

Pig gene mapping : assignment of the genes for mannosephosphate isomerase (MPI) and nucleoside phosphorylase (NP) to chromosome no. 7

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Summary

Ten hybrid cell lines (pig × a3 Chinese hamster) have been investigated with respect to their content of pig chromosomes and the enzymes MPI and NP. The pig chromosomes have been identified by their Q-band pattern. The enzymes were analysed by cellulose acetate gel electrophoresis. The results indicate that the genes for MPI and NP are located on chromosome no. 7.

Key words : Pig, gene mapping, mannosephosphate isomerase (MPI), nucleoside phosphorylase (NP).

Résumé

Carte génique du porc : assignation des gènes de la mannosephosphate isomérase (MPI) et de la nucléoside phosphorylase (NP) au chromosome n° 7

La présence de chromosomes porcins et des enzymes MPI et NP a été recherchée dans 10 lignées de cellules hybrides (porc × a3 hamster chinois). Les chromosomes porcins ont été identifiés grâce à leurs bandes Q caractéristiques et les enzymes analysés par électrophorèse sur gel d'acétate de cellulose. Les résultats indiquent que les gènes codant pour MPI et NP sont situés sur le chromosome n° 7.

Mots clés : Porc, carte génique, mannosephosphate isomérase (MPI), nucléoside phosphorylase (NP).

I. Introduction

The present state of the pig gene map includes roughly 40 genes. Most of them belong to linkage groups, whereas only a few genes have been assigned directly to a chromosome. The genes for HPRT, G6PD, PGK and GLA have been located on the

X-chromosome by means of somatic cell hybrids (FOERSTER *et al.*, 1980 ; GELLIN *et al.*, 1980 ; LEONG *et al.*, 1983a). The diseases « splayleg » (LAX, 1971) and « tremor AIII » (HARDING *et al.*, 1973) are also associated with the X-chromosome. Autosomal assignments were made by the methods of family analysis, *in situ* hybridization and mostly somatic cell hybridization. The gene for the G blood group has been assigned to chromosome no. 15 by family studies (FRIES, 1982 ; TIKHONOV & NIKITIN, 1983 ; TIKHONOV *et al.*, 1983 ; FRIES *et al.*, 1984). This implies that the linkage group GPI-HAL-S-H-PO2-PGD could also be located on chromosome no. 15, as a close linkage between the loci for the G and the H blood groups (TIKHONOV *et al.*, 1983) and a weak linkage between the locus for GPI and the centromere of chromosome no. 15 (FRIES *et al.*, 1982) have been found. By *in situ* hybridization the loci for the SLA could be assigned to chromosome no. 7 (GEFFROTIN *et al.*, 1984 ; RABIN *et al.*, 1985 ; ECHARD *et al.*, 1986). Therefore the linkage group C-J (MUIR & RASMUSEN, 1974) is likely to be located on the same chromosome, since a close linkage has been found between the loci for the blood group J and the SLA (HRUBAN *et al.*, 1976). The following genes have been assigned by the approach of somatic cell hybridization. The syntenic group MPI-NP-PKM2 (GELLIN *et al.*, 1981) has been located on chromosome no. 3 (ECHARD *et al.*, 1982 ; 1984) and the gene for SOD1 on chromosome no. 9 (LEONG *et al.*, 1983b). FOERSTER & HECHT (1984) assigned the genes for MDH1 to chromosome no. 3, for LDHA to chromosome no. 4, for LDHB to chromosome no. 5, for PEPB to chromosome no. 11, for PGM1 to chromosome no. 10 and for ME1 to chromosome no. 1 or no. 17. Further they confirmed the assignment of the gene for SOD1 to chromosome no. 9. However, they found the loci for MPI and NP to be on chromosome no. 7, which is in contrast to the assignment by ECHARD *et al.* (1984).

