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The *Sgp3* Locus Derived from the 129 Strain is Responsible for Enhanced Endogenous Retroviral Expression in macroH2A1-deficient Mice

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Abstract

The endogenous retroviral envelope glycoprotein, gp70, implicated in murine lupus nephritis is secreted by hepatocytes, and its expression is largely regulated by the *Sgp3* (*serum gp70 production 3*) locus derived from lupus-prone mice. Because of the localization of the *macroH2A1* gene encoding macroH2A histone variants within the *Sgp3* interval and of an up-regulated transcription of endogenous retroviral sequences in macroH2A1-deficient C57BL/6 (B6) mice, we investigated whether *macroH2A1* is a candidate gene for *Sgp3*. macroH2A1-deficient B6 mice carrying the 129-derived *Sgp3* locus, which was co-transferred with the 129 *macroH2A1* mutant gene, displayed increased levels of serum gp70 and hepatic retroviral gp70 RNAs comparable to those of B6.NZB-*Sgp3* congenic mice bearing the *Sgp3* locus of lupus-prone NZB mice. In contrast, the abundance of retroviral gp70 RNAs in macroH2A1-deficient 129 mice was not elevated at all as compared with wild-type 129 mice. Furthermore, *Sgp3* subcongenic B6 mice devoid of the NZB-derived *macroH2A1* gene displayed an *Sgp3* phenotype identical to that of B6.NZB-*Sgp3* congenic mice carrying the NZB-derived *macroH2A1* gene, thus excluding *macroH2A1* as a candidate *Sgp3* gene. Collectively, our data indicate that enhanced transcription of endogenous retroviral sequences observed in macroH2A1-deficient B6 mice is not a result of the *macroH2A1* mutation, but due to the presence of the 129-derived *Sgp3* locus. In contrast, the effect of a *macroH2A1* knockout mutation on the expression of several non-retroviral cellular genes was very similar on the B6 and 129 backgrounds, indicating that these effects were due to the *macroH2A1* knockout.

Keywords

Systemic lupus erythematosus; Endogenous retrovirus; macroH2A histone; Genetics

1. Introduction

Endogenous retroviruses are classified as ecotropic, xenotropic or polytropic according to the host range dictated by their respective envelope gp70 proteins [1]. Furthermore, based on

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differences in their gp70 nucleotide sequences [1], the polytropic proviruses have been divided into two subgroups, termed polytropic (PT) and modified PT (mPT). The retroviral *env* (*envelope*) gene encodes a precursor polyprotein, which is cleaved to produce two subunits; a surface gp70 protein and a membrane-anchored p15E protein. Retroviral gp70 expression depends on the site of integration of retroviruses into the mouse genome and on the differentiation state of the cells [2]. Indeed, gp70 is a constituent of the surface of various epithelia, thymocytes and peripheral lymphocytes, and shares immunological and biochemical properties with the thymocyte differentiation antigen GIX [2–6]. In addition, gp70 is secreted by hepatocytes into the circulation and behaves as an acute phase protein [7,8]. Significantly, lupus-prone (NZB x NZW)F1, MRL and BXSB mice spontaneously develop autoimmune responses against gp70. gp70-anti-gp70 immune complexes are detected close to the onset of renal disease in the circulation and found as immune deposits within glomerular lesions of lupus mice [9,10]. This underlines the pathogenic role of gp70-anti-gp70 immune complexes in murine systemic lupus erythematosus (SLE).

The expression of serum gp70 is controlled by multiple structural and regulatory genes [11], and its concentrations are highly variable among different strains of mice [9,10,12]. Genetic studies identified at least two loci, *Sgp3* (*serum gp70 production 3*) on mid-chromosome 13 and *Sgp4* on distal chromosome 4, which control basal serum levels of gp70 [13–19] through the regulation in *trans* of the abundance of multiple endogenous retroviral gp70 transcripts [11]. Serological and tryptic peptide mapping analyses showed that the serum gp70 molecule resembles the gp70 protein of xenotropic viruses isolated from NZB mice [20,21]. However, recent analysis of the abundance of retroviral gp70 RNA in livers from different strains, including *Sgp* congenic mice, indicated that PT and mPT gp70s closely related to xenotropic gp70 are additional important sources of serum gp70 [11].

It has previously been shown that the *Gv1* (*Gross virus antigen 1*) locus controls the levels of endogenous retroviral sequences in different tissues, including the liver [22], and regulates the abundance of the thymocyte differentiation GIX gp70 antigen [4], the expression of which is closely correlated with serum levels of gp70 [23,24]. Since the *Gv1* locus, identified in the 129 strain [25], directly overlaps with the *Sgp3* locus [14,16,18,26], *Gv1* and *Sgp3* are likely to be identical or related genes regulating the transcription of retroviral sequences, and the GIX⁺ 129 strain having high serum gp70 [11] may share the *Sgp3* allele with lupus-prone mice. However, our recent studies revealed that many strains of non-lupus mice, including the 129 strain, expressed not only intact mPT *env* transcripts but also one or two defective mPT *env* transcripts, while lupus-prone mice predominantly expressed abundant levels of the intact mPT *env* RNA at the near exclusion of the defective transcripts [27]. The analysis of *Sgp* congenic mice revealed that this specific pattern of expression was regulated by the *Sgp3* locus derived from lupus-prone mice. These results suggest that the 129 strain might carry an *Sgp3* allele different from that in lupus-prone mice, or alternatively, the *Sgp3* locus regulates only a fraction of mPT proviruses, which are absent in the 129 strain.

