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Inherently variable responses to glucocorticoid stress among endogenous retroviruses isolated from 23 mouse strains

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Abstract

Active participation of endogenous retroviruses (ERVs) in disease processes has been exemplified by the finding that the HERV (human ERV)-W envelope protein is involved in the pathogenesis of multiple sclerosis, an autoimmune disease. We also demonstrated that injury-elicited stressors alter the expression of murine ERVs (MuERVs), both murine leukemia virus-type and mouse mammary tumor virus (MMTV)-type (MMTV-MuERV). In this study, to evaluate MMTV-MuERVs' responses to stress (*e.g.*, injury, infection)-elicited systemic glucocorticoid (GC) levels, we examined the GC-stress response of 64 MMTV-MuERV promoters isolated from the genomes of 23 mouse strains. All 64 promoters responded to treatment with a synthetic GC, dexamethasone (DEX), at a wide range from a 0.6- to 85.7-fold increase in reporter activity compared to no treatment. An analysis of the 10 lowest and 10 highest DEX responders revealed specific promoter elements exclusively present in either the three lowest or the two highest responders. Each promoter had a unique profile of transcription regulatory elements and the glucocorticoid response element (GRE) was identified in all promoters with the number of GREs ranging from 2 to 7. The three lowest DEX responders were the only promoters with two GREs. The findings from this study suggest that certain MMTV-MuERVs are more responsive to stress-elicited systemic GC elevation compared to the others. The mouse strain-specific genomic MMTV-MuERV profiles and individual MMTV-MuERVs' differential responses to GC-stress might explain, at least in part, the variable inflammatory responses to injury and/or infection, often observed among different mouse strains.

Keywords

endogenous retrovirus; mouse mammary tumor virus; glucocorticoid; glucocorticoid response element; injury; inflammation

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The authors have nothing to disclose.

1. Introduction

Certain retroviruses, which infect host germline cells and inherently integrate into the germline genomes, are called endogenous retroviruses (ERVs); ERVs are passed down to the offspring in a Mendelian fashion [1, 2]. ERVs account for ~8 % of the human genome and ~10 % of the mouse genome [3]. At least eight different types of endogenous retroviral elements, including MMTV (mouse mammary tumor virus)-type murine ERV (MuERV) (MMTV-MuERV) and MLV (murine leukemia virus)-type MuERV (MLV-MuERV), have been identified in laboratory mouse strains [4]. Among them, only MLV-MuERVs and MMTV-MuERVs have closely related exogenous counterparts, type C and type B retroviruses, respectively. In the mouse genome, the proviral sequences of the majority of MuERVs are related to MLVs [5].

Two types of MMTVs exist in mice: exogenous and endogenous. Exogenous and infectious MMTVs can be transmitted to newborn pups through milk, which contains virions released from infected mammary glands [6-8]. In contrast, endogenous MMTVs (MMTV-MuERVs), which are permanently integrated into the germline, are transmitted to the offspring like any other host genes. Findings from mixed lymphocyte reactions between different, but MHC compatible, strains of mice led to the identification of a unique protein coded from the MMTV genomes [9]. Initially, the antigens responsible were named “minor lymphocyte stimulating (Mls) antigens” [10, 11]. Further studies showed that the Mls antigens, now called superantigens (SAgs), are encoded by MMTV-MuERV open reading frames [12]. The hypervariable region near the C-terminus of SAgs is responsible for the T cell receptor (TCR) Vβ-specificity during interaction with T cells [13, 14]. Recent studies reported that injury-elicited stress signals are associated with the activation of specific T cell subsets by restriction of TCR Vβ usage [15]. The impact of the induction of MMTV-MuERV expression accompanied by SAg activation on the immune system might be extensive, primarily due to activation, anergy, and/or deletion of a substantial fraction of the T cell repertoire in a TCR Vβ-specific manner.

MuERV expression is primarily controlled by two main components: the host's transcription machinery and MuERVs’ own transcription regulatory elements, such as the glucocorticoid response element (GRE) residing on the U3 promoter sequences within the 5’ long terminal repeat (LTR) [5]. MuERVs are also capable of controlling the expression of neighboring host genes via various regulatory elements, such as enhancers, negative regulatory elements, and splicing and polyadenylation signals [16-18]. In addition, alterations in the transcriptional environment, such as elevated cytoplasmic glucocorticoid (GC) levels following stress-activation of the HPA (hypothalamus-pituitary gland-adrenal gland) axis (Figure 1), may directly affect MuERVs’ expression via specific regulatory elements (*e.g.*, GRE) on the promoter and/or indirectly through a network of feedback mechanisms. Our studies identified changes (activation or repression) in the expression of MuERVs in various distant organs of C57BL/6J mice after burn injury, which triggers an increase in systemic GC levels [19, 20].