In the following we present our own findings. They confirm the assignment of the genes for MPI and NP to chromosome no. 7 as also do the results of CHRISTENSEN *et al.* (1985).

II. Materials and methods

A. Hybrid cell lines

Cells of the a3 Chinese hamster cell line (WESTERVELD *et al.*, 1971) were fused with pig fibroblasts in one experiment and pig leucocytes in 2 other experiments. The cell suspension was then distributed to petri dishes (10^5 cells/petri dish). The clones were selected in HAT medium (hypoxanthine 8.8×10^{-5} M, aminopterin 4×10^{-7} M, thymidine 3.3×10^{-5} M), to which ouabain (10^{-5} M) was added in the case where pig fibroblasts were involved. Between 9 and 18 days after the fusion the clones were isolated. Per petri dish one single clone, that had no other clones in its neighbourhood, was chosen. By adding one drop of 0.25 p. 100 trypsin solution, a chosen clone was detached and with a pasteur pipette transferred to a small culture flask. After isolation the hybrid cell lines were kept in Ham's F10 medium, supplemented with 10 p. 100 fetal calf serum. The procedures of hybridization, selection and isolation used in this investigation have already been described in detail (DOLF & STRANZINGER, 1982 ; DOLF, 1984).

The 10 hybrid cell lines investigated resulted from 3 independent fusion experiments. In 5 hybrid cell lines the donor cells were pig fibroblasts and in the other 2 and 3, respectively, they were pig leucocytes.

The hybrid lines were kept deep frozen when they were not under investigation. At any time no more than 5 hybrid lines were investigated. Cell preparations for both chromosome and enzyme analysis were made out of the same culture flask at the same passage, but not at the very same time. Therefore the procedure was repeated for each hybrid cell line.

B. Chromosome analysis

The cultures were visually checked several times a day in order to establish the moment when the number of dividing cells was the greatest. The dividing cells were harvested by adding Dispase (0.8 U/ml) to the culture and gently shaking the flask. The resulting cell suspension was centrifuged for 10 min at $450 \times g$. The cell pellet was resuspended in a KCl solution (0.071 M) and incubated at 37°C for 20 min. After 10 min of centrifugation at $450 \times g$, fixative (methanol : acetic acid 3 : 1) was added to the pellet. The fixation was repeated twice. Slides were prepared the following day. The metaphase chromosomes were Q-banded according to the procedure by CASPERSON *et al.* (1969) and identified according to the Reading Conference (1976) in the case of pig chromosomes and RAY & MOHANDAS (1976) in the case of hamster chromosomes.

C. Enzyme analysis

The preparation of the cell lysates and the electrophoresis on cellulose acetate gels were carried out as described by MEERA KHAN (1971) and VAN SOMEREN *et al.* (1974). The following enzymes were investigated :

Glucosephosphate isomerase	EC 5.3.1.9	(GPI)
Lactate dehydrogenase	EC 1.1.1.27	(LDH)
Mannosephosphate isomerase	EC 5.3.1.8	(MPI)
Nucleoside phosphorylase	EC 2.4.2.1	(NP)
Peptidase A		(PEPA)

III. Results

A. Chromosome and enzyme analysis

The pig chromosomes were identified within the metaphases without arranging them in karyotypes (fig. 1). On the average 40 metaphases were analysed per hybrid cell line. In most cases the metaphases were incomplete. As this loss of chromosomes is due to the influence of the chromosome preparation, that is physical factors, rather than biological factors, we assumed that it occurred randomly. Pig chromosomes were considered to be present in a hybrid cell line, when they could be found in at least 10 p. 100 of the investigated metaphases. The pig Y-chromosome has been omitted, as it is highly unlikely to harbor the genes of the investigated enzymes. Also it has not been found in this investigation. The observations concerning the pig chromosomes and the pig enzymes are summarized in table 1. As an example, a zymogram of NP is

