BRIEF REPORTS

The X–Y Homologous Gene Amelogenin Maps to the Short Arms of Both the X and Y Chromosomes and Is Highly Conserved in Primates

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The localization of amelogenin to Yq (7, 8) remains controversial as there are reports that suggest that the Y homologue is located on Yp (5). To resolve this question, we have examined the map location of X- and Y-linked amelogenin sequences by analysis of a series of somatic cell hybrids carrying deleted X chromosomes and a panel of individuals possessing aberrant Y chromosomes. We have carried out this analysis by means of the polymerase chain reaction (PCR) and two primers (AMELF and AMELR) we designed from the X and Y sequences published by Nakahori *et al.* (8). Two primers (identical in sequence on the X and Y) that span the first intron result in different sized X (542 bp) and Y (358 bp) fragments because of differences in the sizes of the introns on the X and Y. Figure 1A shows the analysis of a series of somatic cell hybrids carrying deleted X chromosomes and includes DNA from a normal male and Y-only somatic cell hybrid as controls. The region of the X present in hybrids is indicated schematically above each track of the figure. The pattern of deletion maps the X (AMELX) fragment in the interval Xp22.1–p21.2 [present in the hybrid EHA9 deleted for Xpter–Xp22.1 but missing from the hybrid W5A9 deleted for Xpter–p21.2—see (6)]. This location on the X is similar to the position determined by Lau *et al.* (7).

The location of the Y homologue (AMELY) has been determined by analyzing DNA from a series of XX male patients (generated by X–Y interchange) and individuals with aberrant Y chromosomes. The regions of the Y short arm present in these patients have been determined previously by analysis with a large panel of Y-specific DNA sequences (1–3) and are shown in Fig. 1B, together with PCR analysis for amelogenin of some of these patients. Patient WC possesses a monocentric iso-Yp chromosome and is positive for AMELY, clearly mapping the Y homologue to Yp. The region of the Y chromosome present in WC is shown aligned against the PCR results. AMELY is present in XX male patient PW but absent from XX male patient DR. The presence of AMELY in patients RW, KS, and the XY female patient AM (generated by a reciprocal of the X–Y interchange leading to XX males and resulting in a large deletion of distal Yp) confirms the location of the Y-linked gene in the interval containing the X–Y homologous sequences GMGXY8, p2F2, and pDP34 since all these patients cover this region. The patient ED (and a second identical case, TM—not shown) excludes AMELY from Yq. ED possesses two normal X chromosomes and two isodicentric Yq chromosomes containing an intact Y long arm and extensive deletion of Yp including the interval containing AMELY. The regions of the Y chromosome present in ED and AM are shown aligned against the PCR results.

These findings are at variance with the reports mapping AMELY to proximal Yq (7). It is possible to reconcile these different localizations by postulating the existence of pericentric inversion polymorphisms on the Y in the general population. Donlon and Muller (4) have supported this with indirect evidence from analysis of XX male patients shown to carry Yq sequences and have accounted for their deposition on Yp as a consequence of a pericentric inversion in the paternal Y and subsequent transfer to the X. It should be possible to test this hypothesis by using *in situ* hybridization to examine the Y localization of AMELY on a large series of normal Y chromosomes. It is interesting to note that the other X–Y homologous sequences mapping to the same interval on the Y do not map to the X short arm but to proximal Xq. This may reflect rearrangements on the Y that led to a short arm location for the Y homologue of amelogenin.

We have used PCR analysis to examine a series of male and female primate DNA samples and have revealed a remarkable degree of conservation of the primers and PCR product size among the great apes and old world monkeys. This is shown in Fig. 1C, where it can be seen that the primer sequences and the size of the intron on the X or Y or both appear to be remarkably conserved in all the primates shown in the figure. The primers failed to amplify with the more distant new world male and female marmoset monkey, indicating that their amelogenin sequences are more divergent. The failure to detect a Y product in the male talapoin and baboon may reflect a specific loss of the Y homologue or a high degree of conservation between the X and Y gene structure in these species. These primers should prove useful for sexing of not only human samples but also other primate species.

