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Cloning of Y derived DNA sequences of bovine origin in *Escherichia coli*

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Abstract. We have constructed a partial library of Y chromosome derived DNA sequences of bovine origin in *Escherichia coli.* That, the recombinants arc Y derived and Y specific was ascertained by differential colony hybridization using male and female DNA probes. Out of 1000 recombinants analysed, 17 were found to be Y derived as well as Y specific and were of repetitive nature. Restriction analysis revealed that most of them had short DNA inserts.

Keywords. Cloning; repetitive DNA; bovine Y chromosome; hybridization.

Introduction

The sexually differentiated state in mammals is determined by ′Y' chromosome and it appears that sex determining genes of Y chromosome behave as dominant genetic trait. Role of histocompatibility Y antigen in male differentiation is envisioned although its precise function is not yet clear (Silver and Wachtel, 1977; Wachtel, 1977; McCarey and Abbot, 1979). The first step towards analysing Y encoded genes involved in sexual differentiation would be to isolate and clone the chromosomal DNA. A variety of approaches have been used for cloning Y encoded sequences of human, murine and reptile origin (Kunkel *et al.,* 1976; Cooke, 1976; Jones and Singh, 1981; Lamer and Palmer, 1984). Repetitive as well as single copy DNA sequences specific to human male DNA have been identified (Kunkel *et al.,* 1976; Cooke, 1976; Bishop *et al.,* 1983) and their localization on Y chromosome has been assigned. Y derived sequences of human origin have provided powerful analytical tools to probe directly the structure of Y chromosome (Cooke and Mackay, 1978; Kunkel, *et al.,* 1979; Cook, 1982; Bostock *et al.,* 1978) and also have been used for fetal sex determination (Gosden *et al.,* 1982; Gosden *et al.,* 1984). Relatively little information is available on Y specific determinants in cattle. Cross reactivity of human Y sequences with cattle has not been reported. In fact the unique sequences of human origin, when tested, did not hybridize with cattle DNA (J. Weissenbach, unpublished results). In this communication we report the construction of a partial library of bovine Y derived sequences.

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Materials and methods

All restriction endonucleases, T_4 DNA ligase, bacterial alkaline phosphatase, DNA polymerase and DNase I were from Bethesda Research Laboratory, Gaithersburg, Maryland, USA. Protease and Ribonuclease A were from Sigma Chemical Corporation, USA. Ampicillin, tetracycline and chloramphenicol were bought from Sigma Chemical Co., St. Louis, Missouri, USA. [α-^{32}P]-dCTP and [α-^{32}P]-dTTP (800 Ci/mmol) were from new England Nuclear.

Isolation of DNA

DNA was isolated from bovine leucocytes according to method described (Vergnaud *et al.,* 1984). For preparation of donor (hybrid) DNA, Sau III A digested male DNA (5 μg) was hybridized in phosphate buffer with 500 μg of sheared female DNA (average size, 500 bp) to a Cot value of 1000 followed by fractionation by chromotography on hydroxylapatite as described (Eden *et al.,* 1978). Plasmid DNAs were prepared as described by Clewell and Helinski (1972).

Construction of recombinant plasmids carrying Y derived sequences

Male-female hybrid DNA was cloned into *Escherichia coli,* strain HB 101 using *Bam* H1 site in plasmid pBR 322 (Mandel and Higa, 1970). Recombinants scored on the basis of ampicillin resistance and tetracycline sensitivity were further analysed by differential colony hybridization technique.

Radiolabelling of bovine DNAs

DNA from male and female cattle (2 μg) was radiolabelled with [α- ^{32}P] -dCTP, [α-^{32}P] -dTTP by nick translation procedure (Rigby *et al.,* 1977). On an average, specific activity obtained was 1–2 × 10^8 cpm/μ g of DNA.

Colony hybridization

Recombinant colonies grown on nitrocellulose membrane filters were lysed by treatment with 0·5 M NaOH and colony hybridization was done essentially as described by Maniatis *et al.* (1982).

Results and discussions

Although Y chromosome specific repetitive DNA sequences of human origin were identified by analysis of male-female DNA restriction digests on agarose gel (Cooke and Mackay, 1978), this was not found to be useful for cattle DNA. Comparison of male DNA restriction digests with female DNA restriction digests for restriction endonucleases *E*coRI, *Hae*III and *Hind*III did not reveal any specific bands on male DNA digest (figure 1). Instead, strong bands of varying intensity were visible over background smear, migrating with same mobility in male as well as female DNA digests. This may suggest that organization of Y derived repetitive sequences for cattle may not be the same as that of human.