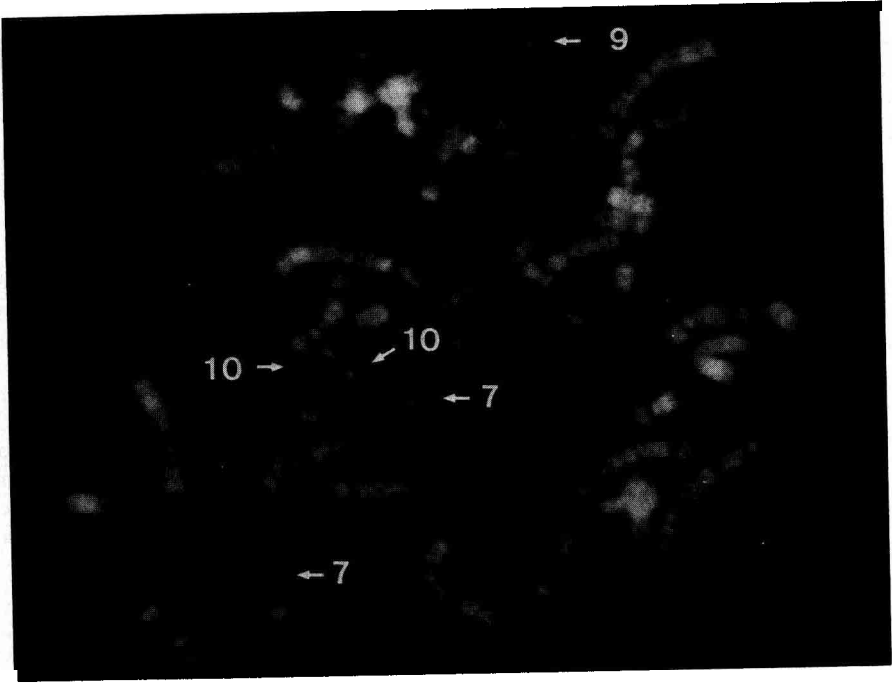


FIG. 1
Q-banded metaphase of hybrid line no. 7. The arrows point to pig chromosomes.

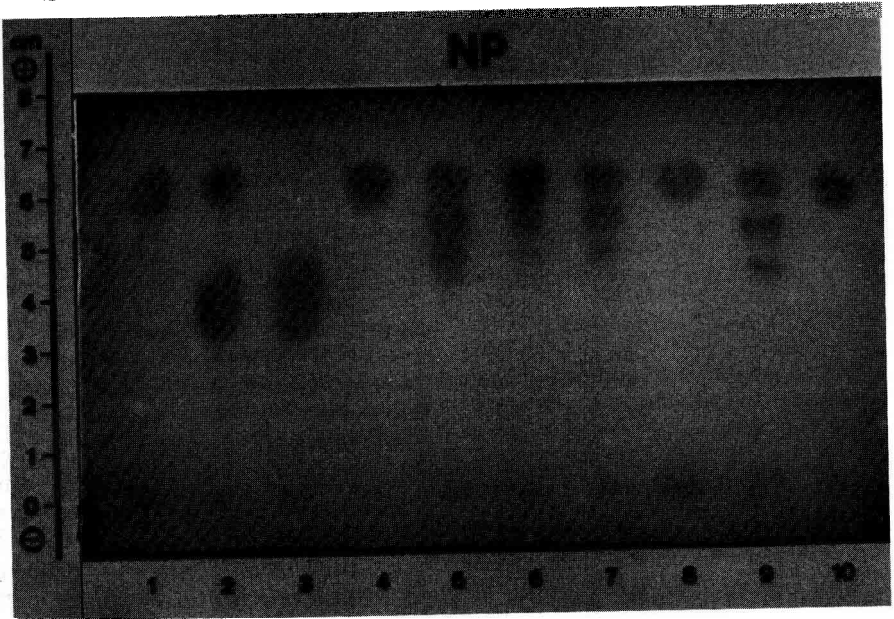


FIG. 2
Zymogram of NP: 1 hamster, 2 pig + hamster, 3 pig, 4 - 10 hybrid lines: 5, 6, 7 and 9 showing pig NP.

