En: de la Peña E, Burguete I, Guadaño A (1999)
Evaluación Mutagénica y Genotóxica. Dirección General de Enseñanza Superior e Investigación Científica, Sociedad Española de Mutagénesis Ambiental, MRCIA98. Madrid. 249-260

MULTICOLOR FISH USING TANDEM PROBES TO DETECT CHROMOSOME ALTERATIONS IN HUMANS CELLS AND POPULATIONS EXPOSED TO GENOTOXIC AGENTS

DAVID A. EASTMOND[1], DOPPALAPUDI S. RUPA[1], MAIK J. SCHULER[1], MICHELLE N. MURG[1] AND EDUARDO DE LA PEÑA[2]

[1]*Environmental Toxicology Graduate Program. University of California Riverside, U.S.A.*
[2]*Consejo Superior de Investigaciones Cientificas. Centro de Ciencias Medioambientales.*

INTRODUCTION

Fluorescence *in situ* hybridization (FISH) with chromosome- or region-specific DNA probes is being increasingly used in cytogenetic studies to detect aneuploidy in interphase human cells [for review see (Eastmond and Rupa, 1995)]. This technique utilizes chemically modified DNA sequences (probes) which hybridize to distinct regions, often blocks of repetitive DNA, located on specific chromosomes. Hybridization with these probes in situ results in the staining of a compact chromosomal region which can be easily detected on metaphase chromosomes or within interphase nuclei. The number of chromosomes within a given cell is then determined by counting the number of hybridized regions. Where conventional cytogenetics is limited to actively proliferating cells or those which could be stimulated to divide *in vitro* such as peripheral blood lymphocytes, FISH studies with centromeric probes can be conducted on interphase cells, significantly increasing the types of cells and tissues available for analysis (Eastmond and Pinkel, 1990; Pinkel, et al., 1986).

However during interphase FISH, the actual chromosomes are not visible so that hybridization and cellular artifacts can give misleading results (Eastmond, et al., 1995). For example during interphase analysis using a single probe, cells containing 3 or more hybridization signals (called hyperdiploid cells) cannot be distinguished from those diploid cells in which one of the regions targeted by the DNA probe contains a break, translocation or inversion (Eastmond and Rupa, 1995).

This can be a particular problem when using probes that target the pericentric heterochromatic regions of human chromosomes 1, 9 and 16. A number of studies have shown that these regions often exhibit elevated frequencies of breakage following treatment with mitomycin C, triethylenemelamine and other clastogenic agents (Aurias, 1993; Brogger, 1977; Meyne, et al., 1979).

