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Mapping the H-Y gene

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Summary

This paper uses cytotoxic and proliferative T cell clones specific for H-Y and restricted by MHC molecules to type mice and humans inheriting incomplete portions of the Y chromosome. The data have allowed us to map the H-Y antigen gene *Hya* in mouse to a position closely linked with, but separable from, *Tdy* on the *Sxr* fragment and thus presumably to a position of the normal mouse Y chromosome near the centromere. The human H-Y gene maps between deletion intervals 4B and 7, separate from *TDF* which is on interval 1. We are currently testing cells from a number of additional patients who have inherited different portions of the Y chromosome to pinpoint the mapping more closely. It is of interest that in mouse a Y-linked gene controlling spermatogenesis (*Spy*) maps near *Hya* on the *Sxr* fragment: they could be the same or closely linked genes. In man, a gene controlling spermatogenesis maps to Yq and the data so far do not exclude that it could be coincident with the H-Y gene.

Key words H Y gene cytotoxic T cell, sex reversed mice sex reversed humans

Introduction: T cell recognition of H-Y

The male specific transplantation antigen, H-Y, is controlled by a gene located on the Y chromosome in both humans and mice H-Y is a member of a family of minor histocompatibility (H) antigens, each characterized by their ability to stimulate certain immune responses of T lymphocytes (Loveland & Simpson, 1986) At one time, the examination of H Y expression was limited to grafting experiments but since the advent of methods for generating specific cytotoxic and proliferative T cell responses *in vitro* and of maintaining these as cloned lines following the introduction of T cell growth factors, H Y expression can be tested *in vitro* as well (Simpson McLaren, Chandler & Tomonari, 1984, Simpson *et al* 1987) This approach has been particularly useful for examining the H-Y phenotype of individuals from outbred populations who are not so amenable to the grafting approach One constraint on such *in vitro* testing with H-Y specific T cells is the need to identify the major histocompatibility complex (MHC HLA in man, H-2 in mouse) alleles of the individual to be typed, since the recognition of H-Y, like other minor H antigens, is MHC restricted (Simpson & Gordon 1977) T cells recognize H Y only when it is associated with a particular self-MHC allele, so an appropriate panel of H Y-specific T cells in necessary to H Y type individuals of different MHC allotypes

H-Y expression in sex-reversed mice

H-Y typing of mice is simpler than that of man because of the ease of preparing H-Y specific T cells restricted by all of the common H-2 haplotypes using inbred mouse strains (Simpson, 1982) Female mice of inbred strains of appropriate H 2 type can be selected for immunization with H-2 compatible male cells and from these either *in vitro* bulk cultures of cytotoxic T cells or T cells cloned from these can be prepared for H-Y phenotyping the mice of interest Examples of the MHC restriction and H Y specificity of cytotoxic T cells from mixed lymphocyte cultures (MLC) of C57BL/10 (H 2^b) and C57BL/10 ×

CBA)F_1 (H-$2^{b/k}$) females immunized with (C57BL/10(H 2^b) and CBA (H 2^k) male cells, respectively, are given in Table 1 (Simpson, 1982) Table 2 shows the MHC restriction and H-Y specificity of proliferative T cell clones isolated from similar MLC using spleen cells from C57BL/6 (H-2^b) and C3H (H-2^k) female mice immunized with syngeneic male cells (Simpson, 1985) H-Y specific cytotoxic T cells and clones were used to type cells from a panel of mice carrying the sex-reversing mutation *Sxr* (Table 3) These include XX*Sxr* males and T16HX*Sxr* females carrying the T16H, X-autosome translocation, which is invariably active, so that the X*Sxr* of paternal origin is inactive This permits the female development of these individuals, since *Sxr* is presumably inactive in the majority of cells, at least during gonadogenesis (McLaren & Monk, 1982) The results in Table 3 indicate that each of the XX*Sxr* and XY males were H-Y positive with the cytotoxic T cells and T cell clones appropriate for their H-2 haplotype These mice are from a noninbred colony in which H-2^k and H-2^b are segregating Each of the XX females is H-Y negative, whilst of the nine T16HX*Sxr* females, eight are clearly H-Y positive, indicating that in adult life, at least, the gene controlling expression of the H-Y antigen, *Hya*, on *Sxr* is expressed in some spleen cells The ninth mouse, number 39, was phenotypically H Y negative she was subsequently progeny tested (T16H*Sxr* females, unlike XX*Sxr* males, are fertile) and since all of the non-XY progeny inheriting her *Sxr* were H-Y negative, it was clear that a mutation had altered her *Sxr* fragment This variant is now designated *Sxr'* (McLaren *et al* 1984) XO*Sxr'* male mice are also H-Y negative when tested by T cells *in vitro* so that XX*Sxr'* and T16HX*Sxr'* mice are not H-Y negative merely because Sxr' in them is inactivated

