

A Physical Map around the WAGR Complex on the Short Arm of Chromosome 11

M. GESSLER AND G. A. P. BRUNS

Genetics Division, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

Received December 9, 1988; revised February 9, 1989

A long-range restriction map of part of the short arm of chromosome 11 including the WAGR region has been constructed using pulsed-field gel electrophoresis and a number of infrequently cutting restriction enzymes. A total of 15.4 Mbp has been mapped in detail, extending from proximal 11p14 to the distal part of 11p12. The map localizes 35 different DNA probes and reveals at least nine areas with features characteristic of HTF islands, some of which may be candidates for the different loci underlying the phenotype of the WAGR syndrome. This map will furthermore allow screening of DNA from individuals with WAGR-related phenotypes and from Wilms tumors for associated chromosomal rearrangements.

© 1989 Academic Press, Inc.

INTRODUCTION

The WAGR syndrome, composed of Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation, is frequently associated with deletions of the short arm of chromosome 11 including band p13 (Francke *et al.*, 1979; Riccardi *et al.*, 1980; Shannon *et al.*, 1982; Turleau *et al.*, 1984). Deletions of functional alleles of the Wilms tumor gene or unmasking of a recessive mutant allele is proposed to result in the formation of Wilms tumor, a childhood nephroblastoma (Knudson and Strong, 1972; Fearon *et al.*, 1984; Koufos *et al.*, 1984; Orkin *et al.*, 1984; Reeve *et al.*, 1984). The large hemizygous deletions found in constitutional DNA of WAGR patients are thought to eliminate additional genes, giving rise to the developmental anomalies of the syndrome affecting the eye, the genitourinary tract, and the brain. These different features may also arise independently through localized mutational events, leading to singular symptoms like aniridia or perhaps genitourinary malformations (Simola *et al.*, 1983; Moore *et al.*, 1986; Porteous *et al.*, 1987). Most Wilms tumors also occur without concomitant WAGR stigmata (Breslow and Beckwith, 1982), consistent with the hypothesis that several independent genes are located in the WAGR region on chromosome 11p13. The analysis of different WAGR-related deletions and

translocations with probes for catalase and the β -subunit of follicle-stimulating hormone has regionalized the WAGR complex between these two genes in an area estimated at several million base pairs in size (Glaser *et al.*, 1986; Porteous *et al.*, 1987).

To characterize this region molecularly, we and other groups have characterized a large number of DNA probes from various chromosome 11-enriched libraries that map to different WAGR deletions (Bruns *et al.*, 1987; Porteous *et al.*, 1987; Davis *et al.*, 1988; Lewis *et al.*, 1988). Through the use of 30 probes together with 6 reference loci, a deletion map of the WAGR region has been established from analysis of 13 partially overlapping deletions which define 16 intervals, several of which could be correlated with phenotypic features (Gessler *et al.*, 1989). New deletions can be mapped more precisely with this ordered bank of probes and the identification of rearrangements or very small deletions in subregions may lead to the genes underlying particular phenotypic features of the WAGR syndrome. Mapping of deletions on conventional Southern blots, however, cannot reveal translocations or microdeletions located between probes that do not affect the probe segments themselves.

Physical mapping of large chromosomal regions is now possible by pulsed-field gel electrophoresis (PFGE), which can separate DNA molecules up to several million base pairs in size (Schwartz and Cantor, 1984; Carle and Olsen, 1984). Restriction enzymes with CpG dinucleotides in their recognition sequence cut infrequently in mammalian DNA and produce fragments in this size range. These enzymes are sensitive to methylation and many of their sites are clustered in hypomethylated HTF islands (Brown and Bird, 1986). Furthermore, these islands are frequently associated with expressed sequences, making them prominent landmarks for a certain class of human genes (Bird, 1986; Lindsay and Bird, 1987). The application of long-range restriction mapping techniques has proven very useful in the molecular analysis of Duchenne muscular dystrophy and cystic fibrosis (Burmeister and Lehrach, 1986; Burmeister *et al.*, 1988; Kenwick *et al.*, 1987; van Ommen *et al.*, 1986; Drumm *et al.*, 1988; Poustka *et al.*, 1988).

Our probe bank for the WAGR deletion region has

already been ordered in a highly resolving deletion map (Gessler *et al.*, 1989). To anchor the probes in a detailed physical map and to create a framework for targeting individual loci associated with specific features of the WAGR syndrome, we developed a long-range restriction map that links all probes into three large clusters spanning more than 15 Mbp and reveals a number of potential HTF islands.

MATERIALS AND METHODS

Cell Lines and DNA Probes

The lymphoblastoid cell line 6697, derived from a karyotypically normal male, has been used to establish the map. For particular subregions additional cell lines were used. GM3809, GM5297, GM4613, and GM7427 were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). H.V., a lymphoblastoid cell line from an individual with familial aniridia and the translocation $t(4;11)(q22;p13)$, was provided by Dr. K. Simola (Simola *et al.*, 1983). After informed consent, peripheral blood leukocytes for DNA isolation were obtained from C.L. (Russell and Weisskopf, 1986).

Probes for an HBV integration site on chromosome 11p14 (HVBS1, Rogler *et al.*, 1985) were provided by Dr. Rogler. Probes ES1-2 (D11S9, Gusella *et al.*, 1980) and J19.4 (D11S17, Housman *et al.*, 1985) were obtained from D. S. Gerhard. The 0.5-kbp *HincII/SacI* fragment of pRS1.2 (Watkins *et al.*, 1987) was used as a probe for FSHB. Plasmid p32-1 (D11S16, Feder *et al.*, 1985) was obtained from the ATCC (Rockville, MD). Probe pTTR is identical to p0.6EH in Boehm *et al.* (1988) and was subcloned from a λ phage obtained by screening a human *MboI* partial digest library in EMBL3 with synthetic oligonucleotides prepared according to the published sequence of p0.6EH. The identity of the clone was verified by comparison of restriction sites and mapping against hybrid cell panels.

All other clones used in this study were isolated from different phage libraries and have been described elsewhere (Bruns *et al.*, 1984, 1987; Gessler *et al.*, 1989).

Pulsed-Field Gel Analysis

Preparation of DNA in agarose blocks and restriction enzyme digestions were performed according to protocols obtained from D. Barlow and H. Lehrach (Herrmann *et al.*, 1987) as described previously (Gessler and Bruns, 1988). One percent agarose gels were run in an LKB pulsaphor unit in $0.5\times$ TBE using the double inhomogeneous field electrodes (Schwartz and Cantor, 1984; Carle and Olsen, 1984) or the CHEF electrode array described by Chu *et al.* (1986). In some experiments the Rotaphor electrophoresis unit (Immunetics, Cambridge, MA) was used. DNA was transferred to GeneScreen membranes in $0.5 N$ NaOH, $1.5 M$ NaCl and immobilized by baking and uv crosslinking (Church and Gilbert, 1984).

Hybridizations were carried out in $0.5 M$ sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA, and $50 \mu\text{g/ml}$ salmon sperm DNA at 65°C with subsequent washing in $40 mM$ sodium phosphate, 1% SDS at 65°C (modified from Church and Gilbert, 1984). Before rehybridization, the filters were stripped in $0.1\times$ TE, 0.1% SDS for 30 min at $75\text{--}80^\circ\text{C}$.

To optimize sizing of fragments, gels were run under a number of different conditions to shift bands into the upper part of the gel where resolution is far greater (region 3 in Vollrath and Davis, 1987). In some instances the switching time was increased during the run time in a linear or quadratic fashion to ensure good resolution over a wider size range (Sor, 1988). Phage λ multimers and chromosomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* were used as size markers (Carle and Olsen, 1985; Vollrath and Davis, 1987; Smith *et al.*, 1987). To measure fragment sizes, the filters were hybridized with radioactively labeled λ DNA or a fluorescent ruler was placed alongside the ethidium bromide-stained gel during photography to compare the migration distance of fragments with the positions of size markers.