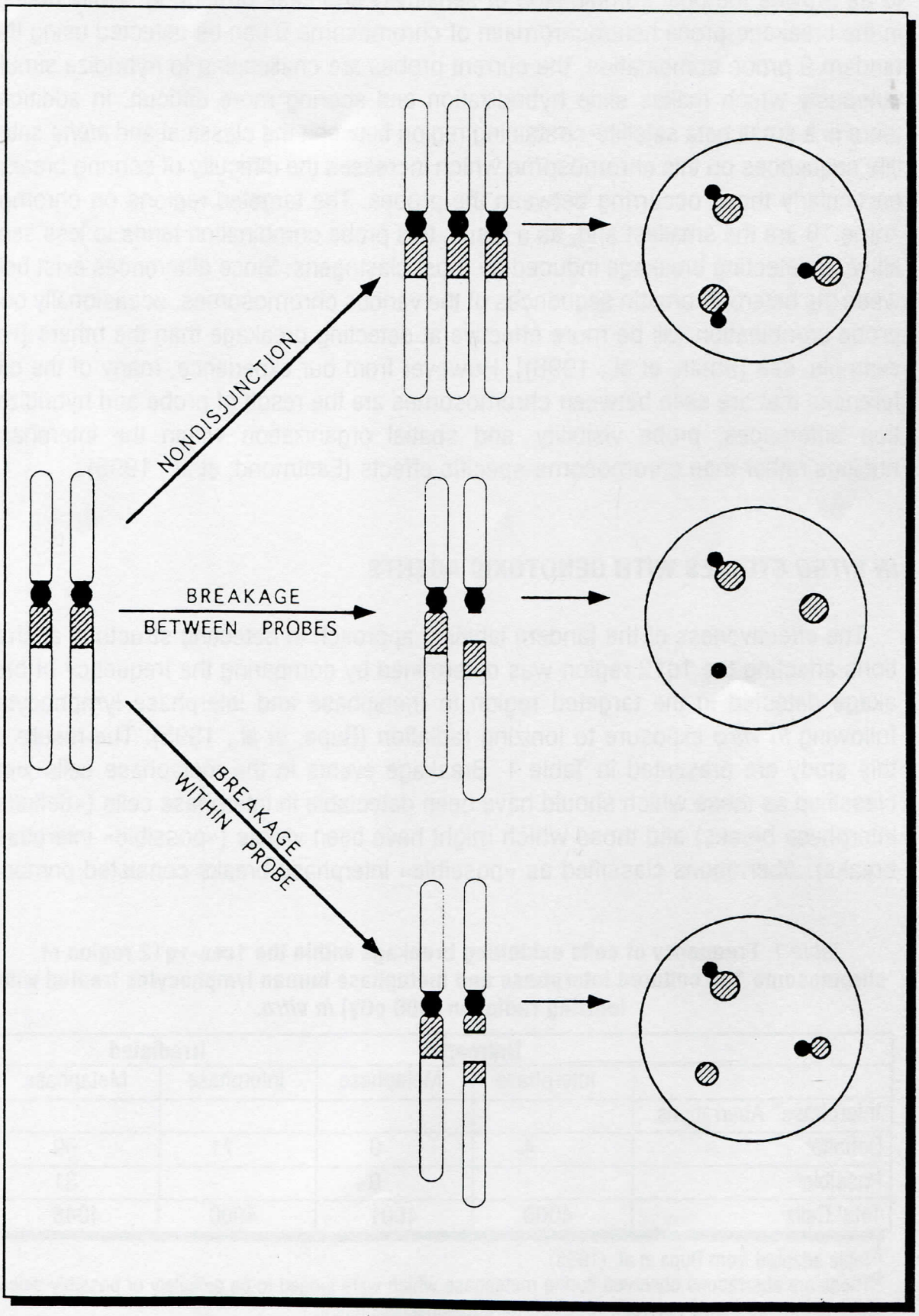
To overcome this limitation, we have recently developed a multicolor FISH approach which uses adjacent or tandem probes to differentiate between hyperdiploidy and breakage affecting the centric/pericentric regions of chromosomes 1, 9 and 16 in interphase human cells (Eastmond, et al., 1994; Hasegawa, et al., 1995; Rupa, et al., 1995; Schuler, et al., 1998). The objective of this paper is to briefly describe the tandem label FISH technique, provide an overview of our studies applying this approach in vitro using model aneuploidy-inducing and clastogenic compounds, and briefly review the published *in vivo* studies in which the tandem label technique has been used to assess chromosomal alterations in human populations.

HYBRIDIZATION STRATEGY AND PROBES

As indicated above, we developed a multicolor FISH approach using two adjacent or «tandem» probes to distinguish hyperdiploidy from breakage affecting the targeted chromosomal region. The principles underlying this tandem label strategy are illustrated in Figure 1. The pericentric heterochromatin region which is large and prone to breakage is labeled using a classical satellite probe. An adjacent centromeric region which is smaller and less prone to breakage is labeled using an *alpha* satellite probe. Typically we have labeled the classical satellite probe with a Texas Red or Cy3 red fluorochrome and the *alpha* satellite probe with a yellow-green fluorescein label. Hybridization regions that contain both the *alpha* (yellow/green) and the classical satellite (red) signals are scored as one intact copy of the chromosome. A nucleus containing 3 or more intact copies of a chromosome are considered hyperdiploid. Since probes for only one chromosome are used in this approach, the hyperdiploid cells identified in this assay represent both cells hyperdiploid for chromosome 1 as well as polyploid cells. A cell containing a separate red classical satellite region in addition to two intact red and green chromosome regions is scored as a cell with a break within the heterochromatin. A cell with a clear separation between an alpha and classical satellite regions is scored as a cell with a break between the two regions.

To date, we have developed tandem probes for centromeric regions of human chromosomes 1, 9 and 16 (Hasegawa, et al., 1995; Rupa, et al., 1995; Schuler, et al., 1998). Some of these probes such as the classical satellite probes for chromosomes 1 and 9 can be obtained commercially or through repositories (see above references for sources). In addition, these and the *alpha* satellite probes can be generated by PCR from genomic DNA isolated from hamster/human hybrid cells containing individual human chromosomes in a hamster background (Hasegawa, et

Figure 1. **Hybridization strategy using adjacent or tandem probes to detect chromosome breakage and hyperdiploidy in metaphase and interphase cells. [adapted from Rupa et al., 1995, with permission].**



al., 1995; Schuler, et al., 1998). The unlabeled probe DNA is then labeled by PCR and PCR/nick translation procedures using modified nucleotides.

