CORRESPONDENCES BETWEEN HUMAN AND PORCINE HOMOLOGOUS CHROMOSOME SEGMENTS USING BIDIRECTIONAL CHROMOSOME PAINTING

CORRESPONDENCIAS ENTRE SEGMENTOS CROMOSÓMICOS HOMÓLOGOS HUMANOS Y PORCINOS UTILIZANDO COLORACIÓN E HIBRIDACIÓN *IN SITU* BIDIRECCIONAL

Goureau, A[1,3], M. Yerle[1], A. Schmitz[2], J. Riquet[1], D. Milan[1], P. Pinton[1], G. Frelat[2] and J. Gellin[1]

[1]Laboratoire de Génétique Cellulaire. Centre de Recherches. INRA de Toulouse-Auzeville, BP 27. 31326 Castanet Tolosan Cédex. France.

[2]Laboratoire de Cytométrie. DPTE. DSV. Centre d'Etudes Nucléaires, BP 6. 92265 Fontenay-aux-Roses. France.

[3]Ecole Nationale de Formation Agronomique, BP 87. 31326 Castanet Tolosan Cédex. France.

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SUMMARY

To localize candidate genes, QTLs, or to use marker-assisted selection, integrated gene maps need to be drawn up. As gene mapping in humans is significantly more advanced, a human/swine comparative karyotype indicating the location and extent of the regions of homology would allow better advantage to be taken of the human map. To determine correspondences between human and porcine homologous chromosome segments, we used whole chromosome painting probes from both species in bidirectional heterologous hybridization experiments. This strategy allows the determination of segment to segment homologies between the chromosomes of these two species. Chromosome-specific painting probes from both species were all, except one, obtained by DOP-PCR (commercial Cambio probes) or PARM-PCR amplification of flow sorted chromosomes. 95 per cent of the total length of porcine chromosomes were labelled by human painting probes and 60 per cent of the human chromosomes were painted in the reverse experiments. Thirty nine homologous segments were determined on autosomes. Five of them concerned entire chromosomes in both species. There was a close agreement between comparative gene mapping data and 27 of the identified homologous segments; this comparison enabled orientation of the segments. In addition, eleven homologous segments constitute new comparative mapping informations. We demonstrate that bidirectional heterologous chromosome painting is a highly efficient way of generating comparative cytogenetic maps between distantly related mammalian species.

RESUMEN

Para localizar genes candidatos, QTLs, o para usar la selección asistida por marcadores se necesi-

tan mapas génicos integrados y desarrollados. Como la cartografía génica humana está significativamente más avanzada, un cariotipo comparativo entre la especie humana y la porcina, que indicara la localización y extensión de las regiones de homología permitiría obtener beneficios del mapa humano. Para determinar las correspondencias entre segmentos cromosómicos homólogos humanos y porcinos, utilizamos sondas para coloración e hibridación *in situ* de cromosomas completos de ambas especies, en experimentos bidireccionales de hibridación heteróloga. Esta estrategia permite determinar las homologías segmento a segmento entre los cromosomas de ambas especies. Las sondas para coloración e hibridación *in situ* específica de cromosomas de ambas especies fueron obtenidas en todos los casos excepto en uno por DOP-PCR (sondas comerciales Cambio) o amplificación PARM-PCR de cromosomas seleccionados en flujo. El 95 p. cien de la longitud total de los cromosomas porcinos fue marcado por sondas de coloración e hibridación *in situ* humanas, mientras que el 60 p. cien de los cromosomas humanos fue tratado por este procedimiento en los experimentos inversos. Treinta y nueve segmentos homólogos fueron detectados en los autosomas. Cinco de ellos abarcaban cromosomas completos en ambas especies. Existe un elevado nivel de concordancia entre los datos de cartografía comparativa y 27 de los segmentos homólogos identificados por lo que esta comparación hizo posible la orientación de los segmentos. Además, once segmentos homólogos aportaron información novedosa acerca de la cartografía comparativa. Queda demostrado que la coloración e hibridación *in situ* heteróloga bidireccional es un método de elevada eficacia para generar mapas citogenéticos comparativos entre especies de mamíferos distantes evolutivamente.

