DETERMINATION OF CHROMOSOMAL AREA OF DOUBLE MINUTE CHROMOSOMES AND A HOMOGENEOUSLY STAINING REGION IN HL-60 HUMAN LEUKEMIA CELLS BY THE USE OF A COLOR IMAGE ANALYZER

Shinichi MISAWA,¹ Tatsuo ABE,¹ Tetsuro TAKAMATSU,²
Setsuya FUJITA,² and Joseph R. TESTA³

¹*Departments of Medicine and* ²*Pathology, Kyoto Prefectural University of Medicine,*
Kyoto 602, Japan and ³*Section of Cancer Genetics, University of Maryland*
Cancer Center, Baltimore, Maryland 21201, U.S.A.

Summary The surface area of chromosomes was measured in two sublines of HL-60 human leukemia cells using a color image analyzer. One subline, HL-60 (dmin), had double minute chromosomes (dmin) and the other line, HL-60 (HSR), showed an abnormal chromosome #8 (8q+) which contained a homogeneously staining region (HSR) within the long arm at band q24. Dmin were not seen in the latter cells. The area of individual chromosomes analyzed from metaphase plates of Giemsa-stained photographic prints proved to be reasonably accurate and reproducible. Moreover, the relative area of each chromosome correlated well both with the relative length of corresponding chromosomes and the relative DNA content. The results indicate that the mean area of a single dmin represents 0.16% of the total genomic area of HL-60 (dmin) cells. The average number of dmin per cell was 8.14, and the total area of all dmin in a representative cell was 1.32% of the entire genome. The 8q+ chromosome had 1.46 times the area of the normal homologous chromosome #8, and the HSR itself had 1.30% of the total chromosomal area of HL-60 (HSR) cells. Thus, perhaps coincidentally, the relative amount of DNA in the HSR in an HL-60 (HSR) cell was similar to the total found in multiple dmin in HL-60 (dmin) cells.

INTRODUCTION

Homogeneously staining regions (HSR) are novel chromosome abnormalities which stain uniformly instead of the pattern of alternating pale and dark bands seen in normal chromosomes when stained with either trypsin-Giemsa or other

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banding procedures (Biedler and Spengler, 1976). Double minute chromosomes (dmin), another type of unusual abnormality (Spriggs *et al.*, 1962), are small, paired chromatin bodies whose number and size vary from one cell to another (Barker and Hsu, 1979; Cox *et al.*, 1965). These chromosome aberrations are not uncommon in primary cells or cell lines of human or animal tumors. Individual cells of a line with HSR or dmin, as a rule, contain one or the other abnormality but not both (Biedler *et al.*, 1983). These observations suggest that HSR and dmin could be alternative cytological manifestations of the same biological phenomenon (Biedler *et al.*, 1983; Cowel, 1982; Hamlin *et al.*, 1984).

The human leukemia cell line HL-60, established from a patient with acute promyelocytic leukemia (Collins *et al.*, 1977), showed dmin in early passage cells as well as in primary leukemic cells (Gallagher *et al.*, 1979). In this cell line an HSR appeared during culture, with a concurrent disappearance of dmin (Nowell *et al.*, 1983). Interestingly, the oncogene *c-myc* has been shown to be amplified in HL-60 cells regardless of whether the particular subline examined has either of these chromosome aberrations (Gallagher *et al.*, 1984; Nowell *et al.*, 1983; Wolman *et al.*, 1985).

The present experiments were designed to measure the surface area of dmin and HSR found in HL-60 cells, using a color image analyzer. The results showed that the HSR comprised 1.3% of the total chromosome area per HL-60 cell which has HSR, whereas dmin had an average 0.16% of the total genomic area of HL-60 cells which have dmin.

MATERIALS AND METHODS

Materials consisted of two sublines of HL-60 human leukemia cells. One subline which has dmin, designated here HL-60 (dmin), was a gift of Dr. A.B. Mukherjee (Fordham University, New York, NY). The other subline which has an abnormal chromosome #8 but not dmin, HL-60 (HSR), was generously provided by Drs. G. Rovera and J.R. Selden (Wistar Institute, Philadelphia, PA). The latter cell line was previously referred to by Nowell *et al.* (1983) as HL-60-79B.

