Cell Research (1992), **2**; 25—34

Construction of the physical map of yeast (*Saccharomyces cerevisiae*) chromosome V[1]

ZHU YIWEN[2] AND DARREN KUANG[3]
Shanghai Institute of Cell Biology, Academia Sinica. Shanghai, China

ABSTRACT

There are about 17 chromosomes in yeast *Saccharomyces cerevisiae*. A middle sized chromosome, chromosome V, was chosen in this work for studying and constructing the physical maps. Chromosome V from strain A364a was isolated by pulsed-field gradient gel electrophoresis (PFGE). Gel slices containing chromosome V DNA were digested with two rare cutting enzymes, NotI and SfiI, and three 6-Nt recognizing enzymes, SmaI, SstII and ApaI. Several strategies—partial or complete digestions, digestion with different sets of two enzymes, and hybridizabion with cloned genetically mapped probes (CAN1, URA3, CEN5, PRO3, CHO1, SUP19, RAD51, RAD3)—were used to align the restriction fragments. There are 9, 9, 15, 17, and 20 sites for NotI, SfiI, SmaI, SstII and ApaI respectively in the map of the A364a chromosome V. Its total length was calculated to be 620 Kb(Kilo-bases).The distributions of the cutting sites for these five enzymes through the whole chromosome are not uniform. A comparison between the physical map and the genetic map was also made.

Key words: *yeast chromosome V, macro-physical map, D N A structural or ganization, PFGE.*

INTRODUCTION

Eukaryotic chromosome DNAs organize a huge amount of information, such as structural and regulatory genes, transcription-regulating sequences and other non-coding cis-acting elements [1]. Structure and function studies about these coding and regulatory sequences have been made by molecular cloning, sequencing, DNA-protein reactions, etc. However, most of these studies were based merely on

1 Project supported by Nationa Sciences Foundation of China.
2 Submitted this work in partial fulfillments for the requirements of the Ph.D. dissertation at Shanghai Institute of Cell Biology, Academia Sinica.
3 Corresponding author.

analyses of cloned individual DNA, and on comparison of structures and functions of similar, but different DNA clones, without studying on DNA structural organization in the whole or the part of a chromosome. Construction of DNA macro-physical maps, as a way of understanding the whole chromosome DNA organization, has become feasible since the development of PFGE [2—4].

Several methods have been developed to align the restriction fragments, such as partial and complete digestions. Smith-Birnstiel strategy, application of 'linking' or 'jumping' libraries, and 'link-up' method [5—11]. With these methods, macro-physical maps of *E. ccli, S. pombe* and some mammalian chromosome regions have been successfully constructed [12—15].

In this report, we constructed a macro-physical map of A364a chromosome V with two rare cutting restriction enzymes, NotI and SfiI, and three 6-Nt recognizing enzymes. SmaI, SstII and ApaI. The recognization sequences of the last three enzymes contain only G and C, and may thus cut yeast chromosome DNAs into larger fragments because bhe G+C content of S. *cerevisiae* genomic DNA it about 40%, lower than that of A+T [16]. Comparison of this physical map with the genetic map of A364a chromosome V was also made.

MATERIALS AND METHODS

1. Restriction enzymes

The enzymes used in physical mapping are listed in Table 1. Other enzymes are prepared by our lab. or purchased from Sino-American Biotechnology Company.

2. Probes

Table 2 shows the chromosome V-specific probes and their genetic distances from centromere 5. The restriction enzyme cutting sites for NotI, SfiI, ApaI, SmaI or SstII within the probes are also shown.

3. Electrophoresis

PFGE was carried out as described previously [17].