As described by other investigators, size differences due to overloading in parts of the gel and dependence on switching times were observed (Bernards *et al.*, 1986; Burmeister *et al.*, 1988). These were most prevalent when the fragments analyzed were migrating in the lower, more dense and compressed region of the gel. All measurements were therefore taken from gels on which the specific fragments were localized in a less dense and well-resolved region close to size standards. This resulted in reproducible size determinations and consistent partial and double digest size data with an approximate error of 10–15% between different gels. The paucity of well-defined size markers and the summated lesser experience with fragments greater than 1.5 Mbp may produce larger errors in this size range.

RESULTS

Strategy of PFG Mapping

All the probes used in this study had been mapped on a series of WAGR-related deletions isolated in hybrid cell lines or by gene dosage analysis. This high-resolution deletion map allowed us to order the probes into 16 intervals spanning proximal 11p14 to distal 11p12 (Gessler *et al.*, 1989). Starting from this framework, a number of these probes were hybridized with PFG blots prepared from normal lymphoblastoid DNA digested with several rare-cutting restriction enzymes. Size distributions varied considerably among individual probes but generally the enzymes *NotI*, *Sfi I*, *SacII*, *BssHIII*, *MluI*, *NruI*, and *SalI* proved to be the most valuable and reliable, giving fragments in the range of 100 kbp to 3 Mbp in most instances.

To obtain almost complete coverage of the chro-

mosomal region and to accurately determine the position of the probes, two types of experiments were performed. Double digests with different combinations of enzymes were used to determine the relative order of specific fragments. This identifies the most distal reaching fragments and also narrows the interval for localization of the probe on the shortest single or double digest fragment. Single digests with enzymes producing long fragments were then used to verify linkup between different probes if they shared at least two separate fragments. For the latter type of experiment, the analysis of DNAs from different tissues or cell types proved to be very useful, as all rare-cutting enzymes containing CpG dinucleotides in their recognition sequence show a high degree of polymorphism, likely due to differential methylation in various cell types.

To show identity of fragments recognized by two probes, we employed different sets of electrophoresis conditions to shift the fragments into the regions of high resolution on the gel and hybridized the probes sequentially against the same filter. Compatibility of the local maps from double digests for both probes corroborated the linkup. Double digests furthermore excluded the presence of any sizable interspersed fragments in a number of cases in which continuity between two fragments was not shown by partial digests. In both cases, however, very small fragments may go undetected.

The Proximal Linkup Region

A summary of the fragment sizes obtained with the probes in different single and double digests is shown in Table 1. Only the two most frequently used double digest combinations are shown. Almost all possible permutations of two enzymes have, however, been useful in at least some instances to precisely determine the relative position of specific fragments. The largest continuously linked region spans approximately 6 Mbp and extends from 508 to E243 (Fig. 1a). The evidence for this is based on multiple shared fragments between adjacent probes. The most valuable long linkup fragments were produced by *MluI* (508 to CAT, E97 to 151), *NruI* (540 to E97), and *SalI* (Cat to TTR, 302 to E243). One example of a sequential hybridization demonstrating the linkup of six probes is shown in Fig. 2. All bands marked by arrows in this figure matched when the autoradiograms were superimposed. This was also seen when the fragments were separated under different electrophoretic conditions to shift their relative positions. The order of probes starting from 508 can be deduced from the pattern of fragments shared with adjacent probes. These results are corroborated by a number of additional linking fragments obtained from digests with *SfiI* and *SalI*, listed in Table 1.

Probe 508 had been placed most centromeric on the basis of gene dosage analysis in GM8785 and other cell lines (Gessler *et al.*, 1989). It could be localized close

to the centromeric end of a long *MluI* fragment which extends far telomeric and includes the catalase gene. An *MluI* partial digest fragment of 3.5 Mbp, also recognized by these probes, confirmed the linkup in this case.

Probe 540 could not be distinguished from J77-PH on the basis of the PFG pattern observed, but it is located centromeric to J77-PH as deduced from gene dosage analysis in GM7736 (Gessler *et al.*, 1989). Both probes share *NotI* and *NruI* fragments with the catalase gene. Double digests of *SalI* with *NotI* and *SacII* place CAT close to the telomeric end of the 1.5-Mbp *NotI* fragment.

SalI proved to be very informative to link the catalase gene with probes 277, E97, and TTR. They share three of four to five different fragments in *SalI*-digested DNA (Table 1). A similarly complex pattern of bands was observed with *MluI* and probes E97, TTR, E61, 302, 502, and 151. Different combinations of four major fragments were found in the DNA samples used in Fig. 3 with additional weaker fragments that were not further evaluated. These variations, which are reproducible in multiple DNA preparations from different cell lines, are highly informative for establishing or corroborating linkup between probes. They are most likely due to partial methylation at a number of consecutive sites which inhibit complete digestion. The possibility of a true RFLP hidden in these patterns cannot, however, be excluded.

Probes 302 and 502 produced identical hybridization patterns and their relative order remains unknown. These probes share a *SalI* fragment and *NaeI* partial digest fragments with probes 257 and 282, located on the telomeric side of a cluster of rare-cutting enzyme sites. Probes 257 and 282 have been linked by chromosome walking. Likewise the *SacII* and *BssHII* fragments identified by E243 have been reached from 282 (M. Gessler, unpublished results). The clusters of rare-cutting enzyme sites between 277/E97, E97/TTR, and 151/257 have been mapped extensively on short-range gels and appear to represent true HTF islands with a localized, marked overrepresentation of unmethylated CpG dinucleotides. The most telomeric extension from E243 is seen with *MluI* giving a partial digest fragment of 1.3 Mbp, similar in size to that of a variant *NruI* fragment seen in the H.V. cell line and to *NruI* partial digest fragments (not shown).

The Linkup Cluster around the FSHB Gene

A second large continuously linked region extends from probe 495 to 25 and includes the FSHB locus. Probe 495 is located close to a region where most of the rare-cutting enzymes employed have sites (Fig. 1b). These sites seem to be cut very efficiently or alternatively there may be additional sites close by, as we have been unable to obtain partial digest fragments extending significantly centromeric. The linear order of probes

TABLE 1
Summary of Restriction Fragment Sizes^a

Probe	Enzymes								
	<i>NotI</i>	<i>MluI</i>	<i>NruI</i>	<i>SacII</i>	<i>BssHII</i>	<i>SaII</i>	<i>SfiI</i>	<i>SaII/SacII</i>	<i>SaII/NotI</i>
508	650	2500 3500	n.d.	>2500 ^b	160	250	n.d.	180 210	210
540 J77-PH	1500	2500 3500	1700	300	300	630	420	250	n.d.
575	1500	2500 3500	1700	530	620	630	420	460	n.d.
CAT	1500	2500 3500	1700	530	620	340 710 780 820	50 170	90	160
277	280	210	1700	280 330	330	400 490 710 780 820	40	120 170 280 330	130
E97	50	230 730 1350 1450	1700	50 330	330	as 277	n.d.	50	50
TTR	200	230 730 1350 1450	1000	140	200	as 277	n.d.	140	200
E61	580	500 730 1200 1350 1450	1000	<50	<50	110 150 680 850	240	<50	110 150
302 502	300	700 1200 1350 1450	300	250	140	570	200	140	140
151	160	100 700 1200 1450	300	140	160	570	150	140	160
257 282	550	400 1300	550	70 350	70	570	170	70 240	240
E243	550	400 1300	550	280 350	280	570	170	170 240	240

