Recombinational resolution in primate cells of two homologous human DNA segments with a gradient of sequence divergence

Mariza Gomez-Pedrozo, Wei-Shau Hu⁺ and Che-Kun James Shen*

Department of Genetics, University of California, Davis, CA 95616, USA

Received June 8, 1988; Revised and Accepted November 4, 1988

ABSTRACT

Human α -thalassemia-2 genotype $-\alpha 4.2$ is the result of meiotic recombination between two 1.3 kb long, homologous DNA segments, X($\alpha 2$) and X($\alpha 1$), located in the adult α globin locus. The two segments can also undergo intramolecular recombination on extrachromosomal vectors transfected into mitotically dividing primate cells (COS 7). The existence of a gradient of sequence divergence between X($\alpha 2$) and X($\alpha 1$) makes them an interesting system to study the relationship between efficiencies of homologous DNA recombination and the extent of dispersed and localized base mismatches. By partial restriction mapping and DNA sequencing of plasmids recombined in COS 7 cells and rescued from bacteria HB 101, we have determined the distribution of recombinational resolution sites along the two X blocks. Most, if not all, of the homologous recombination events between the two X blocks appear to be single crossing-over without efficient gene correction or repair of base mismatches. The distribution of the sites of recombinational resolution is inversely correlated with that of the gradient of sequence divergence, with only approximately 7% of the X recombinants resolved within the 3' third of the X blocks where two diverged Alu family repeats reside. The Alu sequence within which one of the X recombinants resolved is homologous to a previously characterized α thalassemia deletion point.

INTRODUCTION

The molecular details of homologous, or general DNA recombination processes are still far from being fully understood despite several basic working models (1-3) and extensive experimental studies (for examples, see ref. 4). Homologous DNA recombination in both mitotically dividing cells, and during meiosis is believed to play an important role in the maintenance of DNA sequence homogeneity among members of multi-gene families of the eukaryotic genomes (5-9). It has also been implicated in various genomic rearrangement activities such as sister chromatid exchange (reviewed in ref. 10), genetic recombination between mammalian X/Y sex chromosomes (reviewed in ref. 11), and unequal crossing over events that lead to several inherited human diseases (11) including α -thalassemias (12-14) and familial hypercholesterolemia (15).

Because of the lack of genetic studies of mammals in comparison to bacteria and fungi, a number of DNA mediated gene transfer systems have been constructed in cell cultures for the studies of intra- and interchromosomal gene conversion and of DNA recombinations in mammalian cells (4, 16-18 and references therein). In these studies, the

efficiencies of gene conversion and/or recombination have been followed as a function of the lengths of the homologous units (19, 20), the distance between the recombining units (21, 22), or topology of the recombining DNA molecules (23,24). However, unlike in prokaryotes (25-29; also see Discussion), very few studies have aimed at the effects of the sequence divergence, or base mismatches, on the rate of homologous recombination of the mammalian genomes (30).

The intrachromosomal recombination system we have set up recently (31) seems to be ideal for the above study. This system utilizes recombinant plasmids containing an SV40 replication origin and the human α globin gene duplication units (Fig. 1). After transfection into monkey COS 7 or COS 1 cells, efficient recombination occurs between the X blocks or Z blocks, but not the Y blocks (31). These deletions mimic the chromosomal recombination events that occur in the human germ line cells, which result in either the leftward ($-\alpha^{4.2}$) or rightward ($-\alpha^{3.7}$) α -thalassaemia-2 genotype (12, 13). These recombinational processes have also been thought to be responsible for gene correction of the primate α globin gene duplication units (32, 38). Analysis of the recombination behavior in COS 7 cells of different plasmid constructs has suggested the existence of hot spots for initiation of homologous DNA recombination within the first 300 bp of the X blocks (31). Deletion of this DNA region resulted in a 50% decrease in the recombination efficiency *in vivo* of the X blocks, which are 1.3 kb long (31). This data is consistent with sequence analysis of the two X blocks, which showed that they differ by a gradient of sequence divergence (37). The nucleotide differences were found to be essentially zero in the 5' portions, and increase gradually towards the 3' ends where two Alu family repeats reside (37).

In this communication, we report the structural analysis of plasmids generated in COS cells via homologous recombination between the two X blocks (X recombinants). The system has allowed us to investigate the actual distribution of recombinational resolution sites in a primate cell culture along a pair of homologous DNA segments with a well-defined gradient of base mismatches.