Table 1. Restriction enzymes used in physical mapping

Enzyme	Recognition sequence	Source
Not I	GC↓GGCCGC	BioLabs
Sfi I	GGCCNNNN↓NGGCC	BioLabs
Sma I	CCC↓GGG	Promega
Set II	CCGC↓GG	BRL
Apa I	GGGCC↓C	BRL

4. Digestion of DNA embedded in agarose gel

Gel slices containing chromosome V DNA were cut out from gel PFGE gel under longwave UV light, washed twice with 30% isopropanol in TE and then with TE, and used for digestion. Insert gels containing total DNA were washed with 1m*M* PNSF in TE followed several times with TE, and then used for restriction enzyme digestion. DNA in agarose gel was digested in enzyme assay buffer, 0.1mg/ml BSA and restriction enzymes at appropriate temperature. In general, we used a 10-20:1 ratio (Units of enzyme: μg of DNA) for complete digestion, and 1-4: 1 ratio for partial digestion.

Table 2. Chromosome V-specific probes used in this paper

Names of plasmids or recombinant λ	Names of genes	Fragments used as probes	Restriction sites (NotI, SfiI, SmaI, SstII, ApaI) in probe fragments	Genetic distances to centromere(cM)	Source
pPM924	can1	4.6kb B-frag.	no	52.2	M. V. Olson
pFL1	ura3	1.1kb H-frag.	ApaI, SmaI	8.0	LaCroute
pDP24	cen5	0.7kb RI/Sfrag.	no	0	Philippsen
pCBY203	pro3	1.85kb H/Pfrag.	no	28.0	our Lab
pPM972	cho1	2.8kb RI/Sfrag.	SmaI	25.0	M. V. Olson
H742(λ)	SUP19	H742/RI	?	52.5	M. V. Olson
YEp13-RAD51-23	rad51	3.2kb B-frag.	no	80.0	Contopoulou
pPM921	rad3	3.2kb S-frag.	2SmaI	163.5	M. V. Olson

B: BamHI; H: HindIII; RI: EcoRI; S: SalI; P: PstI.

5. Removal of hybridized probe from nitrocellulose filter for re-use of the filter

Filers with radioactive probes were soaked in 0.2N NaOH for 20minutes at 42℃ and nutralized with 0.1 ×SSC-0.5% SDS-0.2 *M* Tris (pHT.5) for 30 mintes at 42℃ The filters can then be used immediately or another hybridization or stored in a vacuum container at room temperature after air drying.

RESULT

1. Restriction and Southern analysis of A364a chromosome V

Most data for construction of macro-physical map were obtained from complete and partial digestions of intact chromosome DNAs, and Southern hybridization with cloned DNA probes.

Yeast *S. cercvisiae* A364a and five restriction enzymes (NotI, SfiI, SmaI, ApaI and SstII) were used in constructing the physical map of chromosome V. Enzyme digested DNAs were seperated by PFGE (two examples are shown in Figure 1). Ethidium bromide-stained results of PFGE show that most of DNA fragments generated by SmaI, ApaI and SstII digestion are 50-150 Kb, smaller than those generated by Not I and SfiI. Because some fragments, especially those SmaI,ApaI

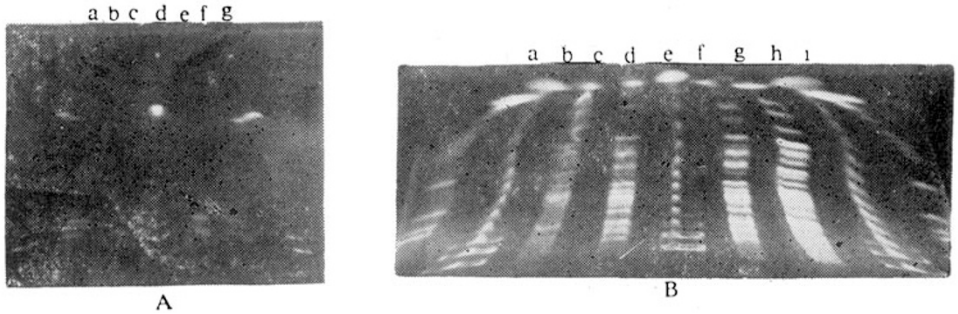


Fig. 1 EB stained PFG electrophoresis. The intact chromosome DNA of A364a. was digested by different restriction enzymes. PFGE was performed at 30—40 160V 21h 15℃.
A) Chromosome V DNA digested by SmaI (b, c, e and f). a, d and g, are molecular weight standard of λ DNA ladder.
B) Genomic DNA digested by rare cut enzyme NotI. a, and i,: genomic DNA of strain 7209—1A; b, e, and h: λDNA ladder; c, and g,: NotI digested genomic DNA of A364a; d, and f,: NotI digested genomic DNA of 7209—1A.

