Delineating Rearrangements in Single Yeast Artificial Chromosomes by Quantitative DNA Fiber Mapping

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Abstract: Cloning of large chunks of human genomic DNA in recombinant systems such as yeast or bacterial artificial chromosomes has greatly facilitated the construction of physical maps, the positional cloning of disease genes or the preparation of patient-specific DNA probes for diagnostic purposes. For this process to work efficiently, the DNA cloning process and subsequent clone propagation need to maintain stable inserts that are neither deleted nor otherwise rearranged. Some regions of the human genome, however, appear to have a higher propensity than others to rearrange in any host system. Thus, techniques to detect and accurately characterize such rearrangements need to be developed. We developed a technique termed 'Quantitative DNA Fiber Mapping (QDFM)' that allows accurate tagging of sequence elements of interest with near kilobase accuracy and optimized it for delineation of rearrangements in recombinant DNA clones. This paper demonstrates the power of this microscopic approach by investigating YAC rearrangements. In our examples, high-resolution physical maps for regions within the immunoglobulin lambda variant gene cluster were constructed for three different YAC clones carrying deletions of 95 kb and more. Rearrangements within YACs could be demonstrated unambiguously by pairwise mapping of cosmids along YAC DNA molecules. When coverage by YAC clones was not available, distances between cosmid clones were estimated by hybridization of cosmids onto DNA fibers prepared from human genomic DNA. In addition, the QDFM technology provides essential information about clone stability facilitating closure of the maps of the human genome as well as those of model organisms.

Keywords: Physical mapping, recombinant DNA, yeast artificial chromosomes, stability, rearrangements, clone validation, *in situ* hybridization, DNA fiber mapping.

INTRODUCTION

The positional cloning of disease genes as well as high-throughput production sequencing using a directed approach demand high-resolution physical maps for megabasepair (Mbp) size genomic regions [1-3]. The assembly of such maps typically followed a top-to-bottom approach beginning with low-resolution framework maps comprised of yeast artificial chromosome (YAC) clones [4-6]. The YACs allowed for cloning and propagation of Mbp size inserts of contiguous DNA, but the use of YAC libraries was compromised by a high rate of chimerism and deletions as well as other forms of rearrangements [7-10]. Coverage of the human genome by clones from other large insert DNA libraries such as the bacterial artificial chromosome (BAC) clones is steadily increasing [11, 12], but YAC clones and the well characterized CEPH/Genethon YAC library, in particular, have been very useful resources for rapid construction of maps with Mbp resolution [13-15]. Technologies providing information about the presence and nature of rearrangements in any type of large insert, recombinant DNA clones should be very useful, since they help to avoid costly mapping errors through an early identification of unsuitable clones.

Using 'Quantitative DNA Fiber Mapping' (QDFM) which allows the delineation of deletions and clone overlaps by direct visualization, we constructed high resolution physical maps for several regions of the human genome, among them a chromosome 22-specific region covered by three different sub-clones of an unstable YAC clone [16-20]. In a nutshell, QDFM is based on the hybridization of probe DNA prepared from one or several clones onto stretched DNA molecules isolated from another, often larger clone, such as a YAC clone or even genomic DNA [16]. As demonstrated below, this method can be applied for the rapid characterization of various types of rearrangements with near kilobase accuracy. The study described here focuses on the region of the human genome containing part of the immunoglobulin (Ig) lambda variant (IGLV) gene cluster located proximal to the lambda constant gene region (IGLC) on chromosome 22q11 [17, 21]. While rearrangements involving this region are found frequently in human tumors, little is known about the stability of the Ig lambda region in recombinant *in vitro* systems. The similarly rearranged immunoglobulin heavy (IGH) and kappa (IGK) light chain genes are known to be rich in large stretches of repetitive

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sequences [22, 23]. They may therefore represent 'critical sequences' for cloning. Interestingly, the IGH locus was shown to be unstable in yeast, whereas IGK sequences were stable [24]. The immunoglobulin genes, in general, are believed to have developed from ancestral duplication events [25], and the IGLC-complex, in particular, from the duplication of a smaller Alu-free segment [26]. The IGLC complex is comprised of 7 clustered and tandemly repeated genes [26, 27], while the IGLV gene cluster consists of between 52-90 individual variant genes assigned to 10 families [28-30]. Sequence analysis of some IGLV segments suggested high homology between members of the same family (>76%), but lower homology (<75%) between members of different families [31]. These differences allowed the construction of two restriction maps for the IGLV region [28, 29].