Metaphase cells were harvested after a short exposure (10 min) to Colcemid (0.05 $\mu\text{g/ml}$), treated with hypotonic solution of 75 mM KCl, then fixed with ethanol-acetic acid mixture (3 : 1). Chromosome preparations were made by the usual air drying method. The slides were stained conventionally with Giemsa (5% in phosphate buffer, pH 6.8) to show well delineated chromosomes. Well spread metaphase plates were photographed, and the slides were destained through an ethanol series and then submerged briefly in fresh fixative. The same cells were then restained with quinacrine (quinacrine mustard, 50 $\mu\text{g/ml}$ in McIlvaine buffer, pH 5.5) for Q-bands. Chromosome identification and karyotype designation were done on the basis of the nomenclature of the ISCN (1985).

The surface area of all chromosomes was measured on 10 metaphase plates

Table 1. Relative area of chromosomes in HL-60 (HSR) cells.

Chromo- some No.	14S-21	14S-25	14S-27	14S-28	14S-29	Cell Number			15B-7	15B-8	15B-9	No. of copies	mean	S.D.
						14S-32	15B-1							
1	4.34	3.94	4.00	4.33	4.42	4.29	3.97	4.46	4.21	4.37		20	4.22	0.24
2	4.57	3.99	3.98	4.15	3.99	4.20	4.72	4.51	4.10	3.98		20	4.28	0.23
3	4.29	4.16	4.42	4.09	3.94	4.42	4.41	4.40	3.94	4.06		20	3.39	0.19
4	3.49	4.73	4.31	3.85	4.37	4.54	4.53	4.30	4.19	4.19		20	3.47	0.40
5	3.50	3.27	3.47	2.93	3.20	3.42	3.72	3.46	3.29	3.39		20	3.14	0.15
6	3.53	3.15	3.20	3.26	3.57	3.30	3.53	3.65	3.48	3.50		20	3.01	0.20
7	3.30	4.28	3.17	3.21	3.23	3.27	3.49	3.34	3.55	3.33		20	2.68	0.24
8	2.98	3.19	3.05	4.03	3.45	3.64	4.60	3.13	3.27	3.14		20	2.41	0.15
9	2.96	2.80	3.15	2.64	3.33	3.38	3.14	3.41	3.17	3.17		10	2.33	0.22
10	3.21	2.84	2.96	2.97	3.31	2.98	2.97	3.07	2.95	3.06		20	2.21	0.15
11	2.65	2.77	2.96	2.40	2.35	2.53	2.81	2.45	2.72	2.67		20	3.01	0.20
12	2.77	2.56	2.77	3.37	2.36	2.61	2.90	2.66	2.53	2.66		20	2.68	0.24
13	2.58	2.31	2.37	2.59	2.11	2.43	2.45	2.52	2.29	2.42		10	2.41	0.15
14	2.14	2.00	2.43	2.12	2.64	2.19	1.91	2.33	2.43	2.40		10	2.33	0.22
15	2.25	2.16	2.19	2.44	2.00	2.51	2.05	2.35	2.22	2.27		10	2.21	0.15
16	2.42	2.11	2.25	2.05	2.31	2.60	2.31	2.26	2.17	2.50		20	2.28	0.16
17	2.31	2.28	2.56	1.97	2.09	2.37	1.82	2.23	2.48	2.29		20	2.24	0.19
18	2.25	2.07	2.47	2.11	2.08	2.17	2.46	2.33	2.33	2.18		20	1.92	0.15
19	1.88	1.88	1.96	1.73	2.02	1.88	1.95	1.99	1.90	1.72		20	1.73	0.12
20	1.72	1.88	2.07	2.32	2.08	2.05	1.64	1.87	1.97	1.87		10	1.67	0.10
21	1.66	1.82	1.56	1.78	1.92	1.86	1.55	1.69	1.75	1.71		10	1.47	0.11
22	1.52	1.70	1.80	1.50	1.76	1.73	1.70	1.68	1.73	1.62		10		
X	1.32	1.44	1.32	1.64	1.47	1.59	1.36	1.55	1.54	1.43		10		
5q-	1.28	1.51	1.49	1.76	1.16	1.24	1.11	1.31	1.36	1.28		30		
8q+	1.39	1.29	1.38	1.43	1.53	1.21	1.43	1.40	1.60	1.31		20		
9p+	1.29	1.29	1.40	1.48	1.62	1.34	1.44	1.36	1.44	1.43		20		
10p+	1.01	1.16	1.16	0.97	0.97	1.03	0.99	1.09	1.14	1.04		20		
14q-	0.90	1.24	1.05	0.98	0.99	1.07	0.97	1.05	1.22	1.15		20		
16q+	1.00	1.35	1.10	0.98	1.02	1.06	1.01	0.87	1.22	1.09		20		
16q+*	0.65	0.94	0.63	0.71	0.99	0.95	1.03	1.00	1.14	1.07		20		
16q+**	0.72	0.96	0.75	0.67	0.85	0.53	0.57	0.71	0.70	0.72		20		
	0.83	0.83	0.77	0.91	0.91	0.55	0.77	0.86	0.85	0.64		20		
	0.83	0.90	0.97	0.66	0.75	0.85	0.80	0.70	0.96	0.95		20		
	2.76	2.64	2.41	2.69	2.80	2.50	2.57	2.48	2.20	2.54		20		
	1.89	2.04	1.89	2.03	2.26	2.01	1.86	2.03	1.88	2.03		10		
	3.50	3.47	3.63	3.20	3.43	3.53	3.51	3.18	3.49	4.22		10		
	1.76	1.76	1.67	1.97	1.64	1.68	1.49	1.65	2.08	1.69		10		
	3.55	3.27	3.21	3.15	3.77	3.68	3.50	3.41	3.41	3.27		10		
	1.48	1.61	1.68	1.90	1.59	1.68	1.64	1.65	3.42	0.20		10		
	1.74	1.89	1.80	1.60	1.60	1.69	1.64	1.91	1.62	0.13		10		
	2.09	1.97	1.98	1.83	2.20	2.04	2.10	1.91	1.72	0.11		10		
									2.01	0.12		10		

