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RESEARCH

An integrated Map of Human Chromosome 6p23

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The human chromosomal band 6p23 is a Giemsa-negative (light) band that may be expected to be relatively gene rich. The genes for spinocerebellar ataxia type 1 (SCA1), guanosine monophosphate reductase (GMPR), DEK involved in a subtype of acute myeloid leukemia (AML), and the folate-sensitive fragile site FRA6A, have already been mapped to 6p23. Recent linkage data have suggested evidence for a susceptibility locus for schizophrenia in the region. We have constructed a single YAC contig of ~100 clones spanning the entire 6p23 band from 6p22.3 to 6p24.1 and covering 7.5–8.5 Mb of DNA. The YAC contig contains 55 markers including genetically mapped STSs, physically mapped STSs, anonymous STSs, anonymous ESTs, and ESTs from the genes mapped to the region. The order of the genetically mapped STSs is consistent with their order in the contig and some of the markers not resolved on the genetic map have been resolved by the YACs. Four of the YACs from 6p23 and covering ~3 Mb of DNA have been used to isolate ~300 cosmids from a flow-sorted human chromosome 6 cosmid library, which have been organized into pockets. The proposed susceptibility locus for schizophrenia is most closely linked to D6S260, which is located within the YAC contig along with genetic markers ≤5 cM on either side. Therefore, the presented materials are valuable reagents for characterization of the genomic region implicated in schizophrenia.

The short arm of chromosome 6 has evoked much interest because of the presence of the major histocompatibility complex (MHC) in the chromosomal band 6p21.3 (Spring et al. 1985; Senger et al. 1993), which contains >110 genes and gene fragments (Campbell and Trowsdale 1993), and the spinocerebellar ataxia type 1 (SCA1) locus in 6p23 (Orr et al. 1993). 6p23 is a Giemsa-negative (light) or R band, the "flavor" of which has been described as rich in Alu repeat sequences but not very rich in GC content (Holmquist 1992). In general, R bands have a high gene density, a high number of CpG islands, are early replicating, relatively GC rich and SINE rich (Craig and Bickmore 1993). Therefore, 6p23 may be expected to have a high gene density.

Several genes have already been assigned to this region of 6p. The SCA1 CAG repeat locus has been assigned to 6p22–p23 (Orr et al. 1993) and 6p23.05–p24.2 (Volz et al. 1992). The DEK gene is involved in a t(6;9)(p23;q34) balanced translocation responsible for a specific subtype of acute nonlymphocytic leukemia (von Lindern et al. 1992) and the guanosine monophosphate reductase (GMPR) gene has been assigned to 6p23 by fluorescence in situ hybridization (FISH) analysis (Murano et al. 1994). The FIM1 locus, the human homolog of the mouse proviral integration site for the Friend murine leukemia virus, Fim1, which is involved frequently in the early stages of myeloblastic leukemogenesis, has been mapped to 6p23 by in situ hybridization (Van Cong et al. 1989). The endothelin 1 (EDN1) locus and the transcription factor AP-2 (TFAP2) have also been mapped by in situ hybridization to 6p23–p24 (Arinami et al. 1991) and 6p22.3–pter (Gaynor et al. 1991b), respectively, and their locations refined to 6p24 (Davies et al. 1995), whereas the

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zinc finger DNA-binding protein (ZNF40/HIV–EP1/MBP–1/PRDII–BF1) has been mapped to 6p22.3–p24 (Gaynor et al. 1991a). The renal sodium phosphate transport protein (NPT1/SLC17A1) has been assigned to 6p21.3–p23 in a somatic cell hybrid panel (Chong et al. 1993). The rare folate-sensitive fragile site FRA6A has also been localized by cytogenetic techniques to 6p23 (Sutherland et al. 1983).

Genetic (Weissenbach et al. 1992; Gyapay et al. 1994) and physical (Orphanos et al. 1994) maps of human chromosome 6 and the process of mapping the SCA1 locus (Kwiatkowski et al. 1993) produced many anonymous microsatellite markers, a number of which were in the 6p23 region. Several anonymous expressed sequence tags (ESTs), previously mapped to chromosome 6 (Polymeropoulos et al. 1992), have been further sublocalized within chromosome 6 using a somatic cell hybrid panel, some of which are localized to 6p21.3–p23 (Pappas et al. 1995).

The central portion of 6p has been studied by several groups searching for linkage with cleft lip with or without cleft palate (CL/P) and cleft palate (CP). Original data studying linkage to the blood clotting factor XIIIa (Eiberg et al. 1987) were not confirmed (Hecht et al. 1993; Vintiner et al. 1993), but more recently linkage has been shown to D6S89 in 6p23 (Carinci et al. 1995). However, detailed investigation of individuals with CL/P associated with cytogenetic abnormalities provides evidence for a locus in 6p24 (Davies et al. 1995). D6S89 is ~10 cM from D6S470, the closest marker proximal of the balanced chromosomal breakpoints, and may be detecting linkage to the locus affected by the cytogenetic abnormalities.

Another linkage study has provided evidence for a susceptibility locus for schizophrenia on 6p (Wang et al. 1995). Although their findings support a model for locus heterogeneity, their strongest linkage was obtained with the marker D6S260.

The critical region for the SCA1 gene in 6p23 was cloned in two yeast artificial chromosome (YAC) contigs, both spanning ~2.5 Mb from D6S274 to D6S443 (Banfi et al. 1993; Nemani et al. 1994). We have constructed a single YAC contig spanning from D6S422 at the distal end of 6p22.3 to connect with the YAC contig including TFAP2 and EDN1 at the proximal end of 6p24.1, thereby covering the entire 6p23 chromosomal band. The 7.5- to 8.5-Mb long contig includes 55 markers comprising genes, anonymous ESTs, and anonymous markers from all the available genetic and physical maps of the region allowing the integration of information from these different sources. The chromosomal localization of YACs across 6p23 has been verified by FISH analysis on metaphase chromosomes and the order of some markers not resolved in the YACs has been resolved by interphase FISH analysis. Toward a more detailed map of 6p23, we have also isolated ~300 cosmids from a flow-sorted chromosome 6 cosmid library derived from ~3 Mb of the chromosomal band. These cosmids have been further sublocalized by the generation of a "cosmid-pocket map" (Nizetic et al. 1994a) and the assignment of markers to cosmid clones.

RESULTS

Construction of a YAC Contig Spanning the 6p23 Chromosomal Band

The Centre d'Etudes du Polymorphisme Humain (CEPH) (Dausset et al. 1992), Imperial Cancer Research Foundation (ICRF) clones prefixed by AM (Larin et al. 1991) and Imperial Chemical Industries PLC (ICI) clones prefixed by RA (Anand et al. 1991) YAC libraries were screened with seven sequence tagged sites (STS) from the 6p23 region (Litt and Luty 1990; Weissenbach et al. 1992) (D6S89, D6S259, D6S260, D6S274, D6S285, D6S288, D6S289) and 47 clones were isolated. These were suplemented subsequently with 132 additional YACs identified from the first data release by Genethon (Cohen et al. 1993) using the Quickmap program to identify YACs mapping to 6p22.3–p24.1. The resulting sublibrary of 179 YACs was picked into two 96 well microtiter

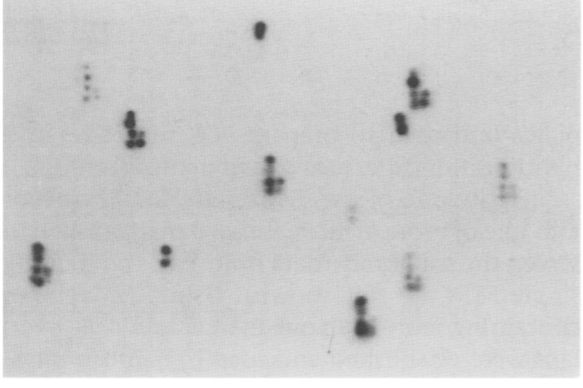

Figure 1 An example of the result of hybridization of an Alu-PCR probe 757_f_7/a8 to a YAC sublibrary filter. The yeast colonies were spotted in different characteristic patterns from each 96-well microtiter plate to facilitate accurate scoring of positive clones.

