

# Chromosome Translocations: A Biomarker for Retrospective Biodosimetry

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We discuss here the results from our studies demonstrating that simple translocations detected by chromosome painting can be used to reconstruct radiation doses for workers exposed within the dose limits and for individuals with past exposure. To be useful, a biomarker for exposure and risk assessment should employ an end point that is highly quantitative, stable over time, and relevant to human risk. Recent advances in chromosome staining using fluorescence *in situ* hybridization facilitate fast and reliable measurement of simple translocations, a type of DNA damage linked both to prior clastogenic exposure and to risk. In contrast to other biomarkers available, the frequency of simple translocations in individuals exposed to whole-body radiation is stable over time postexposure, has little interindividual variability, and can be measured accurately at low frequencies. — *Environ Health Perspect* 105(Suppl 6):1433–1436 (1997)

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## Introduction

Fast and reliable methods are needed to assess past radiation exposures and risk. This is particularly relevant for a large number of individuals exposed to various levels of ionizing radiation as a result of nuclear accidents such as Chernobyl, atmospheric nuclear testing prior to the early 1960s, past human experimentation by federal government agencies, the atom bombs dropped on Hiroshima and Nagasaki, various medical radiologic procedures, occupational exposures, and a variety of other radiation-related exposures for which good dosimetry information may not exist.

Over the last decade, efforts in our laboratory have been focused on the development and validation of a technology known as chromosome painting used in human exposure and risk assessment. This technology employs fluorescence *in situ* hybridization (FISH) with whole chromosome probes to rapidly and accurately detect chromosome abnormalities such as stable translocations in human cells. A

detailed description of FISH can be found in Lucas et al. (1) and Pinkel et al. (2). The development of this technology began at the Lawrence Livermore National Laboratory during the mid-1980s and has now developed into the method of choice worldwide for the detection of chromosome translocations in humans (1,3–6).

## Chromosome Painting Methodology

In principle, chromosomes in any cell can be stained by FISH. However, the method usually uses peripheral blood lymphocytes obtained from the individual to be evaluated. The lymphocytes are cultured and metaphase spreads are deposited on glass slides using standard cytogenetic methods (1,7). A cocktail of composite chromosome-specific DNA probes can be used in combination with pancentromeric probes to discriminate between translocations and dicentric (1,8). To visualize interchromosomal exchanges, target chromosomes are stained yellow with specific chromosome

probes and all other chromosomes are counterstained red [see Figure 1 from Lucas et al. (1)]. A chromosome exchange is distinguished by a bicolor red/yellow pattern. With the additional application of blue pancentromeric probes, the discrimination between translocations and dicentrics is made possible. Thus, exchange aberrations are recognized as bicolor (part red and part yellow) chromosomes and are scored as reciprocal (simple) translocations if the two derivative chromosomes each have one blue-stained centromere and as dicentric if one derivative chromosome has two centromeres and the other is a bicolor acentric fragment. This criteria is equivalent to that described by Simpson and Savage (9).

Chromosome painting is generally performed using DNA probes specific only for a subset of the genome, e.g., painting chromosomes 1, 2, and 4 (22% of the genome) results in the detection of 35% of all translocations (1). Here, genome refers to the full complement of chromosomes in a cell. Comparisons with results from conventional cytogenetic methods requires scaling the chromosome-painting translocation frequencies up to full genome equivalents. Assuming that radiation results in a random distribution of chromosome breaks, such scaling can be performed accurately. Thus, full genomic translocation frequencies are accurately obtained after selectively painting only a small fraction of the genome. This important finding was determined by comparing reciprocal translocation frequencies in the same individuals as measured by FISH and G-banding (1). The frequencies that were measured using FISH for chromosomes 1, 2, and 4 were converted to full genome equivalents and then plotted against translocation frequencies measured by G-banding for all chromosomes for the same individuals. The results demonstrated that FISH provided reciprocal translocation frequencies that did not differ significantly from those measured by the standard G-banding method.

The formula for scaling up to full genome has been published by Lucas et al. (1). The translocation frequency measured by FISH,  $F_p$ , and the genomic translocation frequencies,  $F_G$ , bear this simple relationship to the fraction of the genome covered by the probes,  $f_p$ :

$$F_G = F_p / (2.05 f_p (1 - f_p)) \quad [1]$$

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Abbreviations used: EPR, electron paramagnetic resonance; FISH, fluorescence *in situ* hybridization; Gy, gray; LET, linear energy transfer; Sv, sieverts; U.S. DOE, U.S. Department of Energy.

Universally accepted as an accurate method of detecting chromosome translocations, G-banding is much too labor intensive for exposure and risk-assessment applications. The FISH method is much faster and demonstrates identical results when scaled to full genome, providing a practical new biomarker for applications that require the scoring of large numbers of cells and individuals.

Much of our recent work has centered on the validation of the FISH technology for radiation-dose reconstruction and the development of the data tools required to translate a measured frequency into a dose. *In vitro* calibration curves provide a relationship between translocation frequency and dose, and therefore must be obtained for relevant exposure conditions. Most of these efforts have been summarized in Straume and Lucas (5) and are further expanded here.

