R. U. Sorenson, M. Berger, M. S. Hershfield, *ibid.* **27**, 162A (1990); I. Santisteban *et al.*, *J. Clin. Invest.* **92**, 2291 (1993).
19. Y. Levy *et al.*, *J. Pediatr.* **113**, 312 (1988).
20. The protocol was reviewed and approved by the Clinical Research Subpanels of the NCI and NHLBI, the NCI Cancer Treatment and Evaluation Program (CTEP), the NIH Biosafety Committee, the Human Gene Therapy Subcommittee, the Recombinant DNA Advisory Committee, the Director of NIH, and the U.S. Food and Drug Administration. Informed consent was obtained from the parents of each patient.
21. Beginning with culture 9 for patient 1 and culture 5 for patient 2, the patients' lymphocyte populations obtained by apheresis were fractionated by adherence to flasks coated with CD8 monoclonal antibodies (Applied Immune Sciences) following the manufacturer's instructions. This protocol modification for CD8 depletion was introduced because both patients were developing a progressively inverted CD4-CD8 ratio. This effect was apparently the result of preferential growth of $CD8^+$ cells during the last 4 to 5 days of culture and the subsequent persistence of these infused $CD8^+$ cells in the circulation. Consequently, each subsequent apheresis sampled the recently increased number of $CD8^+$ cells, and thus the skewing of the ratio of CD4 to CD8 cells became compounded with each additional treatment. By partially depleting the apheresis sample of $CD8^+$ cells by an immunoaffinity selection process, the later treatments for each patient consisted of cells with a more balanced phenotype. The perturbation in normal CD4-CD8 cell proportions did not have detectable untoward effects for either patient.
22. C. A. Mullen, K. W. Culver, R. A. Morgan, W. F. Anderson, R. M. Blaese, unpublished results.
23. K. Weinberg, unpublished observations; R. M. Blaese, unpublished observations.
24. D. B. Kohn *et al.*, *Nature Med.* **1**, 1017 (1995).
25. The ADA enzyme activity assay was performed in duplicate as described (*13*). Positive control cells were obtained from healthy normal donors and had a mean of 82 U (normal range, 66 to 102 U). Duplicate samples were run in the presence of the ADA enzyme inhibitor EHNA (30 μM). Specific ADA activity was calculated as total adenosine deaminating activity minus EHNA-resistant activity. EHNA-resistant activity represents metabolic activity of a nonspecific aminohydrolase present in human cells.
26. R. A. Morgan, K. Cornetta, W. F. Anderson, *Hum. Gene Ther.* **1**, 135 (1990).
27. B. A. Bunnell, L. M. Muul, R. R. Donahue, R. M. Blaese, R. A. Morgan, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7739 (1995).
28. Southern hybridization analysis for LASN vector consisted of the following: 10 μg of DNA was digested with Sst I and hybridized with a 728-bp Nco I fragment from LASN corresponding to the SV40 promoter and *neo* gene. DNA from K562-LASN cells served as a positive control.
29. RT-PCR analysis for LASN vector transcripts was as follows: 3 μg of polyadenylated RNA was treated with deoxyribonuclease and reverse-transcribed. The cDNA (0.3 μg) was amplified with LASN vector–specific primers in a 30-cycle PCR reaction. The oligonucleotides 5′-CAGCCTCTGCAGGGCAGAAC-3′ (corresponding to the 3′ end of the ADA gene in LASN) and 5′-GCCCAGTCATAGCCGAATAG-3′ (complementary to 5′ end of the *neo* gene in LASN) were used as primers. After electrophoresis and blotting, the sequences were hybridized with a 527-bp probe corresponding to the entire length of the predicted PCR product.
30. M. Clerici *et al.*, *J. Clin. Invest.* **84**, 1892 (1989).
31. M. Clerici *et al.*, *J. Immunol.* **146**, 2214 (1991); W. E. Biddison, F. E. Ward, G. M. Shearer, S. Shaw, *ibid.* **124**, 548 (1980).
32. T. A. Waldmann *et al.*, *Lancet* **ii**, 609 (1974).
33. H. Kirchner *et al.*, *J. Immunol.* **122**, 1310 (1979).
34. *AABB Technical Manual*, R. H. Walker, Ed. (American Association of Blood Banks, Bethesda, MD, ed. 11, 1993), pp. 622–623.
35. S. W. Fong, B. Y. Qaqundah, W. F. Taylor, *Transfusion.* **14**, 551 (1974).
36. The authors acknowledge superb technical and nursing support by C. Able, K. Snitzer, A. Roop, M. Brown, F. May, R. Gutieriez, M. Yu, H. Goetzman, C. Wannebo, B. Sink, and L. Top. In addition, we thank R. Sorensen for providing the initial patient blood samples, Applied Immune Sciences for CELLector T-150 flasks, and Genetic Therapy for clinical-grade LASN vector. Finally, we also thank our earlier collaborators D. B. Kohn, E. Gilboa, P. W. Kantoff, M. A. Eglitis, R. Moen, K. Cornetta, A. Gillio, R. J. O'Reilly, and C. Bordignon for their contributions that helped prepare the way for this clinical trial.

