Transcriptional Activation by an URE4-like Sequence in the *EhPgp1* Gene Core Promoter

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**Abstract**

*EhPgp1* is one of the multidrug resistance genes expressed in drug resistant trophozoites from *Entamoeba histolytica*. Previous studies in our laboratory have demonstrated that two C/EBP sites participate in the transcriptional activation of this gene. However there is other relevant region that also governs the regulation of *EhPgp1* expression in clone C2. In this report we provide evidence that transcription of the *EhPgp1* gene is at least partly regulated by the cis-acting R9 repeated sequences and EhEBP1 protein. Structural analysis of the region from -234 to -197 bp shows the presence of two repeated sequences of 9 bp [R9(1) and R9(2)] located at -226 to -203 bp. Deletions and mutations analysis of the R9 motifs significantly reduced promoter activity in trophozoites from clone C2. EMSA experiments revealed specific binding of nuclear proteins from *E. histolytica* to the R9 sequence. While competition assays showed that the presence of more than one R9 sequence is necessary for a strong DNA-protein interaction. Moreover, Western blot experiments with partially-purified proteins interacting with the R9 motif and antibodies against EhEBP1, recognized a 28 kDa protein. Interestingly, this antibody in supershift assays prevented the DNA-protein interactions formation, of the R9 sequences and nuclear proteins from amoeba, indicating that one of the proteins that interact with the R9 element is an EhEBP1-like one. In conclusion, we demonstrate that R9 motifs are recognized by an EhEBP1 protein and activate the *EhPgp1* gene expression.

**Keywords:** *EhPgp1* gene; MDR; *E. Histolytica*; Cis-acting element

**Introduction**

*Entamoeba histolytica* is the protozoan responsible for human amoebiasis, it kills 70,000 humans each year around the world and is considered fourth in mortality after malaria, Chagas disease and leishmaniasis [1]. The parasite presents the multidrug resistance phenotype (MDR), due to the expression of a surface P-glycoprotein that transports the drug outside the cell, avoiding its therapeutic effects. In amoeba, there are four genes that code for Pgp proteins, *EhPgp1*, *EhPgp2*, *EhPgp5*, and *EhPgp6*. The *EhPgp1* and *EhPgp6* genes are constitutively expressed in drug-resistant mutants (clone C2). The *EhPgp5* gene is induced by the presence of the drug while the *EhPgp2* gene transcript is not detected [2,3]. Differential *EhPgp* genes expression suggests a specific control mechanism of the MDR phenotype in this parasite.

Cloning and transcriptional characterization of the *EhPgp1* and *EhPgp5* gene promoters from drug-sensitive and drug-resistant trophozoites showed that these were 99.7% identical, however differential complexes were formed when nuclear extracts from sensitive and resistant clones were used. These results suggest that specific transcriptional regulators may be involved in the expression of the *EhPgp* genes in drug-resistant cells [4,5]. Until now only some cis-regulatory elements [6-11], and very few transcription factors have been identified and characterized in gene expression of this parasite [9,12-16].

Analysis of the core promoter of 37 protein encoding genes of *E. histolytica* revealed three conserved regions: i) the putative TATA element located approximately at -30 (GTATTTAAA(G/C)), ii) the GAAC sequence, located between the TATA box and Inr sequence, and iii) the putative Inr region overlapping the transcription initiation site (AAAAATTCA) [7]. Five major upstream regulatory elements (UREs) are present in the *hgl5* gene promoter, four of them act as positive regulatory elements: URE1, URE2, URE4, URE5, whereas the URE3 motif performs a negative regulatory activity [7]. However URE3 function as a positive regulatory element in the ferredoxin (fdx1) promoter region [17]. Additionally URE1-like sequence was reported as a cis-acting element in the *EhRabB* gene promoter [18], and recently was identified as the protein that specifically binds to the URE1 sequence (EhURE1BP), which contains five SNase domains and one Tudor motif [19].

Gilchrist et al. [14] identified the protein that binds to the URE3 element (URE3-BP) and recently demonstrated that several genes of *E. histolytica* are regulated by URE3-BP. The URE3 motif was found in 54% and 39% of promoter regions of the genes modulated by URE3-BP in vitro and in vivo, respectively [20,21]. On the other hand, Schaeffer et al. [13] reported that the URE4 sequence, composed of two 9 bp repeats, functions as an enhancer in the *hgl5* gene and it interacts with two URE4 enhancer-binding proteins of 18 and 28 kDa called EhEBP1 and EhEBP2 [22].

In the *EhPgp1* gene promoter, we identified by homology to consensus sequences reported so far, C/EBP, HOX, GATA-1 and OCT regulatory elements. Some specific oligonucleotides for these elements were able to compete against the DNA promoter in electrophoretic mobility shift assays [4]. Specific deletions of C/EBP elements, demonstrated that two CCAAT/enhancer binding protein sites (-54 to -43 bp and -198 to -186 bp), were cis-acting elements of *EhPgp1* gene expression in both drug-sensitive and resistant trophozoites [9]. In addition, two nuclear proteins of 25 and 65 kDa that were specifically binding to C/EBP probe, share epitopes with the human C/EBP tran...
scription factor. However functional activities of the EhPgp1 promoter demonstrated that other sequences within -259 to -206 bp besides C/EBP are crucial for promoter activity [9]. Previously, we demonstrated that in the -234 to -197 bp region, putative cis-activator sequences for GATA-1, GAL 4, NIT-2 and C/EBP transcription factors are present [23]. Here, we report the presence of a cis-acting element located at -226 to -218 bp of the EhPgp1 gene promoter and the putative transcription factor with which it interacts.