macroH2A core histones have an N-terminal H2A domain and a C-terminal non-histone domain, known as the macrodomain [28]. The variants macroH2A1.1 and macroH2A1.2 are formed by alternative splicing of *macroH2A1*, and macroH2A2 is encoded by a separate gene [28–31]. macroH2As are preferentially associated with transcriptionally repressed or silent chromatin domains, including the inactivated X chromosome [30,32], centromeric heterochromatin [33] and senescence-associated heterochromatic foci [34], suggesting a role in repressing gene expression. This idea was supported by studies of gene expression in the livers of *macroH2A1* knockout mice [35]. Interestingly, recent studies have shown that macroH2A1 nucleosomes were enriched on endogenous retroviruses, the expression of which was markedly up-regulated in livers from C57BL/6 (B6) mice bearing the *macroH2A1* null mutation [36]. Since the *macroH2A1* gene is located within the *Sgp3* interval, one attractive

hypothesis is that *macroH2A1* is the *Sgp3* gene. However, one cannot exclude the possibility that the observed effect of the *macroH2A1* null mutation in B6 mice could be due to the presence of the 129-derived *Sgp3* locus co-transferred with the *macroH2A1* mutant gene, since *macroH2A1*-deficient B6 mice were established by backcrossing the mutated 129 interval into B6 mice.

To define the implication of the *macroH2A1* gene in *Sgp3*-mediated regulation of endogenous retroviral expression, we determined the abundance of endogenous retroviral gp70 RNAs in livers from *macroH2A1* knockout and control mice bred into the B6 or 129 background. Our results demonstrate that enhanced expression of endogenous retroviruses observed in *macroH2A1*-deficient B6 mice is due to the presence of the 129-derived *Sgp3* locus but not the *macroH2A1* mutation itself. This conclusion is consistent with our studies of an *Sgp3* subcongenic line that excluded *macroH2A1* as the *Sgp3* gene.

2. Materials and methods

2.1. Mice

macroH2A1-deficient 129 mice were generated by gene targeting in 129-derived ES cells, and *macroH2A1*-deficient mice with a B6 background were established by backcrossing the former with B6 mice for 10 generations, as previously described [36]. The production of B6 mice congenic for the NZB-derived *Sgp3* locus (B6.NZB-*Sgp3*) was previously described [11]. A B6.NZB*Sgp3a* subcongenic line was generated by backcrossing the NZB chromosome 13 interval into B6 mice using microsatellite markers polymorphic between NZB and B6 mice. All studies were carried out in female mice, and had been approved by the Ethical Committee for Animal Experimentation of the Faculty of Medicine, University of Geneva and the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2. Genotype analysis

Genotypes were determined by PCR using selected microsatellite markers either purchased from Research Genetics (Huntsville, AL) or Invitrogen (Carlsbad, CA). DNAs were extracted from tail biopsies kept at -70°C before use. PCR amplification was conducted with RED *Taq* DNA polymerase (Sigma-Aldrich, Saint Louis, MO) using a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA), as described [16]. The positions of the microsatellite markers with respect to the centromere were obtained from the Ensembl Genome Browser database (www.ensembl.org/Mus_musculus/index.html).

2.3. Quantitative real-time RT-PCR

RNA from livers was purified with TRIzol reagent (Invitrogen AG, Basel, Switzerland) and treated with DNase I (Amersham Biosciences Corp., Piscataway, NJ). The abundance of xenotropic, PT and mPT *env* RNAs (genomic RNA and mRNA) was quantified by real-time RT-PCR, as previously described [27]. For the amplification of xenotropic gp70 cDNA, Xeno1098F forward and Xeno1298R reverse primers were used. For PT and mPT viral gp70 cDNAs, a common PT/mPT730F forward primer, and PT892R and mPT880R reverse primers specific for PT and mPT viruses, respectively, were used. For the amplification of *macroH2A1.1* and *macroH2A1.2* cDNAs, a common forward primer (5'-TCTCCACCAAGAGCCTCTTCC-3'), and *macroH2A1.1*-specific (5'-ATGGCCTCCACCTCAAAGC-3') and *macroH2A1.2*-specific (5'-CAGTGTTTGTCGGGTGAACG-3') reverse primers were used. PCR was performed using the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Philadelphia, PA) and iQ SYBR green Supermix (Bio-Rad). Results were quantified using a standard curve generated with serial dilutions of a reference cDNA preparation from NZB liver and normalized using TATA-binding protein (TBP) mRNA. In addition, the expression of select non-retroviral genes (*Lpl*,

Serpina7, *Krt1-23*, *Scd2* and *Atp11a*) was similarly analyzed by real-time RT-PCR, as described [35].

2.4. RT-PCR and genomic PCR

The gp70-p15E junction region of mPT *env* cDNAs was amplified with an mPT-specific mPT858F gp70 forward primer and a common p15E-R reverse primer, as described [27]. For the amplification of wild-type (WT) and two deletion mutants (D1 and D2) of mPT *env* RNAs, an mPT858F forward primer and reverse primers specific for WT (mPT1447R) or deletion mutants (D1-R and D2-R) were used [27]. Using these sets of primers, the abundance of three different species of mPT *env* RNAs was semi-quantified with 5-fold serially diluted cDNA templates. As a control, the abundance of GAPDH cDNA was semi-quantified in a parallel assay. The presence of ecotropic gp70 RNA was detected by RT-PCR, using a forward primer (5'-AGGCTGTTCCAGAGATTGTG-3') and a reverse primer (5'-TTCTGGACCACCACATGAC-3'). The presence of mPT proviruses carrying the WT and mutant *env* genes in the genome was determined by PCR on genomic DNA prepared from livers, using mPT858F forward primer and mPT1447R, D1-R or D2-R reverse primers. PCR products were visualized by staining with ethidium bromide after electrophoresis on 3.5% polyacrylamide or 2% agarose gels.