16q+*:der(16)t(16;?) (q22;?), 16q+**:der(16)t(16;?) (q23;?).

Table 2. Relative area of chromosomes in HL-60 (dmin) cells.

Chromo- some No.	18E-1	18E-3	18E-5	18E-7	Cell Number				No. of copies				mean	S.D.
					18E-10	18E-12	18E-15	18E-17	18E-18	18E-20				
1	3.84	3.71	4.10	4.26	3.96	4.30	4.45	3.60	4.09	4.09	20	4.15	0.24	
2	4.26	4.27	4.08	4.35	4.39	3.93	4.21	4.51	4.25	4.35	20	4.15	0.24	
3	3.61	3.74	4.20	3.95	3.68	3.56	4.22	3.79	3.46	3.81	20	3.90	0.25	
4	4.17	3.81	3.54	3.99	4.29	3.94	3.96	4.32	4.03	3.73	20	3.90	0.25	
5	3.34	3.51	3.40	3.13	2.97	3.63	3.22	3.36	3.36	3.20	20	3.35	0.26	
6	3.51	3.77	2.83	3.43	3.21	3.68	3.29	2.94	3.63	3.62	20	3.35	0.26	
7	3.22	3.08	3.70	3.42	3.17	3.33	3.41	2.78	3.44	3.46	20	3.32	0.24	
8	3.63	3.19	3.35	3.53	3.32	3.38	3.42	2.91	3.03	3.67	20	3.32	0.24	
9	3.30	3.28	2.87	3.44	2.67	3.10	3.36	3.36	3.21	3.23	10	3.18	0.24	
10	2.71	2.94	2.53	2.94	3.12	2.68	3.09	3.47	3.10	3.05	20	2.96	0.23	
11	2.91	2.85	3.01	2.72	3.09	2.64	2.95	3.32	2.89	3.15	20	2.96	0.23	
12	2.75	2.70	2.80	2.63	2.51	2.76	2.36	2.31	2.53	2.87	20	2.62	0.18	
13	2.76	2.83	2.77	2.37	2.71	2.58	2.23	2.72	2.65	2.61	20	2.62	0.18	
14	2.24	2.47	2.20	2.26	2.63	2.84	2.27	2.32	2.39	2.36	20	2.42	0.17	
15	2.66	2.57	2.43	2.08	2.61	2.53	2.51	2.48	2.50	2.37	20	2.42	0.17	
16	2.03	2.14	2.24	2.42	2.40	2.43	2.45	2.13	2.51	2.42	10	2.32	0.17	
17	2.22	2.11	2.24	2.46	2.26	2.11	2.32	2.10	2.12	2.33	10	2.22	0.12	
18	2.25	2.04	2.20	2.31	2.25	2.63	1.87	2.26	2.23	2.28	20	2.24	0.20	
19	2.59	2.19	2.41	1.88	2.31	2.07	2.43	2.06	2.17	2.41	20	2.24	0.20	
20	2.14	2.21	2.50	2.26	2.50	2.40	1.95	2.44	2.47	2.26	20	2.32	0.17	
21	2.16	2.13	2.41	2.23	2.42	2.25	2.47	2.51	2.56	2.21	20	2.32	0.17	
