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Self/nonself perception, reproduction and the extended MHC

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Self/nonself perception governs mate selection in most eukaryotic species. It relies on a number of natural barriers that act before, during and after copulation. These hurdles prevent a costly investment into an embryo with potentially suboptimal genetic and immunological properties and aim at discouraging fertilization when male and female gametes exhibit extensive sharing of alleles. Due to the fact that several genes belonging to the extended major histocompatibility complex (xMHC) carry out crucial immune functions and are the most polymorphic within vertebrate genomes, it is likely that securing heterozygosity and the selection of rare alleles within this gene complex contributes to endowing the offspring with an advantage in fighting infections. Apart from MHC class I and II antigens, the products of several other genes within the xMHC are candidates for participating in mate choice, especially since the respective loci are subject to long-range linkage disequilibrium which may aid to preserve functionally connected alleles within a given haplotype. Among these loci are polymorphic odorant receptor genes that are expressed not only in the olfactory epithelium, but also within male reproductive tissues. They may thus not only be of importance in olfaction-influenced mate choice, by recognizing MHC-dependent individual-specific olfactory signals, but could also guide spermatozoa along chemical gradients to their target, the oocyte. By focusing on the human HLA complex and genes within its vicinity, we show here that the products of several xMHC-specified molecules might be involved in self/nonself perception during reproduction. Although the molecular details are often unknown, the existence of highly diverse, yet intertwined pre- and post-copulatory barriers suggests that xMHC-encoded proteins may be important for various stages of mate choice, germ cell development, as well as embryonic and foetal life in mammals and other vertebrates. Many of these genes should thus be regarded as crucial not only within the immune system, but also in reproduction.

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Introduction

Charles Darwin's statement that "the females...select the more agreeable partners"[1] has greatly influenced our concepts about how two potential mating partners evaluate their capability to create viable offspring. Although religious, social, dynastic and pecuniary considerations have had an influence on human mate choice for thousands of years, up to the present,[2] visual, auditory and chemical (e.g., olfactory) cues play a role in humans as well[3-11] and govern mate choice in other vertebrates,[12-20] invertebrates,[21-25] plants[26] and even in fungi.[27]

Birds provide particularly instructive examples: the elaborate tail feathers of male peacocks signal to hens that the male is the possessor of 'good genes' that allowed him to survive despite the obvious disadvantage of having to carry his fancy tail around. Similarly, the sophisticated songs of the males in some bird species and the way in which these songs are displayed appear to allow choosy females to enhance their breeding success by selecting males according to their 'personalities.'[28] And even in a flying mammal such as the sac-winged bat (*Saccopteryx bilineata*), males use their wings in a fan-like fashion to direct mixtures of individual-specific odors from glands below their wings towards females in order to catch their attention.[29] In mice, chemical signals received via olfaction play a decisive role in pre-copulatory mate choice: in a seminal publication, Yamazaki and colleagues demonstrated that these animals can sense the type of genes belonging to the major histocompatibility complex (MHC) of another mouse through MHC-dependent odors.[30] Mice exploit this 'knowledge' for choosing an MHC-dissimilar mate, and comparable results have been obtained in several further vertebrate species, from fish to man.[31-36] Therefore, apart from its well-established, crucial role in innate and adaptive immunity,[37-39] the MHC appears also important in mate choice in the vast majority of vertebrates which have to date been investigated regarding this aspect. The exceptional degree of polymorphism exhibited by several MHC loci is thus not only advantageous in Self/Nonself recognition processes during T cell development[40] and presentation of foreign antigens by MHC molecules to receptors on effector cells,[41-43] but also in reproduction, where a number of barriers help in securing the creation of genetically and immunologically optimal offspring.

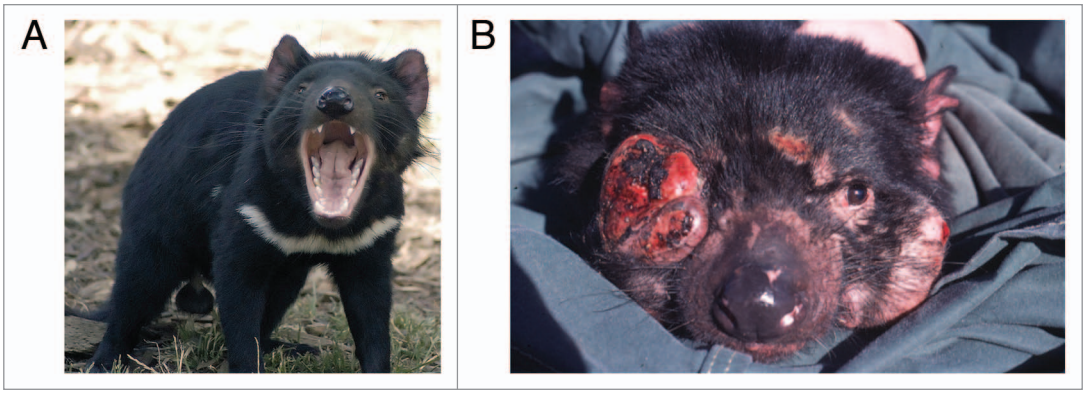

Figure 1. Limited MHC diversity in the Tasmanian devil facilitates the spread of a contagious tumor. (A) Tasmanian devil. (Source: wikipedia.org, Photo: Wayne McLean). (B) Tasmanian devil with devil facial tumor disease. Molecular genetic and karyotypic evidence indicates that this unusual infective cancer originated in a single individual about 15 years ago, and can thus be regarded as 'a clonally reproducing mammal that is an obligate parasite'.[49] (Source: ref. 224. Photo: Menna Jones).

Not all vertebrate species, however, appear to adhere to the idea that it is desirable to combine dissimilar MHC alleles in the offspring to fight infections effectively. Instead, they may prefer to endow their young with an 'optimal' number of alleles within the MHC,[44,45] or rely on other criteria.[46,47] These results indicate that although individuals with identical or similar MHC types are usually not attracted by each other, additional factors can modulate these aversions and preferences or replace them altogether.[48] On the other hand, possessing an MHC with a low degree of polymorphism[15] can be associated with very severe problems, as demonstrated by the Tasmanian devil (*Sarcophilus harrisii*) (**Fig. 1**), whose population is threatened by a clonal tumor (devil facial tumor disease)[49] exhibiting exceptional properties: since there are only marginal differences between the MHC antigens on the malignant cells and those on normal cells of this marsupial,[50] the tumor cells cannot be recognized as 'nonself' by the immune system of infected animals. Therefore, the tumor is not rejected, in consequence of which more than half of the Tasmanian devil population has already succumbed to the disease.[51]

In the following sections, we will initially discuss which barriers have to be surmounted in mate choice, followed by a description of the products of those MHC genes that are likely to participate in the various stages of reproduction, focusing mainly on the situation in man. We will then examine to what extent pre- and post-copulatory mate selection relies on MHC genes. In particular, we will explore possible molecular mechanisms which might help females to evaluate the suitability of potential mates. However, we will not restrict ourselves just to the MHC, but extend the analysis to its immediate vicinity as well, both telomeric and centromeric. The several hundred genes present within this chromosomal segment, in man 7,800 kilobasepairs (kb), have collectively been termed the 'extended MHC' (xMHC; **Fig. 2**)[52] due to the exceptional level of linkage disequilibrium (LD) observed over the entire region in selected haplotypes.[53] The presence of polymorphic odorant receptor (OR) genes in close linkage to the MHC[54-60] and the possible existence of a functional relationship between OR and MHC loci have even led to the suggestion to designate the xMHC an 'immuno-olfactory supercomplex'.[61]

Reproductive Barriers in Vertebrates

Barriers that influence mate choice exist before, during and after copulation (**Table 1**). As mentioned above, pre-copulatory barriers in vertebrates may be of a visual, an auditory or an olfactory nature. Whereas an involvement of the xMHC in auditory contexts is as yet unlikely, despite the presence of the *COL11A2* gene within the extended class II subregion (xclass II) (**Fig. 2**) which is associated also with dominant autosomal deafness,[52] there is evidence that visual and, in particular, olfactory cues are employed in mate selection, and that the latter are dependent on polymorphic proteins specified by the MHC. This is in contrast to the products of *OR* genes within the xMHC, for which a role in pre-copulatory mate choice has so far not been demonstrated. As there are several excellent reviews on pre-copulatory mate choice,[10,11,15,16,62-66] we will discuss these issues only briefly in one of the following sections.