The probes for chromosome 1 are immediately adjacent to each other and tend to be provide the best combination of sensitivity and ease of scoring. While breaks in the breakage-prone heterochromatin of chromosome 9 can be detected using the tandem 9 probe combination, the current probes are challenging to hybridize simultaneously which makes slide hybridization and scoring more difficult. In addition, there is a small beta satellite-containing region between the classical and *alpha* satellite sequences on this chromosome which increases the difficulty of scoring breaks, particularly those occurring between the probes. The targeted regions on chromosome 16 are the smallest and, as a result, this probe combination tends to less sensitive at detecting breakage induced by most clastogens. Since differences exist between the heterochromatin sequences of the various chromosomes, occasionally one probe combination will be more effective at detecting breakage than the others [For example, see (Smith, et al., 1998)]. However from our experience, many of the differences that are seen between chromosomes are the result of probe and hybridization differences, probe visibility, and spatial organization within the interphase nucleus rather than chromosome-specific effects (Eastmond, et al., 1995).

IN VITRO STUDIES WITH GENOTOXIC AGENTS

The effectiveness of the tandem labeling approach in detecting structural aberrations affecting the 1q12 region was determined by comparing the frequency of breakage detected in the targeted region in metaphase and interphase lymphocytes following *in vitro* exposure to ionizing radiation (Rupa, et al., 1995). The results of this study are presented in Table 1. Breakage events in the metaphase cells were classified as those which should have been detectable in interphase cells («definite» interphase breaks) and those which might have been visible («possible» interphase breaks). Aberrations classified as «possible» interphase breaks consisted primarily

Table 1. **Frequency of cells exhibiting breakage within the 1cen-1q12 region of chromosome 1 in cultured interphase and metaphase human lymphocytes treated with ionizing radiation (300 cGy) *in vitro*.**

	Untreated		Irradiated	
	Interphase	Metaphase	Interphase	Metaphase
"Interphase" Aberrations				
Definite[b]	4	0	71	69
Possible[b]	-	0	-	31
Total Cells	4000	4001	4000	4046

[a]Table adapted from Rupa et al. (1995).
[b]These are aberrations observed during metaphase which were judged to be definitely or possibly detectalble during interpahse analysis. (See text for additional description.)

of breaks within the targeted region but where the acentric fragment was not present in the metaphase cell. In the untreated cultured cells, the frequency of breakage in the interphase cells was 1‰ which did not differ significantly from that observed in the Colcemid-treated metaphase cells (0‰). The frequency of 1q12 breakage detected in the interphase lymphocytes (18‰) irradiated in culture with 300 cGy was similar to the frequency of «definite interphase breaks» (17‰) detected in the metaphase cells but somewhat lower than the frequency of combined «definite» plus «possible» breaks (25‰). These results indicated that interphase analysis accurately identified the majority (70 to 100%) of the breakage events induced within the labeled region. A similar study using the tandem probes for chromosome 9 also showed comparable breakage frequencies for the labeled interphase and metaphase cells (Hasegawa, et al., 1995).

The ability of this multicolor FISH assay to detect structural and numerical aberrations involving chromosome 1 and the 1q12 region was determined in a series of experiments in which cultured human lymphocytes were treated with various clastogenic and aneuploidy-inducing agents in vitro (Rupa, et al., 1997b; Schuler, et al., 1998). The results of these published and unpublished studies are shown in Figure 2. As can be seen in the Figure, in cultures treated with the aneuploidy-inducing agents, colchicine (COL, 0.075 μM), vincristine sulfate (VIN, 0.06 μM), diethylstilbestrol (DES, 30 μM) and estradiol (E_2, 75 μM), the majority of the cells with multiple hybridization regions exhibited the pattern indicative of hyperdiploidy for chromosome 1. In contrast, the cells treated with the potent clastogens, ionizing radiation (RAD, 300 cGy), mitomycin C (MMC, 0.3 μM), melphalan (MEL, 5 μM), etoposide (ETOP, 0.2 μM), diepoxybutane (DEB, 20 μM), exhibited a pattern consistent with breakage within or between the labeled regions. Three agents, sodium arsenite (ARS, 9 μM), hydroquinone (HQ, 100 μM) and epoxybutene (EB, 300 μM), gave more modest results with weak, but significant, increases in both hyperdiploidy and brea-kage being seen for both arsenite and hydroquinone ($p<0.05$). Although slightly elevated, neither the increase in breaks or hyperdiploidy seen with epoxybutene was significant when compared with its concurrent controls (breakage frequencies were 5‰ vs. 3‰, respectively; $p>0.05$).

