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Regulation of *ERGIC-53* Gene Transcription in Response to Endoplasmic Reticulum Stress*[S]

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Accumulation of unfolded proteins within the endoplasmic reticulum (ER) activates the unfolded protein response, also known as the ER stress response. We previously demonstrated that ER stress induces transcription of the ER Golgi intermediate compartment protein ERGIC-53. To investigate the molecular events that regulate unfolded protein response-mediated induction of the gene, we have analyzed the transcriptional regulation of *ERGIC-53*. We found that the *ERGIC-53* promoter contains a single *cis*-acting element that mediates induction of the gene by thapsigargin and other ER stress-causing agents. This ER stress response element proved to retain a novel structure and to be highly conserved in mammalian *ERGIC-53* genes. The ER stress response element identified contains a 5′-end CCAAT sequence that constitutively binds NFY/CBF and, 9 nucleotides away, a 3′-end region (5′-CCCTGTTGGCCATC-3′) that is equally important for ER stress-mediated induction of the gene. This sequence is the binding site for endogenous YY1 at the 5′-CCCTGTTGG-3′ part and for undefined factors at the CCATC 3′-end. ATF6α-YY1, but not XBP1, interacted with the *ERGIC-53* regulatory region and activated *ERGIC-53* ER stress response element-dependent transcription. A molecular model for the transcriptional regulation of the *ERGIC-53* gene is proposed.

In eukaryotic cells, protein misfolding within the ER[3] activates a signaling pathway known as the unfolded protein response (UPR) or ER stress response (1–3). The UPR rapidly induces transcription of ER stress genes (1–3), attenuates protein synthesis by activating PERK-dependent phosphorylation of the translational initiation factor eIF2α, and intensifies the proteasomal degradation pathway (4, 5). In mammalian cells, UPR stimulates the expression of proteins ERGIC-53, MCFD2, and VIP36, which exert functions in the post-ER compartments of the secretory pathway (6–8). ERGIC-53 is a highly conserved calcium-dependent lectin that shares structural and functional homology with VIP36 (9). ERGIC-53 is mainly localized within the ERGIC; it cycles continuously between the ER and the Golgi complex and exports a defined number of glycoproteins from the ER (9–16). The adaptor protein MCFD2 forms a complex with ERGIC-53 to transport newly synthesized clotting factors V and VIII to the Golgi complex (17–19). ERGIC-53 and MCFD2 accumulate in response to distinct signaling pathways of the cell stress response. In particular, heat shock stimulates preferential translation of ERGIC-53 and MCFD2 mRNAs (7), whereas UPR induced by either thapsigargin or nitric oxide activates the transcription of both genes (7, 8).

Transcriptional activation by the UPR requires the presence of *cis*-acting ER stress response elements (ERSE) on the promoter region of target genes. Three types of ERSE have been identified: ERSE-I and ERSE-II (20–22, 23) and the mammalian unfolded protein response element (UPRE) (23–25). Multiple copies of the ERSE-I control the UPR-mediated induction of the ER-resident proteins Grp78/BiP, Grp94, calreticulin, and protein-disulfide isomerases (20, 21). ERSE-I contains a CCAAT site at its 5′-end for the constitutive transcription factor NFY/CBF (20, 26, 27), a 9-bp spacer containing the CGG triplet that is the TFII-I binding site (28), and a CCACG motif at the 3′-end that is required for ATF6α (activating transcription factor 6α) recruitment (20, 26, 28–31). ATF6α is an ER transmembrane protein that, during ER stress, can be recruited by transport vesicles destined for the Golgi complex, where, consequent to intramembrane proteolysis, a 50-kDa cytosolic form is generated that activates transcription of UPR genes (26–32). A single copy of the ERSE-II type regulates the ER stress response of the *Herp* gene, which encodes an ER integral membrane protein that is involved in the ER-associated degradation pathway (22, 33, 34). ERSE-II (5′-ATTGGNCCAC(G/A)-3′) retains a reversed NFY/CBF binding site at its 5′-end and a flanking ATF6α site (22). The UPRE, which controls the expression of a subset of ER-resident chaperones (35, 36), contains the ATF6α binding site on its complementary strand and is also the preferred binding site for the transcription factor XBP1 (X-box-binding protein 1). XBP1 is under the control of

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

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[3] The abbreviations used are: ER, endoplasmic reticulum; ERGIC, ER Golgi intermediate compartment; TG, thapsigargin; DTT, dithiothreitol; UPR, unfolded protein response; UPRE, UPR element; ERSE, ER stress response element; MEF, mouse embryonic fibroblast; RT, reverse transcription; ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay; EBC, ERSE-binding complex; nt, nucleotide(s); HA, hemagglutinin.

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the ER membrane nuclease IRE1α (inositol-requiring enzyme 1α), which in response to protein misfolding triggers the processing of XBP1 mRNA, thereby leading to the synthesis of the transcription factor (35, 37, 38). Interestingly, XBP1 binds both the UPRE and ERSE sequences of target genes (23, 37) independently of NFY/CBF, whereas ATF6α binds UPRE albeit with a lower affinity compared with ERSE (23, 37).

We have studied the regulation of the *ERGIC-53* promoter in an attempt to shed light on the transcriptional mechanisms that control *ERGIC-53* expression. The transcriptional regulation of *ERGIC-53* by the UPR requires a single *cis*-acting element (*ERGIC-53* ERSE), which is highly conserved in mammals and is different from the other types of ERSE identified.

EXPERIMENTAL PROCEDURES

Antibodies—The following antibodies were used in EMSA and ChIP assays: mouse monoclonal anti-ATF6α (Active Motif), mouse monoclonal anti-YY1 and anti-HA epitope, and rabbit polyclonal anti-NFY-CBF/A, anti-XBP1, anti-Sp1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

Cell Culture and Induction of ER Stress—HeLa cells, mouse embryonic fibroblasts (MEFs) derived from wild type or IRE1α knock-out mice (38), were grown at 37 °C in a humidified 5% CO_2, 95% atmosphere in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 4.5 g/liter glucose, and 2 mM glutamine. To induce ER stress, we incubated actively growing cells for 8 h (unless otherwise indicated) with 300 nM thapsigargin (TG), 2 mM DTT, or 10 μM MG132 (Sigma) before harvesting.

RNA Extraction and Real Time RT-PCR Analyses—Serial dilutions of total RNA obtained from HeLa, wild type, and IRE1$^{-/-}$ MEF cells (RNeasy kit; Qiagen) were reverse transcribed (Invitrogen) and subjected to real time RT-PCR using the iCycler Apparatus (Bio-Rad) and analyzed by the SybrGreen method. The following primers were used to amplify the corresponding transcripts in human cells: *ERGIC-53*-forward, 5′-GGG CAG CAT GGG CAG ATT AC-3′; *ERGIC-53*-reverse, 5′-CAT AGA CGC CTC CAG CAG AGC-3′; *GAPDH*-forward, 5′- GAA GGT GAA GGT CGG AGT C-3′; *GAPDH*-reverse, 5′-GAA GAT GGT GAT GGG ATT TC-3′. The following primers were designed to amplify the corresponding transcripts in murine cells: *ERGIC-53* forward, 5′-GGA CAG CCT GGG CAG GTC TC-3′; *ERGIC-53* reverse, 5′-GGG TGC TGG ATG CCA CTC A-3′; c-*ABL* forward, 5′-GGT ATG AAG GGA GGG TGT ACC A-3′; and c-*ABL* reverse, 5′-CAC TTG ATT GAG TCG GTC TCA CAA CT-3′. The following primers were used to amplify the *Grp94* transcripts from both human and murine cells: *Grp94* forward, 5′-TCC GCC TTC CTT GTA GCA GAT A-3′; *Grp94* reverse, 5′-TGT TTC CTC TTG GGT CAG CAA T-3′. Expression level was calculated according to the $2^{-\Delta\Delta CT}$ method (39) by using either *GAPDH* or c-*Abl* mRNA as a control gene.

Construction of Plasmids—A 1084-bp fragment spanning from nt −874 to +202 of the human *BiP/Grp78* promoter (EMBL Data Library accession number X59969) (40) and a 1036-bp fragment corresponding to the *LMAN1/ERGIC-53* gene (NCBI ref/NT_025028) from nt −1003 to +33 were isolated by PCR amplification from genomic DNA extracted from HeLa cells, and PCR fragments were gel-purified and cloned by using the pGEM-T Easy Vector system (Promega). The *Bip/Gpr78* promoter region spanning from nt −838 to −16 was PCR-amplified with synthetic oligonucleotides having KpnI-XhoI flanking restriction sites and subcloned in the pGL3Basic vector (Promega) to drive the expression of firefly luciferase reporter gene. Similarly, the *ERGIC-53* promoter region (nt −1000 to −1) was ligated to the KpnI-HindIII site of the pGL3Basic vector (Promega) upstream from the luciferase reporter gene. Progressive deletion of the 5′-flanking region of the *ERGIC-53* promoter was obtained by PCR amplification using forward primers containing the 5′-KpnI site and common reverse primers with the 3′-HindIII site. The −149 to −1 and −149 to −63 fragments were obtained by PCR, digested, and inserted in the KpnI-BglII site of the pGL3 promoter vector (Promega), which contains the SV40 minimal promoter upstream from the luciferase coding sequence (SV40Luc). Synthetic oligonucleotides corresponding to the ERSE-I consensus sequence, the −32 to −1 and the −65 to −31 region of the *ERGIC-53* ERSE, and cassette and point mutations of *ERGIC-53* ERSE were synthesized, *in vitro* annealed, and inserted in the KpnI-BglII site of the pGL3 promoter vector and controlled by sequencing. The pCGN-ATF6-(1–373) and pCGN-ATF6-(1–273) expression vectors (31, 41) were kindly provided by A. S. Lee. The pCGN-ATF6-(1–373)m1 was already described (25).