TABLE 1—Continued

Probe	Enzymes								
	<i>NotI</i>	<i>MluI</i>	<i>NruI</i>	<i>SacII</i>	<i>BssHII</i>	<i>SaII</i>	<i>SfiI</i>	<i>SaII/SacII</i>	<i>SaII/NotI</i>
495	1400	>4000 ^b	1700 2000	340 1400	1400	340 360	410	340	340
582	1400	>4000 ^b	1700 2000	1100 1400	1400	230 350	170	n.d.	n.d.
403	1400	>4000 ^b	1700 2000	1100 1400	1400	130 350	130	n.d.	n.d.
570	1400	>4000 ^b	1700 2000	1100 1400	1400	200	350	n.d.	n.d.
D11S16	1400	>4000 ^b	1700 2000	1100 1400	1400	120 190	520	120 140	120 140
239	3100	>4000 ^b	1700 2000	120 2200	2600	70 190	520	50 70	50 70
1 FSHB	3100	>4000 ^b	380 2000	2100 2200	2600	310	850	310	n.d.
202	3100	>4000 ^b	380 2000	2100 2200	2600	430 550	850	n.d.	n.d.
74	3100	>4000 ^b	n.d.	2100 2200	2600	120 190 210	850	120 190 210	n.d.
291	3100	>4000 ^b	n.d.	2100 2200	2600	80 100 190 210	850	80 100 190 210	n.d.
181 475	3100	>4000 ^b	n.d.	2100 2200	2600	390 560	360	n.d.	n.d.
164	3100	>4000 ^b	n.d.	900	2600	550 800	660	550 650	n.d.
25	3100	>4000 ^b	— ^c	900	820	240 280	660	210 250	230 270
E136	>4000 ^b	3500	— ^c	1300	350	340 900	370	340 440 520	340 520
HVBS1	>4000 ^b	3500	— ^c	1300	2100 2400	125	210	125	n.d.
109	>4000 ^b	3500	— ^c	1300	2100 2400	500	150	380	n.d.
113	>4000 ^b	3500	— ^c	>4000 ^b	2100 2400	360	420	360	n.d.
D11S17	>4000 ^b	3500	— ^c	>4000 ^b	330 2400	220 330	570	220 330	220 330

^a Fragment sizes are given in kilobase pairs. All measurements are derived from cell line 6697, with the exception of additional variant bands for *MluI* (E97-151) and *SacII* (239-181/475) shown in Figs. 3 and 4.

^b No distinct fragment could be resolved. Hybridization to loading slots or limiting mobility region was observed to a varying degree.

^c Accurate *NruI* fragment sizes have not yet been determined.

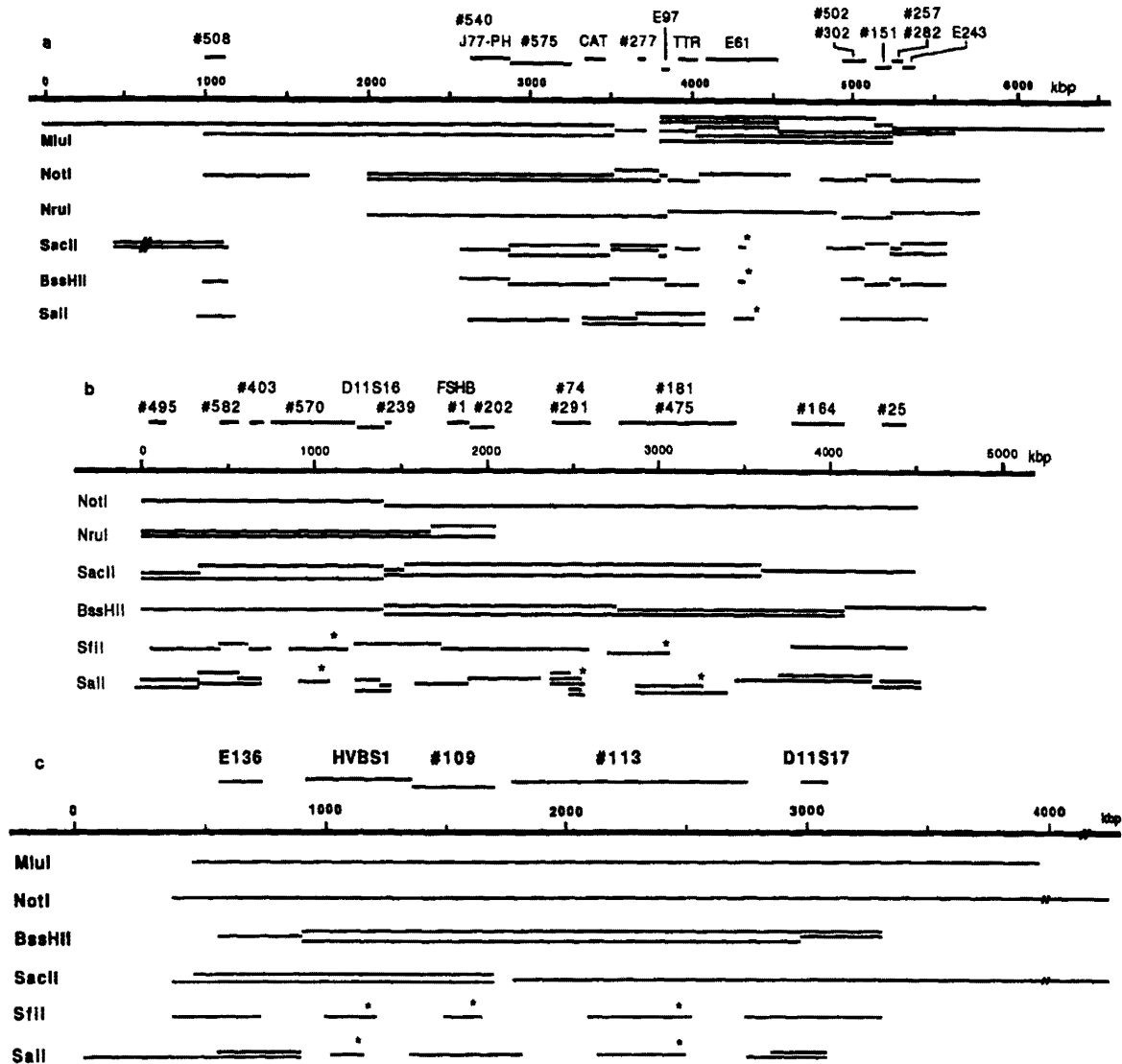


FIG. 1. Long-range restriction maps around (a) CAT, (b) FSHB, and (c) HVBS1. Fragment sizes for 6697 DNA as listed in Table 1 were used together with sizes of a large number of fragments produced by double digests (not shown) to construct these maps. The probe numbers are listed in the order from centromere to telomere, with solid bars indicating the shortest possible interval for their location as deduced from double digests. Horizontal lines below the scale bar represent restriction fragments generated by the respective enzymes listed on the left side. In several instances additional fragments that were only seen in other DNA samples are included (*MluI* E97/151; *BssHII* 239/164; see Figs. 3 and 5). For fragments marked by asterisks the precise position could not be determined due to the lack of informative double digests. The 1.7-Mbp *NruI* fragment for CAT was seen in 6697 DNA. Other cell lines have a 650-kbp fragment that was not mapped further. The orientation of the different *SalI* fragments recognized by 74/291 is not known. For 181/475 the positions of the *SfiI* and *SalI* fragments relative to each other were determined by double digests. Their orientation with respect to surrounding probes, however, is based on preliminary evidence from deletion mapping and remains provisional. The multiple *SalI* partial digest products detected by CAT/TTR and E61 have not been mapped.

495, 582, and 403 has been established from *SalI* partial digests and shared *ClaI* and *PaeR7I* fragments. These probes hybridize to *NotI*, *BssHII*, and partial *SacII* fragments of the same size (1.4 Mbp) which are corecognized by 570 and D11S16. An HTF island separates D11S16 and 239, which are linked by *SfiI* and *SalI* fragments. From there, long *NotI*, *BssHII*, and *SacII* fragments extend telomeric for 2.2 to 3.1 Mbp (Figs. 4 and 5). The variation in partially digested fragments

with *SacII* in DNA samples from different cell lines provides information about the relative order of probes. This has been confirmed and extended with other enzymes. Five of these probes are located on a 850-kbp *SfiI* fragment that can be subdivided using *NruI* and *SalI* in single and double digests. For 1/FSHB, 74/291, and 181/475, however, the order within each pair could not be established. Although different on conventional Southern blots, these probe pairs shared most or all