In a previous study, we used YAC clones and a set of minimally overlapping cosmid clones to prepare a high resolution map of the IGLV region [17]. High molecular weight YAC DNA molecules were isolated by pulsed field gel electrophoresis (PFGE), deposited and stretched on glass slides. We then hybridized cosmid clones along individual YAC molecules and recorded their respective map positions. The work presented here was prompted by the discovery of deletions in some of the YAC clones. We hypothesized that QDFM analysis of several subclones showing different deletions will allow us to reconstruct the history of the deletions, i.e., to discriminate stepwise deletions and thus the progressive loss of insert DNA from events in which different subclones are derived independently from the same parental clone. We were thus interested in pinpointing the exact locations of the rearrangements within the cloned portion of IGLV to study the progression of deletions. As shown below, we mapped cosmid clones along 3 subclones of YAC 400B5 and recorded differences in their representation. Where YAC coverage was absent, cosmids were hybridized onto DNA fibers prepared from partially overlapping cosmids to determine the orientation as well as the extent of overlaps. Deletions within the YAC inserts were characterized by mapping the YAC clones onto high molecular weight DNA fibers prepared from human genomic DNA.

MATERIALS AND METHODOLOGY

Preparation of High Molecular Weight (HMW) DNA

Three subclones of CEPH YAC clone 400B5 [4] mapping to the IGLV cluster [29] were studied. Information about the exact size of the non-deleted clone was not available. Agarose plug preparation was performed according to standard protocols. PFGE was carried out using a CHEF electrophoresis system (BioRad, Hercules, CA) under standard conditions. Fifteen individual colonies were tested. The YAC DNA was recovered by excising the YAC bands from the gel. High molecular weight DNA was then prepared by β-agarase digestion (New England Biolabs, Beverly, MA) of the gel slices as described [16, 32].

Human genomic HMW DNA was isolated from C32 melanoma cells (cat. #CRL-1585, ATCC, Rockville, MD) according to the procedure described by Birren and Lai [33]. Briefly, about $5x10^5$ cells were washed once in phosphate buffered saline (PBS) and resuspended in 0.5 ml of PBS. Meanwhile, 0.5 ml of 1.2% low melting point agarose in PBS (Invitrogen, Gaithersburg, MD) was melted and cooled down to 43°C. Agarose was added to the cell suspension, the solution was mixed thoroughly, and 100 µl aliquots were dispensed into plug molds (BioRad) [20]. Agarose blocks were then placed into 0.5M EDTA (pH 8.0), 1.0% N-lauroylsarcosine (Sigma, St. Louis, MO), 0.5 mg/ml proteinase K and incubated overnight at 50°C. Finally, the plugs were washed 4 times for 30 min each in 50 mM Tris, 1 mM EDTA and stored at 4°C. The HMW DNA was prepared by digestion of the plugs with β-agarase [32].

Glass Modification and Preparation of DNA Fibers

Microscope slide preparation followed our published procedure [17]. Briefly, slides were cleaned mechanically, repeatedly rinsed with and boiled in distilled water, and then treated with sulfuric acid. Slides were derivatized using a 0.1% solution of 3-aminopropyltriethoxy silane (APS, Sigma) in 95% ethanol [17,18,32]. Two microliter of YOYO-1 (1 µM; Molecular Probes, Eugene, Oregon) - stained HMW DNA solution was spread onto an untreated coverslip, before the coverslip was placed on an APS-treated microscope slide. The DNA concentration and binding was checked by fluorescence microscopy using a fluorescein filter set (Chroma Technology Corp., Brattleboro, VT).

Coverslip preparations followed the protocol used for glass slides [18]. Briefly, coverslips were rinsed with distilled water, dehydrated in 100% ethanol and air dried. The coverslips were derivatized using a 0.1% APS solution in 95% ethanol. Next, 2 µl of genomic DNA was applied to an untreated cover slip, which was then placed upon the APS treated coverslip. After a short (1 min) incubation at 20°C, the treated coverslip was removed slowly from one end, allowing the meniscus between the two coverslips to pull the DNA fibers in one direction by hydrodynamic action [16]. Coverslips carrying the DNA fibers were allowed to dry at room temperature.

DNA Probes

Cosmid contigs covering the YAC clone 400B5 [17, 28, 29] were derived from the Lawrence Livermore National Laboratory chromosome 22-specific cosmid library LL22NC03 [34]. Cosmid DNA extraction was performed by alkaline lysis [19-20]. DNA isolated from plasmids pJs97 and pJs98 (Invitrogen) containing the YAC cloning arms was used to determine the orientation of the YAC insert. Plasmid DNA was extracted using a kit (Plasmid Mini Kit; Qiagen, Chatsworth, CA). Both, cosmid and plasmid probes were labeled by random priming reactions using a commercial kit (BioPrime kit, Invitrogen) incorporating biotin-dCTP (bio-dCTP). Random priming incorporating digoxigenin-11-dUTP (dig-dUTP) or fluorescein-12-dUTP (FITC-dUTP) (Roche Molecular, Indianapolis, IN) was done as described [16].