During copulation, optimal complementarity between male and female sexual organs is helping to secure reproductive success, as shown in closely related species of spiders.[67] However, there is evidence for comparable mechanisms also in vertebrates. For example, in species of birds without forced copulations, such as the harlequin duck (*Histrionicus histrionicus*) or the African goose (*Anser cygnoides*), the males have short phalli and the females simple vaginas. On the other hand, genital co-variation has led to long phalli and very elaborate vaginas in species with high levels of forced copulations like the long-tailed duck (*Clangula hyemalis*) and the mallard (*Anas platyrhynchos*).[68] In primates, differences in the anatomy of sexual organs can also be linked to particular sexual behaviors, e.g., to promiscuity.[69] To our knowledge, a relationship to genes within the xMHC has not been described for any of these characteristics and we will not consider them further.

Finally, post-copulatory barriers are part of 'cryptic female choice' mechanisms that involve hidden female effects that impact on the success of males in fertilizing ova. They can be

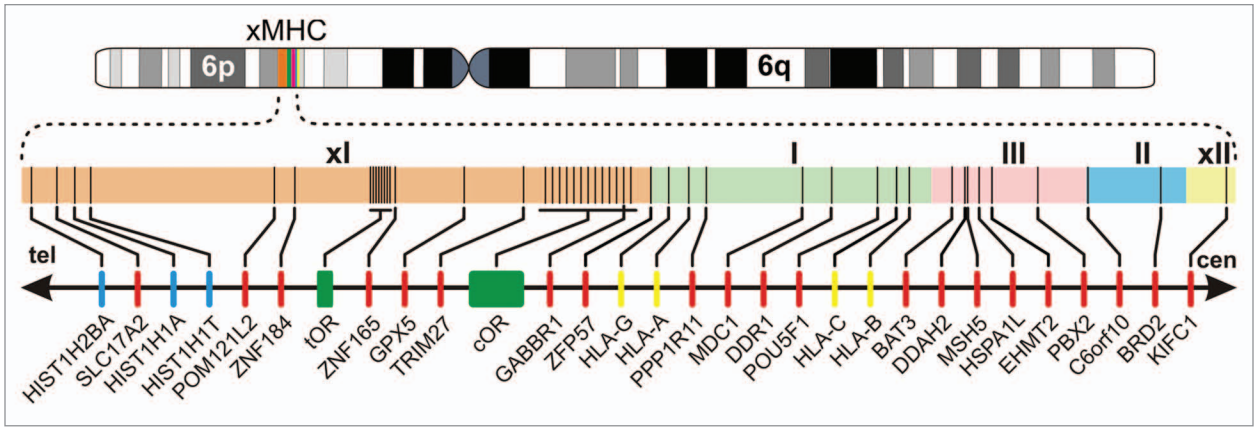

Figure 2. The human extended MHC. Human chromosome 6 is shown with the short (6p) and the long arm (6q). A schematic map of the xMHC is depicted below, with the extended class I (xI, ~3,900 kb), class I (I~1,900 kb), class III (III~700 kb), class II (II~900 kb) and the extended class II (xII, ~200 kb) regions indicated by different colors. Nearly all genes mentioned in the main text are shown, with their approximate locations within a given subregion indicated by vertical lines. Histones are shown in blue, the two odorant clusters with a total of 34 genes in green, selected HLA class I genes in yellow and all other genes in red. The directions towards telomere (tel) and centromere (cen) are also given. In the mouse, all genes telomeric of (and including) *TRIM27* in the xclass I region are not linked to the MHC, but form a syntenic group of loci on chromosome 13.

grouped in mechanisms that are effective before oocyte fertilization, during the fertilization process itself or after sperm—oocyte interaction in the interval preceding the formation of the zygote. Furthermore, these barriers may also affect the pre- or post-implantation embryo and the foetus. Cryptic female choice includes seemingly exotic phenomena such as sperm dumping,[70] but also hurdles that must be surmounted by spermatozoa that traverse towards an oocyte within the female reproductive tract. In the latter process, there may well be functions for MHC class I antigens and other xMHC-encoded proteins, particularly OR.

However, a recent study[71] demonstrates that it may sometimes be difficult to separate pre- and post-copulatory aspects from each other. Gillingham and colleagues found that, when presented with two MHC-distinct females, male red junglefowl (*Gallus gallus*) allocated more sperm to that mate with the most pronounced MHC dissimilarity. This behavior is likely to influence fertilization success, since it has been shown that the relative number of sperm inseminated by competing males into a female chicken reliably permits to predict fertilization success.[72] How a male bird perceives that its MHC type is dissimilar from that of a female is, however, currently enigmatic. Chickens do have numerous *OR* genes, but next to nothing is known of their genomic organization and ligands[73] as well as the avian sense of smell in general, and it appears equally plausible that these birds rely on other sensory modalities, including post-copulatory mechanisms, for MHC-influenced mate choice. Mate choice is thus not only dependent on decisions made by the female, but, at least in selected cases, on male assessments as well.

In the next section, we will point out a number of xMHC loci whose products have either been shown to play a role or are likely to be involved in mate selection, as well as, more generally, in reproduction.

Table 1. Reproductive barriers in vertebrates

(A)	Before copulation
	visual
	auditory
	olfactory
(B)	During copulation
	optimal complementarity between male and female sexual organs
(C)	After copulation
	before oocyte fertilization
	during sperm—oocyte interaction
	after oocyte fertilization, but *before* zygote formation
	after zygote formation

Participation of xMHC Genes in Reproduction

It may not be surprising that some proteins encoded by the xMHC participate in reproduction, but it seems striking how many of them execute functions that are specifically devoted to pre-copulatory mate choice, germ cell differentiation and function, as well as to embryogenesis (**Table 2**). However, it must be pointed out that the genes which are part of the human xMHC are not always part of this chromosomal segment also in other species. For example, the human *TRIM27* locus (xclass I region) and genes telomeric of it are part of a syntenic assembly on chromosome 13 of the mouse, while the MHC and genes centromeric to *TRIM27* are located on chromosome 17 in this species.[60] It seems therefore unlikely that possible functional relationships between the MHC and, for example, histone genes are necessarily dependent on a genetic linkage. The fact that 157 tRNA genes, also requiring intense transcription, are intermingled with more than fifty histone loci in the vicinity of the human MHC, the human leukocyte antigen (HLA) complex, points to another explanation: it might be that co-clustering of these genes serves to maximize their transcriptional levels.[52]

Table 2. Genes within the human xMHC with a role in reproduction

Region, gene designation	Function
xclass I region	
HIST1H2BA and others	Testis-specific histones; destabilization of nucleosome
SLC17A2	Solute carrier protein
POM121L2	Nucleoporin; nuclear envelope formation
ZNF184, ZNF165	Testis-specific zinc-finger proteins; unknown function
GPX5	Glutathione peroxidase; protects against peroxide damage
TRIM27	Member of tripartite motif gene family of zinc-finger proteins; transcriptional regulation of sperm differentiation (?)
tOR and cOR	Telomeric and centromeric OR clusters; involvement in mate selection
GABBR1	Subunit of the receptor for γ-amino butyric acid; regulation of ion channels
class I region	
ZFP57	KRAB zinc-finger protein; imprint maintenance
HLA-G	Expressed on extravillous cytotrophoblast; interaction with maternal NK cells
HLA-A	Involvement in sperm receptor selection (?)
PPP1R11	Complex formation with protein phosphatase γ1, actin and PPP1R7; involved in germ cell morphogenesis; absence causes male infertility
MDC1	Involved in repair of DNA double strand breaks
DDR1	Tyrosine kinase; essential developmental functions
POU5F1	Master regulatory transcription factor; maintenance of germ cell lineage
HLA-C	Expressed on extravillous cytotrophoblast; interaction with maternal NK cells
HLA-B	Involvement in sperm receptor selection (?)
class III region	
BAT3	Deficiency leads to male infertility through HSPA1L degradation
DDAH2	Regulates levels of nitric oxide synthase inhibitors; maintains myometrial quiescence during gestation
MSH5	Involvement in meiotic recombination
HSPA1L	Testis-specific variant of heat shock protein 70; absence leads to male infertility
EHMT2	Repressor of imprinted genes in trophoblast
PBX2	TALE homeodomain protein; non-essential function (?)
class II region	
C6orf10	Testis-specific basic protein with unknown function
BRD2	Bromodomain-containing transcription factor with a possible role in spermatogenesis
xclass II region	
KIFC1	Motor protein involved in acrosome biogenesis; expressed also in the syncytiotrophoblast of the placenta

The description will begin at the telomeric end of the human xMHC (**Fig. 2**). We will omit, however, the majority of genes that are not specifically expressed in reproductive tissues.