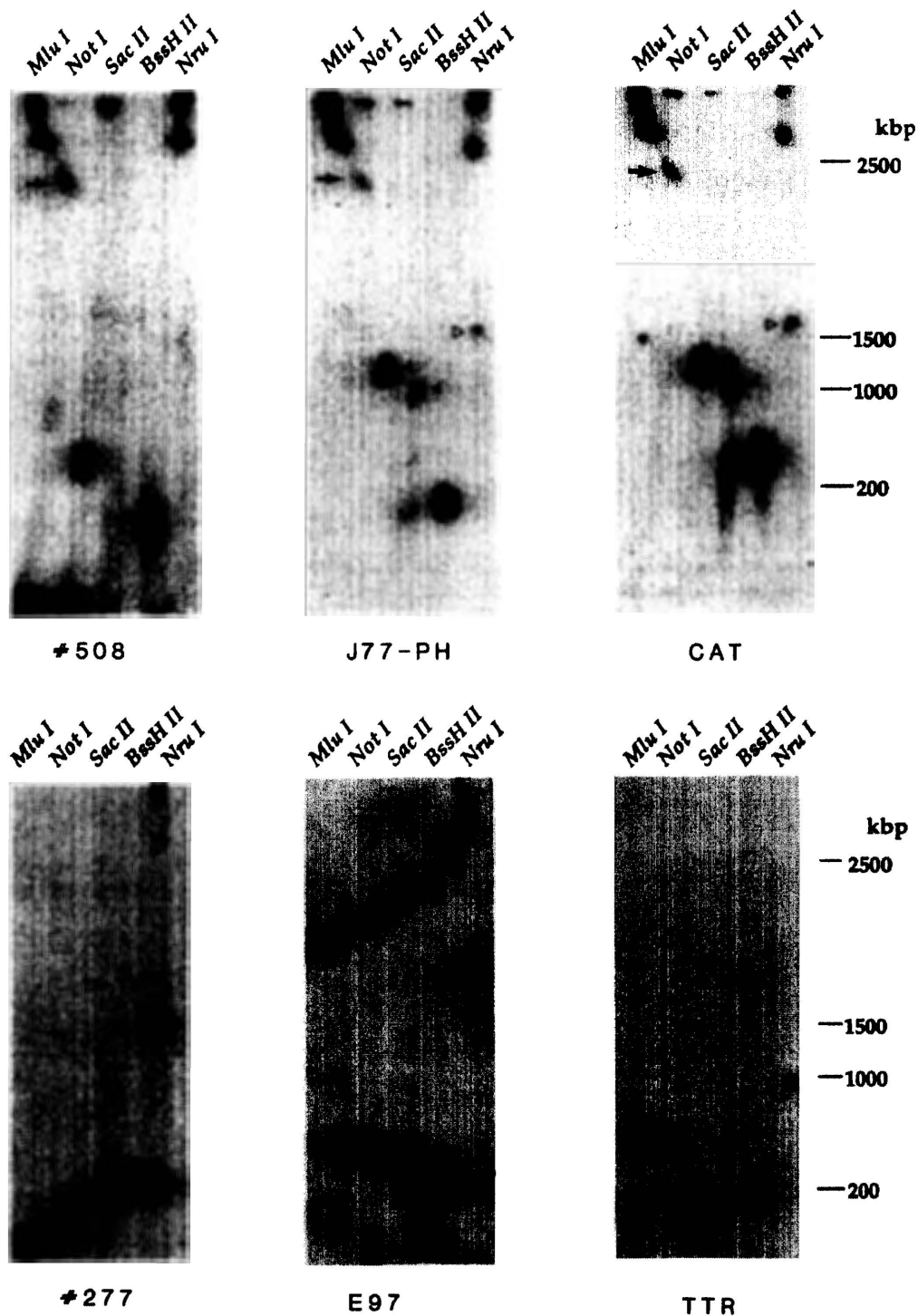


FIG. 2. Linkup of centromeric probes surrounding CAT. DNA from 6697 cells, digested with the enzymes indicated, was separated in an LKB pulsaphor apparatus at 220 V with 300-s switching time at 15°C for 2.5 days and transferred to GeneScreen membranes by alkaline blotting. Hybridizations with the probes listed below the autoradiograms were carried out sequentially and the filters were stripped before reuse to remove radioactive probe. Arrows point to restriction fragments corecognized by different probes when the autoradiograms were superimposed. The enzyme *Sac*II did not cut to completion here and revealed partial digest products with most probes.

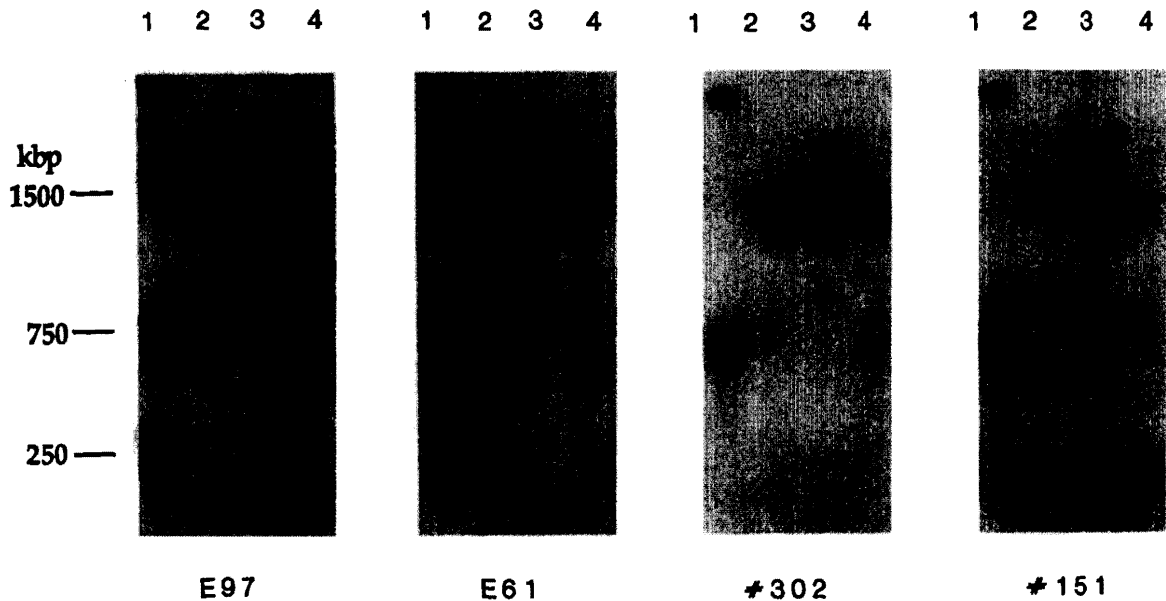


FIG. 3. Linkup with variant *Mlu*I fragments. Four DNA samples, 6697 (1), GM3809 (2), C.L. (3), and GM5297 (4), were digested to completion with *Mlu*I. Electrophoresis was carried out in an LKB pulsaphor apparatus at 220 V with alternating switching times of 100, 200, and 300 s at 10°C for 48 h. After transfer to GeneScreen, the filter was sequentially hybridized with probes E97, E61, 302, and 151. The probes detect approximately 1450 kbp of DNA, represented by the top band in lanes 2-4, that can be cut into shorter subfragments depending on the accessibility of three major internal *Mlu*I sites located between the probes as shown in Fig. 1a. The additional weakly hybridizing fragments of 700-1000 kbp seen in lanes 2-4 were not further evaluated. Patient C.L. is hemizygotously deleted for this entire region (21), thus ruling out a RFLP accounting for the variant fragments. The slightly lower mobility of the top band in that sample is likely due to the preparation of the DNA from a blood sample.

