Dissimilarity between Human and Bacterial DNA

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사람의 DNA와 박테리아의 DNA 사이의 非類似性

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摘 要

DNA-agar 법에 의한 hybridization에 의하여 사람의 DNA와 박테리아의 DNA 사이의 유사점을 찾아보고자 시도하였다. HeLa DNA를 agar에 고착시키고, *Xanthomonas pelargonii*의 ^{14}C-DNA를 절단하여 용액상태로 이용하였다.

사람의 DNA와 박테리아의 DNA 사이에는 그 유사성을 발견할 수 없었다. 만일 두 DNA 사이에 유사성이 존재한다고 하더라도 인간의 染色體의 0.01% 미만 밖에 안될 것으로 본다. 이것은 한 cistron이 포함하는 鹽基双의 수를 1,000이라 가정한다면, 2×10^5의 염기쌍, 즉 200의 박테리아의 cistron이 사람의 DNA에 보존되어 있는 셈이 된다.

INTRODUCTION

The evolutionary origins of human DNA are expressed in the fact that some 20 per cent of it is identical with the DNA of other mammals, 8 per cent with that of birds and 5 per cent with that of fish (Bolton *et al.*, 1964; Hoyer *et al.*, 1964). A search for possible remnants of the bacterial genome in human chromosomal DNA by DNA-hybridization is complicated by the greatly unequal size of their genome; the human genome contains about 3.5×10^9 nucleotide pairs, whereas the bacterial genome, for example, that of *Escherichia coli*, has only some 5×10^6 nucleotide pairs. If any similarity exists it will therefore involve less than 0.2 per cent of the human genome. If carbon-14 labeled human DNA is used with ordinary bacterial DNA in agar, such low values are below the sensitivity of the DNA-agar column method as was employed by McCarthy and Bolton (1964). In these conditions no similarity has been found between labeled DNA from HeLa cells and *E. coli*-DNA (Hoyer *et al.*, 1964).

This approach can be refined by (1) reversing the situation and labeling the DNA of the smaller genome (the bacterial DNA); (2) saturating any homologous sites which may exist on the human DNA by using a ten-fold or twenty-fold excess of labeled fragments of bacterial DNA; (3) using as much human DNA as possible on the agar, if possible in the milligram range; (4) measuring complete homology with normal bacterial DNA of the same strain in amounts equimolar with those of the human DNA (and thus 600 times lighter than the human DNA); and (5) determining the absorption of the labelled DNA on agar alone.

MATERIALS AND METHODS

For the preparation of ^{14}C-DNA from *Xanthomonas pelargonii*, the cells were grown for 3 days at 25°C in a medium containing ^{14}C-uracil, of which the composition was 1% peptone(Difco), 1% beef extract, 1.5% sodium chloride, and 2.5% agar. This medium was selected for low production of polysaccharides. DNA was prepared according to Marmur (1961). Ordinary HeLa DNA was

obtained through the courtesy of Department of Virology, Faculty of Medicine, State University, Gent, Belgium. Donated HeLa DNA was repurified by removing the proteins and RNA through the repeated precipitation of DNA with ethyl alcohol and ribonuclease treatment.

DNA-agar gels from the HeLa DNA was prepared by the method of Bolton and McCarthy (1962). The hot solution of sheared, single-stranded DNA was mixed with a hot solution of agar and the mixture was chilled quickly in ice-water. The resulting slab of DNA-agar gels was forced through a screen and then reduced to granules. The agar-gels particles were thoroughly suspended in 2 ×SSC buffer (1×SSC buffer, 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0).

We used pure sheared denatured ^{14}C-DNA from *Xanthomonas pelargonii* (specific activity 640 cpm/μg) prepared as described above. One gram of moist agar-gel contained about 600 μg HeLa DNA. In a series of experiments 10 -114 μg of bacterial ^{14}C-DNA was incubated with an eighteen-to thirty-fold excess (by weight) of HeLa DNA embedded in agar corresponding to a molar ratio bacterial DNA of 12-20:1. Hybridization and DNA-column elution was carried out according to the method of McCarthy and Bolton (1964). A variety of concomitant controls with HeLa-free agar in the same conditions were carried out.

RESULTS AND DISCUSSION

The results of several experiments are summarized in Table 1. In both sets (with and without HeLa DNA) of experiments the amount of ^{14}C-DNA absorbed ranged between 150-340 cpm out of the 6,400-73,000 cpm added; no significant differences existed between the ^{14}C-DNA bound to HeLa DNA agar and the amount absorbed to the agar alone. Control experiments in which normal denatured bacterial DNA from the same strain were substituted in equimolar amounts for the HeLa DNA (5-10 per cent of the ^{14}C-DNA), the hybridization ranged from 750 to 1,280 cpm. Although the hybridizatien experiments by the method of DNA-agar are useful for the estimation of DNA homology amongst organisms, its sensitivity is not sufficiently high to detect the DNA homology of less than 5 per cent.

No homology between human and bacterial DNA was detected. If homology exists at all, it can be estimated from the sensitivity of the method and assuming some 1,000 nucleotide pairs per cistron, that not more than 2×10^5 base pairs or 200 bacterial cistrons would be preserved in human DNA, corresponding to less than 0.01 per cent of the total human genome.

Table 1. *Xanthomonas pelargonii* ^{14}C-DNA bound to HeLa-DNA. 3.65 g of HeLa DNA-agar containing 1,990 μg was incubated with 114 μg of *X. pelargonii* ^{14}C-DNA for 16 hours at 60°C. The values are the average from four experiments.

DNA in agar		% ^{14}C-DNA fragments bound
HeLa		2.6±1.4
Control	*X. pelargonii*	2.4±2.2
	agar alone	2.4±1.7

SUMMARY

An attempt was made to estimate the possible homology between human and bacterial DNA by the method of DNA-agar gel hybridization. HeLa DNA was embedded in the agar and ^{14}C-DNA *Xanthomonas pelargonii* was used as bacterial DNA for the sheared fragments.

No homology between human and bacterial DNA was detected. If homology exists at all, it can be estimated from the sensitivity of the method and assuming some 1,000 nucleotide pairs per cistron, that not more than 2×10^5 base pairs or 200 bacterial cistrons would be preserved in human DNA, corresponding to less than 0.01 per cent of the total human genome.

REFERENCES

Bolton, E.T., R.J. Britten, T.J. Byers, D.B. Cowie, B. Hoyer, Y. Kato, B.J. McCarthy, M. Miranda, and R.B. Roberts, 1964. *Carnegie Inst. Ann. Rep.* 366.

Bolton, E.T. and B.J. McCarthy, 1962. *Proc. Nat. Acad. Sci., U.S.A.* **48**, 1390.

Hoyer, B.H., B.J. McCarthy, and E.T. Bolton, 1964. *Science* **144**, 959.

Marmur, J., 1961. *J. Mol. Biol.* **3**, 208.

McCarthy, B.J. and E.T. Bolton, 1964. *Proc. Nat. Acad. Sci., U.S.A.* **50**, 156.