2.5. Serological assays

Serum levels of gp70 were quantified by ELISA, as described [37]. Results are expressed as µg/ml of gp70 by referring to a standard curve obtained from a serum pool of NZB mice.

2.6. Statistical analysis

Unpaired comparison for levels of RNAs and mRNAs in livers was analyzed by Student's *t* test. Analysis for serum levels of gp70 was performed with the Mann-Whitney *U*-test. Probability values <5% were considered significant.

3. Results

3.1. Presence of the 129-derived *Sgp3* locus in macroH2A1-deficient B6 mice

Using selected simple sequence length polymorphism markers of the chromosome 13, we first defined the 129 interval present in B6 mice carrying the 129 *macroH2A1* mutant gene, which is located at 56.18–56.24 Mb from the centromere. A ~24 Mb 129-derived segment flanked by markers *D13Mit248* (53.04 Mb) and *D13Mit99* (76.94 Mb) was co-transferred with the *macroH2A1* mutant gene into B6 mice (Fig. 1). This 129 segment directly overlaps with the NZB-derived *Sgp3* interval encompassing markers *D13Mit139* (51.86 Mb) and *D13Mit254* (76.12 Mb) of B6.NZB-*Sgp3* congenic mice. Notably, this region also corresponds to the *Gv1* locus, which was previously identified on the 129 chromosome 13 within a ~24 Mb segment between markers *D13Mit248* and *D13Mit231* (77.19 Mb) and peaked close to the *D13Mit39* marker (62.97 Mb) [26]. This indicates that macroH2A1-deficient B6 mice likely carry the *Sgp3* and *Gv1* loci derived from the 129 strain.

3.2. Enhanced hepatic expression of xenotropic, PT and mPT gp70 RNAs in macroH2A1-deficient B6 mice at levels comparable to B6.NZB-*Sgp3* congenic mice

Serum levels of gp70 in macroH2A1-deficient B6 mice were comparable to those in B6.NZB-*Sgp3* congenic mice, and approximately 6-fold higher than those in WT B6 mice ($P < 0.001$; Table 1). We measured the abundance of different retroviral gp70 RNA transcripts in livers of macroH2A1-deficient B6 mice in comparison with B6.NZB-*Sgp3* and WT B6 mice. Quantification of gp70 RNAs in macroH2A1-deficient B6 mice revealed marked (4- to 25-fold) increases in xenotropic ($P < 0.005$), PT ($P < 0.0001$) and mPT ($P < 0.0001$) gp70 RNAs

compared with WT B6 mice (Table 1). Notably, levels of these three different gp70 RNAs in macroH2A1-deficient B6 mice were comparable to those of B6.NZB-*Sgp3* congenic mice. In contrast, ecotropic gp70 transcripts were hardly detectable in B6 mice, independently of the presence of the *macroH2A1* null mutation and the *Sgp3* locus (data not shown).

3.3. Enhanced hepatic expression of xenotropic, PT and mPT gp70 RNAs in B6.NZB-*Sgp3a* subcongenic mice lacking the NZB-derived *macroH2A1* gene

To determine whether *macroH2A1* is a candidate gene for *Sgp3*, we generated a B6.NZB-*Sgp3* subcongenic line, designated B6.NZB-*Sgp3a*, carrying a ~13 Mb NZB interval flanked by markers *D13Mit283* (63.40 Mb) and *D13Mit254*, which excluded the NZB-derived *macroH2A1* gene (Fig. 1). This subcongenic line had high serum levels of gp70 similar to those of B6.NZB-*Sgp3* mice (Table 1). In addition, the abundance of retroviral gp70 RNA transcripts in livers of B6.NZB-*Sgp3a* subcongenic mice was essentially identical to that of B6.NZB-*Sgp3* mice (Table 1). This indicated that *macroH2A1* itself is not the *Sgp3* candidate gene. Notably, quantification of *macroH2A1.1* and *macroH2A1.2* mRNAs in livers of B6.NZB-*Sgp3a* subcongenic mice showed no significant modulation as compared with WT B6 mice (Table 1). This ruled out the possibility that *Sgp3* down-regulates the expression of the *macroH2A1* gene, thereby up-regulating the transcription of endogenous retroviral sequences.

