RESTRICTION MAP OF GENETIC TRANSFER FACTOR pAP42

R. Perera, E. V. Shcherbakova
and A. P. Pekhov

UDC 575.133:577.212.3+579.252.52

KEY WORDS: plasmid; restriction endonucleases; genetic mapping.

Genetic transfer factor pAP42 is a F-like plasmid of average molecular size and belonging to the FIX incompatibility group [1]. Since this plasmid contains many recognition sites for restriction endonucleases, this complicates the structure of its restriction map. Yet such a map is essential for the further study of the physical and genetic organization of this plasmid.

The aim of the present investigation was automation of mapping of the sites of plasmid pAP42 for restriction endonucleases EcoRI, HindIII, and SalI in accordance with an algorithm designed for cases when the plasmid DNA contains many recognition sites for the restriction endonucleases used.

EXPERIMENTAL METHOD

DNA of plasmid pAP42 was isolated by the method in [2] with some modifications, using lysates of *E. coli* AP115 cells containing this plasmid, clarified with Triton X-100, and centrifuged in a CsCl density gradient. Restriction of plasmid DNA was carried out with restriction endonucleases EcoRI, HindIII, and SalI, added separately (single restrictions) or in pairs to the incubation mixtures.

The restriction fragments were fractionated (separated) in 0.65% agarose gel by horizontal slab electrophoresis.

The size of the restriction fragments of DNA of plasmid pAP42 was determined by the use of restriction fragments of DNA of phage λ as molecular weight standards [3].

The program for finding the optimal dependence of molecular weights of the fragments on the length of their tracks in agarose gel was written in BASIC language and the program of mapping on the basis of the calculated molecular weights was written in PASCAL language. Both programs were read on a CM-4 computer.

EXPERIMENTAL RESULTS

The work began with restriction of DNA of plasmid pAP42 by restriction endonucleases EcoRI, HindIII, and SalI separately and in pairs. Electrophoresis of DNA of plasmid pAP42, treated wth different restriction endonucleases, is illustrated in Fig. 1. To determine the size of the restriction fragments, the piecewise-exponential dependence of molecular weight on the coordinate (length of track) m (x), which best (in the sense of least discrepancy) approximated the experimental data, was found. In this case the relationship has the form:

$$m(x)=\begin{cases}\exp((90-x)/19{,}6), & x\leqslant 43,\\ \exp((91-x)/20), & 62\geqslant x\geqslant 43,\\ \exp((120-x)/40), & x\geqslant 62.\end{cases}$$

The molecular weights of the restriction fragments of plasmid pAP42 obtained by the action of each of the enzymes EcoRI, HindIII, and SalI (single restriction), calculated by the formula, are given in Table 1. Table 1 also gives the results of determination of molecular

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 61-63, July, 1985. Original article submitted November 1, 1984.

0007-4888/85/0007-0909$09.50

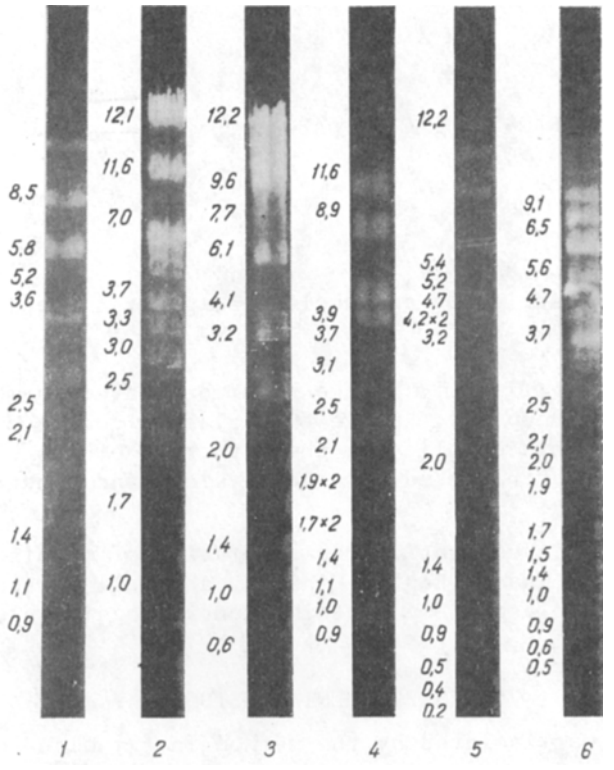

Fig. 1. Electrophoresis of single and double restriction fragments of DNA of plasmid pAP42. Tracks from left to right: 1) EcoRI, 2) HindIII, 3) SalI, 4) EcoRI + HindIII, 5) EcoRI + SalI, 6) HindIII + SalI.