The YAC DNA probes for counterstaining of DNA fibers were generated by PCR using mixed-based oligonucleotide primers [35]. An aliquot of the respective HMW YAC DNA obtained by PFGE for fiber preparation was PCR amplified for a total of 42 cycles. In the first seven amplification

cycles, we used the mixed-base primer JUN1 (5'-CCCAA GCTTGCATGCGAATTCNNNNCAGG-3', N=ACGT) and Sequenase™ (USB, Cleveland, OH) [35]. Briefly, 3 µl of HMW DNA solution were removed from the bottom of each tube and PCR amplified using the following conditions: denaturation at 92°C for 3 min, primer annealing at 20°C for 2 min and extension at 37°C for 6 min. Sequenase™ had to be added after each denaturation [35-37]. Then, 20 µl of the reaction product were resuspended in a 200 µl Taq amplification reaction buffer and amplified with primer JUN15 (5'-CCCAAGCTTGCATGCGAATTC-3') under the following PCR conditions: denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, and extension at 72° for 2 min, repeated for 35 cycles [35]. After ethanol precipitation, the product was resuspended in 30 µl TE, and 1.5 µl aliquots were labeled in 25 µl random priming reactions incorporating FITC-dUTP [16].

Fluorescence *In Situ* Hybridization (FISH)

All hybridizations were carried out overnight at 37°C in a moist chamber following our published conditions for DNA fiber hybridizations or mapping of DNA probes on metaphase spreads [16, 38, 39], i.e., in 55% formamide (FA), 10% dextran sulfate, 2X SSC (300 mM NaCl, 30 mM Na citrate), pH 7.0. A typical fiber FISH hybridization mixture contained 1 µg of COT-1™ DNA (Invitrogen) and 20-30 ng of each of the probes. When hybridizing cosmids onto YAC fibers, a fluorescein-labeled probe to counterstain the YAC fibers was applied at a comparatively low concentration allowing competitive displacement by the cosmid probes [17]. Additionally, one of the two YAC cloning vector probes was included to assess the orientation of the insert. Following hybridization and prior to triple color antibody detection, the slides were washed in 2X SSC (pH 7.0) and blocked with 100 µl PN buffer (0.1 M sodium phosphate buffer, pH 8.0, 1% Nonidet-P40 (Sigma)) supplemented with 5% non-fat milk powder (Carnation, Wilkes-Barre, PA) [37]. Detection was carried out as described [16-18]. Slides were mounted in 1% p-phenylenediamine antifade solution (Sigma) in glycerol [38] and stored at -20°C.

Microscopy and Image Analysis

Images were acquired with a Zeiss fluorescence microscope equipped with a 63x, 1.25 NA objective, quadruple color fluorescent filters (84000, Chroma Technology Corp.) and a cooled CCD camera (CH250; Photometrics Inc. Tucson, AZ) connected to a SUN SPARC workstation [38, 40]. Image analysis for quantitative mapping was performed using the program 'Fibermap' developed at the LBNL [41]. Distances were recorded in micrometers. In each experiment, 4 to 12 individual fibers were analyzed and the map positions of the cosmids in relation to the YAC vector probes and/or to other cosmids were recorded. All measurements were performed in triplicate; the results provided in the form of lists were imported into spreadsheets (MS Excel, Microsoft Corp., Redmond, WA) for further calculations. We used a factor of 2.3 kb/µm for the conversion of the length of fibers or hybridization domains (measured in micrometers) to DNA fragment sizes (reported in kb) [16-18].

RESULTS

DNA fibers of different molecular weight were derived from three subclones of YAC clone 400B5 streaked out on agar plates and used as hybridization targets. Analysis of 15 subclones of YAC 400B5 by PFGE revealed five different YAC bands ranging in size from <200 kb to 350 kb. For our studies, we selected the largest subclone with a size of about 350 kb which was found in two of 15 subclones, a slightly smaller subclone of about 330 kb found in one of 15 colonies, and a 250 kb deleted clone present in two of 15 subclones. All three of these subclones produced sharp YAC bands in PFGE analysis (Fig. **1**). The DNA probes for FISH mapping of YACs were prepared by mixed-based oligonucleotide primer PCR followed by random priming. Hybridization to normal human metaphase spreads gave specific signals on chromosome 22q11, i.e., the IGLV locus [27, 29], for all three YAC probes.

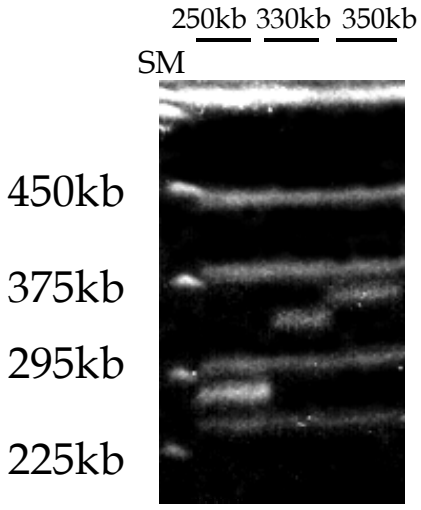

Fig. (1). Pulsed field gel electrophoretic analysis of three subclones of YAC clone 400B5. The sizes of YACs were 250kb, 330 kb and 350 kb in lanes A, B and C, respectively. (SM = yeast chromosome size marker bands with sizes indicated to the left).

