment of such a chromosome could take part with the same probability in translocation events. Other models predict a higher order of three-dimensional chromosome territory organization (Cremer et al. 1993, 1995a,b, Zirbel et al. 1993), where genes are possibly positioned preferentially at chromosome domain surfaces. In such a scenario the surface organization of a chromosome territory may change during the cell cycle during differentiation events, and possibly also during repair processes. As a consequence translocation breakpoints formed at a given time should not be randomly distributed along a chromosome (Folle and Obe 1995) and differ during cell cycle and in different cell types. The total frequency with which a chromosome territory would become involved in translocation events would depend on the territory surface available for contact with other chromosome territories. This surface will likely be affected by the DNA content, the condensation state and the shape of a given chromosome territory. Notably, in this model surface areas attached to the nuclear envelope cannot participate in translocation events in a given cell at a given time. It is not clear to which extents nuclear envelope attachment sites include specific chromosomal subregions or occur at random. Finally, the intranuclear positioning of chromosome territories should affect the frequencies of translocation events. Firstly, this positioning is affected by geometrical constraints resulting from the size and shape of a given chromosome territory. Accordingly, the distribution even of point like targets belonging to a given territory is affected by such geometrical constraints (Münkel et al. 1995). Their effects on the frequencies with which such targets may become involved in translocation events is difficult to predict. Secondly, evidence has been accumulated that chromosome positioning may differ in different cell types (for a review, see Manuelidis 1990), and at least certain chromosome territories may occupy preferential positions within human lymphocyte nuclei (Hager et al. 1982, Dietzel et al., 1995). Recently, volume, shape and surface measurements of chromosome territories have become possible using three-dimensional confocal laser scanning fluorescence microscopy (Eils et al. 1995, 1996, Rinke et al. 1995). Such measurements will provide a basis to test the hypothesis that the translocation frequency of a chromosome territory is correlated with the size of the surface area available for contact with other chromosome territories.

As a general consequence of the above considerations concerning topological and other parameters involved in the induction of chromosome translocations by ionizing radiation, comparisons between translocation rates obtained by chromosome painting of different chromosomal subsets should be performed with appropriate caution.

Acknowledgements

We thank Dr J. A. Aten and Professor A. T. Natarajan for stimulating discussions, and Dr Joe W. Gray for the chromosome-specific DNA libraries.

References


Radiation-induced translocations detected by FISH


EILS, R., DIETZEL, S., BERTIN, E., GRANZOW, M., SCHROECK, E., SPEICHER, M. R., RIED, T., ROBERTNICOUT, M., CREMER, C. and CREMER, T., 1996, Three-dimensional reconstruction of painted human interphase chromosomes: active and inactive X-chromosome territories have similar volumes but differ in shape and surface structure (submitted).


p 98, Figure 1, K. Tanaka et al

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p 101, Figure 2, K. Tanaka et al

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