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Physical Map and Organization of *Arabidopsis thaliana* Chromosome 4

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A physical map of *Arabidopsis thaliana* chromosome 4 was constructed in yeast artificial chromosome clones and used to analyze the organization of the chromosome. Mapping of the nucleolar organizing region and the centromere integrated the physical and cytogenetic maps. Detailed comparison of physical with genetic distances showed that the frequency of recombination varied substantially, with relative hot and cold spots occurring along the whole chromosome. Eight repeated DNA sequence families were found in a complex arrangement across the centromeric region and nowhere else on the chromosome.

Arabidopsis thaliana has been adopted as a model organism for the analysis of complex plant processes by means of molecular genetic techniques (*1*). The increase in map-based cloning experiments makes the generation of a complete physical map of the *Arabidopsis* genome a high priority. In addition, the availability of such a map would enable the organization of the chromosome to be studied in more detail. Little is known about the organization of plant chromosomes, but the general picture is that of chromosomes carrying large numbers of dispersed [often retrotransposons (*2*)] and tandemly repeated DNA sequences (*3*). The relatively small (100 Mb) *Arabidopsis* genome has a much smaller number of repeated DNA sequences than do most other plant species; its five chromosomes contain ~10% highly repetitive and ~10% moderately repetitive DNA (*4*). The dispersion of most of these sequences among the low-copy DNA is unknown.

We discuss here a physical map, which we have presented on the World Wide Web (WWW) at URL: http://nasc.nott.ac.uk/JIC-contigs/JIC-contigs.html, of *Arabidopsis* chromosome 4, one of the two chromosomes carrying nucleolus organizing regions. The construction of this map allowed us to analyze the frequency of recombination along the whole chromosome, the integration of the physical with the cytogenetic map, the interspersion pattern of repeated and low-copy DNA sequences over the whole chromosome, and the arrangement of repeated DNA sequences over the centromeric region.

We generated the physical map by hybridizing probes to four yeast artificial chromosome (YAC) libraries (*5*), using colony hybridization experiments (*6*). The probes consisted of 112 markers genetically mapped to chromosome 4, 20 previously unmapped genes, random genomic fragments and sequences flanking transposable elements, and the 180–base pair (bp) repetitive element carried in pAL1 (*7*). Southern (DNA) blot analysis of YAC clones confirmed the colony hybridization results and revealed common restriction fragments in the different YAC clones hybridizing to a given marker. This demonstrated overlap between the inserts of the YAC clones. On the basis of these results, the YAC clones could be placed into 14 YAC contigs with a high degree of redundant YAC cover, ensuring an accurate map despite the presence of chimeric clones in the YAC libraries.

We generated YAC end fragments, using either inverse polymerase chain reaction (IPCR) or plasmid rescue (*8*), from YAC clones lying near the ends of each of the 14 contigs. The fragments were hybridized to Southern blots of YAC clones from adjacent contigs. In addition, YACs, as well as some of the end fragments generated by IPCR, were used to identify clones from a cosmid library of the Columbia ecotype (*9*). The cosmids were then used as new markers on the YAC libraries. These experiments reduced the number of contigs to four. In all but two instances, the end fragments revealed that the contigs were already overlapping. Experiments aimed at closing the last three gaps have been attempt-

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ed but so far have been unsuccessful. Two of the gaps map to the centromeric region, where repetitive sequences severely limit walking experiments. Sparse YAC coverage as well as chimeric YAC clones have made the walking experiments in the area between contigs III and IV particularly difficult. Neither the generation of end fragments nor the hybridization of YAC clones to cosmid libraries has detected an overlap or extended the contigs. The physical map of 374 YAC clones is represented in a format in which each marker hybridized to the YAC libraries is shown a unit length away from the next; thus, the sizes of the YAC clones only reflect their marker content, not their physical size. Multiple YAC coverage has been achieved for the majority of the chromosome. Only 12 of the 148 links are spanned by one YAC clone.

