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Structure of Amplified DNA, Analyzed by Pulsed Field Gradient Gel Electrophoresis

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Pulsed field gradient electrophoresis allows the separation of large DNA molecules up to 2,000 kilobases (kb) in length and has the potential to close the resolution gap between standard electrophoresis of DNA molecules (smaller than 50 kb) and standard cytogenetics (larger than 2,000 kb). We have analysed the amplified DNA in four cell lines containing double minute chromosomes (DMs) and two lines containing homogeneously staining regions. The cells were immobilized in agarose blocks, lysed, deproteinized, and the liberated DNA was digested in situ with various restriction endonucleases. Following electrophoretic separation by pulsed field gel electrophoresis, the DNA in the gel was analysed by Southern blotting with appropriate probes for the amplified DNA. We find that the DNA in intact DMs is larger than 1,500 kb. Our results are also compatible with the notion that the DNA in DMs is circular, but this remains to be proven. The amplified segment of wild-type DNA covers more than 550 kb in all lines and possibly up to 2,500 kb in some. We confirm that the repeat unit is heterogeneous in some of the amplicons. In two cell lines, however, with low degrees of gene amplification, we find no evidence for heterogeneity of the repeats up to 750 (Y1-DM) and 800 kb (3T6-R50), respectively. We propose that amplicons start out long and homogeneous and that the heterogeneity in the repeat arises through truncation during further amplification events in which cells with shorter repeats have a selective advantage. Even if the repeats are heterogeneous, however, pulsed field gradient gels can be useful to establish linkage of genes over relatively short chromosomal distances (up to 1,000 kb). We discuss some of the promises and pitfalls of pulsed field gel electrophoresis in the analysis of amplified DNA.

Key words: dihydrofolate reductase, gene amplification, double minute chromosomes, multi-drug resistance, Kirsten-ras, homogeneously staining regions

Many tumour cells contain amplified DNA in the form of double minute (DM) chromosomes or chromosomes with homogeneously staining regions (HSR) or abnor-

Abbreviations used: ABR, abnormal banding region; CHO, chinese hamster ovary; DHFR, dihydrofolate reductase; DM, double minute (chromosomes); HSR, homogeneously staining region; MTX, methotrexate.

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mal banding regions (ABR). In some cells amplification is induced and maintained by drug selection and the products of amplified genes are involved in drug metabolism. In other cells amplification of oncogenes is found, probably maintained by the selective advantage of oncogene overexpression in the tumour or tissue culture [see refs. 1–12 for recent reviews].

The analysis of amplified DNA by standard gel electrophoretic techniques is complicated by the large size of the amplified units, which exceeds by far the limit of resolution of this technique (about 50 kilobases [kb]). We have therefore turned to pulsed field gradient (PFG) gel electrophoresis, a novel technique for the size-fractionation of large DNA molecules, developed by Schwartz and Cantor [13]. This technique allows the sizing of DNA molecules up to 1,500 kb and probably even larger. We have used this to study two questions:

1. What is the size and size heterogeneity of the amplicon?
2. What is the size and structure of DMs?

We summarize here the results obtained in the past 2 years with this approach in our lab. Some of the results have been briefly reported elsewhere [14,15].

MATERIALS AND METHODS

Cell Lines

The MTX-resistant EL4/8 and EL4/12 mouse lymphoma cell lines [16,17] were obtained from Dr. C.J. Bostock, MRC Mammalian Genome Unit, Dept. of Zoology, University of Edinburgh (Edinburgh, Scotland).

The MTX-resistant 3T6-R50 mouse cell line [16,17] was obtained from Dr. R.T. Schimke, Dept. of Biological Sciences, Stanford University, (Stanford, CA).

The Y1-DM and Y1-HSR sub-lines of the mouse adrenocortical tumour [18–20] were obtained from Dr. D.L. George, University of Pennsylvania, Dept. of Human Genetics, The School of Medicine/G3 (Philadelphia, PA).

The multi-drug resistant Chinese hamster ovary cell line CH^RC5 [8,21] was obtained from Dr. V. Ling, The Ontario Cancer Institute (Toronto, Canada).

All cell lines were grown in the medium and with the drug concentrations specified by the suppliers.

Lysis and Deproteinization of Cells in Agarose

The procedure developed by Schwartz and Cantor [13] for yeast, was adapted to mammalian cells as described by Van der Bliek et al [15].

PGF Gel Analysis In-Gel Digestion, and DNA Blotting and Hybridization Procedures

This was done essentially as described by Schwartz and Cantor [13] with small modifications [15]. In some experiments long pulse times (60, 70, 90, or 150 sec) were used as described by Johnson and Borst [22]. In-gel digestion with restriction endonucleases was done as described by Bernards et al [23]. Blotting and hybridization was done as described by Van der Bliek et al [15].

