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Identification of incomplete coding sequences for steroid sulphatase on the human Y chromosome: evidence for an ancestral pseudoautosomal gene?

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Summary

A cDNA clone (p422) containing about 200 bp of coding sequences for steroid sulphatase (STS) has been isolated from a λgt11 expression library by antibody screening and has been assigned by mapping with a somatic cell hybrid panel and by *in situ* hybridization to Xp22.3; a localization coincident with the previously identified locus for STS expression. Although no significant hybridization of this clone to the Y chromosome was observed, p422 has been used to isolate a longer cDNA clone and genomic sequences which do recognize Y-specific restriction fragments. An abbreviated *STS* gene has been localized to Yq11.2. The coding sequences for the human enzyme shows little homology to sequences in mice.

Key words: steroid sulphatase, X chromosome, Y chromosome, human, pseudoautosomal gene.

Introduction

The gene for the enzyme steroid sulphatase (STS) is of particular interest in the context of sex chromosome organization and evolution as it is the only well-characterized X-linked locus in humans which has no functional, Y-linked equivalent and yet escapes from inactivation (Craig & Tolley, 1986). STS deficiency is the primary defect of X-linked icthyosis (XLI), a skin disorder often associated with late parturition. A gene necessary for the expression of human STS has been assigned by somatic cell hybrid studies, deletion mapping and linkage analysis to the region Xpter-Xp22.3 (Mohandas, Shapiro, Sparkes & Sparkes, 1979; Muller *et al.* 1980; Tiepolo *et al.* 1980; Wieacker *et al.* 1984) and in common with other loci in the same region, e.g. the blood group *Xg* gene (Weatherall *et al.* 1970) and the *MIC 2X* gene (Goodfellow, Pym, Mohandas & Shapiro, 1984), it has been found to escape X-inactivation (Shapiro, Mohandas, Weiss & Romeo, 1979). Recent evidence suggests that the *STS* gene on the inactive X chromosome is only partially inactivated (Migeon *et al.* 1982). Although there is no evidence for a functional Y-linked allele in man, the *STS* gene in the mouse behaves pseudoautosomally with an apparently functional homologue on the Y chromosome (Keitges, Rivest, Siniscalco & Gartler, 1985). In humans, the *STS* gene is thought to be proximal to the pseudoautosomal region (Craig & Tolley, 1986) and a homologous, but inactive, gene on the Y chromosome has been hypothesized (Polani, 1980).

The availability of a cDNA probe for the *STS* structural gene (Ballabio *et al.* 1987*a*) has enabled the localization of the coding sequences by *in situ* hybridization and by studies on somatic cell hybrids to Xp22.3. This confirms both the previous assignment for *STS* based on enzyme activity measurements and that the locus involved is the structural gene for the enzyme.

Although no Y-chromosomal sequences were detected with this probe, it has been used to isolate more extensive cDNA clones and genomic sequences. These have enabled further investigation of the localization of *STS* coding sequences in humans and an evaluation of their homology to those present in primates and rodents. Our observations demonstrate the existence of a Y-linked incomplete coding