In this study, we examined the stress response of 64 MMTV-MuERV promoters isolated from 23 different mouse strains in order to evaluate MMTV-MuERVs' differential responses to stress (*e.g.*, injury, infection, hormonal imbalance)-elicited systemic GC elevation.

2. Materials and Methods

2.1 Cloning, sequencing, and reporter construction

Genomic DNAs from 23 laboratory mouse strains were obtained from the Jackson Laboratory (Bar Harbor, ME). A set of primers, MTV-1B (forward: 5'-TGC CGC GCC TGC AGC AGA AAT G-3') and MTV-2A (reverse: 5'-TGT TAG GAC TGT TGC AAG TTT ACT C-3'), were designed to amplify the MMTV-MuERV U3 promoter regions from the genomic DNAs [21]. PCR products were gel purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA) and cloned into the pGEM-T Easy vector (Promega, Madison, WI). Plasmid DNAs with the promoter regions were prepared using the QIAprep spin miniprep kit (Qiagen) for sequencing. To identify unique promoter sequences, multiple alignment analysis was performed using the Vector NTI AlignX program (Invitrogen, Carlsbad, CA). Subsequently, 64 unique MMTV-MuERV promoters were cloned into the pGL4.10[*luc2*] luciferase reporter system (Promega).

2.2 Survey of transcription regulatory elements

Profiles of transcription regulatory elements within the individual promoters were determined using the MatInspector program (Genomatix, Munich, Germany). The core similarity was set to 0.90 and the matrix similarity was optimized within the vertebrate matrix group.

2.3 Transfection and luciferase assay

Human embryonic kidney (HEK) 293 subclone cells (ATCC, Manassas, VA) were seeded at a density of 2×10^4 cells per well in a 96-well format. The MMTV-MuERV promoter-luciferase reporter constructs were transfected into the cells using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN). Twenty-four hours post-transfection, cells were treated with 50μM dexamethasone (DEX) (Sigma-Aldrich, St. Louis, MO). After a 5-hour incubation in DEX, cells were lysed and the luciferase assay was performed per the manufacturer's recommended protocol (Agilent, La Jolla, CA), and analyzed using the Wallac MicroBeta TriLux luminometer (PerkinElmer, Waltham, MA). The luciferase assay was performed in triplicate and repeated at least twice and up to five times. All luminescence values from the repeat experiments were used to calculate the fold increase (DEX treatment relative to no DEX treatment) and the mean fold increase ± SD for each promoter.

2.4 Comparison of promoter sequences of different DEX responders

The sequences of the 10 lowest DEX responders were compared to the 10 highest DEX responders by multiple alignments using the Vector NTI AlignX program (Invitrogen).

3. Results and Discussion

3.1 Highly variable GC-stress responses of MMTV-MuERV promoters

Sixty-four unique MMTV-MuERV promoters were isolated from the genomic DNAs of 23 laboratory mouse strains and their response to GC treatment was examined to identify putative MMTV-MuERVs, which may be highly activated in response to stress-elicited elevation of systemic GC levels (Figure 1). Treatment with DEX resulted in changes in luciferase reporter activity in all 64 promoters examined. The levels of DEX response varied widely ranging from 0.6-fold to 85.7-fold increases compared to no treatment controls (Figure 2). There were four promoters with a greater than 50-fold increase in luciferase activity in response to DEX treatment and one of them (MOLF/EiJ-13) had a more than 80-fold increase. The two promoters with decreased luciferase activity, 0.6-fold and 0.8-fold, in response to DEX treatment originated from the strains of DBA/1J and AKR/J, respectively. Within the strains of 129P1/ReJ, ALR/Lt, and C57BL/6J, all promoters (at least four different promoters were tested per strain) had relatively low levels (less than 10-fold) of DEX response. The results from this experiment suggest that the promoters of all three MMTV-MuERVs, *Mtv-8*, *Mtv-9*, and *Mtv-17*, present on the C57BL/6J genome may not respond to elevated GC levels as strongly as the MOLF/EiJ-13 promoter. The results from this study demonstrate that there are substantial polymorphisms in the MMTV-MuERV profile among different mouse strains, and individual MuERVs' DEX response was highly variable.

