Fig. 4 The effect of spatial frequency on phase discrimination. *a*, Foveal phase discrimination thresholds: O, $F = 4$ c per deg, $C_F = 10\%$; □, $F = 8$ c per deg, $C_F = 12.5\%$; △, $F = 12$ c per deg, $C_F = 15\%$. Sensitivities to 0–180 and 90–270 shifts were approximately similar at all spatial frequencies. *b*, Discrimination thresholds at 40°: O, $F = 0.5$ c per deg, $C_F = 10\%$; □, $F = 0.167$ c per deg, $C_F = 15\%$. Lowering spatial frequency does not alter the pattern of results. In particular, it was impossible to discriminate a 90–270° phase shift at both spatial frequencies. Note the change of scale on both axes between *a* and *b*.

mechanism, and a corresponding inability to pick up one class of phase information. This hypothesis is difficult to test currently because recognition and identification models are not sufficiently detailed to make quantitative predictions. However, even and odd mechanisms have been postulated in quantitative models of foveal grating detection and discrimination[15-20], motion discrimination[21-23], and the perception of Mach bands[24]. If the mechanisms proposed in these models are the same as the even and odd mechanisms of our two-channel model, we predict that particular peripheral deficits will be observed in these visual processes. Such a prediction for the perception of Mach bands was confirmed recently[24]. It remains to be seen if the other predictions are confirmed.

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Separation of the genetic loci for the H–Y antigen and for testis determination on human Y chromosome

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The mammalian Y chromosome encodes a testis-determining factor (termed TDF in the human), a master regulator of sex differentiation. Embryos with a Y chromosome develop testes and become males whereas embryos lacking a Y chromosome develop ovaries and become females. Expression of H–Y, a minor histocompatibility antigen, may also be controlled by a gene on the Y chromosome, and it has been proposed that this antigen is the testis-determining factor[1]. We have tested the postulated identity of H–Y and TDF in the human. H–Y typing with T cells was carried out on a series of sex-reversed humans (XX males and XY females), each shown by DNA hybridization to carry part but not all of the Y chromosome. This deletion analysis maps the gene for H–Y to the long arm or centromeric region of the human Y chromosome, far from the TDF locus, which maps to the distal short arm.

H–Y typing of sex-reversed mice has shed light on the relationship of this antigen to gonadal sex determination. XX *Sxr* (sex reversed) mice are both male and H–Y positive owing to the presence of a small portion of the Y chromosome[2,3], consistent with a testis-determining role for H–Y. However, a derivative of *Sxr* known as *Sxr'* produces XX *Sxr'* and XO *Sxr'* mice that are phenotypically male but H–Y negative[4,5], demonstrating that the antigen is not required for testis determination.

In humans, DNA hybridization studies using Y-specific probes of individuals with only parts of the normal Y chromosome have yielded an eight-interval deletion map of the Y chromosome. Such studies of XX males and XY females that carry part but not all of the Y chromosome, have demonstrated that interval 1, representing a portion of the distal short arm of the Y, contains *TDF*[6,7]. Similar studies of another series of XX males, using a different set of Y-DNA probes, likewise show them to possess sequences both within and distal to Yp11.2 (refs 8 and 9). We reasoned that if there is a Y-chromosomal gene responsible for H–Y antigen expression then it should be possible to map that gene by H–Y typing individuals with well-characterized Y deletions.

H–Y-specific T-cell clones and lines have been derived *in vitro* from transfused human female aplastic anaemia patients[10]. These H–Y-specific T cells, whose response is HLA restricted, were used to H–Y type Epstein–Barr-virus-transformed B-cell lines from deleted-Y individuals. Because only HLA-A2 or B7-restricted H–Y-specific T-cell clones were available at the time of this study, we were able to H–Y type only those deleted-Y individuals who were HLA-A2 and/or -B7. Variants of HLA-A2 and -B7 which cannot be recognized by allogeneic or HLA-restricted T cells have been described[11]. Accordingly, in each case, the HLA type was determined serologically and confirmed using alloreactive cytotoxic T cells.

