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SOMATIC CELL HYBRIDIZATION USED IN REGIONAL MAPPING OF STRUCTURAL GENES CODING FOR HUMAN GLUTATHIONE PEROXIDASE-1 (GPX1) AND ARYLSULFATASE B (ARSB) [1]

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Summary. High frequency of cell fusion was observed while crossing several mammalian cells of different origin by means of PEG 6000. The technique applied to fuse human and rodent cells yielded several hundreds independent human-rodent somatic cell hybrid clones. The analysis of hybrids from fusion experiments between a3 (TK^-) and human cells carrying different reciprocal balanced translocations involving chromosome 3 and 5 permitted regional assignment of the structural gene for GPX1 to chromosome 3p23 - 3q13 and for ARSB to 5q11 - 5qter.

Human gene mapping involves several methods, but as a rule they are based on family studies, somatic cell hybridization and molecular hybridization. Classical family studies were limited by number of available markers, generation time and size of sibships, and so somatic cell hybridization played the most important role in human gene mapping until recent development of recombinant DNA technology. During a few years several hundreds genes were assigned to human chromosomes using somatic cell hybridization (McKusick 1982). In recent years the use of recombinant DNA techniques has allowed to clone genes and determine their nucleotide sequence. The number of cloned genes which was 22 at Sixth International Workshop on Human Gene Mapping in 1981, reached 132 in 1983, and there are still other DNA segments which are mapped to chromosomes, the sequence of which is already known (Skolnick et al. 1984). The construction of a molecular map of a man seems to be the goal of the near future. The best approach to the human gene mapping for the time being seems to be a combination of classical Mendelian genetics with somatic cell genetics and DNA recombinant methodology. Family studies have become enriched now by a possibility to use restriction fragment length polymorphisms as genetic markers.

In this work human-Chinese hamster somatic cell hybrids were used to assign two structural genes for human glutathione peroxidase -1(GPX1) and arylsulfatase

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B (ARSB) to the regions of chromosomes 3 and 5. Hybrids were selected after fusion of a3 cells with human cells carrying reciprocal balanced translocations involving the chromosomes mentioned above. Separate fusion experiments were made using several mammalian cell lines and cultures, as the fusion itself plays a very important role not only in somatic cell hybridization, but also in complementation studies and in some virological techniques, and a high fusion efficiency seems to be necessary to achieve all these goals.

MATERIAL AND METHODS

Cells. Different human and animal cells were used in the experiments.

Human cells. 1. Lymphocytes and leukocytes were separated from erythrocytes using Ficoll-Hypaque or dextran (Davidson, Gerald, 1976). The blood was taken from normal volunteers and patients carrying the reciprocal balanced translocations, namely: t (3; 4) (p23; q35), t (3; 9) (q13; q12 or 13), t (5; 21) (q11; q22). 2. Skin fibroblasts were grown in Eagle's MEM supplemented with 10% calf serum. Skin fibroblasts carrying a reciprocal balanced translocation t(5; 16) (p11; p11) were kindly supplied by professor M. A. Ferguson-Smith (Duncan Guthrie Institute of Medical Genetics, Glasgow), and were maintained in F10 medium supplemented with 10% fetal calf serum (FCS). 3. WI-38, a fetal lung diploid fibroblast cell line (Microbiologics Assoc., Bethesda) was grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS. 4. RAJI, a lymphoblastoid cell line derived from Burkitt's lymphoma (ATCC, Rockville) growing in cell suspension was fed with RPMI 1640 medium supplemented with 10% FCS.

Animal cells. 1. Rhesus brain primary and secondary cell cultures were prepared as described by O'Brien et al. (1972). 2. Vero cell line derived from a vervet monkey kidney (Bureau of Biologics, FDA, Bethesda). 3. Rat brain primary and secondary cell cultures kindly supplied by Dr. Hiroshi Sato (Bureau of Biologics, FDA, Bethesda). 4. C6, a rat glial tumor cell line (ATCC, Rockville). Both simian and rat cells were grown in Dulbecco's modified Eagle's medium, supplemented with

Table 1. Cells used for crosses in fusion

Human leukocytes	×	a3 (TK$^-$)
		sg3 (HPRT$^-$)
		It22 (TK$^-$)
		skin fibroblasts
Skin fibroblasts	×	skin fibroblasts
		a3 (TK$^-$)
		sg3 (HPRT$^-$)
Vero cells	×	WI-38
		human lymphocytes
		RAJI
		Rhesus brain cultures
		rat brain cultures
		C6

10% FCS. 5. Chinese hamster and mouse mutant cell lines a3 (TK^-), SG3 ($HPRT^-$), It22 (TK^-) were obtained by courtesy of Dr. Z. Stęplewski (The Wistar Institute of Anatomy and Biology). They were maintained in Eagle's MEM with calf serum and 30μg/ml of BudR (for cells TK^-) or 10 μg/ml of 8-azaguanine ($HPRT^-$).