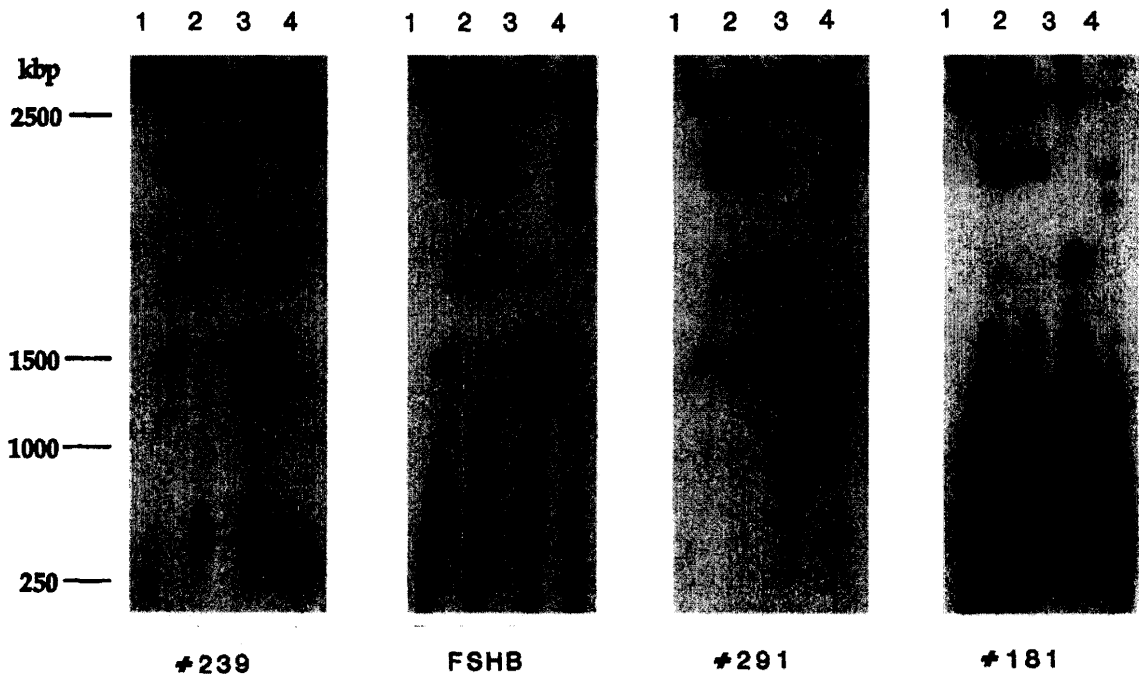


FIG. 4. Variation in *Sac*II cutting sites orders probes around FSHB. DNA from GM7427 (1), 6697 (2), H.V. (3), and GM4613 (4), digested with *Sac*II, was separated as described in Fig. 2. Hybridization with the probes indicated below the autoradiograms revealed major variant fragments in H.V. and GM4613 that establish the order from 239 to 181. The largest fragment detected in lane 3 is corecognized by 239, FSHB, and 291. The next smaller fragment is only shared by 239 and FSHB. Probe 239 detects a very small fragment in lane 4 (120 kbp on short-range blots), whereas the three other probes detect a 2100-kbp fragment in addition to the 2200-kbp fragment, also seen in lanes 1 and 2. The exact position of the variant *Sac*II sites in H.V. and other weakly hybridizing fragments have not been determined.

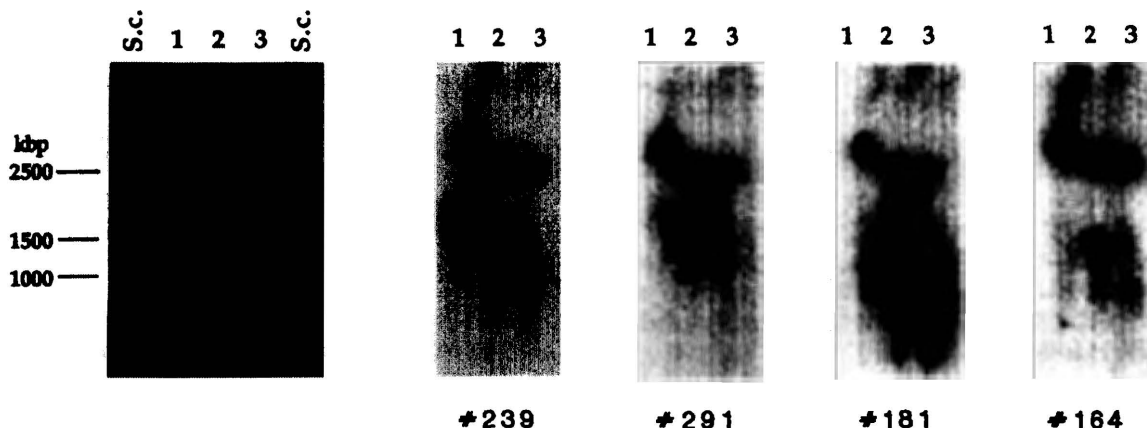


FIG. 5. Linkup with *BssHII*. DNA from the human-hamster hybrid line G156E5, containing a normal human chromosome 11 homolog (1), H.V. (2), and 6697 (3) was digested with *BssHII*. PFGE was performed with 170 V and 600-s switching time for 24 h, followed by 900 s for 36 h at 15°C with *S. cerevisiae* chromosomes (S.c.) as size markers. The ethidium bromide staining of the gel is shown on the left side. All four probes used in sequential hybridizations detect the same band of approximately 2600 kbp. The upshift of the fragment in lane 1 was seen reproducibly with other combinations of probes and enzymes and is characteristic of this hybrid line under the conditions used. The additional fragment seen in lane 1 with 239 represents cross-hybridizing hamster DNA. Variant bands were again seen in H.V. DNA (lane 2). Probes 239 and 291 detect the same fragment, whereas 181 and 164 detect a different, slightly smaller fragment that orients these probes.

rare-cutter fragments and did not produce informative double digest fragments. The restriction map around 164 and 25 on the telomeric end of this cluster has been described (Gessler and Bruns, 1988).

The Telomeric Linkup Cluster Including HVBS1

The HVBS1 marker is localized on a 3.5-Mbp *MluI* fragment (Fig. 6), which is corecognized by four other probes (E136, 109, 113, and D11S17). One of these, E136, is close to the centromeric end of this fragment where a number of sites for other rare-cutting enzymes were found (Fig. 1c). The *SacII* fragment for 113 and D11S17 and the *NotI* fragment for this entire area are

likely larger than 4 Mbp. Resolving these fragments on gels with extremely long switching times (Orbach *et al.*, 1988) may permit scanning of a large fraction of 11p14 for gross rearrangements.

Distribution of Unmethylated CpG Dinucleotides

The entire mapped area covers approximately 15.4 Mbp, reaching from proximal 11p14 to distal 11p12. An enormous variation in average fragment sizes and therefore in the frequency of sites is observed for enzymes containing CpG dinucleotides. As these enzymes are sensitive to cytosine methylation, a combined pat-

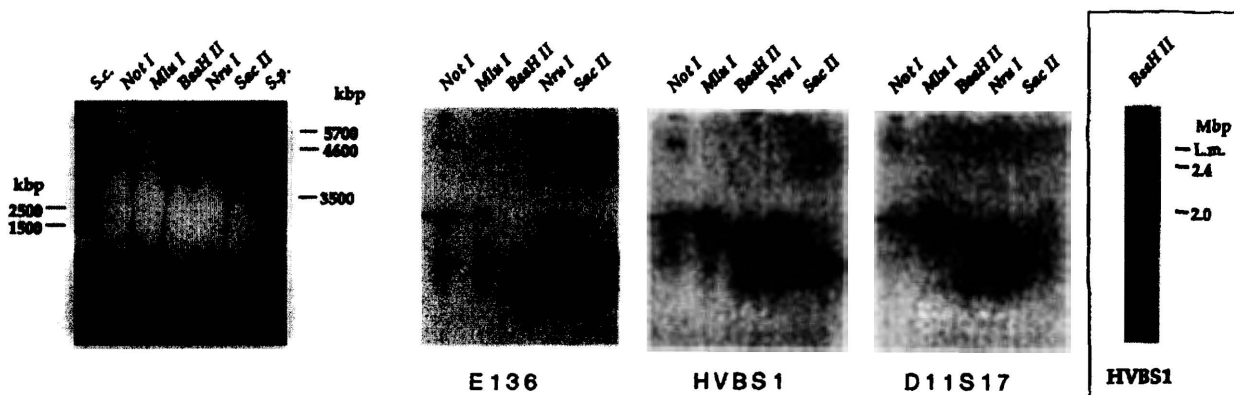


FIG. 6. Linkup of probes around the HVBS1 locus. To resolve larger fragments, 6697 DNA cut with the enzymes indicated was separated in a Rotaphor electrophoresis unit at 50 V with 3600-s switching time at 15°C for 7 days. Size markers were *S. cerevisiae* (S.c.) and *S. pombe* (S.p.) chromosomes. All three probes used detected an *MluI* fragment of 3.5 Mbp in size marked by arrows. Probes E136 and HVBS1 also share a *SacII* fragment of 1.3 Mbp. HVBS1 identifies a tight *BssHII* doublet that can be resolved using electrophoresis conditions described in Fig. 2 (see insert on the right side). Fragments of 2.0 and 2.4 Mbp are separated below the region of limiting mobility (L.m.). Probes HVBS1 and D11S17 corecognize the *BssHII* partial digest fragment of 2.4 Mbp. Although poorly reproduced in the D11S17 picture, the partial digest is clearly seen on the original autoradiogram and in repeat experiments. Digests with *NruI* frequently did not produce distinct bands with these probes.

tern resulting from restriction site occurrence and local degree of methylation is actually observed. The highest density of unmethylated sites is found between the catalase gene and probe E243 and could perhaps extend telomeric for a short stretch, as mapping is incomplete in this region (Fig. 7). The average *NotI* fragment size in this region is only a few hundred kilobase pairs; *SacII* and *BssHII* frequently produce fragment sizes of 250 kbp or less. Outside this region and especially distal to 239, the density of unmethylated sites for rare-cutting enzymes is much lower, resulting in fragment sizes of several megabase pairs.