MATERIALS AND METHODS

Recombinant Plasmids.

The plasmid used to transfect COS 7 cells in this study is the plasmid A (or pA2A1) which contains the majority of the human adult alpha globin gene region subcloned in a psvod vector. See Hu and Shen (31) and Fig. 1 for details.

DNA Transfection.

Monkey COS 7 cells (39) were cultured and transfected by the DEAE-dextran method (40). Low molecular weight DNAs were isolated and purified from COS cells at 72 hours post-transfection by the Hirt procedure (41).

DNA Transformation and Plasmid Isolation.

The low molecular weight DNA samples prepared from seven independent transfections were transformed into recA-HB101 bacteria cells. Small-scale preparations of plasmid DNAs were isolated from individual ampicillin-resistant bacterial colonies by the alkaline lysis method (42). The unrearranged plasmids and the recombinants were identified by restriction enzyme digestion with EcoRI or Bgl II (31).

Restriction Site Mapping and DNA Sequencing.

Mapping of restriction sites in the rescued X recombinant plasmids was done by ^{32}P -end-labelling, and subsequent partial restriction digestions (43). The nucleotide sequences were determined by the chemical method (44).

RESULTS**Rescue of Plasmids Generated via Homologous Recombination in COS 7 Cells.**

Plasmid A has been constructed previously, and contains a major portion of the human adult α globin gene region including both X blocks (31; Fig 1). It was transfected

Figure 1. Structural relation between X recombinants and plasmid A. Plasmid A was constructed previously (31). It contains a major portion of the human adult α globin gene duplication units (thin line) cloned in psvod (bold line). The boxes outside the circular maps represent the three pairs of homology blocks X, Y, and Z. The transcription directions of the $\alpha 2$ and $\alpha 1$ globin genes are clockwise. After transfection into COS 7 cells, the X recombinants, as shown in the figure, and Z recombinants were generated via homologous recombination processes. B: Bgl II; H: Hind III; S: Sal.

into COS 7 cells by DEAE-dextran sulfate method. At 72 hr post-transfection, small molecular weight DNAs were isolated by the Hirt procedure, and transformed into recA⁻ bacterial strain HB101. Plasmid DNAs were then prepared from individual ampicillin-resistant bacterial colonies, and analyzed by restriction digestion.

From seven independent transfections and subsequent transformations, we have rescued a total of 445 plasmid DNAs. Restriction cleavage with Bgl II or EcoRI has identified 340 of them to be wild type plasmid A, 40 to be X recombinants, 21 to be Z recombinants, and 45 to be aberrantly recombined ones. Although the ratio of the X to Z recombinants is consistent with previous results obtained by Southern blot hybridization (31), their absolute percentages are two fold over. This is probably due to the presence of the aberrantly recombined molecules of heterogeneous sizes, which would not have been detected on Southern blots (31). Again, no Y recombinants could be detected. The X and Z recombinants are the result of recombination in COS cells but not in HB101 cells (31).

Restriction Mapping of Recombinational Resolution sites of the X Recombinants.

Each one of the 40 X recombinants is the result of homologous but unequal crossing-over between the two X blocks of the wild type plasmid A in COS 7 cells. We have first examined the distribution of the recombinational resolution sites along the X blocks by partial restriction mapping. This strategy was used because the two X blocks, X(α 2) and X(α 1), differ from each other by 4% overall sequence divergence (37; Fig. 3), and consequently also differ in several restriction sites such as Hpa II, Cfo I, and Rsa I, most of which are located in the 3' portions of the two blocks (Fig. 2B).

The 40 X recombinants were each digested with Bgl II, end-labeled with ³²P, and re-cleaved with Sal I. The 1.98 kb Bgl II-Sal I fragments were then eluted and subjected to partial digestion by Hpa II, Cfo I, or Rsa I, electrophoresed in 2% agarose gels, and autoradiographed on Kodak X-ray films. Some examples of the partial digestion patterns are shown in Fig. 2A. The presence or absence of specific restriction fragments labeled at the Bgl II sites (Fig. 2) thus has allowed us to locate the range within which each of the forty intrachromosomal recombination events has occurred in the monkey cells.