Source of DNA Probes

The DHFR gene was detected with a mouse cDNA probe-pR400-12 obtained from Dr. A.D. Levinson, Dept. of Molecular Biology, Genentech Incorporated (South

San Francisco, CA). In the hybridization we used the gel-purified insert containing the complete DHFR encoding sequence with a single point mutation [24].

The c-Ki-ras gene was detected with a probe derived from the Ki-MusV clone HiHi-3 obtained from Dr. R.W. Ellis, Lab Tumor Virus Genetics, NCI (Bethesda, MD), via Dr. R. Nusse of this institute. A 380 base-pair (bp) SacII/XbaI fragment, devoid of repetitive sequences [25], was gel-purified and used in the hybridizations.

RESULTS

General Features of PFG Gel Analysis of Mammalian (Amplified) DNA

To study the structure of amplified DNA, we have lysed and deproteinized cultured cells in agarose blocks, which allows handling of pure DNA without degradation by shear. The DNA in the blocks was then digested with a restriction endonuclease, the blocks were inserted into the slots of a 1% agarose gel, and the digests subjected to PFG gel electrophoresis. For most experiments we used the modified Schwartz-Cantor [13] box depicted in the left half of Figure 1. In this setup the DNA runs approximately in the diagonal, but with occasional twists owing to the field gradient. In some experiments the modified Carle-Olson [26] box depicted in the right half of Figure 1 was used.

To size the DNA, we have primarily used oligomers of phage lambda DNA [23,26]. Under optimal conditions this provides size marking up to 1,000 kb and, with some luck, up to 1,500 kb. With a pulse time of 35 sec good separation is obtained up till 650 kb; larger DNA migrates in a compression zone. Very large linear DNA (and circular DNA > 10 kb) is trapped in the slot. By increasing the

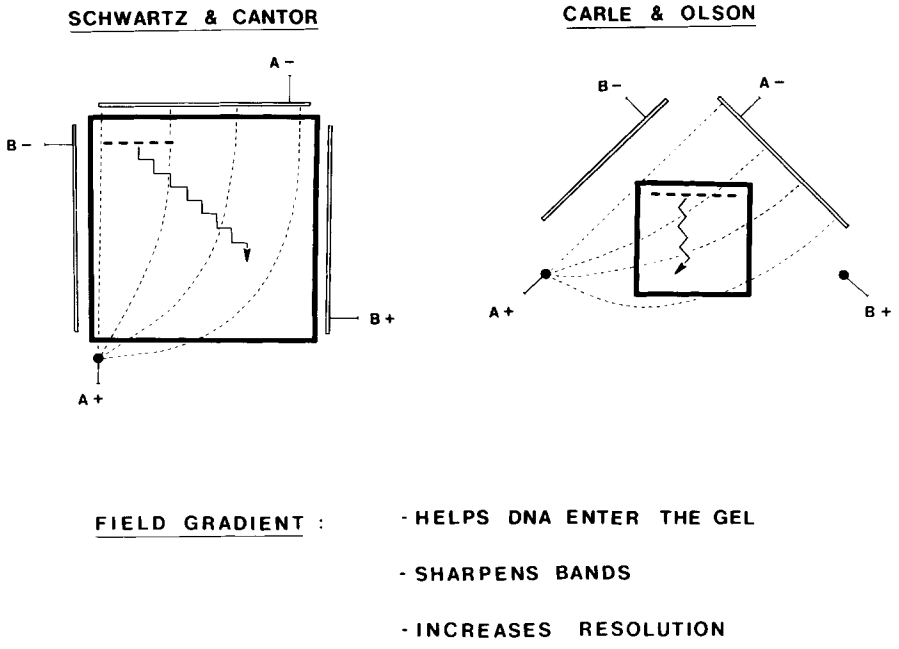

Fig. 1. Gel boxes used for PFG gel electrophoresis. The Schwartz-Cantor box has a field gradient only in the N-S direction (A^- -A^+), the Carle-Olson box in both directions. See text for further explanation.

pulse time, the compression zone can be shifted to higher DNA molecular weights. As reliable markers > 1,500 kb are not available, it is not known what the upper limit of the technique is. It could possibly be up to 3,000 kb at pulse times of 150 sec and longer [see ref. 22].

Undigested mammalian DNA of the cell lines studied was completely retained in the slot at all pulse frequencies. This is illustrated in Figure 2 for Y1-DM DNA. Treatment of the DNA in agarose with pancreatic DNAse I in the presence of Mn^{2+}, which introduces duplex breaks at semirandom positions [27], initially results in the appearance of DNA in the compression zone, even at long pulse times when the compression zone is well separated from the 1,000-kb area (Fig. 2). The same result is obtained when the fate of the DM DNA is analysed by blotting the gel onto nitrocellulose and hybridizing the blot with a probe for the amplified c-Ki-ras gene, located in the DMs [18–20]. At longer DNase incubations all DNA is removed from slot and compression zone and appears as smear lower in the gel.