Transfection Experiments for Transient Expression of Reporter Plasmids—Actively growing HeLa and MEF cells were seeded onto 60-mm plates at ~20–30% of confluence and

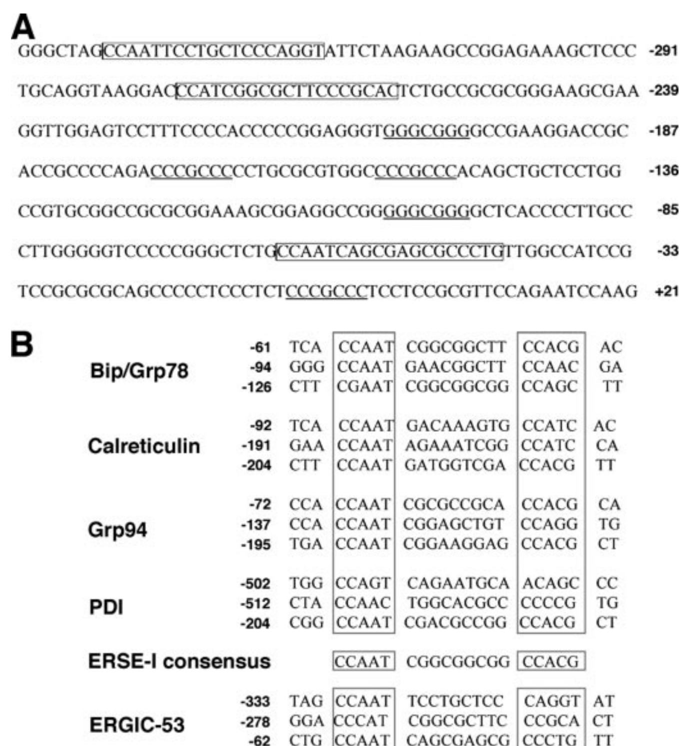

FIGURE 1. **Sequence analysis of the *ERGIC-53* promoter region.** *A*, nucleotide sequence of the 5′-region of the *ERGIC-53* gene. The *numbers* on the *right* indicate the nucleotide position with respect to the transcription start site (+1). CCAAT and 3′-flanking sequences indicative of putative ERSEs are *boxed*. Putative binding sites for transcription factors of the SP family are *underlined*. *B*, sequence alignment of the ERSE-like elements of the *ERGIC-53* promoter with the ERSE consensus sequence and with ERSE sequences of human ER stress-regulated genes.

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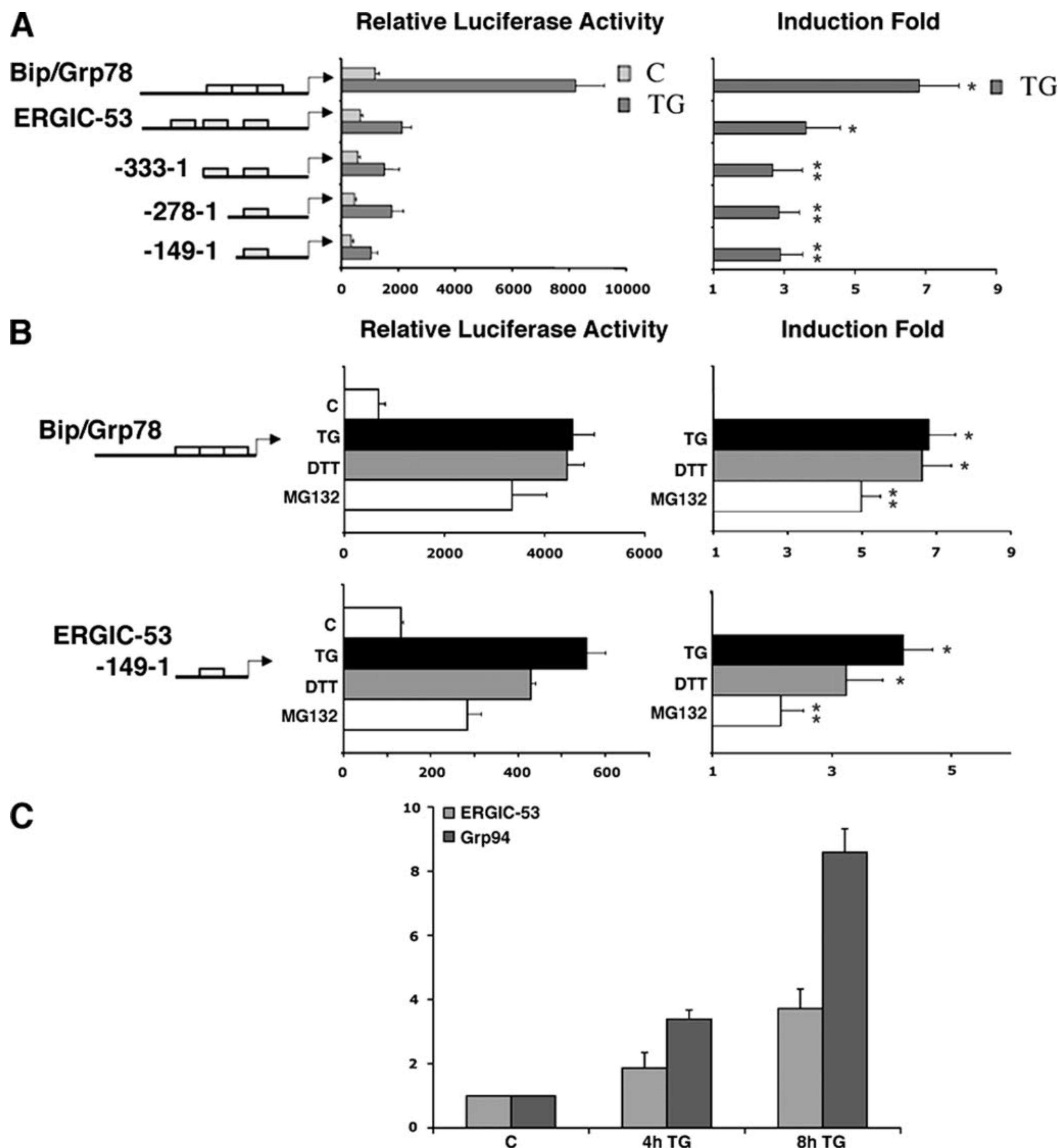

FIGURE 2. **Isolation of the minimal ER stress-inducible region of the *ERGIC-53* promoter.** *A*, activity of luciferase reporter gene driven by the undeleted *ERGIC-53* promoter (1036 bp) or by deletion mutants lacking each of the three ERSE-like sequences. HeLa cells transiently transfected with the recombinant ERGIC-53 promoter were unexposed (*C*) or exposed to 300 nM thapsigargin (*TG*) for 8 h. Luciferase activity obtained with the reporter vector driven by the undeleted *Bip/Grp78* promoter served as positive control. *B*, activity of the −149 to −1 deletion mutant assayed in cell extracts of unexposed cells (*C*) or HeLa cells exposed to 300 nM TG, 2 mM DTT, and 10 μM MG132 for 8 h. The *histograms* represent relative luciferase activity normalized for β-galactosidase activity and protein concentration in the cell lysates. -Fold induction is calculated as the ratio between the enzyme activity of induced cells and noninduced cells. Values represent the average of at least three independent experiments performed in duplicate. Statistical analysis was performed using Student's *t* test ($n = 6-8$; *, $p < 0.001$; **, $p < 0.005$ relative to the control, respectively). *C*, real time RT-PCR analysis of *ERGIC-53* and ER chaperone gene *Grp94* mRNAs accumulation in response to ER stress. Total RNA fractions obtained from HeLa cells exposed for 4–8 h to 300 nM TG were analyzed by RT-PCR to measure the level of the indicated mRNAs. The values were normalized to *GAPDH* mRNA. The relative -fold induction was calculated as the ratio of treated cells divided by untreated cells according to the $2^{-\Delta\Delta CT}$ method. Each value represents the mean ± S.D. of three independent experiments.

transfected with 1 μg of luciferase reporter plasmids and 0.5 μg of the RSV-β-Gal reporter control plasmid (Promega) by using the FuGene Transfection Reagent (Roche Applied Science). Cells were incubated with the Fugene-DNA complex for 16 h at 37 °C, washed with cold phosphate-buffered saline, and lysed in reporter lysis buffer (Promega) 48 h after transfection. To

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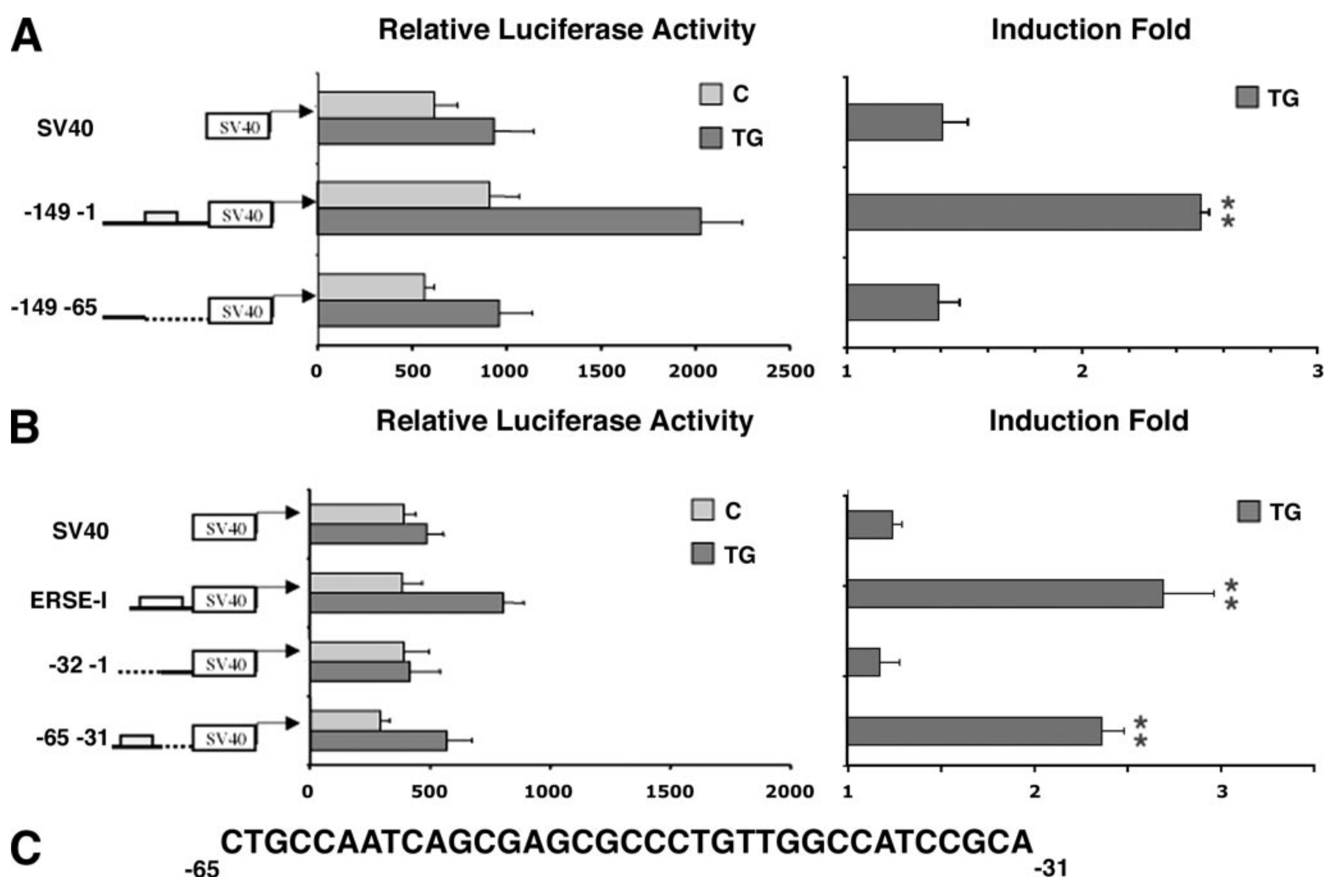