We determined the physical distance between 77 markers by integrating several types of data: the size of the YAC inserts, the minimum size of a YAC clone spanning two markers, and whether each YAC clone ended within or spanned a complete marker (*10*). Because of the redundancy of YAC clones covering each interval and the inclusion of small as well as large insert YAC clones, we estimate the error for these distances to be only ±10%. The total size of the four YAC contigs is approximately 17 Mb, and they cover a minimum of 82.5 centimorgans (cM), or 90% of the chromosome 4 genetic map (Fig. 1).

A comparison of genetic with physical distance over 75 intervals spanning the whole chromosome is shown in Fig. 1. The genetic distances were derived from up to 100 lines of a single recombinant inbred mapping population (*11*) and so can be compared directly. The ratio of physical to genetic distance between markers varied significantly along the length of the chromosome, with the average value for the four contigs being 185 kb/cM. Relative cold spots (>550 kb/cM) were distributed throughout the chromosome, in intervals close to the centromere (mi233 to g2616 to m506), but also in intervals in other chromosomal locations (g3883 to JGB9 and 06455 to g3088) (Fig. 1). Relative hot spots (30 to 50 kb/cM) (mi51 to mi204, m518 to BIO216, mi128 to g6837, and m214 to ap2) were also distributed throughout the chromosome (Fig. 2). There was no distinct association of higher recombination frequencies with certain chromosomal regions. This is different from the situation reported for yeast chromosome 3 (*12*), in which recombination was lowest close to the centromere and highest midway down each arm. It also contrasts with the picture emerging for tomato and wheat chromosomes (*13*), in which recombination is strongly suppressed over large intervals at the centromere, with maximum recombination occurring in the proximal regions.

In order to integrate the physical and cytogenetic maps, it was necessary to place the centromere and the nucleolar organizing region (NOR) that maps to chromosome 4 (*14*, *15*) on the physical map. An end fragment from YAC clone CIC5C11 (CIC5C11LE) was found to contain ribosomal DNA (rDNA) sequences. The YAC also hybridized to markers BIO217 and mi51. Southern blot analysis of two additional YAC clones (yUP2A9 and yUP7C3) hybridizing to these markers showed that they also contained rDNA sequences. These results positioned the NOR locus distal to marker BIO217 and all known chromosome 4 markers (Fig. 1). We did not attempt to obtain YAC clones covering the NOR locus because of the complication of the instability of YAC clones carrying rDNA sequences (*16*). NOR 4 has recently been genetically mapped as the most terminal marker on chromosome 4; it is in close physical proximity to the telomere (*17*). Thus, the NOR locus defines one end of the physical map of chromosome 4.