22	1.68	2.27	1.77	2.15	1.78	2.08	2.11	2.15	1.82	1.81	20	1.96	0.21	
23	1.52	2.21	1.74	2.00	1.72	2.00	2.00	2.15	2.09	2.02	20	1.96	0.21	
24	1.94	1.75	1.62	1.68	1.91	1.52	1.90	1.68	1.69	1.58	20	1.75	0.20	
25	1.90	2.26	1.69	1.93	1.72	1.81	1.29	1.57	1.81	1.75	20	1.75	0.20	
26	1.67	1.58	1.85	1.61	1.84	1.50	1.72	1.65	1.57	1.62	20	1.63	0.12	
27	1.46	1.63	1.53	1.90	1.64	1.48	1.54	1.60	1.66	1.66	20	1.63	0.12	
28	1.52	1.55	1.51	1.55	1.36	1.58	1.55	1.40	1.27	1.34	10	1.46	0.11	
29	1.63	1.33	1.55	1.09	1.37	1.27	1.48	1.47	1.44	1.42	30	1.42	0.14	
30	1.64	1.37	1.50	1.63	1.45	1.18	1.32	1.57	1.39	1.33	30	1.42	0.14	
31	1.59	1.61	1.44	1.53	1.24	1.45	1.16	1.52	1.46	1.38	20	1.42	0.14	
32	1.15	1.12	1.15	0.97	1.26	1.17	1.10	1.23	1.20	1.10	20	1.16	0.07	
33	1.18	1.12	1.18	1.26	1.23	1.09	1.22	1.19	1.25	1.17	20	1.16	0.07	
34	1.22	1.15	1.26	1.21	1.07	1.34	1.06	0.96	1.09	1.05	20	1.14	0.10	
35	1.25	1.27	1.17	1.23	1.07	1.03	1.07	1.16	1.09	1.16	20	1.14	0.10	
36	0.95	0.92	0.86	0.91	0.92	0.96	0.95	0.88	0.84	0.69	20	0.90	0.11	
37	0.72	0.88	1.08	0.87	0.96	0.82	1.02	1.13	0.97	0.81	20	0.90	0.11	
38	0.88	0.96	0.92	0.83	1.02	0.99	1.01	0.94	1.11	0.80	20	0.95	0.08	
39	0.90	0.98	0.99	1.01	0.90	0.84	1.08	0.85	0.96	0.99	20	0.95	0.08	
40	2.82	2.45	2.69	2.28	2.33	2.37	2.59	2.49	2.74	2.74	10	2.55	0.19	
41	1.72	1.85	2.13	1.95	1.98	2.11	2.06	2.16	2.00	1.68	10	1.96	0.17	
42	59-1	1.61	1.54	1.84	1.61	1.74	1.71	1.83	1.79	1.73	10	1.75	0.17	
43	9P-	2.78	2.81	2.85	2.86	3.50	3.21	2.79	3.45	2.93	10	3.00	0.29	
44	10P*	1.85	1.74	1.72	1.61	1.89	1.89	1.96	1.89	1.80	10	1.78	0.13	
45	16G**	2.11	2.01	1.90	2.18	1.92	1.86	1.97	1.92	1.98	10	1.97	0.11	