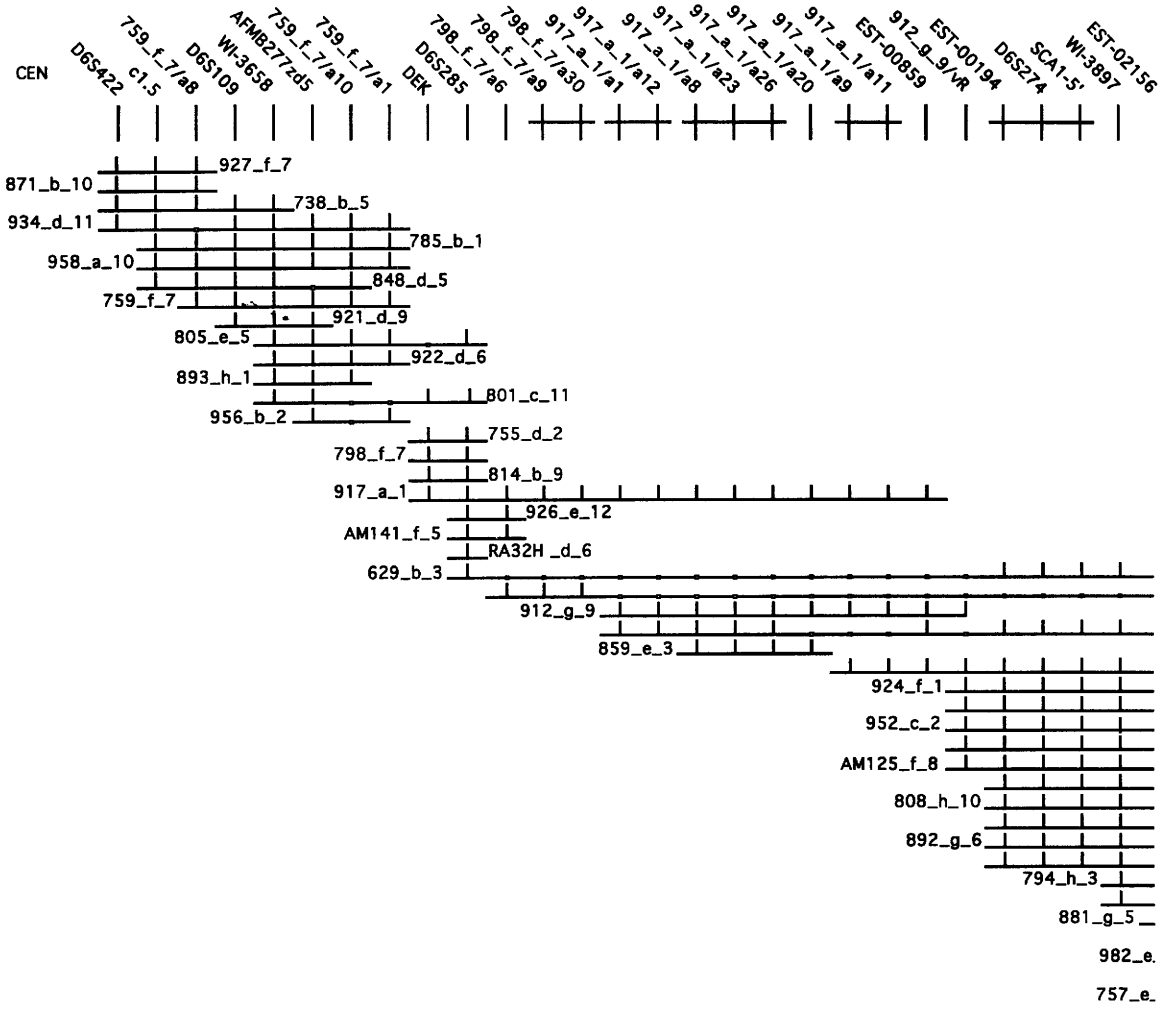

Figure 2 A schematic map of the YAC contig spanning 6p23 showing marker content of the clone inserts. The order of the markers was determined by colocalization to YAC clones and generating the minimum number of internal deletions within the YAC inserts. Horizontal lines show the regions spanned by the YAC inserts. Vertical lines show the positions of markers and their presence within YAC inserts. Small circles show the absence of markers in YACs, confirmed by repeated PCR screening, indicating the presence of internal deletions within YAC clones. Markers grouped together with short horizontal lines could not be resolved from each other in the YAC clones.

plates and used to prepare PCR templates and hybridization filters for subsequent screening.

Eighty-one of the YACs were found to contain one or more of 40 PCR-based markers used to screen the sublibrary (data from YACs containing single STSs are not shown). Using YAC clones containing more than one marker, multiple overlaps were established and used to confirm physical linkage between the markers and generate contigs. Expansion of the number of overlapping clones resulted in the construction of two contigs, the proximal contig covering the region from D6S422 in the distal part of 6p22.3 to D6S285 and the more distal contig from EST00859/D6S1394E to the TFAP2 and EDN1 genes in the proximal part of 6p24.1 (Davies et al. 1995). A single gap was present between D6S285 and D6S274, which are separated by 2 cM on the genetic map (Weissenbach et al. 1992; Gyapay et al. 1994). FISH analysis of interphase nuclei using 798_f_7 and 923_h_11 (which contain D6S285 and D6S274, respectively) revealed that the two YACs are separated by 450 ± 61 kb (Fig. 3E below).

To generate additional markers centromeric of D6S274 YACs 759_f_7, 798_f_7, and 917_a_1 were used as templates for inter-Alu PCR and the