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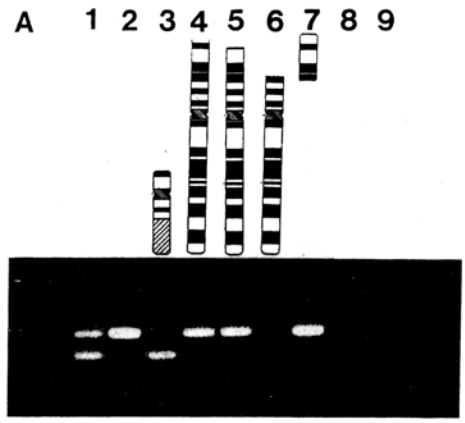

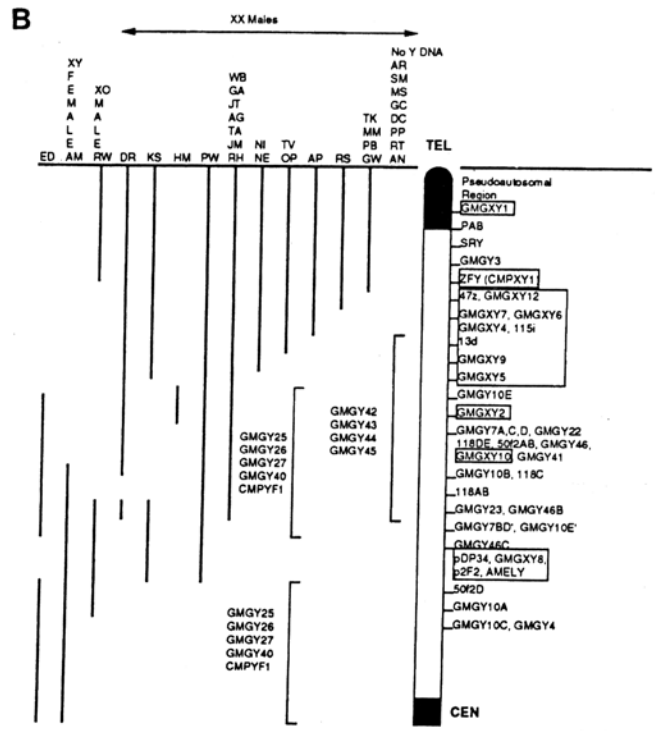

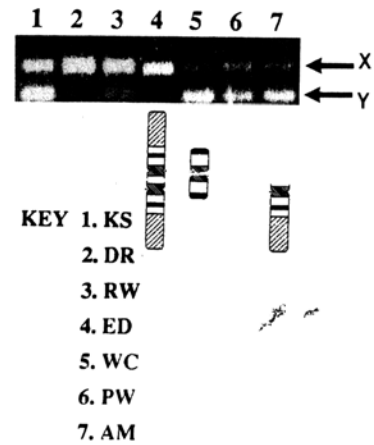

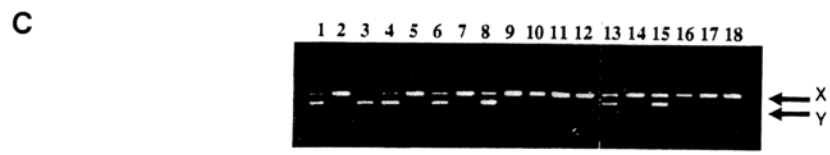

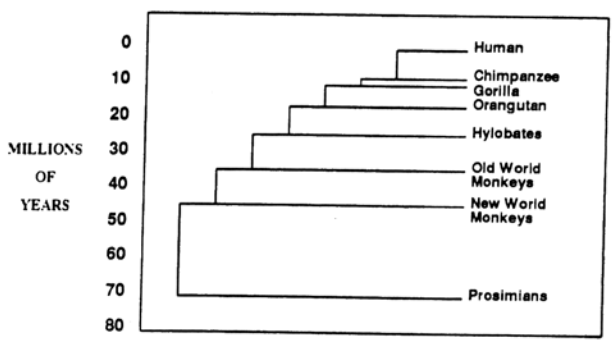

KEY TO PCR WITH AMELOGENIN PRIMERS

1. male
2. female
3. Y only hybrid
4. male chimp
5. female chimp
6. male gorilla
7. female gorilla
8. male orang
9. female orang
10. male baboon
11. female baboon
12. female gibbon
13. male rhesus
14. female rhesus
15. male crab -eating macaque
16. female crab-eating macaque
17. male talapoin
18. female talapoin

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Human DNA Polymerase δ Gene Maps to Region 19q13.3–q13.4 by *in Situ* Hybridization

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DNA polymerase δ[DNA pol δ (EC 2.7.7.7, proposed HGM symbol POLD)] plays a major role in mammalian chromosomal DNA replication. It is distinguished from DNA polymerase α (DNA pol α) by an intrinsic 3′ to 5′ exonuclease activity that provides a proofreading function that contributes to the fidelity of DNA replication [for reviews see (3, 6)]. Current models for eukaryotic DNA replication include the participation of both DNA pol δ and DNA pol α. More specifically, it has been proposed that DNA pol δ is primarily responsible for the synthesis of the leading DNA strand at the replication fork (8).

