SHORT COMMUNICATION

Characterization of a YAC Contig Spanning the Pseudoautosomal Region

KARIN RIED,* ANNELYSE MERTZ,* RAMAIAH NAGARAJA,† MICHELLE TRUSGNICH,† JOHN H. RILEY,‡ RAKESH ANAND,‡ HANS LEHRACH,§ DAVID PAGE,¶ JAY W. ELLISON,‖ AND GUDRUN RAPPOLD*[,1]

*Institute of Human Genetics, University of Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany; †Center for Genetics in Medicine, Washington University School of Medicine, St. Louis, Missouri 63110; ‡Zeneca, Mereside Alderley Park, Macclesfield SK 104T6, United Kingdom; §Max-Planck-Institute of Molecular Genetics, Ihnestrasse 73, 14195 Berlin, Germany; ¶Howard Hughes Research Laboratories at Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142; and ‖Department of Pediatrics, University of California, San Francisco, California 94143

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Due to its unique biology of partial sex linkage and high recombination rates, the pseudoautosomal region (PAR1) on both X and Y chromosomes has attracted considerable interest. In addition, an extremely high level of YAC instability has been observed in this region. We have derived 82 YAC clones from six different YAC libraries mapping to this 2.6-Mb region. Of these a subset of 22 YACs was analyzed in detail. YAC contigs were assembled using 67 pseudoautosomal probes, of which 64 were unambiguously ordered. All markers are well distributed over the entire region, including the middle part of the region, which has previously been found difficult to contig. Two gaps of less than 50 kb within the genomic locus of CSF2RA and around XE7 remain, which could not be covered with YACs, cosmids, or phages. This YAC contig anchored on the physical map of PAR1 represents one of the best characterized large regions of the human genome with a map completion greater than 90% at 100-kb resolution and has permitted the accurate localization of all known genes within this region.
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The human pseudoautosomal region (PAR1) covers 2.6 Mb of DNA and is situated at the tip of the sex chromosomes in Xp22.3 and Yp11.32 (for a review see 15). Interest in the pseudoautosomal region has been stimulated by its unique biology and its link between the different X and Y chromosomes. The development of a high-resolution physical YAC map of PAR1 will provide a framework for defining the structure and function of this unusual part of the genome. Previous physical maps anchored to the genetic map of the region (9, 14) and previous YAC maps (7, 16) have already been proven useful. However, the middle part of this region has been found difficult to contig, with findings of unstable clones or no clones at all.

To try to extend the cloned coverage of the region and to refine the analysis of gaps, we have initiated the construction of a YAC contig based on single-copy marker content mapping, using 67 pseudoautosomal probes well distributed over the entire region (see Fig. 1). A total of 82 YAC clones for contig construction were derived from St. Louis libraries (5, 13), an X-specific library (11), ICI library (3), ICRF library (10, 12), and CEPH library (2) in addition to the clones of Foote *et al.* (7). Of these, a subset of 22 YACs found to give the best fit to the probes was analyzed in greater detail (shown in Fig. 1 and Table 2A). Results in Table 2B of the 60 remaining YACs appear to be in good agreement with Fig. 1.

Since many previously mapped markers were used in the screening, YACs could be assembled initially based on the existing physical map. Overlaps between YACs were established by shared loci. FISH analysis and insert size of YACs, determined by pulsed-field gel analysis also assisted in determining the size of overlaps between the YACs (see Table 1). Insert sizes of the YACs ranged between 100 and 1400 kb of DNA (see Table 1); 9 of 22 YACs (41%) were chimeric. Deletions were revealed by inconsistencies in the presence or absence of markers of known position (see Table 2A) and 8 of the group of 22 YACs (36%) had detectable deletions (see Tables 1 and 2A). For at least 2 of the YACs (yOX44 and yOX225) changes in YAC marker content occurred over time or with passaging compared with earlier reports (7).

Despite intensive efforts to cover the entire region, two minor gaps remain. One is a gap of less than 50 kb within the genomic locus of CSF2RA, which could not be filled with YACs, cosmids, or phages; a further gap of similar size around XE7 was also intractable. In another case in which an overlap by YACs could not be proven (between DXYS211 and DXYS60), overlapping cosmids were used to fill the gap of approximately 15 kb. The telomere-adjacent region also was covered by a contig of cosmids (data not shown). Although we cannot

[1] To whom correspondence should be addressed. Telephone: 49/6221-563897 or -563890. Fax: 49/6221-565332.

