H-Y Antigen: Behavior and Function

A cell surface component of vertebrates may be directly involved in primary sex determination.

Willys K. Silvers and Stephen S. Wachtel

Through repeated brother-sister matings one is able to develop genetically uniform strains of laboratory animals, and it has become a rule of transplantation biology that grafts of skin and other tissues are accepted when exchanged among the members of such strains (*1*).

In many species there is an exception to this rule. This exception, which was first noted in mice (*2*), is due to the presence of histocompatibility Y antigen (H-Y) in the tissues of males. Thus, when females are challenged with intrastrain (syngeneic) male grafts (also called male isografts) their capacity to react against the "foreign" male antigen often culminates in the destruction of the graft. Because H-Y is a representative "weak" transplantation antigen, and because it has been implicated in sex determination, it has been investigated extensively (*3*). The following is a summary of some of these investigations and their biological implications.

Influence of Male Hormone on H-Y

Although rejection of intrastrain male-to-female grafts is probably due to a histocompatibility antigen determined by the Y chromosome, it could be argued that the antigen is determined by an autosomal gene that functions only in the presence of male hormones. If H-Y were autosomally determined, one might expect that it would be synthesized in female skin transplanted to adult males, but there is little evidence that this occurs. If skin from newborn females is transplanted to adult males of the same strain and maintained there for 100 days, a period which should afford ample opportunity for H-Y to develop (assuming that hormonal factors are involved in its induction), the antigen is not synthesized in a form or quantity sufficient to elicit graft rejection. When these long-standing neonatal female grafts are excised from their male hosts and transferred to normal syngeneic females, or to specifically sensitized (male-grafted) females, the grafts are permanently accepted (*4*). The hormonal hypothesis is also inconsistent with (i) the observation that male hemopoietic cells which colonize lethally irradiated female mice continue to express H-Y (*5*) and (ii) with the observation that H-Y is expressed on 50 percent of eight-cell mouse embryos (*6*). Moreover, attempts to induce H-Y expression in tolerant females by implanting testes have failed (*7*).

Further evidence against male hormone playing a direct role in the expression of H-Y comes from studies of the X-linked testicular feminization mutant (*Tfm*) in the mouse (*8*). Since this condition is caused by a failure of target tissues to respond to testosterone, mice with this mutation (X^{Tfm}/Y) when typed should be H-Y positive ($H\text{-}Y^+$) only if expression of H-Y is independent of androgen. To test this hypothesis Bennett *et al.* (*8*) challenged C57BL/6 (B6) female mice (known rejectors of male skin) with X^{Tfm}/Y skin grafts. After these grafts were sloughed, the females were challenged with skin grafts from B6 males. The accelerated destruction (second set rejection) of the B6 male skin grafts showed that the X^{Tfm}/Y skin possessed H-Y. Other (serological) assays confirmed the $H\text{-}Y^+$ status of X^{Tfm}/Y mice. Perhaps the best evidence that expression of H-Y is not dependent on male hormone is its presence on female cells in those species in which the female is the heterogametic (XY) sex, as is discussed below (*9*).

Dr. Silvers is professor of human genetics at the University of Pennsylvania, School of Medicine, Philadelphia 19174, and Dr. Wachtel is an associate at the Memorial Sloan-Kettering Cancer Center, New York 10021.

Genetic Determination of H-Y

The experiments cited above indicate that male hormone is not essential for expression of H-Y antigen, but they provide no evidence whether the *H-Y* gene is Y-linked or autosomal. Although the first experiments concerned with this question were inconclusive, they did rule out the possibility that expression of H-Y antigen resulted from the presence of only one X chromosome, or that its synthesis was suppressed by two X chromosomes. In these experiments (*10*) spleen cells from an XXY male mouse and from XO female mice were inoculated into normal females from a rejector strain. The rejector females were then challenged with syngeneic male spleen cells from donors that had been sensitized to rat erythrocytes. The impaired production of antibody to erythrocytes by these transferred male cells, an indication of their accelerated destruction, was to be taken as evidence of prior sensitization of the hosts to H-Y. Cells from the XXY male immunized these females to syngeneic male spleen cells, but cells from XO females did not.

The best early indication that a genetic determinant for H-Y is Y-situated was the report that testicular teratomas that retain the Y chromosome express H-Y, whereas those which lose it do not (*11*).