Each cosmid from a minimal tiling path defined earlier by cosmid fingerprinting or DNA fiber mapping [17, 28] was hybridized on to metaphase chromosomes to verify its map position and check each clone for potential chimerism. All 14 cosmid clones used in this study (including clone 61E11) gave unambiguous signals on the proximal long arm of chromosome 22. The cosmids were subjected to PFGE to independently determine their size and to compare the results to sizes measured by QDFM. Table **1** shows results of QDFM measurements for 13 cosmids on the different YAC subclones as well as the sizes determined by PFGE. All subsequent experiments were carried out by hybridization of cosmids on to stretched DNA fibers prepared from either one of the three different deletion forms (350 kb, 330 kb, 250 kb) of YAC clone 400B5.

In the actual mapping experiments, two cosmids, one labeled with biotin (visualized in blue), the other labeled with digoxigenin (detected in red), the respective FITC-labeled YAC probe for counterstaining the entire target molecule (green), and one of the digoxigenin-labeled YAC

Table 1. Cosmid Mapping Results for Three Different Subclones of YAC 400B5*

Cosmid Clone	PFGE Size	350kb-YAC			330kb-YAC			250kb-YAC		
		QDFM Size	OL with Previous Clone	Gap to Previous Clone	QDFM Size	OL with Previous Clone	Gap to Previous Clone	QDFM Size	OL with Previous Clone	Gap to Previous Clone
67B3	~40	25.2 (2.8)	n.a.	n.a.	n.d.	n.a.	n.a.	28.9 (4.4)	n.a.	n.a.
102D1	~40	42.1 (2.6)	9.9 (2.0)	0.0 (0)	n.d.	n.d.	n.d.	39.1 (3.7)	10.4 (1.6)	n.a.
52F2	~40	41.9 (2.9)	32.2 (2.2)	0.0 (0)	n.d.	n.d.	n.d.	42.8 (3.0)	29.2 (2.4)	n.a.
31F3	~40	39.2 (2.7)	16.1 (2.5)	0.0 (0)	n.d.	n.d.	n.d.	41.5 (1.7)	13.1 (2.7)	n.a.
75H1	~40	38.7 (1.4)	6.8 (1.4)	0.0 (0)	18.0 (1.8)	n.d.	n.d.	22.9 (4.1)	8.7 (2.0)	n.a.
63E9	~40	not repr.	n.a.	n.a.	19.1 (2.5)	0.0 (0)	0.0 (0)	not repr.	n.a.	n.a.
85H5	~40	not repr.	n.a.	n.a.	16.6 (2.6)	16.6 (2.6)	0.0 (0)	not repr.	n.a.	n.a.
50D10	~15 †	16.6 (0.8)	0.0 (0)	17.6 (2.0)	8.2 (1.4)	0.0 (0)	0.0 (0)	not repr.	n.a.	n.a.
127C9	~40	36.5 (3.1)	0.0 (0)	33.6 (5.2)	38.3 (4.2)	0.0 (0)	32.4 (2.6)	38.3 (4.2)	n.a.	0.0 (0)
107H8	~40	38.9 (2.8)	11.6 (0.9)	n.a.	n.d.	n.d.	n.d.	40.2 (3.3)	n.d.	n.d.
47D10	~40	40.2 (3.1)	9.0 (1.6)	n.a.	n.d.	n.d.	n.d.	45.6 (2.6)	8.2 (1.2)	n.a.
4G11	~40	42.7 (3.3)	6.3 (0.9)	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
95D3	n.d.	20.4 (2.4)	5.4 (1.0)	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

*The physical sizes in kb and standard deviations (in kb, where applicable) were measured by either PFGE or QDFM. The extent of overlaps (OL) with the previous clone as well as of gaps between adjacent clones are listed. [n.d. = not done, n.a. = not applicable, not repr. = cosmid not represented on DNA fiber]

†: Verified unstable cosmid clone.

vector probes, pJs98 and pJs97, (red) were hybridized simultaneously. The YAC vector probes represent the respective ends of the YAC DNA molecules. Applied individually, they served as reference point allowing us to distinguish between the pJs97 (yeast telomere) and pJs98 (yeast telomere and centromere) end of the fibers. Fig. (**2A**) shows a typical DNA fiber representing part of the distal end of the 250 kb subclone of YAC 400B5 after hybridization with the pJs98 vector probe (red) and cosmids 47D10 and 107H8 shown in red and blue, respectively.

Interestingly, QDFM analysis of the 350 kb subclone revealed two gaps of cosmid coverage between the cosmid contigs roughly in the center portion of the YAC (Fig. **3C**), one of which had been described previously [28]. This previously known cosmid contig gap, flanked by cosmid clones 50D10 and 127C9, extended for about 33.6 kb (Table **1**) and was also found in the 330 kb subclone of YAC 400B5 (Fig. **3B**). Furthermore, the QDFM analysis of our 250 kb-sized YAC subclone showed this gap as falling into a larger, deleted region between the map position of cosmid clones 75H1 and 127C9 (Fig. **3A**).