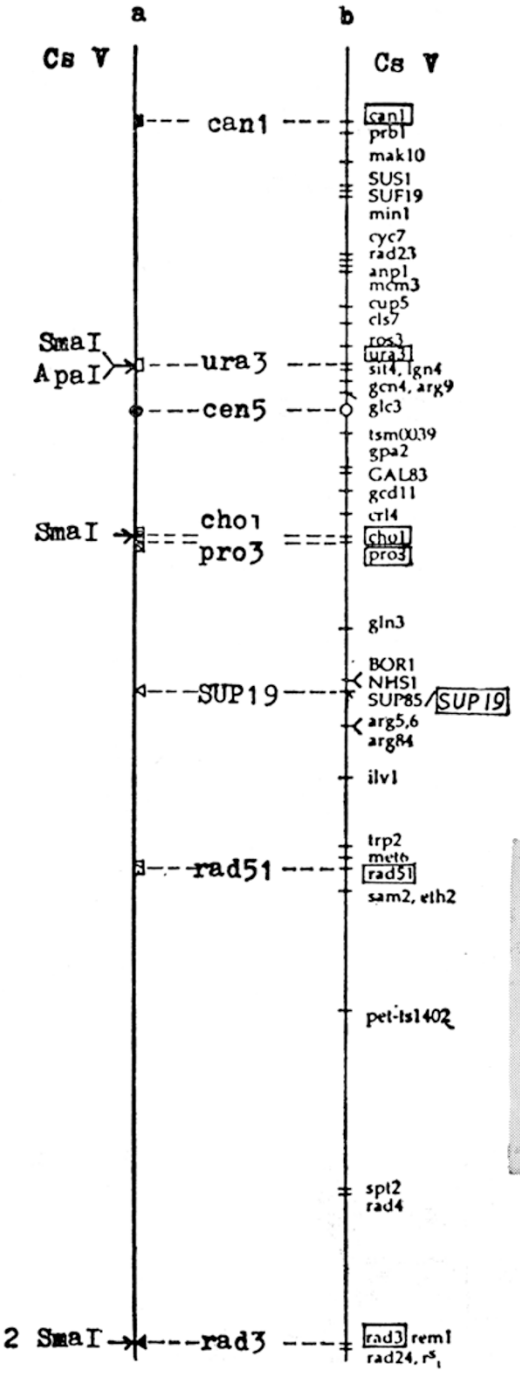
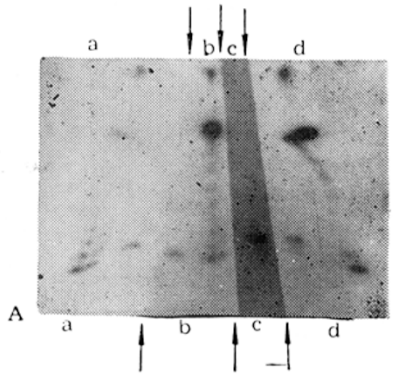


Fig. 2. Locations of the chromosome V-specific DNA probes in genetic map.
(a) Probes and restriction sites known; (b) Genetic map of chromosome V of *Saccharomyces cerevisiae* (quoted from Mortimer, R. K. et al. 1989. Genetic map of Saccharomyces cerevisiae, edition 10. in 'Program and Abstracts-1989 Yeast Genetics and Molecular Biology Meeting' June 27-July 1, Atlanta, Georgia. pp. 188—189).