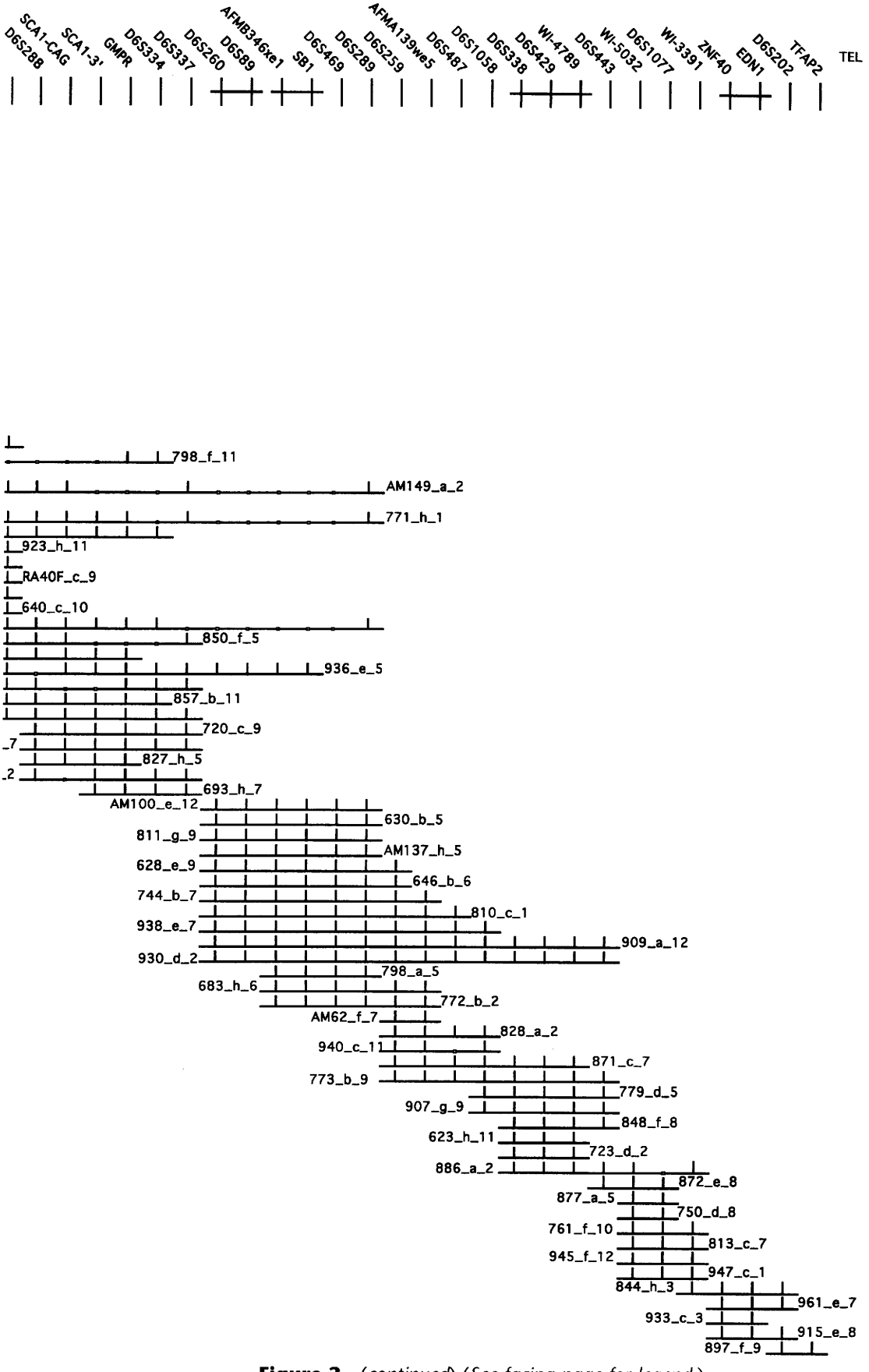

Figure 2 (*continued*) (*See facing page for legend.*)

products were subcloned to isolate individual products. A number of these DNA fragments were then used as hybridization probes (denoted by the name of the YAC from which they were derived and the clone number) to increase the marker density between D6S422 and D6S285 and to "walk" between D6S285 and D6S274 (Fig. 1). The progress of gap closure was monitored by FISH analysis of pairs of YACs on interphase nuclei (Fig. 3E,F below). An additional probe was generated from YAC 912_g_9 by vectorette PCR to confirm the overlap between this YAC and others containing D6S274.

The single YAC contig consists of 94 clones containing one or more markers from the 6p23 region; only the YACs containing two or more markers are included in Figure 2. All the markers are present in at least one YAC used for FISH analysis and mapped to 6p22.3–p24.1 on metaphase chromosomes (Table 1), thereby confirming their localization to this region. The entire set of markers are "doubly linked" to adjacent markers within the contig with four exceptions and all of these markers are present in additional YACs containing material derived exclusively from the 6p23 region as determined by FISH analysis. The order of markers was established by the presence of two or more markers within a single YAC and by building up the number of overlapping clones. However, some YACs contain internal deletions and in some cases created ambiguities as to the marker order. The order presented in Figure 2 generated the minimum number of internal deletions irrespective of the number of markers missing in each case.

The order of genetic markers in the contig is consistent with the CEPH genetic map order of CEN-D6S422-D6S285-D6S274-D6S288-(D6S89, D6S260, D6S289, D6S469)-(D6S259, D6S429)-D6S443-TEL (Gyapay et al. 1994) and others CEN-D6S109-D6S274-D6S288-(D6S89, D6S260, D6S289)-D6S259-EDN1-TEL (Jodice et al. 1993) and CEN-D6S109-AM10GA-D6S89-SB1-D6S202-TEL (Kwiatkowski et al. 1993). The high number of markers compared with the number of YACs and internal deletions detected within 15 of the clones resulted in a number of markers that could not be resolved from one another or positions where the order may be ambiguous. Unresolved markers are: 798_f_7/a9 and 798_f_7/a30, 917_a_1/a1 and 917_a_1/a12, 917_a_1/a8 and 917_a_1/a23 and 917_a_1/a26, 917_a_1/a9 and 917_a_1/a11, EST00194 and D6S274 and SCA1-5′, D6S89 and D6S260, AFMB346xe1 and SB1, D6S338 and D6S429 and WI-4789, ZNF40 and EDN1. Pairs or sets of markers that could be exchanged are 917_a_1/a9 and 917_a_1/a11 and EST00859, D6S288, and SCA1-CAG, SCA1-3′ and GMPR,

Table 1. Cytogenetic localization by FISH and STS content of YAC clones, ordered by STS content

YAC	STS Markers in YAC	Chromosome 6 localisation	Other signals
844_h_3	D6S202, EDN1, ZNF40, WI-3391	6p24.1/p23	
933_c_3	EDN1, ZNF40	6p24.1	
813_c_7	WI-3391, D6S1077, WI-5032	6p23	
761_f_10	WI-3391, D6S1077, WI-5032	6p23	
886_a_2	WI-3391, WI-5032, D6S443, WI-4789, D6S429, D6S338	6p24.1/p23	
779_d_5	D6S443, WI-4789, D6S429, D6S338, D6S1058	6p24.1/p23	
AM62_f_7	AFMA139we5, D6S259	6p23	
930_d_2	D6S443, WI-4789, D6S429, D6S338, D6S1058, D6S487, AFMA139we5 -	6p23	
	- D6S259, D6S289, D6S469, SB1, AFMB346xe1, D6S89, D6S260		
AM137_h_5	D6S289, D6S469, SB1, AFMB346xe1, D6S89, D6S260	6p23	8q13
892_g_6	GMPR, SCA1-3', SCA1-CAG, D6S288, EST02156, WI-3897, SCA1-5' -	6p23	
	- D6S274, EST00194		
AM125_f_8	EST02156, WI-3897, SCA1-5', D6S274, EST00194	6p23	
923_h_11	EST02156, WI-3897, SCA1-5', D6S274, EST00194	proximal 6p23	
AM149_a_2	D6S289, D6S337, SCA1-CAG, D6S288, EST02156, WI-3897, SCA1-5' -	6p23	
	- D6S274, EST00194, EST00859		
912_g_9	EST00859	6p23	
928_e_5	EST00859	6p22.3/p23	4q25, 10p13
798_f_11		6p23	
859_e_3		proximal 6p23	4q23
917_a_1	DEK, D6S285	proximal 6p23	
814_b_9	DEK, D6S285	6p23	
798_f_7	DEK, D6S285	distal 6p22.3	2p21, 3p13, proximal 4q22, 6q23
AM141_f_5	D6S285	6p23	
RA32H_d_6	D6S285	proximal 6p23	
759_f_7	AFMB277zd5, WI-3658, D6S109	6p23/p22.3	
738_b_5	WI-3658, D6S109, D6S422	6p22.3	1q44

AFMB346xe1 and D6S289. The physical map of the SCA1 region (Orr et al. 1993) showed that D6S288 is proximal of the SCA1 $(CAG)_n$ repeat and the mapping of cosmid clones containing SCA1-CAG, SCA1-3′, and GMPR allowed us to position GMPR distal of the 3′ end of the SCA1 gene. Two hybridization markers for FIM1 (Van Cong et al. 1989) and solute carrier family 17 (sodium phosphate) member 1 (SLC17A1/NPT1) (Chong et al. 1993), mapped to 6p22.3–p23 and 6p21.3–p23, respectively, were not present within any of the YACs in the sublibrary and probably lie proximal to the contig.

An estimate of the size of the YAC contig can be made by summing the sizes of the smallest overlapping and nonoverlapping YACs with no known deletions across the contig. By these means the region covered is 7.5–8.5 Mb and from the YACs mapped by FISH analysis of metaphase chromosomes, the 6p23 band is 6.5–7.5 Mb.

Some of the YACs exhibited instability during clonal growth as shown by variation in YAC sizes after demonstration of the presence of 6p23 markers in individual clones from the library plate wells (Table 2) in agreement with previous observations (Nemani et al. 1994) and with regrowth of some clones. In addition, mixtures of yeast clones taken from plate wells were used to prepare DNA to test for STS content to avoid the problems associated with clonal growth. Nevertheless, 15 YACs clearly exhibited internal deletions. All PCR markers apparently deleted from any YACs and any solitary markers, possibly caused by false-positive or false-negative results, were rechecked. In a model system (Kouprina et al. 1994) it has been shown that YACs exhibit a greater level of internal deletion during transformation (33%) compared with the rate of deletion in mitotically growing yeast (0.01%) and some of the YACs from the 6p23 region may have undergone deletion during construction of the YAC libraries. Our observations and those of others (Nemani et al. 1994) suggest that the mitotic instability of some of the YACs from 6p23 is >0.01%, but it is not known whether this is a feature of the sequences present in the YACs and the level of instability may vary from one region of the genome to another.