Table 1 shows the results of HLA and H–Y typing of eight deleted-Y individuals (six 46,XX males and two 46,XY females). All six XX males were negative for H–Y. These include three

Table 1 HLA and H–Y typing of B-cell lines from XX males, XY females and normal controls

Experiment	Karyotype/sex	Individual	HLA serology*	Specific lysis (%)		H–Y phenotype
				αA-2	αH–Y/A-2	
1	XX♂	RH	A2	18	9	–
	XX♂	JT	A2	24	3	–
	XX♂	LGL 105	A2	13	4	–
	XY♂	Normal male	A2	20	38	+
	XX♀	Normal female	A2	17	8	–
2	XX♂	WB	A2	37	0	–
	XY♂	Normal male	A2	25	17	+
	XX♀	Normal female	A2	17	3	–
				αB7	αH–Y/B7	
3	XX♂	WHT950	B7	76	9	–
	XX♂	JM	B7	62	1	–
	XY♂	Normal male	B7	ND	40	+
	XX♀	Normal female	B7	54	0	–
4	XY♀	WHT 1003 (case 1)	B7	55	70	+
	XY♀	WHT 715 (case 2)	B7	57	69	+
	XY♂	Father of case 2	B7	52	61	+
	XX♀	Mother of case 2	B7	36	6	–

The H–Y-specific cytotoxic T cells were T-cell lines or clones derived from the peripheral blood of transfused female aplastic anaemia patients[10]. The alloreactive cytotoxic T cells were derived from the peripheral blood of healthy individuals[11]. They were used in the cytotoxic assays 5–7 days after aliquots previously stored in liquid nitrogen had been thawed and restimulated *in vitro* with appropriate antigen bearing irradiated peripheral blood cells in the presence of interleukin-2 (IL-2) in RPMI medium containing penicillin, streptomycin, glutamine, HEPES and 10% fetal calf serum (FCS) or human serum. The target cells were Epstein–Barr virus (EBV)-transformed B-cell lines grown *in vitro*. Immediately before the assay, aliquots were labelled with ^{51}Cr-labelled sodium chromate, washed and dispensed at 5×10^3 cells per well in round-bottomed microtitre wells. Triplicate wells were used at each A: T ratio[3,4,14]. Per cent specific lysis given is that at an attacker-to-target ratio of 10:1, the figure being taken from a regression analysis of a three or four point titration curve. Underlined figures are positive values on titration curves whose r^2 value lay between 0.70 and 1.00 (refs 3, 4 and 14). ND, Not done.
   * HLA serology of the B-cell lines from sex-reversed patients and the parents of one was performed either by Lorna Kennedy at the ICRF Lincoln's Inn Field, or by Donald Palmer in the Department of Immunology, Hammersmith Hospital. Normal male and female cells in experiments 1–3 were EBV-transformed B-cell lines from Professor A. Rickinson of Birmingham: the individuals from which they had been obtained were serologically identified as A2 or B7 by him.

XX males, LGL105, WHT950 and WB, who carry intervals 1, 2 and 3 of the Y chromosomal short arm (refs 6 and 12; D.C.P. unpublished results; see class 3 XX males in Fig. 1) and three XX males, RH, JT and JM, with similar portions of the Y short arm detected using a different set of Y-DNA probes[8,9]. Whereas *TDF* is in interval 1 of the Y chromosome[6,7], these results indicate that the gene for H–Y lies outside intervals 1, 2 and 3.

This conclusion is reinforced by the finding that both XY females are positive for H–Y. XY female WHT1003 has a deletion of intervals 1, 2 and 3 on the Y short arm, whereas XY female WHT715 has a deletion of intervals 1, 2 and 4A (refs 7 and 13; see class 1 and 2 XY females in Fig. 1). Taken together, these results with XX males and XY females strongly suggest that the gene for H–Y maps somewhere in intervals 4B to 7 of the Y chromosome, that is, near the centromere or on the long arm (refs 6 and 7; Fig. 1).