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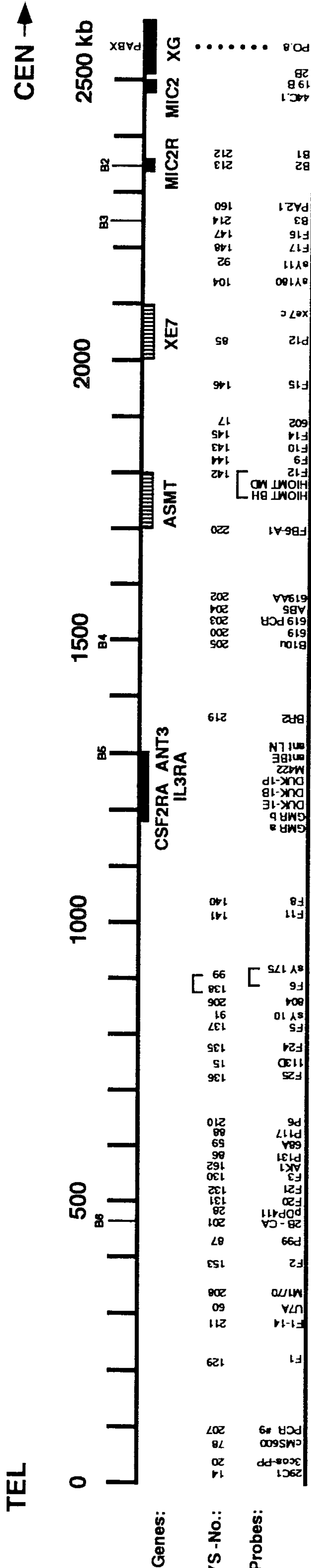
TEL
CEN
0
500
1000
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2000
2500 kb
Genes:
Probes:
YACs:
XG
MIC2
MIC2R
XE7
ASMT
CSF2RA ANT3
IL3RA
YM1000
yOX225
yOX222
yOX223
yWXD4241
yWXD5055
D1116
E10111
yWXD2607
H1130
124E12
320B7
261G10
yWXD1408
yOX44
yOX88
F10116
28HB9
18ED5
yOX28
yOX224
yWXD4932
Boundary between pseudoautosomal and X-specific region (PABX)
YACs with a deletion
Marker order cannot be determined

TABLE 1

YACs

YAC name	Size (kb)	FISH	Size on PAR (kb)	Deletion
yWXD4932	100	Not chimeric	100	Unknown
28HB9	450	Not chimeric	450	Unknown
18ED5	200	Not chimeric	200	Unknown
F10116	450	Not chimeric	450	Unknown
yOX28	350	Not chimeric	350	Yes
yOX224	100	Chimeric, 2p11–p12	20	Yes
yOX44	700	Chimeric, 5q12	350	Yes
yOX88	230	Not chimeric	230	Unknown
yWXD1408	320	Chimeric, 10qtel	150	Unknown
261G10	500/700	Chimeric, 2p12	375/525	Yes
320B7	250	Chimeric, 2q37	180	Yes
D1116	1100	Chimeric, 8p21	700	Yes
124E12	280	Not chimeric	280	Unknown
H1130	1400	Chimeric, 4qtel	400	Yes
yWXD2607	100	Not chimeric	100	Unknown
E10111	300	Not chimeric	300	Unknown
yOX222	490	Chimeric, 11q22–q23	340	Unknown
yOX225	290	Not chimeric	290	Yes
yOX223	410	Not chimeric	410	Unknown
YM1000	1000	Chimeric, 8p21–p22	500	Unknown
yWXD4241	150	Not chimeric	150	Unknown
yWXD5055	320	Not chimeric	320	Unknown

Twenty-two YAC clones spanning the pseudoautosomal region (PAR1) are listed according to their order along the chromosome. YACs were derived from 6 different sources: We screened four YAC libraries using polymerase chain reaction assays or hybridization. YAC clones yWXD4932, yWXD1408, yWXD2607, yWXD4241, and yWXD5055 were derived from the St. Louis YAC library (5, 13) using DXYS129, CSF2RA, ASMT, and MIC2; YAC clones 28HB9 and 18ED5 were derived from the ICI library (3) using DXYS60; YAC clones F10116 (ICRFy900F10116), D1116 (ICRFy900D1116), H1130 (ICRFy900H1130), E10111 (ICRFy900E10111), and YM1000 were derived from the ICRF YAC library (10, 12) using DXYS59, DXYS200, DXYS17, and MIC2. 124E12 was derived from the CEPH library (2) using DXYS203 as a probe. We analyzed all previously isolated pseudoautosomal YAC clones from Foote *et al.* (7), namely yOX28, yOX224, yOX44, yOX88, yOX225, yOX222, and yOX223, in detail. In addition, we analyzed two YACs 320B7 and 261G10 from the middle part of the PAR1, derived from the CEPH YAC library (2), published in Slim *et al.* (16). Each clone has been analyzed by FISH, PCR, and/or hybridization of probes mapping to the respective subregion (see also Table 2A). Eight YAC clones had deletions as shown graphically in Fig. 1 (yOX28, yOX224, yOX44, 261G10, 320B7, D1116, H1130, and yOX225; yOX225 has deleted its proximal pseudoautosomal and its Y-specific part). Nine different YACs have been shown to be chimeric (yOX224, yOX44, yWXD1408, 261G10, 320B7, D1116, H1130, yOX222, and YM1000).