We then examined whether the varying levels of DEX response observed in these promoters are associated with differences in their structures/sequences. The 10 lowest DEX responders, including the two with a fold increase below 1, and the 10 highest DEX responders were selected from the 64 promoters examined in this study. They were subjected to multiple alignment analyses to identify any unique region(s) present only in either the lowest or highest DEX responders (Figure 3). Seven unique DNA regions, five of them near the 3'-end of the promoter, were identified solely in the three lowest responders, while 20 unique DNA regions were exclusively localized in the two highest DEX responders. It is probable that some of the seven unique regions identified from the lowest responders may serve as negative regulatory elements, networked to a feedback mechanism that follows the initial activation of a group of genes by the DEX-mediated glucocorticoid receptor (GCR)-GRE transactivation pathway. On the other hand, some of the 20 unique regions exclusively present in the two highest responders may serve as enhancers and/or positive regulatory elements through direct interactions with by-product signaling molecules, such as IκBα, following the initial GCR-GRE interaction [22].

In 1951, Fraser and Fainstat reported that there are variable occurrence frequencies of cortisone-induced cleft palate among different mouse strains [23, 24]. Among the mouse strains examined, two strains, A/J and C57BL/6J, were studied the most; A/J is more sensitive to cortisone treatment with regard to cleft palate formation in comparison to C57BL/6J. Interestingly, in our study, following GC (DEX) treatment, activities of the four MMTV-MuERV promoters from the A/J strain were determined to be markedly stronger (Figure 4) than the five promoters of the C57BL/6J strain. This finding implies that the A/J

strain's MMTV-MuERV promoters are more responsive to GC stress than the ones of the C57BL/6J strain, in parallel with the A/J strain's higher occurrence frequencies of cortisone-induced cleft palate. It is likely that the GC-responsive MMTV-MuERVs contribute to the uncharacterized molecular and cellular events underlying stress responses through the function of their own gene products and/or transposition into the affected genomes.

3.2 Differential transcription potential of MMTV-MuERV promoters

It has been documented that GREs are found within promoters of certain MMTVs [25]. Upon binding to GCs in the cytoplasm, the activated GCRs are translocated to the nucleus and directly interact with the GREs on MMTV-MuERV promoters, resulting in their transactivation (Figure 1). In this study, we examined whether GREs were present in the MMTV-MuERV promoters, which were subjected to the reporter assay described above, and also whether there was any correlation between the frequency of GREs on the promoters and the intensity of their DEX response. A comprehensive *in silico* survey to profile the transcription regulatory elements within each promoter revealed that all 64 promoters had at least two GREs and up to seven GREs. The promoters of PWD/PhJ-3 and PWD/PhJ-10 with seven GREs showed relatively high levels of DEX response (56.5-fold and 36.2-fold increases, respectively) (Table 1). There were only three promoters (DBA/1J-8, AKR/J-10, and AKR/J-12) retaining two GREs and they happened to be the three lowest DEX responders. The two highest DEX responders (MOLG/Dn-8 [58.1-fold] and MOLF/EiJ-13 [85.7-fold]) had five GREs. However, some promoters with five GREs, such as CBA/J-14 (2.3-fold) and C57BL/6J-22 (2.4-fold) belonged to the 10 lowest DEX responder group. These findings suggest that in addition to GREs, other transcription regulatory elements and/or proteins, either directly or indirectly, contribute to the varying levels of GC-stress response observed among the MMTV-MuERV promoters.