It is apparent that if a structural or regulatory gene for H-Y is on the Y chromosome of mice, all animals lacking this chromosome should fail to express the antigen. However, XX mice carrying the autosomal dominant "sex-reversed" (*Sxr*) are male in phenotype (although infertile with small testes), and these animals type $H\text{-}Y^+$ (*12*). Those who believe that the *H-Y* gene is autosomal cite this in their favor, but it cannot be ruled out that a piece of the Y chromosome too small to be seen cytologically occurs in the cells of these XX males, perhaps as an undetected Y-to-autosome translocation.

Identity of H-Y in Mice

An interesting feature of H-Y immunology is the interstrain variation in male-to-female graft rejection patterns. Although female mice of some strains regularly reject male skin isografts, females of other strains usually accept such grafts (*3*). This interstrain diversity occurs even though the specificity of H-Y appears to be similar in male mice of all stocks. Grafts exchanged between reciprocal F_1 hybrid males, that is, males

which are genetically identical except for the origin of their X and Y chromosomes, are accepted permanently (*13, 14*) (unless the two strains are known to bear different X-linked histocompatibility alleles). The antigenic similarity of H-Y in all male mice is implied also by the fact that male skin isografts are rejected in second-set fashion by females of a rejector strain when the females are first exposed to male cells from any strain (*15, 16*). Indeed this accelerated reaction served as the basis for the experiments of Bennett *et al.* (*8*) and of Celada and Welshons (*10*) noted above. The technique of immunological tolerance induction in neonatal mice has also been employed to demonstrate lack of variation of H-Y antigen among mouse strains (*17, 18*). When female mice of a rejector strain are inoculated at birth with male (but not female) cells from any of a variety of other strains, the recipients permanently accept male skin isografts (*19*).

Genetic Basis of Response to H-Y

If the specificity of H-Y antigen is the same in all mice, we then must ask what is responsible for the interstrain variation in the rejection of male-to-female grafts. Studies of male graft rejection in several mouse strains indicate that H-Y rejector strains are identical at the major histocompatibility complex (MHC) (*20*), that is, they have the same H-2 genotype. Females of the C57BL/10J, C57BL/6Ss, C57L/J, LP/J, and 129/J inbred strains, all of which are *H-2*^{b}, almost invariably reject male skin grafts (*21*), whereas females of such strains as C3H/HeJ (*H-2*^{k}), CBA/Ss (*H-2*^{k}), C57BR/cdJ (*H-2*^{k}), SWR/J (*H-2*^{q}), AU/SsJ (*H-2*^{q}), A/Ss (*H-2*^{q}), BALB/c (*H-2*^{d}), and DBA/2J (*H-2*^{d}) are much more likely to accept male skin grafts (*13, 17, 21, 22*). Although this indicates that the MHC somehow determines the ability to reject H-Y incompatible grafts, experimental confirmation of this proposal necessitated utilizing congenic strains of mice—that is, strains which were genetically identical, except for that region of their 17th chromosome which includes the MHC. For example, C57BL/10 (B10) and B10.BR mice are genetically identical, except that B10 mice are *H-2*^{b} and B10.BR mice are *H-2*^{k}. By the same standard, C3H and C3H.SW mice are "identical," except that C3H mice are *H-2*^{k} and C3H.SW mice are *H-2*^{b}. It follows that, if the MHC is involved in determining reactivity to H-Y–incompatible grafts, then B10 females should reject male skin isografts more often than B10.BR females and C3H.SW females should reject such grafts more frequently than C3H females. This is the case (*23*). It should be noted that, although the MHC type of the female determines the speed and vigor of rejection of male grafts, H-2 is not the only factor involved. Male skin isografts are rejected much more frequently by B10.BR females than by C3H females even though both are *H-2*^{k} and these grafts are not rejected as readily by C3H.SW females as by B10 females even though both are *H-2*^{b}. The basis for the involvement of the MHC in reactivity to H-Y antigen remains to be clarified. However, this complex includes genes that regulate immune responses (Ir genes) (*20*) and so it seems reasonable to speculate that specific H-Y receptors are coded by MHC-associated Ir genes.

