HeLa CELLS LACK *recA* GENE

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1. Introduction

We have been interested in the mechanism of genetic recombination. A protein coded by the *recA* gene of *Escherichia coli* has been found to facilitate the formation of recombination intermediates; roles of this protein in genetic recombination and repair have been discussed [1–3]. In view of the significant role that the *recA* protein seems to play in genetic recombination, we decided to examine the presence of a gene coding such a protein *(recA)* in eukaryotes using HeLa cells. Radioactively labelled, cloned *E. coli recA* DNA sequence was used as a probe to detect the presence of possible homologous nucleotide sequences in HeLa cell DNA after restriction endonuclease digestion and Southern transfer [4]. *E. coli* chromosomal and hybrid plasmid (pBR322 DNA containing *recA* gene) were used as controls in such experiments. The basis for our experiment lies in the fact that a heterologous DNA probe can be used to detect the presence of a gene in evolutionarily divergent groups of organisms and that cloned *E. coli recA* gene is now available [3] which can be used as probe in such experiments. Heterologous DNA probes have been used to detect the presence of nitrogen fixation genes in a wide variety of organisms capable of nitrogen fixation [5,6]. Also, heterologous DNA sequences have been used to detect the presence of specific genes such as histones, actin and others in a wide variety of eukaryotes [7,8].

2. Materials and methods

Escherichia coli strains (C600) with or without the recombinant plasmid (pDR1453) containing *E. coli recA* gene have been described in [3] and were kindly provided by Dr H. Hoffman-Berling. Hybrid plasmid DNA pDR1453, a pBR322 plasmid containing *recA* gene of *E. coli* [3], was prepared by the standard procedures [9]. *E. coli* and HeLa chromosomal DNA were separately prepared by modification of methods in [10]. All DNA preparations were further purified by banding on CsCl ethidium bromide gradient. DNA from *E. coli* and HeLa cell were separately digested to completion by restriction endonucleases (*Eco*RI and *Bam*H1) following the protocol provided by the supplier (Boehringer-Mannheim). ^{32}P-Labelled hybrid plasmid DNA was prepared by nick translation [11]. Since a human cDNA or mRNA is expected to hybridize with the restriction fragments of HeLa cell DNA, therefore the ^{32}P-labelled human myokinase cDNA was used as a control probe in some of the experiments. The ^{32}P-labelled myokinase cDNA was synthesized in vitro [12] by the enzyme reverse transcriptase using the human myokinase mRNA [13].

DNA (3–6 μg) was electrophoresed on agarose (1%) gel. The gels were then stained in ethidium bromide (0.5 μg/ml) and photographed under UV light. They were denatured in 0.2 M NaOH, 10.6 M NaCl for 1.5 h and neutralized in 1 M Tris–HCl (pH 7.5), then DNA was transferred to Millipore HAWP filters with 20 × SSC by the Southern technique [4]. After overnight transfer, the filters were briefly rinsed in 2 × SSC, baked at 80°C for 80 min in a vacuum oven and then prehybridized at 65°C for 12 h in 250 ml of 6 × SSC containing 0.5% SDS and denatured calf thymus DNA (15 μg/ml). The filters were then transferred to 16 ml fresh 2 × SSC containing ~2 × 10^7 cpm ^{32}P-labelled DNA and the bound DNA was hybridized at 65°C with shaking for 24 h. The filter was then rinsed overnight at 65°C in ⩾3 changes of 6 × SSC/0.5% SDS and finally rinsed 3 times for 1 h each in 200 ml 2 × SSC at room temperature. The dried filter was then autoradiographed

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by standard procedure and the films were developed after 36–46 h.

3. Results and discussion

Our strategy to identify the DNA fragment containing sequences homologous to *recA* gene was to use plasmids carrying defined segments of *E. coli recA* gene. *E. coli* DNA and HeLa cell DNA were digested with 2 different restriction enzymes and the DNA fragments were electrophoresed on an agarose gel and then hybridized to ^{32}P-labelled probe (plasmid pBR322 containing the *recA* gene) DNA. In such an experiment, plasmid DNA (containing *E. coli recA* gene) and *E. coli* DNA were used as controls. The plasmid DNA was similarly hybridized to ^{32}P-labelled probe DNA after electrophoresis and Southern transfer. Fig.1 shows the electrophoretic pattern of plasmid DNA (without digestion with restriction enzyme) and *E. coli* and HeLa cell DNA (the latter 2 after restriction enzyme digestion).