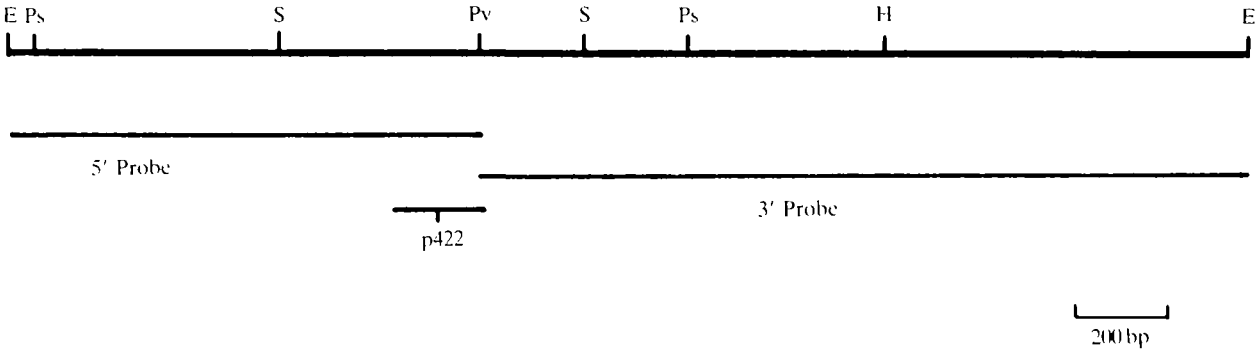

Fig. 1. Restriction map of the cDNA clone P2A7 – a 2·7 kb *Eco*RI fragment. The region showing homology with p422 is indicated, as are the *Eco*RI/*Pvu*II restriction fragments that have been referred to as the 5′ and 3′ probes in the text. (E, *Eco*RI; Ps, *Pst*I; S, *Sst*I; Pv, *Pvu*II; H, *Hin*dIII.)

region at Yq11.2 and the lack of highly homologous sequences in mice.

Isolation of steroid sulphatase clones

We have isolated several cDNA clones from a λgt11 expression library, prepared from human placental mRNA, by screening with polyclonal antibodies raised against purified steroid sulphatase. *Eco*RI inserts from positive plaques have been isolated and subcloned into the vector pUC18 (Ballabio *et al.* 1987). One of these clones, p422, contains a 560 bp insert. However later studies have shown that only 200 bp of this clone correspond to the cDNA for STS, the remaining sequence being of unknown origin, but does not appear to cross-hybridize with human DNA. The insert from p422 has been used to isolate a second larger cDNA from the same λgt11 library. This clone, P2A7, contains a 2·7 kb insert, the restriction map for which is shown in Fig. 1.

X- and Y-chromosomal localization of sequences

P2A7 recognizes four fragments in *Bam*HI restriction digests of normal human female DNA. An additional two fragments are recognized in male DNA. The localization of the genomic sequences recognized by P2A7 has been established by its hybridization to restriction digests of DNAs from somatic cell hybrids with a variable representation of human sex chromosomes and from human cell lines with 1, 2 and 4 X chromosomes (Table 1 & Fig. 2). The decreasing signals of four of the fragments, observed for tracks loaded with the same amount of DNA from 48,XXXX, 46,XX and 46,XY cell lines indicates a clear localization of these to the X chromosome. The analysis of hybrids retaining various portions of the human X chromosome refines this localization to the region Xp22.3. The most precise information is provided by comparison of the hybridization to the DNA of two hybrids which retain human X chromosomes with breakpoints in Xp22.3. One hybrid (817/175) expresses human levels of STS but lacks *MIC2* (Mondello *et al.* 1986), which has been assigned to Xp22.3 (Buckle *et al.* 1985), and the other ($UCLA_{B2}$) lacks both STS activity and the *MIC2* locus (Curry *et al.* 1984). The observation of a positive signal in the former hybrid and not in the latter provides a clear localization of some of the sequences recognized by P2A7 to Xp22.3.

In addition to sequences assigned to Xp22.3, P2A7 recognizes sequences present on the human Y chromosome. This assignment has been established by the observed dosage of signals from 46,XY and 49,XYYYY cell lines and the presence of signals in the Y only hybrid 3E7. Subregional assignment has been established by the presence of Y-specific bands in the hybrid 817/175, known to contain Yq, and by the presence of Y-specific bands in the cell line

Table 1.

Cell line	Description	Human autosomes	Human sex chromosomes
RAG	Mouse	None	None
817/175	Hybrid	Several	Xp22 3-Xqter Yq11-Yqter
$UCLA_{B2}$	Hybrid	Several	Xp22.3-Xqter
FRAG A13R3	Hybrid	Several	Xpter-Xp21 2
WAG 8	Hybrid	Several	Xp21 1-Xqter
MOG T	Hybrid	None	X
3E7	Hybrid	None	Y
GM1416	Human	All	48,XXXX
OX	Human	All	49,XYYYY
Normal female	Human	All	XX
Normal male	Human	All	XY