Histone loci. The cluster of histone genes within the xMHC is the largest within the human genome.[52] Histones are required in enormous quantities not only in mitotic cells, but also in cells undergoing meiosis. In male germ cells, they contribute to large-scale genome compaction which is necessary to enclose the haploid DNA within the small spermatozoal head.[74] The major role of several xMHC-encoded testis-specific histone variants appears to be the creation of less stable nucleosomes, thus preparing the DNA for the interaction with very basic non-histone proteins and eventually protamines. It has been estimated that only 4% of the DNA within a spermatozoon retains nucleosomes.[75] At least three histone genes, *HIST1H2BA, HIST1H1A* and *HIST1H1T* within the xMHC are testis-specific and participate in nucleosome destabilization.

Solute carrier proteins. There are five genes specifying these ion transporters within the human xMHC,[52] but only *SLC17A2* is known to be testis-expressed. It co-transports sodium and phosphate. A specific reproduction-related function has not been determined for this locus.

POM121L2. This polymorphic gene encodes a protein that is essential for the formation of the nuclear pore.[76] It is abundantly expressed in the testis, particularly in pachytene spermatocytes, but its precise function in male germ cells is unknown.

Zinc-finger proteins. There are 36 genes within the xMHC encoding polypeptides belonging to this large family. *ZNF184* and *ZNF165*,[77] are testis-specific, but their functions have not been determined. In case of *TRIM27*, a member of the tripartite motif family of zinc-finger proteins, evidence indicates that it may be involved in the transcriptional regulation of sperm differentiation.[78]

GPX5. Located between *ZNF165* and *TRIM27* in the human xMHC, this locus specifies a polypeptide belonging to the glutathione peroxidase family, but it can be distinguished from other family members because its mRNA does not contain a selenocystein codon. It is strongly expressed in the epididymis within the testis, but also in spermatozoa where it is thought to protect membrane lipids against peroxide damage.

Members of *OR* clusters. Since the work of Parmentier and colleagues, who were the first to demonstrate that transcripts of *OR* genes cannot only be found within the main olfactory epithelium (MOE) within the nose, but also in testicular tissue,[79] a role for these chemoreceptors in reproduction had to be taken into account. In man, there are two clusters of *OR* genes within the xMHC. The centromeric of these is also MHC-linked in most other mammals so far examined,[225] while the telomeric cluster may be found on other chromosomes. Therefore, a species-independent, functional relationship between MHC loci and MHC-linked *OR* genes relying on LD is likely to encompass primarily members of the centromeric *OR* cluster. A prerequisite for the participation of these genes in Self/Nonself recognition is the presence of polymorphisms that affect their function. With the possible exception of a few pseudogenes, all HLA-linked *OR* loci have been found to be polymorphic, with so far up to eight

alleles (*OR12D2*, in the center of the centromeric *OR* gene cluster),[55,58,59,80] and it is known that mouse MHC-linked *OR* loci exhibit variability as well.[60] In man, several HLA-linked *OR* genes are expressed in the testis,[81] in addition to those described previously,[79] although a role in human reproduction has not yet been firmly established for any of them. When reflecting on pre- and post-copulatory mate selection, we will return again to *OR* genes and consider how they might interact with MHC class I molecules.

GABBR1. This polymorphic[82] gene belongs to a family of G-protein-coupled receptors (GPCR) involved in neurotransmission. Together with the $GABA_BR2$ subunit, $GABA_BR1$ constitutes a heterodimeric protein complex in which only the $GABA_BR1$ subunit binds γ-amino butyric acid with high affinity, while $GABA_BR2$ interacts with G proteins.[83] The receptor mediates the coupling to adenyl cyclase, voltage-gated Ca^{2+} channels and inwardly rectifying K^+ channels[84] and is expressed not only in neurons, but also in testicular tissue and spermatozoa.[85,86] An interesting observation concerning *GABBR1* is its apparently obligatory linkage to *OR* genes.[225] This is the case in all mammals so far examined, irrespective of linkage between *OR* loci and the MHC, as well as in amphibia (*Xenopus tropicalis*) and in fish (*Danio rerio*). Since the linkage (between *GABA-B-R1*, *Or33a*, *Or33b*, *Or33c* and *Or35a*) extends even to the fruit fly (*Drosophila melanogaster*), despite the fact that *OR* of this species are evolutionarily unrelated to those of vertebrates,[87] the *GABBR1-OR* linkage is suggestive of an important functional relationship between these genes which requires them to be in close physical proximity that has been retained for at least 990 million years.[88]

ZFP57. This gene belongs to the growing list of xMHC loci with an 'exciting' role in reproduction and development. It was recently detected that it encodes a KRAB zinc-finger protein that is highly expressed in developing oocytes and preimplantation embryos and which is involved in maintaining DNA methylation at specific loci. Its absence leads to a severe phenotype causing early lethality in newborn mice.[89] In man, homozygous mutations affecting ZFP57 function can cause transient neonatal diabetes.[90]

HLA-G. This HLA class I gene exhibits only a low degree of polymorphism. Its product appears to play a role in materno—foetal interactions, where it is expressed on cells of the placental extravillous cytotrophoblast, together with HLA-C antigens.[91,92] HLA-G interacts with receptors on natural killer (NK) cells and has long been suspected to be involved in recurrent spontaneous abortions. However, only a recent analysis demonstrated that promoter variants of this gene are involved in this common pregnancy complication.[93]

HLA-A. This is one of the most polymorphic genes in the human genome. Despite the absence of β_2-microglobulin (β_2m), and the concomitant lack of intact class I molecules, it is expressed in certain spermatogenic cells, but not spermatozoa. Since seminiferous tubules are an immunologically privileged tissue to which immune cells have no access, this site of HLA-A class I heavy chain (HC) synthesis points to a non-immunological function for this molecule. As previously suggested by us,[36,94] certain polymorphic HLA class I HC, including the product of the *HLA-A* locus, might be involved in the selection of OR for expression on spermatozoa that lack reactivity with self-molecules.

PPP1R11. Defects in the protein phosphatase 1 (PP1) γ1 and γ2 isoforms of mice are associated with defective germ cell morphogenesis and apoptosis, but do not cause abnormalities in other tissues. It has recently been detected that the basis for this puzzling observation lies in the association of PP1γ2, actin, and the regulatory subunits (PPP1R7 and PPP1R11) of PP1γ2. Complex formation between these four proteins leads to retention of high levels of PPP1R11 and prevention both of its proteolysis and germ cell apoptosis.[95] This indicates that *PPP1R11* fulfils an important function within the male reproductive system, and it is known that defects of this gene lead to male infertility in mice.[96] The expression of different *PPP1R11* isoforms begins at the pachytene stage of spermatogenesis, but the protein(s) play(s) an important role also in the context of sperm motility.[97,98] By using in silico analyses, we have detected that this locus is always linked to *GABBR1*, and even the opposing transcriptional orientation of the two genes is invariably retained, both in mammals and two species of fish (zebrafish, *Danio rerio* and medaka, *Oryzias latipes*).