So far we have presented evidence that genetic background (*H-2* type), can influence the ability of females to respond to H-Y antigen. There is another way in which genetic background affects the rejection of H-Y incompatible grafts, and that is by influencing the expression of the antigen itself. To study this effect (B6 × CBA) F_1 females were challenged with B6 or CBA male skin grafts. Because H-Y was the only foreign antigen in these transplants and because the evidence presented above indicated its specificity to be the same in all males, these grafts should have been rejected with equal promptitude unless their different genetic backgrounds could influence the expression of H-Y. The fact that the two kinds of parental strain grafts had significantly different survival times (*24*) demonstrated conclusively that expression of H-Y antigen in CBA and B6 males was not the same. Whereas half of the B6 grafts were accepted, all of the CBA grafts were rejected, indicating that H-Y is stronger in CBA male skin than it is in B6 male skin even though CBA females (*H-2*^{k}) are nonrejectors. In other words, the greater frequency with which B6 females destroy male skin grafts, as compared with CBA females, cannot be attributed to a stronger H-Y in B6 males, but rather to the superior ability of B6 females to respond to a cell surface component that is actually less immunogenic in B6 males than it is in CBA males.

This difference in the immunogenicity of H-Y is also associated with the MHC (*16*). This was determined by challenging (B10 × B10.BR) F_1 females with skin grafts from B10 or congenic B10.BR males. The median survival time (MST) of the B10 grafts (20.0 ± 1.4 days) was significantly longer than the MST of the B10.BR grafts (14.5 ± 1.3 days), indicating that H-2 was involved, and that *H-2*^{k} skin grafts are more antigenic than *H-2*^{b} skin grafts when only H-Y incompatibility is involved (*25*).

How the MHC influences the immunogenicity of H-Y antigen is unknown. On the one hand, there could be a functional relation between the different antigens such that the ontogeny of H-Y is dependent on H-2. Alternatively, the differences in survival of B10 and B10.BR male grafts might result from steric interference between H-2 cell surface components and the binding of neighboring H-Y sites to H-Y antibody receptors (*26*). Regardless of the mechanism, the effectiveness of graft rejection, in general, may depend not only on the ability of the host to respond to graft antigens, but also on the genetic background of the transplant donor.

H-Y in Other Species

Soon after the discovery of H-Y antigen in mice, a histocompatibility antigen associated with the Y chromosome was described in rats (*27, 28*). In this species, as in mice, the frequency of rejection of intrastrain male skin grafts varies from strain to strain, even though the specificity of rat H-Y appears to be the same in all strains. Because of the apparent lack of strain variation in the H-Y antigens of mice and of rats, studies were undertaken to determine whether the two antigens were related (*28*). Newborn B6 female mice were injected with male BN rat hemopoietic cells, and then challenged with B6 male skin grafts to determine whether tolerance to H-Y had been induced. In other experiments, adult B6 female mice were inoculated with suspensions of spleen cells from male BN donors and were then challenged with B6 male skin grafts to determine whether these recipients had been sensitized.

Although these experiments did not indicate any similarity between the male-specific antigens of the two species, more recent studies have shown that, if Fischer, Lewis, or BH male (but not female) rat lymphoid cells are inoculated into B6 females, the recipients give a second-set reaction when grafted later with B6 male skin, an indication that mouse and rat H-Y antigens are in fact related (*29*). It is perhaps noteworthy that, with one exception, male cells from all of the rat strains which sensitized female mice to male grafts were alike at their MHC (the *Ag-B* locus), and that

cells from male rats of other strains having different Ag-B genotypes, failed in this respect. The basis for this observation remains to be determined, but it cannot be due to H-Y polymorphism in rats because (BN × Fischer)F_1 hybrid cells (from an animal with a Fischer Y chromosome) were ineffective in sensitizing female mice to male skin grafts.

The fact that (BN × Fischer)F_1 hybrid cells behaved like BN cells may reflect differences in the ability of rat cells of different Ag-B genotypes to survive in mice. According to this hypothesis, *Ag-B*^{1}/*Ag-B*^{1} male cells of Fischer, Lewis, or BH origin persist sufficiently long in B6 female mice to sensitize them against H-Y, whereas male cells from rats of most other Ag-B genotypes are destroyed before sensitization to the relatively weak H-Y antigen can occur.