INTRODUCTION

Our laboratory is involved in the construction of the porcine gene map, genetic map as well as cytogenetic map. Comparison of maps from different species revealed conserved syntenic relationships (O'Brien *et al.*, 1994). Because the human gene map is more advanced than the porcine one, one of our goal was to construct a comparative map between swine and human in order to take advantage of the human map

- to localize quickly porcine genes homologous to previously mapped human genes,

- to choose candidate genes in the homologous human chromosome region when an economical interesting phenotypic trait will be localized on a precise pig chromosome region using linkage analysis.

The construction of a comparative map gene after gene is long and tedious. So, the aim of this work was to determine rapidily, for each segment of porcine chromosome which is the corresponding segment(s) on human chromosomes.

To reach this goal, we used heterologous chromosome painting. Using this strategy relying on coding sequence conservation among species, we hybridized whole chromosome painting probes from one species on chromosomes from an other species in order to detect homologous chromosome segment(s) between the two species. To have correspondences segment to segment, heterologous painting was carried out in a bidirectional way: hybridization of human probes on porcine chromosomes and reverse hybridization of porcine probes on human chromosomes.

Human and porcine flow sorted chromosomes are available and can be used to produce painting probes using random PCR. For human painting probes, we used pricipally commercial Cambio probes (Biosys) produced using the DOP-

PCR technique (Telenius *et al.*, 1992a, b). Porcine painting probes were produced using the PARM-PCR technique (Milan *et al.*, 1993) improved by optimizing the choice of the primer sequence (Milan *et al.*, 1996a). An over-represented repeated motif (GAG), not involved in triplet expansion, was identified in human coding sequences. So a (GAG)7 primer (5',GAGGAGGAGGAGGAGGAGAG-5') was designed to amplify preferentially coding sequences and to produce efficient heterologous painting probes.

MATERIALS AND METHODS

METAPHASE SPREADS

Human and pig chromosome spreads were obtained using standard procedures. They were G-banded and photographed before hybridization in order to identify the labelled chromosomes.

HUMAN PAINTING PROBES

Biotinylated commercial Cambio probes (Biosys, Compiègne, France) were incubated at 42°C for 1 h, then 15 µl were denatured at 65°C for 10 min then prehybridized at 37°C for 1 h prior to hybridization.

PARM-PCR AMPLIFICATION ON FLOW SORTED CHROMOSOMES

Was carried out according to Milan *et al.* (1996a), in two steps. Three hundred chromosome copies were denatured (94°C, 10 min) in 25 µl reaction volume (200 µM dNTP, 300 nM primer, 5 mM $MgCl_2$, 0.5 Unit of Appligene *Taq* polymerase) and three cycles of amplification were carried out (30°C for 1 min, 72°C for 1 min, 94°C for 1 min). Two following cycles were then carried out at 60°C annealing temperature. The reaction volume was increased to 50 µl (200 µM dNTP, 650 nM primer, 2.5 mM $MgC1_2$, 1.5 U Taq) and 30 cycles of amplification (60°C for 1 min, 72 °C for 1 min, 94°C for 1 min) were then performed.

Labeling of probe was done by reamplification of 1 µl of PARM products in a final volume of 25 µl (3 mM $MgC1_2$, 1 µM primer, 200 µM dATP-dCTP-dGTP, 100 µM dTTP, 100 µM Biotin-16-dUTP (Boehringer Mannheim), 1 U Taq). Fifteen cycles (60°C for 1 min, 72 °C for 3 min, 94°C for 1 min) were performed.

Probes were further prepared as previously described (Yerle *et al.*, 1993) then prehybridized during 3 h prior to hybridization in presence of competitor DNA: Cotl DNA of the same species in homologous experiments (controls), a mixture of human and porcine Cotl DNA in heterologous experiments.

IN SITU HYBRIDIZATION AND DETECTION

Fluorescence *in situ* hybridization and detection were performed according to Yerle *et al.* (1993) modified to have lower stringency conditions for heterologous hybridization (40 p. cent deionized formamide in hybridization mixture), increasing hybridization duration (72 h at 37°C), and lower stringency conditions for the post-hybridization washings (40 p. cent deionized formamide in the three firsts baths, 42°C). The same metaphases that were G-banded and photographed prior to hybridization were rephotographed with a color slide film. Labeled chromosomes were identified by comparison with G-banded pictures and painted regions were measured on a screen.

Table I. *Chromosome segments conserved between human and pig.* (Segmentos cromosómicos conservados entre humanos y porcinos).