The finding that XX *Sxr'* male mice were H–Y negative strongly suggested that H–Y is not the testis determinant. However, it might be that the *Sxr'* mutation had affected the antigenicity but not the testis-determining capability of H–Y. There is an earlier report of human XX males with negative H–Y type and an XY female with positive H–Y type with cytotoxic T cells[14]. However, the XX males had not been tested for the presence of Y-chromosomal DNA and the XY female had not been tested for deletions of Y DNA sequences. The results reported here are not subject to such ambiguities of interpretation. In the present study, H–Y typing was carried out on cells from eight sex-reversed individuals with well-characterized deletions of portions of the Y chromosome. The combination of negative and positive findings reported here demonstrates unequivocally that the gene for H–Y maps far from interval 1, which contains *TDF* and that H–Y antigen is not the testis-determining factor. H–Y antigen was originally defined by graft rejection[15]. The *in vitro* T-cell assays of the type used for H–Y

typing in this study have been shown to recognize the same histocompatibility antigen as that defined by graft rejection[16]. In contrast, serological identification of a male antigen assumed to be H–Y (and now called serologically detected male antigen, SDM) has been problematical. In particular, there is doubt concerning whether the SDM antigen is identical to the H–Y histocompatibility antigen[5,17]. The implications of the present study, therefore, are limited to the H–Y antigen and do not extend to the SDM antigen.

We are currently examining additional deleted-Y individuals in an effort to map the H–Y locus more precisely. The possibility that the H–Y gene might be identical to or near a spermatogenesis factor on the long arm of the human Y chromosome[18] is of particular interest, because in mice H–Y (or a closely linked gene) has been implicated in spermatogenesis[19]. Though the results reported here imply that H–Y has no function in gonadal sex determination, it may be involved in testis function at a later stage of development.
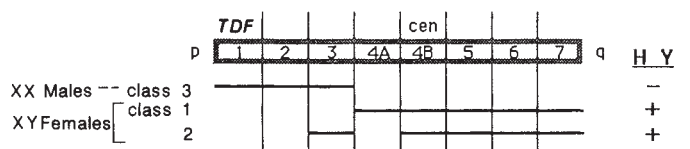

Fig. 1 Eight-interval deletion map of the human Y chromosome (based on refs 7 and 13). The results of H–Y typing of individuals (from Table 1) are summarized. The short arm, centromere and long arm of the Y chromosome are denoted by, respectively 'p', 'cen' and 'q'. *TDF* gene for testis determining factor. Horizontal lines, portions of the chromosome present in the designated individual.

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Tumour prevention and rejection with recombinant vaccinia

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Tumour-specific antigens (TSA; ref. 1) have been exploited in the diagnosis[2] and imaging[3] of human cancer and anti-TSA antibodies have therapeutic potential[4]. Vaccination with TSA[5–8] or anti-idiotypic (TSA) antibodies[9,10] has also been used to control tumour growth in model systems. An effective immune response nevertheless demands copresentation of antigen with host histocompatibility determinants[11]. We therefore examined whether live vaccinia virus recombinants expressing TSA in cells of the vaccinated host might better elicit tumour immunity. Polyoma virus (PY) is tumorigenic in rodents; because killed PY-transformed cells can elicit tumour immunity, a PY-specific TSA has been postulated[12–14]. Tumorigenesis involves expression of three early PY proteins[15–17], large-T (LT), middle-T (MT) and small-T (ST), but their role as TSAs is unclear. We therefore expressed the three T proteins in separate vaccinia recombinants. Rejection of PY tumours was observed in rats immunized with recombinants expressing either LT or MT. Further, tumour-bearing animals could be induced to reject their tumours by inoculation of recombinants.