Individual controls were performed for each set of the experiments and the analyses were based upon comparing each treatment group with its concurrent control. For simplicity, the results for the controls have been pooled in Figure 2. In these interphase analyses, the frequencies of hyperdiploidy for chromosome 1 ranged from 0‰ to 4‰ with an average frequency of 1.2‰. The breakage frequencies varied from 1‰ to 5‰ with an average frequency of 2‰.

In addition to the above studies which were conducted on cultured lymphocytes, a second series of studies was conducted to determine whether cell culture was required for breakage to be detected using the tandem assay (Rupa, et al., 1997a). The ability to detect chromosome breakage in non-dividing cells could be valuable for human monitoring studies as most of the cells in the body would not be dividing at the time of exposure or sampling. To address this question, whole blood obtai-

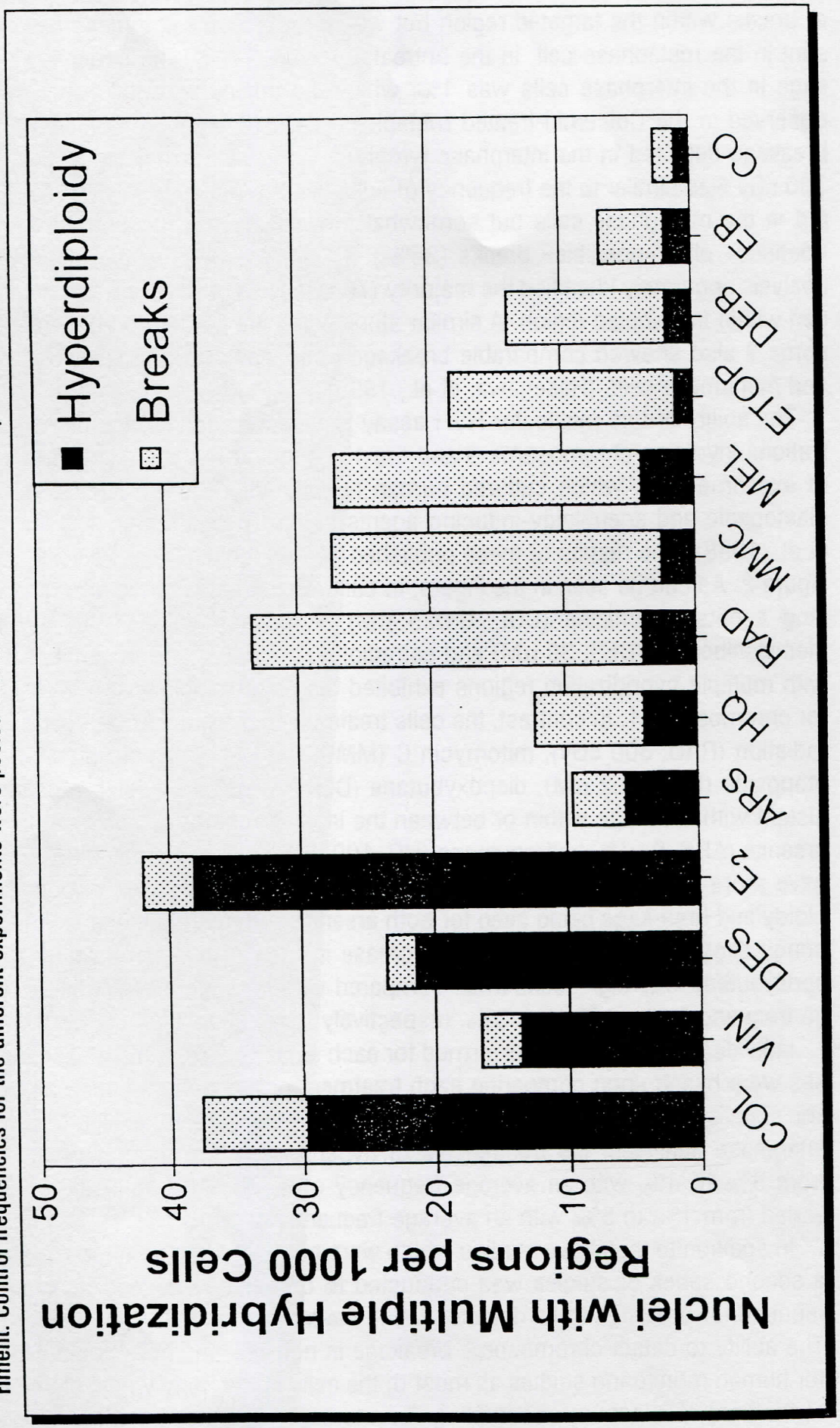


Figure 2. Classification of nuclei containing 3 or more hybridization regions following tandem labeling for the 1cen-1q12 region of chromosome 1 in human lymphocytes. The frequencies presented represent the mean of two separate experiments with at least 1000 cells per experiment. Control frequencies for the different experiments were pooled with the combined results representing over 25,000 untreated cells.