MDC1. The product of this polymorphic[52] gene fulfils an essential function in the repair of DNA double strand breaks. It is not expressed in spermatogonia and preleptotene spermatocytes in mice, possibly explaining the radiosensitivity of these early germ cells, but the MDC1 protein is detectable at later stages of spermatogenesis. In mice with a defect of this gene, all spermatocytes enter apoptosis in epithelial stage IV, pointing to a crucial role of MDC1 also in the production of male gametes.[99]

DDR1. This gene specifies a receptor tyrosine kinase that exerts multiple functions in mice. Major sites of its expression are the skeletal bones, skin and the urogenital tract, but also in the uterus during implantation. Mice defective for *DDR1* gave birth to offspring that were smaller in size than their normal littermates. Furthermore, the majority of mutant females could not give rise to offspring due to a lack of appropriate blastocyst implantation into the uterine wall, and they exhibited also defects of mammary gland architecture.[100]

POU5F1. Although this gene does not seem to play a role in the immune system, it is clearly one of the most crucial in the MHC. It is a highly polymorphic[101] master regulatory transcription factor that is present in the genomes of all mammals and plays a critical role in the maintenance of pluripotency in various cell lineages of the embryo as well as in germ cells.[102-105] A striking finding is its conservation within the mammalian xMHC, where *POU5F1* is an obligatory neighbor of another transcription factor, *TCF19*.[52] The *POU5F1-TCF19* linkage is retained also in a lizard, the Caroline anole (*Anolis carolinensis*), together with the opposing transcriptional orientation of these genes.[106] However, a functional interaction of the products of these loci has so far not been detected. Being right in the middle of the human MHC, *POU5F1* alleles are part of a region that is subject to marked LD,[80] linking them to distinct *HLA-B* alleles in most populations and in some (e.g., Caucasians) also to *HLA-A* alleles. Obviously, *POU5F1* alleles are also part of 'ancient' HLA haplotypes, such

as *A1-B8-DR3* which are characterized by very strong LD. Although there is no reason to assume that these considerations play any role during spermatogenesis, they could become important after fertilization and during embryonic development, and we will later return to them.

HLA-C. This moderately polymorphic class I gene is expressed on cells of the extravillous cytotrophoblast of the placenta, where it can interact with suitable receptors on maternal NK cells.[91,92]

HLA-B. With more than 1,000 alleles, this is the most polymorphic gene in the human genome and, like *HLA-A*, it is expressed at late stages of spermatogenesis, but not on sperm. The deliberations made with respect to *HLA-A* apply also to this locus.

BAT3. This is a polymorphic[52] gene whose targeted inactivation leads to complete male infertility due to apoptosis of meiotic male germ cells. BAT3 deficiency is accompanied by degradation of the protein product of the *HSPA1L* gene, also resident within the xMHC, a testis-specific chaperone, through polyubiquitinylation and subsequent degradation.[107] Therefore, BAT3 must be regarded as a crucial regulator of HSPA1L activity.

DDAH2. This locus encodes a dimethylarginine dimethylaminohydrolase, an enzyme which hydrolyzes inhibitors of endogenous nitric oxide synthase and modulates their intracellular concentrations. It is involved in regulation of myometrial contractivity during pregnancy in the rat, thereby controlling delivery at term.[108]

MSH5. This polymorphic gene encodes a repair protein that is involved in meiotic recombination. Its expression begins at the early primary spermatocyte stage and ends when elongated spermatids form. The C85T polymorphism is associated with male infertility, specifically azoospermia or severe oligozoospermia, due to an anomalous chromosome synapsis and meiotic failure.[109,110]

HSPA1L. There are three heat shock protein 70 genes, and all map to the class III region of the xMHC. *HSPA1L* is the most basic of the three and represents a testis-specific variant.[111] As described above in the context of *BAT3*, its absence leads to male infertility. The pleiotropic functions of the HSPA1L protein offer a plausible explanation for this finding. It is interesting to note that this particular chaperone has also been found in the MOE of mouse and man, and there is evidence that it helps the expression of *OR* in heterologous cells.[112] Whether HSPA1L fulfils this function also in spermatogenic cells, remains to be determined. Nothing is known regarding the consequences of its polymorphism. *HSPA1A* and *HSPA1B* (not shown in **Fig. 2**, immediately centromeric of *HSPA1L*) are two distinct genes, but encode proteins with identical sequence.[113] *HSPA1A* expression is activated by oxidative stress and hyperosmotic shock; it may be the first gene that is physiologically activated and tightly regulated after fertilization in mammals in consequence of the osmotic stress experienced by very immature embryos.[114]

EHMT2. This is a gene within the xMHC that is, like *ZFP57*, involved in imprinting. EHMT2 however, acts by carrying out the majority of dimethylation of histone H3 at lysine 9. In the mouse, placenta-specific imprinting appears to be dependent on this gene to repress developmentally regulated genes during embryonic stem cell differentiation.[115]

PBX2. This is one of four mammalian genes that encode TALE homeodomain proteins. They serve as DNA binding partners for a subset of Hox transcription factors. While other members of the PBX family fulfil essential roles in mammalian development, the PBX2 protein may be dispensable, although it is strongly expressed during embryonic development as well.[116] The authors conclude that it is likely that other PBX members might compensate for the loss of PBX2 in the mutant animals.

C6orf10. This polymorphic locus encodes a testis-specific basic protein with no known function in spermatogenesis.

BRD2. This gene specifies a double bromodomain protein which is part of a family of transcription factors that associate preferentially with hyperacetylated chromatin of transcribed genes. In addition, BRD2 has intrinsic histone chaperone activity and is a transcriptional co-activator/co-repressor.[117,118] The gene is ubiquitously expressed,[119] is polymorphic[120] and may play a role also during spermatogenesis in the context of chromatin modifications, in addition to a related gene (*BRDT*) that encodes a testis-specific double bromodomain protein.[121]

KIFC1. Members of the kinesin superfamily are motor proteins that perform diverse functions. Depending on the cell type, these may include the transport of various cellular organelles, vesicles, chromosomes, protein complexes, entire nuclei and the regulation of microtubule dynamics. KIFC1 belongs to the class of C-terminal kinesins in which the motor activity is directed towards the microtubule *minus* end. In mammals, this gene is vital for normal spermatogenesis[122] and concerned with acrosome biogenesis, by maintaining the structure of the manchette close to the nuclear membrane.[123,124] The protein first associates with vesicles produced by the Golgi apparatus, then with the growing acrosome of the spermatid and with the caudal end of the nucleus in elongated spermatids. The role of KIFC1 in spermatogenesis has also been investigated in an invertebrate, the Chinese mitten crab (*Eriocheir sinensis*), resulting in similar findings.[125] This points to an extreme conservation of the function of this molecule, and the retention of its chromosomal location in the xclass II region within the xMHC of mammals points into the same direction (**Fig. 1**).[126]

Facial Attractiveness, Individual-Specific Odors and the Extended MHC

There can be no doubt that the choice of a sexual partner is crucially affected by facial attractiveness.[3,4,10] It has been suspected that the MHC might be involved in other sensory modalities apart from olfaction,[127] but it came as a surprise that the MHC appears to influence also facial preferences. In a typical study, females had to assess the attractiveness of men from photographs, followed by a comparison of the female and male HLA types. Other than in odor assessment studies such as that carried out by Wedekind and colleagues,[32] where preferences were more pronounced when women and men carried dissimilar HLA types, Roberts and co-workers observed a tendency that similar HLA types correlated with facial attractiveness.[128] Likewise, the level of heterozygosity at the HLA complex was found to be associated positively with this characteristic.[129] It must be mentioned,

however, that comparable findings were not reported by all investigators, most likely due to differences in methodological design (reviewed in ref. 10). Nevertheless, the majority of these studies indicate that genes of the HLA complex do not only contribute to facial features, but suggest also that the preference for a particular facial characteristic correlates with a similar HLA constitution.