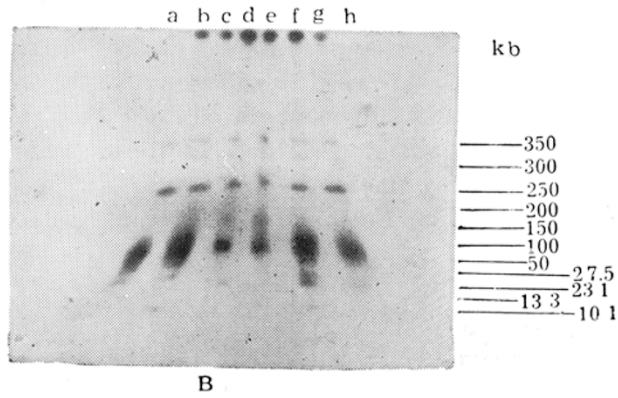


Fig. 3. Southern hybridization with chromosome V-specific probes.
A) chromosome V DNA of A364a was digested with SmaI. PFGE blot was cut into four pieces, each of which was detected by a chromosome V-specific probe, a, URA3 probe; b, Cen5 probe; c, PRO3 probe; d, RAD51 probe. Molecular weight standard is λDNA ladder.
B) Genomic DNA of A364a was digested with restriction enzymes and hybridized with probe Canl.a, and h: λDNA ladder+ λ /BglII + λ/HindIII; b, and c: ApaI digested DNA; d, and e,: SmaI digested DNA; f, and g,: SstII digested DNA.

and SstII digested fragments comigrate in PFGE, and because DNA quantity and the resolution of EB stain are limited, we couldn't count directely the number of DNA fragments generated by each of the five enzymes from EB-stained PFGE gels. Therefore we used cloned DNA segments, which have been precisely mapped on *S. cerevisiae* chromosome V genetically, as probes to determine the length and the number of restriction fragments and their alignment.

Eight chromosome V-specific probes used in this research (Table 2) locate at different loci throughout the chromosome V (Figure 2), which allow us to study all regions of this chromosome. Hybridization of these probes to PFGE gel blot of partially or completely digested DNAs(for example, see Figure 3) gave us the information for mapping.

2. Physical map of A364a chromosome V

Smith and Birnstiel used a strategy for ordering DNA fragments [18]. To sum up their strategy, DNA is completely cut with the first enzyme, then partially digested with the second enzyme which cuts more frequently than the firsL A probe that is near the end of the rare cutting site by the first enzyme will detect the fragments extending from the site of the first enzyme to all the sites of the second enzyme. The length of these fragments will provide a map of all the cutting sites. Because the probes Can1 and Rad3 are genetically mapped adjacent to the left and right end of chromosome V respectively, we analyzed the data of Southern hybridization regarding the two ends of chromosome V as two cutting sites of the first enzyme just like the way in Smith-Birnstiel method. The other probes mapped between Can1 and Rad3 on chromosome V will detect the fragments around probes, and proofread the map aligned by probes Can1 and Rad3. The macro-physical map of A364a chromosome V (Figure 4) was thus constructed. The map includes 9, 9, 15, 17, and 20 sites for NotI, SfiI, SmaI, SstII and ApaI respectively. The total length of A364a chromosome V was calculated to be about 620 Kb according to PFGE and its physical map.

3. Evidences from double digestion of intact chromosome V DNA

If the physical map we constructed is reliable, the fragments generated by digestion with any two of the five enzymes will be able to be explained by this physical map. In order to examine the reliablity of our physical map, we used different combinations of two enzymes to digest A364a chromosome DNAs. Southern hybridization with chromosome V-specific probes not only pick up the fragments the lengths of which are the same as or similar to that of fragments generated by single enzyme digestion, (also probably, different fragments of same length, data not shown) but also show some distinct fragments which do not appear in single enzyme digestion. For example, as shown in Figure 5, the probe Cen5 hybridized with NotI-SfiI digested A364a chromosome DNAs, and a 140 Kb fragment is detected. The 140 Kb fragment containing Cen5 sequence does not appear when A364a chromosome DNA was digested with either Notl or SfiI, but a fragment of 140 Kb appears between N3 site (70 Kb) and S3 site (210 Kb) in the

