MINIREVIEW

Regulated Expression of the Major Histocompatibility Complex Class I Genes

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Abstract. **The major histocompatibility complex (MHC) class I gene products are known to play a fundamental role in foreign antigen presentation to the cellular immune system. Much attention has been directed to the study of MHC class I gene expression as a means to understanding the processes by which MHC class I-mediated immune responses are regulated. Two areas of considerable interest have emerged, including regulation at the levels of transcriptional control and antigenic peptide-induced transport of MHC class I molecules. With an emphasis on these major areas of research, we review recent developments on the molecular and biochemical mediators and events of MHC class I gene regulation.** [P.S.E.B.M. 1993, Vol 203]

Classical transplantation antigens, encoded by the class I genes of the MHC, are responsible for the presentation of endogenously processed foreign antigen to the cellular immune system (1, 2). Although first described for their role in allogeneic homograft rejection (3), the MHC class I molecules are known to play their principle role in host immune defense against virally infected or otherwise antigenically modified syngeneic cells. Thus, the class I molecules enable antigen-specific $CD4^{-}8^{+}$ cytotoxic T lymphocytes (CTL), through their expressed T cell receptors (TcR), to detect cell-associated peptide antigens. Upon interaction of CTL with a peptide-modified MHC class I molecule on a target cell, a signal is transduced through the TcR, as well as other T cell-associated accessory molecules, which results in cytotoxic damage to the target cell. The expression of the class I molecules on the majority of mammalian cells, except for neural cells, acinar cells of the pancreas, and embryonal cells (4), enables virtually all cell types to be recognized by CTL (1, 2), and thereby eliminated from the host in the event of viral infection (5, 6). These same mechanisms are also likely to play a pivotal role in tumor immunity, whereby tumor cells present tumor-specific peptides to CTL in the context of MHC class I molecules (6, 7).

MHC class I antigens consist of a 41- to 45-kDa heavy chain that is noncovalently associated with an 11.6- to 12-kDa light chain, referred to as β2-microglobulin (β2m) (8, 9). In contrast to β2m, which is relatively invariant, a great degree of polymorphism is exhibited by the classical MHC class I heavy chains. Sequence analysis of heavy chains reveals divergence to the extent of 15–20% among allelic products for each locus and between locus-specific products (reviewed in Refs. 10 and 11). The polymorphism in the class I heavy chains is largely associated with the N-terminal extracellular domains, α1 and α2, while the membrane proximal third extracellular domain (α3) is significantly less polymorphic and displays sequence homology with the immunoglobulin superfamily. The polymorphism

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associated with the $\alpha 1$ and $\alpha 2$ domains underlies the role of MHC class I antigens in allorecognition and graft rejection (2, 12), whereas the $\alpha 3$ domain is engaged by the CD8 molecule to provide a costimulatory signal for recognition by and activation of CTL (13, 14). Each classical class I molecule is anchored in the plasma membrane by a hydrophobic membrane spanning domain which precedes the C terminus residing in the intracytoplasmic space (15). Several sites of possible phosphorylation are present in the cytoplasmic domain that may be involved in MHC class I-mediated signal transduction events (16).

The genes encoding MHC class I heavy chains and $\beta 2$m are nonlinked and present, respectively, on chromosomes 17 and 2 in mice and 6 and 15 in humans (17). MHC class I heavy chain genes of the mouse, designated *H-2*, are encoded within three functional loci, K, D, and L, and those of humans, designated *HLA*, are encoded by the A, B, and C loci. A significant feature of the MHC class I genes is the diversity that exists for each locus, with more than 30 alleles proposed for several *H-2* and *HLA* loci (11). The MHC is further broadened by the existence of the nonclassical class I loci, which are not known at present to play a major role in histocompatibility, but which may comprise the majority of class I genes for any given MHC haplotype (17, 18). These latter genes, also referred to as class Ib, encoded within the Qa/Tla region of the mouse, are much less diverse than the classical class I genes and their products, with few exceptions, are expressed with restricted tissue distribution. Although the function of the Qa/Tla molecules is not fully understood, recent evidence suggests that nonclassical class I products may participate in presentation of antigen to the $\gamma\delta$-TcR present on a subpopulation of T lymphoid cells (19).

The genetic structure of MHC class I genes appears to be well conserved among species (15, 17). The *H-2K* gene serves as a prototypic example of class I heavy chain genes consisting of eight exons. Exon 1 encodes the leader sequence, exons 2–4 encode the three extracellular domains $\alpha 1$, $\alpha 2$, and $\alpha 3$, each containing 90–92 amino acid residues, exon 5 encodes the hydrophobic transmembrane domain, and the cytoplasmic tail is encoded by exons 6–8 (15, 17). Analysis of the mRNA encoded from the *H-2K* class I genes indicates that alternative RNA splicing, particularly in the region of the RNA that encodes the cytoplasmic tail of the heavy chain molecule, contributes to the variety of forms of class I molecules encoded from this germline organization (20). By comparison, the *B2m* gene consists of four exons. Exon 1 encodes the leader peptide and the first two residues of the mature peptide, exon 2 encodes 93 amino acids of the protein, exon 3 encodes the C-terminal four amino acids and the 540-base pair exon 4 encodes the 3′ untranslated region (21, 22). Well-documented regulatory regions controlling the expression of these genes are present in their 5′ flanking sequences as well as, in the case of *B2m*, transcriptional regulatory intronic sequences (22). The coordinate expression of these genes in adult somatic cells ensures the equal production of the heavy chain molecules and $\beta 2$m for efficient assembly of the heterodimer.