The second more distal gap in the cosmid coverage has not been reported in the literature. In contrast, the respective region was found to be covered by three other cosmids (61E11, 63E9, 85H5), two of which were reported not to overlap (i.e., clones 61E11, 63E9)[29]. Since there was no representation of this area on the 350 kb form of the YAC, we hybridized cosmid probes on to cosmid fibers to determine overlaps. This allowed us to verify that cosmid 50D10 indeed overlapped at its telomeric portion with another clone, 85H5, for about 3 kb (Fig. **3C**). Clone 85H5 itself showed an overlap with the more telomeric clone, 63E9, of about 22 kb (Fig. **2C**). However, a third cosmid clone, 61E11, reported to overlap with clone 75H1(Fig. **3B** in reference [29]), was found to not be represented along any of the YAC subclones used.

When testing the two smaller subclones of the YAC (330 kb and 250 kb), it became apparent that the centromeric portion of the cosmid contig proximal to cosmid clone 127C9 (that is, toward the end marked by probe pJS98) was retained in all three subforms. This indicated that the differences in YAC sizes might have been caused by rearrangements distal of this clone. When compared to the 350 kb subclone, the 250 kb variant was found to lack a region of ~85-90 kb distal to 127C9 including both gaps and part of cosmid 75H1 (Fig. **3**). This deletion in the 250 kb fibers is in good agreement with the PFGE size difference of ~100 kb between this and the 350 kb subsubclone.

In contrast, the 330 kb variant turned out to be the result of more complex rearrangments. In the region of the more distal gap along the 350 kb subform, those two cosmids found to continuously overlap with each other in the cosmid on to cosmid QDFM approach (clones 65E9 and 85H5, see bottom of Fig. (**3C**) labeled 'Cosmid on Cosmid') were represented, although only in part. The closest telomeric and centromeric cosmids on either sides of clones 63E9 and 85H5 seemed to be directly adjacent, exhibiting no overlap, and thus suggesting deletions on either side.

Fig. (**2B**) shows the respective region along a 330 kb YAC molecule after hybridization with cosmids 75H1 (red) and 63E9 (blue). Note that the two signal domains are directly adjacent and extend less than half the length of the other two cosmids shown in Fig. (**2A**), although our PFGE analysis suggested comparable insert sizes. Fig. (**3**) summarizes the different maps suggested by the QDFM