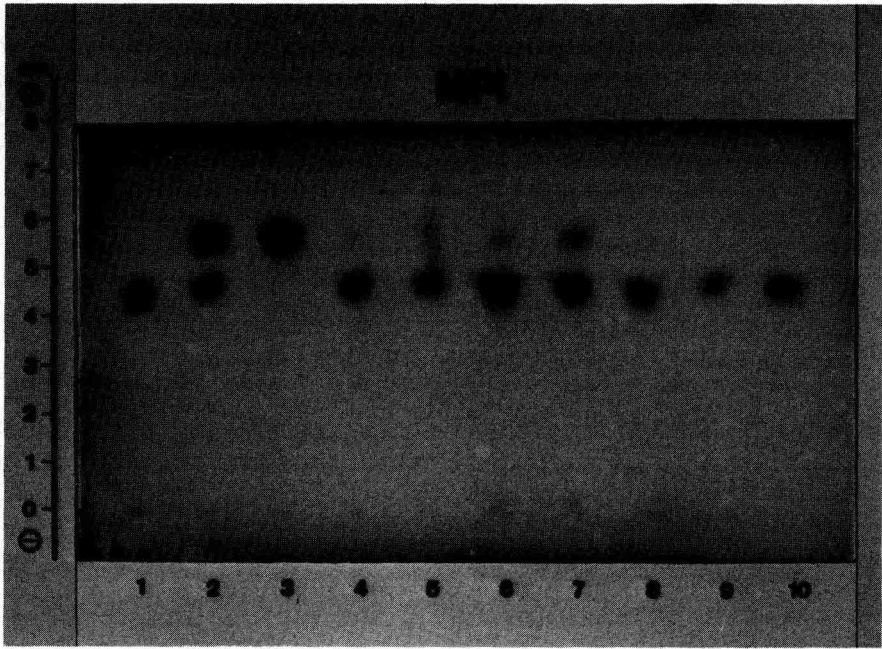


FIG. 3

*Zymogram of MPI : 1 hamster, 2 pig + hamster, 3 pig, 4 - 10 hybrid lines :
4 - 7 showing pig MPI.*

shown in figure 2. As NP is a trimer, 2 intermediate bands can be observed in the cases where both the hamster and the pig NP are expressed. The hybrid lines that express the pig MPI show 2 bands as MPI is a monomer (fig. 3).

B. Chromosomal assignments

There are different approaches to the interpretation of data resulting from investigations in somatic cell hybrids with respect to gene mapping. In this investigation we chose 2 statistical procedures to treat our data (table 1). One procedure is based on the outcome of the values of Phi, Chisq and OR (COWMEADOW & RUDDLE, 1978) and the other on the outcome of the values of the likelihood ratios R_j (DOLF, 1984). Our data did not allow us to assign the genes for GPI, LDH and PEPA. But it was possible to assign the genes for MPI and NP to chromosome no. 7. In this investigation MPI and NP were perfectly syntenic. The values of Phi, Chisq, OR and R_j were calculated for each pig chromosome except the Y-chromosome (table 2).

TABLE 1

The occurrence of pig chromosomes (*p. 100*) and pig enzymes in the investigated hybrid cell lines.

Hybrid cell lines	Enzymes					Chromosomes					
	GPI	LDHA	MPI	NP	PEPA	1	2	3	4	5	6
1		+									8.3
2	+		+	+							35.1
3											76.5
4						33.3		33.3		29.6	
5			+	+				27.5		2.5	
6	+	+	+	+					61.8		1.8
7			+	+		43.6		34.5			
8		+	+	+			50.0	6.3	6.3		
9	+		+	+			2.9		5.7		57.1
10	+	+	+	+				15.6		6.3	28.1

TABLE 2

The results for the enzymes MPI and NP at a pig chromosome level of 10 *p. 100* :
The values of Phi, Chisq, OR and R_i .