ned from male donors was exposed *in vitro* to 0 to 400 cGy of ionizing radiation. Aliquots containing granulocytes and Go lymphocytes from each dose were harvested at various times. In addition, aliquots were also cultured in the presence of PHA and harvested at 48-51 hr of culture. One aliquot was harvested directly for interphase analysis and another was harvested following a 3 hr treatment with colcemid for metaphase analysis. High frequencies of breakage approaching 80‰ for the 400 cGy dose were seen shortly after irradiation in both the granulocytes and the lymphocytes. Repair occurred rapidly with the frequency of breakage declining by approximately 55% within the first 2 hours. The breakage frequencies detected in the granulocytes and Go lymphocytes at the end of the rapid repair phase (~3 hrs post irradiation) were very similar to those observed in the metaphase and interphase preparations of the cultured lymphocytes indicating that unrepaired damage could persist for relatively long periods in a cell.

The results of these studies indicated that the tandem label assay can be effectively used to detect agents inducing hyperdiploidy of chromosome 1 and breakage affecting the 1q12 region. In addition, these studies demonstrated that cell culture was not required for breaks to be detected in this assay. Indeed, high frequencies of breaks were detected almost immediately following irradiation with the majority of the damage being repaired quite rapidly. However, it has been shown by Surralles and associates that repair in the chromosome 1 heterochromatin can be much slower than that occurring in the euchromatin and that this may contribute to the high frequencies of breakage detected by the tandem assay following exposure to some clastogenic agents (Surralles, et al., 1997). Although most of the clastogens tested to date have induced breakage affecting the heterochromatin region, clastogens which selectively damage euchromatic areas or where the heterochromatin damage is rapidly and efficiently repaired would probably not be detected using only this assay. As a result, this approach would have limited usefulness as an *in vitro* screen for genotoxic agents. However the ability of the assay to detect aberrations in both dividing and non-dividing interphase cells, indicates that this approach may be a powerful tool for detection of genetic alterations in human populations, particularly those exposed to clastogens known to induce breakage in the heterochromatin regions.

TANDEM LABEL STUDIES IN HUMAN POPULATIONS

Over the past several years, a number of *in vivo* studies have been conducted to determine the ability of the tandem FISH approach to detect alterations in chemically exposed human populations. A summary of the various studies published to date is presented in Table 2. Studies have been conducted using cultured and non-cultured lymphocytes, polymorphonuclear leukocytes (PMN), sperm and oral mucosal cells. The majority of the studies have utilized the probes for chromosome 1 although the tandem 9 probes were used in one study of benzene-exposed Estonian refinery workers.

The initial study, conducted by Rupa and associates in the Guntur region of India, was based upon previous research by these investigators in which they had shown that pesticide applicators in this region exhibited elevated levels of structural chromosomal aberrations and polyploidy when evaluated using conventional cytogenetic analyses (Rupa, et al., 1991a). In a follow-up study using the tandem assay, investigators from our laboratory assessed the frequency of hyperdiploidy and 1q12 breaks in the cultured lymphocytes of 26 cotton field pesticide applicators and compared them with those detected in the cells of 19 non-pesticide workers from the same village (Rupa, et al., 1995). Significant increases were seen at both endpoints. The frequency of hyperdiploidy increased from 2‰ in the controls to 3‰ in the exposed. Additionally, 1q12 breakage increased from 2‰ in the controls to 5‰ in lymphocytes from the applicators. This study demonstrated that the tandem label assay could be used to detect chromosome alterations in the cultured lymphocytes of humans exposed to certain genotoxicants.

As part of their earlier studies, Rupa and associates interviewed the wives of over 1000 applicators and controls from this agricultural region and found that the wives of the pesticide applicators exhibited increased incidences of reproductive dysfunction including infertility and spontaneous abortions (Rupa, et al., 1991b). To investigate the potential contribution of chromosome alterations transmitted through the sperm to these effects, multicolor FISH using the tandem probes for chromosome 1 was used to determine the frequencies of breakage and hyperploidy affecting the 1cen-1q12 region in the sperm of 21 workers and 18 controls from this region. Significant increases in the frequency of sperm exhibiting 2 copies of chromosome 1 (~2-fold) as well as breakage affecting the 1cen-1q12 region (~9-fold) were seen in the samples obtained from the applicators as compared to the controls. These observations that both hyperploidy/polyploidy and breakage occur at increased frequencies in the sperm of pesticide-exposed workers support the hypothesis that chromosomal alterations occurring in the sperm of the father can contribute to adverse reproductive performance.