A clustering of sites is already obvious from the maps and most prominent between CAT/TTR, 151/257, D11S16/239, and close to 508, 495, and E136 (Figs. 1a, 1b, and 7). More precise mapping on short-range gels using double digests with *SalI* or *SfiI* indicated that most of these clustered sites are within less than 20 kbp. It is possible that they could be within 2 kbp as described for a number of sequences identified as HTF islands by Brown and Bird (1986). The enzymes *SfiI* and *SalI* are particularly useful for precise mapping of CpG clusters because their cutting sites are not over-represented in HTF islands.

DISCUSSION

Pulsed-field gel electrophoresis is a powerful tool with which to take the step from cytogenetic analysis

and deletion mapping to a physical mapping of a chromosomal region. We have previously isolated a large number of random probes that map to a cytogenetically detected WAGR deletion that had been isolated in a somatic cell hybrid line (Bruns *et al.*, 1987). By analysis of additional overlapping deletions, the WAGR deletion region has been subdivided into 16 individual intervals, each definable by one or more deletion boundaries as well as by particular probe subsets, and specific subregions have been correlated with phenotypic features (Gessler *et al.*, 1989).

To define the physical relationship of the probes and to develop a molecular framework of this region, a long-range restriction map has been constructed by PFGE analysis. Twenty-nine probes plus the reference loci CAT, D11S16, FSHB, HVBS1, and D11S17 and the 11p13 T-cell translocation breakpoint region (Boehm *et al.*, 1988) have been incorporated into this map, which consists of three large clusters spanning more than 15 Mbp in total. With few exceptions, the 35 probes produced unique size patterns for the seven enzymes used throughout the study. Linkup between probes was considered to be established only if they shared at least two fragments consistent with local restriction maps derived from double digests and with probe order derived from prior deletion mapping.

The construction of the map is based on the fragment sizes observed in DNA from the lymphoblastoid cell

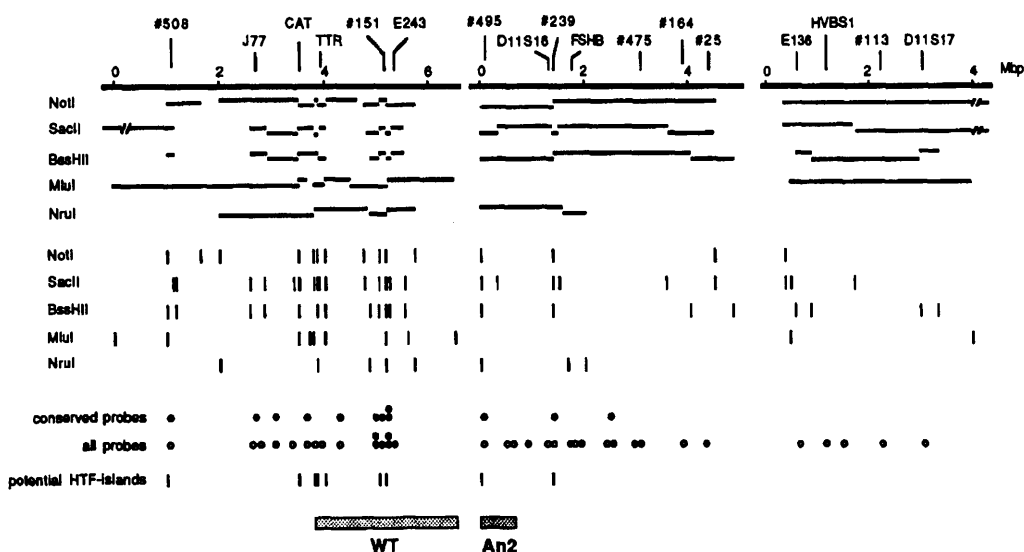


FIG. 7. The physical map around the WAGR complex. Simplified versions of the restriction maps shown in Fig. 1 are lined up in order from centromere to telomere. The restriction fragments are drawn as horizontal lines to show the degree of coverage for each of the enzymes. Only enzymes with preferential cutting in HTF islands (34) are shown. Below the fragments the observed cutting sites for all enzymes are indicated to highlight the uneven distribution of these sites. As seen from the upper part of the figure, there may be additional sites, especially for *SacII* and *BssHII*, in areas where complete coverage was not obtained (e.g., 508/J77, E61, and distal to E243). The positions of all probes used are marked by open circles. Generally, the center of the localization bars in Fig. 1 was used for positioning. All probes that show conservation in rodent DNA (8, 21) are indicated by solid circles. Potential HTF islands were defined as areas where at least three of the five enzymes used have sites within 20 kbp or less, depending on the resolution achieved. The shaded boxes represent the smallest region of overlap for the Wilms tumor and aniridia genes as defined by deletion mapping (21).

line 6697, supplemented in places with additional information derived from six other lymphoblastoid and fibroblast cell lines. At least 85% of the map has been confirmed in these cell lines, with minor variations likely due to methylation differences and possibly RFLPs.

In only two instances were we unable to find linking fragments between neighboring probes. With an average spacing of only 300–400 kbp between the random probes, large gaps in this region would not be expected, although the probe distribution does not appear to be completely random. Rather, the discontinuities may be the result of clustered restriction sites for the enzymes used, which makes it difficult to find fragments spanning these sequences. This type of clustering is especially obvious on the centromeric side of probe 495 and the telomeric side of probe 25. It is quite possible that the 1.3-Mbp *Mlu*I fragment seen by probe 243 and the variant or partial *Nru*I fragments of similar size seen in a number of DNA samples may terminate at this cluster of sites near probe 495.

Most of the probes used in this study were isolated from recombinant phage initially identified by hybridization with labeled total human DNA. No significant bias against a certain region may be expected with this strategy. However, a higher probe density is found close to CAT, between 302 and E243 and around FSHB. This skewing may have been introduced during construction of the library or in subsequent amplification steps. It is also possible, however, that this reflects a difference in the abundance of highly repetitive sequences, used to identify the clones, along the chromosome as suggested by the *in situ* hybridization studies of Korenberg and Rykowski (1988).

Interestingly, the distribution of sites for the rare-cutting enzymes used in this study reveals a markedly higher density in certain places, particularly between the catalase gene and probe E243 or 239 (Fig. 7). In a number of instances several sites appear to be located in very close proximity as judged from short-range PFGE blots, suggesting that they identify putative HTF islands. Localization of these clusters is a powerful method to target expressed sequences, as HTF islands frequently represent the 5' ends of certain types of genes (Bird, 1986; Lindsay and Bird, 1987). We may, therefore, have localized several potential genes on our map. One of these is likely to be the aniridia gene, as we have localized the breakpoint of a chromosome 11 translocation associated with familial aniridia (Simola *et al.*, 1983) close to probe 495 using PFGE (Gessler *et al.*, submitted).

A second interesting area is defined by the TTR probe, which marks a frequent site for translocation breakpoints involving the T-cell receptor locus observed in certain types of T-ALL (Boehm *et al.*, 1988). This localizes a tumor-specific translocation breakpoint

within the region frequently affected by deletions in WAGR patients. The TTR probe is surrounded by close HTF islands, one of which may be the 5' end of a gene involved in T-ALL tumorigenesis.

