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Assignment of the gene for human neutral alpha-glucosidase C to chromosome 15

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Abstract. Human neutral α-glucosidase C (GANC) can be separated from the homologous mouse isozyme by starch gel electrophoresis at pH 6.5. A total of 40 clones (13 primary and 27 secondary) were derived from eight separate hybridization experiments between the mouse HPRT deficient RAG cell line and eight different human long term lymphoid cell lines or fetal cells.

The thirteen primary clones showed 100% concordance between the expression of the human enzyme and the presence or absence of human chromosome 15. Analysis of the 27 secondary clones showed only two subclones discordant for segregation of human GANC and enzyme markers for 15. The two apparently discordant clones for human GANC were both derived from the same RAG $\times$ human fetal lung primary clone, and both lacked GANC activity, while retaining a 15. Since human *GANC* is polymorphic with a null allele at high frequency (MARTINIUK and HIRSCHHORN, 1980), it is possible that these subclones carried one chromosome with a null allele for GANC. Alternatively there could been an undetected chromosome break between the *GANC* locus and the loci of the marker enzymes. Whatever the reason for the two apparently discordant subclones, combined data from all 40 clones show 95% concordant segregation for human GANC and No. 15.

Human $\times$ rodent somatic cell hybrids have been used to study the chromosomal location of genes coding for many proteins. The ability to distinguish human from rodent proteins by electrophoretic or immunological methods in conjunction with cell fusion has made possible the mapping of a large number of genes coding for these proteins (MCKUSICK and RUDDLE, 1977). In the course of our investigations of the neutral α-glucosidases, we were able to identify human and mouse neutral α-glucosidase C (GANC) by electrophoresis on starch gel and staining with the artificial substrate, 4-methylumbelliferyl-α-

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D-glucopyranoside. We present evidence for assignment of the locus for human neutral α-glucosidase C *(GANC)* to chromosome 15.

Materials and methods

Cell lines and hybridization procedures

The mouse cell line used for fusion was the HPRT deficient RAG line (KLEBE et al., 1970). The parental human lymphoid lines used and the clones generated and analysed were: ODY: one primary and five of its secondary clones plus three secondary clones from a different primary clone that had not been analysed; RPMI 1788: one primary clone; Cali: two primary clones; and NB 103: one primary and two secondary clones. Human fetal cells used for fusion were derived from primary cultures of lung, kidney, and liver from two different fetuses. The source of the cells and clones generated were fetus-1 kidney: five primary clones and six secondary clones from one of the primary clones; fetus-1 liver: one primary clone; fetus-2 liver: two primary clones; and fetus-1 lung: eleven subclones from a single primary clone that had not been analysed.

Cells were fused with inactivated Sendai virus as described previously (SMITH et al., 1975), or using a polyethylene glycol gradient (VAUGHAN et al., 1976). A total of 40 clones (13 primary and 27 secondary) derived from eight separate hybridizations between the mouse HPRT deficient RAG cell line and human long term lymphoid lines or fetal cells were isolated using the cloning method described by HAM and PUCK (1962) and characterized.

Sample preparation

Cells from one 75 cm^2 flask were harvested and frozen at $-60°$ C. The cells were suspended in 70 μl H_2O and disrupted with three 5 sec ultrasonic pulses (Heat Systems-Ultrasonics Inc.) in 400 μl Eppendorf tubes. The extract was centrifuged for 5 min at 4° C in a Beckman microfuge; the supernatent was used for electrophoresis. Cell extracts for analysis of enzymes other than GANC were prepared in distilled water (0.1 to 0.3 ml per one 75 cm^2 flask) either by five cycles of freezing and thawing or by sonication.

For visualization of GANC in the absence of neutral α-glucosidase AB, extracts of parental lines were treated with sepharose-Con A (Pharmacia); 20 μl of sepharose-Con A washed in 0.2 M glycine–2 mM $MgCl_2$, pH 7.0, was incubated in 400 μl Eppendorf tubes with 20 μl extract for 60 min at 25° C with constant rocking. The mixture was centrifuged in a Beckman microfuge for 5 min at 4° C, and the supernatant electrophoresed.