FIGURE 3. **Setting the boundaries of the *ERGIC-53* ERSE.** *A*, relative luciferase activity of the heterologous SV40 promoter in the presence or the absence of deletion mutants of the *ERGIC-53* minimal inducible region. HeLa cells transfected with reporter vectors were untreated (*C*) or treated (*TG*) with 300 nM thapsigargin for 8 h when indicated. *B*, HeLa cells were transfected with luciferase reporter plasmids containing the ERSE-I consensus element, the −32 to −1, and −65 to −31 regions of the *ERGIC-53* promoter. Relative luciferase activity and -fold induction was calculated as indicated in Fig. 2 ($n = 6–8$; **, $p < 0.005$ relative to the control; Student's *t* test). *C*, nucleotide sequence of the −65 to −31 region of the *ERGIC-53* promoter.

measure LacZ reporter gene activity (β-galactosidase), we incubated cell extracts for 1 h at 37 °C in β-galactosidase assay buffer (200 mM sodium phosphate buffer, pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol, 1.33 mg/ml *o*-nitrophenyl-β-D-galactopyranoside). The reaction was blocked by adding 1 M sodium carbonate, and absorbance was measured at 420 nm. We measured luciferase activity with a Berthold luminometer in 40 μl of cell lysate supplemented with 100 μl of luciferase assay reagent (Promega). "Relative luciferase activity" is defined as the luciferase-to-β-galactosidase activity ratio and normalized for the protein concentration of each sample. "-Fold induction" is defined as the ratio between the enzyme activities of treated cells with respect to that of untreated cells. In all experiments, values are reported as the average and S.D. of at least three independent experiments carried out in duplicate. Statistical analysis was performed using Student's *t* test ($n = 6–8$).

Chromatin Immunoprecipitation Assays—1×10^6 HeLa cells were treated or not for 2–4 h with 300 nM TG for the UPR induction. For the ChIP analysis of overexpressed ATF6α, HeLa cells were transfected with 6 μg of the pCGN-ATF6-(1–373) and pCGN-ATF6-(1–273) expression vectors (31, 41) and harvested 24 h after transfection. Experiments were performed according to the instructions of the manufacturer (Upstate Biotechnology, Inc.). Briefly, cells were exposed to 1% formaldehyde for 10 min at 37 °C to obtain protein-DNA cross-linking. The nuclear fraction was sonicated to obtain chromatin fragments of 200–1000 bp; an aliquot (5% of the total volume) was removed from each sample and used as the input fraction. Chromatin was precleared by preincubation with a DNA salmon sperm/protein A-agarose 50% slurry (Upstate Biotechnology) for 1 h at 4 °C. The agarose was centrifuged, and the precleared chromatin supernatant was then incubated with the indicated antibodies overnight at 4 °C. The protein-DNA-antibody complexes were collected by the addition of the salmon sperm DNA-protein A-agarose (2 h at 4 °C) and washed, and protein-DNA cross-linking was reversed (4 h at 65 °C). DNA was purified by phenol/chloroform extraction and ethanol precipitation, and aliquots (25%) of the purified materials underwent PCR (5 min at 94 °C; 1 min at 94 °C, 1 min at 52 °C, 1 min at 72 °C for 40 cycles; 5 min at 72 °C). The following primers were used in the PCR: 5′-TGAACCAATGGGACCAGC-3′ and 5′-CTCACCGTCGCCTACTCG-3′ to amplify a 254-bp fragment of the human *Bip*/*Grp78* promoter spanning from nt −267 to nt −13; 5′-AAGCGAAGGTTGGAGTCC-3′ and 5′-CGCCATCTTGGATTCTCC-3′ to amplify a 271-bp fragment of the *LMAN1/ERGIC-53* promoter extending from nt −226 to nt +45.

Preparation of Nuclear Extracts and Electrophoresis Mobility Shift Assays—5–6×10^6 HeLa cells were either treated for 2 h with 300 nM TG to induce ER stress or transiently transfected

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TABLE 1
Conservation of the ERSE ERGIC-53 element

The DNA sequence of the human *ERGIC-53* ERSE (NT_025028) was used as a probe in BLAST analysis. The table shows the similar *ERGIC-53/LMAN1* orthologue promoter sequences identified in monkeys (NW_119156.1), mice (NT_039674), rats (NW_047514), and dogs (NC_006583.2) as reported in the NCBI genomic data base (available on the World Wide Web). 5′-CCAAT motifs are in boldface type, and nonidentical nucleotides are underlined. Numbers on the right are the relative positions of ERSE with respect to the transcription start site. No significant similarity has been found in: *Caenorhabditis elegans*, *Drosophila melanogaster*, or *Gallus gallus*.

Species	Sequence	Position
Homo sapiens	**CCAAT**CAGCGAGCGCCCTGTTGGCCATCCGC	−62 to −32
Pan troglodytes	**CCAAT**CAGCGAGCGCCCTGTTGGCCATCCGC	−79 to −49
Mus musculus	**CCAAT**CAGTGAGCGCCCTGTTGGCCATCCGC	−68 to −38
Rattus norvegicus	**CCAAT**CAGTGAGCGCCCTGTTGGCCATCCGC	−61 to −31
Canis familiaris	**CCAAT**CAGCGCGCGCCCTGTTGGCCGTCCGC	−62 to −42

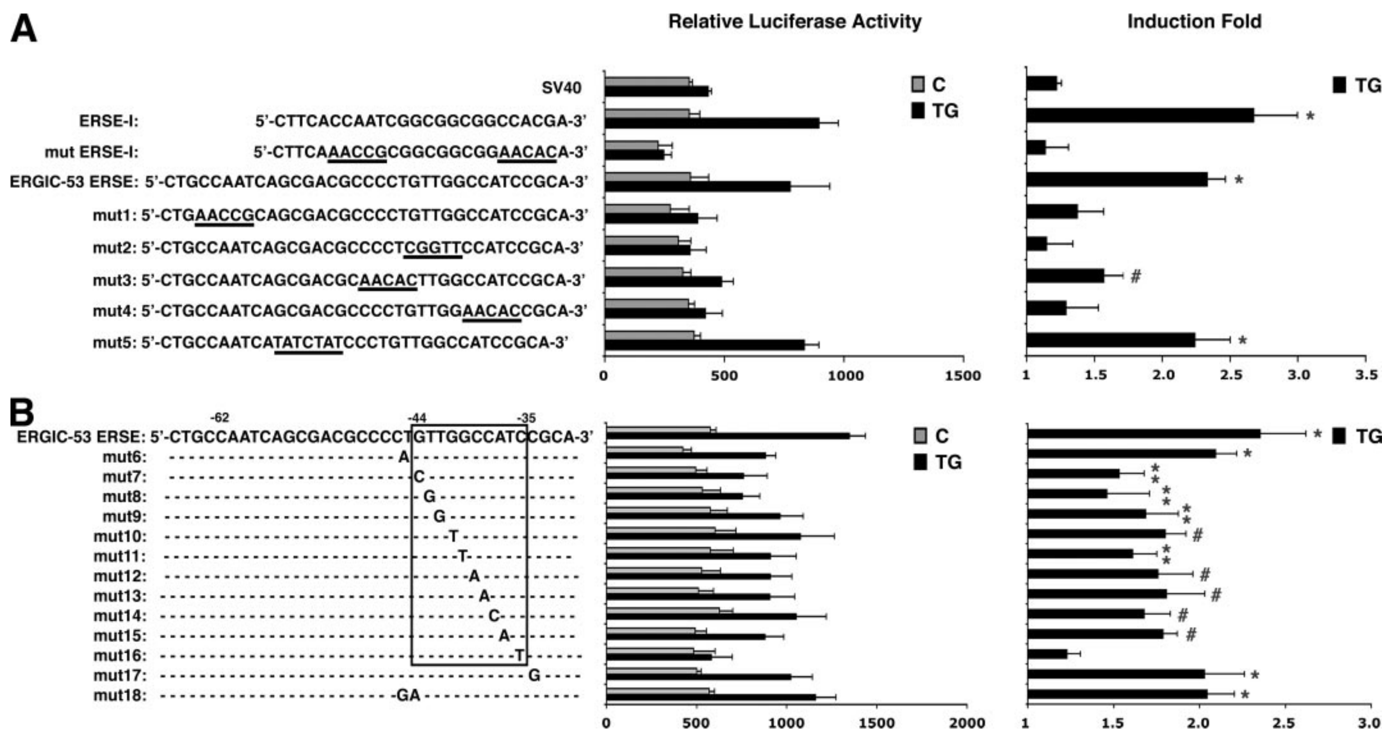

FIGURE 4. **Mutational analysis of the *ERGIC-53* ERSE.** *A*, effect of clusters of nucleotide substitutions on *ERGIC-53* ERSE activity. Synthetic oligonucleotides corresponding to the *ERGIC-53* ERSE or to *ERGIC-53* ERSE mutants (mut 1–5) were inserted in the PGL3 promoter vector, and the basal and thapsigargin-dependent luciferase activities (n = 6–8; *, $p < 0.001$) were measured as described in the legend to Fig. 2. Synthetic oligonucleotides corresponding to the consensus ERSE-I or to its inactive form served as control. Mutated nucleotide sequences are *underlined*. *B*, effect of single point mutations (mut 6–17) in the −34 to −45 segment of the *ERGIC-53* ERSE. Mutated fragments were inserted in the PGL3 promoter vector, and the corresponding reporter activity was assayed as reported in the legend to Fig. 2. The activity of a double substitution (mut 18) is also shown. The *graphs* represent the average ± S.D. of three independent experiments. Statistical analysis was performed using Student's *t* test (n = 6; *, $p < 0.001$; **, $p < 0.025$; #, $p < 0.01$, respectively).