Cell fusion. Fusion experiments were carried out in the cell suspension using 55% solution of polyethylene glycol (PEG) 6000 according to the method described previously (Fidziańska et al. 1979). Cells used for crosses are presented in Table 1. To count the number of fused cells the cultures were stained with neutral red, acridine orange and Giemsa after 2 - 6, 24, 48 and 72 hours and in some cases after 4 and 5 days.

Cloning. Hybrid clones were derived from crosses between rodent (hamster and mouse) and human cells. The clones were isolated 5 - 14 days after cell fusion and maintained in selective HAT medium (Littlefield 1964), supplemented with strophantine 10^{-6}M in the case of human fibroblasts used for fusion. Human and rodent chromosomes were identified in hybrid clones using the Giemsa banding technique. Marker enzymes were tested according to routine methods. These studies were partially performed at State University in Leiden (Fidziańska et al. 1980).

RESULTS

A. CELL FUSION AND ISOLATION OF SOMATIC CELL HYBRIDS

The highest number of fused cells was observed when using Vero cells to cross them with human, simian and rat cells (fusion efficiency was 60 - 70%), but the effective cell fusion was also seen while crossing human and hamster cells (60 - 65%) or two different types of human cells (50 - 60%). The number of multinucleate cells was high; cells with ≥ 4 nuclei per cell constituted about 30 - 38% of all the fused cells. Polykaryons, however, remained viable as shown by neutral red staining. Harvest of hybrid clones was more than satisfactory. Several hundreds colonies could be isolated from each hybridization experiment 5 - 14 days after fusion. The hybrid clones were isolated from crosses between human leukocytes and skin fibroblasts with a3, SG3 and It22 cells. They were passaged from a few to several times and checked for the presence of human chromosomes and enzyme markers. Human chromosomes were rapidly eliminated from hybrid cells, especially when using hamster cells. After 10 passages usually a few human chromosomes remained in the cells. A preferable loss or retention of some human chromosomes was not observed, and several different sets of human chromosomes were obtained. The mouse-human cell hybrids segregated human chromosomes more slowly than hamster-human clones, but they usually had from over 100 to about 250 chromosomes in their cells. For that reason they were very difficult to analyze. Thus, in further experiments on gene mapping the fusion was carried out using only a3 line which gave the best and most reproducible results.

B. REGIONAL LOCALIZATION OF STRUCTURAL GENES FOR GPX1 AND ARSB

1. Mapping of a gene coding for GPX 1

Fusion was carried out between Chinese hamster, a3 (TK^-) line and leukocytes from individuals carrying reciprocal balanced translocations t(3; 4) (p23; q35) and t(3; 9) (q13; q12 or 13). About 250 hybrid clones were isolated after fusion. 50 of them were screened for the presence of human marker enzymes and chromosomes. The results of these studies were presented previously (Fidziańska et al. 1979). Among the tested hybrids 10 informative clones were found. They are presented in Table 2.

Table 2. Segregation of human marker enzymes and chromosomes in 10 informative clones of human-Chinese hamster somatic cell hybrids used in mapping of GPX1 gene

Clone	Number of metaphases scored	Chromosomes[1]				GPX1	Enzyme	PGM2[4]
		3	4	3qter-3p23:: 4q35-4qter	3pter-3p23:: 4q35-4pter			
1	17	–	–	35.0	–	+		–
27	30	–	–	73.0	–	+		–
44	17	–	–	76.4	–	+		–
45	16	–	–	100.0	–	+		–
			9	3qter-3q13:: 9q12 or 13--9qter	3pter-3q13:: 9q12 or 13--9qter		ACO1[2]	AK1[3]
15	22	–	36.4	–	68.2	+	+	+
16	31	–	9.7	–	62.2	+	not tested	+
20	22	–	72.7	22.7	77.3	+	+	+
31	24	–	12.5	8.3	–	–	+	+
32	25	–	80.0	92.0	–	–	+	+
60	27	–	81.5	11.5	–	–	+	+

[1] Percentage of metaphase with the chromosome listed; [2] ACO1 – aconitase soluble; [3] AK1 – adenylate kinase-1; [4] PGM2 – phosphoglucomutase-2.