Transcriptional Control of MHC Class I Gene Expression

Transcription of the MHC class I genes is regulated by the positive and the negative cis-oriented elements located in their 5′ flanking regions (Fig. 1) (18, 23–25). These regulatory sequences were initially identified by deletion mutagenesis of the 5′ flanking region of *H-2* genes that were aligned upstream of the chloromphenicol acetyl transferase (CAT) reporter gene. Based on studies using these constructs in *in vitro* analysis, two major enhancer regions have been defined. These include the class I regulatory element (CRE) or enhancer A, which maps −203 to −161 base pairs upstream of the transcription initiation site (23, 25–27) and the interferon concensus sequence, involved in interferon activation of MHC class I genes, which spans −167 to −139 base pairs upstream of the transcription initiation site (28, 29). In addition to these sites, analysis by *in vivo* footprinting of endogenous *H-2* and transgenic *HLA-B7* genes has identified site α, a DNA sequence element (core sequence TGACGC) spanning nucleotide positions −107 to −121 that enhances transcription of MHC class I genes in a tissue-specific manner, e.g., this site is occupied in lymphocytes but not brain tissue (30).

The CRE has been shown to act either as a positive or negative regulatory element, depending upon the differentiation status of the cell type (26). In undifferentiated embryonal carcinoma cells, for example, CRE serves as a negative regulator, whereas, when these cells are allowed to differentiate in the presence of retinoic acid, this element acts as a positive regulator (26). This phenomenon correlates well with the expression of MHC class I antigens on the surface of these cells. Similarly, as the expression of class I mRNA and antigens starts to increase during embryonic development, the CRE differentiates from a negative regulator to an enhancer. The CRE is itself divided into three subregions, designated CRE I (−173 to −161), CRE II (−203 to −185), and CRE III (−189 to −161), based on the distinct binding properties of these DNA elements (31, 32). CRE region I binding factors are present in tissues with high levels of class I antigen expression and absent in adult brain or fetal tissues where MHC class I antigen expression is very low or absent (32). CRE region II binding activity is found in nuclear extracts of tissues irrespective of MHC class I antigen expression (32). The nuclear factor RXRβ or H-2RIIBP, a member of the nuclear hormone receptor family of transcription

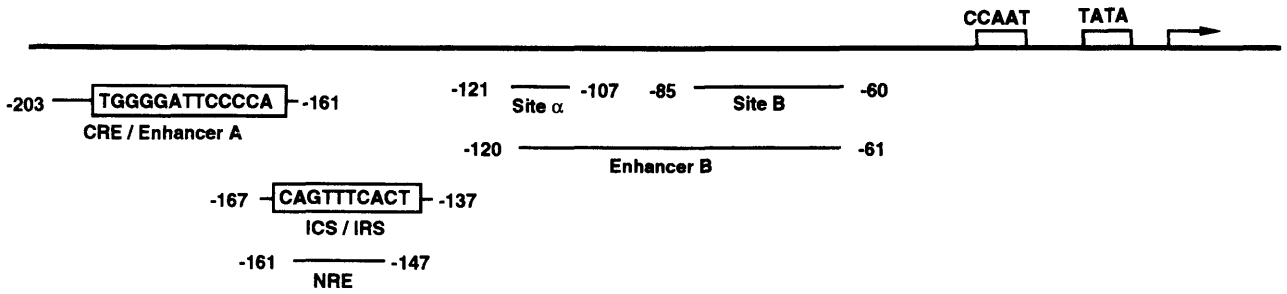

Figure 1. Enhancer elements mapping within the MHC class I promoter. Mapping of the enhancer elements was performed by an extensive series of *in vivo* and *in vitro* studies as described in the text. The position of each element, relative to the transcription start site, is indicated. The region identified as CRE/enhancer A contains the core element TGGGGATTCCCCA, which serves as the site of binding by several nuclear factors, including NF-κB. The ICS/IRS contains the core element CAGTTTCACT and is directly involved in the effect of interferon on the inducible expression of MHC class I genes by serving as a DNA binding site for nuclear transcription factors. *In vitro* transcription assays indicate that the sequence designated negative regulatory element (NRE) maps within the ICS/IRS region of the promoter and is involved in transcriptional repression. Site α was identified from *in vivo* footprint analysis as that promoter element showing strongest protection (within 660 base pairs of the upstream sequence of MHC class I genes) and is associated with MHC class I transcription in a lymphocyte-specific manner (30). The region identified as Enhancer B, defined by Kimura *et al.* (23), and site B, defined by Driggers *et al.* (36) from *in vitro* transcription assays, also plays a major role in controlling the expression of the MHC class I genes. The CCAAT and TATA boxes are identified.