Deletion mapping in different WAGR cell lines has delineated the smallest region of overlap for the loci implicated in Wilms tumor and genitourinary abnormalities, spanning probes E97 to E243 (Gessler *et al.*, 1989). There are a number of potential HTF islands and conserved sequence probes located in this region. Analysis of DNA samples from individuals with genitourinary abnormalities or from Wilms tumors by PFGE analysis for small deletions or other cytogenetically undetected rearrangements in this region may ultimately lead to the identification of the underlying genetic loci. The feasibility of this approach has been documented already by the cloning of the breakpoints of two related chromosome rearrangements, an AGR deletion (Gessler and Bruns, 1988) and a translocation associated with familial aniridia (Gessler *et al.*, submitted), after detection by PFGE analysis.

The detailed restriction map permits a very precise positioning of chromosomal breakpoints. This facilitates their identification by molecular cloning techniques and provides estimates of physical sizes for deletions. The multiplicity of restriction site and probe distance relationships defined by the map establishes a framework for future cloning of the entire chromosomal region.

Some clues about the functional organization of whole chromosomal regions can be drawn from the current map. The overabundance and clustering of rare cutter sites between CAT and 239, an area corresponding to chromosome band p13, are consistent with the hypothesis that Giemsa-light bands like 11p13 are more G/C rich and contain a higher density of HTF islands (Bernardi *et al.*, 1985; Holmquist, 1987; Burmeister *et al.*, 1988). On the other hand, Giemsa-dark bands like 11p14, represented by the region distal to FSHB, are more A/T rich and have only few rare-cutter sites.

The distribution of conserved segments among the set of random probes used appears to show a similar distortion. Within the proximal region more than half of the probes show conservation, whereas for probes from the telomeric part of the map this is almost never observed. These data suggest that specific chromosomal areas contain a higher density of genes or at least of genes that have been conserved during evolution. A higher concentration of expressed and conserved sequences within early replicating DNA and G/C-rich isochores had been previously described (Goldman *et al.*, 1984; Bernardi *et al.*, 1985). As G/C-rich and A/T-rich isochores have recently been correlated with Giemsa-light and -dark bands, respectively (Korenberg and Rykowski, 1988), the skewed distribution of conserved sequence probes along the map of the WAGR

deletion region likely further reflects the chromosome banding structure of the area.

After completion of this manuscript, Compton *et al.* (1988) reported a physical map of the Wilms tumor-aniridia region prepared with the enzymes *NotI* and *MluI*. Although independent probe banks and unrelated cell lines were used, the *NotI* and *MluI* fragment sizes, as well as the fragment arrangements, match well in the critical area of overlap of WAGR deletions. The data of Compton *et al.* (1988) support the speculation that the *MluI* fragment recognized by probe E243 (Figs. 1a and 7) extends telomeric up to the region of rare-cutter sites proximal to 495. The two linkup clusters described in this report can thereby be joined into a detailed and continuous map of 11.5 Mbp which encompasses the WAGR deletion region.

ACKNOWLEDGMENTS

We thank Dr. L. Russell for providing a venous blood sample from patient C.L. and Dr. M. Lalonde for *S. pombe* chromosomes embedded in agarose. This work was supported by NIH Grant GM 34988. M.G. is a recipient of a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft through the University of Giessen F.R.G.

REFERENCES

- BERNARDI, G., OLOFFSON, B., FILIPSKI, J., ZERIAL, M., SALINAS, J., CUNY, G., MEUNIER-ROTTVAL, M., AND RODIER, F. (1985). The mosaic genome of warm-blooded vertebrates. *Science* **228**: 953-958.
- BERNARDS, A., KOOTER, J. M., MICHELS, P. A. M., MOBERTS, R. M. P., AND BORST, P. (1986). Pulsed field gradient electrophoresis of DNA digested in agarose allows the sizing of the large duplication unit of a surface antigen gene in trypanosomes. *Gene* **42**: 313-322.
- BIRD, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature (London)* **321**: 209-213.
- BOEHM, T., BULUWELA, L., WILLIAMS, D., WHITE, L., AND RABBITTS, T. H. (1988). A cluster of chromosome 11p13 translocations found via distinct D-D and D-D-J rearrangements of the human T cell receptor delta chain gene. *EMBO J.* **7**: 2011-2017.
- BRESLOW, N. E., AND BECKWITH, J. B. (1982). Epidemiological features of Wilms' tumor: Results of the National Wilms' Tumor Study. *J. Natl. Cancer Inst.* **68**: 429-441.
- BROWN, W. R. A., AND BIRD, A. P. (1986). Long-range restriction site mapping of mammalian genomic DNA. *Nature (London)* **322**: 477-481.
- BRUNS, G. A. P., GLASER, T., GUSELLA, J. F., HOUSMAN, D. E., AND ORKIN, S. H. (1984). Chromosome 11 probes identified with a catalase oligonucleotide. *Amer. J. Hum. Genet.* **36**: 25S.
- BRUNS, G. A. P., BARNES, S. D., GESSLER, M., BRENNICK, J. B., AND WEINER, M. J. (1987). DNA probes for chromosome 11 and the WAGR deletion. *Cytogenet. Cell Genet.* **46**: 588.
- BURMEISTER, M., AND LEHRACH, H. (1986). Long-range restriction map around the Duchenne muscular dystrophy gene. *Nature (London)* **324**: 582-585.
- BURMEISTER, M., MONACO, A. P., GILLARD, E. F., VAN OMMEN, G. J., AFFARA, N. A., FERGUSON-SMITH, M. A., KUNKEL, L. M., AND LEHRACH, H. (1988). A 10-megabase physical map of human Xp21, including the Duchenne muscular dystrophy gene. *Genomics* **2**: 189-202.
- CARLE, G. F., AND OLSON, M. V. (1984). Separation of chromosomal DNA from yeast by orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Res.* **12**: 5947-5664.
- CARLE, G. F., AND OLSON, M. V. (1985). An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci. USA* **82**: 3756-3760.
- CHU, G., VOLLRATH, D., AND DAVIS, R. W. (1986). Separation of large DNA molecules by contour-clamped homogeneous electric fields. *Science* **234**: 1582-1585.
- CHURCH, G. M., AND GILBERT, W. (1984). Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81**: 1991-1995.
- COMPTON, D. A., WEIL, M. M., JONES, C., RICCARDI, V. M., STRONG, L. C., AND SAUNDERS, G. F. (1988). Long range physical map of the Wilms' tumor-aniridia region on human chromosome 11. *Cell* **55**: 827-836.
- DAVIS, L. M., BYERS, M. G., FUKUSHIMA, Y., QIN, S., NOWAK, N. J., SCOGGIN, C., AND SHOWS, T. B. (1988). Four new DNA markers are assigned to the WAGR region of 11p13: Isolation and regional assignment of 112 chromosome 11 anonymous DNA segments. *Genomics* **3**: 264-271.
- DRUMM, M. L., SMITH, C. L., DEAN, M., COLE, J. L., IANUZZI, M. C., AND COLLINS, F. S. (1988). Physical mapping of the cystic fibrosis region by pulsed-field gel electrophoresis. *Genomics* **2**: 346-354.
- FEARON, E. R., VOGELSTEIN, B., AND FEINBERG, A. P. (1984). Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumors. *Nature (London)* **309**: 176-178.
- FEDER, J., YEN, L., WIJSMAN, E., WANG, L., WILKINS, L., SCHRODER, J., SPURR, N., CANN, H., BLUMENBERG, M., AND CAVALLI-SPORZA, L. L. (1985). A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. *Amer. J. Hum. Genet.* **37**: 635-649.
- FRANCKE, U., HOLMES, L. B., ATKINS, L., AND RICCARDI, V. M. (1979). Aniridia-Wilms' tumor association: Evidence for specific deletion of 11p13. *Cytogenet. Cell Genet.* **24**: 185-192.
- GESSLER, M., THOMAS, G. H., COULLIN, P., JUNIEN, C., MCGILLIVRAY, B. C., HAYDEN, M., JASCHEK, G., AND BRUNS, G. A. P. (1989). A deletion map of the WAGR region on chromosome 11. *Amer. J. Hum. Genet.*, **44**: 486-495.
- GESSLER, M., AND BRUNS, G. A. P. (1988). Molecular mapping and cloning of the breakpoints of a chromosome 11p14.1-p13 deletion associated with the AGR syndrome. *Genomics* **3**: 117-123.
- GLASER, T., LEWIS, W. H., BRUNS, G. A. P., WATKINS, P. C., ROGLER, C. E., SHOWS, T. B., POWERS, V. E., WILLARD, H. F., GOGUEN, J. M., SIMOLA, K. O. J., AND HOUSMAN, D. E. (1986). The beta-subunit of follicle-stimulating hormone is deleted in patients with aniridia and Wilms' tumor, allowing a further definition of the WAGR locus. *Nature (London)* **321**: 882-887.
- GOLDMAN, M. A., HOLMQUIST, G. P., GRAY, M. C., CASTON, L. A., AND NAG, A. (1984). Replication timing of genes and middle repetitive sequences. *Science* **224**: 686-692.
- GUSELLA, J. F., KEYS, C., VASARANYI-BREINER, A., KAO, F.-T., JONES, C., PUCK, T. T., AND HOUSMAN, D. (1980). Isolation and localization of DNA segments from specific human chromosomes. *Proc. Natl. Acad. Sci. USA* **77**: 2829-2833.
- HERRMANN, B. G., BARLOW, D. P., AND LEHRACH, H. (1987). A large inverted duplication allows homologous recombination between chromosomes heterozygous for the proximal t complex inversion. *Cell* **48**: 813-825.
- HOLMQUIST, G. P. (1987). Role of replication time in the control