The survey of transcription regulatory elements within individual MMTV-MuERV promoters revealed a unique profile for each promoter examined. In particular, the highest prevalence of two transcription regulatory elements (two copies of GHF-1 [pituitary specific pou domain] and 14 to 15 copies of TBP [TATA-binding protein]) was found in the lowest three DEX responders (blue shade in Table 1). On the other hand, the highest occurrence of IRF (interferon regulatory factor - four copies) and RU49/Zipro1 (two copies) was identified in the two highest DEX responders (orange shade). Interestingly, two other regulatory elements (AIRE [autoimmune regulatory element binding factor] and v-ERB [erythroblastic leukemia viral oncogene]) were present in all promoters except for the two highest responders (orange shade). We then looked for any correlative trends between the frequencies of selective regulatory elements on the promoters (GRE, ETS1 [E26 transformation-specific sequence], and TBP) vs. the intensities of their DEX response (Table 1 and Figure 5). These elements were selected based on their frequency of occurrence, either high or low, in the three lowest compared to the two highest DEX responders (Table 1). It seems that an inverse relationship exists between the frequency of TBP element and the level of DEX response whereas there might be a parallel trend between GRE frequency and DEX response.

3.3 Conclusions

In summary, all 64 unique MMTV-MuERV promoters examined had at least two GREs and showed a response (repressed or activated) to DEX treatment, ranging from a 0.6- to 85.7-fold difference compared to no treatment controls. *In silico* profiling of the transcription regulatory elements and alignment analysis revealed several putative transcription regulatory elements and a number of unique sequence regions highly specific for either the three lowest or the two highest DEX responders. Each promoter had a unique set of transcription regulatory elements and it is probable that in addition to GREs, there are other regulatory elements and/or proteins, both known and uncharacterized, which modulate the GC-stress response. A potential inverse correlation between the frequency of the TBP elements and the intensity of DEX response was observed.

Activation of the HPA axis in response to injury/infection-elicited stress signals leads to the release of GCs. The injury/infection-induced GCs participate in system-wide molecular and cellular events, which contribute to a wide range of pathologic courses (*e.g.*, local inflammation, sepsis) that are variable depending on mouse strain or patient. The findings from this study indicate that the MMTV-MuERVs, which reside in the genomes of all cells in mice, differentially respond to elevated systemic GC levels stemming from various stressors (*e.g.*, injury, infection, hormonal imbalance). Considering the fact that certain MMTV-MuERV genes are capable of producing functional proteins, the variable GC sensitivity among the MMTV-MuERV promoters may result in strain-specific profiles of post-stress MMTV-MuERV proteins (expression levels and isoforms) [12-15]. For example, stressor-triggered activation of certain MMTV-MuERVs will lead to the increased production of various TCR-Vβ-specific SAg proteins which are capable of altering the systemic immune configuration. Additionally, for each mouse strain, the unique set of GC-sensitive MMTV-MuERVs and their gene products may contribute to a range of strain-specific pathogenic events (*e.g.*, inflammation, sepsis) in response to initial stressors, such as injury and infection. To increase our understanding of the mechanisms, one future study may focus on identifying GC-sensitive MMTV-MuERVs from various mouse strains followed by the characterization of the MMTV-MuERV genes' involvement in injury/infection-related disease courses (*e.g.*, sepsis). Furthermore, in contrast to conventional genes, which may serve as common stress-sensors among the mouse species, MMTV-MuERVs and other types of MuERVs may function as strain-specific stress-sensors; therefore, producing strain-specific actuators in response to the same stress signal (Figure 6).

The findings from this mouse study can be directly translated into understanding the roles of inherently diverse ERVs on polymorphic inflammation and sepsis events in humans and other species by interrogating ERVs' differential responses to stressors (*e.g.*, injury, infection).

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Abbreviations

ERV	endogenous retrovirus
MuERV	murine ERV
MMTV	mouse mammary tumor virus
MLV	murine leukemia virus
GC	glucocorticoid
DEX	dexamethasone
GRE	glucocorticoid response element
GCR	glucocorticoid receptor
TCR	T cell receptor
SAg	superantigen
LTR	long terminal repeat
HPA	hypothalamus-pituitary gland-adrenal gland
CRH	corticotropin releasing hormone
ACTH	adrenocorticotropic hormone
GHF-1	pituitary specific pou domain
TBP	TATA-binding protein
IRF	interferon regulatory factor
AIRE	autoimmune regulatory element binding factor
v-ERB	erythroblastic leukemia viral oncogene
ETS1	E26 transformation-specific sequence

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Highlights

- Inherently diverse MMTV-MuERVs' responses to glucocorticoid stress are variable
- Post-stress MMTV-MuERV activation may contribute to strain-specific disease courses
- Individual's response to injury and infection may be fine-tuned by its ERV profile
- ERVs may function as individual-specific stress-sensors vs. genes as common sensors