Table 1. *H-Y responses in H-2^b homozygotes and H-$2^{b/k}$ heterozygotes*

Responder female	H 2 K	A	E	D	Priming and boosting antigen	Target cell	H 2 K	A	E	D	Corrected* % lysis	Restricting specificity for H Y recognition
B10	b	b	(b)	b	B10♂	B10♂	b	b	(b)	b	*33 3*	
						B10♀	b	b	(b)	b	2 5	
						C3H♂	k	k	k	k	7 3	
						C3H SW♂	b	b	(b)	b	*38 5*	H 2D^l
						B10 A(2R)♂	k	k	k	b	*30 6*	
						B10 A(2R)♀	k	k	k	b	2 2	
						B10 A(5R)♂	b	b	b	d	3 9	
(B10 × CBA)F_1	b	b	(b)	b/	CBA♂	CBA♂	k	k	k	k	*31 1*	
	k	k	k	k		CBA♀	k	k	k	k	2 4	
						B10 A♂	k	k	k	d	4 6	H 2D^k
						C3H OH♂	d	d	d	k	*35 1*	
						B10♂	b	b	b	b	1 2	

Per cent specific lysis of target cells at A T/4 1 as determined from a four point regression curve

Table 2. *Proliferative responses of H-Y-specific T cell clones*

Stimulating cells (KID)		Clone (origin and restriction specificity) 2 1 1(B6 A^l)	10 2(B6 D^l)	C 3(C3H D^l)
None		199	541	
C 57BL/6♂	bbb	*26637*	*241 455*	
C57BI /6♀	bbb	389	1 988	
B10 A(5R)♂	bbd	*31 085*	3 558°	
B10 A(4R)♂	kkb	219		
B10 A(2R)♂	kkb		*270 175*	
bm12♂	bb b	177	*172 956* †	
bm14♂	bbb		5 025	
CBA♂	kkk			*108 970*
CBA♀	kkk			557
C 3H OH♂	ddk			*176 428*
B10 A♂	kkd			648

Data from a separate experiment in which the stimulation by C 57BL/6♂ was 65 434 and medium only was 1575
† From a separate experiment in which addition of C 57BL/6♂ gave 287 737 cts min^{-1} and medium alone gave 1910
Data from Tomonari (1983)

(Simpson, 1986) XX*Sxr'* and T16HX*Sxr'* mice are also H-Y negative when tested for its presence by transplantation, arguing for the identity of H-Y detected by these two methods, one *in vitro* and one *in vivo*, and for the absence of H-Y antigen from all cells in the body (Simpson *et al* 1986) *Sxr'* has lost *Hya* or the ability to express this gene, but still causes sex reversal in XX*Sxr'* males, therefore the Y-chromosome-associated testis determining gene *Tdy* on *Sxr* is clearly separated from *Hya* by this mutation, although the two genes are closely linked on *Sxr* and therefore presumably on the portion of the normal Y chromosome, close to the centromere, where *Tdy* and *Hya* are normally located (Simpson, 1986) Another mutation which provides evidence for the linkage of *Tdy* and *Hya* is Y^{h} described by Eicher & Washburn (1986) Y^{1} is apparently a rearranged Y chromosome in which the pairing region is located close to the centromere amongst the sperm generated by carrier males is an X^{Y}, bearing a paternal X to which the greater part of the Y is attached The XX^{Y} mice created by the fertilization of a normal X bearing ovum with such a sperm are H Y positive and phenotypically male, with aspermatogenic testes (like XX*Sxr* Simpson *et al* 1983)