The utility of this FISH assay to detect breakage in buccal mucosal cells was also demonstrated by comparing the frequencies of 1q12 breakage and hyperdiploidy in oral cells obtained from the mouths of 19 Indian betel quid chewers and compared to that of 23 non-chewing controls (Rupa and Eastmond, 1997). The frequency of breakage increased from a median of 0‰ in the controls to 2‰ in the chewers. The frequencies of hyperdiploidy were very low with a median of 0‰ for both groups. However, an increase in hyperdiploidy with time was seen in those individuals who had chewed quid for many years. Interestingly, a significant correlation between breakage and duration of chewing was seen, suggesting that the tandem assay was detecting stable alterations such as translocations and inversions that had occurred in the oral stem cells. In a second more recent study published by Ramirez and associates, the frequency of alterations in the oral mucosal cells of patients with hyperthyroidism and thyroid cancer was assessed with samples taken prior to treatment with ^{131}Iodine and 3 to 4 weeks following treatment (Ramirez, et al.,

1999). No differences in the frequencies of hyperdiploidy or breakage were seen. However, it should be noted that due to the highly selective accumulation of ^{131}Iodine in the thyroid gland, it is likely that the dose received by the buccal cells was not sufficiently high or prolonged to result in detectable effects.

An additional study was undertaken by Au and associates in which the frequency of 1q12 breakage and hyperdiploidy 1 was determined in peripheral lymphocytes of lung cancer patients, smoking and non-smoking controls (Conforti-Froes, et al., 1997). No differences in hyperdiploidy between the groups were seen, with a pooled frequency of 1.5‰ being reported. The frequencies of breakage for the 3 groups averaged 14‰, 12‰ and 4‰, respectively. The frequency of breakage among the smokers (combined cancer patients and smokers) was significantly elevated as compared to the non-smoking controls. In addition, a significant correlation between duration of smoking and breakage frequency was seen for the lung cancer patients. The frequency of breakage in the cancer patients also appeared to be significantly influenced by the glutathione-S-transferase genotypes, with those possessing one or more non-functional alleles having higher levels of breakage than patients possessing both alleles for GSTT1 and GSTM1.

In a joint study involving several groups of European investigators and those at the University of California, Riverside, the tandem label approach was used to detect chromosome alterations in the blood cells of benzene-exposed workers, cokery workers and rural controls from a shale oil petrochemical facility in Estonia (Carere, et al., 1998; Marcon, et al., 1999). Analyses were performed using the tandem 1 probes on blood smear polymorphonuclear leukocytes, Go lymphocytes and 48 hr cultured lymphocytes. In addition, analyses on the 48 hr cultured lymphocytes were also performed using the tandem 9 probes. For all samples, the frequencies of hyperdiploid cells were very low and no differences were seen between the 3 groups. For the blood smear PMN and Go lymphocytes, the 1q12 and 9q12 breakage frequencies among the 3 groups did not differ significantly, although the values among the benzene-exposed group tended to be higher. A modest but significant increase in 1q12 and 9q12 breakage was seen among the benzene-exposed workers. The frequency of 1q12 breaks increased from 2‰ in the controls to 6‰ in the benzene workers. A similar increase in breaks at 9q12 from 6‰ in the controls to 10‰ in the benzene workers was seen in the tandem 9 analyses. Interestingly, a good correlation was seen between the breakage frequencies reported for the two chromosomes, in spite of the fact that the hybridizations were performed for different chromosomes, using different probes and scored independently in two different laboratories.

These studies have demonstrated the tandem FISH approach can be used to detect chromosomal alterations in humans exposed to genotoxic agents. In five of the six human studies published to date, significant increases in breakage or hyperploidy were detected in the exposed group using the tandem assay. This is encouraging considering that the studies were conducted using a variety of cell types by a number of research groups in different countries. However, most of these studies

have been conducted on highly exposed populations. As additional studies are conducted on groups with lower exposures and exposed to different agents, increasing numbers of negative studies will be reported. We have recently completed a couple of studies on highly exposed benzene workers in which weak or no increases in alterations were seen using the tandem label approach (Eastmond et al., unpublished results). In this instance, the tandem FISH assay appears to be inefficient in detecting benzene-induced genotoxic effects. Similar results will undoubtably be seen with other types of genotoxic agents.