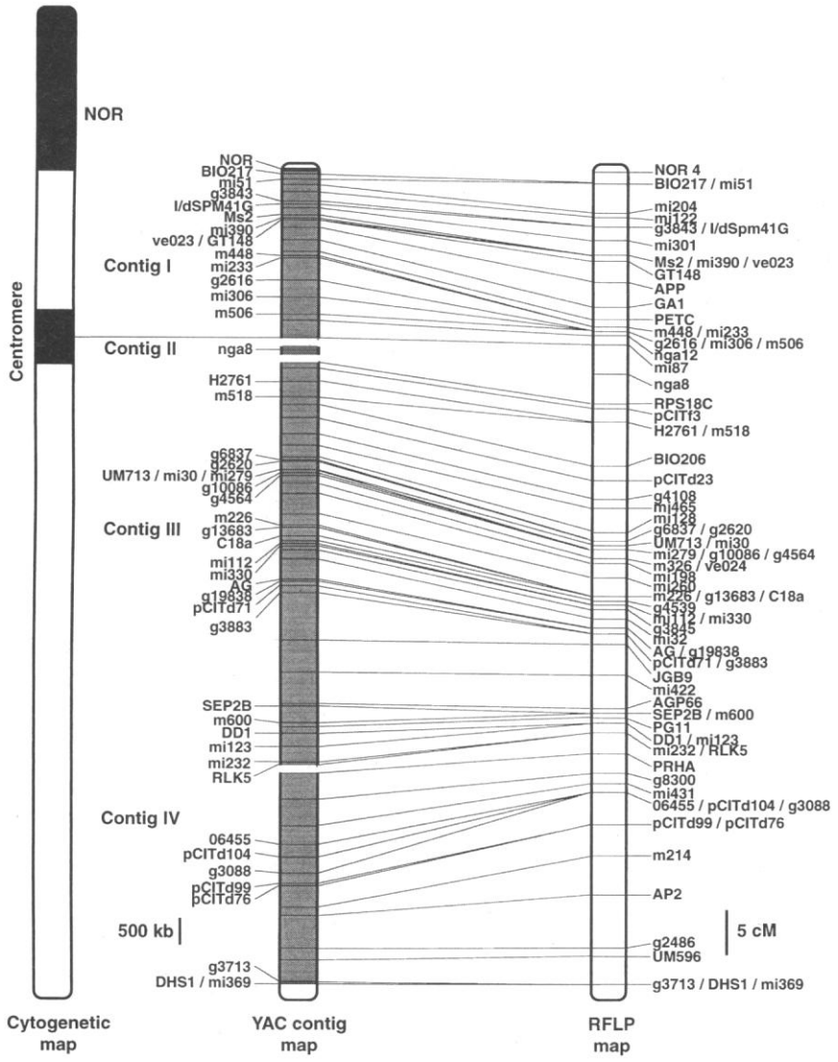

Fig. 1. The alignment of the cytogenetic, YAC contig, and RFLP maps of chromosome 4. The schematic drawing of the cytogenetic map [adapted from (*26*)] approximately reflects the actual physical sizes of the various chromosomal regions. The RFLP map shows positions for 80 loci. These loci were mapped on up to 100 recombinant inbred lines derived from a cross between the ecotypes Landsberg *erecta* and Columbia (*11*). The most likely marker order as determined empirically with the MAPMAKER program (version 3.0) (*27*) differed in four places from the order as derived from the physical map. These intervals and the log of the likelihood ratio of the most likely order predicted by MAPMAKER to the order as determined by physical mapping are as follows: nga12 to mi87 (0.57); m226 to g3845 (4.23); ag to g3883 (0.21); and agp66 to mi232 (0.73). Because the physical ordering of these markers was unambiguous, map distances were calculated [with the use of the Kosambi mapping function (*28*)] by MAPMAKER on the basis of the order determined from the physical map. The relative positions of all loci on the YAC contig and genetic map are indicated by lines across the chromosomes. Only markers that cosegregate with adjacent markers on the RFLP map are shown next to the YAC contig map. The four YAC contigs are shown as gray boxes. Contig II and the gaps between the contigs are not drawn to scale.