Hybrid 817/175 is derived from a human cell line 46,X,t(X,Y) (Xqter-Xp22 3.. Yq11-Yqter) (Ropers *et al.* 1985). $UCLA_{B2}$ retains the chromosome associated with chondrodysplasia punctata (Curry *et al.* 1984). See Boyd *et al.* (1987) and Buckle *et al* (1987) for details of other cell lines and hybrids

TRAN and their absence in the cell line TAP (Fig. 3). Both TRAN and TAP contain Y chromosomes that have extensive, terminal deletions of Yq. TRAN has been shown to lack the locus *DYS20*, which has been mapped to Yq11.2 by *in situ* hybridization (Buckle *et al.* 1987), suggesting that the breakpoint of Yq in TRAN must occur proximal to this band. TAP also lacks the locus *DYS20* and appears to contain a more extensive deletion of Yq. STS homologous sequences can thus be mapped proximal to the locus *DYS20*, but distal to the breakpoint in TAP. The Y-specific sequences are not detected by p422.

It would appear that the majority of the exons recognized by P2A7 are present in the 5′ end of the cDNA, as are the Y-specific sequences. If this orientation is correct, P2A7 can be cleaved by *Pvu*II, to give a 5′ and 3′ probe (see Fig. 1). The 3′ probe detects only one fragment which corresponds to neither of the Y-specific fragments (Fig. 4A,B).

Cross-hybridization to sequences present in other mammals

Neither p422 nor P2A7 show cross-hybridization with sequences present in rodents or lagomorphs under conditions in which significant homology can be detected with human factor IX coding sequences (Fig. 5B,C). Even under conditions of reduced stringency there is a lack of any significant hybridization (Fig. 5A).

Subsequent experiments have shown that P2A7 detects homologous sequences in primates as distantly related as the brown lemur. Weakly cross-hybridizing sequences have since been detected in lagomorphs, but significant cross-hybridization with rodent DNA has still not been observed (unpublished results).

Discussion

The localization of the P2A7 cDNA clone, which contains 2·7 kb of coding sequence, provides confirmation of the subregional assignment of the structural gene for STS. It is also consistent with the existence of a region escaping from inactivation at the distal terminus of the X chromosome short arm (Craig & Tolley, 1986). It has been presumed that the region escaping from inactivation would correspond to that portion of the X chromosome sharing homology with the Y chromosome and which pairs and recombines with the Y chromosome at meiosis. Our observations make it clear that the *STS* sequences cannot be

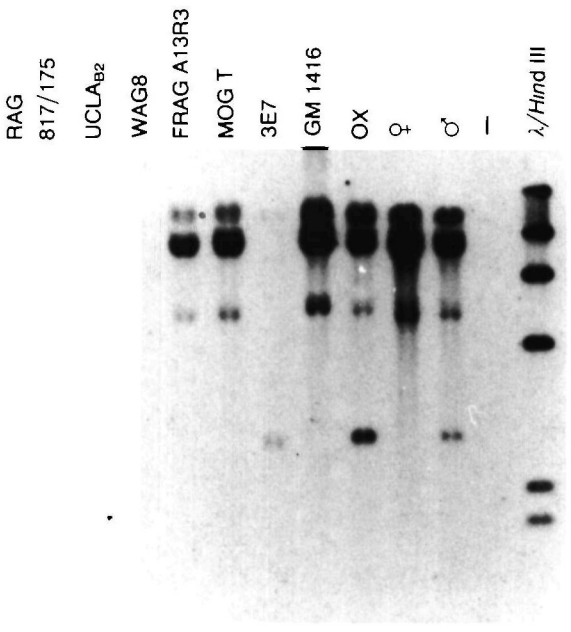

Fig. 2. Localization and dosage analysis in human cell lines and human–mouse somatic cell hybrids. The type of cell line and content of human sex chromosomes are indicated in Table 1. The Southern blot of the *Bam*HI digested DNA samples was hybridized with P2A7 and washed under stringent conditions (0·1 × SSC, 64°C). Y-specific restriction fragments are visible at ≃15 kb and ≃3 kb. The sizes of the λ/*Hin*dIII marker fragments are ≃23 kb, 9·4 kb, 6·6 kb, 4·4 kb, 2·3 kb and 2 kb.