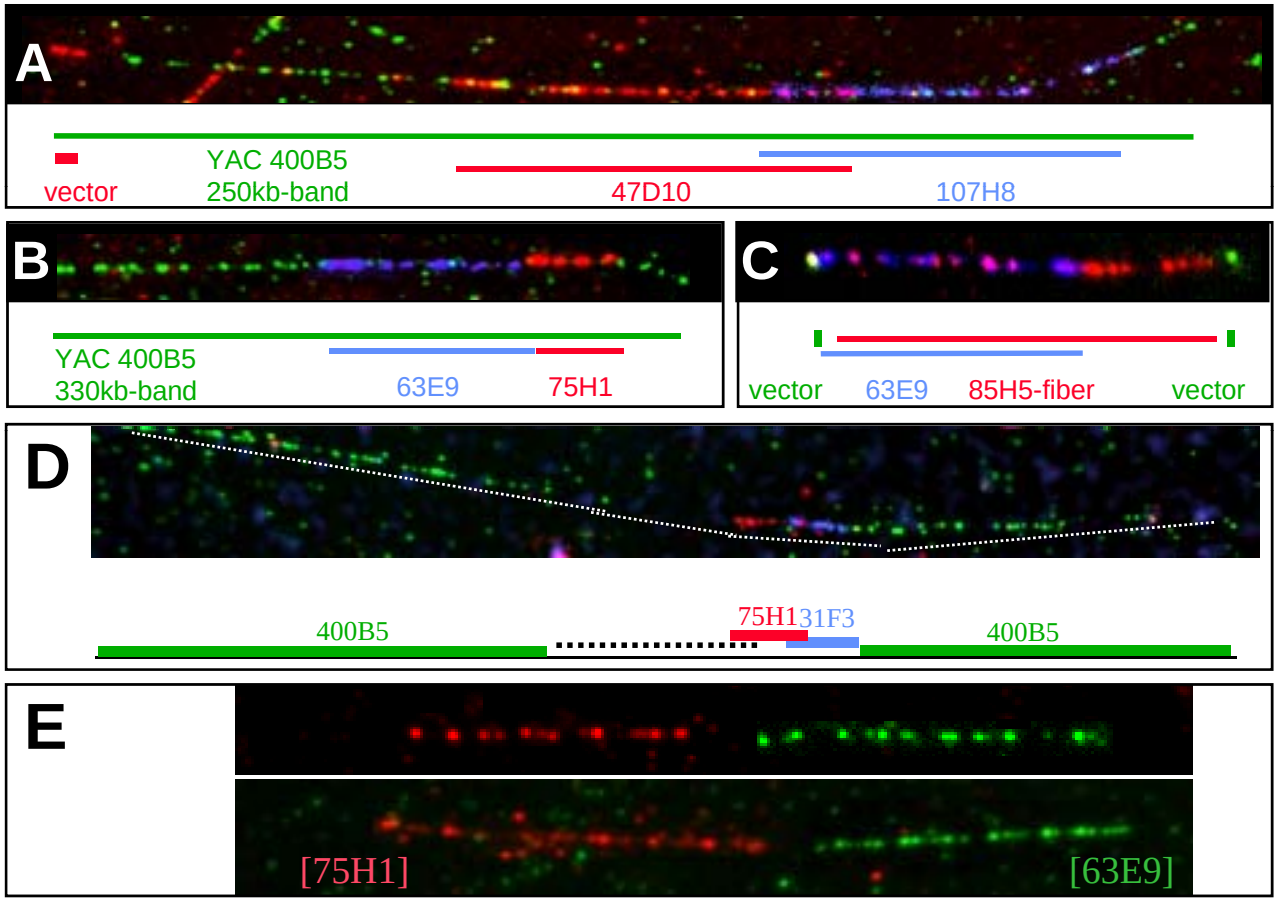

Fig. (2). DNA fiber mapping results. (**A**) Partial 400B5-250kb fiber counterstained with a FITC-labeled YAC probe derived from the 350kb YAC band (green). The fiber was hybridized with two overlapping cosmid clones, 47D10 (red) and 107H8 (blue), and the YAC vector probe, pJs 98 (red), marking the left (centromeric) end of the molecule. (**B**) Partial 400B5-330kb fiber after hybridization with the cosmid clones 75H1 (red) and 63E9 (blue) which map to a region frequently found rearranged. Both signal domains appear short compared to the domains shown in panel A, even though the cosmids have about the same insert sizes in the PFGE. The two domains are directly adjacent indicating a deletion on either side of the two cosmids within the YAC. (**C**) A cosmid 85H5 fiber (red) hybridized with a probe from the partially overlapping cosmid clone 63E9 (blue). DNA isolated from an unrelated cosmid clone was labeled in green and co-hybridized to mark the vector part of this cosmid DNA fiber molecule. This approach enabled us to determine overlaps between cosmids that contained genomic DNA fragments not or only partially represented in YACs. (**D**) Results of hybridization of one of the deleted forms of 400B5 YAC (green), and cosmids 75H1 (red) and 31F3 (blue) to fibers prepared from genomic DNA. The deleted region of the 400B5 YAC can be visualized as a gap in the green signal. (**E**) Results of hybridization of cosmid 75H1 (red) and 63E9 (green) to genomic DNA fibers show these two cosmids in close proximity and non-overlapping with a 2-3 kb gap between them. Top and bottom of this figure represent two different fibers from the same slide.

experiments using the three different YAC hybridization templates and cosmid fibers 85H5 and 63E9.

An elegant approach to validate the integrity of clone inserts is the use of fibers prepared from genomic DNA. Fig. (**2D**) represents the results of the hybridization of three probes to human genomic DNA, isolated from the C32 cell line. The deletion in this subclone of YAC 400B5 can be directly visualized as a gap in the track of green signals along the YAC molecule (indicated by the dotted white line). This experiment also provided direct experimental confirmation of the fact that DNA fragments cloned in cosmids 75H1 and 31F3 are deleted in this subclone of YAC 400B5. Fig. (**2E**) demonstrates the results of the hybridization of the cosmids 75H1 and 63E9 to human genomic DNA fibers. It is apparent that the germ line configuration carries a gap between cosmids 75H1 and 63E9 estimated to be 3-5 kb (Fig. (**2E**) and '?' in Fig. (**3C**), bottom). These results also support the use genomic DNA fibers as 'gold standard' for the verification of gaps, clone overlaps and clone contigs.

Interestingly, cosmid 50D10 maps in the center of the rearranged region and was found deleted, too, as evidenced by its small insert size (QDFM: ~ 17 kb, PFGE: ~15 kb) (Table **1**). The more distal gap along the 350 kb subform, which was not covered by any of the clones represented along other fibers, could not be further characterized by our method. The region distal of cosmid 75H1 was maintained in all three subclones, thus all rearrangements seem to be internal. Very detailed mapping of cosmids was performed along the 350 kb subclone and most of the 250 kb subclone