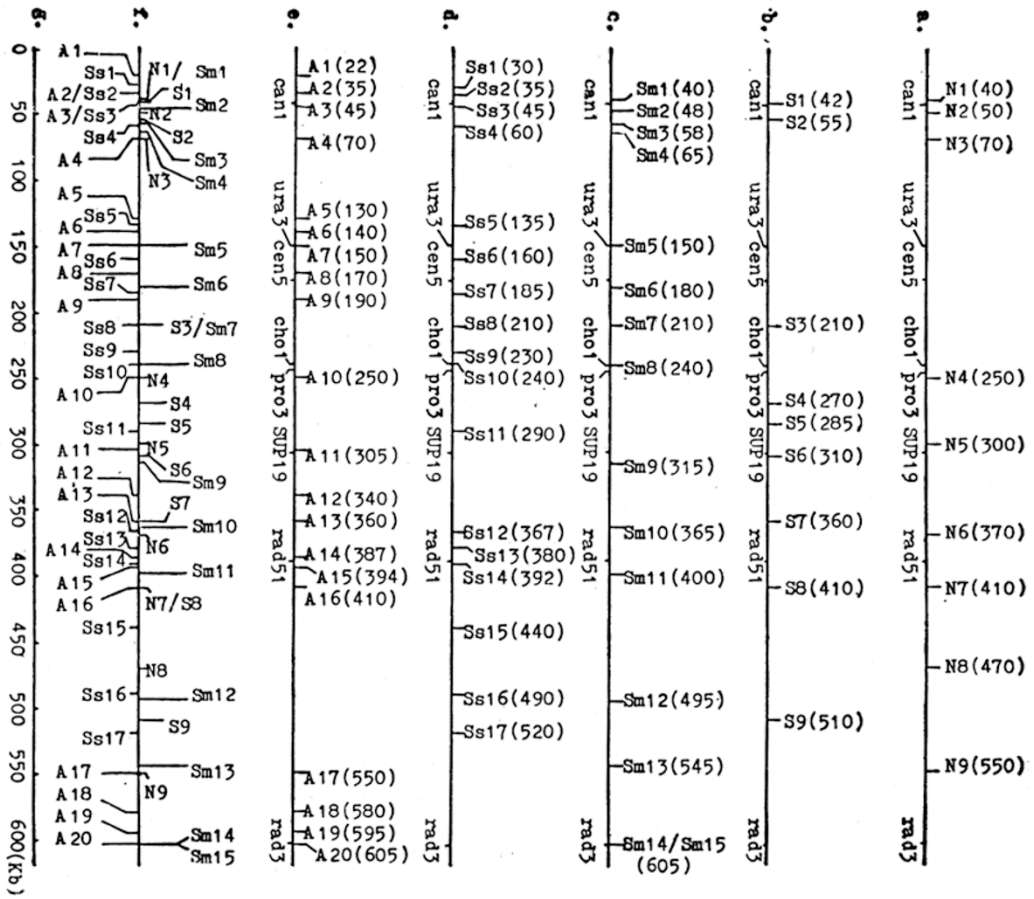


Fig. 4. Physical maps of the A364a chromosome V.
a) NotI restriction map of the A364a chromosome V; b) SfiI restriction map of the A364a chromosome V; c) SmaI restriction map of the A364a chromosome V; d) SstII restriction map of the A364a chromosome V; e) ApaI restriction map of the A364a chromosome V; f) Restriction map of the A364a chromosome V; g) Size scale in Kb. There is about ±5 Kb variation in length.