The few analyses carried out so far do not permit to pinpoint a particular gene within a subregion of the human xMHC as being responsible for the observed effects. However, the possibility that properties of the skin might be influenced by polymorphic genes within the HLA complex should be taken into consideration. There are loci between *DDR1* and *POU5F1* as well as just centromeric of the latter, within the HLA class I region, that are associated with disorders of this organ. Among them are corneodesmosin (*CDSN*; hypotrichosis simplex of the scalp) and several genes associated with psoriasis (*PSORS1C1*, *PSORS1C2* and *PSORS1C3*). At least *CDSN* and *PSORS1C1* are known to be polymorphic, and might thus contribute to distinct skin phenotypes in individuals with different HLA haplotypes. Polymorphisms have also been established for *COL11A2* (xclass II region) which, apart from being associated with a hearing disorder,[52] is a candidate locus for shaping facial characteristics.[10,130] However, additional studies will have to aim at identifying the xMHC gene(s) responsible for facial attractiveness. As pointed out before, the extensive LD within the human xMHC is a severe hurdle for such detailed studies.[52]

Much more, however, is known about MHC-influenced odor preferences.[10,11,15,16,30-36,131-138] These studies concentrated initially on rodents, but have, since the pioneering work of Wedekind and his colleagues,[32] also included humans. A number of facts appear to be relatively well established, across species: (1) genes that are part of the MHC play an important role in determining individual differences in body smell; (2) based on olfaction alone, males and females mostly prefer mates possessing dissimilar MHC types; (3) there may be a female preference for males exhibiting MHC heterozygosity. In species such as mouse and man, several additional results have been obtained. For example, β_2m-deficient mice possess an individual-specific odor (odortype) that is distinct from that of wild type mice carrying the same MHC, and is apparently connected to the greatly reduced number of molecules (among them MHC class I antigens) that normally associate with β_2m on the surface of cells and within body fluids.[139] Furthermore, the natural MHC mutant mouse strains *bm1* and *bm3*, whose H-2K molecules are distinguished by only five amino acid exchanges, are characterized also by distinct odortypes that can be smelled by untrained mice.[134]

This suggests that peptides (or other ligands) bound to these proteins might play a role in olfactory recognition of an MHC type. In support of this contention, it has been found that peptides bound to MHC class I molecules exert effects on the MOE in which *OR* genes are expressed.[135] These effects are only observed when the peptides retain their anchor residues, i.e., those amino acids with which they are firmly bound to a given MHC class I antigen. For example, in case of the human class I molecule HLA-B*27:05, arginine at peptide position 2 is an obligatory anchor, while Leu, Val, Phe, Tyr, Arg and Lys have been established as secondary anchors at the C-terminus.[140-142] The exchange of such anchors precludes high-affinity binding not only to MHC class I molecules,[143] but also to receptors on neurons of the MOE[135] and of the vomeronasal organ (VNO) in rodents (this organ is present only in vestigial, non-functional form within the human nose). Although the precise identity of nearly all of these receptors remains to be determined, they are commonly thought to be typical *OR* within the MOE,[144-146] while the VNO expresses a variety of distinct GPCR, at least in mice (reviewed in ref. 145, 147–149), and may thus be more complex.

Since MHC-bound peptides provide, by way of their anchor residues, fairly accurate mirror images of the pockets that serve to anchor them within an MHC class I molecule's binding groove, they inform the recipient indirectly about the peptide donor's MHC type and could thus provide the basis for self/nonself perception outside of the immune system.[36,146,150,151] In assessing the ability of cells carrying a particular chemoreceptor (V2r1b) to bind individual peptides within the VNO, the conclusion was reached that receptors within this organ are likely to distinguish peptides derived from MHC class I and II antigens.[151] As there is evidence in some species that also MHC class II alleles influence the outcome of pre-copulatory mate choice (reviewed in ref. 16), these findings in mice provide a general conceptual framework for the involvement of peptides derived from both types of antigen presenting molecules within the MHC. Although the interactions between peptides and any of the chemoreceptors within the MOE or VNO are clearly not yet understood at a molecular level, the very existence of these interactions must be regarded as supporting a role for peptide-presenting MHC molecules in precopulatory mate choice.

In this context, could there be a role for *OR* genes within the xclass I region in these processes? Following the discovery of MHC-linked clusters of *OR* loci telomeric of the HLA complex (xclass I region),[54,55,58,59] MHC-linked *OR* clusters have been found in nearly all mammals investigated.[225] Remarkably, it was observed that many of the HLA-linked *OR* are polymorphic,[55,58,80] although the degree of polymorphism exhibited by *individual OR* genes was not comparable to that observed for *HLA-A*, *-B* or *-C* loci. The diversity of *entire OR* haplotypes, however, comprising 34 *OR* loci in case of the human xMHC, was found to match or even exceed that of HLA haplotypes.[58,80] It is still unclear to what extent these two groups of haplotypes are associated with each other through LD, since the analyses are so far limited. Nonetheless, very strong LD is found in the case of the 'ancient' HLA haplotypes *A1-B8-DR3* and *A3-B7-DR15*,[152,153] and our in silico analyses of eleven ethnically diverse human populations with more than 1,000 individuals (http://hapmap.ncbi.nlm.nih.gov/) demonstrate also the existence of LD between alleles within the *OR* clusters and loci within the HLA complex in all analyzed populations.[80] Despite the recent publication of a study with an inbred Caucasian population which arrived at the opposite conclusion,[154] these observations indicate that a functional connection between HLA class I molecules and the products of HLA-linked *OR* genes is a reality.[61]

Although still unproven, we suggest that HLA-linked *OR* will have been selected such that their interaction with HLA molecules that are part of the same xMHC (expressing 'self-HLA' molecules)

will be minimized, while interaction with nonself-HLA molecules would be favored. Such interactions might be accomplished in molecular terms through peptides or their fragments with affinity for both types of proteins.[135,146] As previously pointed out by us,[36,94] this scenario is strikingly similar to mechanisms employed by certain fungi to prevent self-fertilization. For example, in the ink cap (*Coprinopsis cinerea*, formerly *Coprinus cinereus*), the products of genetically linked, polymorphic loci for pheromones and pheromone receptors are only able to interact with each other when they are specified by unrelated haplotypes.[27] This sophisticated system contributes to the existence of a large number (~20,000) of genetically distinct 'mating types' in this mushroom, thus resembling the vast number of HLA haplotypes in human beings. From a structural genetics perspective, it appears plausible that an interdependence of xMHC gene products, e.g., in reproduction, is one of the prerequisites that favors an exceptional, long-range LD such as that observed in the human xMHC.

The relatively high molecular weight of peptides that are presented by MHC class I or II molecules (~1,000 Dalton or more) poses the problem of how these almost certainly non-volatile substances traverse from one individual to another. Other than in aquatic animals, this is not immediately obvious in case of terrestrial vertebrates. It seems that close bodily contact between two potential mates is necessary to ensure an efficient exchange of peptides that signal the MHC type to OR and other GPCR. However, it is very likely that other ligands are part of these pre-copulatory signals as well. For example, since dogs can distinguish individual-specific scents of humans even when they are not in close physical contact,[155,156] volatile substances that permit the animal's chemoreceptors to respond to the scent of individual human beings must exist. Following the identification of 373 candidate substances, Penn and co-workers found evidence for both individual and gender-specific axillary compounds.[138] These included alcohols, phenols, aldehydes, ketones, esters, hydrocarbons and other organic substances of low molecular weight. Although the origin of the observed inter-individual differences is currently unclear and could include genetic as well as environmental factors, such as commensal bacteria which colonize an individual in an HLA type-dependent manner, it appears reasonable to assume that the MHC type of an individual might influence the composition of axillary sweat (in man) or other body fluids (vertebrates in general), thereby influencing individual-specific odors.