Starch gel electrophoresis

Electrophoresis was carried out in horizontal gels of 10% hydrolyzed starch (Connaught Lab., Ltd.) in 0.01 M sodium phosphate, pH 6.5 or pH 7.0, at 10 V/cm for 30 min, followed by 11.5 V/cm for 4.5 h on cooling plates kept at 4° C. Extracts were applied to Whatman 17 chromatography paper inserts. The gel was stained by overlaying with Whatman 17 chromatographic paper saturated with 4-methylumbelliferyl-α-D-glucopyranoside (0.5 mg/ml, dissolved by briefly heating in a small volume of ethanol and then diluted with 0.1 M sodium phosphate, pH 7.5) on the gel. Enzyme activity appeared as fluorescent bands under long wave UV light. On occasion, NH_4OH was sprayed on the gel to increase the intensity of the fluorescence (SWALLOW et al., 1975).

Photography

Bands of GANC activity were photographed with 35 mm Kodacolor II film (ASA 100) with a yellow filter and two UV lamps (UltraViolet Products, Blak-Ray lamp, 366 nm) giving side illumination. Black and white prints were made from the color negatives.

Marker enzyme and chromosome analysis

Enzymes were analyzed by horizontal starch-gel or cellogel electrophoresis using the methods described by HARRIS and HOPKINSON (1976). The enzymes analysed and their chromosomal assignments are listed in table I. Five hybrid clones (RAG/ODY LB 11, 11-A, D, H and I) were analyzed for human hexosaminidase B (human 5) using Ouchterlony double diffusion with rabbit anti-Hex B (a generous gift of Dr. D.M. SWALLOW) (SWALLOW et al., 1977). Metaphase spreads for chromosome analysis were prepared as previously described (SMITH et al., 1975). Chromosomes were banded using quinacrine hydrochloride fluorescence and trypsin-Giemsa treatment. The Giemsa-

Table I. Segregation of GANC and chromosomes and enzyme markers

Chromosome	Enzyme marker[3]	No. of primary clones[1]			No. of secondary clones[2]			Total % concordant
		Concordant	Discordant	% concordant	Concordant	Discordant	% concordant	
1	PEPC, PGD	5	6	45	14	13	52	50
2	MDH1	3	9	25	11	3	74	52
3	–	7	6	54	5	2	71	67
4	PGM2	5	3	62	10	9	53	56
5	HEXB	8	3	73	13	6	68	70
6	ME1, SOD2	4	8	33	12	6	67	57
7	GUSB	5	8	38	13	3	81	62
8	GSR	5	4	56	6	8	43	48
9	ACO1, AK1, 3	8	3	73	7	10	41	54
10	GOT1	6	5	54	12	1	92	75
11	LDHA	7	4	64	10	4	71	68
12	PEPB	9	3	75	12	9	57	64
13	ESD	2	3	40	9	7	56	52
14	NP	7	3	70	13	12	52	57
15	MPI, HEXA	13	0	100	25	2	92	95
16	–	7	6	54	3	4	43	50
17	–	4	9	31	4	3	57	40
18	PEPA	8	3	73	3	9	25	48
19	GPI	7	2	78	8	13	38	50
20	ADA	6	6	50	12	7	63	58
21	SOD1	3	5	38	18	7	72	67
22	ACO2	1	2	33	1	4	20	25
X	G6PD	6	6	50	12	7	63	58

[1] Thirteen independent primary clones derived from seven separate fusions of RAG with seven different human parental lines.

[2] A total of 27 secondary clones were examined. Sixteen subclones were derived from the primary clones listed in (1). Subclones identical to their primary clone or other subclones from that fusion were not included. No discordant clones were eliminated. Eleven secondary subclones were derived from a primary clone from an additional fusion that was not analysed. The two discordant clones were derived from this primary clone. Exact secondary clones generated from individual primary clones are described in Materials and methods.

[3] See INTERNATIONAL SYSTEM for HUMAN GENE NOMENCLATURE (ISGN, 1979) for symbol names.

11 staining method was used to distinguish mouse and human chromosomes (FRIEND et al., 1976). Five to ten karyotypes were analysed from each clone. The clone was considered to be positive for a specific human chromosome if the chromosome was present in 20% or more of the karyotypes and the clone exhibited the human marker enzyme. For most chromosomes, determination was based on both karyotype and enzyme analysis. However, Nos. 3, 16, and 17 were identified by karyotype alone. We had difficulty in unequivocally distinguishing human acrocentric chromosomes (13–15, 21, and 22) from each other when preparations which had been stained by the G-11 procedure

were then stained with quinacrine. Therefore the presence of these chromosomes was considered to be definitive only when combined with enzyme analysis. The chromosomes of the secondary hybrid clones were determined mostly by isozyme analysis.