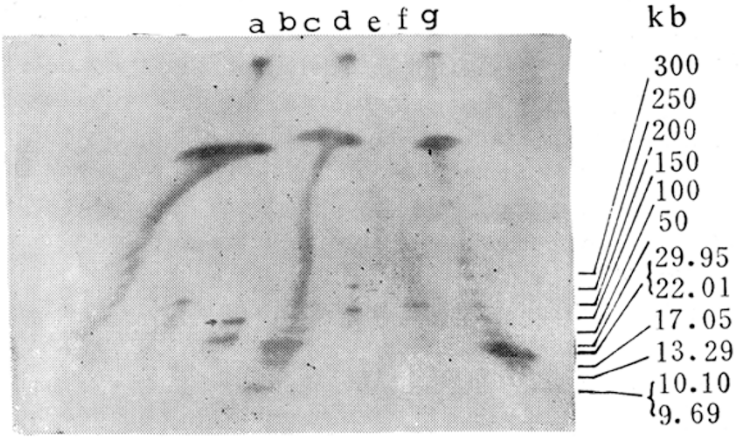


Fig. 5. Southern blot of enzyme digested A364a genomic DNA with probe Cen5. a), d) and g): λDNA ladder+ λ/PglII+ λ/KpnI; b): NotI digested DNA; c) and e): NotI and SfiI digested DNA; f) : SfiI digested DNA. The arrow shows the 140 Kb fragment.

physical map established by us(Fig. 4). Many results from double digestion of intact chromosome DNAs also defined some other restriction sites in tho physical map. Therefore, it can be seen that the macro-physical map of A364a chromosome V established mainly by complete and partial digestion and Southern hybridization is correct on the whole.

However, arrangement of some small fragments, especially those smaller than 20 Kb, is still not sure not only because length determination is coarse in PFGE pattern, and difference of several kilobases between partially digested fragments may thus not be resolved; but also because some small fragments do not have any homology to the probes and may not be detected either in PFGE gel(due to low DNA quantity) or by hybridization. Hence construction of higher resolution map will be desirable. We have constructed a chromosome V library of A364a (to be published soon), andwe are going to walk the chromosome V using DNA fragments from the library as probes to establish a fine physical map of chromosome V.

4. Comparison of physical distance and genetic distance

In the physical map of A364a chromosome V, the distribution of restriction sites are not uniform. We calculated the physical distances(Kb)between chromosome V-specific probes according to physical mapping data (Fig. 6b), and corresponding genetie distances (Kb) based on total length of 233 cM (conti-Morgan) and average length of 2.66 Kb per contiMorgan (620kb/233cM. Fig. 6a). Comparisons between physical distance and gcnetie disbance, and between physical distance per centiMorgan and 2.66 Kb per centiMorgan do not show distinct difference except the difference in a small region from cho1 locus to pro3 locus (Table 3). That is, if the corresponding regions compared are large, there is no distinct difference between physical map and genetic map. The similarities between physical distance and genetie distance are also seen in *E. coli*[15] and fission yeast *S. pombe*[6]. This indicates that recombination hotspots or coldspots in A364a chromosome V, if present, might not be reflected by our coarse macro-physical map.

Table 3. The ratio of physical distance to genetic distance

Interval	Physical distance (Kb)	Genetic distance [23] (cM)	Ratio of P. D./(G. D. (Kb/cM)	Average ratio for the whole Cs (Kb/cM)
can1-ura3	107	44.2	2.421	
ura3-cen5	25	8.0	3.125	
cen5-cho1	65	25.0	2.600	
cho1-pro3	5	3.0	1.667	2.66
pro3-SUP19	62	24.5	2.531	
SUP19-rad51	83	27.5	3.018	
rad51-rad3	215	83.5	2.575	

P,.D.: physical distance; G.D.: genetic distance; Cs: chromosome.