Cytogenetic Analysis of YACs Derived From the 6p22.3–p24.1 Region

The cytogenetic localization of 24 YACs was determined by FISH analysis of metaphase chromosomes. All of the clones tested that lay within the YAC contig were assigned to the 6p23 region (Table 1, Fig. 3A–C). Five of the YACs (21%) produced additional signals derived from other parts of the genome indicating chimerism in the YACs. One possible exception to this is the region of 4q22–q25, detected by YACs 928_e_5, 859_e_3, and 798_f_7, which may represent a region of homology between the 6p22.3–p23 boundary and this region of 4q. However, careful examination of the images showed that the signals on 4q are clearly distinct from one another.

Two subsets of the YACs picked from the Genethon data mapping to 6p22.3–p24.1 were shown to contain markers EST00301/D6S315E (743_c_2, 755_c_6, 769_f_10, 776_h_10, and 808_h_5) and AFM268vh5 (759_f_4 and 977_g_2), but could not be incorporated into the contig. Representative YACs from each set were used for FISH analysis. YACs 759_f_4 and 977_g_2 were localized to 6q21, whereas YACs 769_f_10 and 808_h_5 were localized to 6p21.3. The two YACs 769_f_10 and 808_h_5 are also present in the Genethon tile path data for 6p21.3 and are included within a YAC contig of the region (R.V.F. Mason and J. Ragoussis, unpubl.).

All of the PCR markers in the contig were present in at least one of the YACs used for FISH analysis and mapped to 6p22.3–p24.1. Careful examination of the results was used to position the signals within the chromosomal bands (see Table 1; Fig. 3A–C). Arrangement of the YACs in order according to their STS content with respect to the order of markers in the contig was used for comparison with the cytogenetic localizations (see Table 1). Examination of the cytogenetic localizations revealed that at the proximal end of 6p23 two YACs (928_e_5 and 798_f_7), localized to the 6p22.3–p23 boundary, overlap, on the distal side of, four YACs localized to 6p23. At the distal end two YACs, spanning ~1.5 Mb, were localized to the 6p23–p24.1 boundary and overlap, on the proximal side of, two YACs localized to 6p23 (see Table 1). Therefore, it appears that there is no clearly defined linear cytogenetic boundary along the DNA; instead of the expected order of 6p22.3-p22.3/p23-p23-p23/p24.1-p24.1, the order observed for the assignments is 6p22.3-(p23 or p22.3/p23)-p23-(p24.1/p23 or p23)-p24.1. This apparently nonlinear relationship between cytogenetic localization and clone order along the chromosome may represent looping of the DNA in the chromatin structure that results in the observed discrepancies between cytogenetic

Table 2. Sizes of YAC clones from the 6p22.3–p24.1 region in kb

YAC Name	YAC SIZES	YAC Name	YAC SIZES
927_f_7	1020 [1]	982_e_7	1500 [1]
871_b_10	1750 [1]	827_h_5	850 [1]
738_b_5	800 [2]	757_e_2	1250 [1] 1790 [1]
934_d_11	1500 [1]	693_h_7	780 [1]
785_b_1	1670 [1]	AM100_e_12	640 [2]
958_a_10	1690 [1]	630_b_5	900 [2]
848_d_5	800 [1]	811_g_9	1350 [1]
759_f_7	1420 [1]	AM137_h_5	1050 [2]
921_d_9	490 [1]	628_e_9	620 [1, 3]
805_e_5	1400 [2] 1500 [1]	646_b_6	360 [3] 890 [1] >1000 [3]
922_d_6	1220 [1] 1430 [1]	744_b_7	550 [2] 600 [2] 800 [2] 1320 [1]
893_h_1	1150 [1]	810_c_1	300 [2] 920 [2]
801_c_11	1580 [1]	938_e_7	300 [3] 700 [1,3] 1000 [1,2] 1600 [3]
956_b_2	1250 [1]	909_a_12	950 [2] 1000 [1] 1300 [2] 1600 [3]
755_d_2	1150 [1]	930_d_2	780 [2] 1000 [1] 1600 [2,3]
798_f_11	450 [1]	798_a_5	1570 [1]
814_b_9	1400 [1]	683_h_6	700 [2] 850 [2]
917_a_1	1350 [1]	772_b_2	350 [2] 400 [1,3] 520 [2] 700 [1,3] 900 [1,3]
926_e_12	1550 [1]	AM62_f_7	500 [2]
AM141_f_5	430 [2]	828_a_2	1100 [2] 1200 [2] 1300 [1,3]
RA32H_d_6	500 [2]	940_c_11	450 [1] 1730 [1] 1790 [1]
629_b_3	210 [1]	871_c_7	1000 [3]1210 [1]
798_f_7	1200 [2] 1400 [2] 1540 [1]	773_b_9	650 [2] 760 [3] 800 [1]
912_g_9	1340 [1]	779_d_5	880 [1]
AM149_a_2	1300 [2]	907_g_9	230 [1] 600 [1]
859_e_3	1270 [1]	848_f_8	870 [1]
771_h_1	760 [2] 800 [3] 1400 [2] 1780 [1]	623_h_11	>1000 [3]
924_f_1	800 [1,3] 1040 [2] 1500 [2]	723_d_2	430 [3] 450 [1]
923_h_11	1000 [1,2,3]	886_a_2	150 [1] 1600 [3]
952_c_2	600 [2]	872_e_8	1220 [1]
RA40F_c_9	620 [2]	877_a_5	970 [1]
AM125_f_8	575 [2]	750_d_8	1180 [1] 1770 [1]
640_c_10	700 [2]	761_f_10	1510 [1]
808_h_10	660 [2] 880 [1,3] 900 [2]	813_c_7	1750 [1]
850_f_5	900 [1] 920 [2] 950 [3]	945_f_12	1610 [1]
892_g_6	900 [2]	947_c_1	470 [1]
936_e_5	650 [2] 1250 [2] 1500 [1,3] 1800	844_h_3	1150 [1]
794_h_3	890 [1]	761_e_7	1340 [1]
857_b_11	820 [1]	933_c_3	1580 [1]
881_g_5	760 [1]	915_e_8	1500 [1]
720_c_9	1150 [2]	897_f_9	1750 [1]

[1] Data from Cohen et al. [2] Data from present study [3] Data from Nemani et al.

and clonal orders or may reflect the difficulty of accurate assignments at the lowest limits of resolution of metaphase mapping.

Generation of a Cosmid Pocket Map Within 6p23

To begin detailed molecular analysis of the 6p23 chromosomal band we have started to convert the 6p23 YAC contig into overlapping cosmids. This will facilitate detailed physical mapping and provide a primary resource for transcript mapping.