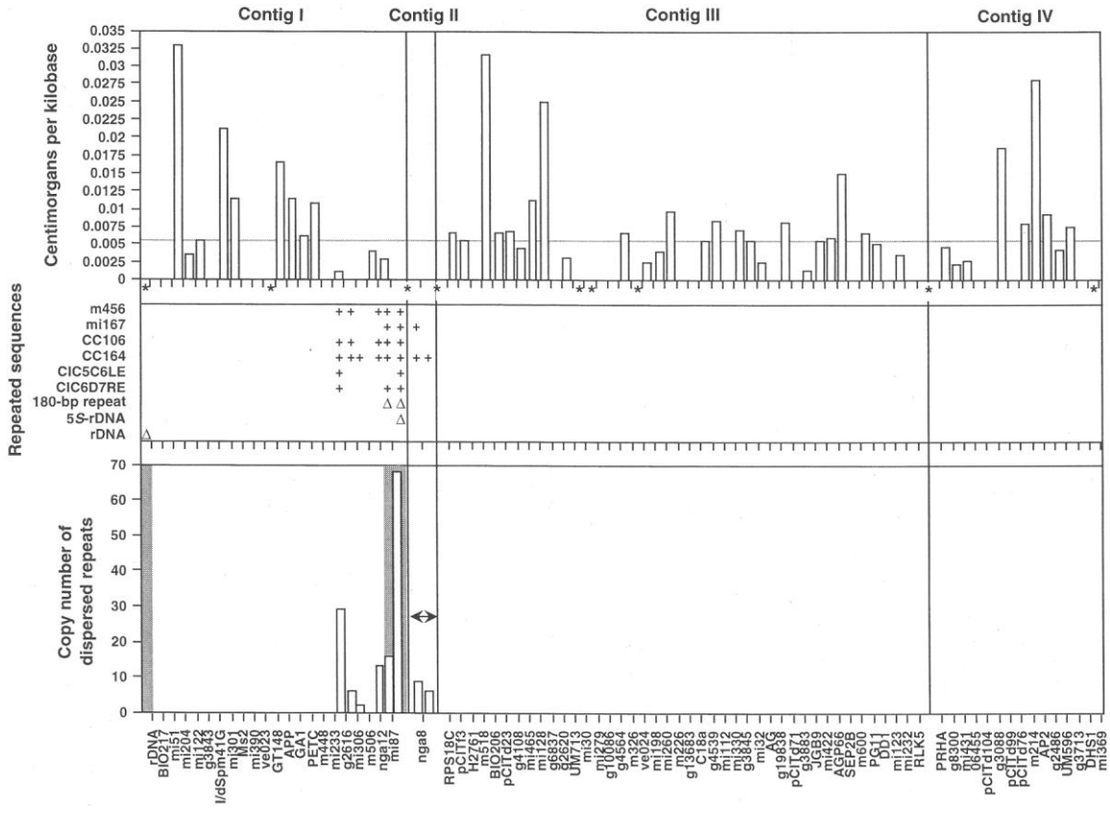

Fig. 2. Distribution of recombination hot spots and nine families of repetitive elements on chromosome 4. All genetically mapped markers are shown a unit length away from each other at the bottom of the figure. Vertical lines represent the boundaries of the YAC contigs. The top panel shows the ratio of centimorgans to kilobases for the intervals between markers. The average value for chromosome 4 is indicated by a horizontal line. Intervals for which the physical distance between markers could not be established, either because markers could not be separated or because of gaps between contigs, are marked by asterisks. The middle panel shows the presence of several classes of repeated sequences along the length of the chromosome. All sequences noted were analyzed across all chromosomal intervals noted. Dispersed repeat sequences are indicated by plus signs; tandemly repeated sequences are indicated by triangles. The bottom panel shows the cumulative copy number of the six dispersed repeated sequence families (indicated by plus signs in the middle panel) in each interval. To determine the copy number, we hybridized Southern blots carrying YAC clone DNA–digested by Eco RI–Bam HI to the various dispersed repeats, and the number of hybridizing restriction fragments was determined for each of the repeated sequences. The presence of tandemly repeated sequences is indicated as gray boxes. Double-headed arrow indicates that the orientation of contig II has not been determined.

Due to the lack of suitable genetic stocks, the centromere had not been mapped relative to phenotypic or restriction fragment length polymorphism (RFLP) markers. The centromeric region of chromosome 4 could be positioned by identification of YAC clones that hybridized to the tandemly repeated 180-bp sequence contained in plasmid pAL1 (*18*). This family of tandemly repeated DNA sequences is present in arrays of >50 kb, makes up 1 to 1.6% of the *Arabidopsis* genome, and has been shown to co-localize with the heterochromatin surrounding the centromeres of all five *Arabidopsis* chromosomes (*14*, *18*, *19*). CIC YAC clones hybridizing to pAL1 as well as to two markers (mi87 and nga12) mapping to chromosome 4 were identified. Southern blot analysis of DNA from these YAC clones digested with several restriction enzymes revealed the presence of two different hybridization patterns corresponding to two 180-bp repeat loci (each carrying a large number of tandemly repeated copies) flanking mi87 (Fig. 3). Maluszynska and Heslop-Harrison (*14*) found in in situ hybridization experiments that the 180-bp tandemly repeated sequence hybridized equally to both sides of the centromeres on all five chromosome pairs. Taken together, one could conclude that the YAC contig shown in Fig. 3 covers at least the core of the centromere.

The mapping of NOR 4 and the centromere allowed the integration of the physical and cytogenetic maps (Fig. 1). YAC contig I covers the short arm of chromosome 4, with most of the genetic map residing on the long arm and being contained in YAC contigs II, III, and IV.