with 6 μg of the pCGN-ATF6-(1–373) expression vector and harvested 48 h after transfection. Cells were washed with cold phosphate-buffered saline and harvested by scraping. The cell pellet was resuspended in extraction buffer containing 10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM DTT, 0.5 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, passed through a needle, kept on ice for 45 min, and centrifuged (15 min at 14,000 rpm at 4 °C). The nuclear pellet was then resuspended in high salt extraction buffer containing 10 mM HEPES, pH 7.9, 0.4 mM NaCl, 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin and incubated for 45 min at 4 °C. The nuclear extract supernatant was obtained by centrifugation (30 min at 14,000 rpm at 4 °C), protein concentration was determined, and 5-μg aliquots were stored at −80 °C until used. Double-stranded synthetic oligonucleotides were radiolabeled using [γ-^{32}P]ATP (3000 Ci $mmol^{-1}$; Amersham Biosciences) and T4 polynucleotide kinase (Fermentas). The binding reaction was carried out for 20 min at room temperature with 5 μg of nuclear proteins in 4% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, pH 8.0, 0.5 mM DTT, 50 mM Tris-HCl, pH 7.5, 0.250 μg/μl poly(dI-dC) containing 30,000 cpm of radiolabeled probe and a 50–100-fold molar excess of unlabeled competitor oligonucleotide when indicated. For supershift experiments, 4 μg of specific anti-NFY/CBF-A and 10 μg of either anti-YY1- or anti-HA-specific antibody was added to the binding reaction and incubated for 30 min before the addition of the radiolabeled probe. DNA-protein complexes were separated by 5% nondenaturing polyacrylamide gel and revealed by PhosphorImager analysis (Typhoon 8600; Amersham Biosciences).

RESULTS

Isolation of the ERGIC-53 Minimal Inducible Promoter—To identify ERSE elements in the ERGIC-53 promoter, we looked for transcription factor binding sites (TESS analysis) on about 1000 bp of the 5′-region of the ERGIC-53 gene. Three

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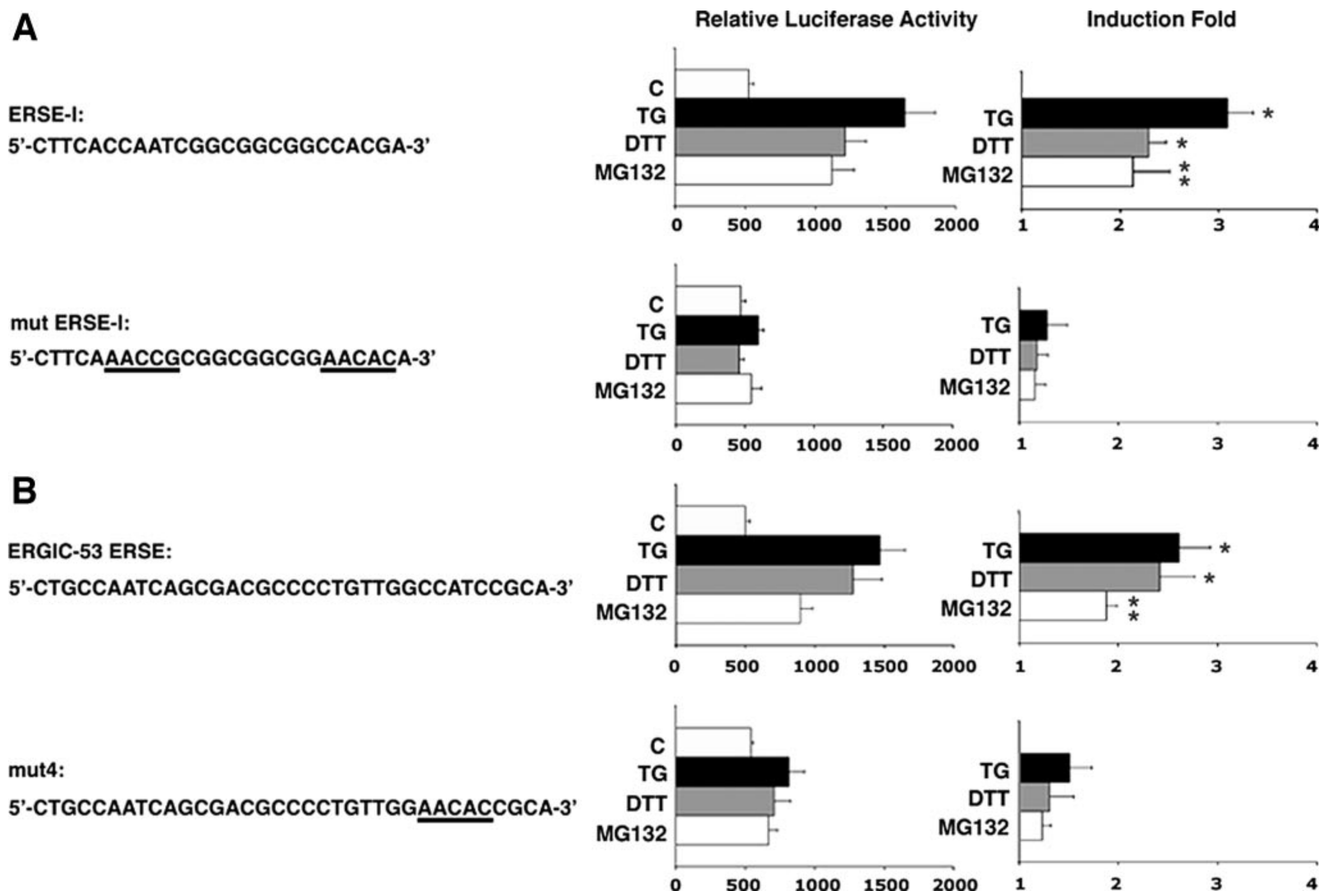

FIGURE 5. ***ERGIC-53* ERSE is a general sensor of ER stress.** *A*, activity of the consensus ERSE and its mutated form (mut ERSE) assayed in cell extracts derived from unexposed cells (*C*) or HeLa cells exposed to 300 nM TG, 2 mM DTT, or 10 μM MG132 for 8 h. *B*, activity of *ERGIC-53* ERSE and its mutated form (mut 4) assayed in cell extracts derived from HeLa cells untreated (*C*) or exposed for 8 h to 300 nM TG, 2 mM DTT, or 10 μM MG132. The *histograms* represent relative luciferase activity normalized for β-galactosidase activity and protein concentration in the cell lysates. Induction -fold is calculated as the ratio between the luciferase activity of induced cells with respect to noninduced cells. Plotted values represent the average of at least three independent experiments performed in duplicate ($n = 6-8$; *, $p < 0.001$; **, $p < 0.005$, respectively).

5′-CCAAT motifs scored high as binding sites for the transcription factor NFY/CBF. This result suggested the presence of three putative ERSEs located at positions −334 to −316, −277 to −259, and −62 to −44 (Fig. 1*A*). Alignment of the three *ERGIC-53* ERSE-like sequences with ERSE of human genes responsive to ER stress (Fig. 1*B*) revealed differences in the downstream region (excluding the 5′-CCAAT box). In addition, five sites had a high affinity for transcription factors of the SP family of proteins (Fig. 1*A*), and the *ERGIC-53* promoter lacked known transcription initiation sequences (Fig. 1*A*).

Thus, we attempted to isolate the *ERGIC-53* minimal inducible promoter by measuring the activity of the firefly luciferase reporter gene driven by deletion mutants lacking the three ERSE-like sequences we had identified (Fig. 2*A*). Expression vectors were transfected in HeLa cells treated or not with TG. The 1036-bp region containing all three putative ERSE enhanced luciferase expression 3.61 ± 0.98-fold in TG-treated cells (Fig. 2*A*, *right*). Deletions of the −335 to −317 and −278 to −259 ERSE-like sequences reduced the basal activity in untreated cells, but the -fold induction was retained in the −149 to −1 region (Fig. 2*A*, *right*) that contains the −62 to −35 ERSE-like motif (Fig. 1*A*). With the three intact ERSE-Is, TG caused a 6.2 ± 1.12-fold increase in the expression of the control *Grp78*/*BiP* promoter. Thus, the minimal inducible region was retained in the −149 to −1 region. This region could initiate transcription in response to various ER stress inducers (Fig. 2*B*), giving a -fold induction of the reporter gene of 4.2 ± 0.49 with TG, 3.24 ± 0.62 with the reducing agent DTT, and 2.14 ± 0.38 with proteasome inhibitor MG132. In contrast, the undeleted *Grp78*/*BiP* promoter enhanced luciferase activity in response to TG and DTT by 6.79 ± 0.72- and 6.72 ± 0.78-fold, respectively and by 4.98 ± 0.52-fold in response to MG132. Finally, the activity of the −149 to −1 *ERGIC-53* promoter was consistent with the -fold *ERGIC-53* mRNA induction by TG as measured by real time RT-PCR analysis (Fig. 2*C*), indicating that the −149 to −1 region (Fig. 2*A*, *right*) that contains a single ERSE-like is sufficient to ensure higher *ERGIC-53* mRNA levels in response to TG treatment.