Live vaccinia recombinants[18,19] expressing foreign antigens have been used successfully in immunization experiments (see ref. 20 for review) and we have previously applied this approach to a rabies vaccine[21,22]. Vaccinia seemed appropriate for the expression of tumour antigens because recombinants can prime a cell-mediated immune response[22–25] important in tumour cell elimination[26,27].
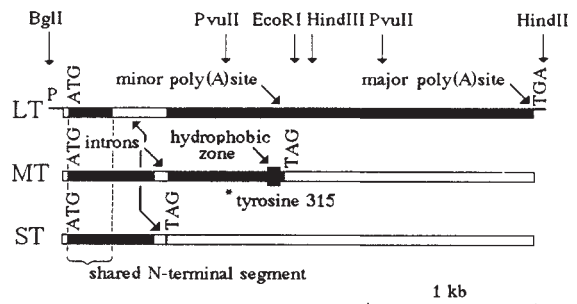

Fig. 1 Polyoma early region and cloning of coding sequences into vaccinia virus. Schematic representation of the PY early region extending from the unique *Bgl*I site to the *Hind*II site (adapted from ref. 17). The promoter for early transcription (P), the translation initiation (ATG) and termination (TGA,TAG) signals and the polyadenylation sites are shown. Solid bar, coding regions; open bar, introns (excised from mature messenger) and untranslated regions. The zone encoding the carboxy-terminal hydrophobic, potentially transmembrane, region of MT is indicated. Tyr 315 is the major site at which MT is phosphorylated in PY-transformed cells.

Methods. For construction of recombinants, intron-free coding sequences for the three T proteins were excised from plasmids pPY-LT1, pPY-MT1 and pPY-ST1 (ref. 29) with *Bgl*I at one end and *Hind*II, *Eco*RI and *Pvu*II respectively at the other. Using a single-stranded oligonucleotide adaptor converting the *Bgl*I extremity to compatibility with ends generated by *Bam*HI cleavage, the three coding sequences were separately introduced between the *Bam*HI-*Sma*I, *Bam*HI-*Eco*RI and *Bam*HI-*Sma*I sites respectively of plasmid ptg186-poly[47], which is ptg1H-TK-7.5K (ref. 21) in which a polylinker has been introduced downstream of the vaccinia 7.5K gene promoter in the body of the viral thymidine kinase (TK) gene. Procedures for the propagation and purification of vaccinia virus and for transfer of coding sequences and accompanying promoter to the vaccinia genome were as described[21].

In the PY genome the genes for LT, MT and ST overlap and messenger RNAs are generated by differential splicing (Fig. 1). DNA sequences separately encoding the three T proteins have been constructed[28,29] and we introduced these separately downstream of the promoter from the vaccinia 7.5K gene. Recombination *in vivo* was used for transfer to the vaccinia genome, generating strains labelled VVpyLT, VVpyMT, and VVpyST according to the T protein concerned.

To confirm the authenticity of the recombinant T species, proteins synthesized on infection of tissue-culture cells with the recombinants were examined by Western blotting for reaction with anti-T serum. Protein bands recognized by anti-T serum had electrophoretic mobilities expected for authentic PY proteins (Fig. 2). In all cases the recombinant T proteins possess biochemical characteristics associated with the cognate PY polypeptides (P.C., I.G., M.P.K. and R.L., in preparation).

To demonstrate appropriate sub-cellular distribution of the three T proteins, infected cells were examined for reaction with fluorescently labelled anti-T serum. Despite alterations in cellular morphology on vaccinia infection, the LT protein was, as expected[30,16], observed only in the nucleus (dark nucleoli, Fig. 3a) whereas the ST species was cytoplasmic (Fig. 3c). Fluorescence in cells infected with VVpyMT revealed cytoplasmic aggregates (Fig. 3b) as well as perinuclear staining that may suggest an association with intracellular membranes[31,32]. Cell-surface MT[14,33] was not observed.

Rats inoculated subcutaneously with PY-transformed syngeneic rat cells rapidly develop tumours. We sought to determine whether prior administration of the vaccinia/polyoma recombinants blocked tumour development. Animals were inoculated with recombinant virus, boosted, and seeded with syngeneic PY-transformed cells (Table 1 legend). In view of a possible cumulative action of the different T proteins (see, for