The availability of YAC clones covering most of chromosome 4 enabled the distribution of low- and high-copy sequences to be examined. Pruitt and Meyerowitz (*20*) analyzed a random set of lambda clones for the presence of repetitive DNA. They showed that the average single-copy sequence length would be 125 kb if the repeated DNA sequences were randomly distributed. Thus, the sequence interspersion pattern of *Arabidopsis* is extremely long relative to that of other plant genomes. Southern blots carrying a representative set of YAC clones covering the chromosome 4 contigs were hybridized with nine different repeated DNA sequences. As described above, rDNA sequences were localized on YAC clones at the top of the map and the 180-bp repeated sequence was localized on YAC clones at the bottom of contig I (Fig. 2). They were not detected elsewhere on the chromosome. 5*S*-rDNA sequences (*21*) hybridized to a number of YAC clones that contained the 180-bp repeated sequence as well as marker mi87. Thus, one of the 5*S*-rDNA loci in *Arabidopsis* (*21*) maps to the centromeric region of chromosome 4 (Figs. 2 and 3).

At least six other repeated DNA sequences were identified during the course of this work. Two RFLP markers, m456 and mi167, were found to hybridize at high stringency to ~ 50 to 100 fragments in *Arabidopsis* DNA. In addition, two cosmid clones carrying Columbia DNA, CC106 and CC164, were identified that hybridized strongly to total *Arabidopsis* DNA but did not cross-hybridize with any previously characterized repetitive sequence. They hybridized to ~ 300 and 150 *Arabidopsis* fragments, respectively. Sequence analysis of mi167 and subclones from CC106 and CC164 revealed no significant homology to sequences in all available databases (*22*). We have not yet established how many different families of repeated DNA sequences are carried on m456, mi167, CC106, and CC164. Two other repeated DNA sequence families were identified as end fragments from YAC clones CIC5C6LE and CIC6D7RE.

The six clones were hybridized to the

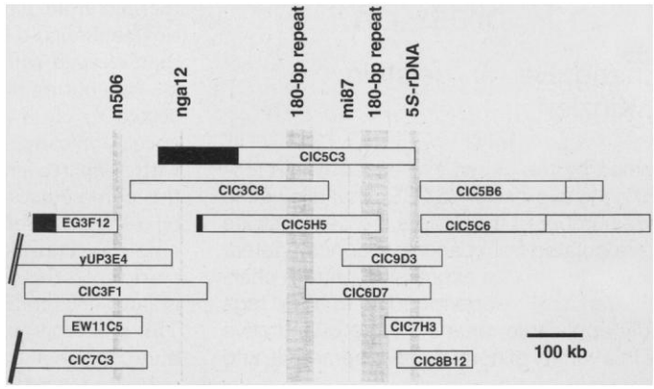

Fig. 3. Contig covering the centromeric region, showing the YAC clones covering this part of the chromosome. The sizes of all the YAC inserts are drawn to scale. The arrangement of the YAC clones shown is consistent with data on hybridization to the markers shown at the top and to the dispersed repetitive elements shown in Fig. 2, as well as with data from a limited number of chromosome walking experiments (*29*). The sizes shown for the 180-bp repeat (pAL1) and 5S-rDNA are not drawn to scale. Noncontiguous sequences in known chimeric clones are shown as black boxes. Slashes at left indicate that only part of these clones is shown. Instability was observed in some of the YAC clones containing the tandemly repeated 180-bp Hind III (pAL1) sequence (*16*).

Southern blots containing the representative set of YAC clones covering the chromosome 4 contigs. They all hybridized to multiple fragments on YAC clones mapping to the bottom of contig I and to contig II (Fig. 2). The copy number of all the repeated sequences was largest in the YAC clones closest to the 180-bp repeated sequence loci. Unlike the 180-bp repeated sequences and 5S-rDNA sequences, these dispersed repeats were also found in several positions up to 1.5 Mb from the centromere in contig I and in YAC contig II (Fig. 2). Long stretches of complex repeated DNA sequences are required for centromere function in *Shizosaccharomyces pombe* (*23*) and *Drosophila* (*24*), and an α-satellite DNA has been implicated in centromere function in human chromosomes (*25*). A functional analysis will be required to establish whether the tandem 180-bp repeated sequence and the dispersed repeats contribute to centromere function on chromosome 4. At the relatively high stringency used in the experiments, YAC clones mapping to the rest of chromosome 4 were devoid of sequences that hybridize to these clones (Fig. 2).