SSC		HSA		SSC		HSA	
Segment	Appr. length p. cent	Segment	Appr. length p. cent	Segment	Appr. length p. cent	Segment	Appr. length p. cent
1p	3.5	(6q)	(3.4)			14q22-qter	1.4
1q12-q14	0.7	(18)		8	6	4p-q31.3	5.1
1q12-q22	2.5	(15)		9p	2.7	(11q14-qter)	(1.8)
1q23-q24	0.7	(14)		9q	3	(7pter-p15.2)	
1q24-q2.12	2.6	(9)				(7q11-q31.2)	
2p	2.4	11p-q13	2.6	9q23-qter	1	(1q31-qter)	
2q11-q21	1.5	19p	0.8	l0p	2.9	?	
2q22-q28	2.1	5q14qter	3.2	10q12-qter	1.8	(10p)	(1.5)
3p14-p11	1.4	16p	1.2	11	4	13q	2.6
3q13-qter	3	2p-q21.2	4.5	12	3.5	17	2.7
4p-q14	3.4	(8q)	(3.1)	13	7.6	3	6.5
4q14-qter	2.6	(1)		13q46-qter	0.9	21	1.5
5pter-p14	0.6	22q12-qter	0.6	14q11-q16	1.7	8p	1.5
5p14-q24	4.1	12pter-q24.1	3.7	14q21-q22	0.9	12q22-qter	1.3
6p	2	16q	1.7	14q21-q23	1.3	22q11-q13.1	0.9
6q11-q21	0.8	19q	1.1	q23-qter	2.9	10q	2.9
6q22-q26	1	1pter-p31	2.5	15q13-q14	0.6	2q	4.8
6q31-qter	2			15q22-qter	2.4		
6q27-q31	1.2	18q11-q12	0.6	16	3	(5p-q13)	
7p-q14	2.7	6p	2.1	17	2.4	20q	1.1
7q13-q22	1.9	15q24-qter	0.9	18	2.3	7p15.2-p12	0.8
7q21-q25	1.9	14q11-q13	0.6			7q31.3-qter	1.4

Note. The different segments are given according to pig chromosome regions labelled by human probes. The approximative length is given in percentage of the total length of the haploid set of chromosomes. Data in parentheses were not obtained by FISH but are deduced from other results.

RESULTS

As shown in **figure 1a**, the porcine chromosome 5 was painted by two human probes: 5pterpl4 region by HSA 22 probe and 5p14-q24 region by HSA 12 probe. The reverse hybridization **(figure 1b)** carried out with SSC 5 probe on human metaphases gave expected signals on HSA 12 (12pter-q24.1) and HSA 22 (22q12-qter). HSA 12 gave an other signal on SSC 14q21-q22 region, and HSA 22 probe gave also a signal on SSC 14q21-q23 region **(figure 1c)**. The reverse hybridization with SSC 14 probe gave expected signals on HSA12, in the 12q22-qter region, and on HSA 22, on 22q11-q13.1 region **(figure 1d)**.

These results allows us to construct a syntenic region map as represented in **figure 1e**.

When a porcine probe failed, the bidirectional way allows us to nevertheless determine segment to segment correspondences. For example, SSC 16 was entirely painted by HSA 5

