CONF-870701--8

UCRL--97276

DE88 000785

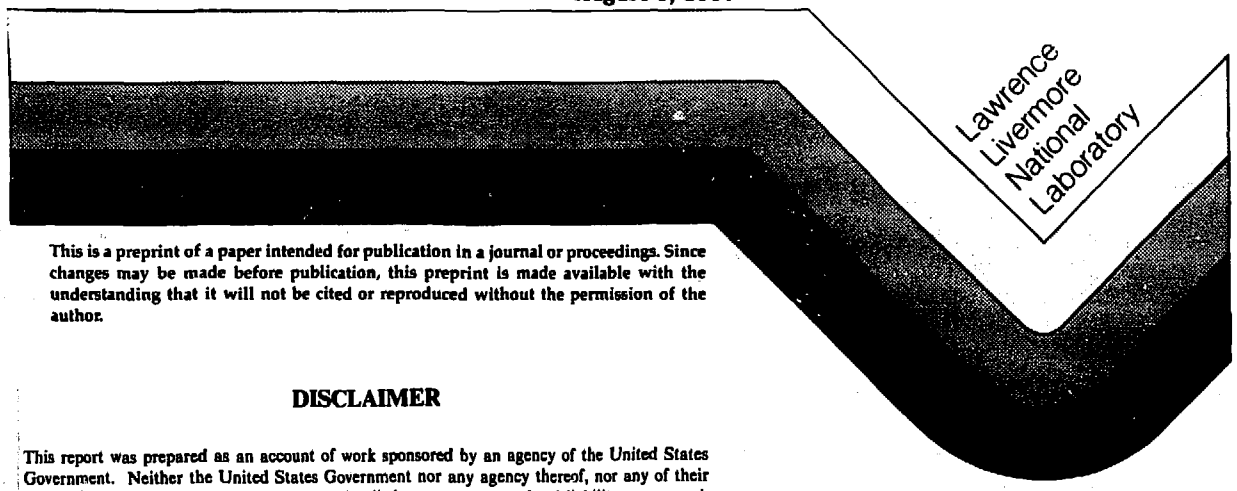
ANALYTICAL CYTOLOGY APPLIED TO DETECTION OF INDUCED CYTOGENETIC ABNORMALITIES

J.W. Gray, J. Lucas, T. Straume, and D. Pinkel

BIOMEDICAL SCIENCES DIVISION

This paper was prepared for submittal to
Proceedings of the 8th International Congress of
Radiation Research
July 19-24, 1987 - Edinburgh, Scotland

August 6, 1987



DISCLAIMER

MASTER

ANALYTICAL CYTOLOGY APPLIED TO DETECTION OF INDUCED CYTOGENETIC ABNORMALITIES

J.W. Gray, J. Lucas, T. Straume, and D. Pinkel

Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

INTRODUCTION

Radiation-induced biological damage results in formation of a broad spectrum of cytogenetic changes such as translocations, dicentrics, ring chromosomes, and acentric fragments. A battery of analytical cytologic techniques are now emerging that promise to significantly improve the precision and ease with which these radiation induced cytogenetic changes can be quantified. This report summarizes techniques to facilitate analysis of the frequency of occurrence of structural and numerical aberrations in control and irradiated human cells.

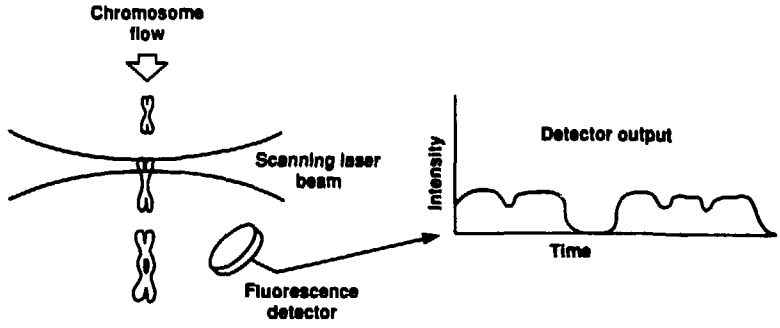
DICENTRIC CHROMOSOME ANALYSIS

Dicentric chromosomes are formed by the end to end fusion of two chromosome fragments, each of which contains a centromere. The frequency with which such aberrations occur in an irradiated cell population increases with increasing radiation exposure. This, plus the fact that dicentric chromosomes can be readily identified in metaphase spreads without banding analysis, has led to the development of assays in which dicentric chromosome frequencies are measured as an indication of radiation-induced biological damage (Lloyd *et al.*, 1975; Lloyd, 1984; Awa *et al.*, 1978) However, measurement of dicentric chromosome frequencies is limited by the low frequency with which dicentric chromosomes occur (approximately 1 dicentric per 2000 cells in unirradiated human lymphocytes; Lloyd, 1984). As a result, thousands of mitotic cells must be scored to allow statistically precise detection of low-level radiation-induced increases in the frequency of occurrence of dicentric chromosomes.