Results

We observed two major forms of neutral α-glucosidase activity after electrophoresis of extracts of mouse RAG cells or human cells in starch gel and visualization of enzyme activity with 4-methylumbelliferyl-α-D-glucopyranoside at neutral pH. In the human, these isozymes have been called neutral α-glucosidase AB, and neutral α-glucosidase C (SWALLOW et al., 1975). Alpha-glucosidase AB appears to consist of two related isozymes which differ in degree of sialation and which migrate rapidly towards the anode. Neutral α-glucosidase C (GANC) is unaffected by treatment with neuraminidase and migrates anodally less rapidly (SWALLOW et al., 1975). Preliminary studies, based upon comparison of binding to sepharose-Con A, indicate that the most anodally migrating rodent neutral α-glucosidase enzyme was glycosylated, and therefore apparently homologous with the most anodally migrating human glycosylated neutral α-glucosidase AB (SWALLOW, 1977; personal observation). The more slowly migrating rodent α-glucosidase therefore appears to be homologous with human GANC. We could not detect any clear differences in mobility between human and rodent neutral α-glucosidase AB under the conditions used. In contrast, we could easily distinguish the human and RAG isozyme of GANC, since the human enzyme migrated anodally more rapidly than did the homologous mouse enzyme (fig. 1). The RAG cells exhibited a double band of neutral α-glucosidase C activity. The human lines exhibited a single band of GANC activity in most cases. One human parental line (ODY) exhibited a double band of GANC activity. There are four alleles at the *GANC* locus, including a silent or null allele (MARTINIUK and HIRSCHHORN, 1980).

Starch gel electrophoresis of human × RAG hybrid clones revealed clones both positive and negative for the human enzyme (fig. 1). In hybrid clones positive for the human enzyme, no heteromeric enzyme forms were observed. No neutral α-gluco-

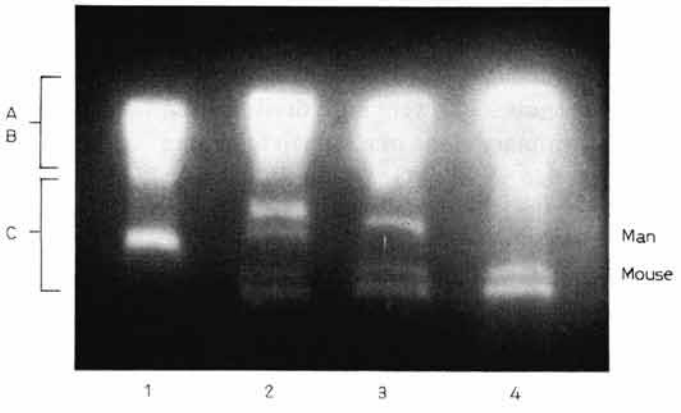

Fig. 1. Photograph of starch gel electrophoresis of GANC. Channel 1 contains extract of human lymphoid cells, Channel 4 contains extract of mouse RAG cells, Channels 2 and 3 show human×RAG hybrids positive for human GANC. Channels 1 and 3 show the usual single band for the human isozyme, while Channel 2 shows the double banded (ODY) human isozyme in a positive human×RAG hybrid.

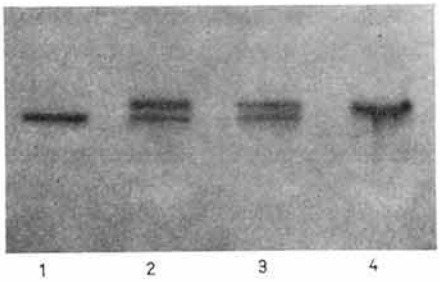

Fig. 2. Photograph of starch gel electrophoresis of MPI in human lymphoid cells, mouse RAG cells, and human×RAG hybrids. Channel 1 contains extract of human lymphoid cells, Channel 4 contains extract of mouse RAG cells, and Channels 2 and 3 contain extracts of hybrids positive for both human and mouse MPI and GANC.

Table II. Mapping of *GANC* to chromosome 15

	Primary clones		Secondary clones	
	15^+	15^-	15^+	15^-
$GANC^+$	8	0	17	0
$GANC^-$	0	5	2	8

sidase isozyme other than α-glucosidase AB and α-glucosidase C were seen in any of the hybrid clones, and specifically no band of activity with mobility like that of the renal α-glucosidase (DREYFUS et al., 1972; personal observation) was observed.

Forty hybrid clones (13 primary and 27 secondary) of RAG and various human cell lines (fetal liver, kidney, lung, and long term lymphoid lines) were examined for the segregation of GANC. The 13 primary clones (table I) showed 100% concordant segregation of human GANC and No. 15, detected by MPI (fig. 2) or HEXA. Analysis of the 27 secondary clones (table I) revealed two discordant clones (fig. 3). These apparently discordant subclones were both derived from the same primary clone of a human fetal lung parental line × RAG fusion, and both contained the 15 but did not have GANC (table II).