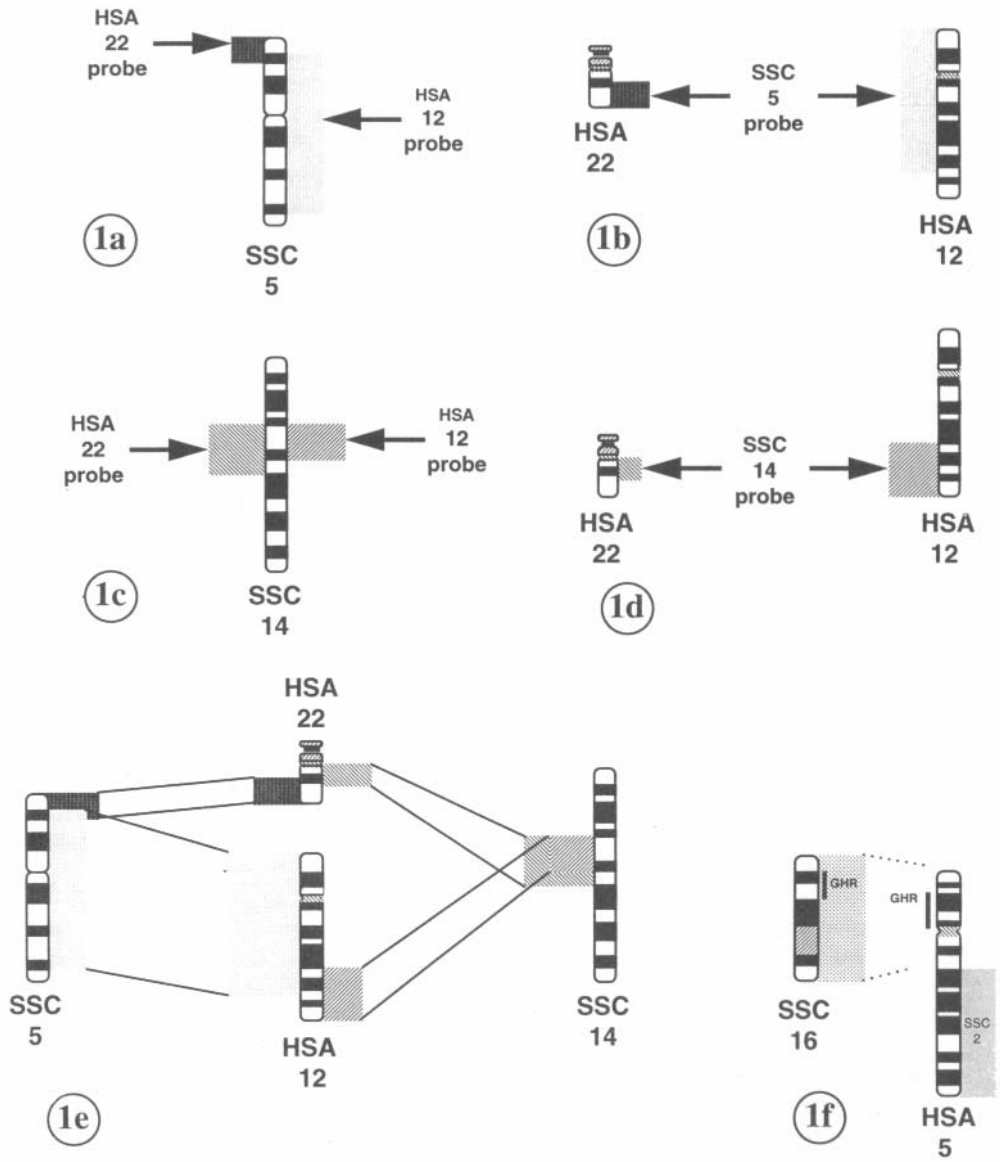

***Figure 1**. Examples of heterologous chromosome painting results.* (Ejemplos de los resultados de tinción de cromosomas heterólogos).

probe. Reverse hybridization with SSC 16 gave no signal on HSA 5 nor other human chromosome. But, as HSA 5 was painted by SSC 2 probe in the 5q14-qter region, we deduced that there is correspondence between SSC 16 and HSA pter-q 13 **(figure 1f)**. This correspondence is according to the localization of the GHR gene to HSA 5p14-p12 and to SSC 16p12-p13.

Painting probes for all human chromosomes gave signal(s) on porcine chromosomes, so around 95 p. cent of a haploid set of porcine autosomes were labeled. Because SSC 4 and SSC 9 probes are not available separately and because three porcine probes failed (SSC 1, SSC 10 and SSC 16), only about 65 p. cent of a haploid set of human autosomes were covered by porcine probes. Nevertheless, the bidirectional way allows us to obtain quite complete syntenic region map (Goureau *et al.*, 1996). Results are reported in **table I**; 94 p. cent of the data of gene localization both in human and swine are in accordance with conserved syntenies observed using bidirectional heterologous chromosome painting.

Syntenies appear to be conserved along extended segments, if not along entire length of chromosomes. The low level of similarity between karyotype of man and swine compared to these results suggest that most of rearrangements that occured during evolution have been intrachromosomal rearrangements. The syntenic region map obtained will be usefull to choose candidate gene; for example, this map is already used in our laboratory to choose a candidate gene to identify the RN gene, involved in *acid meat* phenotype (Milan *et al.*, 1996b).

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