3.4. Predominant expression of WT mPT env RNA in *macroH2A1*-deficient B6 and B6.NZB-*Sgp3a* subcongenic mice

Our recent analysis of RNA from livers of B6 mice revealed the presence of not only intact WT mPT *env* transcript but also two defective (D1 and D2) mPT *env* transcripts which carry a deletion in the *env* sequence of the 3' portion of the gp70 surface protein and the 5' portion of the p15E transmembrane protein, respectively [27]. In contrast, lupus-prone mice expressed predominantly the WT mPT *env* RNA at the near exclusion of the defective transcripts. Since the *Sgp3* locus derived from lupus-prone mice was responsible for the selective up-regulation of the WT mPT *env* RNA, we assessed the relative expression of the three different species of mPT *env* transcripts in *macroH2A1*-deficient B6 mice. As shown in Fig. 2A, *macroH2A1*-deficient B6 mice displayed a predominant expression of the WT mPT *env* RNA, as was the case in B6.NZB-*Sgp3* congenic and B6.NZB-*Sgp3a* subcongenic mice [27]. The levels of the three different mPT *env* RNAs in livers were semi-quantified by RT-PCR specific for the three different mPT *env* sequences because the remarkable homology in the gp70-p15E junction region between mPT and PT *env* genes precludes the design of a WT-specific mPT primer suitable for real-time RT-PCR [27]. The analysis with serially diluted cDNA samples from *macroH2A1*-deficient B6 mice showed marked and selective ~100-fold increases in WT mPT *env* transcripts, as compared with WT B6 mice, while no appreciable increases in D1 and D2 mPT *env* RNAs were observed (Fig. 2B). Notably, the results obtained with *macroH2A1*-deficient B6 mice were essentially identical to those with B6.NZB-*Sgp3a* subcongenic mice.

3.5. Lack of enhanced hepatic expression of xenotropic, PT and mPT gp70 RNAs in *macroH2A1*-deficient 129 mice

macroH2A1-deficient B6 mice likely carry the 129-derived *Sgp3* locus, and the 129 strain might share the *Sgp3* allele with lupus-prone mice. Thus, the up-regulated expression of endogenous retroviral gp70 RNAs observed in *macroH2A1*-deficient livers of B6 mice could be due to the linked *Sgp3* locus rather than the knockout mutation of *macroH2A1*. To address this question, we determined whether the presence of the *macroH2A1* null mutation could indeed promote the expression of endogenous retroviral gp70 RNAs in 129 mice. The analysis of the abundance of PT and mPT gp70 RNA transcripts in the liver of *macroH2A1*-deficient 129 mice showed no significant up-regulation of these retroviral gp70 RNAs compared with WT 129 mice (Fig. 3A). In addition, the relative expression pattern of the three different species

of mPT *env* transcripts was unchanged in macroH2A1-deficient 129 mice, which failed to display the pattern of predominant expression of WT mPT *env* RNAs (Fig. 3B). Semi-quantitative RT-PCR analysis confirmed that levels of WT and D1 mPT *env* RNAs were not different between macroH2A1-sufficient and -deficient 129 mice (data not shown). The lack of D2 mPT *env* transcripts in 129 mice was due to the absence of the D2 mutant provirus in this strain, as documented by genomic PCR analysis (Fig. 3C). Notably, as previously described [11,27], 129 mice failed to express xenotropic and ecotropic gp70 RNAs because of the absence of both proviruses in their genome.

3.6. Similar effect of macroH2A1 knockout on expression of non-retroviral genes in B6 and 129 mice

B6 *macroH2A1* knockout mice were found to have increased expression in livers of several cellular genes, including *Lpl*, *Serpina7*, *Scd2*, *Krt23* and *Atp11a* [35]. To examine whether these effects on gene expression are related to *Sgp3* or another linked 129-derived gene(s), the expression of these five select genes was compared in the livers of 129 *macroH2A1* knockout and control mice. The effect of the *macroH2A1* knockout on the abundance of these mRNAs in 129 mice was very similar to that previously observed on the B6 background (Table 2), indicating that an up-regulated expression of these five non-retroviral genes was indeed due to the *macroH2A1* null mutation.

4. Discussion

The *Sgp3* locus present on mid-chromosome 13 has been identified as the major genetic locus controlling the levels of serum retroviral gp70 and the expression of endogenous retroviral gp70 RNAs in the livers. Recent findings that the transcription of endogenous retroviral sequences was substantially enhanced in livers from macroH2A1-deficient B6 mice and the fact that *macroH2A1* is localized within the *Sgp3* interval prompted us to investigate the possibility of *macroH2A1* as a candidate for *Sgp3*. Results obtained through comparative analysis of macroH2A1-deficient B6 and 129 mice demonstrate that macroH2A1-deficient B6 mice exhibited markedly enhanced levels of retroviral gp70 RNAs, as compared with WT B6 mice, while this was not the case in macroH2A1-deficient 129 mice. Notably, the analysis of the genomic composition of chromosome 13 revealed that macroH2A1-deficient B6 mice still carry the 129-derived *Sgp3* locus, which was co-transferred with the *macroH2A1* mutant gene during the backcross procedure. Our data thus indicate that elevated levels of endogenous retroviral gp70 RNAs in macroH2A1-deficient B6 mice were not the result of the *macroH2A1* null mutation, but due to the presence of the 129-derived *Sgp3* locus.

Our conclusion is consistent with the finding that B6.NZB-*Sgp3a* subcongenic mice lacking the NZB-derived *macroH2A1* gene displayed the typical *Sgp3* phenotype and were indistinguishable from B6.NZB-*Sgp3* congenic mice carrying the NZB-derived *macroH2A1* gene. Moreover, comparable levels of *macroH2A1* mRNAs between B6.NZB-*Sgp3a* subcongenic and WT B6 mice ruled out a contribution of *Sgp3* to the derepression of endogenous retroviruses through down-regulated expression of macroH2A1. However, it should be stressed that the expression of several non-retroviral cellular genes was similarly enhanced in macroH2A1-deficient B6 and 129 mice, indicating that these effects were indeed mediated by the *macroH2A1* null mutation, independently of *Sgp3* or any other 129 genes.