Both clones exhibited human MPI; one clone tested for HEXA had the human isozyme. The chromosomes present in these clones were examined using the G-11 staining method to distinguish the human and mouse chromosomes. No evidence of a human-mouse chromosomal translocation was seen.

The combined thirteen primary clones and 27 secondary clones showed 95% concordant segregation of GANC with 15.

Because another neutral glycosidase, neutral α-mannosidase maps to 15 (CHAMPION et al., 1978) and because of the wide substrate specificities of these enzymes, we compared the electrophoretic mobility of the two enzymes. GANC migrated more anodally than neutral α-mannosidase on starch gel electrophoresis, indicating that they are distinct and separate enzymes (personal observation).

Discussion

We have found concordant segregation of human GANC with No. 15. Although 100% concordance was observed in the initial 13 primary clones, two of 27 secondary hybrid clones derived from the same primary clone contained the human 15 as determined by MPI and/or HEXA but no human GANC activity.

We have recently found that there are four alleles of *GANC* and that one is a null allele. Its frequency is 0.17, and therefore

approximately one-third of individuals are heterozygous for this allele (MARTINIUK and HIRSCHHORN, 1980), but are not distinguishable by starch gel electrophoresis from homozygotes for one of the other three alleles. Therefore there is approximately a 17% chance that a clone retaining one 15 will carry the null allele. A total of 27 clones expressed chromosome 15 marker enzymes. Two of these (8%) did not express GANC. The two discordant subclones may retain a 15 with the marker enzyme and a null allele, and so synthesize no GANC. Confirmatory gene mapping of *GANC* should utilise parental cells that exhibit a double band of enzyme activity and so do not carry the null allele. One of the cell lines (ODY) used in this study did show a double band of GANC activity, and none of the nine segregating clones derived from fusions using it were discordant. Alternatively, the two discordant secondary clones could simply reflect the greater sensitivity of the MPI staining method relative to that for GANC or an undetected chromosome break between the *GANC* locus and *MPI* or *HEXA*. Examination of all six enzyme markers regionally assigned to 15 might be informative in these discordant clones (SHIMIZU et al., 1977; CHAMPION et al., 1978; HELLKUHL et al., 1978; DONALD et al., 1979). Whatever the reason for the two discordant subclones, we conclude from the combined data (total concordance = 95%) that the gene for human GANC maps to chromosome 15.

The other enzyme loci mapped to 15 are mannose phosphate isomerase, *MPI* (MC MORRIS et al., 1973; VAN HEYNINGEN et al., 1975), cytoplasmic mannosidase, *MANA* (CHAMPION et al., 1978), hexosaminidase A, *HEXA* (LALLEY et al., 1974; GILBERT et al., 1975; NGUYEN VAN CONG et al., 1975), sorbitol dehydrogenase, *SORD* (DONALD et al., 1979), pyruvate kinase-3, *PKM2* (VAN HEYNINGEN et al., 1975), and mitochondrial isocitrate dehydrogenase, *IDH2* (BRUNS et al., 1976; SHIMIZU et al., 1977). One of these enzymes, MANA, is also a neutral glycosidase. There are several other glycosidases that have neutral pH optima, and it will be of interest to determine if the genes coding for these neutral glycosidases are clustered together on a single chromosome. No such clustering appears to occur for the acid glycosidases, since lysosomal acid α-glucosidase, *GAA* (SOLOMON et al., 1979), β-glucuronidase, *GUSB* (GRZESCHIK, 1976; CHERN and CROCE, 1976), α-mannosidase, *MANA* (INGRAM et al., 1977; CHAMPION and SHOWS, 1977), and hexosaminidase A, *HEXA* (LALLEY et al., 1974; GILBERT et al., 1975; NGUYEN VAN CONG et al., 1975) and B, *HEXB* (LALLEY et al., 1974; GILBERT et al., 1975) have each been assigned to a different chromosome. There also would appear to be no clustering of acidic and neutral isozymes exhibiting similar substrate specificities since the neutral and acidic isozymes of α-mannosidase (CHAMPION et al., 1978; INGRAM et al., 1977) and now of α-glucosidase, have been assigned to different chromosomes (SOLOMON et al., 1979).

Finally, *GANC* is the first polymorphic enzyme marker assigned to 15, and therefore it may be useful in family linkage studies.

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