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Short Communication

LOCALIZATION OF 24 COSMID CLONES ON THE HUMAN Y CHROMOSOME

Shigeo NAGAFUCHI,[1,**] Yutaka NAKAHORI,[2] Yusuke NAKAMURA,[3] Sarah B. ENGLAND,[1] Takashi TAMURA,[1,4] Hironao NUMABE,[1,5] and Yasuo NAKAGOME[2,*]

[1]*Department of Congenital Abnormalities Research, National Children's Medical Research Center, Setagaya-ku, Tokyo 154, Japan*
[2]*Department of Human Genetics, School of International Health, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*
[3]*Division of Biochemistry, Cancer Institute, Toshima-ku, Tokyo 170, Japan*
[4]*Department of Clinical Genetics, School of Health Sciences, Kyorin University, Mitaka, Tokyo 181, Japan*
[5]*Department of Pediatrics, Tokyo Medical College, Shinjuku-ku, Tokyo 160, Japan*

Summary Twenty-four novel cosmid clones were cloned and mapped on the human Y chromosome using a panel consisting of DNA from seven individuals each having a different segment of the Y chromosome. Eight were assigned to the short arm, 15 to the long arm and 1 to the both short and long arms.

Key Words Y chromosome, deletion mapping, cosmid clone

INTRODUCTION

Deletion mapping of the Y chromosome has seen significant progress in the past 2 years (Nakahori *et al.*, 1991; Bardoni *et al.*, 1991). The latest one identified 43 intervals within the non-fluorescent segment of the Y chromosome using STSs (sequence-tagged sites, Vollrath *et al.*, 1992). On the other hand, cosmid clones are useful for *in situ* hybridization in the analysis of structurally abnormal Y chromosomes.

We have successfully mapped 24 novel cosmid clones, 8 were assigned to the

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*To whom correspondence should be addressed.
**Present address: Department of Human Genetics, Roswell Park Cancer Institute, 666 Elm Street, Buffalo, New York.

short arm, 15 to the long arm and 1 to both short and long arms of the Y chromosome.

MATERIAL AND METHODS

General procedures for molecular cloning are according to Sambrock *et al.* (1989). The cosmid library had been constructed from a human Chinese hamster hybrid cell line R244-3A which has a intact human chromosome 11 and a non-fluorescent human Y chromosome. The isolation of the hybrid cell line and construction of a cosmid library were described by Tokino *et al.* (1991).

The DNA used for the Y chromosome mapping panel were prepared from the established cell lines of clinical samples previously reported by Nakahori *et al.* (1991). The cell line 1411 was added in the panel because it has a small interstitial deletion different from others (Nagafuchi *et al.*, submitted). The Southern hybridization were carried out under the existence of excessive human placental DNA to decrease the back ground due to repetitive sequences (Litt and White, 1985).

RESULTS

The library was screened with radio-labeled total human DNA to find out the cosmids harboring human inserts. Among 3,500 positive clones, 130 clones were randomly picked up. Each cosmid DNA was radio-labeled and used as probes for the hybridization with genomic DNA prepared from normal human males and females. For the first stage, 36 clones showed the differences between males and females. After excluding the overlapping clones and clones showing autosomal RFLPs, 24 independent cosmids revealed male specific hybridization pattern.

To determine the Y-chromosome localization of these cosmids we have performed the Southern hybridization to a Y-chromosome mapping panel. The location of cosmid clones on the Y chromosome is shown in Fig. 1 with the orders of the DNA markers we have previously reported. Among 24 cosmids, 8 are assigned to the short arm and 15 to the long arm and 1 (CTBP10) to both short and long arms. None of the clones was derived from heterochromatic region.

DISCUSSION

None of the 24 novel clones were mapped to the heterochromatic region of the long arm of the Y chromosome. This is in agreement with the finding that hybrid R244-3A contained no intact Y chromosome with quinacrine-positive heterochromatic region (data not shown). The selection of clones showing male specific hybridization patterns would have excluded clones in the pseudo-autosomal region.