Our data showed that the recombination resolution sites of 37 of the rescued X recombinants are mapped upstream the Cfo I site located at position 986 of the X (α 1) block (lanes a of Fig. 2A). The remaining three rescued X recombinants have been generated by recombinational resolutions near the 3' portions of the X blocks. Their partial restriction patterns (lanes b, c, and d of Fig. 2A, respectively) indicate that these three resolution events occurred within DNA regions of bases 1223-1251 (in between the most 3' Hpa II sites of the two X blocks), 1198-1220 (in between the Cfo I site of X(α 2) and the most 3' Hpa II site of X(α 1)), and 988-1080 (in between the Cfo I site of X(α 1) and the Rsa I site of X(α 2)), respectively (Fig. 3).

Figure 2. Mapping of recombinational resolution sites of the two X blocks in COS 7 cells. The 1.98 kb Bgl II-Sal I fragments (Fig. 1) of different X recombinant plasmids were labeled at the Bgl II ends, purified by gel elution, partially digested with Cfo I, Rsa I, or Hpa II, electrophoresed in 2% agarose gels, and autoradiographed on Kodak X-ray films.

(A) Examples of the partial digestion patterns. The two sets of lanes (a) show the patterns of two X recombinants derived from recombinational resolution events occurring within the first 986 bp of the X blocks. The digestion patterns in lanes (b), (c), and (d) suggest that these three plasmids have resolved in DNA regions of 1223-1251, 1198-1220, and 988-1080, respectively, downstream the 5' ends of the X blocks (for sequences of the X blocks, see Fig. 3). The numbers to the left of each panel represent the lengths in bp of the restriction fragments as indicated in (B). These ranges of resolution sites have been further mapped by DNA sequencing (see text and Fig. 3).

(B) Top, restriction maps of the two X blocks, X(α2) and X(α1). Only those restriction sites used in (A) are indicated. C:Cfo I; R:Rsa I; H:Hpa II. The lines below the maps represent restriction fragments generated by partial digestion of the Bgl II-Sal I fragments from different X recombinants as exemplified in (A). The lengths in bp of the fragments are indicated. Note that the two 3' Hpa II sites of X(α1) block are very close, and so the two HpaII-Bgl II fragments could not be separated on 2% agarose gels (for example, see Hpa II digestion patterns (A)). Only the length of the shorter Hpa II-Bgl II fragment (373 bp) is indicated in both (A) and (B).

Figure 3. Locations of four sites of recombinational resolution within the X blocks on a DNA sequence level. The nucleotide sequences of the two X blocks shown are identical to those derived previously (37) except that a G is present at position 690 of the X(α 1) block. The five nucleotide differences within the first 840 bp region are boxed. Several restriction sites (Cfo I, Rsa I, and Hpa II) that are different between the two blocks, and have been used for restriction mapping, as exemplified in Fig. 2, are indicated. The 5' ends (nucleotide 1048) of the Alu family repeats located in the 3' portions of the X blocks are indicated by a vertical arrow. The numbers on the left side of each line indicate the distance in nucleotides from the 5' ends of the X blocks. The ranges within which four X recombinants have resolved are bracketed by arrows under the DNA sequences. The range of the X recombinant plasmid c was derived from partial mapping results shown in lane (c) of Fig. 2. The range of X recombinant plasmid b was determined by sequencing from the Hinf I site (position 1240) upstream for 100 bp and downstream for 50 bp. For X recombinant plasmid d, sequencing was done from 100 bp upstream of the Dra I site (position 1028) to 100 bp downstream. For one of the two X recombinants shown in lane(a) of Fig. 2A, sequencing was performed for 90 bp downstream of the Dde I site at position 792.

the frequency of recombination by the presence of 3% of base mismatches within 40 bp long recombining units, while Shen and Huang (28) detected a 40 fold decrease of recombination frequency when the homology is reduced from 100% to 90% for pairs 166-168 bp recombining units. The propagation of a recombination signal initiated from the ends of linear DNA molecules does not seem to be completely blocked by heterologous DNA sequences in *E. coli* cells (29). However, the insertion of heterologous DNA does favor the arrest of the signal, and stimulates recombination at the border of the homology and the heterologous insertion (29). *In vitro* studies of the rec A protein of *E. coli* have demonstrated that base pair mismatches inhibit the extension and migration of the heteroduplex joints (25, 26). By comparison, very few similar studies have been attempted in mammalian systems. By using herpes simplex viral tk genes as the substrates for homologous recombination in mouse L cells, Waldman and Liskay (30) have demonstrated that an extent of 19% base mismatches spread over 1 kb can decrease the frequency of intrachromosomal recombination by 3-15 fold, and that of the interchromosomal recombination by 1000 fold.