factors, binds region II (33) to modulate transcription of MHC class I genes (34). This factor also binds site α (30). Although the functional significance of trans-acting factors binding to CRE III is not clear, CRE I and CRE II have been shown to have a significant role in the regulation of MHC class I gene expression. Two base pair substitutions in CRE I or CRE II, which alter the binding of trans-acting nuclear factors, reduce the activity of the CAT reporter gene (32). Additional evidence for a functional role of the class I regulatory element in class I gene regulation comes from *in vivo* studies in transgenic mice (35) and *in vitro* transcription assays (36). The latter studies identified the region from −209 to −160, containing CRE regions I and II, capable of activating the *H-2L*^{d} promoter in either orientation. This region is also known to be protected *in vivo* as shown by footprint analysis (30). Another sequence, mapping to position −85 to −60 and referred to as site B, is also involved in class I gene regulation, as shown by *in vitro* transcription analysis, and binds a nuclear factor through an NF-1 site (36).

Several nuclear factors bind to the core (−173 to −161, TGGGGATTCCCCA) of the MHC class I promoter CRE I region. The nuclear factor κB (NF-κB, molecular mass 42–44 kDa), identified as the factor binding to the enhancer of the immunoglobulin light chain κ, binds the CRE I region (37). NF-κB is constitutively expressed in mature B cells and is induced in pre-B cells by stimulation with lipopolysaccharide, cycloheximide, and phorbol 12-myristate 13-acetate (PMA) (38) or in cells of the non-B lineage with PMA (38, 39). The functional significance of binding of NF-κB to the MHC class I promoter core region has not yet been elucidated, although its property of activating other eukaryotic genes suggests that it may contribute to MHC class I gene expression (38, 39). Recently, the KBF1 DNA binding protein (molecular mass 48 kDa), which is associated with binding the *H-2K*^{b} promoter, was shown to be identical to the p50 subunit of NF-κB (40). This raises the likelihood that NF-κB, or its subunits, are directly involved in MHC class I gene expression. Another well-characterized factor that binds this region is KBF2 (molecular mass 58 kDa), which is found in a variety of cells, such as mouse fibroblasts, mouse erythroleukemia, and mouse T cell lines and HeLa cells (25, 41, 42). The relationship between KBF1 and KBF2 is not very clear; however, studies with undifferentiated embryonal carcinoma cells indicate that KBF1 may serve a role in upregulation of class I gene expression, whereas KBF2 may act as a repressor or as an inactive precursor form of KBF1 (43). Thus, when allowed to differentiate in the presence of retinoic acid and dibutyryl cAMP, embryonal carcinoma cells exhibit an increase in KBF1 activity and a concomitant decrease in KBF2 activity (43, 44). In addition to NF-κB, KBF1, and KBF2, H-2 transcription factor (H2TF1, molecular mass 110 kDa) also binds this region of DNA. H2TF1 activity is detected in a variety of cell lines such as HeLa, 3T3 fibroblasts, T cell lymphomas, retinoblastomas, and neuroblastomas (25, 37). Nuclear factor H2TF1 is present in very low amounts in pre-B cells, which is induced after stimulation with LPS but, in contrast to NF-κB, not after induction with cycloheximide (37). Other transcription factors such as MHC enhancer binding protein-1, a member of the zinc finger protein family (45), and interferon (IFN)-β second positive regulatory domain binding factor 1 (PRDII-BF1) (46) also bind the same promoter region, but the functional significance of these activities is not well established. AP-2 (molecular mass 52 kDa), a ubiquitously found DNA-binding protein, also binds to CRE (−184 to −173), but not in the core region (47). It has been suggested that AP-2 may con-

tribute an enhancer activity by modifying the binding of other transcription factors, such as KBF1.

Downstream of the CRE is the regulatory element referred to as the interferon consensus sequence (ICS; −167 to −139) (28, 29) or interferon responsive sequence (IRS; −165 to −137) (41, 47, 48). Interferon-mediated activation of MHC class I antigens is regulated by inducible as well as constitutive trans-acting factors binding to this region. A core region (−154 to −145) within ICS is responsible for binding of constitutive and IFN-induced transcription factors. ICS binding factors are present in the nuclear extracts of uninduced fibroblasts and lymphocytes, while at least two other factors have been shown to be induced after IFN treatment (31, 49, 50). Based on the finding that IFN-induced ICS binding factors show differential sensitivity to inhibition of protein synthesis, it is possible that the binding of some IFN-induced factors could be due to posttranslational modification (31). Base pair substitutions within this region abrogate the binding of these nuclear factors as well as IFN-mediated transcriptional induction of MHC class I genes. The consensus sequence motif of the core ICS (CAGTTTCACT) is found in interferon-inducible genes, which include *B2m*, 2–5A synthetase, complement factor B genes, and also IFN genes that are autoregulated by IFN treatment (51). The sequences in the regulatory regions in CRE and ICS are well conserved in various *H-2* alleles as well as *HLA* class I genes, despite the high degree of divergence in the coding sequences. Similarly, the trans-acting nuclear proteins from either mouse or human cells when tested by *in vitro* DNA-protein binding assays react with the homologous sequences from either species (31, 44, 47, 48).