Chr	+/+	-/+	+/-	-/-	Con	Dis	Phi	Chisq	OR	R_i
7	5	0	2	3	8	2	.65	4.29	12.01	27.22
8	3	0	4	3	6	4	.43	1.84	7.01	3.18
9	4	1	3	2	6	4	.22	.48	8.74	2.43
12	5	2	2	1	6	4	.05	.02	13.15	1.86
6	3	1	4	2	5	5	.09	.08	8.02	.83
3	3	1	4	2	5	5	.09	.08	7.07	.83
10	3	1	4	2	5	5	.09	.08	6.92	.83
2	1	0	6	3	4	6	.22	.48	11.91	.37
4	1	0	6	3	4	6	.22	.48	6.91	.37
13	0	0	7	3	3	7	nc	nc	1.91	.13
14	0	0	7	3	3	7	nc	nc	1.91	.13
15	0	0	7	3	3	7	nc	nc	1.91	.13
16	0	0	7	3	3	7	nc	nc	1.91	.13
17	0	0	7	3	3	7	nc	nc	1.91	.13
18	0	0	7	3	3	7	nc	nc	1.91	.13
X	0	0	7	3	3	7	nc	nc	1.91	.13
1	1	1	6	2	3	7	-.22	.48	2.57	.10
5	0	1	7	2	2	8	-.51	2.59	1.14	.03
11	0	1	7	2	2	8	-.51	2.59	1.14	.03

Chr : chromosome ; Con : concordant ; Dis : discordant ; nc : not calculated.

7	8	9	10	11	12	13	14	15	16	17	18	X
15.6	45.5	14.9	16.7 26.0	8.3	16.7 11.7							
40.0		11.1		11.1	22.2							
1.8	20.0	12.5	5.0		20.0							
109.1	3.6		5.5		27.3							
6.3	54.5	54.5	67.3		34.5							
65.7		34.3	31.4	5.7								5.7
31.3			3.1		18.8							

IV. Discussion

In order to reflect on the opposing assignments of MPI and NP one has to consider their origin.

The assignment of the genes for MPI and NP to chromosome no. 7, as presented in this paper, is connected with 20 p. 100 discordance at a donor chromosome level of 10 p. 100 (10 chromosomes per 100 metaphases). FOERSTER & HECHT (1984) had a discordance of 11 p. 100 at a donor chromosome level of 30 p. 100, and CHRISTENSEN *et al.* (1985) had one of 0 p. 100 at a level of 20 p. 100. The assignment to chromosome no. 3 by ECHARD *et al.* (1982 ; 1984) is connected with 0 p. 100 discordancy at a donor chromosome level of 90 p. 100.

A possible explanation of the 2 contradictory assignments could be, e.g. a submicroscopic translocation of the genes concerned, from chromosome no. 7 to no. 3. Such a translocation would imply that the 3 loci investigated are closely linked. This possibility can not be excluded as ECHARD *et al.* (1982 ; 1984) investigated secondary hybrid cell lines which were derived from one single hybrid cell line. But as this hypothesis is based on many assumptions, it cannot fully satisfy.

Another explanation for the different assignments, and especially for the different rates or discordancy, is the differences in the chosen donor chromosome levels. If the chosen donor chromosome level differs too much from the chromosome level really needed for the detection of a certain gene product, chance associations will occur. We considered a donor chromosome to be present at a level of 10 p. 100, whereas ECHARD *et al.* (1982 ; 1984) did not accept a level of 33 p. 100. In order to determine a reasonable donor chromosome level, one would have to proceed as suggested by BURGERHOUT (1978). By applying this method the minimal frequency of the donor chromosomes necessary to detect a certain gene product is evaluated. In his investigation BURGERHOUT (1978) found a minimal frequency around 10 p. 100 for the detection of UMPK.