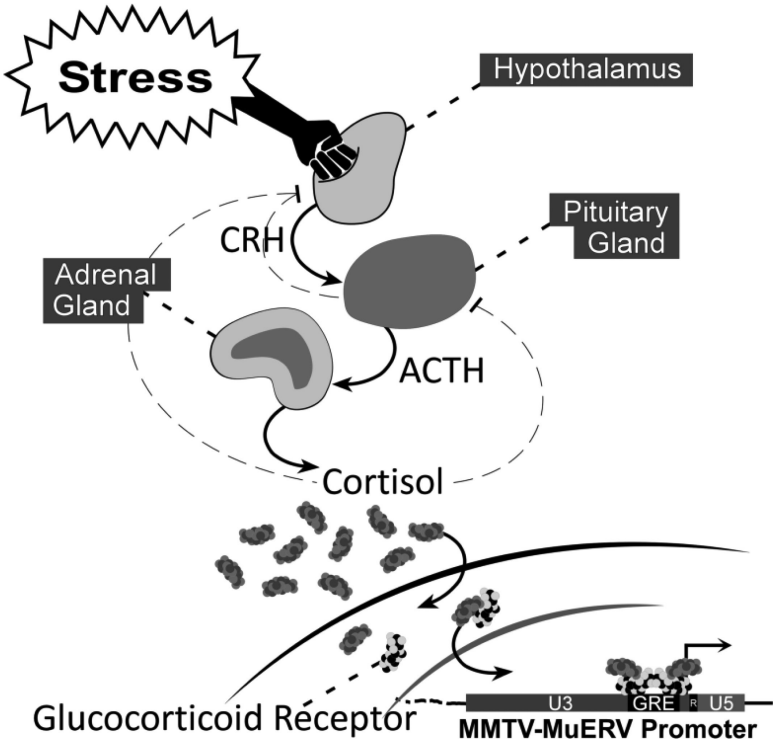

Figure 1. Schematic illustration of stress-elicited activation of MMTV-MuERVs

Reception of stress signals (e.g., injury, infection) by the HPA axis leads to the release of GC (cortisol) into systemic circulation. Upon binding GC, the GCR is activated and translocated to the nucleus. Subsequently, direct interaction between the GCR and GRE transactivates the MMTV-MuERV promoter. CRH (corticotropin releasing hormone), ACTH (adrenocorticotropic hormone)