Taking advantage of the well-defined gradient of sequence divergence between the two X blocks of the human adult α globin gene duplication units, we have investigated, by both restriction mapping and DNA sequencing, the distributions of the recombinational resolution sites in relation to the base mismatches. As summarized in Fig. 4, 37 of the 40 rescued X recombinants have been generated by homologous recombination and resolution between the first nucleotide and nucleotide 986 of the X blocks. Seven randomly selected plasmids out of these 37 X recombinants have been studied by DNA sequencing. The data showed that three were resolved in between nucleotides 45 and 470, three between 470 and 809, and one between 824 and 830 (Figs. 3, 4). Thus, the majority of the X recombinants from COS 7 cells are most likely the results of recombinational resolution spread evenly within the first 800 bp region, where the two X block sequences are essentially identical.

These data strongly suggest that initiation of homologous recombination in the monkey cells between the two X blocks preferentially occurs within the regions of almost perfect homology. This has been hypothesized previously (37) based on the similarity between the gradient of sequence divergence of the human X blocks and the distributions of frequencies of gene conversion along several fungal loci (see 45 and references therein). It is also consistent with recombination studies *in vivo* (31) which suggest that at least 50% of the X recombination events are initiated within the first 300 bp of the X blocks.

The preferential occurrence of the recombinational resolutions within the first 800 bp of the X blocks could be due to preferential synapsis of almost perfectly homologous DNA duplexes, the existence of hot spots for endonuclease cleavages, or both. The resolution events may occur very close to the sites of recombinational initiation. Alternatively, they could occur at relatively distant regions due to the process of branch migration. In the latter case, the recombination intermediates, possibly a Holliday structure, appear not to be able to

migrate easily into the 3' portions of the X blocks where higher amounts of base mismatches exist. In fact, less than 10% (3 out of 40) of the resolution events have occurred beyond the region with a local mismatch of only 5% (nucleotides 986-1100). In an independent experiment, we have demonstrated that the recombination signals initiated within the X blocks and migrating towards the adjacent Z blocks could be stalled by the introduction of a short, localized base mismatches at the X/Z junctions (Hu and Shen, manuscript in preparation). These *in vivo* results of homologous recombination in primate cells are consistent with the *E. coli* rec A protein-mediated recombination reactions *in vitro* (reviewed in 46, 47). In one of the studies (25), recA-mediated branch migrations had a tendency to stop in regions where sequences differed by an average of 10% in a stretch of over 900 bp DNA. Another *in vitro* study showed that fd/M13 heteroduplex phage DNA, containing about 3% base pair mismatches over approximately 7kb, can be formed as well as completely homologous heteroduplex (fd/fd or M13/M13). However, the rate of strand exchange for mismatched substrates is lower than those molecules with perfect homology (26). It appears that mismatches are impediments that stall strand exchange, and perhaps provide greater opportunity for the recombinase or other proteins involved in recombination to dissociate from the DNA. However, it should be noted that in the above studies, the heterologous insertion/deletion or base mismatches did not stall the migration of the heteroduplex joints when single-strand DNA binding protein and ATP regenerating system were included in the *in vitro* reactions (26).

Another purpose for this set of experiments is to search for any possible gene conversions of single base mismatches during the X block recombination *in vivo*. Within the limits of our partial restriction mapping (Fig. 2) and sequencing strategies (Fig. 3), all the recombinants rescued appear to be the results of intramolecular, single reciprocal crossingover events without detectable gene corrections, or repair of base mismatches (Figs. 3, 4). The other half of the reciprocal recombination products are not rescued efficiently since they lack both SV 40 and bacterial plasmid replication origins. Alternatively, this homologous recombination process may occur following exonucleolytic digestion and reannealing of the homologous recombination units, as proposed in (24). In this case, a double-strand DNA cut would have been generated prior to the recombination process, presumably within the 5' portion of the X block(s). Our results, i.e., low frequencies of apparent gene conversion, are consistent with some of the previous studies by others who have used mutated fragments of selectable genes like neomycin resistance gene as the recombination units. For example, Rubnitz and Subramani (48) have found that the frequencies of extrachromosomal, intramolecular gene conversion events in COS cells are at least 10 fold lower than those of single, reciprocal recombinations. However, the base mismatches in those systems are usually not single bases, but are from several to a few hundred bp long. It should also be pointed out that in our system, which has no selection

procedure, it is not easy to determine if gene conversion events have occurred within the first 800 base pairs of the X blocks where most of our plasmids were resolved. Within the more divergent regions, we have only identified three resolution events, which may not be sufficient to detect the occurrence of gene conversion of single base mismatches through the sequencing analysis.