Total yeast DNA was isolated from YACs RA32H_d_6, AM149_a_2, 744_b_7, and 930_d_2 and used individually to probe high density cosmid filter grids of a flow-sorted human chromosome 6 cosmid library (Nizetic et al. 1991, 1994b). Three hundred and ninety-six cosmid clones were identified by one or more of the YAC clone probes, but not by total yeast DNA, and these were

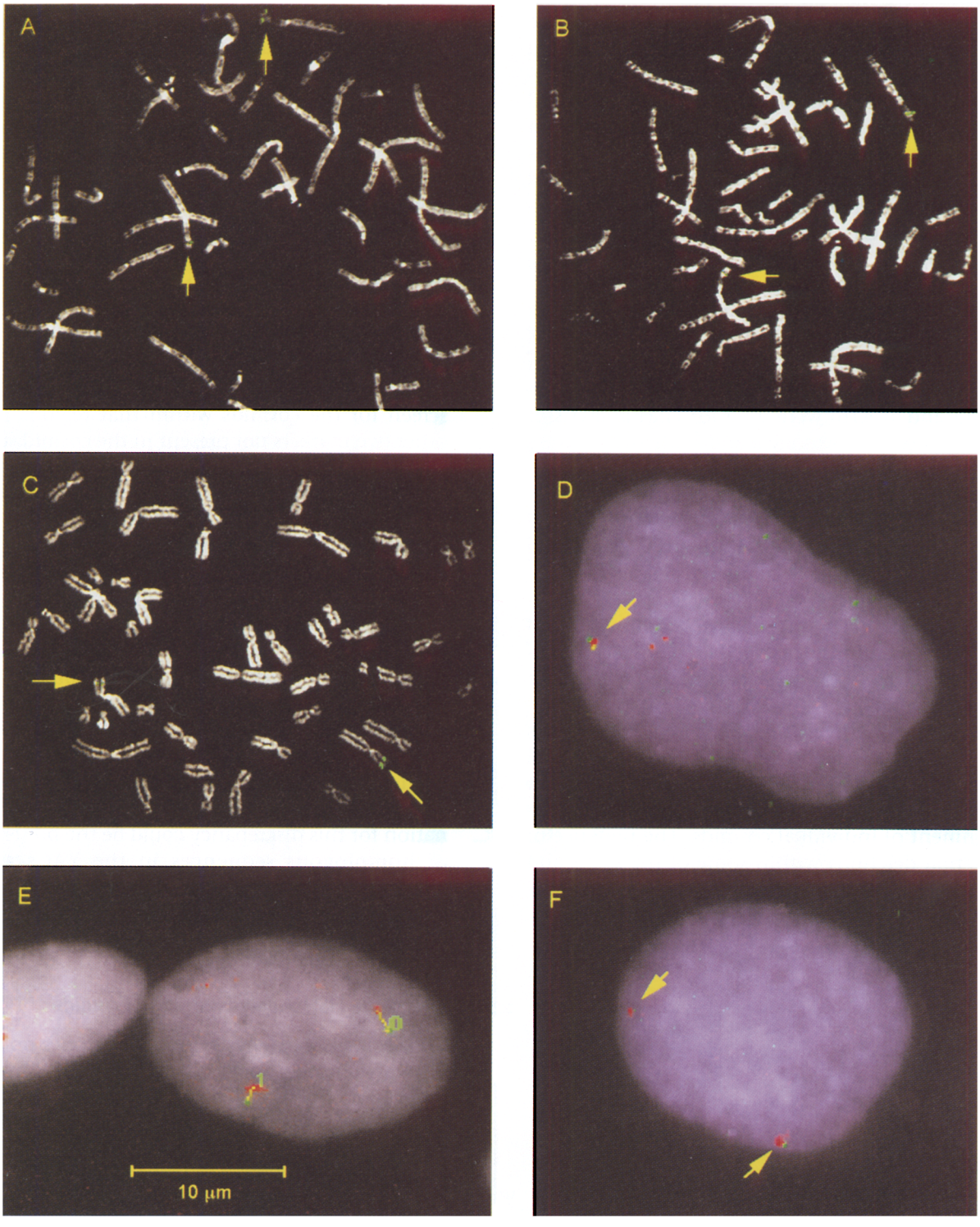

Figure 3 FISH analysis of YAC and cosmid clones. YAC clones were mapped to G-bands at the 850-band level after image enhancement. (*A*) YAC 779_d_5 mapped to the 6p23–p24.1 border; (*B*) YAC 798_f_11 to 6p23; and (*C*) YAC 759_f_7 to the 6p22.3–p23 border. (*D*) Interphase analysis was used to verify the order of cosmid clones 7N14, 23E6, and 45F20 (red, green, and yellow, respectively) and to ascertain the physical distances between them (see Results). Using the same technique, YAC pairs 923_h_11 and 798_f_7 (*E*) and 912_g_9 and 798_f_7 (*F*) were shown to be separated by 450 ± 61 kb and < 50 kb, respectively. *E* also illustrates the on-screen measurement of distances between signals (yellow dotted line between red and green signals) and a scale bar.

picked into 96-well plates. This sublibrary of clones was gridded onto new filters that were probed with the same YACs. Two hundred and sixty-four (67%) of the cosmid clones were rescreened successfully with the original YACs and the names of these rescreened cosmids have been submitted to the Reference Library System Database (Zehetner and Lehrach 1994). The cosmid sublibrary filters were then probed with eight additional YACs (AM137_h_5, 773_b_9, 798_f_7, 827_h_5, 850_f_5, 871_c_7, 938_e_7, and 982_e_7) derived from the same region as the YACs originally used to screen the chromosome 6 cosmid library (Figs. 4 and 5). Eight (3%) of the cosmids were positive with all of the YACs used to screen the cosmid sub-library. Two of these cosmids (8I17 and 10A16) were used for FISH analysis of metaphase chromosomes and both signals were detected on the satellites, but not the centromeres, of all the D- and G-group chromosomes. By grouping the cosmids according to the YACs to which they hybridized it was possible to assign 252 of them to defined intervals overlapping and nonoverlapping between the YACs (Fig. 5; and Table 3). By this means 222 clones (84%) of the successfully rescreened clones were assigned to 18 pockets each containing three or more cosmids.

Using a relatively high density of YACs across the region to screen the cosmid grids increased the resolution of the resulting cosmid pocket map, but also introduced ambiguities in the assignment of individual cosmids. The presence of internal deletions within some of the YACs and additional false-negative results were probably responsible for a number of pockets with only one or two cosmids that could not be positioned accurately on the map (Fig. 5).

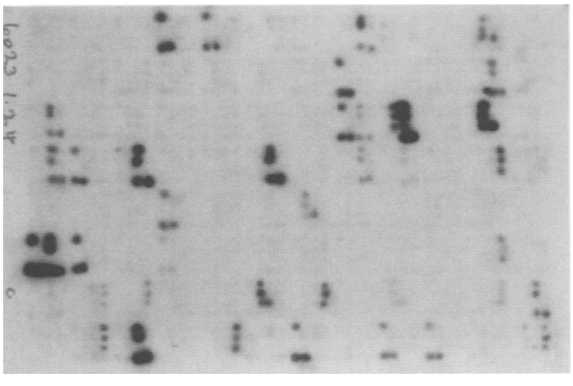

Figure 4 An example of hybridization of YAC 798_f_7 (*E*) in total yeast DNA to a cosmid sublibrary filter. The bacterial colonies from each 96-well microtiter plate were repeatedly spotted in different characteristic patterns to facilitate accurate scoring of positive clones.

To detect clones positive with PCR markers, DNA was prepared from multidimensional pools of the cosmid sublibrary. Twenty-seven PCR markers, from D6S1077 to EST00859 plus D6S285, were used to screen the sublibrary and selected cosmids subcloned from YAC AM137_h_5 (S.J.Broxholme, J.L.Wixon, and R.D. Campbell, unpubl.). Twenty-four of the markers were positive for one or more of the cosmids (Fig. 5). One of the three markers not present in any of the cosmids, D6S334, was also found to be absent from YAC AM149_a_2 that had been used to screen for the cosmids within that region. The other two markers not present in the cosmid sublibrary, D6S337 and EST02156, may be present in a region from which cosmids were not identified in the original screen or may not be present in the entire cosmid library. Some of the cosmid clones were found to contain more than one marker and this has allowed small contigs to be generated at different points across the pocket map. All of the cosmids containing STSs were present within the pockets expected from the STS content of the YACs with three exceptions. Cosmids 7A15, 8P9, and 12A13 contain STSs absent from YACs 982_e_7 (D) and 827_H_5 (G), although the cosmids themselves were positive when screened with the YACs. A possible explanation for this discrepancy could be the presence of homologous sequences in the YACs and cosmids resulting in false assignment of the cosmids to these pockets and is consistent with the observations of others (Nizetic et al. 1994a).

Interphase FISH Analysis of 6p23 Cosmids

Multicolor FISH analysis of cosmid clones on interphase chromosomes was used to establish the order of some of the clusters of markers on the pocket map. Cosmids 23E6 (containing D6S260), 7N14 (containing D6S469 and SB1), and 45F20 (containing D6S259) were used for the analysis (see Fig. 3D). Cosmid c144, which contains D6S289 in addition to D6S469 and SB1, did not yield reproducible signals on interphase chromosomes. The observed order and separation of the probes is 23E6–250 ± 58 kb–7N14–100 ± 50 kb–45F20.