UPR Regulates ERGIC-53 Transcription by Means of a Single ERSE—To identify the ERSE within the −149 to −1 region, we first compared the luciferase activity of this region with that of the −149 to −63 deletion mutant that lacks the putative ERSE (Fig. 3*A*). Deletion mutants were placed upstream from the SV40Luc vector to drive the expression of the luciferase reporter gene. As expected, reporter assays confirmed that the −149 to −1 region conferred thapsigargin-mediated activation of the SV40Luc vector and enhanced the activity

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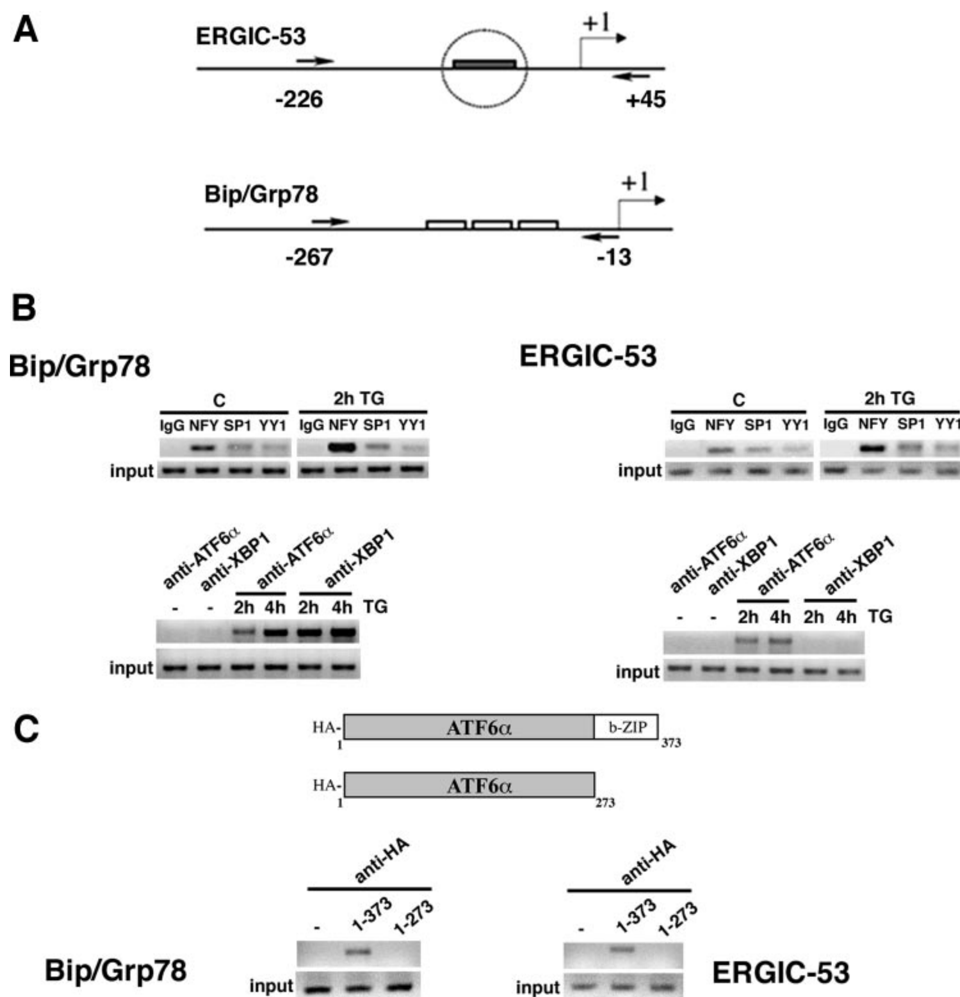

FIGURE 6. **Chromatin immunoprecipitation analysis of nuclear factors interacting with the ERGIC-53 promoter.** *A*, schematic illustration of the *BiP/Grp78* and *ERGIC-53* promoter regions. The *numbers* with *arrows* indicate the relative position of the PCR primers used to reveal DNA protein interaction. *B*, sonicated chromatin obtained from either steady-state (*C*) or thapsigargin-treated (*TG*) HeLa cells was immunoprecipitated by anti-IgG as control or by the indicated specific antibodies recognizing different basal and ER stress-activated transcription factors and revealed by PCR amplification, as described under "Experimental Procedures." *C*, HeLa cells transfected with empty (−), HA-ATF6(373), or HA-ATF6(273) expression vector were cross-linked with formaldehyde subjected to ChIP assay with 10 μg of an anti-HA epitope-specific antibody. PCRs were performed as described above.

of reporter gene by 2.50 ± 0.08-fold. Conversely, the ERSE-lacking mutant (−149 to −63) was inactive in thapsigargin-treated cells, which indicates that the putative *ERGIC-53* ERSE is located within the −63 to −1 region of the promoter (Fig. 3*A*). Thus, we mapped the boundaries of the *ERGIC-53* ERSE, by investigating the capacity of the −65 to −31 and −32 to −1 deletion mutants to confer TG-mediated activation to the SV40Luc reporter vector (Fig. 3*B*). We also compared the activity of *ERGIC-53* promoter deletions with that of the reporter gene under the control of the ERSE-I consensus sequence (20, 21). The results of luciferase assays showed that the *ERGIC-53* ERSE spans from nucleotide −65 to nucleotide −31 of the promoter. In addition, the -fold induction of the SV40Luc vector driven by the *ERGIC-53* ERSE (2.36 ± 0.12) was comparable with that driven by the control vector bearing a single copy consensus ERSE-I (2.69 ± 0.27). The *ERGIC-53* ERSE sequence identified is highly conserved and is located at a similar position along the promoter region of several mammalian *ERGIC-53* genes as shown by BLAST analysis (42) carried out on the NCBI genomic data base (available on the World Wide Web). This suggests that *ERGIC-53* could be similarly regulated by ER stress in different species (Table 1).

Mutational Analysis Reveals the Novel Structure of the ERGIC-53 ERSE—To define the sequence required for the ER stress response of the *ERGIC-53* ERSE (Fig. 3*C*), we inserted oligonucleotides bearing the substitutions shown in Fig. 4*A* in the SV40Luc reporter plasmid and determined their capacity to confer the ER stress response to the minimal SV40 promoter. We also performed these experiments using reporter vectors bearing a single copy of the consensus or a mutated ERSE-I (20, 21). As expected, mutation of the entire 5′-CCAAT box (mut 1) impaired ERSE activity. Interestingly, nucleotide replacement of the −48 to −35 segment (mut 2–4) inhibited the response to TG. Inhibition was greater when we replaced the segment between nucleotides −44 and −35 (mut 2 and 4), which indicates that this sequence is crucial for TG-dependent activation of *ERGIC-53*. Mutation in the segment spanning nucleotides −55 to −49 (mut 5) did not result in a similar reduction (Fig. 4*A*). To verify these findings, we introduced single point mutations into each nucleotide of the −44 to −35 sequence (Fig. 4*B*). Replacement of any of the nucleotides within this sequence impaired -fold induction, albeit to different degrees (Fig. 4*B*, mut 7–16). In particular, the −44 G-to-C transition (mut 7) resulted in a -fold induction decrease comparable with that of the −48 to −44 cassette mutant (mut 3), whereas the −35 C-to-T transversion (mut 16) almost completely abolished induction. Replacement of the outer nucleotides (mut 6 and 17) had little effect on the ER stress-dependent activity of the *ERGIC-53* ERSE.

Therefore, the sequence of the *ERGIC-53* ERSE crucial for UPR-mediated gene induction is constituted by a leader 5′-CCAAT-3′ sequence followed by a novel −48 to −35 sequence (5′-CCCTGTTGGCCATC-3′), both of which are required to confer full inducibility in response to ER stress. The *ERGIC-53* ERSE is a general sensor of ER stress. The induction of the *ERGIC-53* ERSE reporter gene transfected in HeLa cells was 2.61 ± 0.32-fold with TG, 2.42 ± 0.35 with DTT, and 1.88 ± 0.12 with MG132 (Fig. 5). These values were comparable

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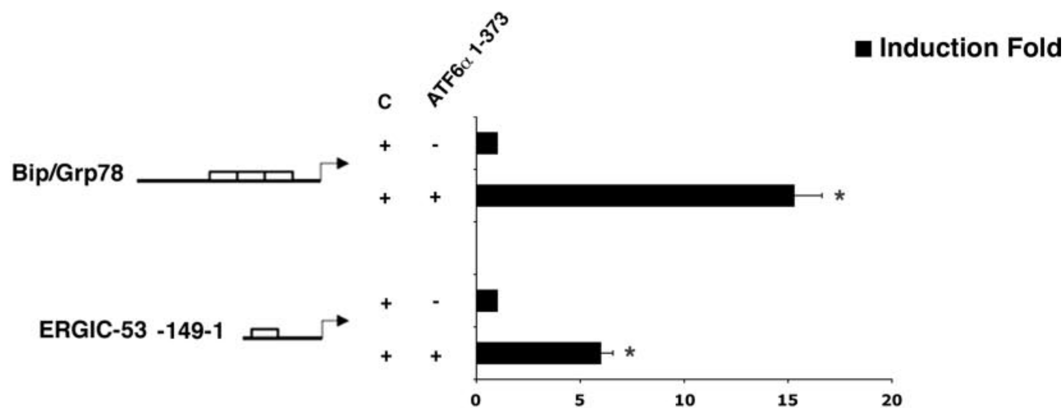