The same result was obtained for all DM-containing cell lines, whether DNase I was used or a restriction endonuclease. We conclude that all DMs studied by us must contain DNA > 1,500 kb and possibly > 3,000 kb. The fact that undigested DNA does not enter the gel at all suggests but does not prove that intact DMs contain circular DNA, as also indicated by the lack of free ends in DM nucleoprotein spread and visualized by EM [28].

To test the size and homogeneity of the repeats contained in these DMs, we have digested the DNA in agarose blocks with restriction endonucleases that cut mammalian DNA infrequently. In our hands MluI (ACGCGT), NaeI (GCCGGC), NotI (GCCCCCGC), SacII (CCGCGG), and SfiI (GGCCNNNNNGGCC) were most useful. This may be related to the long recognition site (NotI and SfiI) and/or the presence of CpG doublets, which are underrepresented in mammalian DNA [29] and

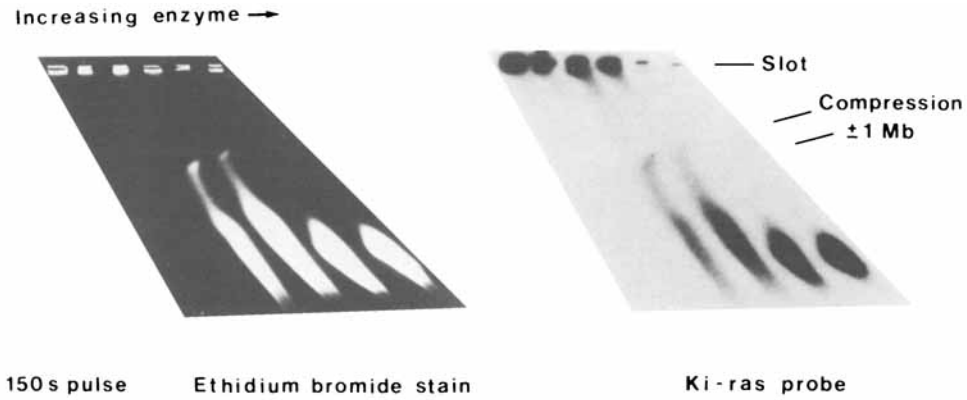

Fig. 2. DNA fragments generated by digestion of Y1-DM DNA with pancreatic DNase I in the presence of Mn^{2+}. Cells of Y1-DM were embedded in agarose, lysed, and deproteinized. The blocks were pre-incubated for 1 hr at room temperature with various DNase I concentrations in 0.05 M Tris-HCl, pH 8.0; 0,1 % gelatine; 0.1 mM phenylmethylsulfonylfluoride (PMSF). After the addition of 0.6 mM $MnCl_2$ the blocks were incubated for 1 hr at 37°C. The DNaseI reaction was stopped by extensive washing with 0.1 M EDTA, pH 8.0, at 4°C. The fragments were size-fractionated by PFG gel electrophoresis using a pulse of 150 sec. The gel was blotted onto nitrocellulose and hybridized with a probe for the c-Ki-ras gene. From left to right the DNaseI concentrations were 0.5-1-5-10-50-100 pg/ml. See Materials and Methods for further details of procedures.

often methylated [30]. The results obtained with this approach are summarized in Table I and briefly discussed in the following sections.

The Mouse EL4/8, EL4/12 and 3T6-R50 Lines (DHFR Gene)

The MTX-resistant lines EL4/8 and EL4/12 were isolated by Bostock and coworkers from mouse EL4 lymphoma cells by stepwise MTX selection. EL4/8 has 750–1,120 times the wild-type DHFR gene copy number; EL4/12 650–1,360 times. In EcoR I digests, EL4/12 only shows the parental DHFR DNA fragments; EL4/8 has additional bands associated with rearrangements close to the 3′ end of the DHFR gene in a subportion of the amplified DNA [1]. Cytogenetically EL4/8 and /12 have "variable numbers of DMs, which vary in size up to forms that appear as rings" [1]. Whether extra DHFR genes are also present in chromosomes is not known, but this is not impossible [cf. ref. 1]. We have verified by cytogenetics (Diamidinophenylindole [DAPI] and Giemsa staining) that EL4/12, as used in our experiments, still contains numerous DMs.

The 3T6-R50 was obtained from a clonal line 3T6-S5 by stepwise selection with MTX. It contains DMs and a 50× elevation of the number of DHFR genes without rearrangements detectable in Southern blots [16,17].