This map is in agreement with the order of markers in the YAC contigs (see Fig. 2) (Banfi et

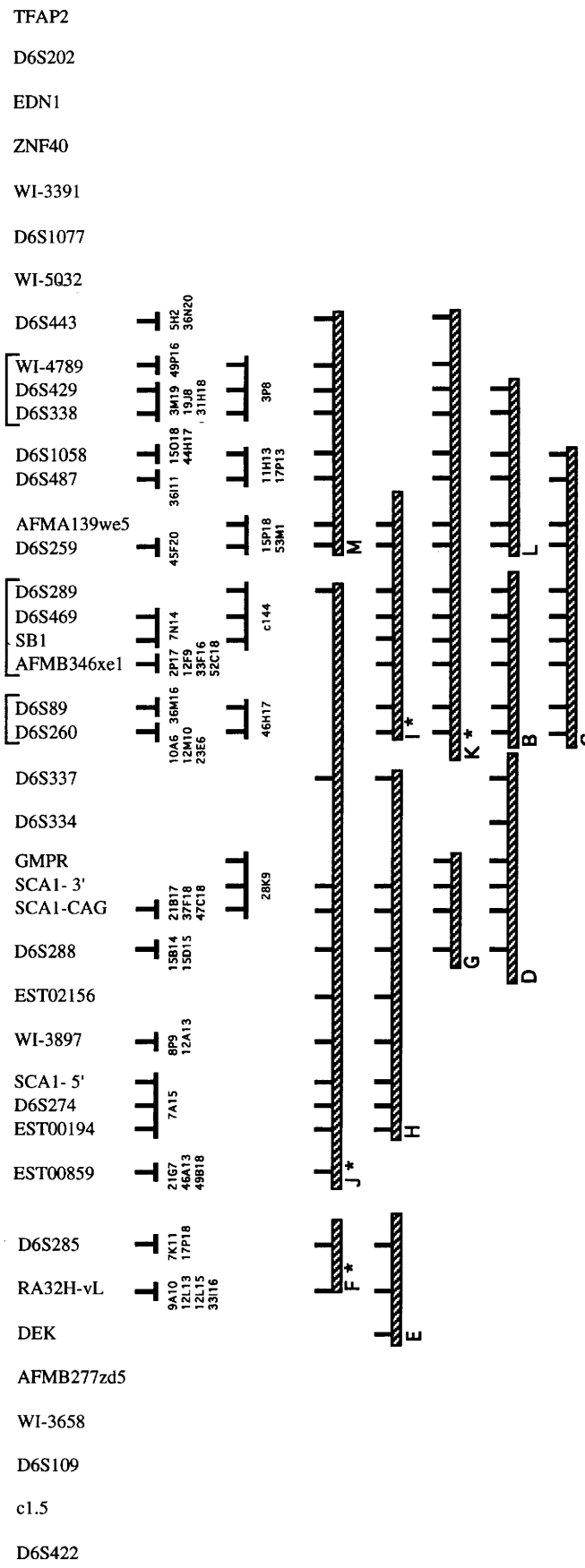

Figure 5 A schematic map of markers, cosmids, and YACs used to generate cosmid pockets in 6p23. (Top) PCR markers and a vectorette PCR product probe from the left arm of YAC RA32H_d_6. Markers are bracketed together where the order or orientation could not be determined. (*Middle*) Marker content of individual cosmid clones is shown by vertical lines and markers colocalizing to the same cosmid are connected by short horizontal lines and labeled with the cosmid names. (*Bottom*) The YACs used to screen the chromosome 6 cosmid sublibrary are shown as hatched horizontal boxes with vertical lines indicating the presence of markers. YAC clones are labeled with single letters (B–M): (*B*) AM137_h_5; (*C*) 938_e_7; (*D*) 982_e_7; (*E*) 798_f_7; (*F*) RA32H_d_6; (*G*) 827_h_5; (*H*) 850_f_5; (*I*) 744_b_7; (*J*) AM149_a_2; (*K*) 930_d_2; (*L*) 871_c_7; (*M*) 773_b_9. The YACs used to screen the original flow-sorted chromosome 6 cosmid library are marked with an asterisk.

Table 3. List of cosmid pockets in 6p23

Cosmid Pocket	Cosmid Names														
BCIK	5F14	7N14	10A6	11D22	12M10	19G15	23E6	27E1	36M16	39C4	46H17	46N3	47N13	48K6	53D5
BK + BCK	2P17	12F9	27J18	33F16	44M19	52C18									
C	1H6	3C19	3D24	4A15	4G16	5M20	6H19	6L11	7D3	7N14	11H13	13G10	14G5	17B11	19L3
	20C10	20N24	20J2	26A10	31A6	37I9	37N20	38E22	43H18	43N8	45G9	46G8			
CIM	15J18	15P18	27G5	28G21	38D13	40P1	44K12	45F20	49H11	51P1	53M1	54P22			
CM	8B11	9O20	10H21	11H13	11N14	13D16	15O18	17B22	17G23	17P13	31B5	31C10	31F10	36I11	44H17
	46H6	46N6	47E5	52I8											
DGHJ	7C6	15B14	15D15	32L5	33B19	37A10	40M17	43O20	52L22						
DGJ	7A15	21J10	28K22	35L3	54G10										
DK	9P1	17C19	22C17	28N8	50O16										
EF	1I7	1I15	3M18	5E1	5N3	7K11	8P13	9A10	12F7	12L13	12L15	14H20	17P18	21F8	25P2
	26A7	28D22	29P9	32H12	32J15	33I16	35A16	36E6	36E8	41G9	47L19	50A17	50J18	50J20	51E11
	52D12	52E22	52K3												
GHJ	21B17	25F8	28K9	37F18	40C15	47C18	49O8								
GJ	1N17	3F7	4F18	4H3	4O18	6G21	6K6	8P9	9I18	10C11	12A13	12A22	13P23	16K14	18C19
	19L13	22D19	23A22	24L15	26A13	28D6	36C17	38B22	38F12	39B10	40K14	45A8	45K14	45L16	45M11
	45O11	45P11	46K12	46N22	47N3	48A11	48A8	48C13	51D10						
IK	14H4	18I7	18K12	19O14	20C8	25K11	32F5	40C14	40I4						
J	10E8	21G7	25A13	27C4	27M23	39G13	39N5	43D23	46A13	47C17	49B18	52F10	52I2	53H17	53O18
JK	6K22	11O13	15A20	18J16	37D17	38J23	39G18	39J1	40E15	42L18	43E18	47M7	47O12	49H12	51C4
	51J4	52H9													
K	1H22	3A14	4H16	8J22	10A10	13I8	21A21	32J4	35G7	39K18	43E19	45F8	45N16	46P2	48A15
	53E20	53L15													
KLM	2I16	3M19													
KM	3P8	5H2	15A15	17I8	19J8	19P1	22B14	31H18	36N20	37E3	38G12	39D19	42P1	43P15	49P16

The pockets are defined by overlapping and nonoverlapping YAC clones (labeled B-M): AM137_h_5 (B), 938_e_7 (C), 982_e_7 (D), 798_f_7 (E), RA32H_d_6 (F), 827_h_5 (G), 850_f_5 (H), 744_b_7 (I), AM149_a_2 (J), 930_d_2 (K), 871_c_7 (L) and 773_b_9 (M).

al. 1993) and the EUROGEM genetic map order (Terrenato et al. 1994) of CEN-D6S260-D6S289-D6S259-TEL, which places D6S289 distal of D6S260 by virtue of a single recombination event. The physical maps (Nemani et al. 1994; Volz et al. 1994) have the order CEN-D6S289-(D6S89, D6S260)-TEL, probably as result of internal deletions within YACs. The direct physical mapping of uncloned DNA by interphase FISH analysis avoids this possible source of error and is in agreement with the genetic mapping data.