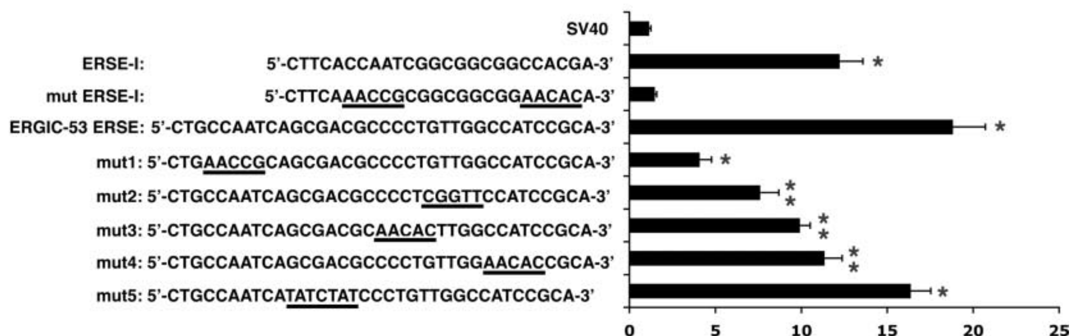

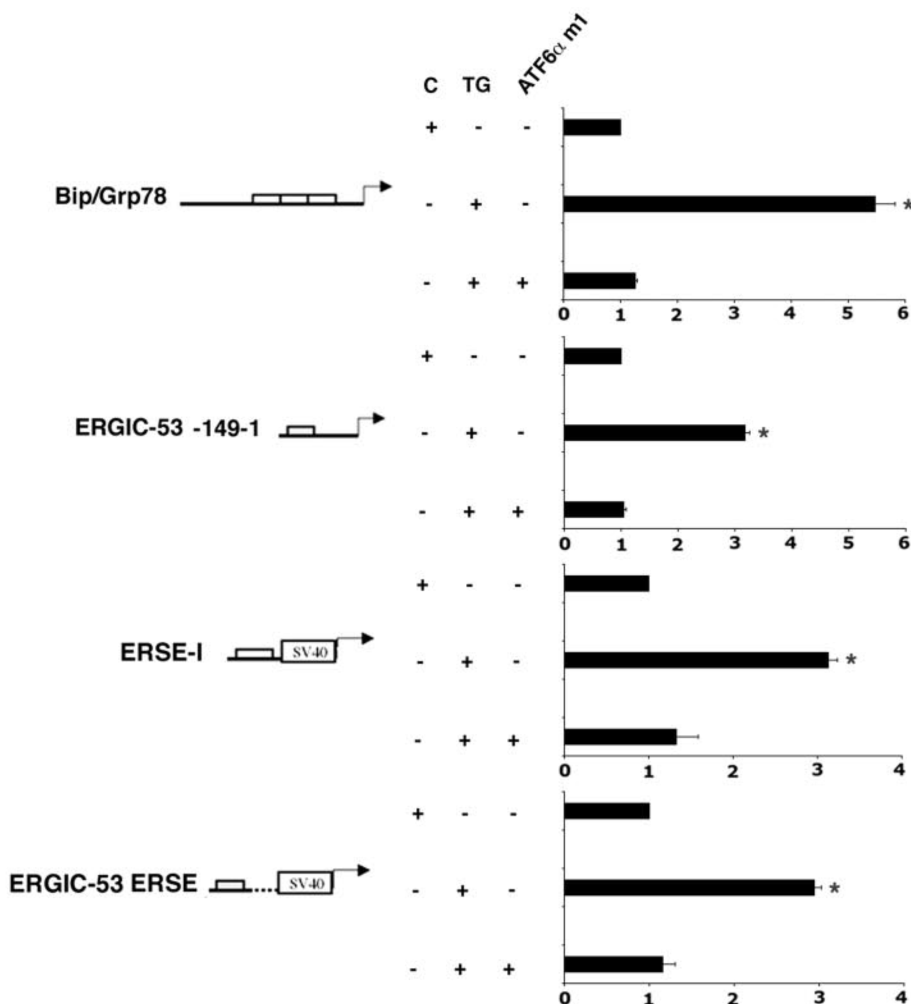

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with those obtained with the *ERGIC-53* deletion mutant −149 to −1 despite the different reporter vector (Fig. 2*B*). mut 4 did not respond to any of the ER stress inducers, thus confirming the regulatory role of the 3′ region (5′-CCATC-3′) of the *ERGIC-53* ERSE.

NFY/CBF and ATF6α-YY1 Interact in Vivo with the ERGIC-53 Promoter—We next asked whether transcription factors involved in the ER stress response could interact with the *ERGIC-53* promoter. Accordingly, we examined DNA-protein complexes from untreated and TG-stressed cells using chromatin immunoprecipitation. The presence of endogenous factors NFY, Sp1, ATF6α, YY1, or XBP1 in the immunoprecipitates was revealed by PCR amplification of a 271-bp fragment of the *ERGIC-53* promoter extending from nucleotide −226 to nucleotide +45, containing a single *ERGIC-53* ERSE (Fig. 6*A*). As a control, we examined the 254-bp fragment of the human *Bip/Grp78* promoter spanning from nucleotide −267 to nucleotide −13, which harbors the three ERSEs (Fig. 6*A*). Untreated cells retained endogenous NFY, YY1, and Sp1 bound on *ERGIC-53*, and NFY binding was more evident in untreated cells on the control *Bip/Grp78* promoter, which contains multiple copies of ERSE (Fig. 6*B*). Consequent to TG-induced ER stress, and on both promoters, NFY binding was more pronounced, whereas Sp1 and YY1 binding was slightly higher than in uninduced cells. In TG-stressed cells, there was endogenous ATF6α binding on the *ERGIC-53* promoter and on the control gene *Bip/Grp78*. In contrast, induction of ER stress raises the interaction of endogenous XBP1 with *Bip/Grp78* but not with the proximal region of the *ERGIC-53* gene (Fig. 6*B*, *bottom*). To verify ATF6α binding to the *ERGIC-53* promoter, we examined DNA-protein complexes from HeLa cells transiently transfected with plasmid vectors expressing two distinct HA-tagged ATF6α nuclear forms (Fig. 6*C*): the active HA-tagged form ATF6(373) and the deletion mutant ATF6(273) that lacked the B-ZIP domain (31). Chromatin immunoprecipitation with anti-HA antibody revealed binding of the ATF6(373) form to the *ERGIC-53* promoter and to the control gene *Bip/Grp78* (Fig. 6*C*). Instead, immunoprecipitation with anti-HA antibody did not reveal binding of the HA-tagged ATF6(273)-deleted form, which confirms recruitment of active ATF6α at the *ERGIC-53* promoter (Fig. 6*C*).

ATF6α but Not XBP1 Stimulates ERGIC-53 ERSE Activity—In transiently transfected HeLa cells, the overexpression of the HA-tagged ATF6α nuclear form was able to stimulate, in the absence of ER stress induction, the activity of the *ERGIC-53* minimal promoter (−149 to −1) by 5.97 ± 0.59-fold and the control plasmid *Grp78/BiP*-driven reporter gene by 15.28 ± 1.35-fold (Fig. 7*A*). To define the sequence required for the ATF6α-dependent transactivation, we compared the effect of the HA-tagged ATF6(373) expression on the wild-type and mutant (mut 1–5) *ERGIC-53* ERSE (Fig. 7*B*) reporter gene induction. Overexpressed ATF6(373) enhanced the *ERGIC-53* ERSE luciferase activity by about 18-fold and enhanced the expression of the ERSE-I control reporter, albeit to a lesser extent (about 12-fold). As we would expect, mutation of the CCAAT box (mut 1) strongly reduced *ERGIC-53* ERSE activity. Nucleotide replacement of the −48 to −35 segment (mut 2–4) inhibited the response to ATF6(373), and the inhibition was greater for mut 2, which confirms the importance of this sequence for ER stress activation of *ERGIC-53* (Fig. 4*B*). We verified further the involvement of ATF6α by analyzing the effect of the overexpression of the ATF6α dominant negative form ATF6-(1–373)m1, which is able to prevent the activity of endogenous ATF6α (25). Expression of the ATF6-(1–373)m1 construct completely inhibited the TG induction of the *ERGIC-53* minimal promoter and *ERGIC-53* ERSE-driven reporter genes; a similar result was observed with the control *Bip/Grp78* and ERSE-I reporter (Fig. 7*C*).

Finally, we tested the requirement of XBP1 for the transcriptional activation of *ERGIC-53* in MEFs derived from wild-type or IRE1α knock-out mice ($IRE1^{-/-}$ MEF) (Fig. 8*A*). TG induction of the *ERGIC-53* promoter and of the *ERGIC-53* ERSE reporter plasmid was similar in wild-type and $IRE1^{-/-}$ MEF cells, which indicates that *ERGIC-53* transcription occurred independently of the IRE1/XBP1 pathway. This conclusion was confirmed by the analysis of *ERGIC-53* mRNA level in response to TG induction in wild-type and in $IRE1^{-/-}$ MEF cells (Fig. 2*C*). Real time RT-PCR assays showed that wild-type and $IRE1^{-/-}$ MEF cells have a similar -fold induction of *ERGIC-53* transcripts in response to TG treatment. Differently, reporter and RT-PCR assays revealed a significant decrease in *Bip/Grp78* and *Grp94* -fold induction upon ER stress, thereby confirming direct involvement of XBP1 in the ERSE-I-mediated transactivation. Thus, our results show that ATF6α requires the −48 to −35 segment of the ERSE to activate the *ERGIC-53* promoter, and, unlike XBP1, it is essential for the transcriptional regulation of *ERGIC-53*.

The ERGIC-53 ERSE Retains Binding Sites for Nuclear Factors NFY/CBF and YY1—The *ERGIC-53* ERSE contained two distinct regulatory regions: a high affinity NFY/CBF binding site located at the 5′-end and a downstream 5′-CCCTGTT-GGCCATC-3′ sequence containing an NFY/CBF-like site in the reverse orientation (5′-GTTGG-3′). To examine the interaction of nuclear proteins with these motifs, we carried

FIGURE 7. **Requirement of ATF6α for full induction of *ERGIC-53* in response to ER stress.** *A*, HeLa cells were co-transfected with either *Bip/Grp78* or *ERGIC-53* reporter plasmids in combination or not with HA-ATF6(373) expression vector and then assayed for luciferase activities. Values reported represent the average ± S.D. of at least three independent experiments performed in duplicate. Statistical analysis was performed using Student's *t* test ($n = 6–8$; *, $p < 0.001$ relative to the mock-transfected control). *B*, effect of cassette mutation on the ATF6α-driven trans-activation of the *ERGIC-53* ERSE. HeLa cells were co-transfected with the pGL3 promoter vector bearing the indicated oligonucleotide sequences and 0.5 μg of the HA-ATF6(373) expression vector. The consensus ERSE-I and its inactive form served as control. Mutated nucleotide sequences are *underlined*. The *histograms* represent the -fold induction calculated as the ratio between the luciferase activity of ATF6(373) and mock-transfected cells. Values are the average ± S.D. of three independent experiments performed in duplicate. Statistical analysis was performed using Student's *t* test ($n = 6$; *, $p < 0.001$; **, $p < 0.005$, respectively). *C*, HeLa cells were transfected with 1 μg of dominant negative ATF6-(1–373)m1 and the indicated reporter vectors, treated or not for 8 h with 300 nM TG, and then assayed for luciferase activities. Values are the average ± S.D. of three independent experiments performed in duplicate; statistical analysis was performed using Student's *t* test ($n = 6$; *, $p < 0.001$ relative to control cells).