Figure 3A compares the fragments obtained when DNA from EL4/8, EL4/12, and 3T6-R50 is digested in agarose with NotI. 3T6-R50 yields a single band of 300 kb and no other DNA; EL4/12 yields approximately equal bands of 250, 350, and 550 kb, but in addition some DNA in the compression zone and in the slot that does not completely disappear even with large excess of enzyme; EL4/8 gives a complex pattern of nonstoichiometric bands with a majority of the hybridization over compression zone and slot.

The interpretation of these patterns is aided by the analysis of two mouse cell lines without DHFR gene amplification, L1210 and Y1-DM, shown in Figure 3B. L1210 only yields a single band at about 1,000 kb, well separated from the compression zone in this 150-sec PFG gel. The same band is also present in the Y1-DM line, but in addition this line yields a 300-kb band which comigrates with the 300-kb band in 3T6-R50. The two bands in the Y1-DM DNA are not equally intense, but this could easily be due to some trapping of the larger DNA in the slot. This is often seen with long pulse times [22]. These results suggest that the two bands in Y1-DM are allelic and that L1210 is homozygous for the long fragment. The 3T6-R50 cell line

TABLE I. Structure of Amplified DNA

Cell line	Selected gene	Estimated size in kb of		
		Intact DMs	Subrepeats	Repeats
DM lines				
Mouse EL4/12	DHFR	>1,500	250, 350, 550	>1,150
Mouse EL4/8	DHFR	>1,500	Complex	?
Mouse 3T6 R5O	DHFR	>1,500	None ?	>800
Mouse Y1 DM	c-Ki-ras	>1,500	None ?	2,500 ?
HSR lines				
Mouse Y1 HSR	c-Ki-ras		Complex	>2,000 ?
Hamster CH^RC5	P-glycoprotein		>800	>1,100
Hamster CHOC 400.5	DHFR			>800