An Integrated Map of 6p23

The construction of the YAC contig spanning the chromosomal region from 6p22.3 to 6p24.1 used a number of markers from diverse sources such as genetic maps (Weissenbach et al. 1992; Jodice et al. 1993; Kwiatkowski et al. 1993; Gyapay et al. 1994), genes and anonymous ESTs mapped to the region (Arinami et al. 1991; Gaynor et al. 1991a,b; von Lindern et al. 1992; Orr et al. 1993; Banfi et al. 1994; Murano et al. 1994; Pappas et al. 1995), and anonymous markers from the Whitehead Institute/MIT Genome Center Database and Genome Data Base (GDB), as well as the subcloned Alu-PCR products. In addition, 25 of the PCR markers were mapped to cosmids within the pocket map thereby increasing the resolution of the overall map and resolving some of the markers not separated by the YACs. All of the markers have been incorporated into a single physical map thereby integrating genetic, physical, and transcriptional mapping data.

The map contains three anonymous ESTs whose location can now be integrated with the genetic maps and positioned relative to the other genes. At least one of the anonymous ESTs

(EST02156) is located between the STSs at the 5′ and 3′ ends of the SCA1 gene and therefore, must lie within the SCA1 gene. Another anonymous EST (EST00194) cannot be resolved from the marker at the 5′ end of the SCA1 gene, or D6S274, and therefore, this expressed sequence may also lie within the SCA1 gene. The sequences of both these ESTs have been compared with the published sequence of the SCA1 gene (Banfi et al. 1994) and no sequence similarity was observed.

DISCUSSION

We have constructed an integrated map of human chromosome 6p23 consisting of YAC and cosmid clones, polymorphic genetic markers, anonymous STSs and ESTs, anonymous subcloned Alu PCR products, and known genes. Both PCR screening and hybridization probing were used to determine the relationships between the YAC clones. This allowed both the previously published STS markers and the new markers, generated by subcloning Alu PCR products and STSs designed from known genes, to be used to characterize the same set of YAC clones.

The cosmid pocket map was constructed by hybridizing whole yeast DNA-containing YACs to arrayed grids of cosmids thereby removing the need to purify the YAC DNA away from the host yeast chromosomes. The map has been used to facilitate the construction of individual region-specific cosmid contigs derived from the pockets (M.G. Olavesen, unpubl.) that can be expanded to generate longer range contigs (S.J. Broxholme, J.L. Wixson, and R.D. Cambell, unpubl.). Meanwhile, some of the cosmids were found to contain one or more markers allowing small contigs to be formed, increasing the resolution of the map, and allowing some markers to be resolved. In addition, the data from the cosmid pocket map has been used to select cosmids distributed across the region for use as probes for long-range mapping of 6p23 (J.L. Wixon and R.D. Campbell, unpubl.).

To verify the physical map derived from the cloned resources of YACs and cosmids we have carried out FISH analysis on uncloned DNA from metaphase and interphase chromosomes. This proved particularly useful to check that the YACs used for Alu PCR to generate additional markers were not chimeric and to confirm that YACs positive for any of the anonymous STSs not genetically linked to chromosome 6 were derived from the region of interest. In addition, analysis of YACs on interphase chromosomes allowed gaps in the contig to be monitored to facilitate closure and also confirmed the physical order of cosmids to be determined on uncloned DNA.

The contiguous resource of YAC clones now spans from 6p22.3 to distal 6p24.3 (Davies et al. 1995; R.J. Stephens and J. Ragoussis, unpubl.). This is an invaluable resource for cytogenetic investigation of distal 6p as well as mapping of new markers in the region. The YAC clones can also be used to extend the YAC to cosmid contig conversion so that the cosmid resources can be used for more detailed physical mapping as well as gene detection and transcript mapping in 6p22.3–p24.3.

The integrated map contains 55 markers, spanning the region from 6p22.3 to 6p24.1 that covers 7.5–8.5 Mb, including the entire 6p23 band. Twenty of the markers on the map are polymorphic genetic markers (Weissenbach et al. 1992; Jodice et al. 1993; Kwiatkowski et al. 1993; Gyapay et al. 1994). Their integration into a single map has increased the marker density and ordered the markers to facilitate the generation of haplotypes for fine genetic mapping of the nonsyndromic CL/P (Carinci et al. 1995) and schizophrenia (Wang et al. 1995) loci mapped to the 6p23 region.

Six genes, DEK, SCA1, GMPR, ZNF40, EDN1, and TFAP2, and three anonymous ESTs are included in the map, thereby generating a preliminary transcription map of 6p23. It is unclear whether all three of the ESTs represent new genes in 6p23 because one lies within the SCA1 gene and another lies in very close proximity to the 5′ end of the SCA1 gene. The distribution of the genes and ESTs appears to be clustered into two groups. The first group of genes is located at the distal end of the contig and consists of ZNF40, EDN1, and TFAP2. The second group is flanked by the DEK and GMPR genes and includes the SCA1 gene. EST00194 lies within the same cosmid as the 5′ end of the SCA1 gene, EST02156 lies within the SCA1 gene, although neither EST appears to be part of the published SCA1 sequence, and GMPR lies immediately adjacent to the 3′ end of the SCA1 gene. The close proximity of these genes and ESTs supports the suggestion that 6p23 may be gene dense.

The locations of the genes and ESTs have been integrated with the genetic markers and this could facilitate their use in any positional cloning studies.

METHODS

Probes, PCR Primers, and Conditions

All STS markers used the same PCR conditions with the exception of the annealing temperature (T_{ann}), which was optimized for each primer set. The standard conditions used were 94°C for 4 min, then 35 cycles of 94°C for 30 sec, 30 sec at T_{ann}, 72°C for 30 sec followed by 72°C for 2 min. The PCR was carried out in 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$ 0.1% Triton X-100, 0.2 mM dNTPs, 1 μM of each primer (unless stated otherwise), and 0.5 units of *Taq* DNA polymerase in a 10-μl reaction.

Primers for D6S89 (T_{ann} = 56°C) (Litt and Luty 1990), D6S109 (T_{ann} = 62°C) using 0.1 μM primer 15a (Ranum et al. 1991), D6S202 (T_{ann} = 52°C) using primer set pHZ-30/2.6 (Le Borgne-Demarquoy et al. 1991), D6S334 (T_{ann} = 56°C) (Orphanos et al. 1994), SB1 (T_{ann} = 56°C), AM10GA/D6S337 (T_{ann} = 61°C), LR40/D6S338 (T_{ann} = 60°C) using primer set A (Kwiatkowski et al. 1993), EST00301/D6S315E (T_{ann} = 56°C), EST02534/D6S316E (T_{ann} = 58°C), EST00798/D6S321E (T_{ann} = 54°C), EST00194/D6S324E (T_{ann} = 58°C), EST00449/D6S328E (T_{ann} = 52°C), EST02156/D6S1385E (T_{ann} = 54°C), EST00859/D6S1394E (T_{ann} = 54°C) (Pappas et al. 1995) and D6S259 (T_{ann} = 58°C) using 0.5 μM of each primer, D6S260 (T_{ann} = 52°C), D6S274 (T_{ann} = 56°C), D6S285 (T_{ann} = 66°C), D6S288 (T_{ann} = 54°C), D6S289 (T_{ann} = 56°C), D6S422 (T_{ann} = 53°C), D6S429 (T_{ann} = 59°C), D6S443 (T_{ann} = 56°C), D6S469 (T_{ann} = 62°C) from the Genethon genetic maps (Weissenbach et al. 1992; Gyapay et al. 1994). Primers for D6S1058 (T_{ann} = 52°C), D6S1077/WI-2369 (T_{ann} = 56°C), D6S1301/WI-4789 (T_{ann} = 58°C), WI-3391 (T_{ann} = 58°C), WI-3658 (T_{ann} = 54°C), WI-3897 (T_{ann} = 56°C), WI-5032 (T_{ann} = 54°C), AFMA139we5 (T_{ann} = 50°C), AFM268vh5 (T_{ann} = 60°C), AFMB277zd5 (T_{ann} = 56°C), AFM346xe1 (T_{ann} = 56°C) were taken from the Whitehead Institute/MIT Genome Center Data Release 7. Data for D6S487 (T_{ann} = 64°C) were obtained from GDB version 5.5.1. Primers for STS c1.5 were c1.5A 5′-CAGATGCTATGCTTCAC-3′ and c1.5B 5′-CCAACAGATGGGTTTAG-3′ (T_{ann} = 52°C (P. Malaspina, A. Novelletto, and L. Terrenato, pers. comm.).

