Studies of the locus for androgen receptor: Localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse

(somatic cell hybrids/complementation analysis/androgen insensitivity syndrome)

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ABSTRACT We have established a cell line from mouse kidney cells expressing the tfm mutation and showed that these cells lack androgen binding activity. A subclone of these simian virus 40 (SV40)-transformed cells (6TG R-SV-tfm) selected in 6-thioguanine and lacking hypoxanthine phosphoribosyltransferase was used to produce a series of mouse-human hybrids containing the normal human X chromosome or various X autosomal-translocation chromosomes (expressing only segments of the human X chromosome). When the androgen receptor locus (AR) was present in the hybrid, the number of receptor sites and kinetics of binding were similar to that in the human parental cells. Analysis of hybrids with partial human X chromosomes by assays for X chromosome-linked enzymes and for the androgen receptor protein indicate that the AR locus on the human X chromosome is near the centromere between Xq13 and Xp11 and is proximal to the locus for phosphoglycerate kinase. Hybrids derived from 6TG R-SV-tfm mouse cells and human labial fibroblasts from an XY individual with the ar - form of androgen insensitivity have no binding activity. The lack of complementation indicates that the X chromosome-linked mutations in mouse and man affect homologous loci and supports the evolutionary conservation of X chromosomal loci in mammals; however, the position of the locus on the human X chromosome indicates that intrachromosomal rearrangement has occurred.

The testicular feminization locus (Tfm), as it is referred to in the mouse, is essential for normal sex differentiation in the male (1, 2). Its presumed homology with the X chromosome-linked androgen receptor locus (AR) in man has contributed to evidence for conservation of the mammalian X (3). The tfm mutation in the mouse is clearly associated with deficient androgen receptor activity (4) and is X chromosome linked, having been mapped to a linkage group that includes Tabby (Ta) and Blotchy (1), which physically occupies the distal half of the murine X chromosome (5). The evidence for X chromosome linkage in man is based on transmission of the mutation in families segregating androgen insensitivity and observations of mutant-type cells that do not bind the androgen dihydrotestosterone in the heterozygous female (6). Furthermore, there is heterogeneity with regard to mutations associated with androgen insensitivity in man (7, 8). In some families the dihydrotestosterone receptor activity is lacking (ar -), whereas in others receptor activity with respect to androgen binding is not obviously abnormal (7). The basis for androgen insensitivity in the second variety has not been established, but the mutation may be allelic with the ar - mutation at the AR locus.

We report studies designed to test the hypothesis that the X chromosome-linked loci for androgen receptor activity in man and mouse are in fact homologous, using complementation analysis. In addition, these studies have permitted the localization of the AR locus to the pericentromeric region of the human X chromosome.

METHODS AND MATERIALS

Strategy. The strategy for mapping the human locus was to hybridize mouse fibroblasts that lack both the androgen receptor and hypoxanthine/guanine phosphoribosyltransferase (HPRT) activity with a variety of human cells having X autosome-translocation chromosomes. These translocation chromosomes lack different segments of the X chromosome, but each has the distal portion of the long arm that contains the gene for HPRT. Only hybrid cells that retain the human X chromosome can proliferate in nutrient medium that makes HPRT essential (8). Those hybrids having HPRT and other appropriate human X chromosome markers were assayed for androgen binding activity. For complementation studies, the mouse cells that are deficient in androgen binding were hybridized with human androgen-insensitive cells (ar -).

Cells. Because methods are not available to distinguish rodent from human androgen receptors, these studies required mouse cells lacking androgen receptor activity. Therefore, we established cultures of kidney cells from the tfm mouse, bred from a female who was heterozygous, in repulsion for both ta and tfm—an incipient inbred strain maintained by E. Eicher at The Jackson Laboratory. The tfm, +/+ kidney cells were transformed with simian virus 40 (SV 40) as described (9). To obtain a selectable marker for cell hybridization studies, we isolated subclones that are resistant to the toxic effects of medium containing 6-thioguanine (6TG R) because they lack HPRT. These cells (6TG R-SV-tfm) have a modal number of 64 chromosomes (acrocentric) and have little detectable androgen binding activity in contrast to kidney cells simultaneously derived from the +, ta/y littermate, and mouse L cells (Fig. 1).