In summary the multicolor FISH using tandem DNA probes has been shown to be a useful technique for detecting chromosomal alterations in human cells. The ability of the assay to detect breakage and hyperdiploidy in interphase cells expands the range of cell types that can be evaluated for genotoxicity and used for human biomonitoring. In addition, the sensitivity of the targeted heterochromatin regions to breakage induced by various clastogens indicates that this approach may prove particularly valuable for detecting damage in humans exposed to these chromosome-breaking agents.

REFERENCES

1 - Aurias, A. (1993) Acquired chromosomal aberrations in normal individuals, in: I.R. Kirsch (Ed.), The causes and consequences of chromosomal aberrations, CRC Press, Boca Raton, pp. 125-139.

2 - Brogger, A. (1977) Non-random localization of chromosome damage in human cells and targets for clastogenic action., Chromosomes Today, 6, 297-306.

3 - Carere, A., R. Crebelli, A. Zijno, P. Leopardi, F. Marcon, S. Lagorio, I. Iavarone, A. Antoccia, C. Tanzarella, D. Eastmond, R. Parks, M. Schuler, K. Peltonen, M. Sorsa and T. Veidebaum (1998) Cytogenetic monitoring of oil shale petrochemistry workers, in: A. Carere and R. Crebelli (Eds.), Biomarkers for Environmental and Occupational Exposure to Aromatic Mutagens and Carcinogens from Emissions of Oil Shale Petrochemistry, Report of the EC PECO Programme. Project CIPA-CT92-3016, Istituto Superiore di Sanità, Rome, pp. 30-46.

4 - Conforti-Froes, N., R. El-Zein, S.Z. Abdel-Rahman, J.B. Zwischenberger and W.W. Au (1997) Predisposing genes and increased chromosome aberrations in lung cancer cigarette smokers, Mutation Research, 3799, 53-59.

5 - Eastmond, D.A. and D. Pinkel (1990) Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence in situ hybridization with chromosome-specific DNA probes, Mutation Research, 234, 303-318.

6 - Eastmond, D.A. and D.S. Rupa (1995) Fluorescence in situ hybridization: application to environmental mutagenesis, in: D.H. Phillips and S. Venitt (Eds.), Bios Scientific Publications, Oxford, pp. 261-290.

7 - Eastmond, D.A., D.S. Rupa and L.S. Hasegawa (1994) Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence in situ hybridization with DNA probes, Mutat. Res. 322, 9-20.

8 - Eastmond, D.A., M. Schuler and D.S. Rupa (1995) Advantages and limitations of using fluorescence in situ hybridization for the detection of aneuploidy in interphase human cells., Mutat. Res. 348, 153-162.

9 - Hasegawa, L.S., D.S. Rupa and D.A. Eastmond (1995) A method for the rapid generation of alpha- and classical satellite probes for human chromosome 9 by polymerase chain reaction

using genomic DNA and their application to detect chromosomal alterations in interphase cells, Mutagenesis, 10, 471-6.

10 - Marcon, F., A. Zijno, R. Crebelli, A. Carere, T. Veidebaum, K. Peltonen, R. Parks, M. Schuler and D.A. Eastmond (1999) Chromosome damage and aneuploidy detected by multicolor FISH in benzene exposed shale oil workers, Mutat. Res. in press.

11 - Meyne, J., L. Lockhart and F. Arrigh (1979) Nonrandom distribution of chromosomal aberrations induced by three chemicals., Mutat Res. 63, 201-209.

12 - Pinkel, D., T. Straume and J.W. Gray (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization., Proc Natl Acad Sci, 83, 2934-2938.

13 - Ramirez, M.J., J. Surralles, P. Galofre, A. Creus and R. Marcos (1999) FISH analysis of 1cen-1q12 breakage, chromosome 1 numerical abnormalities and centromeric content of micronuclei in buccal cells from thyroid cancer and hyperthyroidism patients treated with radioactive iodine, Mutagenesis, 14, 121-127.

14 - Rupa, D.S. and D.A. Eastmond (1997) Chromosomal alterations affecting the 1cen-1q12 region in buccal mucosal cells of betel quid chewers detected using multicolor fluorescence in situ hybridization, Carcinogenesis (Oxford), 18, 2347-2351.

15 - Rupa, D.S., L. Hasegawa and D.A. Eastmond (1995) Detection of chromosomal breakage in the 1cen-1q12 region of interphase human lymphocytes using multicolor fluorescence in situ hybridization with tandem DNA probes, Cancer Res, 55, 640-5.