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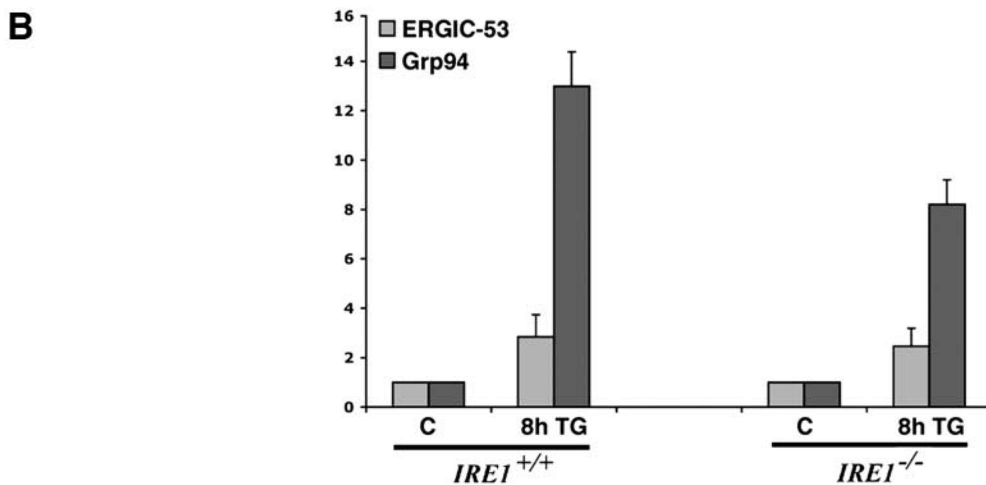

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out mobility shift assays using wild-type or mutated forms of *ERGIC-53* ERSE as probes (Fig. 9*A*). In both uninduced and TG-induced cell extracts (data not shown), there were two distinct ERSE-binding complexes (EBC-I and EBC-II) that specifically interacted with the *ERGIC-53* ERSE (Fig. 9*B* and supplemental materials). Preincubation with anti-NFY/CBF antibody resulted in the supershift of EBC-II in all samples, suggesting that NFY/CBF is the major component of the slower migrating complex (Fig. 9*B*). Preincubation with anti-YY1 antibody resulted in inhibition of the faster migrating complex, suggesting that YY1 is the major component of EBC-I (Fig. 9*C*). The *ERGIC-53* ERSE mut 1, in which the 5′-CCAAT-3′ motif was mutated, retained the EBC-II interaction, which indicates that NFY/CBF could interact with the inner NFY/CBF-like. Mutation of the 5′-CCCTGTTGG-3′ sequence (mut 2 and mut 3) impaired EBC-I activity, which suggests that YY1 interacts with the 5′-CCCTGTTGG-3′ sequence. Furthermore, EBC-II binding to mut 4 (Fig. 9*B*) was severely reduced, thereby confirming that the pentameric 5′-CCATC-3′ motif plays a critical role within the 5′-CCCTGTTGGCCATC-3′ of the ERSE. In conclusion, the results of *in vitro* binding assays strongly support the concept that the *ERGIC-53* ERSE is constituted by the 5′-CCAAT box and two distinct motifs located 9 nt downstream, the 5′-CCCTGTTGG and the CCATC-3′ part, both of which are important for the ERSE induction.

DISCUSSION

In an attempt to identify the transcription mechanisms that control the *ERGIC-53* gene, we have studied the transcription regulation of the *ERGIC-53* promoter. Here we show that a single *cis*-acting element, the *ERGIC-53* ERSE, which has a novel ERSE organization, enhances the UPR-dependent expression of the gene, and favors the formation of a transcriptional complex constituted by ER stress factors.

The Novel ERSE Structure of the ERGIC-53 ERSE—The ERSE found in the *ERGIC-53* promoter region is distinct from all other ERSEs and UPREs so far identified in the promoter regions of UPR-regulated genes (20–25). In the newly identified *ERGIC-53* ERSE, the NFY/CBF CCAAT site, which is a feature of both ERSE-I and -II elements, is functionally coupled to the CCCTGTTGGCCATC ER stress regulatory sequence, located 9 nucleotides downstream from the CCAAT site and equally important for UPR-mediated regulation of the gene.

The CCAAT site of the *ERGIC-53* ERSE constitutively binds NFY/CBF that in all ERSE types previously reported serves as the "foundation" upon which the UPR-induced ER stress factors are assembled (20, 26–30). In our study, replacement of the CCAAT domain impaired the functional activity of the *ERGIC-53* ERSE, which suggests that NFY/CBF exerts a function in the ER stress-induced activation of *ERGIC-53* similar to that exerted in other ERSE types. Interestingly, we showed that the downstream CCCTGTTGGCCATC sequence is equally important for the UPR induction. TESS analysis of the sequence revealed, in the reverse orientation of the DNA sequence, a low affinity binding site for the basal transcription factor NFY/CBF (GTTGG). We found that NFY/CBF could interact with the inner NFY/CBF-like. Interestingly, a double substitution mutant that reconstituted the NFY/CBF consensus binding site (CCAATCG) did not enhance *ERGIC-53* ERSE activity in response to ER stress (Fig. 4*B*, mut 18), which suggests that the NFY/CBF protein is not involved in the UPR regulation of that sequence. The downstream sequence could be divided into two parts: the 5′-end part CCCTGTTGG that is required for the interaction of YY1 and the 3′-end part CCATC that possibly represents the binding site for accessory and as yet unidentified regulatory protein(s).

The ERGIC-53 ERSE Is the Binding Site for Basic Factors NFY/CBF and YY1 and Is Activated by the ATF6 Pathway of the UPR—The results of our ChIP assays showed that *ERGIC-53* is a target gene for nuclear factors commonly involved in UPR-mediated activation of gene expression. Moreover, we found that not only NFY/CBF but also YY1 is recruited by the *ERGIC-53* promoter, and both proteins are immunoprecipitated particularly well in the nuclei of ER-stressed cells. YY1 is a co-activator of the ER stress response in mammalian cells (28–31), and our results suggest that it plays a similar role during UPR-mediated activation of *ERGIC-53*. We also found that SP proteins constitutively interact with the ER stress-responsive region of the *ERGIC-53* gene, presumably by recognizing a high affinity site in the promoter region analyzed. This finding is compatible with the report that nuclear factors of the SP family are essential for the stress-induced response of *Grp78* in which they constitutively bind ERSE sequences (43).

Our experiments show that, differently from the *Grp78* promoter, ER stress-induced XBP1 factor does not enter the ERGIC-53 regulatory region. This result, together with our previous finding that, in response to nitric oxide-induced ER stress, *ERGIC-53* mRNA accumulated independently of XBP1 activation (8), suggested that XBP1 is not required for *ERGIC-53* activation during ER stress. Results of ERGIC-53 expression analyses performed in IRE1 knocked-out cells clearly show that *ERGIC-53* induction does not rely on the IRE1α-XBP1 pathway, suggesting that the UPR modulates *ERGIC-53* expression selectively during the ER stress response.

Earlier evidence showed that induction of *ERGIC-53* during the UPR was dependent on the activation of the ERSE binding factor ATF6α (6). Now we show that ATF6α is

FIGURE 8. **Analysis of *ERGIC-53* gene expression in IRE1α-null MEFs in response to ER stress.** *A*, wild type or IRE1$\alpha^{-/-}$ MEFs cells were transfected with the indicated reporter vector, treated or not for 8 h with 300 nM TG, and then assayed for luciferase activities. The values reported represent the average ± S.D. of at least three independent experiments performed in duplicate. Statistical analysis was performed using Student's *t* test ($n = 6–8$; *, $p < 0.001$; **, $p < 0.005$, respectively, relative to control cells). *B*, real time RT-PCR analysis of *ERGIC-53* and ER chaperone gene *Grp94* mRNA accumulation in response to ER stress. Total RNA fractions obtained from either wild type or IRE1$\alpha^{-/-}$ MEF cells exposed or not for 8 h to 300 nM TG were analyzed by RT-PCR to measure the level of the indicated mRNAs. The values were normalized to c-*Abl* mRNA. The relative -fold induction was calculated as the ratio of treated cells divided by untreated cells according to the $2^{-\Delta\Delta CT}$ method. Each value represents the mean ± S.D. of three independent experiments.

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FIGURE 9. ***In vitro* binding analysis of proteins interacting with *ERGIC-53* ERSE.** *A*, sequence of the oligonucleotides used in the EMSA. Mutated nucleotides are *underlined*. *B*, 5 μg of nuclear extracts obtained from control HeLa cells were incubated with ^{32}P-labeled *ERGIC-53* ERSE oligonucleotide and with either specific or nonspecific unlabeled oligonucleotides, as indicated. EBCs were analyzed by EMSA and revealed by autoradiography. In supershift experiments, binding reactions were incubated 20 min before the addition of the radiolabeled probe with 4 μg of anti-NFY/CBF-A antibody. The positions of EBC are indicated on the *left*. The *asterisk* on the *right* indicates migration of the antibody-supershifted complex. *C*, EMSA of nuclear extracts obtained from untreated HeLa cells incubated with the indicated ^{32}P-labeled *ERGIC-53* ERSE oligonucleotides. In supershift, binding reactions were incubated with 10 μg of anti-YY1 antibody 20 min before the addition of the radiolabeled probe. EBCs are indicated on the *left*.

engaged at the *ERGIC-53* promoter of ER-stressed cells. The nuclear form of ATF6α is actively involved in the transcriptional regulation of *ERGIC-53* and regulates *ERGIC-53* ERSE activity in a sequence-specific manner. These results together with the finding that dominant negative ATF6α exerts an inhibitory effect on UPR induction of *ERGIC-53* conclusively demonstrated that *ERGIC-53* expression is linked to the UPR by the ATF6 pathway.

A Model for the UPR Regulation of the ERGIC-53 Gene—In a model proposed for UPR-mediated activation of *Grp78*, activation of ATF6α by ER stress requires, in addition to NFY/CBF binding to the CCAAT box (26), the presence of the CCACG motif at the 3′-end of the ERSE-I and interaction between the two co-activators YY1 and TFII-I (28, 30). YY1 and ATF6α interact through the b-Zip domain of ATF6α and the region flanking the zinc finger domain of YY1. The integrity of these domains is crucial for activation of the *Bip*/*Grp78* promoter (30, 31). Despite the different organization of the *ERGIC-53* ERSE, this model is probably applicable to *ERGIC-53* transcriptional activation. Fig. 10 illustrates how the transcription factors we identified could converge to form the transcriptional complex required for UPR-mediated control of the gene. We propose that, in response to ER stress, ATF6α is rapidly activated and associates with YY1. The ATF6α-YY1 complex could bind the *ERGIC-53* promoter to the CCCTGTTGG part of the ERSE. The nuclear proteins that form the EBC-I complex in association with ATF6α-YY1 could act as coactivators for NFY/CBF present in the EBC-II complex. In this context, Sp1 proteins could contribute to the formation of the complex by recognizing the high affinity site in the regulatory region.