In conclusion, the size of the four YAC contigs shown in Fig. 1 is ~17 Mb. If the NOR carrying the tandemly repeated rDNA units is 3.5 Mb (*17*) and the gaps are ~1.0 Mb, then the total size of chromosome 4 would be on the order of ~21.5 Mb. The eight repeated sequence families analyzed were clustered around the centromere and most likely constitute the flanking heterochromatin. This finding, along with the relative lack of repeated DNA sequences found in the chromosome walking experiments, suggests that the majority of both arms (except for the NOR locus) is composed predominantly of medium- and low-copy DNA. Recombination frequency varied along the chromosome, with some suppression being detected around the centromere, in the region carrying most of the repeated DNA sequences (Fig. 2). However, other chromosomal regions showing equally low recombination frequencies were not associated with these particular repeated sequence families (Fig. 2). The extreme localization of the repetitive DNA distinguishes the organization of the *Arabidopsis* chromosomes from that so far described for other plant chromosomes.

REFERENCES AND NOTES

1. E. M. Meyerowitz and C. R. Somerville, *Arabidopsis* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994).
2. M. W. Ganal *et al.*, *Mol. Gen. Genet.* **213**, 262 (1988); G. Moore *et al.*, *Genomics* **10**, 461 (1991).
3. C. Dean and R. Schmidt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 395 (1995).
4. E. M. Meyerowitz, in *Methods in Arabidopsis Research*, C. Koncz, N.-H. Chua, J. Schell, Eds. (World Scientific, Singapore, 1992), pp. 100–118.
5. E. Grill and C. Somerville, *Mol. Gen. Genet.* **226**, 484 (1991); E. R. Ward and G. C. Jen, *Plant Mol. Biol.* **14**, 561 (1990); J. R. Ecker, *Methods* **1**, 186 (1990); F. Creusot *et al.*, *Plant J.*, in press.
6. A. Coulson *et al.*, *Nature* **335**, 184 (1988).
7. C. Chang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6856 (1988); H.-G. Nam *et al.*, *Plant Cell* **1**, 699 (1989); J. M. McGrath *et al.*, *Theor. Appl. Genet.* **86**, 880 (1993). We acknowledge the generosity of the following people in sending markers and segregation data, both published and unpublished: ABP: K. Palme *et al.*, *Plant Cell* **4**, 193 (1992); AG, GA1, and PG11: A. Konieczny and F. Ausubel, *Plant J.* **4**, 403 (1993); AGL19: S. Gold and M. Yanofsky (Department of Biology UCSD, La Jolla, CA); AGP66: A. Prescott (John Innes Centre, Norwich, UK); AP2: K. D. Jofuku, B. G. W. den Boer, M. van Montagu, J. K. Okamuro, *Plant Cell* **6**, 1211 (1994); APP: L. Lepiniec, E. Babiychuk, S. Kushnir, M. van Montagu, D. Inzé, *FEBS Lett.* **364**, 103 (1995); AtGβ and AtKC1: K. Palme (Max-Delbrueck Laboratory, Köln, Germany); ATH1: N. Quadvlieg, J. Dockx, F. Rook, P. Weisbeek, S. Smeekens, *Plant Cell* **7**, 117 (1995); ATHSF1: A. Hübel and F. Schöffl, *Plant Mol. Biol. Rep.* **26**, 353 (1994); ATM4: S. Smeekens (Department of Molecular Cell Biology, University of Utrecht, Utrecht, Netherlands); BIO217, BIO219, and BIO206: B. Osborne and B. Baker (PGEC, U.S. Department of Agriculture, Albany, CA); C18a: J. E. Parker *et al.*, *Plant J.* **4**, 821 (1993); CC10, CC114, CC141, CC250, and CC128: R. Schmidt (John Innes Centre); CH-42: C. Koncz *et al.*, *EMBO J.* **9**, 1337 (1990); COP9: N. Wei, D. A. Chamovitz, X. W. Deng, *Cell* **78**, 117 (1994); DD1: G. Vancanneyt (CIT INIA, Madrid, Spain); DHS1 and DHS2: B. Keith, X. Dong, F. M. Ausubel, G. R. Fink, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8821 (1991); ELI3: S. Kiedrowski, P. Kawalleck, K. Hahlbrock, I. E. Somssich, J. L. Dangl, *EMBO J.