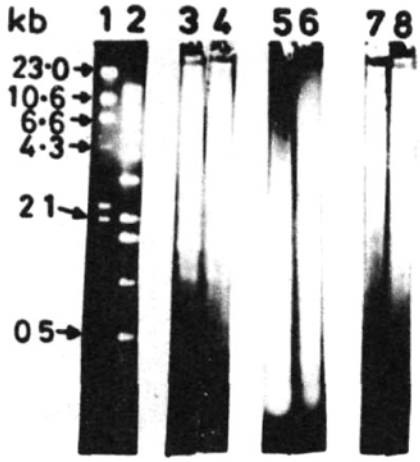

Figure 1. Restriction analysis of male and female cattle DNAs. 10 μg of DNA was digested to completion with restriction endonucleases *Eco* RI. *Hae* III and *Hind* III. **DNA** fragments were separated on 1% agarose gel and visualised after staining with ethidium bromide. Lanes 1 and 2, standard DNA markers; 3 and 4. *Eco* RI digested male and female DNAs; 5 and 6, *Hae*III digested male and female DNAs; 7 and 8, *Hind* III digested male and female DNAs.

Construction of partial library of Y derived DNA sequences in E. coli

To construct Y chromosome derived DNA library, the strategy used by us was similar to that used by Lamer and Palmer (1984). DNA from male cattle was digested to completion with restriction endonuclease *Sau*3A, while female DNA was sheared to an average size of 500 bp fragments. Male DNA was hybridized with large excess of female DNA (see 'materials and methods') to a Cot of 1000. Amongst 3 different kinds of hybrid molecules expected *(i. e.,* male-male, male-female and female-female), male-male hybrids should predominently repre sent Y derived sequences with *Sau*3A sticky ends, while sequences that are common between male and female DNAs will be present in the other two kinds of hybrid molecules. The hybrid DNA, purified free of single stranded DNA by hydroxyl apatite chromatography (figure 2) was used for cloning.

Restriction endonuclease *Sau*3A recognises the base sequence GATC which is common to the internal 4 bases of the sequences recognised by *Bam* Hl ($G^{\downarrow}GATCC$). Thus *Sau*3A derived DNA fragments can be cloned into *Bam* H1 site. Thus male-male hybrid molecules enriched for Y specific sequences and having *Sau*3A sticky ends were cloned into *E. coli* using *Bam* H1 site of plasmid pBR 322. From 10 μg of total hybrid DNA 1000 recombinants were obtained which were subjected to further analysis.

Identification of male specific recombinants

The strategy employed was mainly based on the assumption that there are sequences present on the Y chromosome that are not present in the female DNA. In order to ascertain that the partial library constructed by us has Y derived as well

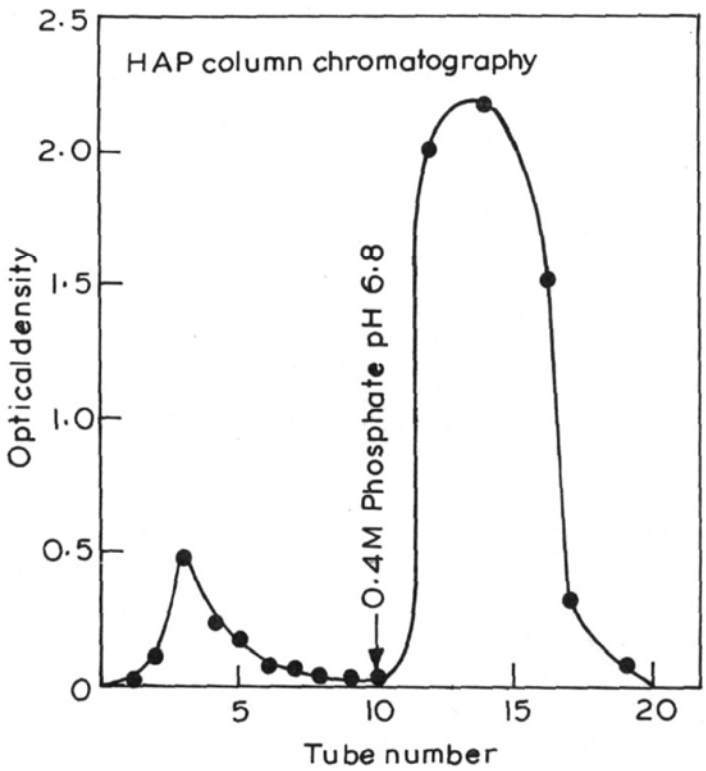

Figure 2. Hydroxylapatite column chromatography of hybrid DNA. Hybrid DNA in 0·12 M phosphate buffer, pH 6·8 was passed through HAP column (2 ml bed volume) equilibriated in the same buffer at 60°C. The column was washed with several volumes of 0.12 M phosphate buffer and the double stranded DNA was eluted from the column with 0.4 M phosphate buffer (fraction size. 0.5 ml).