The human parental fibroblasts (GM3552, GM0073, GM2859, and 297) were those carrying balanced X chromosome/autosome translocations with breakpoints on the X chromosome at Xq26 (10, 11), Xq13 (12), Xq11, and Xpl1 (13)* respectively. Moreover, the X chromosome segments of the translocation chromosomes were genetically active and, therefore, were expressed in the hybrid, whereas the normal X chromosome, if present, was the inactive one. Other parental cells included

Abbreviations: SV40, simian virus 40; 6TG R, 6-thioguanine; 6TG R, 6TCrexistant; LDH, lactate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; PGK, phosphoglycerate kinase; HPRT, hypoxanthine phosphoribosyltransferase; AR, androgen receptor locus; Tfm, testicular feminization locus.

* Studies indicate the breakpoint on X chromosome is halfway between centromere and bright band at Xp21 (14).

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fibroblasts from a normal female with intact X chromosome and those from an XY individual with the $ar^+$ form of androgen insensitivity. The fibroblasts derived from biopsies of labial skin are referred to as "sex skin."

Medium. Cells were maintained in Eagle’s Minimal Essential Medium (GIBCO) enriched as described (14). Selection for hybrids was carried out in medium with hypoxanthine/aminopterin/thymidine (15) that also contained $\mu$M ouabain. Medium containing 60 $\mu$M 6-thioguanine (6TG) was used to select for HPRT-deficient cells.

Cell Fusion. Human fibroblasts were fused with 6TG$^{5}$-SV-tfm cells in a 1:9 ratio by using 50% (wt/vol) polyethylene glycol 6000 in Hanks’ balanced salt solution (14). Hybrids were isolated by using cloning cylinders and were maintained in the selective medium.

Chromosome Studies. Hybrid clones were analyzed for human chromosome content by using alkaline Giemsa banding to identify the human chromosomes in the hybrid (16). Specific identification of the X translocation chromosome was based on sequential quinacrine and alkaline Giemsa banding.

Enzyme Assays. For enzyme assays, cells were removed from the Petri dish with rubber policeman in 15 $\mu$l of water containing 100 $\mu$g of NADP per ml and disrupted in a sonic cleaner. Electrophoresis for glucose-6-phosphate dehydrogenase (G6PD) was performed on cellulose acetate gels in Tris/glycine/sucrose buffer, pH 9.2 (365 V for 25 min). For lactate dehydrogenase (LDH), the gels were treated identically but stained with LDH isozyme substrate (Gelman). Electrophoresis for phosphoglycerate kinase (PGK) was performed at room temperature with Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX). The method was modified from that described by Meera Kahn (17) for Cellox as follows: The buffer was 40 mM Tris/1.8 mM citric acid/3.8 mM acid EDTA/0.26 mM disodium EDTA; the pH was adjusted to 8.0 with citric acid. The gel ran at a constant current of 12 mA for 25 min.

**Androgen Receptor Assay.** [1,2,4,5,6,7-3H]Dihydrotestosterone (131 and 200 Ci/mmole; 1 Ci = $3.7 \times 10^{10}$ becquerels) was purchased from New England Nuclear.

Specific binding to the androgen receptor was measured as described (18). Briefly, cell monolayers were incubated for 45 min at 37°C with [3H]dihydrotestosterone (0.2–2.5 nM). After incubation, the medium was removed and an aliquot was assayed for free radioactivity. The cells were washed, collected by centrifugation, and sonicated in 0.02 M Tris-HCl, pH 7.4/1.5 mM EDTA/0.5 M KCl. The cell lysates were centrifuged at 2,000 × g, and the supernatants were assayed for androgen receptor binding by the adsorption method with 0.05% dextran T70/0.1% calf skin gelatin/0.5% charcoal/0.02 M Tris-HCl, pH 7.4/1.5 mM EDTA. Each lysate was divided into three 250-μl aliquots, one of which was assayed for DNA content (19), another was heated to 70°C for 4 min to denature the receptor protein, and the third remained at 0°C (unheated). Free steroid in the heated and unheated aliquots was adsorbed to 500 μl of the dextran/gelatin charcoal suspension and removed by centrifugation at 2000 × g. The radioactivity remaining in the supernatant was assayed by liquid scintillation spectrometry. Specific binding to the androgen receptor was calculated as the difference between the total binding (unheated aliquot, 0°C) and the nonspecific binding (heated aliquot, 70°C). Maximum binding capacity ($B_{\text{max}}$, fmol/mg of DNA) and the apparent dis-
sociation constant \((K_d \times 10^{-9} \text{ M})\) for the androgen receptor were derived from Scatchard plots (20) by using linear regression analysis (21).

**RESULTS AND DISCUSSION**

**Binding Characteristics of Mouse Parental Cells.** Fig. 1A shows the Scatchard plots for the parental 6TG-R-SV-tfm cells and for the cells similarly derived from the tabby littermate. We interpret these data as indicating little, if any, binding activity in the tfm mouse cells.

**Binding Characteristics of Mouse Intraspecific Hybrid Cells.** Hybrids between these cells and thymidine kinase-deficient L cells (LM tk- e11D) were obtained to determine if the hybrid cells would have binding activity. Fig. 1B shows that the hybrid phenotype is intermediate between the mutant and the wild-type parent cell. However, the apparent difference between hybrid and parental cell most likely merely reflects the increased DNA content of the hybrid.

**Binding Characteristics of Human Parental Cells.** The human parental fibroblasts with balanced X chromosome/autosome translocations had significant dihydrotestosterone binding activity; however, some of the parental cells had small numbers of binding sites (Fig. 2) as expected for fibroblasts derived from non-sex-skin, which have a lower \(B_{\text{max}}\) (7). The androgen binding pattern in cells with the X/11 translocation containing the X chromosome segment q11-qter was unusual because the affinity constant, \(K_d\), was too high.

**Characteristics of the Mouse–Human Somatic Cell Hybrids.** Hybrids derived from human parental cells with an intact normal X chromosome or various X chromosome/autosome translocations and 6TG-R-SV-tfm were isolated, propagated in selective medium and analyzed for their human X chromosome enzyme content (Table 1). Those hybrids that expressed human G6PD and PGK (when Xq13 was present) were analyzed for their androgen receptor phenotype. Fig. 2 shows Scatchard plots describing the binding that characterized each set of hybrids. In contrast to the mouse parental cells, hybrid cells having the entire X chromosome or the Xp11–Xqter segment of the human X chromosome had androgen binding activity (Table 1; Fig. 2A). This binding activity was lost along with other human X chromosome markers when the hybrid was back-selected in 6TG (Table 1; Fig. 2A). On the other hand, hybrid cells containing only the distal half of the long arm of the X chromosome (Xq13–Xqter or Xq25–Xqter) had no binding activity (Fig. 2 C and D; Table 1).

**Characteristics of Mouse–Human Hybrids Derived from Sex Skin Fibroblasts.** Because the dihydrotestosterone binding activity of the hybrid appeared quantitatively less than that of the human parental cell, we were concerned that lack of binding in hybrids might be artifactual, attributable to low activity in non-sex-skin. Therefore, when cells were available, hybrids were prepared from fibroblasts of sex skin origin because of their greater dihydrotestosterone binding activity. Fig. 2C shows the Scatchard plot of hybrids derived from labial fibroblasts with a 14/X reciprocal translocation (only Xqter–Xq13 present in the hybrid) and confirms the negative binding observed in hybrids derived from non-sex-skin (Table 1).

**Regional Localization of Androgen Receptor Locus on the Human X Chromosome.** Although it is clear that hybrids with only Xqter–Xq13 do not have androgen receptor binding activity whereas hybrids with Xqter–Xp11 do, the phenotype of hybrids having the segment Xqter–Xq11 was more difficult to assess. The human parental cells were derived from a 3-yr-old female with a reciprocal X/11 translocation with breakpoints at

### Table 1. Characteristics of hybrids derived from SV-tfm mouse cells and various human fibroblasts

<table>
<thead>
<tr>
<th>Human parent fibroblasts</th>
<th>Human X chromosome segment</th>
<th>Source of fibroblasts</th>
<th>Hybrid clone ref. no.</th>
<th>Human Enzymes</th>
<th>DHT binding*</th>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal female</td>
<td>Entire</td>
<td>Non-sex-skin</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>36 0.76 0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>52 0.43 0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 6TG-R</td>
<td>–</td>
<td>–</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>27 0.96 0.85</td>
</tr>
<tr>
<td>GM 3552</td>
<td>Xq26–Xqter</td>
<td>Non-sex-skin</td>
<td>11</td>
<td>+</td>
<td>–</td>
<td>Negative</td>
</tr>
<tr>
<td>GM 0073</td>
<td>Xq13–Xqter</td>
<td>Non-sex-skin</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>Sex skin</td>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>GM 2859</td>
<td>Xq11–Xqter</td>
<td>Non-sex-skin</td>
<td>I1–10</td>
<td>+</td>
<td>+</td>
<td>Negative†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 6TG-R</td>
<td>–</td>
<td>–</td>
<td>Negative†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>+    +</td>
<td>–</td>
<td>142 3.67 0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 6TG-R</td>
<td>–</td>
<td>+</td>
<td>Negative‡</td>
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<td></td>
<td></td>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>161 3.34 0.85</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5 6TG-R</td>
<td>–</td>
<td>–</td>
<td>Negative‡</td>
</tr>
<tr>
<td>GM 297</td>
<td>Xp11–Xqter</td>
<td>Non-sex-skin</td>
<td>2a</td>
<td>+</td>
<td>+</td>
<td>55 1.40 0.76</td>
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<td></td>
<td></td>
<td></td>
<td>5c</td>
<td>+</td>
<td>+</td>
<td>42 0.88 0.71</td>
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<td></td>
<td></td>
<td></td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>58 1.08 0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 6TG-R</td>
<td>–</td>
<td>–</td>
<td>Negative</td>
</tr>
<tr>
<td>Androgen insensitivity</td>
<td>Entire</td>
<td>Non-sex-skin</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>(ar–)</td>
<td></td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Sex skin</td>
<td></td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* \(B_{\text{max}}\) is expressed as fmol of dihydrotestosterone (DHT) bound per mg is not DNA; \(K_d\) is expressed \(\times 10^{-9}\)nM; \(r\) is the correlation coefficient from linear regression analysis.

†I, II, clones observed from first and second hybridizations, respectively.

‡Some radioactivity remaining after nonspecific activity was subtracted. However, no regression line can be obtained.
Xq11 and 11p11. These cells bound significant amounts of [3H]dihydrotestosterone, but the kinetics of binding characterized by high \( K_d \) values (ranging from 1.45 to 3.53 nM) were not that expected for the androgen receptor protein (Fig. 2B). To determine if the atypical binding pattern was attributable to the X chromosome, we obtained hybrids between these cells and the 6TG\(^{SV}\)-SV-tfm mouse cells from two separate matings. The androgen binding pattern of several of these hybrids was also atypical and, in fact, nearly identical to that of the human parental cells (Fig. 2B). When these hybrid cells were back-selected in 6TG to obtain derivative cells that had lost the human X chromosome, the binding activity did not completely disappear; however, the pattern changed. Although a significant amount of radioactivity remained in the specific fraction, it was not saturable, so that no significant regression line could be obtained. The loss of the segment Xqter–Xq11 was confirmed by studies of the X chromosome-linked enzymes G6PD and PGK and by cytogenetic studies. The absence of LDHA in these subclones indicated that the reciprocal product of the translocation was also not present. The persistence of some binding activity despite loss of the human X chromosome indicates that the atypical pattern is not entirely attributable to that chromosome.