Evidence that H-Y might not be limited to laboratory rodents (*30*) came from the finding that chickens also have a sex-specific transplantation antigen (*31*). In this class of vertebrates, it is the female which expresses the antigen. However, in birds the female is the heterogametic sex (ZW) and the male is homogametic (ZZ). Thus skin grafts exchanged among inbred strains of chickens are rejected most often when the donor is a hen and the recipient a rooster. Once the occurrence of an antigen associated with the Y (W) chromosome of mice, rats, and chickens was demonstrated, it seemed reasonable to ask whether similar antigens might exist in the heterogametic sex of all species of vertebrates and, if so, whether these antigens might be related to H-Y of the mouse and of the rat. The lack of inbred strains made it impossible to assay for H-Y with skin grafts, and heterogametic cells from distant species, such as the chicken, could not be expected to survive long enough in mice to sensitize females to male skin grafts. Therefore cross-reaction between H-Y antigens of mouse and outbred species such as the chicken could not be demonstrated by means of the traditional assays of transplantation biology. If H-Y were to be demonstrated as a widely occurring antigen, newer procedures for its detection would have to be devised.

Serological assays based on the activity of antiserums to mouse H-Y with mouse sperm have proved particularly useful in this respect. This chapter in the history of H-Y opened in 1971 when Goldberg (*32*) was exploring technical modifications of the cytotoxicity assay that would allow its routine application to sperm cells. Having accomplished this for antiserums against MHC antigens of the mouse, she screened all available mouse antiserums to find out what known antigens might be expressed on sperm, including, in her survey, serums from B6 female mice that had rejected grafts from B6 males.

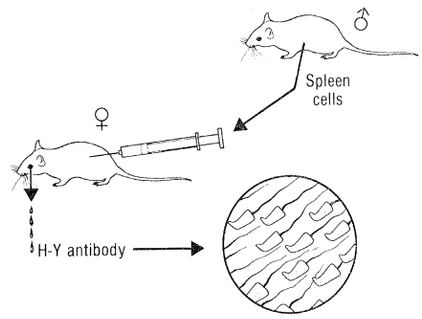

Fig. 1. Preparation of mouse H-Y antibody. Female C57BL/6 mice were injected intraperitoneally with cell suspensions prepared from the spleens of C57BL/6 males. After weekly inoculations for 4 to 5 weeks, the recipients were bled from the retroorbital plexus, and the serum was separated and stored at −70°C. In the standard cytotoxicity test, mouse sperm were incubated for 50 minutes with both H-Y antiserum and rabbit complement. Trypan-blue dye was added during the last 10 minutes of incubation to stain dead sperm (*44*).

Previous tests for H-Y antibody in such serums had been reported by others (*33*), but the reaction was either too weak to be reliable or too cumbersome for use in routine serology. However Goldberg *et al.* (*34*) found sperm to be satisfactorily and consistently sensitive to H-Y antibody in cytotoxicity assays with highly selected complement (a serum component necessary for antibody-mediated cell lysis). Specificity for H-Y was readily established by showing that male tissues absorbed out all cytotoxicity for sperm whereas female tissues did not (*35*).

The cytotoxicity assay with sperm remains one of the two standard means of H-Y typing (Fig. 1). The second is the MHA-HA (mixed hemadsorption–hybrid antibody) test, which is concerned with formation of "rosettes" rather than with lethality of target cells (*36, 37*).

The antiserum for the MHA·HA assay is the same as that used in the cytotoxicity tests, produced generally by inoculating B6 females with B6 male spleen cells. Sperm are exposed first to antibody to H-Y antigen (H-Y antibody), then to a hybrid antibody [made by uniting Fab fragments of rabbit antibody to mouse Ig, to Fab fragments of rabbit antibody to sheep red blood cells (SRBC)], and finally to SRBC. The SRBC link themselves to the anti-SRBC arm of the hybrid antibody, which is bound to H-Y antibody on the sperm by its antimouse Ig arm, thus forming a rosette. The result of the test is expressed in terms of counts of rosetted and nonrosetted sperm.

The development of the MHA-HA test was greatly facilitated by the technique of incorporating the reagents in three layers of a discontinuous density gradient interspersed with wash layers, and centrifuging the sperm down through the gradient (*36*). The repeated washings and centrifugations which would otherwise be necessary, and which are so detrimental to sperm, were thereby obviated.