totally exclude that this array still includes any as yet undetectable gaps, they must be small.

In conclusion, we have analyzed 22 of a total of 82 pseudoautosomal YACs in detail and precisely positioned them on the physical map of the region. The estimated chimera frequency of pseudoautosomal YACs (41%) is comparable to the rates described for YACs from other chromosomal subregions (4). Deletion rates (36%), however, seem high within pseudoautosomal YACs and may be attributable to the particular structure of this region.

In general our results agree well with published

FIG. 1. Schematic representation of 22 YAC clones spanning the pseudoautosomal region on Xp/Yp (PAR1). The PAR1 is bounded by the telomere (TEL) on its distal site and the boundary to the X-specific region (PABX) on its proximal site and covers approximately 2560 kb (9). YACs are drawn proportional to their size as horizontal black bars with the YAC identification number below. Their position can vary within a range of ±50 kb. Internal deletions within YACs are shown as broken lines between black bars. Hybridization of probes against Southern blots of YAC DNA and PCR analysis has been used to identify overlaps. Several of the YACs are chimeric, and only the portion within the PAR is drawn. Probes and respective DXYS numbers are indicated below the scale. Thirty-two probes represent STS markers and 35 hybridization probes. Order of probes with respect to distance from the telomere (zero) has been derived by a combination of PFGE analysis, YAC ordering, or the underlying cosmid map and is unambiguous for 64 of 67 probes used; physical distance from the telomere is approximate. Probes were derived from different sources: New probes are from our group (DXYS160, 162, 200–208, 210–214, 219, 220, and ANT3), probes with an initial F are STS-derived probes from Slim *et al.* (16), and probes with an initial sY are STS-derived probes from Vollrath *et al.* (17). Further probes were derived from 5′ and 3′ portions of genes mapping to this region or from classical pseudoautosomal markers. Six genes that are known within the pseudoautosomal region have been mapped to this contig. The mapping position of the colony stimulating factor receptor subunit (CSF2RA), the interleukin receptor subunit (IL3RA), the adenine nucleotide translocase 3 (ANT3), the cell surface antigen (MIC2), the MIC2-related pseudogene (MIC2R), and the blood group factor (XG) are indicated as a black bar below the scale. The detailed map position of the acetyl-serotonin-methyl-transferase (ASMT) and XE7 is new, indicated by a vertical dashed line, and was deduced from the presence or absence of probes in all studied YACs and from pulsed-field gel electrophoretic analyses (data not shown). B2–B6 represent *Bss*HII containing CpG islands (15).

TABLE 2

Analysis of the Presence or Absence of Pseudoautosomal Markers in YACs

A. Loci (DXYS)	yWXD4932	28HB9	18ED5	F10116	yOX28	yOX224	yOX44	yOX88	yWXD1408	261G10	320B7	D1116	124E12	H1130	yWXD2607	E10111	yOX222	yOX225	yOX223	YM1000	yWXD4241	yWXD5055
78	−		−																			
129*	+	−	−	−	−																	
211	+	−	−	−	+																	
60*		+	+	−	−	−																
208	−	+		−	−	−																
153*	−	+	+	+	−	−																
87	−	+		+	(+)																	
28*		+	+	+	+	+																
131*		+	+	+	+	+																
132*		+	+	+	+	−																
130*		+	+	+	+	+																
162		+	(+)	+	+	−																
86*		+	−	+	+	−																
59		+	−	+	+	−																
210		+	−	(+)	+																	
136*		+	−	+	+																	
15*		+	−	(+)	+	−	−	−	−													
135*		+	−	+	+	+																
137*		−	−	+	−		+	−	−	−	−											
91*				−			+	+	−	−	−											
138*		−		−			−	+	−	+	−											
99*				−			−	+	−	+	−											
141*							+	+	−	+	+											
140*							+	−	−	+	+	−	−									
CSF2RA							−	−	+	−	−	−	−									
IL3RA							−	−	−	+	+	−	−									
ANT3							−	−	−	+	+	−	−									
219										−	+	+	−	−								
200										−	−	+	+	+	−							
220												−	+	+	−	−						
ASMT*												−	−	−	+	−						
142*													−	−	+	−						
144*													−	−	+	+						
143*												−	−	−	+	+						
145*												−	−	+	−	+	−	−	−	−		
17*												−	−	+	−	+				−		
146*													−	+	−	+	−	−	−	−		
85													−	−		+	−	−	−	−		
XE7												−		−		−	−	−	−	−		
104*												+		−		−	+	+	+	+		
92*												+		−		−	+	+	+	+		
148												+		−		−	+	+	+	+		
147*												+					+	+	+	+		
214												+					+	+	+	+	−	−
213																	(+)	(+)	(+)	+	−	−
MIC2*												+					−	−	+	+	+	+
PABX*												+					−	−	−	+	+	+