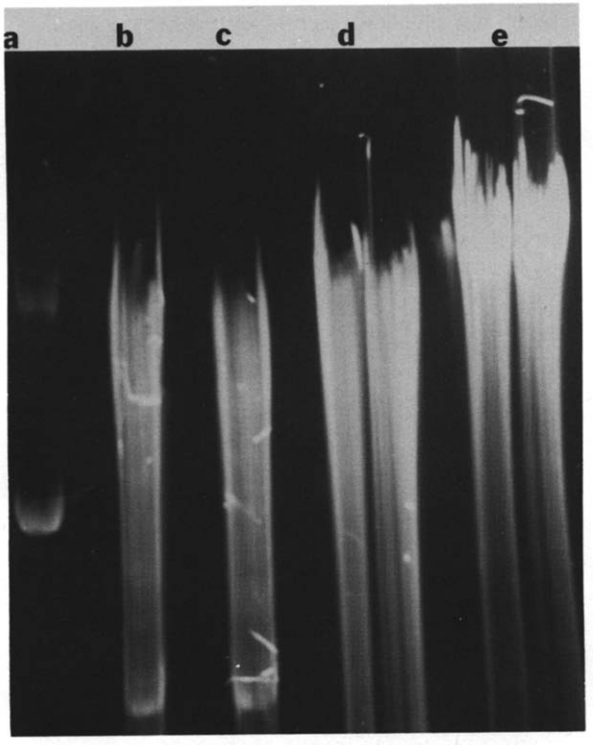

Fig.1. Agarose gel electrophoresis of DNA: hybrid plasmid (pBR322 with *recA* gene) (a); *E. coli* DNA after digestion with *Eco*R1 (b); and *Bam*Hl (c); HeLa DNA after digestion with *Eco*R1 (d); and *Bam*H1 (e).

Fig.2 shows the hybridization of DNA (seen in fig.1) to ^{32}P-labelled probe DNA (containing *recA* gene). As can be seen in fig.2, the probe DNA was able to hybridize with plasmid DNA as well as with *E. coli* DNA (fig.2a,b,c). However, the probe DNA failed to hybridize with HeLa DNA fragments (fig.2d,e). In several repeat experiments, exactly the same hybridization pattern was seen (i.e., positive hybridization with plasmid and *E. coli* DNA but never with HeLa DNA). These data clearly indicated the lack of homology between HeLa DNA and probe DNA.

In separate experiments *E. coli* DNA (100–1000 pg) showed a quantitative hybridization with the ^{32}P-labelled probe DNA (i.e., hybrid plasmid containing *recA* gene). Similar positive hybridization was also seen when *E. coli* DNA (1000 pg) was mixed with HeLa DNA (3 μg) and then examined for hybridization with ^{32}P-labelled probe DNA (i.e., hybrid plasmid containing *recA* gene); in this case the hybridization was due to interaction between the probe DNA and the *E. coli* DNA. These data are summarized in table 1. It is known that human genome contains ⩾1000-fold

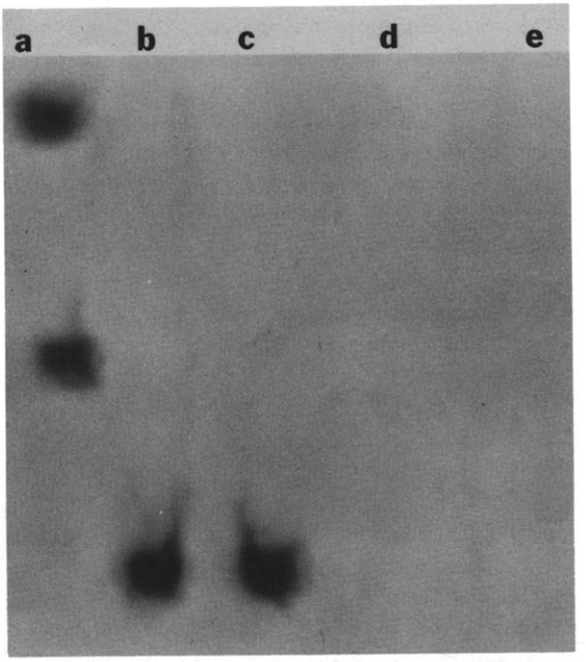

Fig.2. Autoradiograph of the DNA in fig.1 after Southern hybridization with the probe (^{32}P-labelled hybrid plasmid DNA containing *recA* gene): hybridization seen with plasmid DNA (a) and with *E. coli* DNA (b,c); no hybridization seen with HeLa DNA (d,e).