### Stability of Translocation Frequencies in Primates

Retrospective biodosimetry requires stability of chromosome translocation frequency over time. We demonstrated the stability of reciprocal translocations in blood lymphocytes in previously exposed rhesus monkeys (*Macaca mulatta*). Probes for human chromosomes 1 and 4 were used to paint those same chromosomes in *M. mulatta* with no cross-hybridization to other chromosomes (6). As with human chromosomes, translocations between *M. mulatta* chromosomes 1 or 4 and any other chromosome were made distinct by the resultant bicolor derivative chromosomes.

The rhesus monkeys studied were exposed to whole-body (fully penetrating) radiation in 1965 in connection with National Aeronautics and Space Administration studies (10). Twenty-eight years later, in 1993, near the end of the animals' life span, we performed biodosimetry on blood lymphocytes from six of the primates and compared our results with the actual doses delivered to the animals in 1965. These results (6) nearly 30 years postexposure are presented in Table 1 with the treatment dose.

Essentially all chromosome exchange aberrations observed 28 years after irradiation were translocations; only one dicentric was found compared to 230 translocations in 2700 cells scored. As expected from previous studies (1,11-14), cells with dicentrics disappeared as these tissues repopulated. The data show very good agreement between the actual treatment

dose given in 1965 and the dose estimated biodosimetrically from the translocation frequency in 1993. The biological dose estimates are within 15% of the actual doses for all six animals; for four of the six animals our biodosimetry differs by less than 5% from the given doses. As almost 30 years had elapsed since exposure, these results demonstrate a lifetime stability for reciprocal translocations in these animals. The animals in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council).

### Human Studies with Known Dose

The stability of reciprocal translocation frequencies in primates suggests that one should be able to reconstruct the dose to humans exposed to ionizing radiation in the past. In our laboratory we evaluated four individuals previously exposed to penetrating whole-body radiation either accidentally or during normal work situations. Each of the four cases was exposed to different types of radiation, patterns of exposure, and dose rates, but all had in common exceptionally good independent dosimetry against which our biodosimetry results could be compared.

#### Case 1

A U.S. Department of Energy (U.S. DOE) radiation worker occupationally exposed within the DOE dose limits of 0.05 Sv/year during the 1950s, 1960s, and early 1970s was also evaluated biodosimetrically using four different assays, including

**Table 1.** Dose reconstruction for rhesus monkeys irradiated 28 years ago<sup>a</sup> and for humans recently exposed.<sup>b</sup>

Cells analyzed, genomic	Biodosimetry dose, Sv	Treatment dose, Sv	Difference, percent
Rhesus monkeys			
375	0.62	0.56	11
349	1.09	1.13	4
226	1.18	1.13	4
266	1.95	2.25	13
149	2.32	2.25	3
39	2.28	2.00	14
55	2.24	2.25	1
Humans			
725	0.49	0.56	12
1000	0.44	0.43	2
7625	0.30	0.33	9
1767	0.30	0.33	9

<sup>a</sup>Data from Lucas et al. (6). <sup>b</sup>Data from Straume and Lucas (5).

translocations measured by chromosome painting (14). In 1989 the best-estimate dose obtained from the measured translocation frequency was  $0.49 \pm 0.21$  Sv, in agreement with the total integrated dose recorded in the worker's official dosimetry record from badge readings of  $0.56 \pm 0.20$  Sv (5). These results suggested that reciprocal translocations could be detected in workers, even those exposed within the dose limits.

#### Case 2

A tritium worker in Switzerland accidentally inhaled tritium oxide vapors in 1985, which resulted in a whole-body dose of 0.44 Sv based on urinalysis and 0.42 Sv based on dicentric aberrations measured within 1 month of the acute inhalation exposure (15). Our biodosimetry performed for this same individual in 1992 (6 years postexposure) using chromosome painting to measure stable reciprocal translocations (13) resulted in 0.44 Sv, essentially identical to the dosimetry results obtained from urinalysis and dicentrics immediately after the accident. Furthermore, we resampled this individual in 1996 (11 years postexposure) and results showed that the translocation frequency remained essentially unchanged, i.e., 0.044 Sv in 1991 and 0.036 Sv in 1996.

#### Case 3

A U.S. DOE laboratory worker was exposed to photons and particle radiation from high-energy accelerator operations during 30 years of work in that environment. This individual was a dosimetry expert and kept meticulous records of his exposure history. His integrated dose equivalent from personnel dosimeters was  $0.33 \pm 0.04$  Sv, which compared very well with our biodosimetry results ( $0.3 \pm 0.1$  Sv) from the translocation frequency in his blood lymphocytes measured in 1995 (6 years postexposure) (5).

#### Case 4

A Ukrainian radiation worker was exposed over the past decade to external gamma radiation from <sup>137</sup>Cs and some internal contamination from radiocesium. The external and internal exposure resulted in an essentially uniform whole-body radiation dose. Two independent dosimetry methods were used with this worker: *a*) electron paramagnetic resonance (EPR) dosimetry was performed on the individual's tooth enamel by scientists in Ukraine, and *b*) chromosome painting was

performed on the individual's blood lymphocytes. The dicentric frequency had decayed to background. Dosimetry results from the two independent methods showed agreement, i.e.,  $0.3 \pm 0.1$  Sv for EPR and  $0.33 \pm 0.12$  Sv for translocations measured using FISH (5).