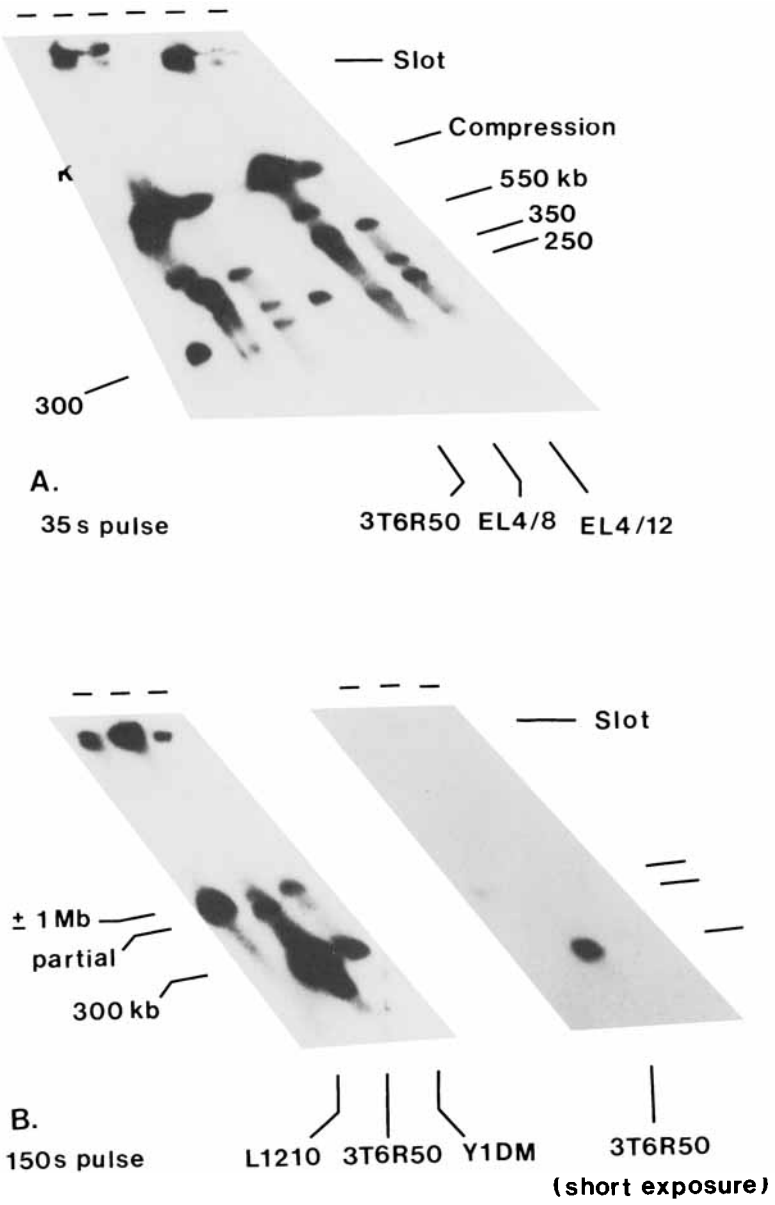

Fig. 3. PFG gel analysis of restriction fragments containing amplified and wild-type DHFR genes. Cells from the lines indicated were embedded in agarose, lysed, and deproteinized and the DNA was cleaved in-gel with endonuclease NotI. The fragments were size-fractionated by PFG gel electrophoresis using pulse times of 35 sec (A) or 150 sec (B). The gels were blotted onto nitrocellulose and hybridized with a probe for the DHFR gene. The figure shows the autoradiograms of this hybridization. In L1210 and Y1-DM the DHFR gene is not amplified; in the other lines it is. The molecular weights indicated were deduced from ladders of lambda DNA oligomers, run in adjacent slots (not shown). **A:** The left three lanes were digested with 10 U NotI for 3 hr at 37°C. The right three lanes were digested with 50 U NotI for 6 hr at 37°C. **B:** The blocks were digested with 50 U NotI for 6 hr at 37°C. The left part was exposed for 5 days at −70°C, the right part was exposed for 1 hr at −70°C.

would then contain an amplicon derived from the allele with the short NotI DHFR fragment. Homogeneity of the amplicons is suggested by the amplification of the intact 300-kb NotI fragment and by the partial digestion product of about 800 kb, visible in Figure 3B. This is the only partial seen in NotI digests of 3T6-R50 DNA. Note that the weak band at 1,000 kb in 3T6-R50 comigrates with the WT allele found in Y1-DM and L1210 DNA. In EL4/8 and EL4/12 DNA the amplified DHFR gene resides on very large NotI fragments (Fig. 3A), which could easily have arisen from the WT long NotI fragment.

We cannot link the polymorphism in the NotI fragments to the observation by Federspiel et al [17] of two alleles of the mouse DHFR gene, which diverge about 50 kb downstream of the gene, because the 3T6 line only contains one of these alleles [17].

We have done many experiments to get more information on the repeats of EL4/12 DNA with only limited success. All enzymes tested, including SfiI, gave fragments < 300 kb that were useless for long-range mapping. Only NaeI gave large fragments, which comigrate with the major fragments obtained with NotI, as shown in Figure 4A,B. It is known from previous work that a GC-rich area with one NotI and two NaeI sites lies directly upstream of the mouse DHFR gene [31]. The simplest interpretation of our results is therefore that the 550-, 350-, and 250-kb fragments actually run from this upstream cluster to the upstream cluster of the DHFR gene in the next repeat. There are various ways in which multiple repeats may have arisen. The simplest model is that the initial amplicon was 550 kb or larger and that shorter repeats were formed during additional rounds of amplification, either by recombination, or deletion.

We have attempted to define the relation between the three major repeats in the amplified DNA by partial digestion analysis. Figure 4 shows examples. At low incubation time the 550-kb band is most prominent. This suggests that the 250- and 350-kb fragments are linked in the amplified DNA and that the expected 600-kb band comigrates with the 550-kb band. This is certainly within the error limits of our size determinations. The larger fragments appearing at shorter digestion times are compatible with a tandem arrangement of 550/350/250-kb fragments in the intact DNA, but the predicted size distribution for the partials expected from other arrangements, eg, runs of 250s, 350s, and 550s, is not sufficiently different from the one observed to exclude these alternatives.

Figure 4 also illustrates two peculiarities in this type of analysis: crooked lanes caused by the field gradient and unusual partials. The partial digests are unusual in that final digestion products appear at the earliest time points and that the relative concentration of partials is never high at any digestion condition. We return to this probelem below.

The Y1 Mouse Adrenocortical Tumour Cell Lines

The Y1 clonal cell line was established in culture in 1966 and two sublines with amplified c-Ki-ras gene are available, the Y1-DM and the Y1-HSR line [see 18–20]. Our results with the Y1-DM line can be summarized in two points: 1) Each restriction endonuclease that cleaves the Y1-DM DNA into large fragments yields a single homogeneous fragment that hybrizidizes with the Ki-ras cDNA probe. The largest fragments are obtained with MluI and NotI, which both give a 750-kb band, showing that the repeat is homogeneous over a large region. The homogeneity over an even