One of the most interesting aspects of pre-copulatory mate preferences is the emerging consensus that facial preferences tend to be assortative (favoring similarity), as opposed to odor-based studies, most of which indicate disassortative preferences (favoring dissimilarity). Although these seemingly contradictory associations were initially considered puzzling, they could indeed make sense, as pointed out and discussed in more depth by Havlicek and Roberts.[10] A plausible hypothesis[128] assumes that potential mates achieve, by relying on two very different assessment systems, an intermediate level of genetic dissimilarity among themselves. This is reminiscent of theoretical considerations[44] as well as experimental studies with three-spined sticklebacks (*Gasterosteus aculeatus*) which have shown that females prefer males that complement their own MHC class II alleles optimally.[33,45]

Pre-copulatory mate choice appears thus as a multi-facetted, highly complex subject in which not only MHC-encoded molecules but also OR are involved. The proven interactions between MHC antigen-derived peptides and OR (or other GPCR within the VNO) continue to be a fascinating research topic. Further work in this area should aim at identifying the OR responsible for binding a given peptide, thereby opening the opportunity to conduct X-ray crystallographic studies of OR involved in mate choice. Furthermore, if additional experiments will reinforce the concept that particular haplotypes of the MHC and those of *OR* clusters near the MHC are connected by LD, as suggested by the existence of ancient HLA haplotypes[53,152] and our recent analyses,[80,153] this would have far-reaching functional implications and provide an explanation for the nearly obligatory proximity of these two chromosomal regions which is found at least in mammals.[225]

Enabling Spermatozoa to Function in Self/Nonself Perception

Most xMHC genes which we have alluded to in the foregoing sections, play roles in human spermatogenesis. This is a very complex process whose details are still being unravelled. It entails the proliferation of diploid spermatogonia, their differentiation and division into haploid spermatids, and finally the formation of spermatozoa, perhaps the most specialized cells in the body. Following spermiogenesis, the last stages of spermatogenesis, mammalian spermatozoa have to undergo additional steps of maturation during their storage in the epididymis until ejaculation. If self/nonself discrimination and female choice were to find their continuation within the mammalian female reproductive tract, and if such processes would rely also on the interactions of polymorphic proteins, as in pre-copulatory mate selection, spermatozoa might have to signal their 'self' in some way to the female. Being part of the most polymorphic region within the genome, certain genes within the xMHC could indeed be involved. Consequently, a participation of MHC antigens has already been suggested,[157] and the authors reflected on the possibility that the presence of these molecules on the surface of male gametes might provide the female with an indication of the MHC haplotype carried by an individual spermatozoon. There is, however, a problem with this theory: it has been established that neither spermatozoa nor oocytes, at least in man, carry MHC class I and II antigens on their surfaces.[158-160]

Although cryptic female choice may appear as a logical complement to pre-copulatory mate selection, how could the former be accomplished in the absence from male and female gametes of the potentially most useful proteins that enable self/nonself discrimination? Prompted by the finding that certain HLA class I genes are abundantly expressed during spermatogenesis, though in seemingly non-functional form due to the lack of β_2m,[94] and the presence of *OR* transcripts in human testis,[79,81] we have previously reasoned that polymorphic OR on the surface of

Table 3. Outcome of selections within thymus and testis

Feature	Type of selection	
	T cell selection in the thymus	**Sperm receptor selection in the testis**
Positive selection on MHC molecules	T cells with low affinity for self-MHC survive	Probably not necessary
Negative selection with self-molecules on/in cells with 'promiscuous' transcription	T cells lacking high affinity to self-MHC:peptide complexes survive	Spermatozoa are fitted with receptors that lack self-reactivity
Consequence	T cells recognize nonself-MHC:peptide complexes	Spermatozoa recognize nonself-ligands

spermatozoa might be the principal molecules that allow to signal the male's MHC type. We proposed the term 'Sperm Receptor Selection' (SRS) hypothesis for a process that suggests a plausible molecular mechanism for the selection of a genetically 'optimal' spermatozoon which is deemed fit by a given female to fertilize an oocyte.[36,94] We postulate that there are a number of prerequisites which would have to be fulfilled in order to accomplish this task:

Before expression on the spermatozoal surface, chemoreceptors (e.g., OR) would have to undergo a testicular selection step during which they are assessed for potential self-reactivity with polymorphic molecules (for simplicity, we will assume that these are specified by MHC haplotypes '*W*' and '*Z*', although non-MHC-encoded molecules could in principal be involved as well).

Receptors with self-reactivity (anti-W or -Z) would have to be eliminated; in this way, only receptors *lacking* self-reactivity will obtain the chance to participate in Self/Nonself discrimination processes during cryptic female choice.

Some of the selected receptors (and, by inference, the spermatozoa on which they reside) might then be able to interact with nonself-molecules (molecules that are specified by haplotypes that are *not W* or *Z*).

Females with at least one haplotype *other* than *W* or *Z* should thus be able to attract spermatozoa using molecules within their reproductive tract.

The SRS hypothesis succeeds in providing a framework for Self/Nonself discrimination that bears some resemblance to the negative selection of T cells within the thymic medulla (**Table 3**).[40,143] In both cases, those receptors that do not interact beyond a certain affinity threshold with self-molecules that are provided in the context of a selection process 'survive', and they offer the cells on which they reside, the opportunity to interact with nonself-molecules (MHC-presented 'foreign' peptides in case of T cells, ligands within the female's reproductive tract in case of spermatozoa). Therefore, comparable to the interaction of MHC-derived peptides with OR within the MOE or chemoreceptors within the VNO,[135,146,151] such nonself-reactive OR would indirectly indicate the polymorphic 'self' of the male and thereby provide the basic prerequisites for participating in Self/Nonself discrimination.

Logical as all this may possibly be, is there also experimental support for the SRS hypothesis? Clearly, most of the evidence is so far circumstantial, but there are also some important facts that must be taken into account:

Transcription within the testis has been termed 'promiscuous',[161] and, to a considerable extent, resembles that observed within epithelial cells of the thymic medulla.[162] These cells are crucial for establishing peripheral T cell tolerance during negative T cell selection and rely on the AIRE protein for the transcription of many genes that are otherwise only transcribed within peripheral organs.[163] Remarkably, the *AIRE* gene is expressed also in the testis, where its prime function appears to assure normal apoptosis during spermatogenesis.[164] As in medullary thymic epithelial cells, promiscuous transcription within sperm precursor cells might serve to enlarge the repertoire of self-proteins for optimal negative selection purposes.

At least the two most highly polymorphic HLA class I loci, *HLA-A* and *HLA-B*, are abundantly expressed in spermatocytes I and II (ref. 94 and Hutter et al. unpublished results). It is very unlikely that these molecules fulfil their established antigen presentation function, since β_2m is not expressed in intratubular cells within the testis. From an immunological point of view, HLA class I antigens serve no meaningful purpose in spermatogenic cells, since there are no effector cells (T cells, natural killer cells) in seminiferous tubules. Although it appears possible that these molecules are only 'accidentally' produced and fulfil no useful function at all, the SRS hypothesis suggests a plausible way to explain their presence within an immunologically privileged tissue.

OR genes start to be expressed in spermatocytes II, are very strongly expressed in spermatids and more weakly on spermatozoa. In human testicular cells, transcripts from approximately fifty *OR* genes, about one third encoded by the xMHC, are present.[79,81] Similar findings have been described in mice,[165-167] rats,[166,168] and dogs.[169] A given *OR* gene is expressed in a variable percentage of mammalian sperm precursors (~30–90%) and spermatozoa (~5–40%).[165,170-172] These percentages imply that a single spermatozoon will express more than one type of OR, in marked contrast to the situation within the MOE, where a given olfactory neuron expresses only a single OR species.[145] Consequently, individual spermatozoa should be able to respond to a larger spectrum of ligands than olfactory neurons.

The combination of promiscuous gene expression, in particular the synthesis of polymorphic HLA class I antigens and a variety of OR, during spermatogenesis, supports the SRS hypothesis, but it would be crucial to demonstrate a direct and functionally relevant interaction between HLA-A or HLA-B molecules and OR within testicular tissue to prove the correctness of its basic assumptions.

Cryptic Female Choice Before Fertilization

These deliberations suggest that spermatozoa can express chemoreceptors that do not exhibit high affinity to any self-molecule

due to negative selection within the testis. They would thus potentially be receptive to foreign ligands supplied by the female. These ligands should also be polymorphic to provide the genetic signature of the female. Again, ideal candidates seem to be MHC class I antigens or the peptides bound to them.