The complete cDNA of human DNA pol δ has been cloned by PCR (1), and by a combination of PCR and screening of human cDNA libraries (9). Because of its central role in DNA replication, localization of the DNA pol δ gene would facilitate studies of its potential linkage to disorders involving abnormalities in DNA replication and/or repair. In this work, we report the chromosomal localization of the gene encoding DNA pol δ using *in situ* hybridization.

Overlapping cDNA clones encoding the complete primary sequence for DNA pol δ were isolated by plaque hybridization as described by Yang *et al.* (9). The largest of these clones, HLgt10-2, contained an open reading frame of 1043 amino acids and was 3.2 kb long. HLgt10-2, containing 94% of the full cDNA sequence, was used for subsequent studies, including *in situ* hybridization.

Metaphase chromosome spreads were prepared by standard cytogenetic procedures from karyotypically normal, PHA-stimulated human peripheral blood lymphocytes. The HLgt10-2 DNA was labeled by the random primer technique (2) with [^{3}H]dCTP, [^{3}H]dGTP, and [^{3}H]dTTP (New England Nuclear) to a specific radioactivity of 2.1×10^9 dpm/μg. Labeled probe DNA was precipitated by the addition of ethanol, recovered by centrifugation, and redissolved in hybridization buffer (100 μg/ml sonicated salmon sperm DNA; 2× SSC, pH 6.0; 1 m*M* NaH_2PO_4; 0.2 mg/ml each of acetylated bovine serum albumin, ficoll, and polyvinylpyrrolidone; 50% formamide; 10% dextran sulfate) at a final concentration of 0.075 μg/ml. *In situ* hybridization was performed essentially as described by Rabin *et al.* (4). After ribonuclease treatment and denaturation of the chromosomal DNA, hybridization mix was applied to the slides. The slides were incubated at 37°C for 18 h. Following hybridization, the slides were washed and autoradiographed (using Kodak NTB2 photographic emulsion) as reported by Rabin *et al.* (4).

Metaphase chromosome spreads were G-banded and photographed prior to hybridization with the HLgt10-2 probe. After hybridization and autoradiography, the distribution of silver grains over 40 previously photographed chromosome spreads was analyzed. The location of each silver grain was plotted on an idiogram of the haploid human karyotype. The corresponding histogram divides the haploid human karyotype into 238 units, each scaled to the diameter of an autoradiographic silver grain (0.35 μm). Of 233 total grains observed overlapping the chromosomes, 33 (14%) were located on chromosome 19. The background label associated with the chromosomes averaged 0.98 grains/unit. An analysis of the distribution of silver grains observed over chromosome 19 revealed that 23 (70%) were localized to 19q13.3–q13.4 (Fig. 1). Statistical evaluation

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FIG. 1. (**A**) DNA from a normal male individual and a series of somatic cell hybrids was subjected to PCR analysis with the primers AMELF, 5′ CTCTGATGGTTGGCCTCAAG 3′ and AMELR, 5′ ACCTTGCTCATATTATACTTGACAAAG 3′. 250 ng of genomic DNA from each sample was amplified under the following conditions: Buffer, 25 m*M* TAPS, pH 9.3, 50 m*M* KCl, 1 m*M* DTT, 2 m*M* $MgCl_2$, 0.05% W1 detergent (Sigma), and 0.4 units of *Taq* polymersase (NBS) in a 50 μl reaction. Cycling was as follows: 1 cycle, 94°C for 5 min; 30 cycles, 94°C for 30 s, 60°C for 1 min, 72°C for 1 min; 1 cycle, 72°C for 10 min. PCR products were then analyzed on a 1% agarose gel and visualized by staining with ethidium bromide. 1, Normal male DNA; 2, normal female DNA; 3, 7631 Y-only somatic cell hybrid; 4, AMIR 2N somatic cell hybrid deleted for Xpter–Xp22.3; 5, EHA9 somatic cell hybrid deleted for the region Xpter–Xp22.1; 6, W5A9 somatic cell hybrid deleted for Xpter–Xp21.2; 7, LUMAGB19R2 somatic cell hybrid deleted for Xp21–qter; 8, mouse DNA; 9, hamster DNA. The primers gave no product with mouse or hamster DNA samples. (**B**) PCR analysis of patients with deleted Y chromosomes. Patients ED and AM contain an intact Y long arm. All other patients possess only segments of the Y short arm. KS, DR, and PW are XX male patients generated by X–Y interchange; RW is an XO male with a fragment Y chromosome; AM is an XY female generated by X–Y interchange; WC is a male with a monocentric iso Yp chromosome; ED is a female with a dicentric iso-Yq chromosome with a breakpoint in proximal Yp11.1. (**C**) PCR analysis of genomic DNA from several primate species. Gorilla, chimpanzee, and orangutan represent the great apes; gibbon, the hylobates, and the remaining primates are all old world monkeys. The baboon is the most primitive of the old world monkeys.