TABLE 1. Molecular Weights of Restriction Fragments of Plasmid pAP42

Restriction enzymes	Restriction fragments and molecular weights, megadaltons																
	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	f11	f12	f13	f14	f15	f16	f17
EcoR1	18.6	8.9	5.8	5.2	3.6	2.5	1.4	1.1	0.9								
HindIII	12.1	11.6	7.0	3.7	3.3	3.0	2.5	2.1	1.7	1.0							
SalI	12.2	9.6	7.7	6.1	4.2	3.2	2.0	1.4	1.0	0.6							
EcoR1+HindIII	11.6	8.9	3.9	3.7	3.1	2.5	2.1	1.9	1.9	1.7	1.7	1.4	1.1	1.0	0.9		
EcoR1+SalI	12.2	5.4	5.2	4.7	4.2	4.2	3.2	2.0	1.6	1.4	1.0	0.9	0.9	0.5	0.4	0.2	
HindIII+SalI	9.1	6.5	5.6	4.7	3.7	2.5	2.3	2.1	2.0	1.9	1.7	1.5	1.4	1.0	0.9	0.6	0.5

weights of DNA fragments of plasmid pAP42, formed by restriction by pairs of enzymes EcoRI + HindIII, EcoRI + SalI, and HindIII + SalI (double restriction).

As Table 1 shows, the number of double restriction fragments was on average 1.5 times greater than the number of fragments obtained as a result of restriction by one enzyme.

The process of mapping was automated (programmed) in accordance with the following algorithm. Any plasmid restriction enzyme obtained by single restriction with plasmid DNA, enzyme I for example, must have a molecular weight equal to the molecular weight of the plasmid fragment obtained by double restriction, by enzymes I and II for example (provided that the chosen fragment does not contain one or more recognition sites of enzyme II), or to the sum of the molecular weights of 2, 3, 4, or more double restriction fragments.

For example, the restriction fragment obtained as a result of restriction of DNA of the test plasmid pAP42 by enzyme EcoRI, with a molecular weight of 18.6 megadaltons, may be represented by fragments formed by double restriction (by two enzymes), viz.:

[18.6]EcoR1+ [5.4+ 12.2+ 1.0]EcoR1+ SalI
[18.6]EcoR1+ [1.7+ 11.6+ 3.7+ 1.0]EcoR1+ Hind III.

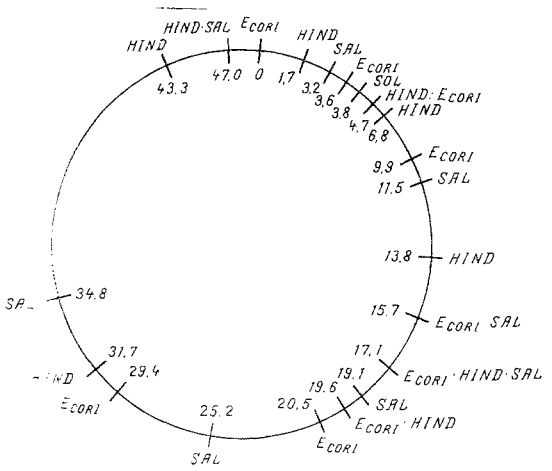

Fig. 2. Restriction map of plasmid pAP42. Sites oriented relative to single site of EcoRI (0), distances from which are given in megadaltons.

This condition, together with equality of the sums of the molecular weights of the fragments obtained by restriction by each enzyme separately, to the molecular weight of the whole plasmid DNA molecule, is essential (but not sufficient) for construction of the restriction map. Meanwhile it may be found that the molecular weights of some fragments obtained by single restriction are the sum of the molecular weights of the different fragments formed by double restriction. In this case, further realization of the algorithm branches into several different versions of construction of the map. In each of these versions of mapping, the sum total of all the fragments must be such that the molecular weights of the fragments obtained by single restriction were the sum of the molecular weights of fragments formed by double restriction. Further mapping was done for each of the possible sets of data consecutively.

Mapping began with choice of the fragment obtained as a result of single restriction, and consisting of the largest number of fragments formed by double restriction. For example, the EcoRI fragment measuring 18.6 megadaltons (MD) was equal to the sum of the fragments 1.7 + 11.6 + 3.7 + 1.0 MD obtained by double restriction by enzymes EcoRI and HindIII. Each of these fragments is contained in the following HindIII fragments (respectively): Fragments measuring 11.6, 3.7, and 1.0 MD are at the same time HindIII fragments, whereas the 1.7 MD fragment is contained in the HindIII fragment measuring 12.1 MD (12.1 = 0.9 + 8.9 + 1.7). The HindIII fragment measuring 12.1 MD, the components of which are at the same time EcoR1 fragments (measuring 0.9 and 8.9 MD), is decoded in the same way.