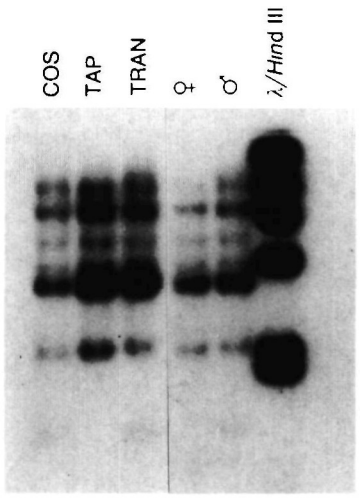

Fig. 3. Subregional assignment of the Y-specific restriction fragments. Details of the cell lines TRAN and TAP are given in the text. COS is a cell line derived from a 46,XY female. The Southern blot of the *Eco*RI digested DNA samples was hybridized with P2A7 and washed under stringent conditions (0·1 × SSC, 64°C). A Y-specific restriction fragment, ≃1·5 kb, is present in the normal male, COS and TRAN tracks, but absent in TAP. The sizes of the λ/*Hin*dIII marker fragments are ≃23 kb, 9·4 kb, 6·6 kb, 4·4 kb, 2·3 kb and 2 kb (filter provided by M. Ross).

exchanged regularly between the sex chromosomes. It is possible, however, that the close proximity of the *STS* gene to the pseudoautosomal region on the X chromosome may predispose rearrangements resulting in deletions which may include this locus. It is therefore of considerable interest that ten unrelated STS-enzyme-deficient patients, including eight cases of classic X-linked icthyosis (XLI), were found to have deletions including the coding sequences detected by the clone p422 (Ballabio *et al.* 1987*a*,*b*).

The exact degree of homology between the human and rodent steroid sulphatase genes and their products remains to be evaluated; however, we have shown that the coding sequences show little cross-

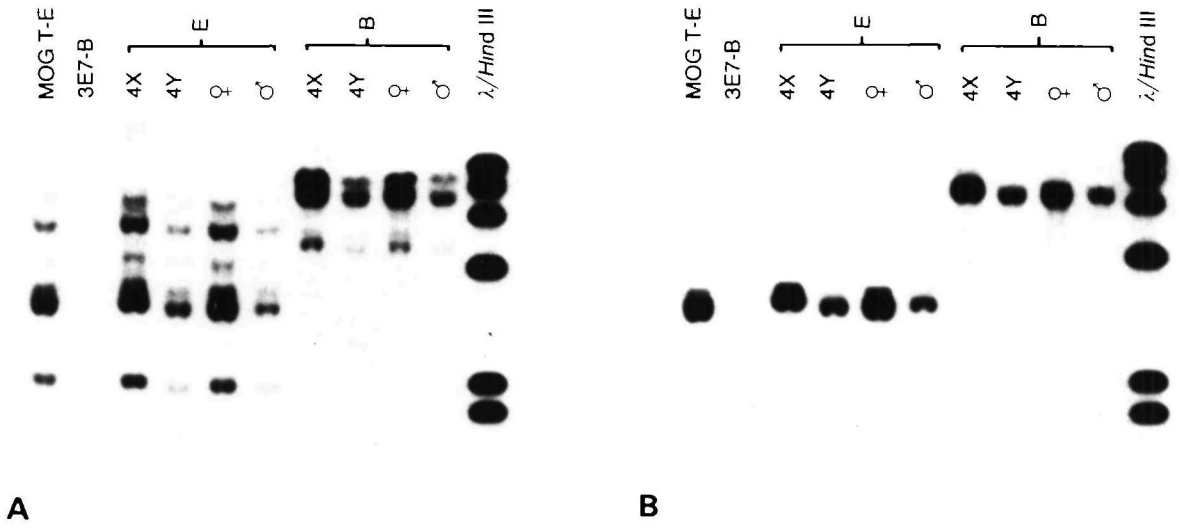

Fig. 4. Human cell line (4X: GM1416, 4Y: OX) and human–mouse somatic cell hybrid DNA digested with *Eco*RI (E) and *Bam*HI (B) and probed with the P2A7 5′ probe (A) or the 3′ probe (B) (see Fig. 1). The 5′ probe recognizes all of the restriction fragments detected by the intact cDNA, P2A7; the 3′ probe detects only one. The sizes of the λ/*Hin*dIII marker fragments are ≈23 kb, 9·4 kb, 6·6 kb, 4·4 kb, 2·3 kb and 2 kb.