as Y specific sequences, a differential colony hybridization experiment was carried out. Recombinants were grown in duplicate on nitrocellulose membrane filters in the presence of ampicillin and hybridized separately with male as well as female DNA probes of very high specific activity (see 'materials and methods" and legend to figure 3). The specific activity for both the probes was of the same order and same number of counts were used in hybridization. On the basis of autoradiog raphic signal the recombinants were grouped into 3 categories: (i) Those that gave strong hybridization signal of same intensity with both the probes, (ii) those which gave very strong hybridization signal with male DNA probe in comparison with female DNA probe and (iii) those with very weak signal and indistinguishable between male and female DNA probes. Out of 1000 colonies analysed, 17 fell into category II, where a stronger signal was observed with male DNA in comparison with female DNA probe. This is indicative of the presence of Y derived and Y specific determinants in them. Y derived repetitive sequences of human are also reported to be present on X chromosome (Cooke and Mackay 1978) and the first category of recombinants may be of this type. Weak hybridization signal observed with the remaining colonies may be due to single copy sequences present in them. Although hybridization of male and female cattle DNA was carried out at a Cot of

1000, where most of the single copy and repetitive sequences were collected in 3 different kinds of hybrid molecules, only highly repetitive sequences were identifiable on the basis of colony hybridization. The probes used in differential colony hybridization experiments were nick translated male and female total DNAs, of same specific activity (see legend to figure 3) and within themselves the sequences of repetitive nature should have higher specific activity in comparison to single copy sequences. The conditions used for hybridization as well as stringent washings and exposure time allowed detection of recombinants carrying highly repetitive sequences (male specific) (figure 3).

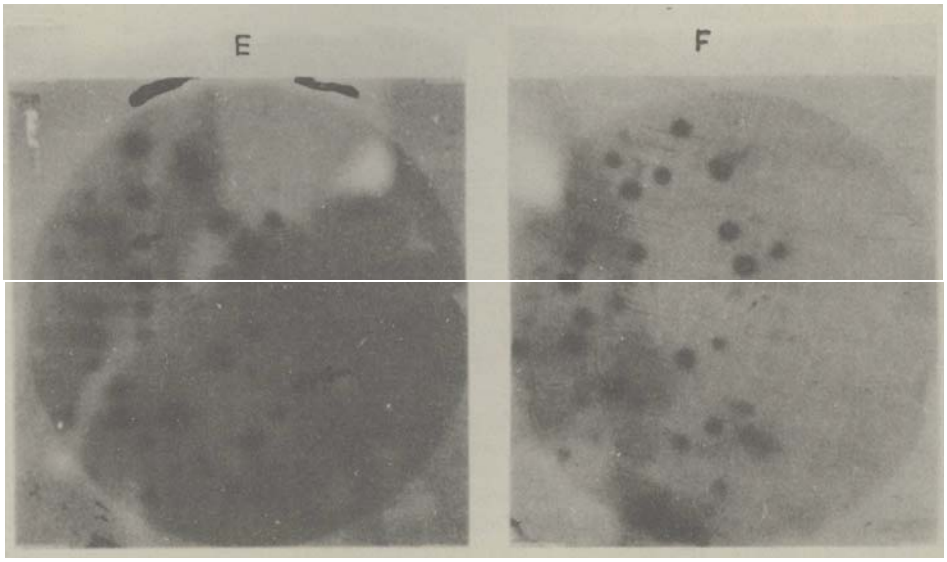

Figure 3. Identification of recombinants by colony hybridization. Recombinants were grown in duplicate on membrane filters (0.45 mμ) at 37°C for 18 h, lysed with 0.5 M NaOH and the denatured DNA was fixed after baking the filters at 80°C for 2 h. Each filter was hybridized separately with 5×10^6 cpm of ^{32}P labelled, nick translated total male or female DNAs (materials and methods). E and F represent hybridization profile with male and female DNA probes, respectively. Specific hybridization signal with male probe is indicated by arrows.

Recombinants of the second catagory were analysed for the presence of DNA insert. Analysis of DNA fragments after *Eco* RI as well as *Hin C*II digestion revealed that inserts were in the range of 200-800 bp (data not shown). Similarly, the nature of inserts as determined by southern blot hybridization experiments revealed coexistence of Y specific and non Y determinants on the cloned DNA (published elsewhere). Such observations are reported in the case of 3·4 kb *Hae* III fragment of human origin (Cooke, 1976). Screening of more recombinants from expanded library as well as their characterization are in progress.

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