H-Y expressed in sex-reversed humans

The investigation of the position of the human H-Y gene on the Y chromosome has produced findings which are in parallel with those of mice, since they clearly separate the testis-determining factor, *TDF*, from the H-Y gene, but in man the linkage between these two genes, unlike mouse, is not at all close (Simpson *et al* 1987)

H-Y typing in man is possible because of the isolation of T cell clones specific for H-Y from transfused spontaneously recovered female aplastic anaemia patients (Goulmy, 1985) Clones currently available are either HLA-A2 or HLA-B7 restricted, so this limits our ability to type cells from individuals carrying one or both of these alleles, fortunately, this includes more than 50 % of the population For the localization of the H Y gene in man, potentially informative patients are those who have inherited a partly deleted paternal Y chromosome or a translocated Y chromosome fragment Such patients are in two phenotypic categories XX males and XY females The six males described here have inherited variable portions of Yp whilst the two females possess Yq and a variable portion of the Yp Table 4 shows the results of HLA and H-Y typing lymphoblastoid B

Table 3. *H Y typing by CML and proliferation of H Y specific clones of normal mice and of mice of both sex phenotypes carrying* Sxr

Cells added from mouse	Proliferation of H Y specific clone (restriction specificity)			CML typing with		H 2 type	H Y type
	C 3(D^{k})	10 2(D^{1})	2 1(A^{1})	anti H Y^{k}	anti H Y^{1}		
None	1 016	301	765				
30 XX♀	2 174	1 078	197	0 9	1 1	k	–
32	929	1 312	489	−1 2	2 0	k	–
33	1 487	552	245	14 4	−7 7	k	–
34	591	649	3 932	3 0	1 7	k	–
4 I 16HX*Sxr*♀	*26 406*	651	3 252	*20 0*	8 5	k	+
13	*55 269*	379	3 086	*30 6*	6 4	k	+
35	*66 205*	826	518	*26 4*	8 4	k	+
36	*42 014*	1 531	2 526	*23 0*	−1 1	k	+
37	*46 753*	586	2 053	*23 3*	5 5	k	+
38	*64 040*	1 255	1 160	*29 4*	2 0	k	+
39	1 004	1 648	1 685	1 3	1 9	k	–
40	*61 249*	1 613	4 149	*25 2*	3 1	k	+
41	*47 145*	778	7 653	*25 8*	6 2	k	+
42 XX*Sxr*♂	*52 529*	1 229	704	ND	ND	k	+
43	*40 797*	341	225	ND	ND	k	+
47	899	*22 400*	ND	−0 7	*12 4*	b	+
31 XY♂	549	*32 610*	*30 472*	−3 7	*29 4*	b	+
45	3 178	1 451	579	*29 3*	2 7	k	+
46	*12 092*	635	30	*16 9*	1 1	k	+

For method of proliferation see legend for Table 2
CML % cytotoxicity at A 1/10 1 from 12 point regression analysis
H 2 type established with allospecific cytotoxic T cells
Summary of H Y typing with H Y specific cytotoxic T cells and H Y specific proliferative clones

cell lines from these patients and appropriate A2 and B7-positive normal male and female controls, with cytotoxic T cells It is important to confirm serological HLA typing with T cells, since variants of A2 and B7 exist which are not distinguishable serologically but which cannot be recognized by allospecific or MHC-restricted T cells (Horai, von der Poel & Goulmy, 1982) A negative H Y typing can thus only be interpreted as such in face of a positive allotyping for the restriction element with T cells (A2 or B7 in the case of individuals shown in Table 4)

The deletion map shown in Fig 1 is based on Vergnaud *et al* (1986), Disteche *et al* (1986) and Page (1986) and includes the summarized H-Y results of Table 4 as well as unpublished data on class I XX males Since six class 3 males were H-Y negative it is clear that the gene for H-Y does not map to deletion interval 1–3 on Yp (*TDF* is in interval 1, see also Affara *et al* 1986) Likewise the gene for H Y is excluded from interval 4A, since the class 2 XY female is H-Y positive and lacks this portion of Yp The H-Y gene thus maps between intervals 4B and 7, far from *TDF* in interval 1