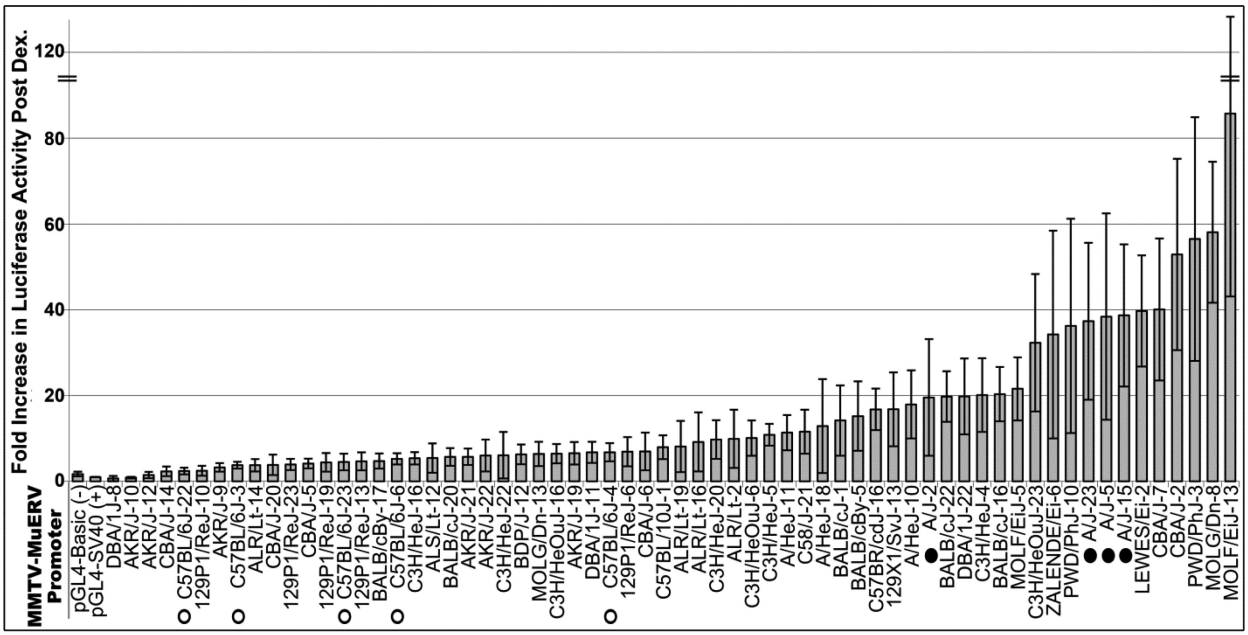

Figure 2. Divergent levels of GC-stress response among 64 MMTV-MuERV promoters

Graph shows fold increase (lowest to highest) in luciferase activity in cells transfected with the individual MMTV-MuERV promoters (64 total) after DEX treatment. First two bars from the left represent two controls (promoter-less pGL4-Basic [negative control] and pGL4-SV40 promoter [positive control]). It is possible that the luminescence values for the pGL4-SV40 control reached the measurement threshold in both DEX treatment and no treatment groups. All luminescence values from the repeat experiments were combined to calculate mean ± SD fold increase for each promoter. The open (C57BL/6J strain) and closed (A/J strain) circles indicate the MMTV-MuERV promoters used in Figure 4.