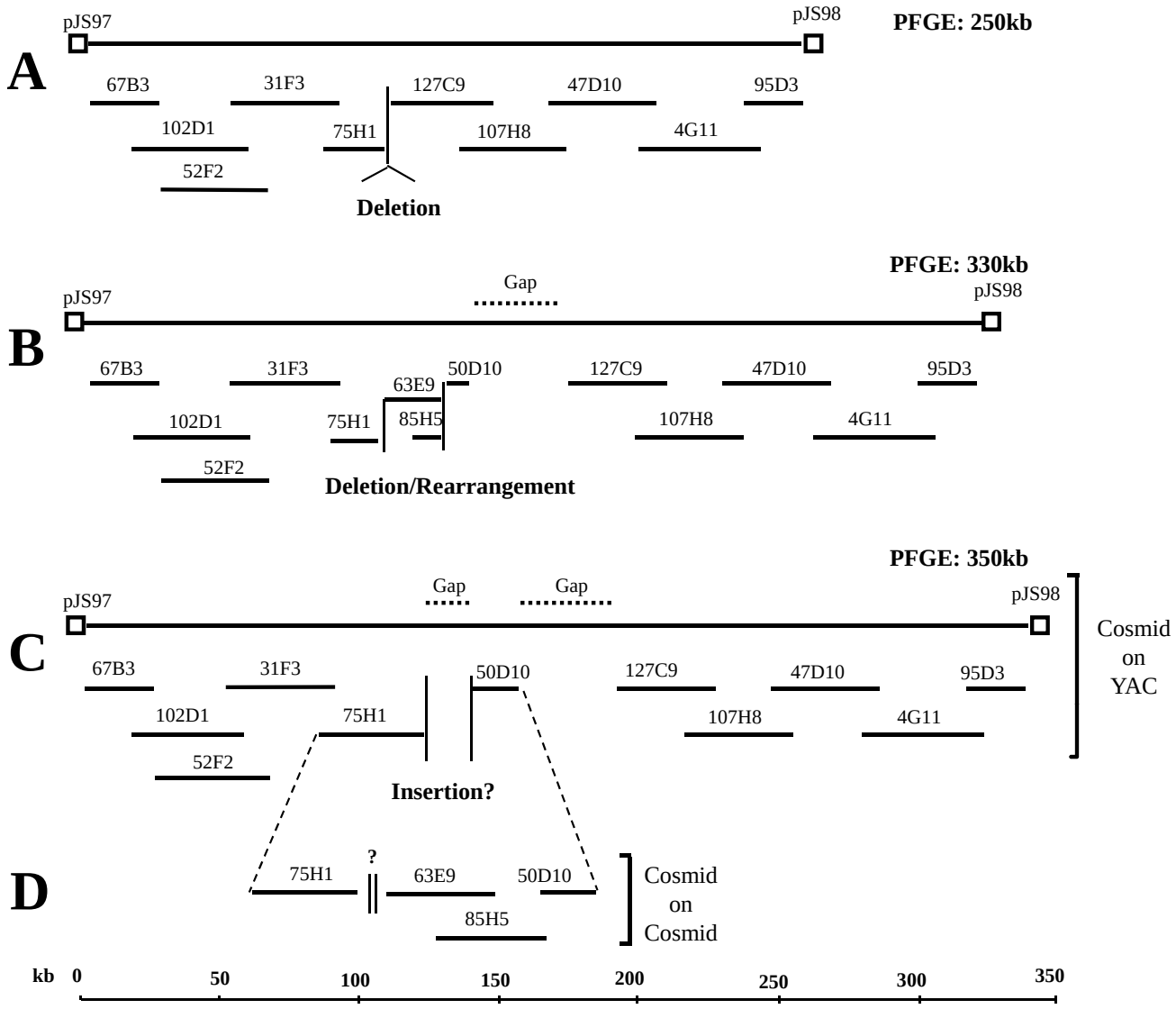

Fig. (3). High resolution physical maps of part of the immunoglobulin lambda variant gene cluster. Cosmid probes were hybridized onto stretched YAC 400B5 DNA molecules of different sizes. Hybridization target fibers were prepared from subclones carrying YACs of 250kb (**A**), 330kb (**B**) or 350 kb (**C**). The cosmid coordinates are given above the lines indicating their map positions; pJs97 and pJs98 indicate the YAC vector probes used for the determination of the YAC fiber orientation. The map displayed in panel (**D**) summarizes the results from hybridizing cosmid probes onto cosmid fibers, since the respective region was found absent in all of the YACs used.

(Table **1**). Since visual inspection of cosmid probes along the 330 kb subclone suggested an equal representation in most parts, we limited our quantitative mapping to the critical region.

DISCUSSION AND CONCLUSION

Yeast artificial chromosomes represent a major development in genome research, allowing the propagation of very large inserts of several Mbp [4]. In combination with PFGE techniques to physically separate large DNA fragments, YACs enabled the assembly of long range physical maps extending over much longer distances than ever achieved before [1-2, 13, 33]. Problems limiting the use of YACs are chimerism, artificially joining non-contiguous portions of the genome, stable deletions and clonally unstable rearrangements [4, 6-8].