- of tissue specific gene expression. *Amer. J. Hum. Genet.* **40**: 151-173.
28. HOUSMAN, D. E., GERHARD, D. S., GLASER, T., AND JONES, C. (1985). Mapping of chromosome 11p by linkage and somatic cell genetic techniques: A comparison of relative map order derived by each method. *Cytogenet. Cell Genet.* **40**: 655.
 29. KENWRICK, S., PATTERSON, M., SPEER, A., FISCHBECK, K., AND DAVIES, K. (1987). Molecular analysis of the Duchenne muscular dystrophy region using pulsed field gel electrophoresis. *Cell* **48**: 351-357.
 30. KNUDSON, A. G., AND STRONG, L. C. (1972). Mutation and cancer: A model for Wilms' tumor of the kidney. *J. Natl. Cancer Inst.* **48**: 313-324.
 31. KORENBERG, J. R., AND RYKOWSKI, M. C. (1988). Human genome organization: Alu, lines and the structure of metaphase chromosome bands. *Cell* **53**: 391-400.
 32. KOUFOS, A., HANSEN, M. F., LAMPKIN, B. C., WORKMAN, L. C., COPELAND, N. G., JENKINS, N. A., AND CAVENEE, W. K. (1984). Loss of alleles at loci on human chromosome 11 during genesis of Wilms' tumor. *Nature (London)* **309**: 170-172.
 33. LEWIS, W. H., YEGER, H., BONETTA, L., CHAN, H. S. L., KANG, J., JUNIEN, C., COWELL, J., JONES, C., AND DAFOE, L. A. (1988). Homozygous deletion of a DNA marker from chromosome 11p13 in sporadic Wilms tumor. *Genomics* **3**: 25-31.
 34. LINDSAY, S., AND BIRD, A. P. (1987). Use of restriction enzymes to detect potential gene sequences in mammalian DNA. *Nature (London)* **327**: 336-338.
 35. MOORE, J. W., HYMAN, S., ANTONARAKIS, S. E., MULES, E. H., AND THOMAS, G. H. (1986). Familial isolated aniridia associated with a translocation involving chromosomes 11 and 22 [t(11;22)(p13;q12.2)]. *Hum. Genet.* **72**: 297-302.
 36. ORBACH, M. J., VOLLRATH, D., DAVIS, R. W., AND YANOFSKY, C. (1988). An electrophoretic karyotype of *Neurospora crassa*. *Mol. Cell. Biol.* **8**: 1469-1473.
 37. ORKIN, S. H., GOLDMAN, D. S., AND SALLAN, S. E. (1984). Development of homozygosity for chromosome 11p markers in Wilms' tumor. *Nature (London)* **309**: 172-174.
 38. PORTEOUS, D. J., BICKMORE, W., CHRISTIE, S., BOYD, P. A., CRANSTON, G., FLETCHER, J. M., GOSDEN, J. R., ROUT, D., SEAWRIGHT, A., SIMOLA, K. O. J., VAN HEYNINGEN, V., AND HASTIE, N. D. (1987). HRAS1-selected chromosome transfer generates markers that colocalize aniridia and genitourinary dysplasia-associated translocation breakpoints and the Wilms tumor gene within band 11p13. *Proc. Natl. Acad. Sci. USA* **84**: 5355-5359.
 39. POUSTKA, A., LEHRACH, H., WILLIAMSON, R., AND BATES, G. (1988). A long-range restriction map encompassing the cystic fibrosis locus and its closely linked genetic markers. *Genomics* **2**: 337-345.
 40. REEVE, A. E., HOUSIAUX, P. J., GARDNER, R. J. M., CHEWINGS, W. E., GRINDLEY, R. M., AND MILLOW, L. J. (1984). Loss of a Harvey ras allele in sporadic Wilms' tumor. *Nature (London)* **309**: 174-176.
 41. RICCARDI, V. M., HITTNER, H. M., FRANCKE, U., YUNIS, J. J., LEDBETTER, D., AND BORGES, W. (1980). The aniridia-Wilms' tumor association: The critical role of chromosome band 11p13. *Cancer Genet. Cytogenet.* **2**: 131-137.
 42. ROGLER, C. E., SHERMAN, M., SU, C. Y., SHAFRITZ, D. A., SUMMERS, J., SHOWS, T. B., HENDERSON, A., AND KEW, M. (1985). Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. *Science* **230**: 319-322.
 43. RUSSELL, L. J., AND WEISSKOPF, B. (1986). Cognition in aniridia-Wilms tumor association: Analysis of karyotype associations. *Amer. J. Hum. Genet.* **39**: A78.
 44. SCHWARTZ, D. C., AND CANTOR, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**: 67-75.
 45. SHANNON, R. S., MANN, J. R., HARPER, E., HARNDEN, D. G., MORTEN, J. E. N., AND HERBERT, A. (1982). Wilms' tumor and aniridia: Clinical and cytogenetic features. *Arch. Dis. Childhood* **57**: 685-690.
 46. SIMOLA, K. O. J., KNUUTILA, S., KAITILA, I., PAKOLA, A., AND POHJA, P. (1983). Familial aniridia and translocation t(4;11)(q22;p13) without Wilms' tumor. *Hum. Genet.* **63**: 158-161.
 47. SMITH, C. L., MATSUMOTO, T., NIWA, O., KLCO, S., FAN, J.-B., YANAGIDA, M., AND CANTOR, C. R. (1987). An electrophoretic karyotype for *Schizosaccharomyces pombe* by pulsed field gel electrophoresis. *Nucleic Acids Res.* **15**: 4481.
 48. SOR, F. (1988). A computer program allows the separation of a wide range of chromosome sizes by pulsed field gel electrophoresis. *Nucleic Acids Res.* **16**: 4853-4863.
 49. TURLEAU, C., DE GROUCHY, J., TOURNADE, M.-F., GAGNADOUX, M.-F., AND JUNIEN, C. (1984). Del 11p/aniridia complex: Report of three patients and review of 37 observations from the literature. *Clin. Genet.* **26**: 356-362.
 50. VAN OMMEN, G.-J. B., VERKERK, J. M. H., HOFKER, M. H., MONACO, A. P., KUNKEL, L. M., RAY, P., WORTON, R., WIERINGA, B., BAKKER, E., AND PEARSON, P. L. (1986). A physical map of 4 million bp around the Duchenne muscular dystrophy gene on the human X-chromosome. *Cell* **47**: 499-504.
 51. VOLLRATH, D., AND DAVIS, R. W. (1987). Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. *Nucleic Acids Res.* **15**: 7865-7876.
 52. WATKINS, P. C., EDDY, R., BECK, A. K., VELUCCI, V., LEVERONE, B., TANZI, R. E., GUSELLA, J. F., AND SHOWS, T. B. (1987). DNA sequence and regional assignment of the human follicle-stimulating hormone beta subunit gene to the short arm of human chromosome 11. *DNA* **6**: 205-212.