Human oocytes do not express these molecules, but granulosa cells that surround them exhibit strong reactivity with antibodies directed at intact HLA class I and II complexes.[159] These HLA:peptide complexes possess only a limited lifespan (hours to days),[143] and will sooner or later disintegrate. Their extracellular domains will also be shed into the follicular fluid. The repertoire of chemoattractants within this fluid[173-175] should thus also contain polymorphic polypeptides or peptides that were once bound to them. Even in case of OR for which an interaction with a specific ligand has been demonstrated,[170] several problems remain.[175] Probably the most important of these is the genuine nature of the ligand for a sperm-expressed OR. For example, more than 90% of sperm respond to the low-molecular weight compound bourgeonal, although only about 10% of the spermatozoa are capacitated.[170,176] The relevance of bourgeonal as a physiological ligand has therefore been questioned,[175,176] and chemotactic experiments with sperm are complicated by the varying functional states of these cells.[175] Nevertheless, in order to prove the basic assumptions of the SRS hypothesis, MHC-derived peptides, MHC class I molecules or their fragments would have to be shown to exert chemotactic effects on sperm from MHC-typed donors.

Additionally, the SRS hypothesis suggests a molecular basis for sperm competition. This term refers to the competition between spermatozoa from two or more males to fertilize a given set of oocytes.[177] In promiscuous species, sperm competition is common and may influence the mobility or shape of spermatozoa.[178] Since soluble MHC class I antigens are present in seminal fluid, at least in man,[179] these self-molecules should not be able to interact with chemoreceptors on sperm from the same male. In contrast, there is a high chance that they will be able to bind to sperm from males that carry other haplotypes. An interaction could possibly prevent a directed movement of these competing sperm towards the oocyte and soluble MHC molecules might therefore be the 'decoy' compounds envisaged by Branscomb and colleagues.[166] Although currently largely speculative, this plausible scenario should at least be taken into consideration, in the absence of another molecular explanation, to clarify sperm competition.

A compelling case for this process can even be made for spermatozoa within one ejaculate, as previously pointed out by us.[36] It involves male mice that are heterozygous for wild type and *t-complex* haplotypes. The latter comprise long variant segments of chromosome 17 that exist as natural polymorphisms.[180] Spermatozoa harboring the wild type allele exhibit a greatly reduced chance to fertilize an egg (down to 1%) in comparison to those carrying a *t-complex* haplotype. It has been found that several *t-complex* distorter genes act additively to enhance the transmission rate of the respective haplotypes by increasing the motility of the *t-complex* bearing spermatozoa, leading to the speculation that not only the *t-complex* distorter genes, but also others involved in male gametogenesis may have the potential to evolve functionally different alleles, causing the phenotypic inequality of male gametes.[181]

Furthermore, the female can influence the outcome of sperm competition not only by providing gradients of chemical attractants to which sperm from different males might respond distinctly, but also by 'adjusting' the length of her oviducts: in promiscuous primates (e.g., the chimpanzee, *Pan troglodytes*), oviducts are longer than in non-promiscuous species (e.g., the gorilla, *Gorilla gorilla*), suggesting that more time can be spent on selecting the 'best' spermatozoon among those available.[69] If also the combination of different OR expressed on a single spermatozoon should be random, as suggested in the previous section,[170,172] a simple calculation indicates that the vast majority of cells in an ejaculate will express a unique 'signature' of chemoreceptors, leading to distinct responses upon chemical stimulation. As there is evidence that cryptic female choice involving the MHC does indeed take place in vertebrates, even in primates,[69,182] we must think of molecular mechanisms that could provide a framework for post-copulatory Self/Nonself discrimination.

Female Choice during Fertilization and in the Prezygotic State

The analysis of several different taxa of invertebrates has shown that these animals have evolved very elaborate systems to ensure that fertilization occurs mainly between unrelated members of a species, thus preventing self-fertilization and inbreeding. In many taxa, in particular in free-spawning animals, the absence of pre-copulatory mate selection and cryptic female choice before fertilization made it necessary to develop other means of selecting compatible gametes. Gamete recognition proteins play decisive roles whose molecular basis is already well understood in sea urchins (in particular, Echinometra species) and a marine mollusc, the abalone (Haliotis sp.). In many species belonging to these taxa, only conspecific spermatozoa are permitted to enter the egg (reviewed in ref. 24). However, gamete interactions may partially fail, as in reef building corals,[183] or may work only one-way: A-species sperm cannot fertilize B-species eggs, but B-species sperm is able to fertilize also A-species eggs.[184] Positive selection of repeated domains in sperm-binding polypeptides, such as the product of the *VERL* gene on the surface of abalone eggs, results in rapid evolution of these proteins.[185]

Furthermore, a sophisticated system of self-incompatibility exists in an ascidian species. In *Ciona intestinalis*, it involves four proteins, two on the sperm surface (s-Themis-A and s-Themis-B) and two that are part of the egg's vitelline coat (v-Themis-A and v-Themis-B). Each of the *v-Themis* genes are located on different chromosomes and within the first intron of the respective *s-Themis* gene, but in opposite transcriptional orientation. All four genes are extremely polymorphic and the regions exhibiting polymorphism in both *s-* and both *v-Themis* loci are near each other. It has been suggested that the interaction of the products of *both* sperm-expressed *Themis* genes with the respective proteins on the egg surface leads to fertilization failure.[23] The basic genetics of this interesting system had already been discovered

more than sixty years ago by Thomas Hunt Morgan, who also proposed a 'Haploid Sperm Hypothesis' to account for his findings.[186] In another ascidian, *Botryllus schlosseri*, self/nonself recognition during a natural transplantation reaction is controlled by three loci and two of the polypeptides specified by these loci (*fester* and *uncle fester*) contain a Sushi domain.[187,188] Such domains have also been detected in a protein found on mouse spermatozoa (sp56) which is thought to be involved in the interaction with the zona pellucida.[189]

We are referring to these systems, because they illustrate beyond any doubt that self/nonself discrimination mechanisms do not have to end once a spermatozoon has made its way into the vicinity of the mammalian oocyte. Even in the absence of MHC antigens from both of these cells,[158-160] there are many other proteins that might enable a highly selective Self/Nonself perception during sperm—oocyte interaction. It is known since several decades that the acrosome reaction in mammalian spermatozoa can be triggered by binding to the zona pellucida (ZP) protein 3,[190,191] and mice lacking the ZP3 protein are infertile.[192,193] Together with ZP2, ZP3 belongs to the 10% of the most polymorphic proteins in mammals and parallels between these polypeptides and gamete recognition proteins in invertebrates have been pointed out.[194] Several low-molecular weight substances are also able to induce the acrosome reaction in vitro, including γ-amino butyric acid, although the relevance of these observations for the situation in vivo has been questioned (reviewed in ref. 195). Presently, it must remain an open question whether xMHC-encoded proteins participate in the direct encounter of male and female gametes, although we have already highlighted some candidates (**Table 2**).

For a number of reasons, our knowledge of the events that directly follow fertilization is fragmentary, particularly so in humans due to ethical considerations. However, the outcome of the second meiotic division in the oocyte, which occurs in many vertebrates after the sperm has penetrated the vitelline membrane, is influenced by the MHC type carried by the spermatozoon and in vitro fertilization (IVF) experiments in mice have provided evidence that MHC-different mice exhibit female choice for distinct male MHC haplotypes also at this stage.[196] In addition, when MHC-heterozygous and -homozygous embryos should have been produced in approximately equal numbers, females carried more heterozygous embryos when the parents were infected with hepatitis virus. This might indicate that parental infection exerts an effect on the degree of MHC heterozygosity in the offspring.[197] At least in mice, MHC-influenced cryptic mate choice during fertilization or even at a later stage should therefore be taken into account.