If as a result of these actions part of the map was found to be "finished," i.e., to have recognition sites for two enzymes at its ends, mapping continued thereafter with the choice of any unused fragments. Similar procedures were carried out based on the results of restriction of plasmid pAP42 DNA by enzymes EcoRI, SalI, EcoR1 + SalI and HindIII, SalI, HindIII + SalI.

In conclusion, the sites of completed parts of the map were determined by consecutive reassembly, as a result of which the desired map is a map coinciding with each of the three maps already obtained, with an accuracy equal to that of permutations of fragments whose ends are recognition sites for two enzymes in combinations EcoR1 + HindIII, EcoR1 + SalI or HindIII + SalI.

The map of plasmid pAP42, constructed by the algorithm described above (Fig. 2), shows that recognition sites of the restriction endonucleases are arranged relatively uniformly in a region measuring 35.8 MD, and their highest density is observed in a measuring 5.7 MD.

The rest of the map of plasmid pAP42, measuring 12.2 MD, contained only one recognition site of HindIII. The nature of this unouniform distribution of restriction sites along the genome of this plasmid requires special investigation.

The map given above can be regarded as a trial map, requiring further clarification. In particular, two fragments of the map (17.1 and 30.9 MD) have recognition sites of all three

restriction endonucleases used at their ends. The precise location of these fragments one relative to the other can therefore be determined once the recognition sites of another (the 4th) restriction endonuclease have been mapped.

LITERATURE CITED

1. E. V. Gubar' and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 4, 85 (1982).
2. R. B. Meagher, R. C. Tait, M. Betlach, and H. B. Boyer, Cell, 10, 521 (1977).
3. M. Thomas, and R. W. Davis, J. Mol. Biol., 91, 315 (1975).

EXPERIMENTAL STUDY OF THE ANTIMUTAGENIC PROPERTIES OF 5-METHYLRESORCINOL

Yu. V. Pashin, L. M. Bakhitova, and T. I. Bentkhen

UDC 575.224.22:612.6.052:665.44

KEY WORDS: benz(a)pyrene; γ-rays; 5-methylresorcinol.

Metabolic activation of premutagens and precarcinogens has been shown to be connected with free radical formation. Among the inhibitors of free-radical processes the phenolic antioxidants are definitely interesting [3]. On the assumption that the antioxidative ability of phenolic compounds may determined their efficacy as antimutagens it was decided to study the effect of simple phenols on the induction of gene mutations in mammals *in vitro* [5]. The most effective compound proved to be 5-methylresorcinol (5-MR).

The antimutagenic activity of 5-MR during the action of benz(a)pyrene (BP), a typical environmental pollutant, and also of γ-rays, as a result of whose action products of free-radical oxidation of hydroperoxides accumulate, was studied *in vivo*.

EXPERIMENTAL METHOD

The action of 5-MR on the mutagenic activity of BP was studied *in vitro* by counting the number of induced direct gene mutations affecting the hypoxanthineguanine phosphoribosyltransferase (HGPRT) locus in cultures of somatic cells from V-79 Chinese hamsters by the method in [7] under conditions of metabolic activation by mouse liver microsomes [4].

BP (from Fluka, Switzerland) was used in a constant concentration of 0.04 mM, inducing a mutagenic effect, and 5-MR (from Merck, West Germany) in concentrations of 0.35 to 3.47 mM. Ethanol in a volume of 0.1 ml was used as the solvent. The toxic effects and mutagenic activity of BP and 5-MR, also used in the form of mixtures, also were investigated.

In an *in vivo* system mutagenic activity of BP and γ-rays was assessed, using as the criterion induction of micronuclei in polychromatophilic erythrocytes (reticulocytes) in adult (CBA × C57BL/6j)F_1 moust bone marrow. The presence of micronuclei in reticulocytes, which are the last precursor cells of mature erythrocytes to divide, reflects damage to chromosomes at the erythroblast stage. To determine the optimal time for recording the frequency of induction of micronuclei samples of bone marrow were taken from the animals 1, 2, 3, and 4 days after a single intraperitoneal injection of the chemicals or after irradiation. BP was injected in sunflower oil in a dose of one-third of the minimal lethal dose for mice [10]. A solution of 5-MR in water was injected in a dose of 0.1 LD_{50}, in the ratio of 1:0.5 (w/w) with BP. Films were stained by the method in [6], using the modification in [9], with fixation in methanol, washing in bidistilled water, and staining in 7% Giemsa solution at pH 6.8.

The animals were irradiated on the GUPOS apparatus with ^{137}Cs source of γ-rays (dose rate 470 R/min) at 0°C, with a total dose of 75 or 150 rads.

N. I. Vavilov Institute of General Genetics, Academy of Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 63-65, July, 1985. Original article submitted October 30, 1984.

0007-4888/85/0007-0912$09.50