We have previously observed that the expression pattern of three different species (WT, D1 and D2) of mPT *env* transcripts in GIX⁺ 129 mice was clearly different from that of B6 and B10 mice bearing the *Sgp3* locus derived from lupus-prone NZB and BXSB mice, respectively [27]. The expression of the thymocyte differentiation GIX gp70 antigen is regulated by the *Gv1* locus [4], and the *Gv1* locus derived from the 129 strain has been reported to regulate the transcription of PT proviruses, but not mPT proviruses [26]. Thus, we speculated that lupus-

prone mice carry different regulatory elements in the *Sgp3* interval which might independently control the levels of mPT, PT and xenotropic proviral sequences, and that the presence of a regulatory element controlling the mPT proviral expression may be unique to lupus-prone mice [27]. However, the analysis of macroH2A1-deficient B6 and 129 mice bearing the 129-*Sgp3* allele revealed that the 129-*Sgp3* allele promotes the predominant and abundant expression of WT mPT *env* transcripts as well as the expression of PT and xenotropic gp70 RNAs in B6 mice. This indicates that the 129 strain likely shares the same *Sgp3* allele with lupus-prone mice. Accordingly, the lack of predominant expression of WT mPT *env* transcripts in 129 mice is probably rather due to the absence of mPT proviruses carrying the intact *env* sequence, the expression of which is strongly promoted by *Sgp3*. This interpretation is consistent with our previous findings in SB/Le mice which also failed to display the predominant expression of WT mPT *env* RNAs [27], although the *Sgp3* allele of BXSb mice is inherited from the SB/Le strain (BXSb is a recombinant strain derived from a cross of B6 and SB/Le mice) [18]. Notably, these results also exclude the possibility that the selectively up-regulated expression of WT mPT *env* RNA in lupus-prone mice and in *Sgp3* congenic mice is the result of the presence in the *Sgp3* region of a unique mPT provirus, which may be especially highly expressed because of its particular integration site.

It is worth noting that other GIX⁺ strains of mice, such as AKR, DBA/2 and C3H/He [23], also displayed an expression pattern of the three species of mPT *env* RNAs similar to that of 129 and SB/Le mice. If we assume that all the GIX⁺ strains of mice carry the same *Sgp3* allele, the copy number of the unique mPT provirus responsive to *Sgp3* and its strain distribution may be very limited. As the estimated copy numbers of mPT proviruses in the genome of NZB, B6, AKR and C3H/He mice are 7–11 [38–40], it is possible that *Sgp3* selectively up-regulates the expression of only a particular fraction of mPT proviruses, while the expression of others is controlled independently of the presence of *Sgp3*. BLAST search analysis confirmed the presence in the B6 mouse genome of 11 mPT proviruses carrying the intact *env* gene, in which 9 different microheterogeneities of the U3 regulatory region in the long terminal repeat were identified. Thus, the genetic locus of the mPT provirus that is selectively up-regulated by *Sgp3* could be identified through an extensive analysis of the U3 sequences of mPT proviruses expressed in B6.NZB-*Sgp3* and macroH2A1-deficient B6 mice. This could help not only to determine whether the selective function of *Sgp3* as a *trans*-activating factor is related to a unique U3 sequence of endogenous retroviruses or to their integration site but also to clarify the pathogenic role of the mPT retrovirus in the development of murine SLE.

We have recently demonstrated that single-stranded RNA-specific TLR7 plays a critical role for the development of autoimmune responses against retroviral gp70 [27,41]. Since it is unlikely that virion-free serum gp70 is able to trigger TLR7, an attractive hypothesis would be that *Sgp3* enhances the production of endogenous retroviral virions carrying single-stranded RNA, which would then promote the development of autoimmune responses against serum retroviral gp70 through the activation of TLR7. This hypothesis is also supported by the finding that the *Sgp3* locus contributes to the production of anti-gp70 autoantibodies [18]. Since endogenous retroviruses can contribute to the development of different pathologies [42–44], a wide variety of mechanisms are used to protect the genome from retroviral elements with one being the control of the transcription of endogenous retroviral sequences [45]. Although our present studies show that macroH2A1 is not involved in silencing of endogenous retroviruses, it is important to identify the *Sgp3* gene, since this will help elucidate a molecular basis for transcriptional suppression of endogenous retroviruses.