Finally, a remark about the involvement of Alu family repeats in homologous recombinations in the primate cells. Several deletions, duplications, and other types of genetic rearrangements causing inherited human diseases have been identified which are the results of recombinations between Alu repeats (14, 15, 49, 50 and references therein). In some cases, recombinations between Alu repeats occur by perfect aligning of the sequences as in the LDL receptor gene, and within the alpha and beta globin gene clusters (14, 15). In other cases, homologous sequences of two Alu repeats may misalign during recombination (49). Our analysis of the X recombinants generated in COS cells showed that, out of the three plasmids analyzed that resolved within the region of frequent mismatches, two were resolved within the second monomer of the Alu repeats, and one around the region where the Alu sequence starts (Fig. 3). Interestingly, the 19 bp Alu sequence within which the X recombinant plasmid b resolved (Fig. 3) contains the recombination sites of the α thalassemia deletion ($\alpha\alpha$)^{RA} (14). Thus, certain regions within the Alu family repeat may undergo homologous recombination more frequently than the rest of the repeat sequences.

Acknowledgement

We thank our colleagues for discussions, and Scott Erdman for helpful suggestions. This research has been supported by an NIH grant (DK 29800), and an NIH Research Career Development Award (DK 01377) to C.-K.J. Shen.

*Present address: McArdle Laboratory, University of Wisconsin, Madison, WI 53706, USA

*To whom correspondence should be addressed

REFERENCES

1. Holliday, R. (1964) *Genet. Res.* **5**, 282-304.
2. Meselson, M.S. & Radding, C.M. (1975) *Proc. Nat. Acad. Sci. U.S.A.* **72**, 358-361.
3. Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J. & Stahl, F.W. (1983) *Cell* **33**, 25-35.
4. Cold Spring Harbor Symp. Quant. Biol.: Recombination at the DNA Level (1984) Vol. **49**, eds. Klar, A.J.S. & Strathern, J.N., Cold Spring Harbor Lab, N.Y., N.Y.
5. Baltimore, D. (1981) *Cell* **24**, 592-594.
6. Egel, R. (1981) *Nature (London)* **290**, 191-192.
7. Klein, H.L. & Petes, T.D. (1981) *Nature (London)* **289**, 144-148.
8. Jackson, J.A. & Fink, G.R. (1981) *Nature (London)* **292**, 306-311.
9. Dover, G. (1982) *Nature (London)* **299**, 111-117.
10. Latt, S.A. (1981) *Annu. Rev. Genet.* **15**, 11-56.
11. Siniscalco, M. (1986) Cold Spring Harbor Symp. Quant. Biol. **51**, 191-194.