Slit-scan flow cytometry (SSFCM) is being developed to allow quantitative analysis of the frequency of dicentric chromosomes with increased statistical pre-

cision (Gray *et al.*, 1984; Lucas *et al.*, 1983; Lucas *et al.*, manuscript in preparation). In this approach, metaphase chromosomes are isolated from large numbers of mitotic cells, stained with a DNA specific fluorescent dye and forced to flow lengthwise through the thin laser beam of a SSFCM (see Figure 1).

Figure 1: Slit-scan flow cytometric detection of dicentric chromosomes. Fluorescence profiles are measured for individual chromosomes as they flow lengthwise through a 1.5 μm thick laser beam. Normal chromosomes exhibit one dip while profiles for dicentric profiles exhibit two dips.



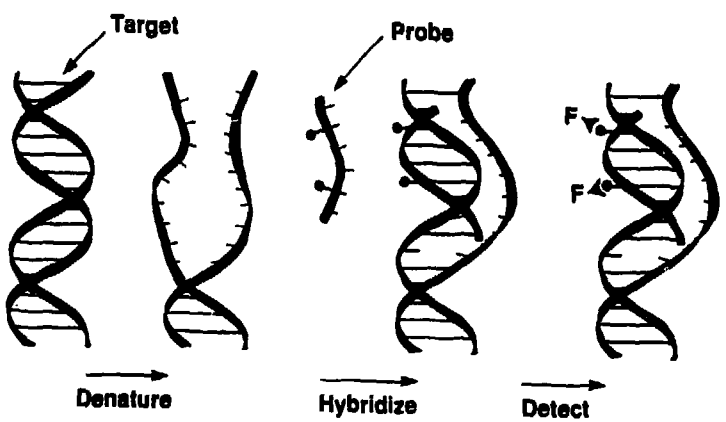
The fluorescence intensity is measured as each chromosome flows through the laser beam and is recorded as a measure of the distribution of DNA content along the chromosome (such distributions are called chromosome profiles). Each chromosome profile typically shows a dip produced as the centromere with its reduced DNA content passes through the scanning laser beam. Thus, profiles for normal chromosomes show one dip and profiles for dicentric chromosomes show two dips. SSFCM has been applied to quantify dicentric chromosome frequencies in Chinese hamster (Gray *et al.*, 1984) and human cells (Lucas *et al.*, manuscript in preparation) exposed to gamma radiation at several doses. In these studies, profiles were measured only for the larger chromosomes in the population. A computer algorithm was applied to the recorded profiles to distinguish between normal profiles and profiles from dicentric chromosomes. The algorithm scored profiles as being from dicentric chromosomes only if they showed two dips that were well separated from each other and from the chromosome telomeres. This algorithm necessarily missed many dicentric profiles. However, it did not seem to be confused by profiles from chromosome clumps or fragments whose profiles might otherwise be mistaken for those from dicentric chromosomes. Dose-re-

sponse curves for Chinese hamster cells irradiated in G1-phase to doses ranging from 0 to 4 Gy and human lymphocytes irradiated in G1-phase to doses ranging from 0 to 1 Gy showed the linear-quadratic shape expected for low-LET radiation (Lloyd *et al.*, 1975; Lloyd, 1984). The advantage of this approach is sufficient speed to allow statistically precise dicentric frequency analysis. Several hundred chromosomes can be scanned each second using SSFCM. Thus, a large number of chromosomes can be scanned at each dose point. The main limitations to routine application of SSFCM currently are the technical complexity of the approach and the relatively poor dicentric chromosome detection efficiency (only about 1/300th of all dicentric chromosomes are detected). Improved dicentric chromosome detection algorithms now under development promise to increase the detection efficiency as much as 10-fold.

TRANSLOCATION ANALYSIS

Assessment of dicentric chromosome frequencies as measures of radiation-induced biological damage are limited by the relative instability of dicentric chromosomes in dividing cells (Carrano & Heddle, 1973). These aberrations are likely to be lost within a few cell divisions so that the assay results become difficult to interpret at long times after irradiation. Chromosome translocations, on the other hand, are more stable (Buckton *et al.*, 1978; Awa *et al.*, 1978). Unfortunately, accurate detection of translocations requires chromosome banding analysis. This is sufficiently time consuming and labor intensive that the use of translocation analysis for routine radiation dosimetry is not practical. The detection of translocations can be greatly facilitated by increasing the distinctness with which selected chromosomes or portions thereof are stained. We have applied fluorescence *in situ* hybridization (Pinkel *et al.*, 1986) and chromosome-specific probes for this purpose. In this approach (see Figure 2), the DNA in the target chromosomes is denatured and incubated with chemically modified chromosome-specific nucleic acid probes under conditions such that the chromosome-specific probes bind only to DNA sequences to which they have high sequence homology. The bound probe is then detected using a fluorescent reagent that binds with high affinity to the chemical modification.

Figure 2: Principles of fluorescence *in situ* hybridization. The DNA in chromosomes to be stained is denatured thermally and then incubated with a biotin labeled chromosome-specific probe under conditions that permit annealing of the probe only to DNA sequences to which it has high homology. The bound probe is detected by treatment with fluorescein-labeled avidin. Thus, the chromosomes or segments thereof to which the probe is homologous are rendered fluorescent (Adapted from Trask, 1985).