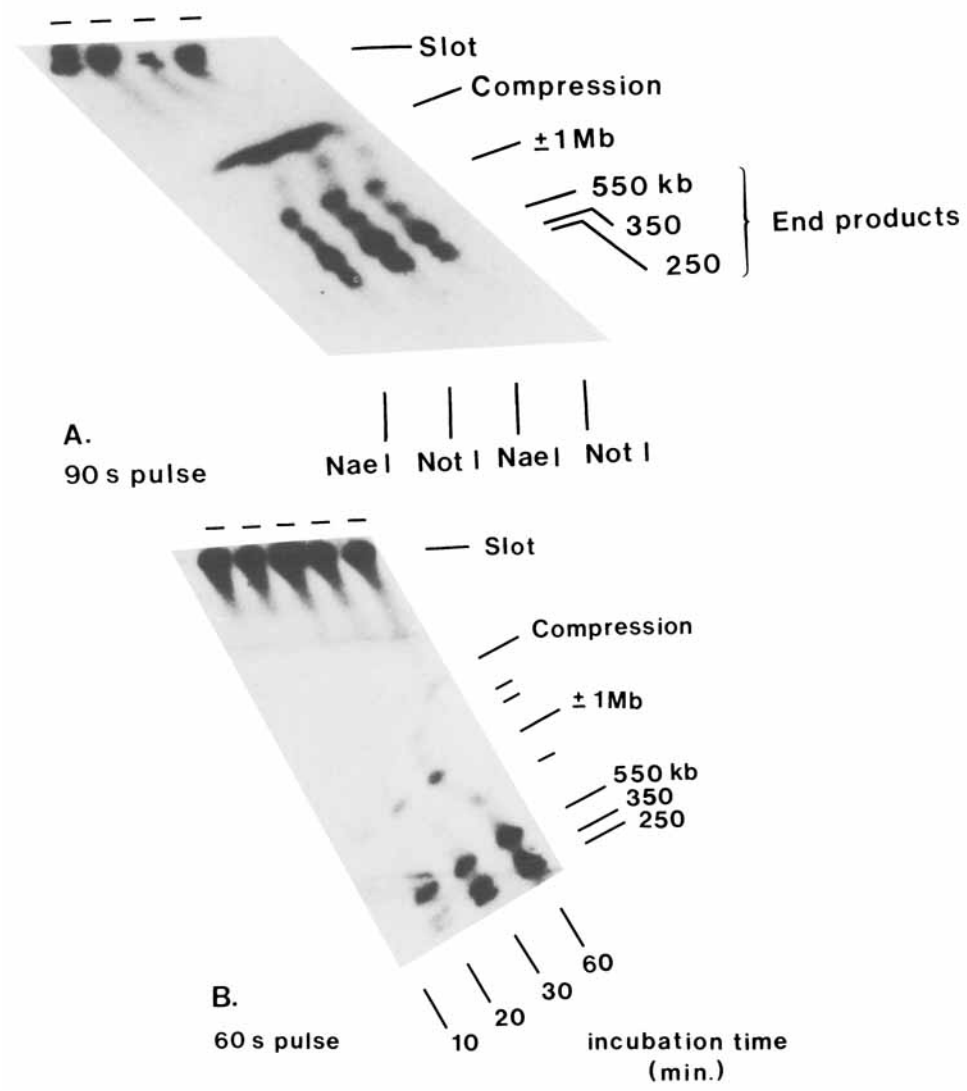

Fig. 4. PFG gel analysis of partial digestion products of EL4/12 DNA cleaved with NaeI or NotI. The experiment was carried out as in Figure 3, but using variable enzyme concentrations or incubation times to generate partial products. In A the pulse time was 90 sec, in B 60 sec. A: From left to right: EL4/12 blocks were digested with 12 U NaeI for 30 min at 0°C; 12 U NotI for 2 hr at 37°C; 12 U NaeI for 60 min at 0°C; 12 U NotI for 4 hr at 37°C. **B:** EL4/12 blocks were digested with 12 U NaeI at 15°C with an incubation time as indicated.

larger segment is indicated by the appearance of defined, partial digestion products, too large to size, at short incubation times. 2) In the ethidium-stained gel of Y1-DM DNA cut with MluI or NotI, DNA bands are visible superimposed on the heterogeneous DNA background. The relative intensity of these bands is proportional to their size; they are specific for Y1-DM DNA; and only one of these bands (the 750-kb band) hybridizes with the Ki-ras probe. We infer that each of these bands is derived from the DM and that the repeat size of the amplified DNA is at least equal to the sum of these bands, ie, $>$ 2,500 kb.

The amplified DNA in the Y1-HSR is more complex and yields multiple nonstoichiometric bands with NotI and MluI. A full account of our work on the Y1-DM and Y1-HSR lines will be published elsewhere.

The CH^RC5 Chinese Hamster Ovary Line

The CH^RC5 line was selected for colchicine resistance by Ling and coworkers and is cross-resistant to a wide variety of other, unrelated drugs. We have isolated DNA probes for five genes that are overexpressed and amplified in the resistant line relative to the sensitive parent and used these probes to construct a map of the amplicon, which is complex and covers at least 1,100 kb in wild-type DNA [15]. Three other multi-drug-resistant hamster lines amplify subsets of these genes that are adjacent in the map [32]. Although we have been unable to construct a precise restriction map of the amplicon, PFG gel analysis has been very useful in this case to establish linkage of genes [15]. In fact, short-range linkage studies may become one of the most useful applications of PFG gel analysis.