16q+*ider(16)t(16;?) (q22;?), 16q+*ider(16)t(16;?) (q23;?) .

Fig. 1. A metaphase plate of the HL-60 (HSR) subline. a) Quinacrine stained chromosomes of the same HL-60 (HSR) cell shown in (b). This metaphase is prestained with Giemsa followed by quinacrine staining; b) Giemsa stained metaphase spread; c) processed image by the color image analyzer of Giemsa stained mitosis shown in (b).

for each subline. The image of chromosomes on black and white photographic prints of Giemsa-stained metaphase cells was taken by a video camera and stored in video RAM ($512 \times 480 \times 8$ bits) in a digital color image analyzer (Olympus CIA, Japan) (Fujita, 1983; Takamatsu *et al.*, 1986). The area of respective chromosomes was measured automatically by counting the number of pixels constituting the chromosome image after segmentation (Fig. 1). Results are presented as a relative area of individual chromosomes: the measured area of each chromosome was divided by the total area of the entire chromosome complement in every cell. The area of each dmin was calculated in the same manner, but the area of dmin was excluded from the total area of the cells because the number of dmin varied from one cell to another.

RESULTS

For each subline 25 metaphase cells were karyotyped. Details are described elsewhere (Misawa *et al.*, submitted). The most distinctive chromosome alterations were the presence of dmin in HL-60 (dmin) and an abnormal chromosome #8 in HL-60 (HSR). This abnormal #8 showed an expanded segment at the distal end of the long arm at band q24.1 (Fig. 2). The segment appeared to be an HSR with Q-banding, that is, a long chromosome segment showing medium fluorescence intensity without any distinct bands. However, with G-banding, narrow, or indistinct bands of a clearly abnormal type are discernible within the region. Dmin were not seen in these 25 cells or 100 additional mitoses examined under the microscope. The modal karyotype was $44, X, -X, -5, -10, -15, -16, -16, -17, +18, +\text{der}(5)\text{t}(5; ?17)(\text{q} ?13; ?\text{q}12), (8)(\text{q}24.1\text{HSR}), \text{del}(9)(\text{p}13), +\text{der}(10)\text{t}(10; 13)(\text{p}13; \text{q}12), \text{del}(14)(\text{q}24\text{q}32), +\text{der}(16)\text{t}(16; ?)(\text{q}22; ?), +\text{der}(16)\text{t}(16; ?)(\text{q}23. ?)$ (Fig. 2). The $\text{der}(10)$, $\text{del}(9)$,

and der(5) correspond to marker chromosomes M1, M2, and M3, respectively, as designated by Gallagher *et al.* (1979).

The other subline contained 1 to 37 dmin in 44 of 50 consecutive mitoses with an average 8.14 per cell (Fig. 3). The karyotype was similar to that of HL-60 (HSR) cells but the cells had two normal chromosomes #8 and #14. The representative karyotype was 45,X,-X,-5,-10,-16,-16,-17,+18,+der(5)t(5;?17)(q?13;?q12),del(9)(p13),+der(10)t(10;13)(p13;q12),+der(16)t(16;?)(q22;?),+der(16)t(q16;?)(q23;?).