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References

1. Stoye JP, Coffin JM. The four classes of endogenous murine leukemia virus: structural relationships and potential for recombination. *J Virol* 1987;61:2659–69. [PubMed: 3039159]
2. Lerner RA, Wilson CB, Villano BC, McConahey PJ, Dixon FJ. Endogenous oncornaviral gene expression in adult and fetal mice: quantitative, histologic, and physiologic studies of the major viral glycoprotein, gp70. *J Exp Med* 1976;143:151–66. [PubMed: 172586]
3. Del Vellano BC, Nave B, Croker BP, Lerner RA, Dixon FJ. The oncornavirus glycoprotein gp69/71: a constituent of the surface of normal and malignant thymocytes. *J Exp Med* 1975;141:172–87. [PubMed: 46909]
4. Stockert E, Old LJ, Boyse EA. The GIX system. A cell surface allo-antigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. *J Exp Med* 1971;133:1334–55. [PubMed: 5576334]
5. Tung JS, Vitetta ES, Fleissner E, Boyse EA. Biochemical evidence linking the GIX thymocyte surface antigen to the gp69/71 envelope glycoprotein of murine leukemia virus. *J Exp Med* 1975;141:198–205. [PubMed: 163885]
6. Morse HC III, Chused TM, Boehm-Truitt M, Mathieson BJ, Sharrow SO, Hartley JW. XenCSA: cell surface antigens related to the major glycoproteins (gp70) of xenotropic murine leukemia viruses. *J Immunol* 1979;122:443–54. [PubMed: 216748]
7. Hara I, Izui S, Dixon FJ. Murine serum glycoprotein gp70 behaves as an acute phase reactant. *J Exp Med* 1982;155:345–57. [PubMed: 7057139]
8. Baudino L, Yoshinobu K, Dunand-Sauthier I, Evans LH, Izui S. TLR-mediated up-regulation of serum retroviral gp70 is controlled by the *Sgp* loci of lupus-prone mice. *J Autoimmun.* 2010 in press. 10.1016/j.jaut.2010.06.001
9. Yoshiki T, Mellors RC, Strand M, August JT. The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J Exp Med* 1974;140:1011–27. [PubMed: 4279268]
10. Izui S, McConahey PJ, Theofilopoulos AN, Dixon FJ. Association of circulating retroviral gp70-anti-gp70 immune complexes with murine systemic lupus erythematosus. *J Exp Med* 1979;149:1099–116. [PubMed: 221610]
11. Baudino L, Yoshinobu K, Morito N, Kikuchi S, Fossati-Jimack L, Morley BJ, et al. Dissection of genetic mechanisms governing the expression of serum retroviral gp70 implicated in murine lupus nephritis. *J Immunol* 2008;181:2846–54. [PubMed: 18684976]
12. Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, McConahey PJ, et al. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J Exp Med* 1978;148:1198–215. [PubMed: 309911]
13. Tucker RM, Vyse TJ, Rozzo S, Roark CL, Izui S, Kotzin BL. Genetic control of gp70 autoantigen production and its influence on immune complex levels and nephritis in murine lupus. *J Immunol* 2000;165:1665–72. [PubMed: 10903778]
14. Kikuchi S, Fossati-Jimack L, Moll T, Amano H, Amano E, Ida A, et al. Differential role of three major NZB-derived loci linked with *Yaa*-induced murine lupus nephritis. *J Immunol* 2005;174:1111–17. [PubMed: 15634937]
15. Santiago ML, Mary C, Parzy D, Jacquet C, Montagutelli X, Parkhouse RME, et al. Linkage of a major quantitative trait locus to *Yaa* gene-induced lupus-like nephritis in (NZW x C57BL/6)F1 mice. *Eur J Immunol* 1998;28:4257–67. [PubMed: 9862363]
16. Laporte C, Ballester B, Mary C, Izui S, Reininger L. The *Sgp3* locus on mouse chromosome 13 regulates nephritogenic gp70 autoantigen and predisposes to autoimmunity. *J Immunol* 2003;171:3872–77. [PubMed: 14500689]