Practical Problems in the PFG Analysis of (Amplified) Mammalian DNA

1) PFG analysis is still somewhat of an art. Although improved classical PFG boxes [22] and the orthogonal field alternation gel system [26] give less crooked lanes and more reproducible results than the original PFG system, good results still require more fiddling, feeling, and repeated experiments than standard electrophoresis. In fact, uninterpretable results are easy to get with this method. It is possible that the new system developed by Carle et al [33], will solve these problems.

A major source of problems in PFG gels is the field gradient, which makes adjacent lanes nonequivalent and therefore difficult to compare. According to Schwartz and Cantor [13] the field gradient is essential for stretching large DNA, ie, $>$ 500 kb. Without gradient this DNA tends to stick in the slot. This is also our experience.

2) The number of enzymes available that cleave mammalian DNA into large fragments is still limited. The enzymes that do, have GC-rich recognition sites. These sites are often clustered, because of the presence of GC-rich areas in mammalian DNA. Potentially the usefulness of such enzymes could be further limited by methylation of C residues in recognition sites. This may be less of a problem in the study of amplified DNA, as active genes are usually undermethylated and amplified genes are selected for activity. Shimada and Nienhuis [34] have verified that in the case of DHFR gene amplification all amplified copies are equally undermethylated.

Ingenious methods have been devised to cut DNA into large fragments by combining the use of specific DNA methylases with a restriction enzyme that only cuts methylated DNA [35]. This approach, however, has been applied with only limited success to DNA embedded in agarose [23].

3) An appealing feature of PFG gel electrophoresis is the high resolution over a large range, because mobility is a linear function of the DNA size (rather than of the logarithm of size as in standard electrophoresis). This is why under optimal conditions lambda DNA 20-mers and 21-mers can be completely separated [23]. Remaining drawbacks are the following: a) The occurrence of compression zones at unexpected positions in the gel; these depend on the exact conditions of the run [23]. b) The fact that mobility is not exclusively dependent on size but that DNA sequence makes a minor contribution [23]. The reasons for sequence dependence remain unknown. c) The uncertaintly of sizing above 1,500 kb because of the lack of suitable markers.

The problems in size calibration complicate the interpretation of partial digestion products and the construction of restriction maps.

4) We have had difficulties in getting good series of partial digestion products in our PFG analysis. Usually we get only minor amounts of partials and complete digestion products at the earliest time points, or the lowest enzyme concentrations. It is possible that the enzyme does not readily diffuse into the agarose blocks and hence tends to completely digest whatever DNA it encounters on its slow influx. We have tried to promote partial digestion by digesting at 0°C for long periods, or by partially preventing digestion with drugs that bind to DNA, like actinomycin D. The results have been unsatisfactory. It should be added, however, that we have had no problems in getting partial digestion in agarose blocks of lambda DNA with MluI, of adeno 12 DNA with NotI and of adeno 5 DNA with SfiI. In view of this we cannot rule out that the problems seen with infrequently cutting enzymes are due to site clusters, making the generation of detectable partials unlikely.

DISCUSSION

Our initial results with PFG gels, summarized in Table I, lead to three tentative conclusions:

1) In several cell lines (EL4/8, EL4/12 and Y1-HSR) we find gross heterogeneity in the size of the amplified unit. This is most pronounced when the copy number of amplified genes is high, but it is also seen in the multi-drug-resistant CHO cell line CH^RC5 in which amplification is 30-fold. This confirms the results of standard gel analysis of highly amplified DNA, which has shown multiple joints and increasing heterogeneity of flanking sequences as one moves away from the gene that has been selected for [see 1,2,17,36].

2) The size of the wild-type segment found amplified in some of the cell lines studied is large, up to 2,500 kb in Y1-DM. Although this also confirms previous estimates [see 1,2,37], we note that these estimates were based on data with a considerably larger margin of uncertainty than our results.

3) Some amplified units are homogeneous over distances of at least 750 kb (Y1-DM) or 800 kb (3T6-R50) and they may well be homogeneous over all of the amplicon. This is unexpected in view of the pronounced heterogeneity reported for all amplicons studied in depth [see 2,17,36] with the possible exception of the short DHFR amplicon in Chinese hamster cell line CHOC 400 [38]. Why this discrepancy? We do not think that lack of resolution of the PFG gel system is responsible, because some amplicons yield bands that are nearly as sharp as bands visualized after standard gel electrophoresis. We also do not think that heterogeneity is a side effect of drug selection. It might be argued that selection with MTX or N-(phosphonacetyl)-L-aspartate inhibits DNA synthesis by substrate limitation and would therefore promote formation of short amplicons, if amplification is mainly due to rereplication of partially replicated DNA segments. The results with the 3T6-R50 line show, however, that drug selection does not necessarily lead to heterogeneous amplicons.