The absence or suboptimal expression of MHC class I gene expression in various cell types has led to investigations on the molecular mechanisms of gene repression. Examples of negative regulatory elements (referred to by Ozato and colleagues as NRE) located in the MHC class I gene promoter region have been reported. Negative regulators have been demonstrated in undifferentiated embryonal carcinoma cells (26, 52), while such factors, binding the sequence from −161 to −147, were undetectable in cells that were induced to differentiate. Introduction of concatenated, double-stranded oligonucleotides containing the negative regulatory element sequence into F9 teratocarcinoma cells abolished the negative regulation by the endogenous class I promoter, thereby supporting the hypothesis that the negative regulatory element binding factor was acting as the repressor. Negative regulatory elements located at −1836 to −1521 of the transcription start site have been detected in cells transformed with oncogenic adenovirus 12 and the trans-acting factors binding to these elements were found only in adenovirus-transformed cells (53). Negative regulatory elements have also been described in the region between −503 and −1091 upstream of the promoter of the swine MHC class I gene *PD1* (54, 55). Although the mechanism(s) of repression is not completely understood, DNA binding factors associated with repression have been described. The potential influence of nuclear oncoproteins in repression of MHC class I gene expression has been shown from studies of N-*myc* in neuroblastoma (56) and c-*myc* in melanoma (57). Overexpression of N-*myc* is associated with a marked reduction in the expression of class I mRNA in rat neuroblastoma cells (56) and correlates with reduced binding by a cognate activating protein, notably the H-2TF1 transcription factor (58). Deficiency in the KBF1 protein, a member of the *rel* family of proto-oncogenes, is also associated with attenuated MHC class I gene expression when combined with a deficiency in NF-κB-like DNA binding activities (59). In Abelson virus-transformed murine pre-B cell leukemia, there is the existence of a labile repressor affecting transcription of the *H-2* class I genes (60). Although its identity is unknown, synthesis of the repressor protein can be affected by treatment of cells with cycloheximide, which augments the steady state mRNA levels for both the *H-2* and *B2m* genes.

Modulators of MHC Class I Gene Transcription

Among several biologic and chemical agents known to modulate the transcription of MHC class I antigens, the interferons are the most widely characterized (51). Interferons are produced by leukocytes (IFN-α), fibroblasts (IFN-β), and activated T cells (IFN-γ). As discussed earlier, interferons act as transcriptional activators by inducing trans-acting nuclear factors to bind the consensus IFN sequences (ICS/IRS) in the promoter of MHC class I genes (28, 29, 41, 47, 48). Sequences unrelated to ICS/IRS, located upstream as well as downstream of the transcription initiation site, have also been shown to be involved in IFN regulation of class I gene expression (29). Although recent evidence indicates that IFN-γ regulates gene expression by affecting phosphorylation of nuclear proteins (61), it is not clear whether interferons act directly as the transcriptional activators of the MHC class I genes or via a second messenger. However, IFN does not appear to influence gene expression in cells that do not express specific surface receptors for IFN and, therefore, binding of interferons to IFN receptors appears to be a prerequisite for IFN-induced class I gene activation (62). Interferons not only upregulate MHC class I antigen expression in a variety of cells that constitutively express these antigens, but they can also induce MHC class I antigens in cells that do not normally express these antigens (63). This is evident from studies of MHC class I antigen expression during embryonal development. Cell lines derived from embryos older than Day 6 that have not begun to express class I molecules

constitutively can be activated to express class I antigens by IFN treatment, suggesting that IFN-induced MHC class I gene expression precedes the constitutive expression of MHC class I antigens (44). Similarly IFN-γ can induce reversible class I antigen expression in F9 embryonal carcinoma cells that lack H-2 antigen expression prior to differentiation (26, 50, 63).

In addition to its property as a transcriptional regulator, it appears that IFN-γ can also modulate surface expression of class I antigens by an effect on posttranslational processing (Fig. 2). IFN-γ induction of the assembly of H-2 class I molecules was first reported for cell lines defective in formation of the MHC class I heterodimer (64). Exocytosis of heavy chains and β2m was rescued by treatment of the cells with IFN-γ. Consistent with this observation, we have observed recently that structural variations in β2m result in reduced maturation kinetics of the HLA class I heavy chains, an effect that is modulated by IFN-γ at the assembly level (65). Such data suggest that IFN-γ can act by neutralizing blocking factors that prevent class I heterodimer assembly as proposed by Klar and Hammerling (64) or by promoting synthesis of factors that enhance assembly, such as peptide transporter proteins (66, 67). It is also possible that due to the transcriptional activation of heavy chain and *B2m* genes by IFN, their increased synthesis may alter the equilibrium in favor of those molecules that are assembly competent.