* **11**, 4677 (1992); EW11E9LE: A. F. Bent *et al.*, *Science* **265**, 1856 (1994); g8802, g16143, g2620, g14587, g13683, g13838, g15064, g7873, and g16224: H. Goodman (Department of Molecular Biology, Massachusetts General Hospital, Boston); GT148: P. S. Springer, W. R. McCombie, V. Sundaresan, R. A. Sundaresan, R. A. Martienssen, *Science* **268**, 877 (1995); H2761: P. Gallois (Laboratoire de Physiologie of Biologie Moleculaire Vegetales, Université de Perpignan, Perpignan, France); HY4: M. Ahmad and A. R. Cashmore, *Nature* **366**, 162 (1993); I/dSpm 27, 41G, 70, 64, 75, and 76: M. Aarts and A. Pereira (Molecular Biology Department, Centre for Plant Breeding Research, Wageningen, Netherlands); JGB9, RJS, SEP2B, and 261a: G. Coupland (John Innes Centre); LD: I. Lee *et al.*, *Plant Cell* **6**, 75 (1994); mi markers: Y. G. Lui, N. Mitsukawa, R. F. Whittier, in preparation; Ms2: S. Tourmente *et al.*, *Nucleic Acids Res.* **22**, 3317 (1994); nga8 and 12: C. J. Bell and J. R. Ecker, *Genomics* **19**, 137 (1994); PETC: M. Walker (Department of Plant Sciences, University of Cambridge); pLM117: F. Ausubel (Department of Molecular Biology, Massachusetts General Hospital, Boston) and S. Rodermel (University of Iowa, Ames, IA); PRHA: U. Korfhage, G. F. Trezzini, I. Meier, K. Hahlbrock, I. E. Somssich, *Plant Cell* **6**, 695 (1994); PRL1 and RNA polymerase II LS: C. Koncz (Max-Planck Institut, Köln, Germany); RLK5: M. Hom and J. C. Walker, *Biochim. Biophys. Acta* **1208**, 65 (1994); RPS18C: M. van Lijsebettens *et al.*, *EMBO J.* **13**, 3378 (1994); TG1C8: T. Osborn (University of Wisconsin, Madison, WI); TSB2: R. L. Last, P. H. Bissinger, D. J. Mahoney, E. R. Radwanski, G. R. Fink, *Plant Cell* **3**, 345 (1991); ve023, ve024, ve025, ve030, and ve031: D. Bouchez (Versailles, France); yUP17E10LE: A. Pepper, T. Delaney, T. Washburn, D. Poole, J. Chory, *Cell* **78**, 109 (1994); 02213-2 and 06455: C. Dean (John Innes Centre, Norwich).
8. R. Schmidt, G. Cnops, I. Bancroft, C. Dean, *Aust. J. Plant Physiol.* **19**, 341 (1992).
9. C. Lister and C. Dean, unpublished data.
10. R. Schmidt *et al.*, in preparation.
11. C. Lister and C. Dean, *Plant J.* **4**, 745 (1993).
12. S. G. Oliver *et al.*, *Nature* **357**, 38 (1992).
13. S. D. Tanksley *et al.*, *Genetics* **132**, 1141 (1992); J. S. Heslop-Harrison, *J. Cell Sci.* **100**, 15 (1991).
14. J. Maluszynska and J. S. Heslop-Harrison, *Plant J.* **1**, 159 (1991).
15. S. M. Albini, *ibid.* **5**, 665 (1994).
16. R. Schmidt *et al.*, *ibid.*, p. 735.
17. G. Copenhaver and C. Pikaard, personal communication.
18. J. M. Martinez-Zapater, M. A. Estelle, C. R. Somerville, *Mol. Gen. Genet.* **204**, 417 (1986).
19. S. Bauwens *et al.*, *Chromosoma* **101**, 41 (1991).
20. R. E. Pruitt and E. M. Meyerowitz, *J. Mol. Biol.* **187**, 169 (1986).
21. B. R. Campell *et al.*, *Gene* **112**, 225 (1992).
22. H. Thompson and C. Dean, in preparation.
23. C. Polizzi and L. Clarke, *J. Cell Biol* **112**, 191 (1991).
24. T. D. Murphy and G. H. Karpen, *Cell* **82**, 599 (1995).
25. T. Haaf, P. E. Warburton, H. Willard, *ibid.* **70**, 681 (1992).
26. D. Schweizer, P. Ambros, P. Gründler, F. Varga, *Arabidopsis Inf. Serv.* **25**, 27 (1987).
27. E. S. Lander *et al.*, *Genomics* **1**, 174 (1987).
28. D. D. Kosambi, *Ann. Eugen.* **12**, 172 (1944).
29. R. Schmidt *et al.*, data not shown.

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