This paper demonstrates a novel application of QDFM: the characterization of rearrangements in cloned DNA molecules, such as YACs, BACs or cosmids. The YACs, widely used for large scale mapping projects, are frequently found rearranged or deleted, which creates problems in the use of such clones for map assembly [29, 42]. The hybridization of labeled PCR products or small insert clones derived from independent libraries which cover the regions of interest on to such rearranged DNA molecules using QDFM allows the direct visualization of various types of rearrangements including DNA deletions, inversions, amplifications or insertions [16-20, 43-45]. Helping to prevent errors in the construction of physical maps or to identify chimeric clones in early stages of a project, QDFM can characterize rearrangements with kilobase accuracy and sub-kilobase sensitivity [16-20] over distances exceeding

one Mbp [45-47]. The procedure is easy to perform, requires no specialized equipment, and at this time, there is no comparably cost- and time-efficient alternative method to uncover alterations in cloned DNA molecules. As shown in the present report, QDFM is especially useful for studies of regions that contain clusters of closely related genes and/or are unusually high in repeated sequences.

In the course of a physical mapping project on the proximal long arm of human chromosome 22, we identified several rearranged YAC clones [17] and decided to characterize these unstable clones in more detail. The present investigation showed that unstable YACs are not only subject to simple deletions, accounting for the presence of more than one size variant of a clone in the PFGE, but rearrangements can be more complex. Like other studies, our results hint that rearrangements may be closely associated with particular genomic sequences or base composition, since a defined region was found rearranged in different subclones. All alterations within three different deletion forms of YAC 400B5 were internal, with retained genomic sequences flanking the rearranged region on either side. One cosmid, located in the center of two gaps in the two larger subforms, also showed a deletion, since it had an insert of about half the expected size when compared to more than 30 other cosmids analyzed in this region (Table **1**) [17]. One of these gaps had been reported previously by others, who were unable to find coverage for these sequences in the cosmid library used (LL22NC03). This may be an indirect hint that this region might be particularly unstable in recombinant DNA *in vitro* systems, as LL22NC03 is reported to be overall 10- to 40-fold redundant for sequences from chromosome 22.

The second gap, found within the largest 350 kb subform, was expected to be covered by three cosmids, according to the restriction map assembled by Kawasaki *et al*. [28]. While this region was deleted entirely in the smallest subclone of 250 kb, these cosmids were, at least in part, represented along the 330 kb subclone. The results indicated deletions on either side of two overlapping cosmid clones (Fig. **3B**). This was further supported by the finding that QDFM using these two cosmids as hybridization templates, as well as PFGE, revealed much bigger insert sizes. This allowed us to conclude that at least two independent deletion events must have occurred within YAC 400B5 prior to analysis.

One cosmid clone, 65E11, previously reported to localize to the unstable region [28, 29], could not be detected along any of our clones, neither YACs nor cosmids. Therefore, in contrast to the published restriction maps, this clone may not map to this region.

As mentioned above, the YAC DNA insert itself, its composition or conformation, may very well be the main reason for the instability of YAC clone 400B5. The region on chromosome 22 band q11.2 from which this clone was derived is known to be involved in a variety of translocations [48, 49]. Genomic instability in this region has been blamed to cause the most common constitutive translocation in humans, t(11;22) [50, 51]. Sequence analyses of the critical region on chromosome 22q11.2 identified a large cluster of breakpoints and suggested the involvement of low copy number repeats in the translocation events [52, 53]. More recently, the DNA composition in the critical region and palindromic AT-rich repeats, in particular, have been suggested as major mediators of translocations [54-56].

Our results demonstrate the capability of QDFM to characterize deletions and rearrangements in recombinant DNA molecules allowing one to compare the integrity or molecular structure of specific genomic regions in different *in vivo* and *in vitro* systems. Findings with cloned DNA can be directly compared to genomic DNA, if appropriate clones from the regions of interest are available. Gaps can be delineated and accurately sized when clones that flank deleted areas are at hand. A significant advantage of QDFM compared to the conventional methods such as restriction mapping and STS-content mapping is that it allows direct visualization of individual DNA molecules. Thus it is not hampered by the presence of closely related tandem repeats or other repetitive sequences.

In conclusion, we present a straightforward experimental method for the detailed characterization of recombinant DNA clones such as YACs or cosmids, and show that unstable YACs may be the product of simple or complex rearrangements. Further analysis of cosmid clones or PCR products that have been mapped with QDFM's kilobase accuracy to regions of the human genome that are unstable *in vitro* and involved in frequent rearrangements in patients will support efforts to elucidate the chromosomal mechanisms underlying translocations and deletions.

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DISCLAIMER

ABBREVIATIONS

APS = 3-Aminopropyltriethoxy silane

BAC = Bacterial artificial chromosome

CEPH = Centre d'Études du Polymorphisme Humain

FISH = Fluorescence *in situ* hybridization

Ig = Immunoglobulin

IGH = Immunoglobulin heavy

IGK = Immunoglobulin kappa

IGLC = Lambda constant gene region

Mbp = Megabasepair

PFGE = Pulsed field gel electrophoresis

QDFM = Quantitative DNA Fiber Mapping

YAC = Yeast artificial chromosome

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