All the four clones from the crossing of a3 line with the leukocytes carrying t(3; 4) (p23; q35) retained the product of translocation 3qter-3p23: 4q35-4qter in their cells and were positive for human GPX1. Hybrids from another fusion experiment between a3 and leukocytes with t(3; 9) (q13: q12 or 13) consisted of both positive and negative clones. The positive ones retained the product of the translocation carrying the segment 3pter-3q13 in the cells. Three other clones with the most important among them, clone No. 32, which had a large portion of 3q13-3qter (92%) in the cells, did not express the human GPX1 activity. The results presented above showed, that GPX1 segregated with 3p23-3qter and 3pter-3q13 and, thus, the gene could be mapped to the region 3p23-3q13.

2. The mapping of the gene coding for ARSB

Two different balanced translocations involving chromosome 5 were used in the experiments. Human cells were obtained from patients carrying t(5; 16) (p11; p11) and t(5; 21) (q11; q22). In the first case the skin fibroblasts were fused with a3 line in

two independent experiments, which were described earlier (Fidziańska et al.1984). 103 hybrid clones were checked for the presence of human chromosomes and marker enzymes. 22 of them were positive for human ARSB, and 6 of them appeared to have as the only portion of chromosome 5 — the segment 5p11-5qter (Table 3). 37 hybrids

Table 3. Segregation of human marker enzymes and chromosomes in 10 informative clones of human-Chinese hamster somatic cell hybrids carrying the products of translocation t (5; 16) (p11; p11)

Clone	Number of metaphases scored	Chromosome[1]				ARSB	Enzyme HEXB	DIA4
		5	16	5qter-5p11:: 16p11-16pter	5pter-5p11:: 16p11-16qter			
3	52	–	65.4	96.2	–	+	+	+
23	30	–	33.3	56.7	–	+	+	+
26	47	–	70.2	87.2	–	+	+	+
31	21	–	71.4	76.2	–	+	+	+
34	31	–	83.9	96.8	–	+	+	+
35	30	–	–	63.3	–	+	+	–
11	67	–	3.0	–	47.8	–	+	+
57	41	–	7.3	–	39.0	–	–	+
54	29	6.9	37.9	–	34.4	–	+	+
58	48	–	14.8	–	41.6	–	–	+

[1] Percentage of metaphase with the chromosome listed.

were positive for human diaphorase-4 (DIA4), a marker for 16q12-16q21. Four of them retained the product of the translocation carrying the segment 5pter-5p11 in their cells. They were ARSB negative. It was obvious from these experiments that ARSB activity was connected with the region 5p11-5qter and with hexosaminidase B (HEXB), a marker for 5q13, which segregated with ARSB. The further step in regional localization of ARSB was to apply the leukocytes from a patient carrying the reciprocal balanced translocation t(5; 21) (q11; q22) to fuse them with a3. A more detailed analysis of the somatic cell hybrids obtained from the crosses was described (Fidziańska et al., submitted for publication). Table 4 shows 9 informative clones, both positive and negative for human ARSB, detected after chromosomal analysis,

Table 4. Segregation of human marker enzymes and chromosomes in 9 informative clones of human-Chinese hamster somatic cell hybrids carrying the products of translocation t (5; 21) (q11; q22)

Clone	Number of metaphases scored	Chromosome[1]				ARSB	Enzyme HEXB	SOD1
		5	21	5qter-5q11:: 21q22-21pter	5pter-5q11:: 21q22-21qter			
218	30	–	60.0	70.0	–	+	+	+
221	30	–	73.3	66.7	–	+	+	+
286	30	–	56.7	93.3	–	+	+	+
254	31	–	77.4	87.1	6.4	+	+	+
228	25	–	76.0	84.0	–	+	+	+
285	25	–	76.0	92.0	–	+	+	+
206	42	–	71.4	–	76.2	–	–	+
207	28	–	71.4	–	53.6	–	–	+
225	34	–	70.6	–	64.7	–	–	+

[1] Percentage of metaphase with the chromosome listed

and a human marker enzyme screening of 90 independent hybrids. There were only 12 hybrids which expressed the activity of human ARSB (6 of them were informative). 27 expressed human superoxide dismutase-1 (SOD1), a marker for 21q22. Six clones retained the region 5q11-5qter in their cells and were positive for ARSB, three others, negative for ARSB, had the fragment 5pter-5q11. The activity of human SOD1 was detected in all the 9 informative clones, but all of them retained an intact 21 apart from the portion 21pter-21q22 in ARSB positive clones. ARSB segregated with HEXB as it had been in the crosses of a3 with t(5; 16). The results obtained in these two experiments permit to assign a gene for ARSB to q11-qter of chromosome 5.