Chromatin remodeling has been demonstrated to be critical for the ER stress-mediated transcriptional activation of *Bip*/*Grp78* (31). In our

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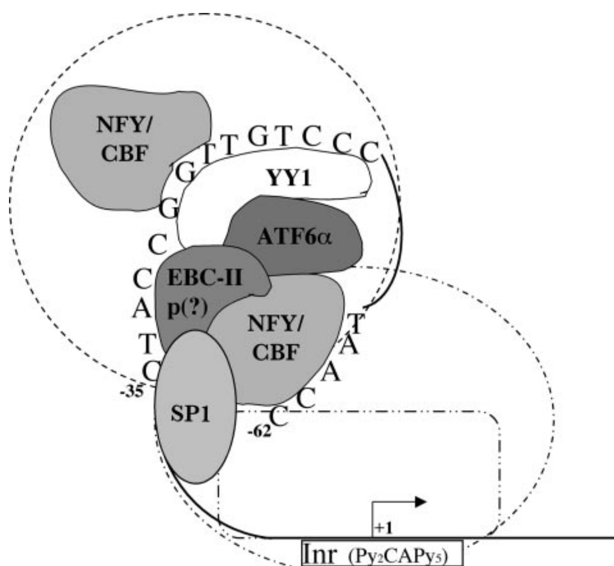

FIGURE 10. **Proposed model for the *ERGIC-53* promoter transactivation in response to ER stress.** In response to ER stress, ATF6α is rapidly activated and becomes with the constitutively expressed YY1 part of a multiprotein complex able to bind to the *ERGIC-53* ERSE (indicated in *boldface type*) and to ensure promoter activation. NFY/CBF and Sp1 are needed as co-activators for ATF6α. Other components (*EBC-II p(?)*) of the transcriptional complex could act as bridging factors between the ERSE and basal transcription machinery (depicted by *dashed shapes*). *Inr* is the initiator sequence required for the transcription of TATA-less promoters.

context, we found that ER stress rapidly stimulated similar modifications of the histone acetylation pattern at both the *ERGIC-53* and *Bip/Grp78* promoter.[4] In particular, we observed a rapid increase of H3 acetylation and decrease of the H4 acetylation on both promoters. Therefore, we believe that the observed modifications of histone acetylation patterns could favor the formation of the transactivation complex at the *ERGIC-53* promoter during UPR. In addition, the recruitment of co-activators that either interact with other transcription factors or modify histones represents a critical event for the achievement of an open chromatin state that has been well described for, among other functions, the regulation of gene expression mediated by YY1 (44, 45), which we show to play a pivotal role in the *ERGIC-53* ERSE complex.

Because the ERSE sequence is highly conserved, *ERGIC-53* could be regulated by the same mechanism in diverse species (Table 1). Our model is consistent with the requirements for conservation of the ER stress response and could explain how the newly identified *ERGIC-53* ERSE is a general sensor for ER stress.

A putative ERSE sequence is present in the promoter of MCFD2, a protein that is functionally related to ERGIC-53. To gain further insight into the role of UPR in the control of genes involved in post-ER functions, it would be interesting to establish whether UPR regulates *MCFD2* transcription by a similar mechanism. A crucial question is the function of ERGIC-53 and MCFD2 during the UPR. We shall address this issue studying the effect of the ER stress on the intracellular trafficking and interaction properties of the two proteins.

[4] M. Renna, S. Bonatti, and P. Remondelli, unpublished results.

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REFERENCES

1. Patil, C., and Walter, P. (2001) *Curr. Opin. Cell Biol.* **13,** 49–55
2. Harding, H. P., Calfon, M., Urano, F., Novoa, I., and Ron, D. (2002) *Annu. Rev. Cell Dev. Biol.* **18,** 575–599
3. Schroder, M., and Kaufman, R. J. (2005) *Annu. Rev. Biochem.* **74,** 739–789
4. Harding, H. P., Zhang, Y., and Ron, D. (1999) *Nature* **397,** 271–274
5. Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000) *Mol. Cell* **6,** 1099–1108
6. Nyfeler, B., Nufer, O., Matsui, T., Mori, K., and Hauri, H. P. (2003) *Biochem. Biophys. Res. Commun.* **304,** 599–604
7. Spatuzza, C., Renna, M., Faraonio, R., Cardinali, G., Martire, G., Bonatti, S., and Remondelli, P. (2004) *J. Biol. Chem.* **279,** 42535–42544
8. Renna, M., Faraonio, R., Bonatti, S., De Stefano, D., Carnuccio, R., Tajana, G., and Remondelli, P. (2006) *Int. J. Biochem. Cell Biol.* **38,** 2040–2048
9. Itin, C., Roche, A. C., Monsigny, M., and Hauri, H. P. (1996) *Mol. Biol. Cell* **7,** 483–493
10. Schweizer, A., Fransen, J. A., Bachi, T., Ginsel, L., and Hauri, H. P. (1988) *J. Cell Biol.* **107,** 1643–1653
11. Itin, C., Schindler, R., and Hauri, H. P. (1995) *J. Cell Biol.* **131,** 57–67
12. Klumperman, J., Schweizer, A., Clausen, H., Tang, B. L., Hong, W., Oorschot, V., and Hauri, H. P. (1998) *J. Cell Sci.* **11,** 3411–3425
13. Hauri, H. P., Appenzeller, C., Kuhn, F., and Nufer, O. (2000) *FEBS Lett.* **476,** 32–37
14. Hauri, H. P., Kappeler, F., Andersson, H., and Appenzeller, C. (2000) *J. Cell Sci.* **113,** 587–596
15. Appenzeller, C., Andersson, H., Kappeler, F., and Hauri, H. P. (1999) *Nat. Cell Biol.* **1,** 330–334
16. Vollenweider, F., Kappeler, F., Itin, C., and Hauri, H. P. (1998) *J. Cell Biol.* **142,** 377–389
17. Moussalli, M., Pipe, S. W., Hauri, H. P., Nichols, W. C., Ginsburg, D., and Kaufman, R. J. (1999) *J. Biol. Chem.* **274,** 32539–32542
18. Zhang, B., Cunningham, M. A., Nichols, W. C., Bernat, J. A., Seligsohn, U., Pipe, S. W., McVey, J. H., Schulte-Overberg, U., de Bosch, N. B., Ruiz-Saez, A., White, G. C., Tuddenham, E. G., Kaufman, R. J., and Ginsburg, D. (2003) *Nat. Genet.* **34,** 220–225
19. Zhang, B., Kaufman, R. J., and Ginsburg, D. (2005) *J. Biol. Chem.* **280,** 25881–25886
20. Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998) *J. Biol. Chem.* **273,** 33741–33749
21. Roy, B., and Lee, A. S. (1999) *Nucleic Acids Res.* **27,** 1437–1443
22. Kokame, K., Kato, H., and Miyata, T. (2001) *J. Biol. Chem.* **276,** 9199–9205
23. Yamamoto, K., Yoshida, H., Kokame, K., Kaufman, R. J., and Mori, K. (2004) *J. Biochem.* (*Tokyo*) **136,** 343–350
24. Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., and Ron, D. (2002) *Nature* **415,** 92–96
25. Wang, Y., Shen, J., Arenzana, N., Tirasophon, W., Kaufman, R. J., and Priwes, R. (2000) *J. Biol. Chem.* **275,** 27013–27020
26. Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. (2000) *Mol. Cell Biol.* **20,** 6755–6767
27. Mantovani, R. (1998) *Nucleic Acids Res.* **26,** 1135–1143
28. Parker, R., Phan, T., Baumeister, P., Roy, B., Cheriyath, V., Roy, A. L., and Lee, A. S. (2001) *Mol. Cell Biol.* **21,** 3220–3233
29. Li, W. W., Hsiung, Y., Zhou, Y., Roy, B., and Lee, A. S. (1997) *Mol. Cell Biol.* **17,** 54–60
30. Li, M., Baumeister, P., Roy, B., Phan, T., Foti, D., Luo, S., and Lee, A. S. (2000) *Mol. Cell Biol.* **20,** 5096–5106
31. Baumeister, P., Luo, S., Skarnes, W. C., Sui, G., Seto, E., Shi, Y., and Lee, A. S. (2005) *Mol. Cell Biol.* **25,** 4529–4540
32. Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999) *Mol. Biol.*

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Cell **10,** 3787–3799
33. Hori, O., Ichinoda, F., Yamaguchi, A., Tamatani, T., Taniguchi, M., Koyama, Y., Katayama, T., Tohyama, M., Stern, D. M., Ozawa, K., Kitao, Y., and Ogawa, S. (2004) *Genes Cells* **9,** 457–469
34. Schulze, A., Standera, S., Buerger, E., Kikkert, M., Van Voorden, S., Wiertz, E., Koning, F., Kloetzel, P. M., and Seeger, M. (2005) *J. Mol. Biol.* **354,** 1021–1027
35. Lee, A. H., Iwakoshi, N. N., and Glimcher, L. H. (2003) *Mol. Cell Biol.* **23,** 7448–7459
36. Yoshida, H., Matsui, T., Hosokawa, N., Kaufman, R. J., Nagata, K., and Mori, K. (2003) *Dev. Cell* **4,** 265–271
37. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001) *Cell* **107,** 881–891
38. Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K., and Kaufman, R. J. (2002) *Genes Dev.* **16,** 452–466
39. Livak, K. J., and Schmittgen, T. D. (2001) *Methods* (*Orlando*) **25,** 402–408
40. Chao, C. C., and Lin-Chao, S. (1992) *Nucleic Acids Res.* **20,** 6481–6485
41. Luo, S., and Lee, A. S. (2002) *Biochem. J.* **366,** 787–795
42. Tatusova, T. A., and Madden, T. L. (1999) *FEMS Microbiol. Lett.* **174,** 247–250
43. Abdelrahim, M., Liu, S., and Safe, S. (2005) *J. Biol. Chem.* **280,** 16508–16513
44. Thomas, M. J., and Seto, E. (1999) *Gene* (*Amst.*) **236,** 197–208
45. Yao, Y. L., Yang, W. M., and Seto, E. (2001) *Mol. Cell Biol.* **21,** 5979–5991

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