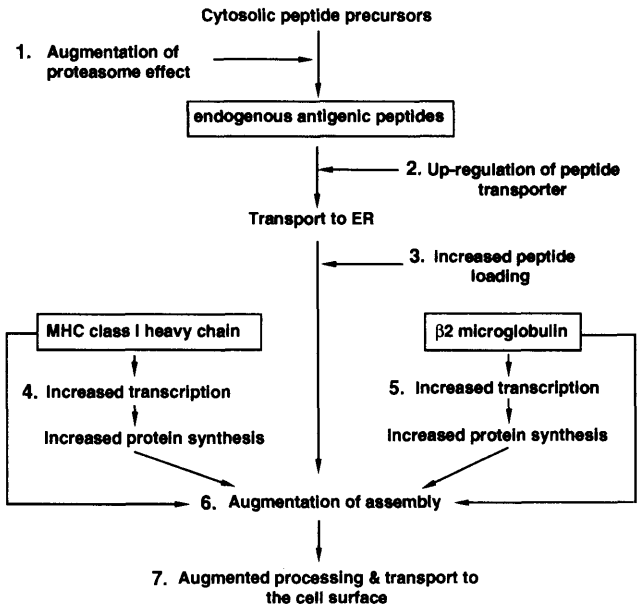

Figure 2. Multiplicity of IFN-γ effects on antigen processing and assembly of MHC class I molecules. Identified in the schematic are the effects of IFN-γ on the processing pathway through which foreign antigen peptide is directed to the plasma membrane in the context of MHC class I molecules. The steps affected by IFN-γ, including: (1) augmentation of the proteasome effect (154); (2) upregulation of peptide transporters, including TAP-1 (previously referred to as HAM-1) (66) and TAP-2 (previously referred to as RING-4) (67); (3) increased peptide loading (129); (4) increased heavy chain transcription (155); (5) increased β2m transcription (88); (6) augmentation of assembly (64); and (7) augmented processing and transport of the ternary complex (65), have each been shown to be involved in the upregulatory effect of IFN-γ on MHC class I expression.

Another well-studied modulator of MHC class I antigen expression is tumor necrosis factor (TNF). TNF is produced by activated mononuclear phagocytes and is cytotoxic for a variety of tumor cells (68). TNF modulates class I antigen expression on the tumor cell surface, but unlike IFN, it does not induce class I expression on class I-negative tumors (44, 69). Studies using a variety of tumor cell lines indicate that TNF is a transcriptional activator of MHC class I genes but not of β2m (47). Investigations into the mechanisms of transcriptional activation of MHC class I genes by TNF suggest that at least two pathways exist which rely on different mediators. In the first pathway, the role of transcription factor AP-1 has been proposed (47). Oncogenes *jun* and *fos*, which encode the major components of AP-1, are transcriptionally activated by TNF. In turn, AP-1 binds to DNA elements found in the promoter region of MHC class I genes. The involvement of transcription factor NF-κB has been suggested in a second pathway of TNF activation of MHC class I genes. TNF-treated HeLa cells show the activation of NF-κB, which binds the CRE I core region of the MHC class I promoter (47).

In addition to transcriptional activation, posttranslational modulation of MHC class I antigens by TNF has also been suggested (69). In human adenocarcinoma cell line Colo 205, TNF induces the cell surface expression of MHC class I antigens without affecting class I mRNA levels. The mechanism responsible for this effect remains to be determined.

Expression of MHC class I antigens is modulated in viral infection, and up- or downregulation of expression depends on the infecting virus. The viruses that upregulate MHC class I expression, by increasing the steady state mRNA levels, are radiation leukemia virus (70) and Moloney murine leukemia virus (71). Virally induced tumors also show reduced MHC class I antigen expression (5). The ability of oncogenic adenovirus to downregulate MHC class I antigen expression has been studied extensively (5, 72–75). Repression appears to be directed to the transcription of MHC class I genes by induction of trans-acting negative regulatory factors (53). Viruses have also been shown to integrate near MHC class I genes, which can affect their transcription (76). In addition to transcriptional control, there may be factors affecting the posttranscriptional translocation of immature RNA, as in the studies of adenovirus 12 infections in which decreased class I mRNA levels were not correlated with either decreased transcription or increased degradation of cytoplasmic RNA (72). Additional effects have been observed for adenovirus 2, which encodes a glycoprotein that noncovalently asso-

ciates with cytoplasmic class I heavy chains and prevents their terminal glycosylation (77–80). Downregulation of MHC class I antigens in cytomegalovirus-infected cells appears to be due to yet another posttranslational mechanism. Translational products of the cytomegalovirus genome share homology with the MHC class I molecules, and it has been proposed that products of the cytomegalovirus virion sequester endogenous β2m, thereby preventing association and subsequent maturation of synthesized cellular MHC class I heavy chains (81).