Table 1
Summary of data from hybridization of *E. coli* and HeLa DNA(s) with ^{32}P-labelled *recA* gene probe on a Southern blott [4]

DNA source	Genomic DNA content (pg)[a]	Southern hybridization		Results	Detectable *recA* gene equivalents (est.)[b]
		DNA used (pg)	Genomic copies present (no.)		
1. *E. coli*	0.004	1×10^2	(25×10^3)	+	0.1
		1×10^3	(25×10^4)	+	1.0
		3×10^6	(75×10^6)	+	3×10^3
2. HeLa	3.2	3×10^6	(9.4×10^5)	–	3.76
		6×10^6	(18.8×10^5)	–	7.52

[a] Values taken from [14]
[b] The number of *recA* gene equivalent is calculated on the assumption that at least one *recA* gene was detectable in the 1000 pg *E. coli* DNA under these experimental conditions

more DNA than the genome of *E. coli* [14]; therefore, the use of HeLa and *E. coli* DNA(s) in a ratio of 3000:1 is consistent with the magnitude of their genome size complexities (see table 1). Furthermore, the minimum amount of HeLa DNA (3 μg) used (here to detect a *recA* like gene by a heterologous probe) can be estimated to contain ⩾4–40 *recA* gene equivalents, if present in HeLa cells. This estimation is based on the assumption that the amounts (100–1000 pg) of *E. coli* DNA used represent the lowest limit of the detectability of the *recA* gene by the radioactive probe under these experimental conditions (section 2). In another experiment, the ^{32}P-labelled cDNA of human origin (such as human myokinase cDNA) when used as a probe showed positive hybridization with HeLa DNA on a Southern transfer. These results suggested that DNA probes were able to interact with the appropriate complementary DNA sequences under the experimental conditions used.

It can be concluded from these data that HeLa cell DNA does not contain a gene homologous to *recA* gene of *E. coli*. This lack of homology may be due to either the absence of *recA* gene in higher eukaryotes or due to divergence in DNA sequences. The fact that all of our controls yielded expected results and that the use of heterologous DNA probe in identifying homologous DNA sequences in evolutionary divergent organisms have been demonstrated [4–7] supports our conclusion regarding the lack of *recA*-like gene in HeLa cell, a eukaryote.

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References

[1] Shibata, T., Dasgupta, C., Cunningham, R. P. and Radding, C. M. (1980) Proc. Natl. Acad. Sci. USA 77, 2606–2609.
[2] McEntee, K., Weinstock, G. M. and Lehmann, I. (1980) Proc. Natl. Acad. Sci. USA 77, 857–860.
[3] McEntee, K. and Epstein, W. (1977) Virology 77, 306–318.
[4] Southern, E. M. (1975) J. Mol. Biol. 98, 503–517.
[5] Mazur, B. J., Rice, D. and Haselkorn, R. (1980) Proc. Natl. Acad. Sci. USA 77, 186–190.
[6] Ruvkun, G. B. and Ausubel, F. M. (1980) Proc. Natl. Acad. Sci. USA 77, 191–195.
[7] Pardue, M. L., Kedes, L. H., Weinberg, E. S. and Birnstiel, M. C. (1977) Chromosoma 63, 135–151.
[8] Gallwitz, D. and Seidel, R. (1980) Nucleic Acid Res. 8, 1043–1059.
[9] Clewell, D. B. and Helinski, D. R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159–1163.
[10] Marmur, J. (1961) Mol. Biol. 3, 208–218.
[11] Maniatis, T., Jeffrey, A. and Kleid, D. (1975) Proc. Natl. Acad. Sci. USA 72, 1184–1188.
[12] Retzel, E. F., Collett, M. S. and Faras, A. J. (1980) Biochem. 19, 513–518.
[13] Mishra, N. C., Schmeidel, U., Rahr, S., Schrimer, R. H. and Vosberg, H. P. (1981) Genetics 97, 574.
[14] Strickberger, M. W. (1976) in: Genetics, 2nd edn, p. 54, MacMillan, New York.