B Loci (DXYS)	yWXD 2540	yWXD 2539	yWXD 2610	yWXD 3806	yWXD 3285	yWXD 1109	yWXD 2541	yWXD 2543	yWXD 2542	yWXD 1108	yWXD 3772	yWXD 2849	yWXD 7066	yWXD 7065	yWXD 7064	yWXD 7059	yWXD 7061	yWXD 3250	yWXD 7067	yWXD 7063	yWXD 7062	yWXD 7057	yWXD 7060	yWXD 3158	yWXD 5526	yWXD 5262	yWXD 7058	yWXD 6406	yWXD 5167	yWXD 2565
129*	+	+																												
60*	+	+	+																											
153*	+	+	+																											
28*		+	+	+	+	+																								
131*		+	+	+	+	+	+																							
132*		+	+		+	+	+																							
130*		+	+		+	+	+																							
86*			+			+	+	+																						
136*		+	+			+	+		+	+	+																			
15*		+	+			+	+		+	+		+																		
135*		+	+			+			+	+																				
137*													+	+	+	+	+	+	+	+	+	+	+	+	+	+				
91*													+	+	+		+	+		+		+	+	+		+	+	+		
138*																													+	+
99*																													+	+

Loci (DXYS)	yWXD 2611	yWXD 2613	yWXD 2607	yWXD 2609	yWXD 3811	yWXD 2608	yWXD 2615	yWXD 2616	yWXD 1974	yWXD 1973	yWXD 3211	yWXD 1976	yWXD 7006	yWXD 7005	yWXD 1975	yWXD 4142	yWXD 1972	yWXD 7004	yWXD 4888	yWXD 1751	yWXD 1977	yWXD 997	yWXD 998	yWXD 976	yWXD 4363	yWXD 5055	yWXD 4241	yWXD 1979	yWXD 1978	yWXD 3530
141*	+	+																												
140*		+																												
ASMT*			+																											
142*			+																											
144*			+	+		+																								
143*			+	+	+	+																								
145*					+	+	+																							
17*						+	+																							
146*					+	+		+																						
104*									+	+	+	+	+	+																
92*									+	+	+	+	+	+	+	+	+	+	+											
147*									+	+	+	+	+	+	+	+	+	+	+	+	+									
MIC2*																						+	+	+	+	+	+	+	+	+
PABX*																						+	+	+		+		+	+	+

(**A**) Twenty-two YACs as shown on Fig. 1. Loci with asterisks have also been used to characterize a second set of YACs as shown in **B.** (+) represents weak hybridization signals. (**B**) Additional 60 YACs, derived from the St. Louis library. All of these YACs are now available through ATCC.

data. In our contig, however, DXYS135 maps proximal to DXYS15, DXYS140 proximal to DXYS141, and DXYS17 proximal to DXYS145 compared to the data of Slim *et al.* (16). As shown in Fig. 1 and discussed above, the yOX YACs do not cover the middle part of the PAR1, a phenomenon also seen by other investigators, using, e.g., YACs from the CEPH YAC library (unpublished; see workshop report of Affara and Lau (1). The further analysis also showed that YACs 320B7 and 261G10 (16) from the middle region are chimeric and contain internal deletions.

Even though more than six collections have been screened and many YAC clones analyzed, several loci are clearly seriously underrepresented. It is striking that these problems were encountered with almost all of the known gene-containing subregions of the PAR1, including the portions containing CSF2RA, IL3RA, ANT3, ASMT, and XE7. Employing this new array of YAC clones, combined with the use of PFGE (data not shown), we can now also assign two pseudoautosomal genes ASMT and XE7 (6, 18) to narrow intervals at 1700–1800 and 2000–2100 kb from the telomere, respectively. It remains to be seen whether the local instability around or within genes is related to sequences with a special function.

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