Conclusion

In summary, these data, using cytotoxic and proliferative T cell clones specific for H-Y and restricted by MHC molecules to type mice and humans inheriting incomplete portions of the Y chromosome, have allowed us to map the H-Y antigen gene *Hya* in mouse to a position closely linked with, but separable from, *Tdy* on the *Sxr* fragment and thus presumably to a portion of the normal mouse Y chromosome near the centromere The human H Y gene maps between deletion intervals 4B and 7, separate from *TDF* which is on interval 1 We are currently testing cells from a

Table 4. *HLA and H-Y typing of B cell lines from XX males, XY females and normal controls*

Exp	Karyotype/ sex	Individual	HLA* serology A	HLA* serology B	αA 2†	αH Y/A 2†	H Y phenotype
1	XX♂	RH	*2 3*	21 40	*18*	9	–
	XX♂	JT	*2*	44 45	*24*	3	–
	XX♂	LGL 105	*2 3*	35 44	*13*	4	–
	XY♂	Normal male	1 *2*	8	*20*	*38*	+
	XX♀	Normal female	*2* 11	8 44	*17*	8	–
2	XX♂	WB	*2* 9	17 18	*37*	0	–
	XY♂	Normal male	1 *2*	8	*25*	*17*	+
	XX♀	Normal female	*2* 11	8 44	*17*	3	–
					αB7	αH Y/B7	
3	XX♂	WHT 950	1 3	*7*	*76*	9	–
	XX♂	JM	3 28	*7*	*62*	1	–
	XY♂	Normal male	9	*7* 44	ND	*40*	+
	XX♀	Normal female	3 24	*7*	*54*	0	–
4	XY♀	WHT1003 (case 1)	3	*7* 13	*55*	*70*	+
	XY♀	WHT 715 (case 2)	3	*7*	*57*	*69*	+
	XY♂	Father of case 2	28 3	*7* 40	*52*	*61*	+
	XX♀	Mother of case 2	*29*	*7*	*36*	6	—

†H A serology performed by Lorna Kennedy at ICRF Lincoln s Inn Fields or Donald Palmer of Dept Immunology RPMS
Per cent specific lysis of target cells at A T/10 1 as determined from a 6 point regression curve
Modified from table 1 Simpson *et al* 1987

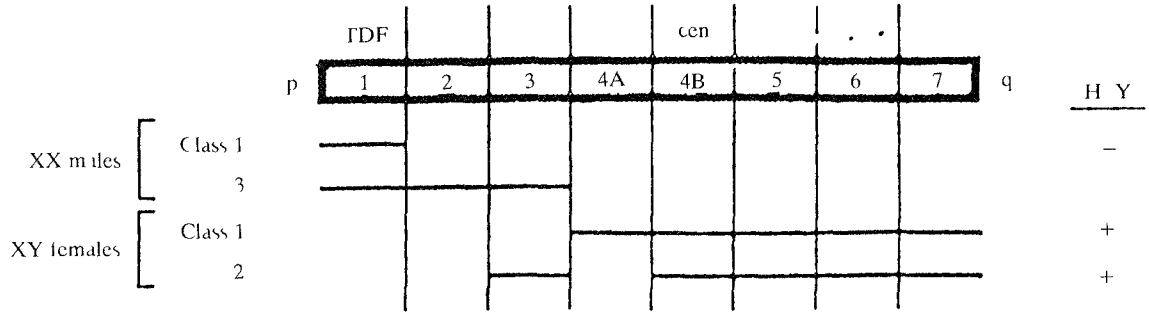

Fig 1 8 interval deletion map of the human Y chromosome (based on Page 1986)

number of additional patients who have inherited different portions of the Y chromosome to pinpoint the mapping more closely It is of interest that in mouse a Y-linked gene, *Spy*, controlling spermatogenesis maps near *Hya* (Burgoyne, Levy & McLaren, 1986, for discussion see Burgoyne, this symposium) on the *Sxr* fragment they could be the same or closely linked genes In man, a gene controlling spermatogenesis maps to Yq (Tieopolo & Zuffardi, 1976), and the data so far do not exclude the possibility that it could be coincident with the H-Y gene

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