DISCUSSION

There are several methods to fuse human and animal cells (Ringertz, Savage 1976), and still there is a strong need to find more efficient techniques. Both inactivated Sendai virus and PEG have proved to be active fusion agents. The present paper presents results of cell fusion in the suspension using 55% solution of PEG 6000. A variety of mammalian cells was used to show that this technique can be safely applied to fuse human cells in complementation studies and animal cells to isolate viruses, as well as rodent and human cells to receive somatic hybrids. In all the crosses, the efficiency of cell fusion was high (50 - 70%), leaving cells viable and able to produce growing clones of hybrids, though the number of multinucleate and giant cells was large. As a matter of fact, the efficiency of hybrid cell formation was very high and several hundreds colonies could be isolated from each experiment. Nevertheless, it seems worth of pointing out, that the newly developed technique of electrical fusion (Rivera et al. 1983), which can be carried out during microscopic observation, offers some advantages as compared to glycol-mediated fusion, for example, in the studies on gene complementation analysis with heterokaryons. The choice of the technique, which leads to extensive cell fusion, and application of PEG 6000 in a cell suspension in that case, were only the first step to receive human-Chinese hamster somatic cell hybrids, which could be used in mapping two structural genes, we were interested in, coding for GPX1 and ARSB. These both genes have been already mapped to human chromosomes: GPX1 to chromosome 3 (Donald et al. 1979, Wijnen et al. 1978), and ARSB to chromosome 5 though their regional localization remained unknown. The use of cells carrying different reciprocal balanced translocations involving chromosome 3 made it possible to assign the gene for GPX1 to 3p23-3q13 by analysis of somatic cell hybrids. The results were in agreement with those described by Johannsmann et al. (1979), who mapped the gene in the region 3p13-3q12. The gene coding for ARSB was mapped to chromosome 5 by Hellkuhl, Grzeschik (1978) and DeLuca et al. (1979). Our studies are the first to deal with the regional localization of gene. The results obtained from the analysis of man-Chinese hamster somatic cell hybrids carrying the product of translocations t(5; 16) (p11; p11) and t(5; 21) (q11; q22) in their cells permit mapping of ARSB to 5q11-

-5qter. Continuation of the studies aimed at a more precise localization of gene should be based on the use of translocations involving the long arm of chromosome 5 and a number of DNA probes representing DNA sequences from chromosome 5.