A great deal of our work now centers on the H-Y typing of tissues from species other than the mouse. For this purpose H-Y typing is invariably done by absorption. Removal of the heteroantibody that hampers direct serological tests across species barriers is not, we find, routinely practicable. However, typing by absorption is entirely satisfactory, provided that proper control tissues, that is, of the opposite sex, are available, and fail to absorb H-Y activity. An advantage of typing by absorption is that contaminating sperm autoantibodies (of unknown specificity) which are reactive with antigens of both male and female mice are automatically removed. Our practice is to test the two absorbed portions of antiserum to H-Y (H-Y antiserum) (one absorbed with the cells to be typed and the other portion absorbed with control cells) in both the cytotoxicity and MHA·HA assays (Fig. 2). If the absorbing cells do not possess H-Y antigen, the activity of the antiserum is unaffected and it reacts with a significant proportion of the sperm as indicated by the uptake of trypan-blue dye by dead cells (in the cytotoxicity test) and by the formation of rosettes (in the MHA·HA test). On the other hand, if H-Y antibodies are removed from the antiserum during the absorption procedure (indicated by a fall in cytotoxic titer or in the frequency of rosettes), the absorbing cells must be H-Y$^+$.

Cytotoxicity tests and MHA·HA tests performed with H-Y antiserums absorbed with cells of the guinea pig, rabbit, human (*37*), wood lemming (*38*), and cattle (*12*) have revealed in each of these species a male-specific cell surface component cross-reactive or identical with H-Y antigen of the mouse. Subsequent tests showed that H-Y also occurs in chickens (*9*), but in this species it is confined to the female rather than the male, an observation of special relevance because, as noted above, it is the female that is the heterogametic sex in birds.

The occurrence of H-Y in birds as well as mammals was informative from an evolutionary perspective because these classes are derived from unrelated species, representing widely divergent pathways of reptilian evolution. This means that H-Y must have been inherited from an early common ancestor of birds and mammals (or, alternatively, that it arose twice in the evolution of the higher vertebrates). The subsequent discovery of H-Y antigen in amphibians (*9*) indicates that it arose in an early common ancestor and may thus be ubiquitous in all higher vertebrates. In amphibians, as in mammals and birds, H-Y is associated with the heterogametic sex. In the leopard frog, *Rana pipiens*, the male is heterogametic and H-Y$^+$, whereas in the South African clawed frog, *Xenopus laevis*, the female is heterogametic and H-Y$^+$. These observations signify that H-Y is concerned with some sex-related function that is operative only at the population level because individuals survive as well without H-Y as with it.

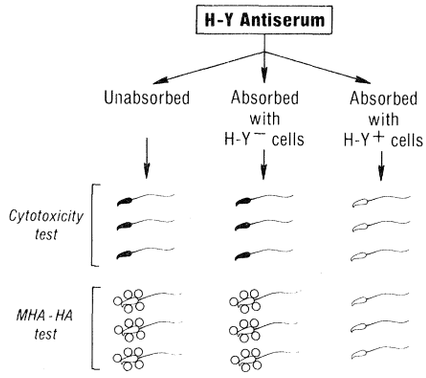

Fig. 2. H-Y typing by absorption. Cells were suspended in selected pools of diluted H-Y antiserum. H-Y$^+$ cells absorb H-Y antibodies, thereby decreasing the ability of the antiserum to react with sperm. Positive absorption was manifested as a fall (i) in the number of sperm killed (stained with trypan-blue dye) in the cytotoxicity test and (ii) in the frequency of rosettes formed in the MHA-HA test (*44*).

H-Y and Sex Determination

In mammals the Y chromosome determines male sex by causing the initially indifferent embryonic gonad to differentiate as a testis. Thus, in both mice and men the male phenotype occurs in the presence of the Y chromosome, and the female phenotype occurs in its absence (*39*). Normally the male phenotype occurs in the presence of the Y regardless of the number of X chromosomes present (although supernumerary X chromosomes lead to a variety of abnormalities including small, azospermic testes) (*40*). Induction of testicular differentiation need be the only function of the Y-chromosomal male-determining gene, because subsequent male differentiation occurs under the influence of androgen that is secreted by the newly formed testis. In the absence of androgen the individual becomes a female. Thus a minimum of two genes is required for sexual differentiation in mammals: the Y-linked gene which determines the primary sex of the gonad, and the X-linked gene which mediates androgen responsiveness and development of secondary sex characteristics (*41*).