We therefore favour the interpretation that the homogeneity or heterogeneity of the amplicon is mainly determined by the degree of amplification, heterogeneity being the result of multiple rounds of amplification imposed by stringent selection. We propose that amplicons start out long and homogeneous and that heterogeneity arises later. If the initial amplicon were 3,000 kb, a 1,000-fold amplification would double

the haploid DNA content of the cell. This would be such an extra load that any deletion that would shorten the amplified unit by removing co-amplified DNA would confer a selective advantage on the cell containing it. The multiple rounds of selection required for high degrees of amplification would obviously provide ample opportunity for deletions to occur. This proposal is compatible with published data and testable.

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REFERENCES

1. Bostock CJ, Tyler-Smith C: Genome Evolution 20:69, 1982.
2. Stark GR, Wahl GM: Annu Rev Biochem 53:447, 1984.
3. Schimke RT: Cell 37:705, 1984.
4. Schmike RT: Cancer Res 44:1735, 1984.
5. Curt GA, Cowan KH, Chabner BA: J Clin Oncol 2:62, 1984.
6. Borst P: Nature 309:580, 1984.
7. Hamlin JL, Milbrandt JD, Heintz NH, Azizkhan JC: Int Rev Cytol 90:31, 1984.
8. Riordan JR, Ling V: Pharmacol Ther 28:51, 1985.
9. Alitalo K: TIBS 10:194, 1985.
10. Schwab M: TIG 1:271, 1985.
11. Sager R, Gadi IK, Stephens L, Grabowy CT: Proc Natl Acad Sci USA 82:7015, 1985.
12. Glanville N: Mol Cell Biol 5:1456, 1985.
13. Schwartz DC, Cantor CH: Cell 37:67, 1984.
14. Van Der Bliek AM, Bernards A, Borst P: J Cell Biochem [Suppl] 9C:32, 1985.
15. Van Der Bliek AM, Van Der Velde-Koerts T, Ling V, Borst P: Mol Cell Biol 6:1671, 1986.
16. Brown PC, Beverley SM, Schimke RT: Mol Cell Biol 1:1077, 1981.
17. Federspiel NA, Beverley SM, Schilling JW, Schimke RT: J Biol Chem 259:9127, 1984.
18. George DL, Francke U: Cytogenet Cell Genet 28:217, 1980.
19. George DL, Scott AF, De Martinville B, Francke U: Nucl Acids Res 12:2731, 1984.
20. George DL, Scott AF, Trusko S, Glick B, Ford E, Dorney DJ: EMBO J 4:1199, 1985.
21. Ling V, Kartner N, Sudo T, Siminovitch L, Riordan JR: Cancer Treat Rep 67:869, 1983.
22. Johnson PJ, Borst P: Gene 43:213, 1986.
23. Bernards A, Kooter JM, Michels PAM, Moberts RMP, Borst P: Gene 42:313, 1986.
24. Simonson CC, Levinson AD: Proc Natl Acad Sci USA 80:2495, 1983.
25. Ellis RW, DeFeo D, Shih TY, Gonda MA, Young HA, Tsuchida N, Lowy DR, Scolnick EM: Nature 292:506, 1981.
26. Carle GF, Olson MV: Nucl Acids Res 12:5647, 1984.
27. Melgar E, Goldthwait DA: J Biol Chem 213:4409, 1968.
28. Hamkalo BA, Farnham PJ, Johnston R, Schimke RT: Proc Natl Acad Sci USA 82:1126, 1985.
29. Setlow P: In Fasman GD (ed): "Handbook of Biochemisty and Molecular Biology." Cleveland, Ohio: CRC Press, 1976, pp 312–318.
30. Razin A, Cedar H: Int Rev Cytol 92:159, 1984.

31. Crouse GF, Simonson CC, McEwan RN, Schimke RT: J Biol Chem 257:7887, 1982.
32. De Bruyn MHL, Van Der Bliek AM, Biedler JL, Borst P: Mol Cell Biol 6:4717, 1986.
33. Carle GF, Frank M, Olson MV: Science 232:65, 1986.
34. Shimada T, Nienhuis AW: J Biol Chem 260:2468, 1986.
35. McClelland M, Kessler LG, Bittner M: Proc Natl Acad Sci USA 81:983, 1984.
36. Roberts JM, Buck LB, Axel R: Cell 33:53, 1983.
37. Cowell JK: Annu Rev Genet 16:21, 1982.
38. Montoya-Zavala M, Hamlin JL: Mol Cell Biol 5:619, 1985.