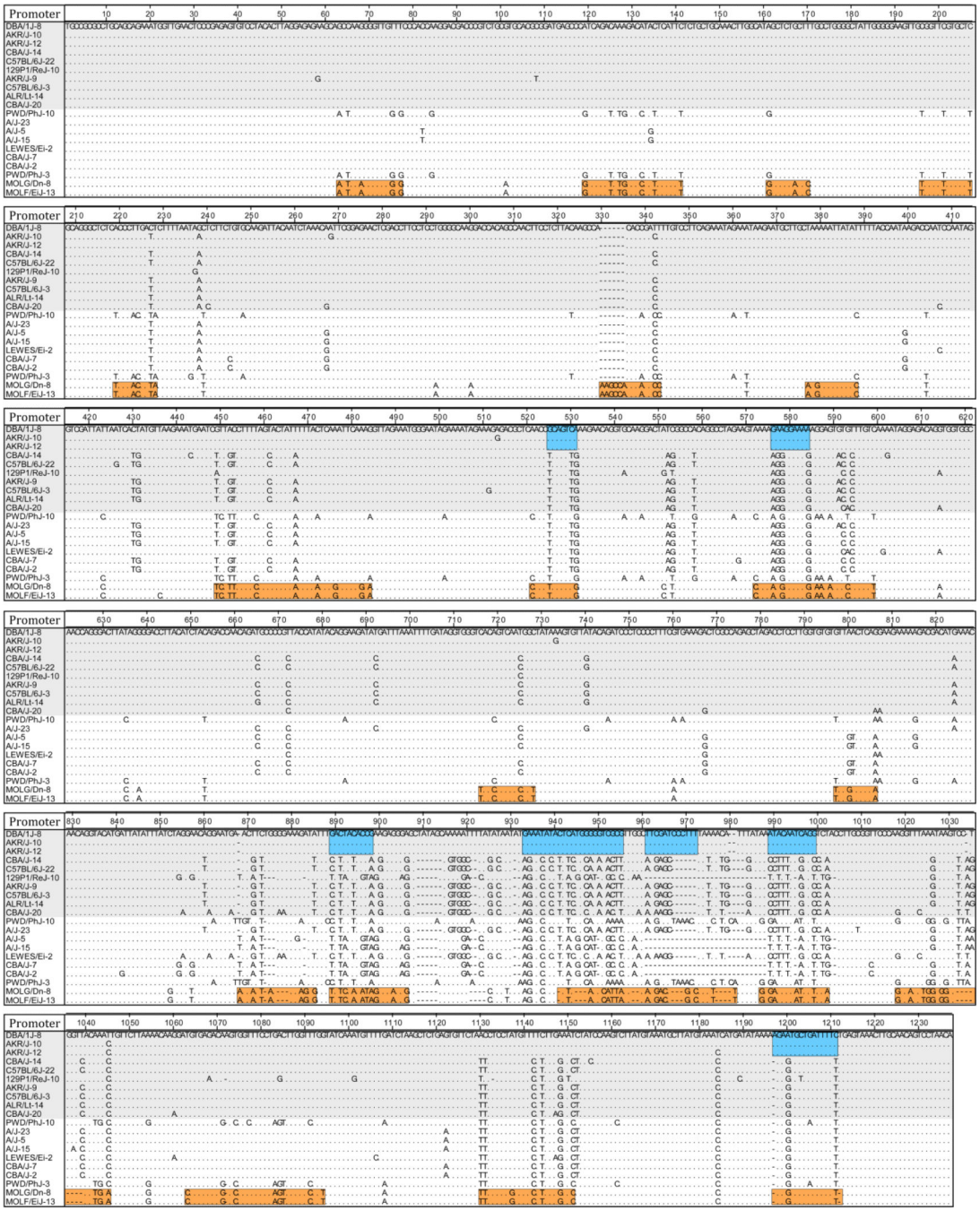

Figure 3. Comparison of MMTV-MuERV promoter sequences (10 lowest DEX responders vs. 10 highest DEX responders)

Multiple alignment analysis was performed for the 10 lowest (gray shade) and the 10 highest DEX responders to compare their sequences. Unique regions identified in the three lowest (blue shade) and in the two highest DEX responders (orange shade) are indicated.

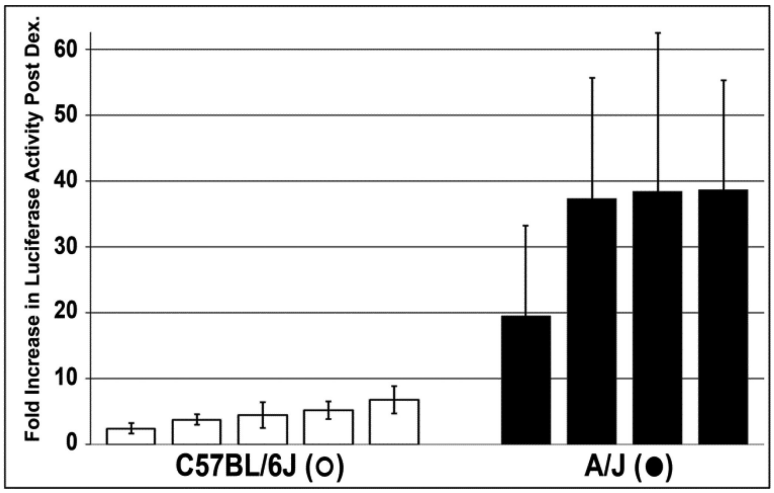

Figure 4. Comparison of GC-stress response of MMTV-MuERV promoters between A/J and C57BL/6J strains

Graph indicates fold increase in reporter activity of MMTV-MuERV promoters after DEX treatment; the five C57BL/6J strain's MMTV-MuERV promoters (marked with open circles in Figure 2) and the four A/J strain's MMTV-MuERV promoters (marked with closed circles in Figure 2) are compiled next to each other for comparison with regard to GC-stress responsiveness.