12. Orkin, S.H., Old, J., Lazarus, H., Altay, C., Gurgey, A., Weatherall, D.J. & Nathan, D.G. (1979) *Cell* **17**, 33-42.
13. Embury, S.H., Lebo, R.V., Dozy, A.M. & Kan, Y.W. (1979) *J. Clin. Invest.* **63**, 1307-1310.
14. Nicholls, R.D., Fischel-Ghodsian, N. & Higgs, D.R. (1987) *Cell* **49**, 369-378.
15. Lehrman, M.A., Goldstein, J.L., Russel, D.W. & Brown, M.S. (1987) *Cell* **48**, 827-835.
16. Smithies, O., Gregg, R.G., Boggs, S.S., Koralewski, M.A. & Kucherlapati, R.S. (1985) *Nature (London)* **317**, 230-234.
17. U. Weiss & Wilson, J.H. (1987) *Proc. Nat. Acad. Sci. U.S.A.* **84**, 1619-1623.
18. Thomas, K.R. & Capecchi, M.R. (1987) *Cell* **51**, 503-512.
19. Rubnitz, J. & Subramani, S. (1984) *Mol. Cell. Biol.* **4**, 2253-2258.
20. Ayares, D., Chekuri, L., Song, K.-Y. & Kucherlapati, R. (1986) *Proc. Nat. Acad. Sci. U.S.A.* **83**, 5199-5203.
21. Folger, K., Thomas, K. & Capecchi, M.R. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 123-138.
22. Rubnitz, J. & Subramani, S. (1985) *Mol. Cell. Biol.* **5**, 529-537.
23. Kucherlapati, R.S., Eves, E.M., Song, K.-Y., Morse, B.S. & Smithies, O. (1984) *Proc. Nat. Acad. Sci. U.S.A.* **91**, 3153-3157.
24. Lin, F.-L., Sperele, K. & Sternberg, N. (1984) *Mol. Cell. Biol.* **4**, 1020-1034.
25. Das Gupta, C. & Radding, C.M. (1982) *Proc. Nat. Acad. Sci. U.S.A.* **79**, 762-766.
26. Bianchi, M.E. & Radding, C.M. (1983) *Cell* **35**, 511-520.
27. Watt, V.M., Ingles, C.J., Urdea, M.S. & Rutter, W.J. (1985) *Proc. Nat. Acad. Sci. U.S.A.* **82**, 4768-4772.
28. Shen, P. & Huang, H.V. (1986) *Genetics* **112**, 441-457.
29. Abastado, J.-P., Darche, S., Godeau, F., Cami, B. & Kourilsky, P. (1987) *Proc. Nat. Acad. Sci. U.S.A.* **84**, 6496-6500.
30. Waldman, A.S. & Liskay, M. (1987) *Proc. Nat. Acad. Sci. U.S.A.* **84**, 5340-5344.
31. Hu., W.S. & Shen, C.-K.J. (1987) *Nucl. Acids Res.* **15**, 2989-3007.
32. Lauer, J., Shen, C.-K.J. & Maniatis, T. (1980) *Cell* **20**, 119-130.
33. Zimmer, E.A., Martin, S.L., Beverley, S.M., Kan, Y.W. & Wilson, A.C. (1980) *Proc. Nat. Acad. Sci. U.S.A.* **77**, 2158-2162.
34. Liebhaver, S.A., Goossens, M. & Kan, Y.W. (1981) *Nature (London)* **290**, 26-29.
35. Hess, J.F., Fox, M., Schmid, C.W. & Shen, C.-K.J. (1983) *Proc. Nat. Acad. Sci.* **80**, 5970-5974.
36. Michelson, A.M. & Orkin, S.H. (1983) *J. Biol. Chem.* **258**, 15245-15254.
37. Hess, J.F., Schmid, C.W. & Shen, C.-K.J. (1984) *Science* **226**, 67-70.
38. Marks, J., Shaw, J.-P. & Shen, C.-K.J. (1986) *Proc. Nat. Acad. Sci. U.S.A.* **83**, 1413-1417.
39. Gluzman, Y. (1981) *Cell* **23**, 175-182.
40. McCutchen, J. & Pagano, J. (1986) *J. Natl. Cancer Inst.* **41**, 351-357.
41. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365-369.
42. Maniatis, T., Fritsch & Sambrook, J. (1982) *Molecular Cloning: A Manual*, Cold Spring Harbor Laboratory, N.Y., N.Y.
43. Smith, H.O. & Bernstiel, M.A. (1976) *Nucl. Acids Res.* **3**, 2387-2395.
44. Maxam, A.M. & Gilbert, W. (1977) *Proc. Nat. Acad. Sci. U.S.A.* **74**, 560-564.
45. Fogel, S., Mortimer, R.K. & Lusnak (1981) *Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, eds. Strathern, J.N., Jones, E. & Broach, J.R. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 289-339.
46. Radding, C.M. (1982) *Annu. Rev. Genet.* **16**, 405-437.
47. Cox, M.M. & Lehman, I.R. (1987) *Ann. Rev. Biochem.* **56**, 229-262.
48. Rubnitz, J. & Subramani, S. (1986) *Mol. Cell. Biol.* **6**, 1608-1614.
49. Lehrman, M.A., Schnieder, W.J., Sudhof, J.C., Brown, M.S., Goldstein, J.L. & Russell, D.W. (1985) *Science* **227**, 140-146.
50. Rougher, F., Simmler, M.C., Page, D. & Weissenbach, J. (1987) *Cell* **51**, 417-425.