Posttranslational Maturation and Cell Surface Expression of MHC Class I Antigens

MHC class I heavy chains are synthesized on membrane-bound ribosomes in the rough endoplasmic reticulum, where they receive core *N*-acetylglucosamine and mannose residues. Heavy chain glycans are then converted into a more complex form within the Golgi complex, after which the class I heterodimer is transported to the cell surface (82, 83). This maturation process is dependent upon heavy chain association with β2m (83–86), which occurs immediately after the synthesis of both molecules (82, 83). In the absence of β2m, the heavy chain molecules are neither terminally glycosylated nor are they transported to the cell surface. This lack of MHC class I antigen expression, due to a defect in the synthesis of β2m, was first observed in the human lymphoblastoid cell line Daudi (87), in which a point mutation was identified in the translation initiation codon of β2m (88). That the failure in expression of HLA class I antigens was due solely to a defect in β2m synthesis, and not a concomitant alteration in heavy chain synthesis, was shown by reconstitution of these cells with the *B2m* gene, which resulted in the expression of HLA antigens on the cell surface (89–92). Similarly, four mouse cell lines derived from the C58 mouse thymoma cell line R1.1 do not express H-2 antigens on the cell surface due to a defect in *B2m* gene expression resulting from genetic recombination (21, 93, 94). Although for all four cell lines the DNA rearrangement was not at precisely the same site, each mapped to within the first intron of the *B2m* gene (93). The β2m null human melanoma cell line FO-1, which also fails to express HLA class I antigens on the cell surface, sustained a defect in the *B2m* gene mapping to intron 1, suggesting that this region may be a recombinational hot spot (95).

In addition to β2m negative cell lines, β2m-deficient mice have been developed in which the *B2m* gene has been disrupted by targeted mutagenesis (96). In the absence of β2m, MHC class I antigens are not expressed by the mutant mice, albeit for low level expression of H-2D^b antigens. Of considerable interest for our understanding of the role of MHC class I antigens in the immunobiology of antigen recognition is the observation that the MHC class I-negative mice fail to form mature CD4$^-$8$^+$ T cells. The absence of these cells is due to a blockade in the T cell selection process associated with the failure of immature CD4$^+$8$^+$ T cells to engage MHC class I molecules in the thymus (96).

Although there is little doubt that β2m plays a major role in the transport of MHC class I molecules to the cell surface, the role of β2m as an *obligatory* component for the cell surface expression of class I antigens has been questioned based on results obtained for the H-2L^d (97) and H-2D^b (98–100) molecules. The β2m-deficient cell line, R1E, when transfected with the H-2D^b gene, express H-2D^b antigens on the cell surface that could be detected by an antibody reactive with the conserved α3 domain of H-2D^b, but not by H-2D^b-specific CTL (98). Similarly, a mutation in H-2K^d, responsible for a defect in association with β2m, did not interfere with appearance of H-2K^d antigens on the cell surface; however, these antigens lacked epitopes for CTL recognition as well as a conformational epitope recognized by antibody against α1/α2 domain (101). When β2m associates with these heavy chains, they are expressed on the cell surface in much higher concentrations and in a distinct conformation (97, 98, 101). Thus, certain H-2 molecules can be expressed on the cell surface in the absence of β2m, but expression of a native conformational epitope imparted by properly folded α1/α2 domains is highly dependent upon the association with β2m (97, 98, 101). In addition to the role of β2m in the formation of the necessary heavy chain conformation for passage through the exocytic pathway, β2m also participates in the formation of stable MHC/antigenic peptide complexes that are recognized by T lymphocytes (102–105).

The protein structural requirements of MHC class I molecules for their efficient exocytosis have been analyzed with the aid of heavy chain structural mutants. Mutations responsible for aberrant glycosylation affect proper folding and intracytoplasmic passage of these proteins (106, 107). Mutations were created in the *H-2L^d* gene, in which codons for N-linked glycosylation were altered by site-directed mutagenesis, that resulted in reduced cell surface expression of the H-2L^d molecule. Reduction in the cell surface expression of the H-2L^d antigens was attributed to the susceptibility of nonglycosylated heavy chains to degradation prior to appearance at the cell surface (108). For the HLA-A2 heavy chain molecule, similar mutations in the consensus glycosylation sequences, Asn 86-X-Ser 88, by substituting Asn 86 with glutamine or aspartic acid, resulted in the synthesis of a 39-kDa nonglycosylated heavy chains with no surface expression (109). Asn 86 may be involved in the conformation of heavy chains and association with β2m, since substitution of Ser 88 with glycine did not result in changes in cell surface expression, even though glycosylation was affected

(109). Additional effects of disulfide bond formation on heavy chain folding in heterodimer formation and intracytoplasmic transport have been studied (110–112). In general, disruption of these bonds by either site-directed mutagenesis (111) or somatic mutation (112) alters the transport of these molecules to the cell surface. A structural variant derived from the R8 Abelson murine leukemia virus-transformed cell line, in which folding of the heavy chain was affected due to the substitution of amino acid Trp 167 with Arg, resulted in almost 80% of the synthesized molecules being retained in the rough endoplasmic reticulum (113). Recently, a variant of HLA-A2 with a mutation in the $\alpha 3$ domain at position 242 (Gln to Lys), was shown to prevent assembly with $\beta 2$m and abrogate transport of the heavy chain to the cell surface at 37°C (114). Subsequent stabilization at the permissive temperature range of 21–30°C resulted in heterodimer formation and exocytosis of the mutant molecule to the cell surface. Such results are consistent with the observation that association of a natural structural variant of $\beta 2$m (i.e., mouse $\beta 2$m) with HLA class I heavy chains is stabilized by reduced temperature incubation of human melanoma cells (65). Thus, the rate of transport of these glycoproteins is decided by their structure, both independent of and resulting from association with $\beta 2$m. Of those heavy chain molecules that remain in the endoplasmic reticulum (ER), association with the ER resident protein, calnexin, is a likely outcome of their failure to egress from the ER. This molecule, a 88- to 90-kDa phosphoprotein (115, 116), has been shown to bind H-2 class I heavy chain molecules that are synthesized in the absence of mouse $\beta 2$m, and, therefore, not folded properly for transport from the ER (117).