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The Use of the amelogenin gene for the determination of sex.

Introduction

The location of X- and Y-linked amelogenin sequences can been determined by means of the polymerase chain reaction (PCR) and two primers (AMELF and AMELR). The forward and reverse primers (identical in sequence on the X and Y) span the first intron but produce different sized X (542bp) and Y (358bp) fragments because of the differences in the sizes of the introns on the X and Y. The Cambio kit contains these two primers and a control DNA of known sex.

Figure A shows the results and analysis of a series of somatic cell hybrids carrying deleted X chromosomes and the results include DNA from a normal male and Y-only somatic cell hybrid as controls. The region of the X chromosome present in each of the hybrids is indicated schematically in line with each track of the figure. The pattern of deletion maps the X (AMELX) fragment in the interval Xp22.1-p21.2 but excludes it from the region Xpter-p21.2.

FIGURE (A) DNA is taken from a normal male in and a series of somatic cell hybrids were subjected to PCR analysis with the primers AMELF and AMELR.

1, Normal male DNA - primers gave both products.
2, Normal female DNA - primers gave only the X product.
3, Y-only somatic cell hybrid - primers gave only the Y product.
4, Somatic cell hybrid deleted for Xpter-Xp22.3 - primers gave only the X product.
5, Somatic cell hybrid deleted for the region Xpter-Xp22.1 - primers gave only the X product.
6, Somatic cell hybrid deleted for Xpter-Xp21.2 - primers gave no product.
7, Somatic cell hybrid deleted for Xp21-qter - primers gave only the X product.
8, Mouse DNA - primers gave no product.
9, Hamster DNA - primers gave no product.

The location of the Y homologue (AMELY) has been determined by analyzing DNA from a series of XX males (generated by X-Y interchange) and individuals with aberrant Y chromosomes. The regions of the Y short arm present in these individuals have been detemined previously. Figure B shows the PCR results for amelogenin of some individuals. WC possesses a monocentric iso-Yp chromosome and is positive for AMELY, clearly mapping the Y homologue to Yp. AMELY is present in XX male PW but absent from XX male DR. The presence of AMELY in RW, KS, and the XY female AM (generated by a reciprocal of the X-Y interchange leading to XX males and resulting in a large deletion of distal Xp) confirms the location of the Y-linked gene. ED possesses two normal X chromosomes and two isodicentric Yq chromosomes containing an intact Y long arm and extensive deletion of Yp including the interval containing AMELY. Hence ED excludes AMELY from Yq.

These primers should prove useful for sexing of not only human samples but also other primate species.

FIGURE (B) PCR analysis of individuals with deleted Y chromosomes. ED and AM contain an intact Y long arm. All other individuals possess only segments of the Y short arm. KS, DR, and PW are XX male individuals generated by X-Y interchange; RW is an XO male with a fragment Y chromosome: AM is an XY female generated by X-Y interchanges; WC is a male with a monocentric iso Yp chromosome; ED is a female with a dicentric iso-Yq chromosome with a breakpoint in proximal Yp11.1

Method:

Take 250ng of genomic DNA from each sample and amplify under the following conditions:

Prepare PCR Buffer containing 25 mM TAPS, pH 9.3, 50 mM KCl, 1mM DTT, 2mM $MgCl_2$, 0.05% (v/v) W1 detergent (Sigma), and
0.4 units of Taq polymerase (Cambio product number) in a 50μl reaction.

Carry out thermal cycling as follows: 1st cycle: 94°C for 5 min; thereafter 30 cycles, 94°C for 30 s, 60°C for 1 min, 72°C for 1 min; 72°C for 10 min.

Analyse PCR products on a 1% agarose gel and visualise by staining with ethidium bromide.

Note:

Use of PCR requires a licensed DNA (Taq) polymerase and purchase of this product from Cambio Ltd is accompanied by a limited license to use it in the PCR process for research in conjunction with an authorised thermal cycler. PCR is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd.