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The regulatory network behind MHC class I expression

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ABSTRACT

The MHC class I pathway, presenting endogenously derived peptides to T lymphocytes, is hijacked in many pathological conditions. This affects MHC class I levels and peptide presentation at the cell surface leading to immune escape of cancer cells or microbes. It is therefore important to identify the molecular mechanisms behind MHC class I expression, processing and antigen presentation. The identification of NLRC5 as regulator of MHC class I transcription was a huge step forward in understanding the transcriptional mechanism involved. Nevertheless, many questions concerning MHC class I transcription are yet unsolved. Here we illuminate current knowledge on MHC class I and NLRC5 transcription, we highlight some remaining questions and discuss the use of quickly developing high-content screening tools to reveal unknowns in MHC class I transcription in the near future.

1. Introduction

The role of MHC (Major Histocompatibility Complex) molecules in adaptive immunity has been extensively studied since their discovery in 1936 (Klein, 1986). MHC presents peptides to T cells leading to initiation of an adaptive immune response in many pathological conditions, such as auto-immunity, cancer, and during infections. While MHC class I molecules generally present peptides to be recognized by the T cell receptor of $CD8^+$ T cells, MHC class II molecules showcase peptides to $CD4^+$ T cells. Though the principles of loading and presenting peptides are similar for both molecules, their different peptide sources require them to follow different transport routes and mechanisms of peptide loading for presentation (Neefjes et al., 2011). MHC class I molecules, in complex with β-2-Microglobulin (β2 M), are loaded with endogenous peptides generated by the proteasome and imported into the ER by the heterodimeric TAP1/TAP2 transporter. In contrast the MHC class II peptide groove is blocked in the ER by Invariant Chain (Ii) and becomes available only in the MIIC (MHC class II loading Compartment), which is connected to the endocytic pathway thus also containing exogenously derived peptides. Another dissimilarity between MHC class I and MHC class II molecules is their expression pattern. MHC class I molecules are ubiquitously expressed while MHC class II molecules are expressed in specialized Antigen Presenting Cells (APCs) like dendritic cells, B cells and macrophages and in several cell types under inflamed conditions. Therefore, it is unlikely that MHC class I is expressed by exactly the same transcriptional machinery as MHC class II. CIITA/NLRCA (Class II TransActivator/NOD-like receptor family and acid domain containing) was identified in 1993 as the master regulator of MHC transcription (Steimle et al., 1993). However, it soon became clear that CIITA, though capable of activating both MHC class I and class II transcription *in vitro* (Martin et al., 1997), could not be the master regulator of class I molecules since its expression is restricted to APCs. Additionally, *CIIta*-deficient mice retain intact MHC class I expression (Chang et al., 1996; Williams et al., 1998). This led to a search for a MHC class I regulator, which was identified in 2010 to be NLRC5/CITA (NOD-like receptor family CARD domain containing 5/Class I TransActivator) (Meissner et al., 2010).

Following this discovery, the characterization of three independently generated knockout mice enabled to detail the contribution of NLRC5 to MHC class I in different tissues (Staehli et al., 2012; Robbins et al., 2012; Biswas et al., 2012; Ludigs et al., 2016). From these analyses it emerged that NLRC5 is not a master regulator of MHC class I, as in fact MHC class I expression is still found in these mice. Moreover, the $CD8^+$ T cell population was largely intact arguing for adequate MHC class I expression during T cell development in the thymus (Staehli et al., 2012; Perarnau et al., 1999). However, MHC class I expression in lymphocytes was strongly reduced in the absence of NLRC5, and T cells in particular. Therefore, under steady state conditions, NLRC5 is a key transcriptional regulator of MHC class I in selected immune cell subsets. In this review we mainly focus on the mechanism of MHC class I expression through NLRC5 by discussing recent findings, including the accessory molecules required during this process. Additionally we will highlight some remaining questions in the field and why high-content screening represents a good approach to

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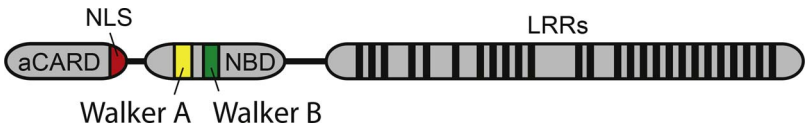

Fig. 1. Schematic view of domain structure of NLRC5. The N-terminus of NLRC5 contains an atypical Caspase Activation and Recruitment Domain (aCARD) in which the Nuclear Localization Signal (NLS) is localized. The aCARD is followed by a central Nucleotide-Binding domain (NBD). The Walker A and B motif are parts of the NBD. The C-terminus of NLRC5 contains irregularly scattered Leucin-Rich Repeats (LRRs).

identify these missing links.

2. NLRC5, one of the expression forces within the NLR-family

Since it became clear that CIITA could impossibly function as the main regulator of MHC class I transcription, the quest to identify the real master regulator of MHC class I began. In 2010, ChIP (Chromatin Immunoprecipitation) analysis revealed that NLRC5 is associated to MHC class I promotors regulating its transcriptional activity (Meissner et al., 2010). NLRC5 is the largest member of the NOD-like receptors (NLRs) family. Most of the currently known 22 members of the NLR-family have been described as regulators of innate immunity by recognizing Microbial- and Pathogen-associated Molecular Patterns (MAMPs and PAMPs) (Meunier and Broz, 2017). In contrast two family-members, NLRC5 and CIITA, function as regulators of adaptive immunity by regulating MHC class I and MHC class II transcription respectively. All NLR-family members share a similar protein structure consisting of three main domains: a variable N-terminal protein-interaction domain, a central Nucleotide-Binding Domain (NBD) and a C-terminal stretch consisting of LRRs (Albrecht and Takken, 2006) (Fig. 1). Variation in the N-terminal domain of the NLRs subdivides them in four groups having either an acidic transactivation domain (NLRA), Baculovirus IAP Repeat (NLRB/NAIPS), a Caspase Activation and Recruitment Domain (CARD/NLRC) or a Pyrin domain (PYD/NLRP). Of note, the NLRC5 N-terminal CARD differs in sequence from the others and is called an atypical CARD (aCARD), showing only little sequence similarity with the CARD of for example CIITA (Benko et al., 2017) (Fig. 1). Why the N-terminus of NLRC5 still belongs to the CARD subfamily is explained by structural alignments of the NMR-resolved N-terminal domain of NLRC5 to a representative of each death-fold subfamily, which showed the best fit to the CARD (Gutte et al., 2014; Motyan et al., 2013). In between its CARD and NBD domain NLRC5 contains a bipartite-type nuclear localization signal (NLS), allowing NLRC5 transport towards the nucleus (Meissner et al., 2010). The NLRC5 NBD contains a Walker A and Walker B region essential for binding to and hydrolyzing nucleotide triphosphate respectively (Fig. 1). The nucleotide triphosphate (NTP)-binding Walker A motif of NLRC5 is required for its nuclear translocation and consequently MHC transactivation, although it is not clear whether NTP-binding is essential for this process (Meissner et al., 2012a; Neerincx et al., 2012). Remarkably, the Walker B motif hydrolyzing NTP is not essential for NLRC5's transactivation function (Meissner et al., 2010). Nuclear export of NLRC5 is mediated through Crm1 (Neerincx et al., 2012; Benko et al., 2010). It seems that constant cytoplasmic-nuclear exchange of NLRC5 is required for its transcriptional activity since both blockade of the Crm1 mediated export by Leptomycin B as well as nuclear targeting of NLRC5 by fusion with a NLS reduced MHC class I transactivation (Meissner et al., 2012a; Neerincx et al., 2012). These data together are indicative of particular functional, but temporary modifications of NLRC5 in the cytosol. Surprisingly, a DNA-binding domain is absent in both NLRC5 and CIITA indicating that these regulators need to orchestrate complex formation with DNA binding proteins once transported into the nucleus (Masternak et al., 2000). Like its N-terminal domain, NLRC5 also has an unusual C-terminal domain containing LRRs which are found in many different classes of proteins to mediate protein-protein interactions (Kobe and Deisenhofer, 1995). NLRC5 contains the largest number of LRRs of all family members, irregularly scattered along the C-terminus (Kuenzel et al., 2010) (Fig. 1). Besides their role in protein interactions, LRRs have been shown to be important for nuclear export (Neerincx et al., 2012; Hake et al., 2000).

Recently also another NLR-family member, NLRP2, has been shown to function as a selective repressor of HLA-C expression in fetal extravillous trophoblasts that lack NLRC5 and CIITA. NLRP2 does not have a nuclear localization domain suggesting it to act as a cytosolic regulator and not as a transcriptional regulator directly (Tilburgs et al., 2017). Yet, this additional evidence for the involvement of NLRs in the regulation of MHC expression is intriguing and suggests that other NLR-family members may also fulfill a regulatory role in MHC expression and antigen presentation transcriptionally or post translationally.

3. MHC class I promotor regions

Before the identification of NLRC5, MHC class I expression was proposed to be controlled via the NF-κB binding Enhancer A region and interferon (IFN)-sensitive response element (ISRE) motif in the MHC class I promotor region (Ting and Baldwin, 1993) (Fig. 2). Whereas these regions certainly contribute to MHC class I expression after stimulation by type I and type II IFNs or engagement of the NF-κB pathway, their contribution to basal MHC class I in various tissues and cell types remains elusive and shall be readdressed by the use of recently generated genetic tools. From *in vitro* approaches, we know that NF-κB-induced MHC class I expression is most prominent for the HLA-A

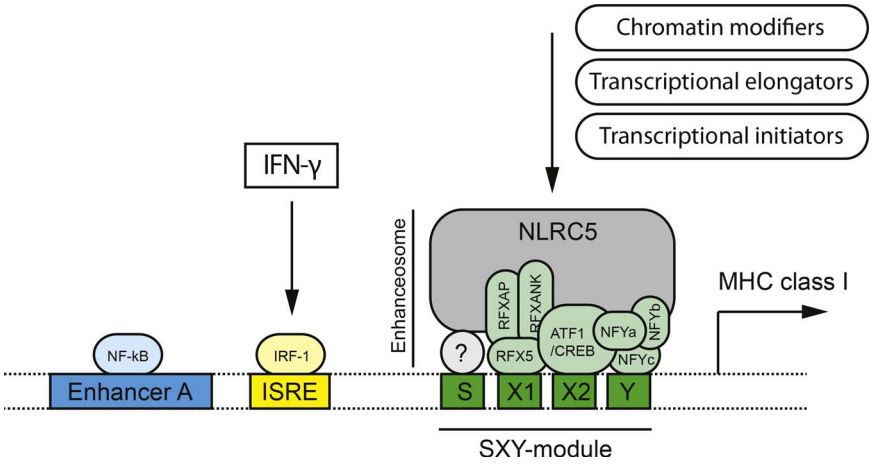

Fig. 2. The MHC class I promotor region. NLRC5 translocates to the nucleus where it forms a MHC class I enhanceosome together with the RFX-complex, ATF1/CREB and the NFY-complex (binding the SXY-module) to activate MHC class I transcription. NLRC5 recruits chromatin modifiers and transcriptional elongation and initiation factors. Additionally, type II interferons (IFN-γ) can activate MHC class I transcription by upregulation of IRF1 which can bind to the ISRE elements of the MHC class I promotor. NF-κB binding to Enhancer A is necessary for both constitutive and induced MHC class I expression.

locus, which contains two NF-κB binding sites in its Enhancer A region. HLA-B contains only one NF-κB binding region supported by a Sp1 binding site (Sp1 is known to interact with NF-κB (Perkins et al., 1993)), while the other alleles are not transactivated by NF-κB at all (Gobin et al., 1998a). IFN Regulatory Factors (IRFs), of which IRF1 is the principle factor for MHC class I transcription, target the ISRE and are upregulated in response to IFN-γ stimulation (Chang et al., 1992). IFNs signal through the JAK/STAT pathway leading to transcriptionally active STAT1 (dimers) binding to *cis*-elements (Gamma interferon Activation Site, GAS) in the IRF promotor region (Chang et al., 1992; Decker et al., 1997). Our expression data obtained from several cell lines and publicly available expression profiles reveal that some IRFs are constitutively expressed at low levels (Wu et al., 2009 and unpublished data), suggesting that IRFs control MHC class I expression under non-inflammatory conditions.

In addition to the above described regions, the MHC class I promotor region contains a SXY-module containing a S, X1, X2 and Y box. As said, NLRC5 lacks a DNA-binding domain and is therefore dependent on other components, called the enhanceosome, to connect to the promotor region (Masternak et al., 2000) (Fig. 2). Most research to the enhanceosome is done on MHC class II transcription (Sachini and Papamatheakis, 2017), but can easily be extrapolated to MHC class I transcription since the SXY-module is highly conserved between the two promotor regions (Neerincx et al., 2012; Meissner et al., 2012b). For MHC class I, the X1 box is bound by the RFX complex (Ludigs et al., 2015), a trimer consisting of the DNA-binding subunit Regulatory Factor X 5 (RFX5), RFX-Associated Protein (RFXAP) and RFX containing three Ankyrin repeats (RFXANK). RFXANK is known to bind NLRC5 via its ankarin repeats (Meissner et al., 2012b; Durand et al., 1997; Gobin et al., 1998b; Masternak et al., 1998; Steimle et al., 1995). The X2 box is bound by X2BP, a complex of members of the CREB/ATF transcription factor family including CREB1, CREM and ATF1 (Moreno et al., 1999; Moreno et al., 1995). The Y box is bound by a NFY-complex consisting of NFYa, NFYb and NFYc subunits (Sachini and Papamatheakis, 2017). Intriguingly, S box binding proteins have not been identified yet for MHC class I and II (Fig. 2).

ChIP-sequencing revealed that NLRC5 is specifically recruited to genes encoding MHC class I and its accessory molecules B2 M, TAP1 and LMP2 (Ludigs et al., 2015), while previous data showed that CIITA induces the expression of MHC class II and its accessory molecules like Ii, HLA-DM and HLA-DO (Kern et al., 1995; Krawczyk et al., 2004). Thus, CIITA and NLRC5 are extremely specific transcriptional regulators even though they function via the same common DNA binding factors of the enhanceosome. In addition, the absence of compensatory mechanisms was shown by the generation of *CIIta/Nlrc5* double-deficient animals (Ludigs et al., 2015). ChIP experiments revealed that variation in the S box of the MHC class I and II promotors are an essential feature granting NLRC5 specificity (Ludigs et al., 2015). Studying the role of the S box, likely by the identification of S box binding proteins, will help to understand the specificity of NLRC5 and CIITA transactivation. Interestingly, a more or less conserved SXY-module has been identified in the promotor region of all NLRC5 target genes, including the MHC class I light chain B2 M, the subunit of the peptide transporter TAP1, and the immunoproteasome subunit LMP2, underlining the strict requirement for this regulatory element by NLRC5 (Ludigs et al., 2015).

4. NLRC5 recruits components regulating methylation and acetylation

Located at the enhanceosome complex NLRC5 is suggested to function, like CIITA, as a scaffold to recruit transcriptional initiation and elongation factors, but also co-activators (like CBP (CREB Binding Protein), p300, GCN5 and PCAF) and chromatin modifiers. his last category is important for transcriptional regulation via methylation or acetylation of transcriptional components. Most current knowledge on methylation and acetylation events regulating MHC transcription comes from studies on CIITA and MHC class II transcription.

4.1. Methylation

CARM1/PRMT4 is one of seven arginine methyltransferases so far identified in mammals and is recruited by CIITA resulting in methylation of histone H3R17, positively affecting MHC class II transcription (Zika et al., 2005). Additionally, CARM1 is known to methylate the closely related co-activators CBP and p300 which have been shown to be required for IFN-γ-induced MHC class II expression (Zika et al., 2005). CARM1 or one of its family members may fulfill a similar role in MHC class I regulation. Strikingly, histone de-methylation on other sites can also increase IFN-γ induced MHC class I expression. NLRC5 has been shown to be essential for the removal of the gene-silencing trimethylation of Lysine 27 on Histone 3 (H3K27me3) on the MHC class I promotor (Robbins et al., 2012). This is likely the result of specific recruitment of de-methylating enzymes by NLRC5. Additionally to histone methylation, DNA methylation of the MHC I promotor region regulates the transcription of some but not all HLA-A alleles. This is based on the fact that HLA-A24 possesses a polymorphism in its promotor region disrupting a methylation site (CpG → TpG), while this site is intact in the lower expressed HLA-A3 lineage, arguing for methylation mediated suppression of expression of the HLA-A locus (Ramsuran et al., 2015). DNA methylation does not regulate HLA-B or –C expression levels directly, since these alleles have been shown to be unmethylated (Ramsuran et al., 2015). Clearly, the details of how methylation and de-methylation processes are involved in MHC class I transcription are still largely unknown.

4.2. Acetylation

Besides the inhibitory function of methylation, acetylation seems to be an important enhancer of MHC transcription. For example, acetylation on Histone 3 (AcH3) activates MHC class I transcription (Robbins et al., 2012). Oppositely, Histone deacetylases (HDACs) are known to negatively affect MHC class II transcription. HDAC4 competes with CIITA for its binding site on the RFXANK ankyrin repeat domain and HDAC1/HDAC2 have been shown to inhibit CIITA enhanceosome assembly (Wang et al., 2005). Both these mechanisms dampen MHC class II expression. The regulation of the NLRC5 enhanceosome may have similarities to this mechanism, also because NLRC5, like CIITA, is an RFXANK interacting protein (Meissner et al., 2012b). Though acetylation is shown to act as a positive effector of MHC transcription, most observations involving acetylation cannot be explained at a molecular level yet.

5. Post-transcriptional regulation of MHC class I expression

MHC class I expression levels are not only regulated at transcriptional level, but also post-transcriptional regulation affects the amount available for peptide loading and presentation. Post-transcriptional regulation of MHC class I levels identified so far occurs via its 3′UTR. The E3 ligase MEX-3C acts as a post-translational repressor by binding to the 3′UTR of HLA-A2 (but not HLA-B), preventing its translation and inducing its degradation in a RING-dependent manner (Cano et al., 2012). Additionally, miR-148a has been shown to reduce specifically HLA-C expression levels via its binding site present in the HLA-C 3′UTR (Kulkarni et al., 2011). In addition, miR-34a interacts with the 3′UTR of NLRC5, downregulating its expression thereby likely affecting MHC class I levels indirectly (Li et al., 2016). Again, several molecular details and knowledge about other post-transcriptional regulatory mechanisms are lacking.

6. Regulation of NLRC5 expression

The importance of NLRC5 in regulating MHC class I transcription suggests that MHC class I expression depends on the NLRC5 levels. Indeed, the expression levels of NLRC5 and MHC class I are highly correlated, with more NLRC5 resulting in more MHC class I expression and increased MHC class I cell surface levels (Neerincx et al., 2012). Therefore, to understand MHC class I transcription, it is likewise important to understand how NLRC5 expression is regulated. The gene encoding for NLRC5 protein is located at locus 16q13 of the human genome. There are several isoforms of NLRC5 reported which differ in length of their C-terminal LRR domains (Neerincx et al., 2010). The expression pattern of the different isoforms is tissue and cell type specific, though their functional roles are not clearly defined yet (Kuenzel et al., 2010). NLRC5 is highest expressed in hematopoietic cells (Meissner et al., 2010; Robbins et al., 2012; Kuenzel et al., 2010; Neerincx et al., 2010) rendering them perfect model systems to search for (DNA-binding) factors involved in NLRC5 transcriptional regulation. Furthermore, NLRC5 is known to be upregulated in response to viral infection mainly as a result of increased type I IFN secretion and subsequent JAK/STAT signaling similar as described for IRF1 above (Staehli et al., 2012; Kuenzel et al., 2010; Katze and He Gale, 2002; Platanias, 2005) (Fig. 3). Unfortunately, more detailed knowledge on NLRC5 transcriptional regulation in particular in the absence of inflammatory signals is so far limited.

7. MHC class I association to disease

Classical MHC class I molecules (HLA-A, −B and −C) are highly polymorphic within the human population (Parham et al., 1988). This variation ensures a large diversity of peptides to be presented by MHC class I. Besides causing variation in the repertoire of loaded peptides, many polymorphisms localize in the promotor and untranslated regions inducing diversity in MHC class I expression levels. These two variables together highly influence the extent of immune responses between individuals, explaining why MHC molecules are often highly associated with diseases like infections, but also auto-immunity and cancer.

In some viral infections, like HIV, high HLA expression levels have been correlated to enhanced control (Apps et al., 2013). The levels of MHC class I expression are thus important to clear virus-infected cells. Therefore various viruses, such as EBV, CMV and HIV itself, developed mechanisms to suppress MHC expression as a mechanism of immune evasion (Hansen and Bouvier, 2009; Yewdell and Hill, 2002; Gainey et al., 2012; Kang et al., 2014).

Next, for auto-immune diseases like Bechterew (linked to HLA-B27), Behçet (HLA-B51), psoriasis (HLA-Cw6) and Birdshot uveitis (HLA-B29), it is unknown why they are very specifically linked to certain HLA-alleles and what the trigger for onset is (Brionez and Reveille, 2008; Nussenblatt et al., 1982; Genetic Analysis of Psoriasis et al., 2010; Harden et al., 2015). Certain alleles of the peptide repertoire-shaping proteases, namely ERAP1 and ERAP2, are epistatically linked to the corresponding HLA-alleles of these diseases (Genetic Analysis of Psoriasis et al., 2010; Tsui et al., 2010; Kuiper et al., 2014; Kirino et al., 2013). Thus the auto-immune responses in these patients are potentially initiated or amplified by a subset of peptides presented by these HLA-alleles. Importantly, there is some evidence that also the expression levels of MHC and antigen presentation machinery may play an additional role in the presentation of these diseases (Fang et al., 2015; Oguz, 2016).

Furthermore, T cell responses against cancer require MHC mediated presentation of peptide antigens often derived from aberrantly or overexpressed proteins. A mechanism of immune escape exploited by tumor cells is inhibition of these T cell activating signals by alteration or downregulation of MHC class I or its interactors. Interference with antigen presentation machinery such as TAP1/2 or B2 M has often been detected in colorectal carcinoma, cervical carcinoma, melanoma and other cancers in the last decades (Cordon-Cardo et al., 1991; Hilders et al., 1995; Kageshita et al., 1993; Dierssen et al., 2007). Interestingly, also NLRC5 expression is found to be suppressed in some tumors (Yoshihama et al., 2016). NLRC5 therefore may even function as an immunotherapeutic target to enhance antitumor immunity (Yoshihama et al., 2016; Chelbi and Guarda, 2016). Indeed, induced expression of NLRC5 was recently correlated with enhanced immunogenicity and reduced tumor progression in a melanoma mouse model (Rodriguez, 2016).

These findings show that factors involved in MHC class I expression can play an important role in various diseases, making it essential to understand the regulation of MHC class I transcription in full detail.

8. Conclusions

8.1. Open questions on MHC class I expression

Seven years after the identification of NLRC5, MHC class I transcriptional regulation remains poorly understood. We have highlighted various shortcomings in the current scientific knowledge on MHC class I expression above. Additionally, we would like to emphasize that current and future research is likely to provide insights in the following points.

(1) Fortunately, the vast majority of CIITA-regulated MHC class II transcription knowledge could be extrapolated to NLRC5-regulated MHC class I transcription. However, because these investigations only focus on similarities, they mainly raise questions about differences in gene specificity as we describe above. The S-box has been suggested to play an important role in the differential specificity of NLRC5 and CIITA for MHC class I and MHC class II transactivation respectively. The identification of the S-box binding

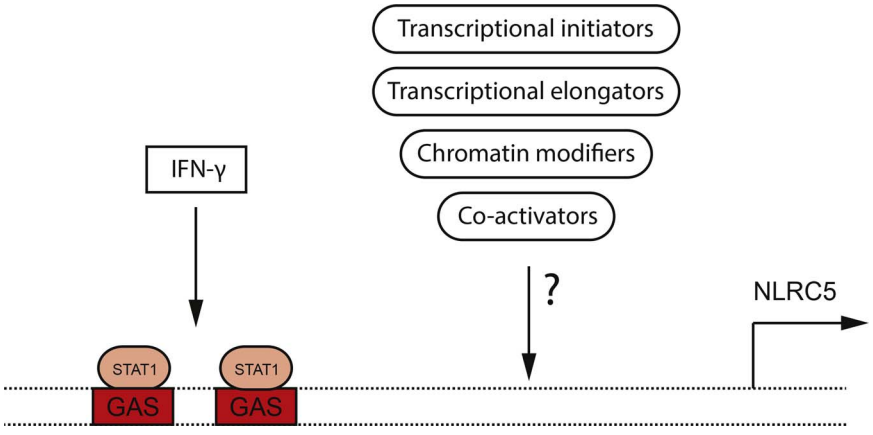

Fig. 3. The NLRC5 promotor region. Knowledge on NLRC5 transcriptional regulation is limited. IFN-γ activates the JAK/STAT route leading to STAT1 binding to Gamma interferon Activation Site (GAS) elements of the NLRC5 promotor region activating transcription. Other co-activators and how chromatin modifiers and transcriptional initiation and elongation factors are recruited is unknown.

factors for both MHC class I and class II promotors would clarify the mechanism of specificity of the two transactivators. Additionally, the restricted gene specificity of NLRC5 itself is also remarkable. TAP1 is regulated by NLRC5, while TAP2 is not. Nevertheless a TAP1/TAP2 heterodimer is required for proper peptide transport into the ER. How is the coordinated expression of TAP1 and TAP2 regulated? This question is not restricted to TAP2. Also Tapasin is expressed independently of NLRC5, though their expression is highly essential for the MHC class I antigen presentation pathway.

(2) The N-terminal domain of CIITA contains acidic and proline/serine/threonine-rich domains required for MHC class II promotor activation (Chin et al., 1997). These domains have been shown to interact with the basal transcriptional machinery (Xiao et al., 1994). Although NLRC5 lacks these domains, the NLRC5 N-terminus is definitely important for MHC class I promotor specificity since a chimeric construct of CIITA with an NLRC5 N-terminus enhanced transcriptional activity towards the MHC class I promotor (Neerincx et al., 2014). These data suggest that NLRC5 requires another set of co-factors to interact with the transcriptional machinery and activate the enhanceosome on MHC class I promotors.

(3) We are starting to understand some of the mechanisms regulating NLRC5-driven MHC class I transactivation. Nevertheless, except from a likely role for the STAT1 driven expression, little is known about factors binding the NLRC5 promotor region inducing and regulating NLRC5 expression.

8.2. Unbiased screening to reveal the unknowns

Complete knowledge of MHC antigen presentation and its regulation is crucial for the understanding of many pathological conditions, and thus potentially for the prevention or treatment of various diseases. One way to identify unknown players of MHC transcription and antigen presentation is by unbiased genome-wide screening. For example an siRNA based genome-wide screen already identified a transcriptional network regulating MHC class II expression (Paul et al., 2011). Another siRNA-based screen focused on the role of ubiquitination in MHC class I transcription and post-transcriptional regulation using a custom siRNA library targeting the E3 ubiquitin ligase family (Stagg et al., 2009). This led to the identification of the post-transcriptional MHC class I repressor MEX-3C (Cano et al., 2012). While siRNA and shRNA screens were a highly popular tool during the last decade, the current state-of-the-art for loss-of-function screens is the use genome-wide CRISPR/Cas9 libraries or random mutagenesis in near-haploid cells (Wang et al., 2005; Carette et al., 2009; Shalem et al., 2014). Moreover these tools are well-suited to screen for functional microRNAs or long non-coding RNAs as well (Wallace, 2016; Zhu et al., 2016). And specifically CRISPR/Cas9 also provides opportunities for gain-of-function screens, as well as screens targeting any genomic regulatory element such as promotors or distant enhancers (Konermann et al., 2015; Korkmaz et al., 2016; Sanjana et al., 2016). In combination with next generation sequencing these high-content screens can be performed at an unprecedented resolution. Genome-wide unbiased screening for MHC related processes will likely lead to hits that would otherwise not be considered in hypothesis-driven strategies. Although the highest resolution screens can identify hits with a very small effect size, a potential caveat of these genome-wide strategies still is the lack of identification of redundant regulators. Therefore, screening should not be limited to genetic approaches. The sensitivity and high-throughput capacity of both mass-spectrometry and sequencing have majorly improved in the past years. Furthermore, several smartly engineered tools to facilitate discoveries on protein-protein and protein-nucleic acid interactions have recently become available (Roux et al., 2012; McHugh et al., 2014; Beck et al., 2014; Liu et al., 2017). Combinations of all the above should be utilized as front line screening approaches to uncover the complete regulatory network behind MHC class I expression.

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