On the other hand, our interpretation of studies of 2859 fibroblasts and hybrids is that there is specific androgen receptor activity in these cells and that at least some of it is associated with the presence of the Xq13–Xq11 segment of the X chromosome. Regression lines could be obtained only from hybrids that had the relevant segment (Table 1, clones 5, 6, and 8). It may be that the presence of unusual background binding activity seen in the 6TG\(^{SV}\) hybrids has obscured most of the specific receptor activity present in these cells—especially as these cells originate from non-sex-skin. Alternatively, the atypical affinity that we observed in both parent and hybrid cells could be explained if the breakpoint at Xq11 had disrupted the activity of the relevant gene, in which case the AR locus could be mapped close to Xq11. Phenotypic abnormalities at other X chromosome loci close to breakpoints of X chromosome/autosome translocations have been observed (22). Further studies of X chromosome/autosome translocations with the Xq11 breakpoint should be informative when such cells are available.

Because of the atypical binding patterns we observed with 2859 fibroblasts, we are unable to assign the locus with confidence to the Xq13–Xq11 segment of the human X chromosome. However, because there is significant specific binding activity associated with the presence of that segment, we believe that it is likely that the locus is on the proximal long arm of the X chromosome, close to the centromere. In any event, the AR locus is pericentric because hybrids derived from 297 cells with an X chromosome breakpoint in the middle of the p11 band have receptor activity (Fig. 3; Table 2).

**Complementation Analysis of ar\(^{-}\) Mutation in Mouse and Man.** To determine if the X chromosome-linked loci for androgen receptor activity are homologous in mouse and man, we hybridized the tfm mouse cells with human fibroblasts from an androgen-insensitive male expressing the X chromosome-linked ar\(^{-}\) mutation. Evidence that the mutation in this individual is X chromosome-linked is based on pedigree data and analysis of fibroblast clones from the heterozygous mother (6). The interspecific hybrids were derived from non-sex-fibroblasts as well as those of labial origin. These hybrids have no binding activity so that complementation has not occurred (Fig. 4).

**Homology of Mouse and Human X Chromosomes.** Our observations are particularly relevant with respect to the degree of homology between X chromosomes in mammals. Ohto (3, 23) has suggested that mechanisms compensating for gene dosage differences between the sexes have resulted in the evolutionary conservation of X chromosome-linked genes, and points out that the available evidence indicates that genes found to be on the X chromosome in one mammalian species are also X chromosome linked in others. The presence of loci on the mouse and human X chromosome that affect androgen receptor activity suggests homology, but our evidence that hybrid cells derived from human and mouse mutants lack receptor activity provides stronger support for homology.

On the other hand, while the gene content of the X chro-

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**Table 2. Binding activities associated with X chromosome/autosome translocations or ar\(^{-}\)**

<table>
<thead>
<tr>
<th>X chromosome segment</th>
<th>Entire</th>
<th>Xq26–Xqter</th>
<th>Xq13–Xqter</th>
<th>Xq11–Xqter</th>
<th>Xp11–Xqter</th>
<th>Entire (ar(^{-}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal segment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11pter–11p23</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>14pter–14q32</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Illustrated in Fig. 3.
mosome may be similar from one mammalian species to another, the arrangement of the material on the chromosome seems to vary considerably. Although size with relation to that of the genome may be relatively constant (3), the morphology of mammalian X chromosomes differs with respect to position of the centromere and the banding patterns are highly variable (24), suggesting that internal rearrangements have occurred. Observations have been reported that suggest the order of two loci on the mouse X chromosome are reversed from that on the human X chromosome (25). Our finding that the locus for androgen receptor (AR) on the human X chromosome is near the centromere, whereas, the homologous locus (Tfm) on the mouse X chromosome is not is further evidence that intrachromosomal rearrangements have occurred during the evolution of the two species.

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