Our results are not really supported by the statistical procedures proposed by COWMEADOW & RUDDLE (1978), as the OR value for chromosome no. 12 is greater than the one for chromosome no. 7. Based on our data the likelihood ratios R_j indicate that the assignment of the genes for MPI and NP to the pig chromosome no. 7 is 30 times more likely than the assignment to the chromosome no. 3. If we go down to a donor chromosome level of 5 p. 100, the procedures by COWMEADOW & RUDDLE (1978) also lead to the assignment of the genes for MPI and NP to the pig chromosome no. 7 (table 3). However, we base our assignment only on the observations at a donor chromosome level of 10 p. 100 since we consider this a reasonable level. Table 3 has been added in order to point out the great influence of the donor chromosome level on the interpretation of the data.

TABLE 3

*The results for the enzymes MPI and NP at a pig chromosome level of 5 p. 100 :
The values of Phi, Chisq, OR and R_j .*

Chr	+/+	-/+	+/-	-/-	Con	Dis	Phi	Chisq	OR	R_j
7	6	0	1	3	9	1	.80	6.43	12.68	46.13
10	5	1	2	2	7	3	.36	1.27	10.85	4.12
8	3	0	4	3	6	4	.43	1.84	6.75	1.84
4	3	0	4	3	6	4	.43	1.84	9.25	1.84
3	4	1	3	2	6	4	.22	.48	8.82	1.41
9	4	1	3	2	6	4	.22	.48	7.41	1.41
12	5	2	2	1	6	4	.05	.02	10.65	1.08
2	1	0	6	3	4	6	.22	.48	4.49	.21
X	1	0	6	3	4	6	.22	.48	3.42	.21
6	3	2	4	1	4	6	-.22	.48	5.86	.13
13	0	0	7	3	3	7	nc	nc	1.99	.07
14	0	0	7	3	3	7	nc	nc	1.99	.07
15	0	0	7	3	3	7	nc	nc	1.99	.07
16	0	0	7	3	3	7	nc	nc	1.99	.07
17	0	0	7	3	3	7	nc	nc	1.99	.07
18	0	0	7	3	3	7	nc	nc	1.99	.07
1	1	1	6	2	3	7	-.22	.48	2.65	.06
5	1	1	6	2	3	7	-.22	.48	3.22	.06
11	1	2	6	1	2	8	-.52	2.74	1.98	.01

Chr : chromosome ; Con : concordant ; Dis : discordant ; nc : not calculated.

Four hybrid lines out of 10 showed the pig GPI but not the pig chromosome no. 15. But, as mentioned in the introduction, it is suspected that the linkage group GPI-HAL-S-H-PO2-PGD could be located on chromosome no. 15. We do not worry about this suspicion, because the data it is based on are very weak. For example, FRIES *et al.* (1982) estimated a recombination frequency between the centromere of chromosome no. 15 and the locus for GPI of 30 p. 100. A similar recombination frequency was found by ANDRESEN (1967) between the loci for the G and the H blood group. But this kind of data, obtained by family analysis, can only be considered a hint, since the underlying investigations are not designed to detect this kind of genetic distance. The results presented by TIKHONOV *et al.* (1983) indicate a close linkage of 3.9 p. 100 between the loci for the G and the H blood group. This is somehow in contradiction

with the findings mentioned above. We think it amazing, that such a close linkage has not yet been confirmed by other researchers, especially since the linkage group GPI-HAL-S-H-PO2-PGD has been investigated thoroughly by many researchers. Our results rather indicate that this linkage group is not located on chromosome no. 15.

No attempt was made to map the gene for thymidine kinase (TK). After isolation the clones were kept in normal medium without aminopterin. Therefore we can not exclude the occurrence of revertants or even the loss of the pig chromosome carrying the gene for TK.

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