The Role of Foreign Antigen Peptide in MHC Class I Antigen Expression

An area of very active study in the posttranslational control of MHC antigen expression is the role of nominal antigen peptide in MHC class I antigen transport. It is now generally accepted that endogenously processed self- and/or viral antigenic proteins are degraded by proteolysis into small peptides within cells (118–121). At this point, it remains unclear whether nascent MHC class I proteins are loaded with the preformed peptides (122, 123), or, based on observations in congenic and MHC class I mutant strains of mice, whether MHC class I molecules may be involved in determining peptide formation (124). Thus, a pre-existing pool of peptides, if present, may be short lived unless protected by binding to the MHC molecules. Once bound by peptide, MHC class I molecules undergo conformational changes as determined by antibody binding measurements (97, 123), which coincide with their transport to the plasma membrane surface. The appearance of MHC class I antigen on the surface of peptide-transporter-defective RMA-S cells cultured at reduced temperature (i.e., 26–32°C) in the absence of antigenic peptide (125), and stabilization of MHC molecules devoid of antigenic peptide by other ligands such as anti-class I mAb (126), supports the conclusion that antigenic peptides serve a role in the transport of MHC class I complex through the exocytic pathway by stabilization of the heavy chain/$\beta 2$m heterodimer. Hence, as discussed earlier, one possible interpretation for the failure of class I heavy chain structural mutants to migrate efficiently to the membrane surface at a nonpermissive temperature is due to a defect in peptide binding. Indeed, MHC class I mutants with altered cell surface expression have been described that have sustained amino acid substitutions within the peptide-binding cleft (112, 113).

It has been proposed that peptides are translocated to a pre-Golgi compartment where they associate with the appropriate MHC class I heavy chain and trigger proper folding for association with $\beta 2$m (127). The first direct evidence for induction of heavy chain and $\beta 2$m assembly by peptides has come from experiments using the RMA-S cell line (123). In these cells, the amount of coprecipitable $\beta 2$m increased when the cells were treated exogenously with peptides from influenza nucleoprotein. Furthermore, a conformational epitope derived from the $\alpha 1/\alpha 2$ domain of H-2 heavy chains conformed with $\beta 2$m was detected after the treatment of these cells with specific antigenic peptide. Another observation that serves as evidence for the induction of assembly of heavy chain and $\beta 2$m by antigenic peptide has been obtained from experiments in which *in vitro* translated products of HLA-B27 and $\beta 2$m were induced to associate in the presence of a nucleoprotein peptide of influenza A virus (128, 129). Assembly was shown to occur in the lumen of microsomal vesicles, which represents the *in vivo* ER compartment, suggesting that formation of the ternary complex (i.e., heavy chain, $\beta 2$m, and peptide) occurs in the ER before transport to the Golgi (128). These observations are further supported by recent evidence that HLA-A2 heavy chains and $\beta 2$m, if separated in the presence of denaturing agents, can reassemble efficiently in the presence of MHC-restricted influenza virus peptide (130). Different peptides derived from either influenza virus nucleoprotein or influenza matrix protein stimulated reconstitution of only those MHC class I molecules with which they were known to interact, as shown by CTL reactivity (131, 132).

Loading of peptides into the MHC class I heterodimer is controlled by the genes that encode for peptide transporter molecules and factors responsible for proteolytic degradation of proteins (133, 134). The molecules associated with peptide transport, referred to as TAP-1 and TAP-2, are encoded in the MHC class II region and are homologous to the ATP-driven mem-

brane transporters that are responsible for energy-dependent pumping of a variety of molecules across cellular membranes (67, 135, 136). Cells defective in either TAP-1 (e.g., mutant 721.134) or TAP-2 (e.g., RMA-S) fail to express MHC class I molecules on the cell surface due to a defect in the transport of peptides from the cytosol into the ER (137–139). Recently, TAP-1-deficient mice were described in which a defect in class I assembly and transport resulted in a marked reduction in cell surface H-2 antigen expression (140). Consistent with studies performed on β2m-negative mice (96), the TAP-1-deficient animals show normal distributions of T cells with the immature $CD4^+8^+$ and mature $CD4^+8^-$ phenotypes, but lack mature $CD4^-8^+$ cells (96, 140). Transfection of the TAP-1 or TAP-2 peptide transporter cDNA into the appropriate peptide-transporter-deficient cell lines induces the transport of peptide-MHC class I complexes to the cell surface (137–139, 141). In at least one cell line, transfection of both the TAP-1 and TAP-2 cDNA was necessary for recovery of class I expression on the cell surface (142), supporting the proposal that TAP-1 and TAP-2 assemble as a heterodimer in order to transport peptides into the ER lumen (67, 135). Although the precise mechanism by which peptide transporter molecules augment peptide loading and stabilization of MHC complex is not yet clear, peptide transporter molecules and proteolytic components encoded by genes within the MHC region are inducible by IFN-γ (66, 67) and may be partially responsible for activation of MHC class I antigen expression after IFN-γ treatment.