REFERENCES

1. Davidson R. L., Gerald P. S. (1976). Improved techniques for the induction of mammalian cell hybridization by polyethylene glycol. Somat. Cell Genet., 2: 165 - 176.
2. DeLuca C., Brown J. A., Shows T. B. (1979). Lysosomal arylsulfatase deficiencies in humans: chromosome assignments for arylsulfatase A and B. Proc. natn. Acad. Sci. USA. 76: 1957 - 1961.
3. Donald J. L., Wang H. S., Hamerton J. L. (1979). Confirmation of the assignment of a glutathione peroxidase locus to chromosome 3 in man. Cytogenet. Cell Genet., 23: 141-143.
4. Fidziańska E., Abramowicz T., Czartoryska B., Głogowska I., Górska D., Mazurczak T., Rodo M., Wehr H., Witkowska J. (1984). The further step in regional localization of the gene coding for human arylsulfatase B (ARSB). Submitted for publication.
5. Fidziańska E., Abramowicz T., Czartoryska B., Głogowska I., Górska D., Rodo M. (1984). Assignment of the gene for human arylsulfatase B, ARSB, to chromosome 5p11-5qter. Cytogenet. Cell Genet., in print.
6. Fidziańska E., Abramowicz T., Pawłowska B., Głogowska I., Zaremba J., Wijnen L. M. M., Meera Khan P. (1979). Regional localization of GPX1 on human chromosome 3. Human Gene Mapping 5 (1979). Cytogenet. Cell Genet., 25: 153.
7. Fidziańska E., Abramowicz T., Pawłowska B., Głogowska I., Zaremba J., Wijnen L. M. M., Meera Khan P. (1980). Human glutathione peroxidase-1 (GPX1) gene mapped to chromosomal region 3p23-3q13 by using human-rodent somatic cell hybrids. Genetica Polonica, 21: 477 - 481.
8. Fidziańska E., Hicks J. T., Kim E., Głogowska I., Abramowicz T. (1979). Polyethylene glycol-mediated somatic cell fusion. Experience with fresh human leukocytes. Genetica Polonica, 20: 135 - 139.
9. Hellkuhl B., Grzeschik K-H. (1978). Assignment of a gene for arylsulfatase B to human chromosome 5 using human-mouse somatic cell hybrids. Human Gene Mapping 4 (1977). Cytogenet. Cell Genet., 22: 203 - 206.
10. Johannsmann R., Hellkuhl B., Grzeschik K-H. (1979). Regional assignment of a gene for glutathione peroxidase on human chromosome 3. Human Gene Mapping 5. Cytogenet. Cell Genet., 25: 167.
11. Littlefield J. W. (1964). Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. Science, 145: 709 - 710.
12. McKusick V. A. (1982). The human genome through the eyes of a clinical geneticist. Human Gene Mapping 6 (1981). Cytogen. Cell Genet., 32: 7 - 23.
13. O'Brien T. C., Albrecht P., Hannah J. E., Tauraso N. M., Robbins B., Trimmer R. W. (1972). Foamy virus scrotypes 1 and 2 in *Rhesus* monkey tissues. Arch. ges. Virusforsch., 38: 216 - 224.
14. Ringertz N. R., Savage R. E. (1975). Cell hybrids. Acad. Press, New York, San Francisco, London.
15. Rivera H., Pohl H. A., Czerski P., Swicord M. L. (1983). Electrical fusion of cells and nuclei in vitro: a preliminary note. J. Biol. Physics., 11: 63 - 65.
16. Skolnick M. H., Willard H. F., Menlove L. A. (1984). Report of the committee on human gene mapping by recombinat DNA techniques. Human Gene Mapping 7 (1983). Cytogenet. Cell Genet., 37: 210 - 273

17. Wijnen L. M. M., Monteba van Heuvel M., Pearson P. L., Meera Khan P. (1978). Assignment of a gene for glutathione peroxidase (GPX1) to human chromosome 3. Cytogenet. Cell Genet., 22: 232 - 235.

ZASTOSOWANIE HYBRYDYZACJI KOMÓREK SOMATYCZNYCH DO REGIONALNEJ LOKALIZACJI GENÓW STRUKTURALNYCH KODUJĄCYCH PEROKSYDAZĘ GLUTATIONOWĄ-1 (GPX1) I ARYLOSULFATAZĘ B (ARSB)

Streszczenie

Wysoką częstość fuzji komórkowej obserwowano w wielu różnych komórkach ludzkich i zwierzęcych po traktowaniu ich glikolem polietylenowym (PEG) 6000. Wydajność hybrydyzacji była również wysoka; po krzyżowaniu komórek ludzkich z mysimi i chomiczymi otrzymywano w każdym doświadczeniu od kilkudziesięciu do kilkuset niezależnych klonów hybryd somatycznych.

Po fuzji komórek linii chomika chińskiego, a3 (TK^-) z leukocytami krwi i fibroblastami skóry nosicieli różnych translokacji zrównoważonych, obejmujących chromosomy 3 i 5, izolowano kilkaset hybryd. Analiza cytogenetyczna i biochemiczna tych hybryd pozwoliła na lokalizację genu strukturalnego GPX1 w odcinku 3p23-3q13, a genu ARSB — 5q11-5q ter.

ГИБРИДИЗАЦИЯ СОМАТИЧЕСКИХ КЛЕТОК, ИСПОЛЬЗУЕМАЯ В РЕГИОНАЛЬНОМ ПЛАНЕ СТРУКТУРНЫХ ГЕНОВ, КОДИРУЮЩИХ ГЛЮТАТИОННУЮ ПЕРОКСИДАЗУ-I (GPX1) И АРИЛСУЛЬФАТАЗУ В (ARSB) У ЧЕЛОВЕКА

Резюме

Высокая частота слияния клеток наблюдалась во время скрещивания некоторых клеток млекопитающих различного происхождения с помощью PEG 6000. В результате техники, используемой для слияния клеток человека и грызунов, получено несколько сотен независимых гибридных клонов соматических клеток человека и грызунов. Анализ гибридов, полученных в экспериментах слияния a3 (TK^-) и человеческих клеток, несущих различные реципрокные балансированные транслокации, включающие хромосомы 3 и 6, позволил отнести структурные гены для GPX1 к хромосоме 3p23-3q13 и гены для ARSB к 5q11-5qter.