Some of the cosmids have been used in the FISH (fluorescence *in situ* hybrid-

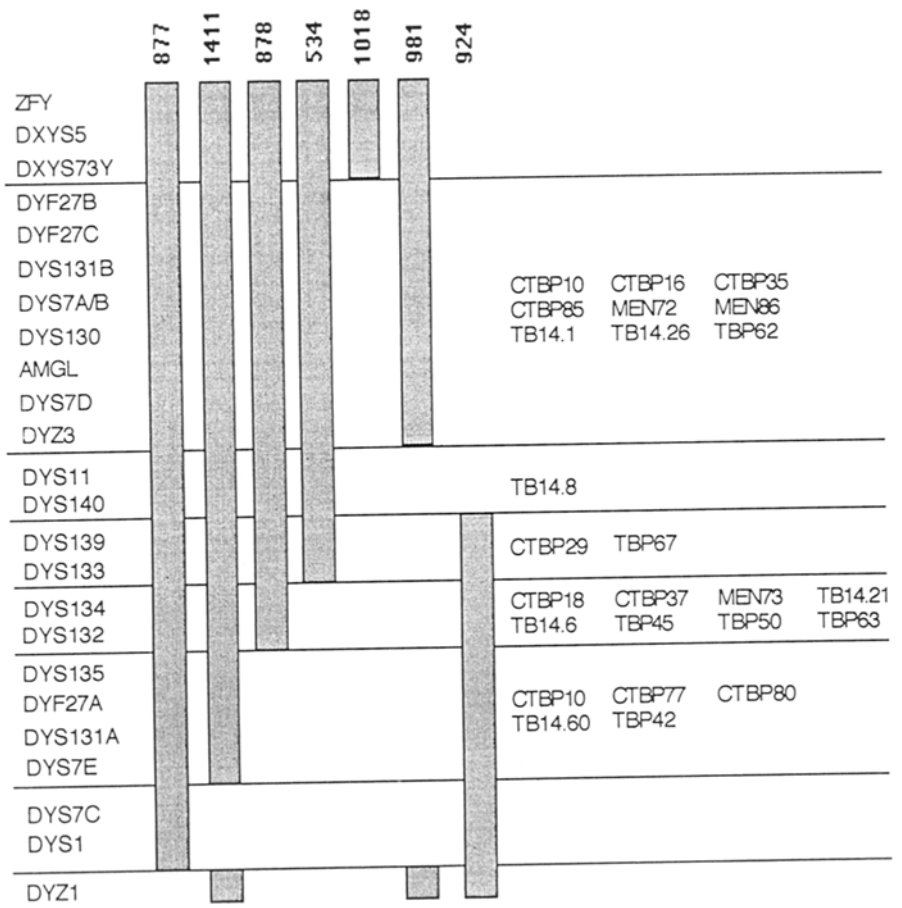

Fig. 1. Localization of 24 cosmid clones, 23 previously described loci by Nakahori *et al.* (1991) and an additional DYZ3 was established in the deletion panel consisted of 7 established cell lines each with different structural abnormality. The locus DYZ3 corresponds to the centromere. Locus number corresponding to cosmid clones are DYS159 (CTBP10), DYS160 (TB14.1), DYS161 (TB14.26), DYS162 (CTBP16), DYS163 (CTBP35), DYS164 (TBP62), DYS165 (MEN72), DYS166 (CTBP85), DYS167 (MEN86), DYS169 (TB14.6), DYS170 (TB14.8), DYS171 (TB14.21), DYS172 (TB14.60), DYS173 (CTBP18), DYS174 (CTBP29), DYS175 (CTBP37), DYS176 (TBP42), DYS177 (TBP45), DYS178 (TBP50), DYS179 (TBP63), DYS180 (TBP67), DYS181 (MEN73), DYS182 (CTBP77), and DYS-183 (CTBP80). The sizes of the intervals are arbitrary. The CTBP10 clone detects a locus on each of the short and long arms.

ization) analysis of patients with structually abnormal Y chromosomes and have been found indispensable in the analysis of those patients when the presence of a normal Y hampered analysis of abnormal Y *via* the PCR/Southern-blot approach (Takano *et al.*, unpublished).

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REFERENCES

Bardoni B, Zuffardi O, Guioli S, Ballabio A, Simi P, Cavalli P, Grimoldi MG, Fraccaro M, Camerino G (1991): A deletion map of the human Yq11 region: Implications for the evolution of the Y chromosome and tentative mapping of a locus involved in spermatogenesis. Genomics **11**: 443–451

Litt M, White RL (1985): A highly polymorphic locus in human DNA revealed by cosmid-derived probes. Proc Natl Acad Sci USA **82**: 6206–6210

Nakahori Y, Tamura T, Nagafuchi S, Fujieda K, Minowada S, Fukutani K, Fuse H, Hayashi K, Kuroki Y, Fukushima Y, Agematsu K, Kuno T, Kaneko S, Yamada K, Kitagawa T, Nonomura M, Fukuda S, Kusano M, Onigata S, Hibi I, Nakagome Y (1991): Molecular cloning and mapping of 10 new probes on the human Y chromosome. Genomics **9**: 765–769

Sambrock J, Fritsch EF, Maniatis T (1989): Molecular cloning I–III, 2nd ed., Cold Spring Harbor Lab., Cold Spring Harbor

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf A, Lovell-Badge R, Goodfellow PN (1990): A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **346**: 240–244

Tokino T, Takahashi E, Mori M, Tanigami A, Glaser T, Park JW, Jones C, Hori T, Nakamura Y (1991): Isolation and mapping of 62 new RFLP markers on human chromosome 11. Am J Hum Genet **48**: 258–268

Vollrath D, Foote S, Hitton A, Brown LG, Beer-Romero P, Bogan JS, Page DC (1992): The human Y chromosome: A 43-interval map based on naturally occurring deletions. Science **258**: 52–59