DISCUSSION

In bacteria (eg. *E. coli*) and bacteriophage (eg. lamda phage), there is a chi site (crossover hotspot instigator site) which can stimulate homologous recombina-

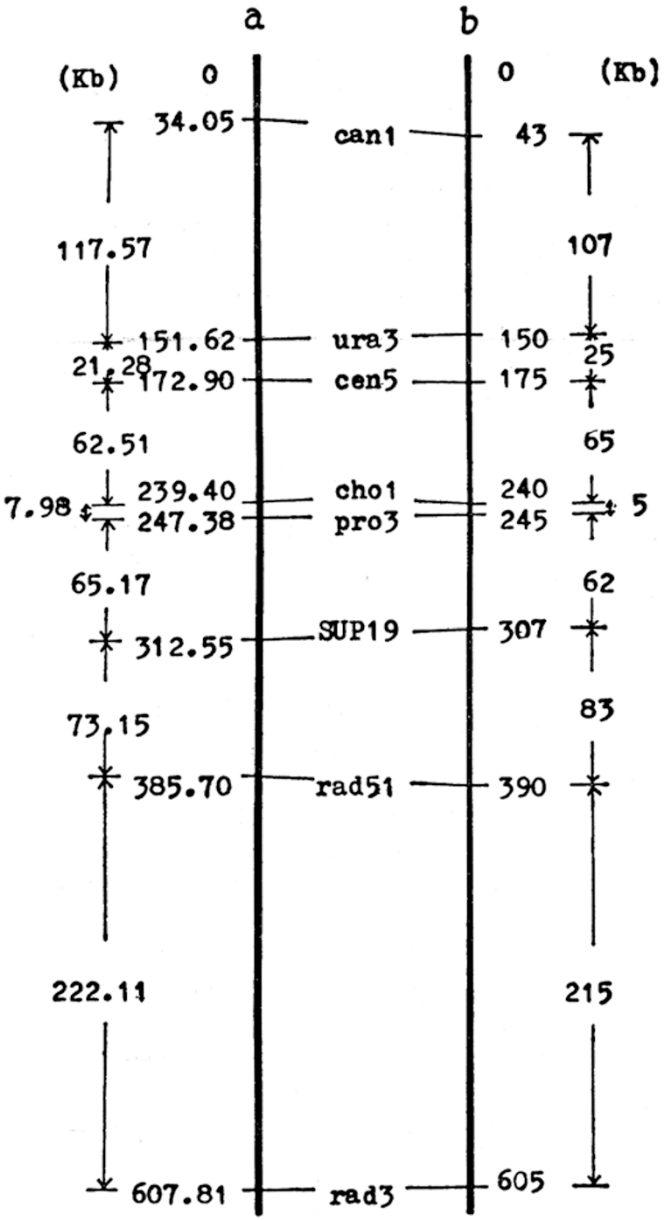


Fig. 6. Comparison of the physical map with genetic map.
a) Genetic distance in Kb [23]; b) Physical distance in Kb. Left arm is on top.

tion[19]. Recombination hotspots similar to chi also present in mammalian genome [20]. In the chromosome XII of yeast S. cerevisiae there is a region where homologous recombination occurs easily [21], while meiotic recombination in rDNA repeat region is infrequent [22]. Therefore there may also be recombination hotspots or coldspots in yeast genome. Recombination hotspots in a genome result in difference between physical map and genetic map. Geneeic distance between two gene loci will become extraordinarily large if there is a hotspot between the

two loci, otherwise, in case of a coldspot, the distance will be smaller than normal. A physical map perfected by other frequently cutting enzymes may be helpful in detecting regions containing a hotspots or coldspot in chromosome V. If the position of a recombination hotspot and its structure are known, one may be able to increase integrating frequency and hence copy number of a foreign gene in this position through homologous recombinabion. This may be important in improving expression efficiency of foreign genes in yeast.

Construction of physical map is also important in defining functional and regulatory sequences (such as ARSs, etc.) in chromosomes and in cloning and aligning of genes located in gapped regions with few gonetically mapped genes.

REFERENCES

[1] Clack-Walker GD. The genome of bakers yeast-The benchmark for a eukaryotic cell. Yeast (special issue) 1989; 5: S247—254.

[2] Smith CL, Warburton, Gaal A, Cantor CR. Analysis of genome organization and rearrangements by pulsed field gradient gel electrophoresis. Genetic Engineering 1986; 8: 45—70.