Results of measurements of chromosomal area for each subline are summarized in Tables 1 and 2. There was good general agreement between the two sublines with regard to area of specific chromosomes. Slight variations occurred due to technical limitations associated with chromosome slide preparation, staining, photography, and measurement procedures, as well as to true differences between homo-

Fig. 2. Karyotype of a HL-60 (HSR) cell shown in Fig. 1. Breakpoints of rearranged chromosomes are indicated by arrows and HSR by a bracket. Marker chromosomes, m1, m2 and m3, are designated according to the original description by Gallagher *et al.* (1979).

Fig. 3. A Giemsa stained metaphase of the HL-60 (dmin) subline showing multiple copies of dmin.

Fig. 4. Correlation between relative surface area of chromosomes and relative chromosome length (a) and relative DNA content (b) in HL-60 (HSR) cells. Bars indicate means $\pm$ S.D.

logues. When the results were compared with the relative chromosome length (ISCN, 1985) or the relative DNA content (Bosman *et al.*, 1977), a clear correlation was observed in both cell lines (Figs. 4 and 5).

The relative area of three rearranged chromosomes, der(10), der(9), and der(5), was $3.42 \pm 0.20\%$, $1.74 \pm 0.17\%$ and $1.99 \pm 0.12\%$, respectively, of the entire chromosome area in HL-60 (HSR) cells, and $3.00 \pm 0.29\%$, $1.75 \pm 0.17\%$ and $1.96 \pm 0.17\%$, respectively, in HL-60 (dmin) cells. The other rearranged chromosome, 8q+, had 3.52% of the total chromosomal area of the HL-60 (HSR) cells, whereas

Fig. 5. Correlation between relative surface area of chromosomes and relative chromosome length (a) and relative DNA content (b) in HL-60 (dmin) cells. Bars indicate means $\pm$ S.D. Relative areas of 132 dmin in HL-60 (dmin) cells are shown in the right marginal space on an expanded scale.

the normal chromosome #8 had 2.41%. The 8q+ chromosome was, therefore, 46% greater than that of the normal homologous chromosome #8 with respect to surface area. In addition, the band 8q24.1 appeared to be included in the HSR because the band could not be distinguished from the HSR. The exact area of the HSR substantially consisted of 1.30% of the total area of the representative karyotype of HL-60 (HSR) cells.

The relative area of dmin ranged from 0.048% to 0.318% (average 0.162%) of the total area of the representative HL-60 (dmin) karyotype (Fig. 5).

DISCUSSION

Metaphase chromosomes exhibit varying degrees of morphological distortion from one chromosome to another even within a single metaphase spread. However, our results show a good correlation between the relative area of chromosomes and previously reported data on relative length (ISCN, 1985) or relative DNA content (Bosman *et al.*, 1977).

With regard to rearranged chromosomes, the deleted segment of a marker chromosome, m2 (Fig. 2) corresponded to 25% of a normal chromosome #9, whereas a marker chromosome, m3 (Fig. 2) represented 50% of the sum of normal homologues #5 and #17. The chromosomal material missing from der(10) comprised approximately 28% of the total area of normal chromosomes #10 and #13 in HL-60 (dmin) cells and 17% in HL-60 (HSR) cells. The possible reasons for this difference are: a) some of the der(10) examined were folded or overly contracted in HL-60 (dmin) cells resulting in smaller surface area, or b) the der(10) in HL-60 (dmin) cells has a further chromosomal alteration leading to an additional loss of chromatin.

Although the banding pattern of the der(10) generally seems quite similar in these two sublines, a few cells appear to have a slightly smaller short arm, especially in HL-60 (dmin) cells.

The abnormal segment on the 8q+ which showed no distinct bands in Q-banding and the G-banding pattern was clearly abnormal and different from any part of the normal chromosome complement. These findings are consistent with that of a homogeneously staining region because HSR are not always uniformly stained with G-banding but sometimes exhibit an abnormal pattern of closely apposed fine bands (Biedler *et al.*, 1980; Cowell, 1982). Such segments are usually referred to as "abnormally banding regions" or "differentially staining regions" and are considered to have the same biological properties as HSR that show no distinctive bands (Biedler *et al.*, 1980, 1983; Cowell, 1982).