How could this be accomplished in molecular terms? The time until a zygote is formed, can vary considerably between species, but takes ~18 hours in case of humans. During this period, the condensed sperm chromatin is unwrapped,[198] spermatozoan transcripts[199] are released and transcription begins. Remarkably, a spermatozoon does not only deliver male transcripts, but also certain transcription factors.[200] Among these is the product of the highly polymorphic *POU5F1* gene (**Table 2**). By forming heterodimeric complexes with proteins specified by other transcription factor genes (*SOX2*, *NANOG*), both of which are not part of the xMHC, POU5F1 critically influences embryonic differentiation.[201,202] The developmental target loci of POU5F1 and NANOG are hypomethylated in sperm DNA, but acquire methylation during development.[75] The authors describe also the localization of certain modified histones to developmental promoters, but we will not further refer to these, because histone genes are not part of the xMHC in all vertebrates. It is unknown whether naturally occurring polymorphisms of *POU5F1*,[101] or of the two other transcription factor genes affect these interactions. If so, this could be taken as an indication that the similarity between fungal and mammalian reproductive barriers does not only comprise the polymorphic pheromone receptor/pheromone system which we have previously mentioned (products of clustered *OR* genes, MHC molecules), but might also include transcriptional control mechanisms. In *Coprinopsis cinerea*, a successful completion of the mating process is dependent also on a transcription factor heterodimer to which both potential mates have to contribute distinct components.[27]

It has been estimated that a single sperm cell contains ~18,000 transcripts that are delivered to the oocyte upon fertilization, and some of these may have a role in the developing zygote.[199] For example, *HSPA1L* transcripts may participate in stress responses and other transcripts have been postulated to be involved in several other processes, including essential functions in early embryonic development,[203] although paternal transcripts have not been observed beyond the four-cell stage.[204] Even if the latter observation would be generally valid, paternal RNA molecules expressed in fertilized oocytes could have a function also as epigenetic modifiers of early embryonic development, by altering the phenotype of the offspring while preserving a wild-type genotype through paramutation.[205-207] Paramutation takes place when two alleles of a single locus interact such that one allele influences the other, yielding a change that can be inherited for more than one generation, even if the allele causing the change is not directly transmitted.[203] It appears possible that the product of the *ZFP57* gene (**Table 2**) is involved in this process.[89,90] The basic prerequisites for a substantial paternal contribution to embryonic development, apart from donating half of the zygote's nuclear chromosomes, do therefore seem to be fulfilled also in mammals, and there are abundant opportunities for executing MHC-dependent female choice as observed by Wedekind's group during their IVF experiments.[196]

A particularly striking case of post-fertilization, but prezygotic cryptic female choice is shown by a marine invertebrate, the ctenophore *Beroë ovata*. This organism has very large oocytes (~1 mm), in which a female pronucleus can migrate considerable distances at a speed of ~0.2 μm/sec. Fertilization occurs usually not only by a single, but by several male gametes. The male pronuclei are stationary within the egg's cytoplasm, but are 'visited' and 'evaluated' by the female pronucleus which seems to travel along microtubules that are provided by the male gamete. This inspection-like process may take several hours before fusion between the female and one of the male pronuclei eventually occurs (http://biodev.obs-vlfr.fr/recherche/biomarcell/ctenophores/beroe.htm).[208]

Female Choice Following Zygote Formation

In this short review, it is impossible to provide a comprehensive and critical summary of embryonic and foetal development and the participation of xMHC genes. However, some of these loci (**Table 2**) deserve particular, though brief, mention.

We have already pointed out that POU5F1 is a master regulatory transcription factor on whose activity early embryonic development is crucially dependent.[102] The *POU5F1* gene is downregulated during gastrulation and in cells of the trilaminar embryo, except in undifferentiated embryonic stem cells and in primordial germ cells. Its level of expression appears to be important for proper function,[209] and the shut-down of its transcription in non-germ cell embryonic tissue seems mainly carried out through methylation.[101] In this context, it is important that there are differences between vertebrate species as to how particular steps during embryonic development are accomplished[210] and which transcription factors are involved.[106]

On the other hand, we have already mentioned the *ZFP57* locus whose expression is essential in the earliest stages of embryonic development to ensure proper DNA methylation within specific genes. Instead of giving a detailed account of the function of the ZFP57 protein, we would like to refer the reader to the excellent review by X. Li.[89] As in case of *ZFP57*, the function of the *DDR1* gene is totally different from that of *POU5F1* and a defect does not lead to global problems as in case of the latter. However, its effects on blastocyst implantation and mammary gland development[100] demonstrate that the xMHC is also involved in these processes. With regard to *HSPA1A*, we have already drawn attention to the function of this nearly ubiquitously expressed gene in very early embryonic development.[114]

Finally, five further xMHC loci deserve mention, because they are expressed in placental or uterine tissue. One of these is *KIFC1*, which specifies a motor protein that is mainly found within the syncytiotrophoblast, both in early and term placentas and in even larger amounts in placentas from women suffering from preeclampsia or diabetes.[211] As the name implies, the syncytiotrophoblast is a multinucleated syncytium with a barrier-like function between the foetus and its mother. Remarkably, HLA class I or II antigens cannot be found in this tissue, possibly due to the immunological incompatibility between mother and child. The function of KIFC1 within the syncytiotrophoblast has not been investigated.

The expression of two MHC class I genes, in man *HLA-G* and *HLA-C*, has been observed on placental cells as well. Human oocytes and preimplantation embryos lack expression of these class I loci,[159,160] but, following implantation, both are strongly expressed on cells of the extravillous cytotrophoblast,[212-215] a site of intense interaction between foetal and maternal tissues. On the maternal side, both molecules appear to be recognized by NK cell receptors and downregulation of the nearly invariant HLA-G molecules on the surface of a trophoblast cell line in RNAi inhibition experiments leads to an abolishment of the resistance to NK cell lysis.[216] HLA-G is also thought to induce local tolerance of maternal immune cells towards the foetus (reviewed in ref. 217). The recent finding that there are *HLA-G* promoter variants that can be grouped into distinct haplotypes revealed that patients with recurrent spontaneous abortions had a significantly higher chance to carry a particular haplotype, while the healthy controls carried another. These results permit to conclude that variants of the *HLA-G* promoter may be associated with the risk to suffer from a spontaneous abortion.[93]

The other HLA class I molecule found on the surface of the extravillous cytotrophoblast, HLA-C, exhibits an intermediate level of polymorphism. Nevertheless, this poses a potential problem, since it opens the possibility for the mother to reject the foetal semi-allogeneic 'transplant'. HLA-C molecules react with polymorphic 2-domain killer immunoglobulin-like receptor (KIR) molecules on maternal cells (reviewed in ref. 217) and it has been found that this interaction is dependent on the expression of suitable foetal and maternal haplotypes whose products recognize each other. Since certain HLA-C/KIR combinations might favor the remodelling of maternal blood vessels, the placenta and the foetus will profit in selected cases through an optimized blood supply.[218] Therefore, even at this stage, self/nonself perception mechanisms appear to be at work.

The product of the *EHMT2* gene, a histone methyltransferase, is crucial for the developing embryo as well, since mouse embryos with a defect of this locus die in utero at ~day 10.[115,219] EHMT2 forms a functional heterodimer and acts to repress imprinted genes in the trophoblast. A better understanding of these results would possibly also be of interest for human reproduction, since the culture of preimplantation embryos is accompanied by a preferential loss of placental imprinting.[220,221] In addition, we have already referred to the *DDAH2* locus which specifies an enzyme that regulates intracellular levels of nitric oxide synthase inhibitors. At least in the rat, this enzyme seems to have an important function in maintaining myometrial quiescence during gestation and is also involved in controlling delivery at term.[108] It is unknown whether it is only of anecdotal relevance or truly important, but it has been reported that the DDAH2 activity is inhibited by tumor necrosis factor-α,[222] whose gene (*TNF*) is located close to *DDAH2*, within the class III region of the xMHC (not shown in **Fig. 2**).[52]

Conclusions

We are aware that these considerations are in most cases still incomplete, often speculative, and certainly influenced by our own preferences and interests. Furthermore, there are several additional genes within the xMHC which we have not mentioned with a single word, although they fulfil indispensable functions in reproduction. For example, the expression of the *TUBB* locus (the centromeric neighbor of *MDC1*) is essential for all cells, as mitosis and meiosis cannot work without microtubules. Our selection of xMHC genes (**Table 2**) is therefore highly biased, and one can take it for granted that the list will grow over the next years. The *EHMT2* locus provides a typical example. It has only recently been found that this protein carries out a crucial role during embryonic development,[115,219] but it has now been observed that it contributes also to proper differentiation and function of $CD4^+$ T helper cells.[223] *EHMT2* can thus be added

to those xMHC genes that act in totally distinct developmental stages. MHC class I genes, *OR* loci or *GABBR1* are well known further examples for the versatility of members of this interesting chromosomal segment. A particular hallmark of several of the encoded proteins is, however, their involvement in self/nonself discrimination processes that are fundamental not only for the functioning of the immune system, but also for the selection of appropriate mates.

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