Differentiation of the testis under the influence of the Y chromosome commences when the primordial germ cells have completed their migration from the yolk sac, where they originate, to the gonadal ridge. At this stage of differentiation, it is likely that direct interactions between the germ cells and somatic elements of the primordial gonad are necessary in order for testicular organogenesis to proceed. Such interaction must occur via the plasma membranes of these cells (*42*), and on this basis the highly conserved plasma membrane component which we recognize serologically as H-Y antigen becomes an excellent candidate for the product of the Y chromosomal gene that induces the indifferent gonad to virilize. From this perspective, the H-Y gene and the primary male-determining gene are identical (*43*). Indeed, an H-Y gene is on the human Y chromosome since males with two Y chromosomes have more H-Y antigen than normal XY males (*44*), and recent unpublished studies by Koo indicate that the *H-Y* gene is found in a region that is known to be male-determining (*45*).

The proposed identity of the Y-chromosomal male-determining gene product and H-Y antigen can best be tested on individuals with intersexual phenotypes, and on those whose gonadal sex and chromosomal sex fail to coincide. In mammals, H-Y antigen always should be associated with formation of at least rudimentary testes regardless of phenotype or karyotype (*46*). The following observations are consistent with this hypothesis:

1) XX male mice sex-reversed by the autosomal dominant *Sxr* are H-Y$^+$ (*12*).

2) XX human males and XX true hermaphrodites are H-Y$^+$ regardless of cytological evidence for or against presence of the Y chromosome (*47*).

3) X^{Tfm}/Y female mice exhibiting the syndrome of testicular feminization develop testes under the influence of the Y, but show no further male development because of mutational deficiency of the androgen receptor (*48*). The X^{Tfm}/Y phenotypic females are H-Y$^+$ (*8*).

4) XY human females with testicular feminization syndrome are also H-Y$^+$ (*12*).

5) In the Scandinavian wood lemming (*Myopus schisticolor*) there is a tendency of some XY individuals to develop as females. Karyologically indistinguishable from normal XY males, XY female wood lemmings are fertile and anatomically indistinguishable from XX females (*49*). This suggests that the male-determining portion of the Y has been inactivated in these animals. The XY female wood lemmings are H-Y$^-$ (*38*).

6) In cattle, chorionic vascular anastomosis between male and female twin fetuses leads to intersexual development in the female twin. In some cases the gonads of the freemartin female are virilized and in extreme cases they produce substantial amounts of androgen (*50*). Male hormones cannot be responsible for the masculine development of the freemartin gonad because large amounts of androgen injected into pregnant cows lead to masculization of the female offspring but *not* of their ovaries (*51*). (Also it would seem unlikely that an organ is induced by its end product.) The discovery that freemartins are XX/XY chimeras led to assays for H-Y antigen in the gonads of developing bovine male, female, and freemartin fetuses. These assays demonstrated that freemartin fetuses are H-Y$^+$. Indeed, H-Y antigen is prominent in the fetal freemartin gonad and to the same extent as in the testis of the fetal bull twin (*52*).

At present all data from animal and human subjects support the hypothesis that H-Y antigen is the product of the primary sex-determining gene of vertebrates. We trust that future studies will shed more light on this hypothesis and provide further information on the precise mode of action of H-Y antigen in the morphogenesis of the heterogametic gonad.

Summary

The factors are reviewed which affect the expression of H-Y antigen, a cell surface component that has been extensively analyzed in mice but which may be ubiquitous in all vertebrates. The phylogenetic stability of this antigen and its association with the Y chromosome indicate an important role in primary sex determination.

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26. Another possibility is that the *H-Y* gene is close ly associated with genes of the MHC. In this context *H-Y*^{b} (B10) and *H-Y*^{k} (B10.BR) could represent alleles of the *H-Y* locus, each coding for a distinct H-Y antigen [D. S. Steinmuller and E. J. Eichwald, in *Immunogenetics of the H-2 System*, A. Lengerová and M. Vojtíšková, Eds. (Karger, Basel, 1971) p. 278].
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