ESTs for TFAP2 and EDN1 genes were as described previously (Davies et al. 1995). Primers for DEK (von Lindern et al. 1992) were DEKL 5′-GAGCTAATTTCTTGAGATAGAGG-3′ and DEKR 5′-GGAACAATTAATGCCATGCAAG-3′ (T_{ann} = 56°C), for ZNF40 (HIV-EP1/MBP-1/PRDII-BF1) (Gaynor et al. 1991a) were PRDII 5′ 5′-CTCATGACTAATCTTTGTGC-3′ and PRDII 3′ 5′-CTTACACAAGGAGGACAGAC-3′ (T_{ann} = 56°C), and for GMPR (Kondoh et al. 1991) were GMPR-5′ 5′-GTTCTATCGTCTTCCAGAGCC-3′ and GMPR-3′ 5′-GAGGTTATGAGTTCTGGGCAG-3′. For the SCA1 gene the $(CAG)_n$ repeat was amplified with the Pre-1 and Rep-2 primers (Orr et al. 1993). Primers for the ends of the SCA1 gene (Banfi et al. 1994) were SCA1 5′f 5′-CAGTGGCGGACGTACAGG-3′ and SCA1 5′r 5′-GTAAATGGATCTGGGGTTGC-3′ (T_{ann} = 59°C) and SCA1 3′f 5′-CTGACATGGCCAGTACAGAG-3′ and SCA1 3′r 5′-CCTACAAATAGACACACCACG-3′ (T_{ann} = 59°C).

Hybridization probes for the human renal sodium phosphate transport protein gene (NPT1/SLC17A1) (Chong et al. 1993) and the human FIM1 gene (Van Cong et al. 1989) were used to screen YAC and cosmid clones isolated from primary library screens.

Isolation and DNA Preparation of YAC and Cosmid Clones

YAC clones were isolated by PCR-only screening of the CEPH (Dausset et al. 1992), ICRF (Larin et al. 1991) and ICI (Anand et al. 1991) YAC libraries based on the strategy of Green and Olson (1990). Agarose blocks of yeast DNA were prepared by the method described in Southern et al. (1987) and total yeast DNA was isolated from the blocks as described in Davies et al. (1995). High density membranes of the flow-sorted chromosome 6 cosmid library (Nizetic et al. 1991, 1994b) were screened with YACs by hybridization of total yeast DNA using total human DNA to suppress signals from repetitive sequences (Sealey et al. 1985). YAC and cosmid clones selected from primary library screens were gridded onto membranes using a Beckman Biomek 1000-robot and processed as described in Bentley et al. (1992). Clones were arrayed in characteristic patterns to facilitate precise identification of coordinates on filters. Cosmid DNA was prepared by alkaline lysis followed by binding of the DNA to silica as described in Carter and Milton (1993). Additional cosmids subcloned from YAC AM137_h_5 into the SuperCos 1 vector (J.L. Wixon, S.J. Broxholme, and R.D. Campbell, unpubl.), prefixed with "c" were used where clones had not been obtained from the flow-sorted chromosome 6 cosmid library.

The clones described are available upon request. In addition, the ICI, ICRF, and CEPH YAC library clones are available from the U.K. Human Genome Mapping Project (HGMP)–Resource Centre, the chromosome 6 cosmids and the ICRF YAC library clones are available through the Reference Library Database System and the CEPH YAC library clones are available from CEPH.

Preparation of Alu PCR and Vectorette PCR Probes for Hybridizations

Inter-Alu PCR was carried out using the ALE1 and ALE3 primers (Cole et al. 1992) in 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 7.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM dNTPs, 1 μM each primer, and 0.5 units of *Taq* DNA polymerase in a 10-μl reaction. The PCR conditions used were: 94°C for 4 min, then 35 cycles of 94°C for 1 min, 63°C for 1 min, 72°C for 3 min, followed by 72°C for 5 min. Amplified products were purified using silica particles (Carter and Milton 1993) and shotgun cloned into the pCR II vector (Invitrogen). Hybridization probes were isolated from individual clones after excision of the inserts from the vector DNA with *Eco*RI. Vectorette PCR was carried out as described (Riley et al. 1990) using the restriction enzymes *Rsa*I, *Alu*I, and *Pvu*II on total yeast DNA isolated from blocks. All Alu PCR and vectorette PCR probes were hybridized in the presence of total human DNA to suppress signals from repetitive sequences (Sealey et al. 1985).

Fluorescence in situ Hybridization

FISH mapping of YACs and cosmids was performed on metaphase chromosomes prepared from phytohemagglu-

tinin (PHA)-stimulated peripheral blood lymphocytes from healthy men. These were synchronized with thymidine, lysed and harvested by standard cytological techniques (Rooney and Czepulkowski 1992).

YAC clones were not isolated from endogenous yeast DNA before FISH. Cosmid clones, total yeast DNA, and Alu PCR products were labeled with biotin-14-dATP or digoxigenin-11-dUTP by nick translation (Bio-Nick Labelling System or Nick Translation System, respectively, BRL Life Technologies, USA). Probes requiring double-labeling were prepared by mixing equal quantities of biotin- and digoxigenin-labeled probe.

In situ hybridization was performed as described (Adinolfi and Davies 1994). YAC or cosmid probes (50 or 100 ng per slide, respectively) were prehybridized with Cot-1 DNA (50 × (wt/wt)) before FISH. Hybridization signals from biotin-labeled probes were developed using alternate layers of avidin–fluorescein isothiocyanate (avidin–FITC) and biotinylated anti-avidin. Those from digoxigenin-labeled probes were developed with a layer of sheep anti-digoxigenin conjugated to tetramethylrhodamine isothiocyanate (TRITC–anti-digoxigenin) followed by one layer of TRITC-conjugated donkey anti-sheep (The Binding Site, Birmingham, UK). Slides were mounted in Vectashield antifading medium (Vector Laboratories, Peterborough, UK) containing 80 ng/ml of 4′, 6-diamidino-2-phenylindole (DAPI) as counterstain. Signals were visualised under a Zeiss Axioplan microscope equipped with a cooled charge-coupled device (CCD) camera (Photometrics, Woburn, MA) and Smartcapture image analysis system (Digital Scientific Instruments, Cambridge, UK). FITC, TRITC, and DAPI images were captured using filters four, three, and two of the Pinkel filter wheel 1, respectively. G-banding was enhanced during image analysis.

YAC and cosmid clones were mapped to specific chromosome bands by examination of a minimum of 10 well-extended metaphases per clone. The quality of the banding was sufficient to allow localization of clones to precise bands and subbands by comparison with the digitized and differentially shaded ideograms, at the 850-band level, as depicted by Francke (1994).

Interphase FISH was performed on nuclei prepared from fibroblasts that had been grown to confluence and, therefore, were in G_1 phase. In the absence of a well-characterized cosmid contig from 6p23 the nuclei were calibrated using cosmid pairs from the human MHC in 6p21.3 of known distance separation (Carroll et al. 1984; Sargent et al. 1989; Kendall et al. 1990) as previously described (Senger et al. 1993) to obtain a calibration curve of physical distance (kb) against measured distance (μm). The calibration curve had a region of linearity between 50 and 500 kb and was therefore, was used to estimate distances between key cosmids and YACs in experiments using two or more differentially labeled clones. Clone pairs separated by distances >500 kb could only be ordered using this technique and especially chosen sets of clones could be used to ascertain contig orientation. Physical distances were estimated from a minimum of 15 measurements per probe set and were normally taken from 25 or more measurements depending on the quality of signals obtained. Possible differences between the region of the genome used to generate the calibration curve and 6p23 may result in some variation between the actual distances and the distances we have estimated.

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