Hybridization with repeat sequence probes that bind to the centromere and short arm telomere of human chromosome 1 allowed detection of translocations involving the 1p that caused a separation between the telomeric and centromeric probes (Lucas *et al.*, manuscript in preparation). Identification of translocations and other rearrangements involving the short arm of human chromosome 1 was sufficiently easy that about 400 metaphase spreads per hour were scored routinely. This technology was applied to analysis of translocations in human lymphocytes. The frequency of translocations involving 1p increased in a linear-quadratic fashion with increasing gamma radiation dose. However, the frequency of translocations involving 1p was approximately 25-fold lower than the frequency for all translocations (Dutrillaux *et al.*, 1985). This reduced frequency was expected since 1p accounts for only about 1/25th of the total cellular DNA.

Translocation analysis using this approach is limited currently by the speed with which metaphase spreads can be found and by the fact that the probes now available allow assessment of translocations only in a small fraction of the total

genome. These limitations may be eased by application of automated metaphase finding to speed the location of metaphase spreads and by increasing the target size by using probes that allow analysis of translocations in more chromosomes or in larger chromosome segments.

ANEUPLOIDY ANALYSIS

Genetic damage may also result in the loss or gain of one or more chromosomes. Fluorescence hybridization with chromosome-specific repeat sequences is proving especially useful for analysis of chromosome loss or gain since it allows detection of these events in interphase nuclei (Pinkel *et al.*, 1986; Gray *et al.*, 1987). Aneuploidy detection in interphase is possible because the domains to which many chromosome-specific repeat sequence probes bind are relatively well localized in interphase nuclei. In addition, the domains for homologous chromosomes seem to be well separated in interphase. Thus, nuclei carrying two copies of a particular chromosome should have two fluorescent domains following hybridizations with a probe specific for that chromosome. The gain or loss of a fluorescent domain signals the gain or loss of one copy of the chromosome carrying the sequence to which the probe is homologous.

Aneuploidy analysis is also facilitated by the finding that the intensity of fluorescence following hybridization with a chromosome-specific repeat sequence probe is proportional to the amount of target sequence to which the probe binds (Trask *et al*, 1987; Pinkel *et al*, 1986). Thus, for example, nuclei carrying an extra chromosome can be detected as having elevated fluorescence intensity following fluorescence hybridization with a probe for that chromosome. Fluorescence intensity measurements can be made using quantitative fluorescence microscopy or by flow cytometry.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48 with support from USPHS grants HD 17665 and GM 25076. The authors thank Ms. Lil Mitchell for expert help in the preparation of this manuscript.

REFERENCES

Awa, A., Sofuni, T., Honda, T., Itoh, M., Neriishi, S. and Otake, M., 1978, Journal of Radiation Research, 19, 126.

Buckton, K., Hamilton, G., Paton, L. and Langlands, A., 1978, Chromosome aberrations in irradiated ankylosing spondylitis patients. In Mutagen Induced Chromosome Damage in Man edited by J. evans and D. lloyd (Edinburgh University Press) p. 77.

Carrano, A. and Heddle, J., 1973, Journal of Theoretical Biology, 38, 289.

Dutrillaux, B., Viegas-Pequignot, E., Prod'homme, M. and Sportes, M., 1985, Mutation Research, 152, 197.

Gray, J., Lucas, J., Yu, L.-C. and Langlois, R., 1984, Flow cytometric detection of aberrant chromosomes, In Biological Dosimetry: Cytometric Approaches to Mammalian Systems edited by W. Eisert and M. Mendelsohn (Springer-Verlag) p. 25.

Gray, J., Pinkel, D., Trask, B., van den Engh, G., Pallavicini, M., Fuscoe, J., Mullikin, J. and van Dekken, H. 1987, In Proceeding of a conference on the Prediction of Tumor Treatment Response (in the press).

Lloyd, D., C., 1984, An overview of radiation dosimetry by conventional cytogenetic methods, In Biological Dosimetry: Cytometric Approaches to Mammalian Systems. edited by W. Eisert and M. Mendelsohn (Springer-Verlag) p. 1.

Lloyd, D., Purott, R., Dolphin, G., Bolton, D. and Edwards, A., 1975, International Journal of Radiation Biology, 28, 169.

Lucas, J., Gray, J., Peters, D. and Van Dilla, M., 1983, Cytometry, 4, 109. Lloyd, D., Purrott, R., Dolphin, G., Bolton, D. and Edwards, A. 1975, International Journal of Radiation Biology, 28, 75.

Manuelidis, J. 1985, New York Academy of Science, 450, 205.

Pinkel, D., Straume, T. and Gray, J., 1986, Procedures of the National Academy of Science USA 83, 2934.

Trask, B., 1985, Studies of chromosomes and nucleii using flow cytometry. PhD Thesis, Leiden University.

Trask, B., van den Engh, G., Landegent, J., Jansen in de Wal, N. and van der Ploeg, M. 1985, Science, 230, 1401.

Trask, B., van den Engh, G., Pinkel, D., Mullikin, J., van Dekken, H. and Gray, J., 1987, Human Genetics (in the press).