The question of how these three components, i.e., heavy chain, β2m, and nominal antigen peptide, become physically associated to form a stable complex is receiving considerable attention. Of the three components necessary for translocation of a stable structure to the cell surface, β2m is the only invariant component and is likely to contact heavy chain in a consistent fashion (10, 143). In contrast to β2m, which contacts heavy chain at each of the extracellular domains, peptides associate with heavy chain in the polymorphic α1/α2 domain "groove" (143–146). Furthermore, peptide is a variant component of the system and, therefore, binding parameters may change with the sequence of the peptide, particularly when substitutions occur at positions that contribute to the binding motifs that have been identified (132, 144, 145, 147, 148). Antigenic peptides have been isolated from MHC class I molecules using acid elution (131, 132, 148), and studies examining the interaction between MHC class I heavy chains and peptide have recently been undertaken (144, 145, 149). Endogenously bound peptides are typically eight to nine amino acids in length and, except for a certain few residues, each position can accommodate many different amino acids. For certain positions, there are characteristic amino acid residues, referred to as anchor residues, that interact with allelic residues within the heavy chain to allow stable binding of peptides. Conserved regions within the peptide binding groove of MHC class I molecules are involved in peptide association, specifically of the NH2- and COOH-termini of the peptide, and a region of polymorphic residues in the middle of the groove is complementary to the anchor residue, providing the structural framework for heavy chain-specific peptide binding (150). In addition to acid elution (131, 132, 148), antigenic peptides from the class I complex can be released by reducing disulfide bonds in the α1 and α2 domains and by unfolding heavy chains using detergents as demonstrated for HLA-A2 and a peptide derived from influenza matrix protein (151). The difficulty of removing the endogenous peptide or an exogenously added peptide from MHC class I molecules suggests that they are bound with high affinity (147, 148, 151). This is supported by experiments in which the binding of radiolabeled peptides to cell surface-associated MHC class I molecules has been shown to be a very rapid process with a slow dissociation rate (152, 153).

Although the stoichiometry of 1:1:1 for the components in the ternary complex is expected, the equilibrium can change according to the concentration of each of the components. The concentrations of β2m and heavy chain can be directly regulated by several modulators, such as TNF and IFN-γ, whereas the concentrations of intracellular peptides would be expected to alter indirectly by IFN-γ induction of the MHC linked multicatalytic proteinase (154) and peptide transporter molecules (66, 67), affecting their intracellular concentrations and the ultimate cell surface expression of the MHC class I molecules.

Summary

Numerous cellular and extracellular factors appear to be involved in controlling the expression of the MHC class I genes and their products. Transcriptional regulation of MHC class I genes correlates with different constitutive and inducible trans-acting factors and multiple DNA regulatory elements. It is evident that many trans-acting factors from cells of different lineages and from various species can bind to the same regulatory sequence *in vitro* and that the same binding factor can bind to multiple regions within the promoter. Thus, the stage of embryonal development, cellular differentiation status, and cellular activation constitute the major components controlling synthesis and DNA binding of the trans-acting factors that participate in regulating the transcription of MHC class I genes. Furthermore, the simultaneous interaction of multiple binding factors to the same or adjacent regions of the MHC class I promoter is likely to control the final outcome of the transcription of MHC class I genes.

There is further indication that tissue-specific expression of MHC class I genes correlates with occupancy of cis elements *in vivo*, and that control of MHC class I gene expression is a product of chromatin structure in relationship to the synthesis of transcription factors (30).

In addition to the regulation of MHC class I antigen expression at the level of transcription, recent evidence indicates that MHC class I assembly and cell surface expression are a product of association with processed antigen. Numerous studies have analyzed the nature of the peptide that associates with specific MHC class I molecules and the structural motif of such peptide molecules (for detailed review, see Refs. 131 and 147). Importantly, transport of the MHC class I heterodimer to the membrane surface is directly affected by the interaction with and stable binding by foreign antigen peptide. Alterations in the peptide transporter pathway have severe consequences in antigen presentation, as do defects in assembly of the MHC class I heavy chain molecules with β2m. Thus, class I transport requires formation of the ternary complex, ensuring that each class I molecule directed to the cell surface is assembled with peptide for presentation of the signal ligand to the TcR.

The ability to modulate these MHC class I responses at the transcriptional and posttranslational levels is now becoming a reality based on information derived from the described variety of approaches aimed at understanding the regulation of MHC class I expression. The potential use of transcriptional regulators to modulate MHC class I gene expression raises intriguing possibilities for altering the course of allogeneic transplantation, viral infection, and tumor immune detection. Exciting possibilities are also becoming a reality in the use of specific MHC class I binding peptides for the immunotherapy of disease by regulation of MHC class I expression and antigen presentation, as well as by altering peptide-dependent, TcR-mediated target cell recognition. Although efforts to understand the regulation of these genes remain relatively early in their developmental stages, a wealth of information is presently available that is likely to prove useful for the effective modulation of MHC class I-mediated immune responses.

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