16 - Rupa, D.S., L.S. Hasegawa and D.A. Eastmond (1997a) Detection of chromosomal alterations affecting the 1cen-1q12 region in irradiated granulocytes and lymphocytes by multicolour FISH with tandem DNA probes, Mutagenesis, 12, 195-200.

17 - Rupa, D.S., P.P. Reddy and O.S. Reddi (1991a) Clastogenic effect of pesticides in peripheral lymphocytes of cotton-field workers, Mutat Res, 261, 177-80.

18 - Rupa, D.S., P.P. Reddy and O.S. Reddi (1991b) Reproductive performance in population exposed to pesticides in cotton fields in India, Environ Res, 55, 123-128.

19 - Rupa, D.S., M. Schuler and D.A. Eastmond (1997b) Detection of hyperdiploidy and breakage affecting the 1cen-1 q 12 region of cultured interphase human lymphocytes treated with various genotoxic agents, Environmental and Molecular Mutagenesis, 29, 161-167.

20 - Schuler, M., L. Hasegawa, R. Parks, M. Metzler and D.A. Eastmond (1998) Dose-response studies of the induction of hyperdiploidy and polyploidy by diethylstilbestrol and 17beta-estradiol in cultured human lymphocytes using multicolor fluorescence in situ hybridization, Environ Mol Mutagen, 31, 263-73.

21 - Smith, L., K.K. Parks, L.S. Hasegawa, D.A. Eastmond and A.J. Grosovsky (1998) Targeted breakage of paracentromeric heterochromatin induces chromosomal instability, Mutagenesis, 13, 435-443.

22 - Surralles, J., F. Darroudi and A.T. Natarajan (1997) Low level of DNA repair in human chromosome 1 heterochromatin, Genes Chromosomes Cancer, 20, 173-84.

Table 2. **Population studies using the tandem label assay to detect chromosomal alterations in humans exposed to genotoxic agents.**

Study Group	N[a]	Cs	Cell Type	Hyperploidy[b]	Breakage[b]	Reference
Indian controls	19	1	Lymphocyte	2 (1-2)	2 (1-3)	Rupa et al. 1995
Indian pesticide applicators	26	1	Lymphocyte	3 (2-4)	5 (4-8)	
Indian controls	18	1	Sperm	2 (1-3)	0 (0-1)	Rupa et al. 1997
Indian pesticide applicators	21	1	Sperm	4 (3-8)	4 (1-5)	
Indian controls	23	1	Oral mucosal	0 (0-0)	0 (0-0)	Rupa et al. 1997
Indian betel quid chewers	19	1	Oral mucosal	0 (0-1)	2 (1-3)	
US controls	13	1	Lymphocyte	1.5[c]	4 ± 1[d]	Conforti-Froes et al. 1997
US smokers	22	1	Lymphocyte	1.5[c]	12 ± 1[d]	
US lung cancer patients	22	1	Lymphocyte	1.5[c]	14 ± 1[d]	
Estonian controls	8	1	PMN[e]	1 (0-2)	8 (4-13)	Carere et al. 1998/
		1	Go lymphocyte	0 (0-2)	5 (3-9)	Marcon et al. 1999
		1	Lymphocyte	0 (0-0)	2 (1-4)	
		9	Lymphocyte	0 (0-0)	6 (4-8)	
Estonian cokery workers	5	1	PMN[e]	2 (0-2)	6 (4-11)	
		1	Go lymphocyte	0 (0-3)	4 (2-6)	
		1	Lymphocyte	0 (0-1)	4 (2-4)	
		9	Lymphocyte	0 (0-0)	7 (6-7)	
Estonian benzene workers	12	1	PMN[e]	2 (1-4)	11 (10-14)	
		1	Go lymphocyte	1 (0-2)	6 (4-11)	
		1	Lymphocyte	0 (0-1)	6 (4-7)	
		9	Lymphocyte	0 (0-1)	10 (6-13)	
Hyperthyroid patients						
before ^{131}I-treatment	16	1	Oral mucosal	0.3 (0-0.5)	2.5 (1.8-3.3)	Ramirez et al. 1999
after ^{131}I-treatment	16	1	Oral mucosal	0.3 (0-0.8)	3.0 (1-3.5)	
Thyroid cancer patients						
before ^{131}I-treatment	15	1	Oral mucosal	0 (0-0.5)	2.5 (1-4.4)	
after ^{131}I-treatment	15	1	Oral mucosal	0.5 (0-0.5)	2.5 (1.5-3.4)	

[a]Number of individuals sampled.; [b]Median (interquartile range).;

[c]Pooled mean reported for the three groups.; [d]Mean ± SEM.; [e]Polymorphonuclear leukocytes