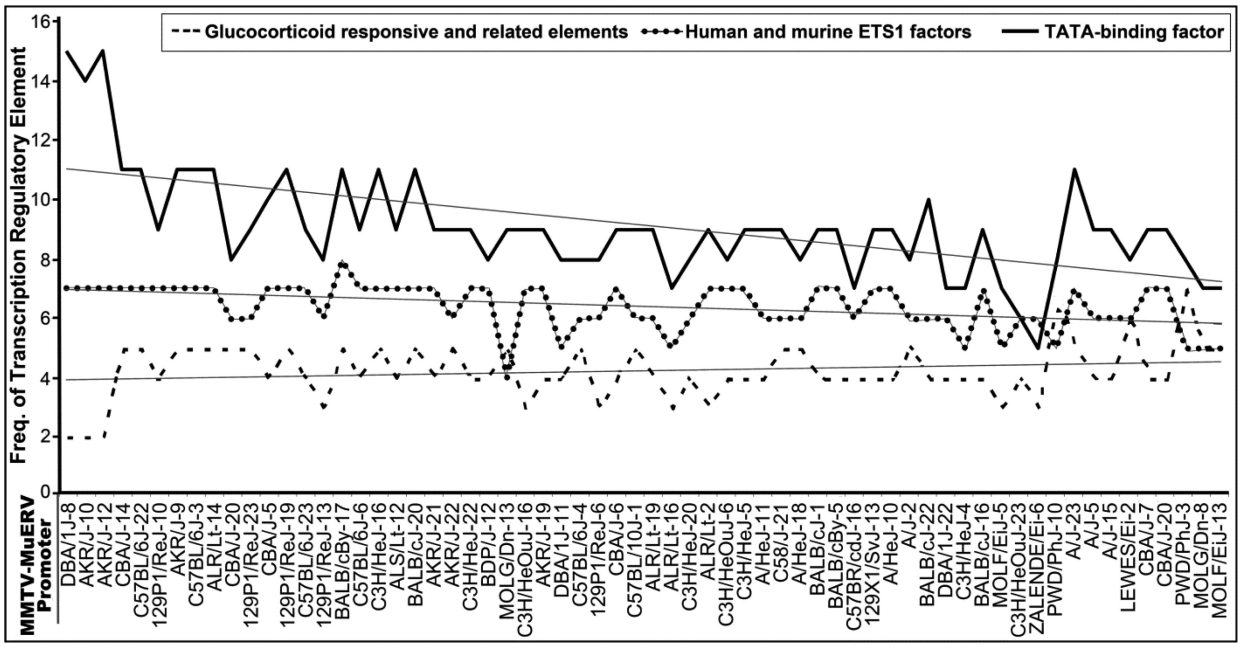

Figure 5. Correlation between copy number of certain transcription regulatory elements and DEX response

Three regulatory elements (GRE, ETS1, and TBP) were analyzed for correlation between their occurrence frequencies in each promoter and respective intensities of DEX response.

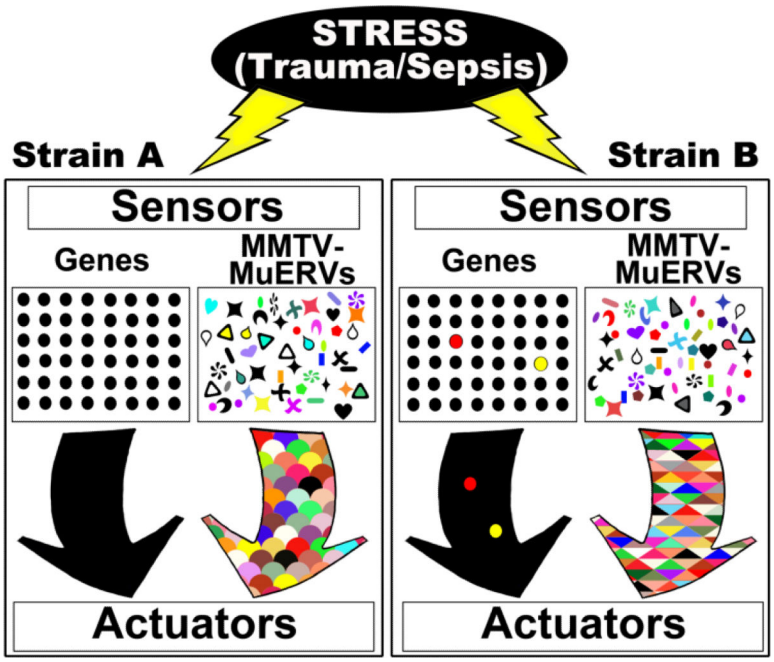

Figure 6. MMTV-MuERVs: "Mouse Strain-Specific Stress-Sensors," in comparison to conventional genes as common stress-sensors

Highly homogeneous conventional genes serve as common stress-sensors among the mouse species. In contrast, the polymorphic MMTV-MuERVs can function as strain-specific stress-sensors. Thus, when two mouse strains are under the same stress (*e.g.*, trauma, sepsis), MMTV-MuERVs produce strain-specific actuators, such as SAg and other proteins as a strain-specific stress-sensing system.

Table 1

Profile of transcription regulatory elements in 64 MMTV-MuERV promoters

MMTV-MuERV promoters are arranged top to bottom in order of lowest to highest DEX response. Blue shade: either highest or lowest frequency of certain elements identified in the three lowest DEX responders.

Orange shade: either highest or lowest (including absence) occurrence frequency of certain elements identified in the two highest DEX responders. *Elements subjected to correlation analysis in Figure 4.