[3] Barlow DP, Lehrach H. Genetics by gel electrophoresis: the impact of pulsed field gel electrophoresis on mammalian genetics. Trends in Genetics 1987; 3: 167—171.

[4] Poustka A, Pohl T, Barlow DP, et al. Molecular approaches to mammalian genetics. Cold Spring Harbor Symp. Qnant. Biol. 1986; 51: 131—139.

[5] Smith CL, Econome JG, Schutt A, Klco S, Cantor CR. A physical map of the Escherichia coli K12 genome. Science 1987; 236: 1448—1453.

[6] Fan JB, Smith CL, Chikasige Y, Niwa O, Yanagida M, Cantor CR. Construction of a NotI restriction map of the fission yeast *Schizosaccharomyces pombe* genome. Nuc1. Acid Res. 1989; 17: 2801—2818.

[7] Lawrance SK, Srivastava R, Chorney M J, et al. The molecular approaches to characterization of megabase region of DNA: Studies of the human major histocompatibility complex. Cold Spring Harbor Symp Quant Biol 1986; 51: 123—130.

[8] Poh1 TM, Zimaer M, MacDonald ME, et al. Construction of a NotI linking library and isolation of new markers close to the Huntington's disease gene. Nucl. Acid Res. 1988; 16: 9185—9195.

[9] Smith CL, Cantor CR. Approaches to physical mapping of the human genome. Cold Spring Harbor Symp Quant Biol 1986: 51: 115—122.

[10] Smith CL, Lawrance SK, Gillespie GA, Cantor CR, Weissman SM, Collins FS. Strategies for mapping and cloning macro-regions of mammalian genomes. Method Enzymol. 1987; 151: 461—489.

[11] Aasland R, Smith CL. An efficient manual method for aligning DNA restriction map data on very large genomic restriction map. Nucl Acid Res 1988; 16: 10392.

[12] Wallace MR, Fountain JW, Brereton AM, Collins FS. Direct construction of a chromosome-specific NotI linking library from flow-sorted chromosomes. Nucl Acid Res 1989; 17: 1665—1677.

[13] Poustka A, Pohl TM, Barlow DP, Frishauf A—M, Lehrach H. Construction and use of human chromosome jumping libraries from NotI-digested DNA. Nature 1987; 325: 353—355.

[14] Poustka A, Lehrach H. Jumping libraries and linking libraries: The next generation of molecular tools in mammalian genetics. Trends in Genetics 1986; 2: 174—179.

[15] Olson MV, Dutchik JE, Graham MY, et al. Random-clone strategy for genomic restriction mapping in yeast. Proc Natl Acad Sci USA 1986; 83: 7826—7830.

[16] Laure CD, Roberts TM, Klotz LC. Determination of the nuclear DNA content of Saccharomyces cerevisiae and implications for the organization of DNA in yeast chromosomes. J Mol Biol 1977; 114: 507—526.

[17] Zhu YW. Electrophoretic karyotype analysis of yeast Saccharomyces cerevisiae. Acta Biologiae Experimentalis Sinica 1988; 21: 23—33.

[18] Smith HO, Birnstiel NL. A simple method for DNA restriction site mapping. Nucl Acid Res 1976; 3: 2387—2395.

[19] Smith GR. Chi hotspots of generalized recombination. Cell 1983; 34: 709—710.

[20] Steinmetz M, Uematsu Y, Lindahl KF. Hotspots of homologous recombination in mammalian genomes. Trends in Genetics 1987; 3: 7—10.

[21] Hohmann S. A region in the yeast genome which favors multiple integration of DNA via homologous recombination. Current Genetics 1987; 12: 519—526.
[22] Petes TD. Meiotic mapping of yeast ribosomal deoxyribonucleic acid on chromosome XII. J. Bacteriol. 1979; 138: 185—192.
[23] Mortimer RK, Schild D. Genetic map of Saccharomyces cerevisiae. Microbiological Reviews 1985; 49: 181—212.

Received 24-4-1990. Revised 21-6-1990. Accepted 29-12-1990.