17. Haywood MEK, Vyse TJ, McDermott A, Thompson EM, Ida A, Walport MJ, et al. Autoantigen glycoprotein 70 expression is regulated by a single locus, which acts as a checkpoint for pathogenic anti-glycoprotein 70 autoantibody production and hence for the corresponding development of severe nephritis, in lupus-prone BXSB mice. *J Immunol* 2001;167:1728–33. [PubMed: 11466397]
18. Rankin J, Boyle JJ, Rose SJ, Gabriel L, Lewis M, Thirumalaiah V, et al. The *Bxs6* locus of BXSB mice is sufficient for high-level expression of gp70 and the production of gp70 immune complexes. *J Immunol* 2007;178:4395–401. [PubMed: 17371996]
19. Rigby RJ, Rozzo SJ, Gill H, Fernandez-Hart T, Morley BJ, Izui S, et al. A novel locus regulates both retroviral glycoprotein 70 and anti-glycoprotein 70 antibody production in New Zealand mice when crossed with BALB/c. *J Immunol* 2004;172:5078–85. [PubMed: 15067091]
20. Izui S, Elder JH, McConahey PJ, Dixon FJ. Identification of retroviral gp70 and anti-gp70 antibodies involved in circulating immune complexes in NZB x NZW mice. *J Exp Med* 1981;153:1151–60. [PubMed: 7252417]
21. Elder JH, Jensen FC, Bryant ML, Lerner RA. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differential antigens encoded by a multi-gene family. *Nature* 1977;267:23–28. [PubMed: 193039]
22. Levy DE, Lerner RA, Wilson MC. The *Gv-1* locus coordinately regulates the expression of multiple endogenous murine retroviruses. *Cell* 1985;41:289–99. [PubMed: 3995585]
23. Obata Y, Ikeda H, Stockert E, Boyse EA. Relation of GIX antigen of thymocytes to envelope glycoprotein of murine leukemia virus. *J Exp Med* 1975;141:188–97. [PubMed: 46910]
24. Hara I, Izui S, McConahey PJ, Elder JH, Jensen FC, Dixon FJ. Induction of high serum levels of retroviral *env* gene products (gp70) in mice by bacterial lipopolysaccharide. *Proc Natl Acad Sci USA* 1981;78:4397–401. [PubMed: 7027259]
25. Stockert E, Boyse EA, Obata Y, Ikeda H, Sarkar NH, Hoffman HA. New mutant and congenic mouse stocks expressing the murine leukemia virus-associated thymocyte surface antigen GIX. *J Exp Med* 1975;142:512–17. [PubMed: 167097]
26. Oliver PL, Stoye JP. Genetic analysis of *Gv1*, a gene controlling transcription of endogenous murine polytropic proviruses. *J Virol* 1999;73:8227–34. [PubMed: 10482573]
27. Yoshinobu K, Baudino L, Morito N, Dunand-Sauthier I, Morley BJ, Evans LH, et al. Selective up-regulation of intact, but not defective *env* RNAs of endogenous modified polytropic retrovirus by the *Sgp3* locus of lupus-prone mice. *J Immunol* 2009;182:8094–103. [PubMed: 19494335]
28. Pehrson JR, Fried VA. MacroH2A, a core histone containing a large nonhistone region. *Science* 1992;257:1398–400. [PubMed: 1529340]
29. Rasmussen TP, Huang T, Mastrangelo MA, Loring J, Panning B, Jaenisch R. Messenger RNAs encoding mouse histone macroH2A1 isoforms are expressed at similar levels in male and female cells and result from alternative splicing. *Nucleic Acids Res* 1999;27:3685–89. [PubMed: 10471737]
30. Chadwick BP, Willard HF. Histone H2A variants and the inactive X chromosome: identification of a second macroH2A variant. *Hum Mol Genet* 2001;10:1101–13. [PubMed: 11331621]
31. Costanzi C, Pehrson JR. MACROH2A2, a new member of the MARCOH2A core histone family. *J Biol Chem* 2001;276:21776–84. [PubMed: 11262398]
32. Costanzi C, Pehrson JR. Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. *Nature* 1998;393:599–601. [PubMed: 9634239]
33. Grigoryev SA, Nikitina T, Pehrson JR, Singh PB, Woodcock CL. Dynamic relocation of epigenetic chromatin markers reveals an active role of constitutive heterochromatin in the transition from proliferation to quiescence. *J Cell Sci* 2004;117:6153–62. [PubMed: 15564378]
34. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, et al. Formation of macroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev Cell* 2005;8:19–30. [PubMed: 15621527]
35. Changolkar LN, Costanzi C, Leu NA, Chen D, McLaughlin KJ, Pehrson JR. Developmental changes in histone macroH2A1-mediated gene regulation. *Mol Cell Biol* 2007;27:2758–64. [PubMed: 17242180]
36. Changolkar LN, Singh G, Pehrson JR. macroH2A1-dependent silencing of endogenous murine leukemia viruses. *Mol Cell Biol* 2008;28:2059–65. [PubMed: 18195046]

37. Merino R, Fossati L, Lacour M, Lemoine R, Higaki M, Izui S. H-2-linked control of the *Yaa* gene-induced acceleration of lupus-like autoimmune disease in BXSB mice. *Eur J Immunol* 1992;22:295–99. [PubMed: 1537372]
38. Frankel WN, Stoye JP, Taylor BA, Coffin JM. A linkage map of endogenous murine leukemia proviruses. *Genetics* 1990;124:221–36. [PubMed: 2155154]
39. Frankel WN, Lee BK, Stoye JP, Coffin JM, Eicher EM. Characterization of the endogenous nonectropic murine leukemia viruses of NZB/B1NJ and SM/J inbred strains. *Mamm Genome* 1992;2:110–22. [PubMed: 1311971]
40. Tomonaga K, Coffin JM. Structure and distribution of endogenous nonectropic murine leukemia viruses in wild mice. *J Virol* 1998;72:8289–300. [PubMed: 9733873]
41. Santiago-Raber ML, Dunand-Sauthier I, Wu T, Li QZ, Uematsu S, Akira S, et al. Critical role of TLR7 in the acceleration of systemic lupus erythematosus in TLR9-deficient mice. *J Autoimmun* 2010;34:339–48. [PubMed: 19944565]
42. Aziz DC, Hanna Z, Jolicoeur P. Severe immunodeficiency disease induced by a defective murine leukaemia virus. *Nature* 1989;338:505–08. [PubMed: 2538760]
43. Cho BC, Shaughnessy JD Jr, Largaespada DA, Bedigian HG, Buchberg AM, Jenkins NA, et al. Frequent disruption of the *Nf1* gene by a novel murine AIDS virus-related provirus in BXH-2 murine myeloid lymphomas. *J Virol* 1995;69:7138–46. [PubMed: 7474134]
44. Laigret F, Repaske R, Boulukos K, Rabson AB, Khan AS. Potential progenitor sequences of mink cell focus-forming (MCF) murine leukemia viruses: ecotropic, xenotropic, and MCF-related viral RNAs are detected concurrently in thymus tissues of AKR mice. *J Virol* 1988;62:376–86. [PubMed: 2826802]
45. Maksakova IA, Romanish MT, Gagnier L, Dunn CA, van de Lagemaat LN, Mager DL. Retroviral elements and their hosts: insertional mutagenesis in the mouse germ line. *PLoS Genet* 2006;2:e2. [PubMed: 16440055]

Fig. 1.