### Summary of Case Studies

Altogether, these case studies suggest that the frequency of reciprocal translocations in human lymphocytes provides an accurate measure of prior exposure to ionizing radiation in whole-body exposed individuals regardless of the temporal pattern of the exposure or the types of radiation involved. Additional individuals with good independent dosimetry are being sought to continue these very important validation studies. These data are listed in Table 1. Human studies were conducted only after informed consent was obtained and after the research was approved by the institutions' internal review boards.

### Human Studies with Unknown Dose

We reconstructed doses for 27 Chernobyl liquidators (cleanup personnel) from the translocation frequencies measured in their blood lymphocytes (J Lucas, unpublished data). Of the 27 individuals, 15 are currently being treated for radiation sickness. The remaining 12 are exposed individuals with no medical symptoms. For each group we calculated both a chronic and an acute dose using an average translocation background frequency of 0.0036 translocations/cell. This value comes from measurements in our laboratory of seven unexposed individuals of similar ages as the liquidators. Chronic dose,  $D$ , was calculated using a linear formula  $D = (Y_i - Y_b) / \alpha$ , where  $Y_i$  is the measured frequency in the individual being evaluated,  $Y_b$  is the background frequency (0.004; J Lucas, unpublished data), and  $\alpha$  is the slope of the calibration curve ( $0.03 \text{ Gy}^{-1}$ ) (16). The acute dose was derived using a linear quadratic model of the form  $Y_i = Y_b + \alpha D + \beta D^2$  ( $\beta = 0.0053 \text{ Gy}^{-2}$ ) (1). A clear preliminary

observation was that the group with radiation sickness has a significantly higher average dose compared to that for the exposed group. The calculated average FISH-based dose was 0.96 Gy chronic and 0.51 Gy acute for the exposed group compared to 6.4 Gy chronic and 1.5 Gy acute for the radiation sickness group. Four individuals in the radiation sickness group had clones but none were found in the exposed individuals. Clones are cells having the same translocation break points on identical chromosome numbers. To calculate final doses for individuals with clones, the clones must be purged (T Straume, in preparation).

### Future Directions

#### FISH in Suspension

The ability to perform *in situ* hybridization on a large number of isolated individual chromosomes in suspension offers the possibility of flow analysis and sorting chromosomes based on FISH signals, and bulk detection of chromosomal exchange rearrangements. However, FISH in suspension has been limited by chromosome loss, breakage, clumping, or aggregation (17,18). In our laboratory we recently demonstrated the ability to hybridize DNA probes to a suspension of chromosomes isolated from cells. After hybridization in suspension, we routinely recover large numbers of free chromosomes with good morphology and low debris. Our recovery of individual chromosomes after hybridization in suspension and washes has been as high as 70%, as compared to a maximum of 6% recovery by others. In a direct comparison of methods, we recovered 2,390,000 of 3,500,000 (68%) compared to 155,000 of 3,500,000 (4.4%) recovered using methods of others (19,20). Our method and results are being prepared for publication (H He, in preparation).

#### Cytogenetic Signature for the Linear Energy Transfer of Ionizing Radiation

A stable, easily measurable biological signature for past exposure to densely ionizing radiation would be of significant value

to radiation biology and biodosimetry. No radiation signature has been demonstrated to date. Simple, complete chromosome exchange aberrations (e.g., translocations) are among the easiest to score using chromosome painting and they are the most abundant at low to moderate doses. The incomplete forms of these aberrations are also easy to score and they occur in relatively large numbers. The  $S$  ratio, a recently developed concept, is the ratio of complete to incomplete chromosome translocations. It constitutes a radiation signature that is stable over time, independent of dose, and varies inversely with the relative biological effectiveness of the radiation (21).

An inverse correlation between radiation linear energy transfer (LET) and the  $S$  ratio was demonstrated for *in vitro*-exposed human lymphocytes to high-, intermediate-, and low-LET radiation. *In vitro* measurements resulted in a ratio of approximately 2 for densely ionizing radiation ( $^{56}\text{Fe}$  and  $^{12}\text{C}$  ions), in contrast to a value of approximately 10 for sparsely ionizing radiation (X- and gamma-rays).  $S$  ratio measurements are simple to conduct because incomplete translocations are as easy to measure as complete translocations, occur in abundance for high-LET radiation, and are measured using the same methods. Moreover, the large difference in  $S$  ratios should be sufficient to separate the high- and low-LET effect contributions in mixed radiation exposures such as the exposure received by A-bomb survivors. Importantly, such a distinctive clastogenic signature should facilitate a method to provide a causal connection between early exposure to densely ionizing radiation and late development of cancer.

### Conclusion

Available biodosimetry data from human case studies together with the results for nonhuman primates provide a strong basis for the use of chromosome translocations detected by FISH to reconstruct radiation dose, regardless of when the exposure occurred or whether the exposure was received acutely or chronically.

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