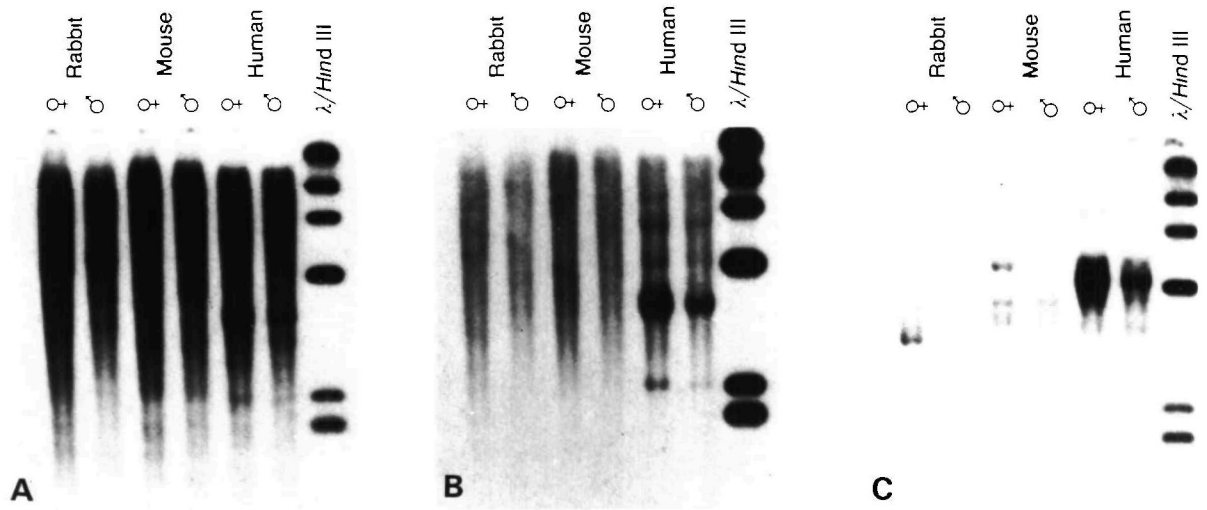

Fig. 5. Southern blot of human, mouse and rabbit genomic DNA digested with *Eco*RI. (A) The blot was hybridized and washed with P2A7 under conditions of low stringency (hybridization was at 55°C and washing was carried out at room temperature using 2 × SSC). (B) Following autoradiography the filter was washed under stringent conditions (0·1 × SSC, 64°C) and re-exposed. (C) The same filter probed with the factor IX cDNA probe cVII (Anson *et al.* 1984) and washed under stringent conditions (0·1 × SSC, 64°C).

hybridization under conditions in which significant homology can be detected between human and mouse factor IX coding sequences.

The observation of *STS* gene sequences located on the long arm of the Y chromosome is intriguing. It is unlikely that they result from viral retroposition as presumptive intron sequences have been detected on the Y chromosome. Furthermore, it appears that there are additional sequences unrelated to *STS* which show a similar distribution on the X and Y chromosomes, i.e. Xp22.3 and Yq11 (Goodfellow, Davies & Ropers, 1985). The simplest explanation for the apparent differences in the organization of the *STS* gene in mice and humans is that the human Y chromosome has rearranged since their divergence and reflects a rearrangement from an ancestral sex chromosome organization in which both the X and Y alleles were functional and whose identities were maintained by crossing-over (Craig, Levy & Fraser, this symposium).

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Since this paper was submitted Yen *et al.* (*Cell* **49**, 443–454 (1987)) have described similar X and Y chromosomally located sequences for STS and their work confirms the surmised orientation of the *STS* gene shown in Fig. 1.

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