Genetic map of the chromosome 13 in B6 macroH2A1^{-/-}, B6.NZB-*Sgp3* and B6.NZB-*Sgp3a* mice. Diagrams indicate the segment of the chromosome 13 derived from macroH2A1^{-/-} 129 mice in macroH2A1^{-/-} B6 mice (left panel), from NZB mice in B6.NZB-*Sgp3* congenic mice (middle panel), and from NZB mice in B6.NZB-*Sgp3a* subcongenic mice (right panel). Black sections indicate the region that is definitely 129 (left panel) or NZB (middle and right panels), and grey sections the region which cannot be defined as B6 or 129 (left panel) and as B6 or NZB (middle and right panels). In each panel, the position of selected microsatellite markers from the centromere is indicated as Mb.

Fig. 2. RT-PCR analysis of WT, D1 and D2 mPT *env* genes in B6 mice deficient in macroH2A1 and B6.*Sgp3a* subcongenic mice

(A) The presence of three different species of mPT *env* RNAs in livers of 2–3 mo-old B6 female mice was determined by RT-PCR with mPT specific gp70 forward and p15E-R reverse primers. Representative results of three individual animals are shown. As a control (Ctl), a mixture of three different plasmids containing WT, D1 and D2 clones obtained from B6 mice was included. Note the predominant expression of WT *env* transcripts in macroH2A1^{-/-} B6 (KO) and B6.*Sgp3a* subcongenic mice, as compared with WT B6 mice.

(B) Semi-quantitative RT-PCR analysis for WT, D1 and D2 mPT *env* RNAs with reverse primers specific for the three different mPT *env* genes (mPT1447R, D1-R and D2-R) and a common forward mPT-specific primer (mPT858F) was carried out with 5-fold serially diluted cDNAs from different B6 mice. As a control, the abundance of GAPDH mRNA was assessed in parallel. Four 5-fold dilutions of cDNAs were examined for WT mPT *env* RNA, while three 5-fold dilutions of cDNAs were examined for D1/D2 mPT *env* RNAs and GAPDH mRNA. Representative results of three individual mice analyzed are shown.

Fig. 3. Analysis of PT and mPT RNAs in macroH2A1^{-/-} and WT 129 mice

(A) Levels of PT and mPT gp70 RNAs from livers of 2–3 mo-old 129 female mice (means $\pm$ SEM of 5 mice) were quantified relative to a standard curve generated with serial dilutions of a reference cDNA preparation and normalized using TBP mRNA. Results are expressed as fold changes of each transcript in macroH2A1^{-/-} mice (KO) relative to WT mice.

(B) The presence of two different species of mPT *env* RNAs in livers of macroH2A1^{-/-} (KO) and WT 129 mice was determined by RT-PCR with mPT specific gp70 forward and p15E-R reverse primers. Representative results of three individual animals are shown. Note the lack of predominant expression of WT *env* transcripts in macroH2A1^{-/-} 129 mice, unlike macroH2A1^{-/-} B6 mice.

(C) The presence of WT, D1 and D2-specific mPT *env* proviral sequences in 129 and B6 mice was analyzed by genomic PCR with reverse primers specific for the three different mPT *env* genes and a common forward mPT-specific primer.

Table 1

Levels of gp70 in sera, and gp70 RNAs and *macroH2A1* mRNA in livers of B6 mice deficient in *macroH2A1* or congenic for the *Sgp3* locus

Mice	Serum gp70 ^a	Xeno gp70 ^b	PT gp70 ^b	mPT gp70 ^b	macroH2A1.1 ^b	macroH2A1.2 ^b
macroH2A1 ^{-/-}	12.7 ± 0.7	13.89 ± 2.72	3.91 ± 0.02	25.04 ± 3.17	NT ^c	NT
NZB- <i>Sgp3</i>	11.1 ± 0.7	12.92 ± 2.93	4.51 ± 0.81	19.15 ± 4.14	NT	NT
NZB- <i>Sgp3a</i>	10.3 ± 0.6	14.12 ± 1.32	4.28 ± 0.40	14.25 ± 2.12	0.77 ± 0.10	0.98 ± 0.07
WT	2.2 ± 0.2	1.02 ± 0.16	1.02 ± 0.13	1.01 ± 0.10	1.00 ± 0.07	1.00 ± 0.06

^aSerum levels of gp70 (µg/ml; mean ± SEM of 7 female mice at 2–3 months of age).

^bLevels of each gp70 RNA and *macroH2A1* mRNA (mean ± SEM of 4 female mice at 2–3 months of age) were quantified relative to a standard curve generated with serial dilutions of a reference cDNA preparation and normalized using TBP mRNA. Results are expressed as fold increases of each transcript relative to B6 WT mice.

^cNot tested.

Table 2

Expression of select non-retroviral genes in livers of macroH2A1-deficient 129 and B6 mice

Gene	129^a	B6^b
<i>Lpl</i>	3.4 ($P < 10^{-5}$)	2.6 ($P < 10^{-4}$)
<i>Serpina7</i>	3.3 ($P < 10^{-6}$)	4.1 ($P < 10^{-4}$)
<i>Krt23</i>	6.5 ($P < 10^{-7}$)	2.4 ($P < 10^{-4}$)
<i>Scd2</i>	3.9 ($P < 0.0002$)	1.5 ($P < 0.05$)
<i>Atp11a</i>	3.5 ($P < 0.0002$)	1.9 ($P < 10^{-4}$)

^aValues are the ratio of levels of each mRNA in macroH2A1-deficient livers (n = 5) relative to control livers (n = 4) of 129 female mice. cDNAs were normalized to equal expression of GAPDH.

^bValues for macroH2A1-deficient (n = 12) and control livers (n = 13) of B6 female mice were taken from Changolkar *et al* [35].