The 8q+ chromosome had $3.52 \pm 0.29\%$ of the total chromosomal area of a representative HL-60 (HSR) karyotype, whereas, the area of a normal chromosome #8 was $2.41 \pm 0.15\%$. The difference, 1.11% (range 0.61–1.80%), corresponds to 46.1% of a normal #8. Balaban-Malenbaum and Gilbert (1980) compared the amount of DNA in HSR-containing chromosomes with that in normal homologues from the same human neuroblastoma cells, and they found that the DNA content of HSR-bearing chromosomes was 50–250% greater. However, in a wide variety of HSR-bearing mammalian cells the length of HSR can range from barely detectable to 15–20% of the entire condensed mitotic genome length (Hamlin *et al.*, 1984).

In situ chromosomal hybridization studies showed amplification of the proto-oncogene *c-myc* in the HSR found in HL-60 cells (Nowell *et al.*, 1983; Wolman *et al.*, 1985). Moreover, labeling of silver grains with the *v-myc* probe distributes randomly over the entire segment (Misawa *et al.*, submitted). The results suggest that this segment originated from band 8q24.1 which is the native site of this proto-oncogene (Neel *et al.*, 1982; Taub *et al.*, 1982), or that dmin, where the *c-myc* is also amplified in HL-60 cells (Misawa *et al.*, submitted), is integrated into band 8q24.1 at the site of sequence homology, although the integration of the extrachromosomal DNA sequences can potentially occur anywhere in the genome (Cowell, 1982; Hamlin *et al.*, 1984).

Dmin vary in size among different metaphases. The number of dmin also varies from cell to cell within a cell population. The largest dmin reported are nearly equal in size to the smallest normal human chromosomes, whereas the smallest dmin are barely detectable at the light-microscope level (Biedler *et al.*, 1983; Cowell, 1982). Typically, dmin are approximately 0.3–0.5 μm in diameter (Barker and Hsu, 1979; Cowell, 1982), and in a given cell most dmin are about the same size. There was a 6.6-fold difference in size between the largest and smallest dmin based on counts of pixels on the video RAM. However, the actual size variation among dmin may be less marked, because black and white photographic prints may tend to make some small pale staining dmin appear even smaller whereas very dark dmin look larger. In any case, the size of most dmin (73.5%) ranged between

0.11% and 0.22% of the total chromosomal area of a representative HL-60 (dmin) cell. By quantitative mass determination of dmin, Bahr *et al.* (1983) showed a four-fold difference of dmin in a human neuroblastoma cell line, NB16. They showed that dmin vary in size from 2×10^{-14} g to 8×10^{-14} g, with a mean of 4.71×10^{-14} g per single dmin. This quantity represents about 6.38×10^6 base DNA per double minute which corresponds to about 0.12% of the DNA in a normal human cell, since the haploid complement has been estimated to contain 2×10^9 bp of DNA (Rees and Jones, 1972). Moreover, the dry mass of a dmin in NB16 cells was about one-seventh of that found in the smallest normal human chromosomes (Bahr *et al.*, 1983). Our results with HL-60 cells show that the mean area of a single dmin corresponds to 1 : 5.6 of a single chromosome #21 or 1 : 5.9 of a #22. Since the average number of dmin per cell in the HL-60 (dmin) line was 8.14, the total area of dmin represents 1.32% ($8.14 \times 0.162\%$) of the total genomic area of the cells. Interestingly, the total area of the average number of dmin in HL-60 (dmin) cells is about the same as the area of the HSR in HL-60 (HSR) cells. This similarity is probably coincidental. However, our results showed that a combination of measuring of surface area by a color image analyzer with banding analysis of the same chromosomes provides useful informations in the study of chromosomes.

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