Materials and Methods

Culture of E. histolytica trophozoites

Trophozoites of E. histolytica clone C2 (emetine-resistant) were axenically cultured in TYI-S-33 medium [24].

Search of putative consensus elements by In silico analysis

To find consensus sequences for transcription factors in the -234 to -197bp EhPgp1 promoter region, we used the transcription factors data base TF Search version 1.3 (http://www.cbrc.jp/research/db/TFSEARCH.html).

Plasmid construction of EhPgp1 promoter

Constructions containing different deletions and mutations of -234 to -197 bp EhPgp1 promoter region were done. We amplified four different regions: I) from -234 to +24, II) from -222 to +24, III) from -211 to +24 and, IV) from -207 to +24bp, using the four different regions: I) from -234 to +24, II) from -222 to +24, III) from -211 to +24 and, IV) from -207 to +24bp, using the sense Pgp1F234 (5’-GCTCTAGATATCTGATATTCTAGTTATCT-3’), Pgp1F222 (5’-GCTCTAGATATCTGATATTCTAGTTATCT-3’), Pgp1F211 (5’-GCTCTAGAATATCTGATATTCTAGTTATCTG-3’) and Pgp1F207 (5’-GCTCTAGATATCTGATATTCTAGTTATCTG-3’) oligonucleotides respectively, and the antisense Pgp1AS-33 (5’-CCACGTAAAACTCTACCTGCTGTTTTTGGG-3’). We generated fragments containing the mutations described below and marked in bold letters, and used the sense R7(3)m4 (5’-GCTCTAGGGGATGTTAATAAGAT-3’), R7(3)m5 (5’-GCTCTAGGAGGGATGTTAATAAGAT-3’), R9(2)m5 (5’-GCTCTAGATATCTGATATTCTAGTTATCTG-3’), and R9(2)m6 (5’-GCTCTAGATATCTGATATTCTAGTTATCTG-3’), oligonucleotides, and the antisense Pgp1AS-33 oligonucleotide. We used 50 ng of the plasmid containing the core promoter (p268Pgp1) as template and 2U of Deep Vent Polimerase (New England Biolabs). The PCR products were cloned into XbaI and HindIII restriction sites of the template and 2U of Deep Vent Polimerase (New England Biolabs). The specific proteins (with a lower affinity constant) did not bind. The specific DNA-binding proteins were eluted from the immobilized particle resulting in long concatamers with hundreds of specific binding sites. Oligonucleotides undergo a self-priming reaction during the PCR, resulting in long concatamers with hundreds of specific binding sites. Concatameric DNA was bound to magnetic particles, then 50 µg of E. histolytica NE was added. The specific proteins were captured by the concatameric oligonucleotide with a high affinity constant, while non-specific proteins (with a lower affinity constant) did not bind. The specific DNA-binding proteins were eluted from the immobilized particle with a high ionic strength buffer. After removal of the elution buffer by filtration with centricin YM-10 (Millipore), the proteins were transferred to nitrocellulose membranes for Western blot assays. The eluted proteins were also evaluated by supershift assays.

DNA binding protein purification

E. histolytica nuclear proteins that bind to the R9 region were partially purified using a DNA-binding protein purification kit (Roche). The Pgp1-226/218R3 double stranded oligonucleotide was used as DNA probe to obtain concatameric DNA using self primer PCR. These oligonucleotides undergo a self-priming reaction during the PCR, resulting in long concatamers with hundreds of specific binding sites. Concatameric DNA was bound to magnetic particles, then 50 µg of E. histolytica NE was added. The specific proteins were captured by the concatameric oligonucleotide with a high affinity constant, while non-specific proteins (with a lower affinity constant) did not bind. The specific DNA-binding proteins were eluted from the immobilized particle with a high ionic strength buffer. After removal of the elution buffer by filtration with centricin YM-10 (Millipore), the proteins were transferred to nitrocellulose membranes for Western blot assays. The eluted proteins were also evaluated by supershift assays.

Western blot assays

Partially purified proteins and NE were separated on a 12% polyacrylamide gel, transferred to nitrocellulose membranes, and immunoblotted under standard conditions. Membranes were blocked with 4% non fat milk in PBS pH 7.4 /Tween 0.05%, for 2-3 h at room temperature, and then incubated with mouse anti-EhEBP1 antibody (1:600) (kindly supplied by Dr. Carol A. Gilchrist) for 1 h at 37°C. As control, we used the rabbit polyclonal antibody against the human C/EBPβ (Santa Cruz Biotechnology) (1:500). Immunoreactivity was detected.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed as described [4]. Briefly, we used a double stranded Pgp1-226/218R3 oligonucleotide containing three copies of the R9(2) region, from -226 to -218 bp, marked in bold face (5’-AAAAATGTTATCTGAAAAATGTTATCTGAAAAATGTTGTTATCTGAAAAATGTTGTTATCTGAAAAATGTT-3’). Oligonucleotides were [γ-32P]-ATP (3000 mCi/mmol) labeled using T4 polynucleotide kinase (Invitrogen). Binding reactions contained 1 ng of radiolabel probe, 20 µg of NE, 1 µg of poly dI-C (Amersham Pharmacia Biotech) and DNA-protein binding buffer. Competition assays were carried out using a 150 fold molar excess of the same unlabeled double stranded oligonucleotide or poly dI-C. Mixtures were incubated 10 min on ice. The complexes were separated by electrophoresis on 6% non-denaturing poly-acrylamide gels in 0.5 X TBE, for 2.5 h at 120 V. Gels were dried and subjected to Phosphor Image analysis (Bio-Rad). To determine the interaction of nuclear proteins with the sequence R9, we used also as a specific competitors three additional oligonucleotides, the Pgp1-226/218R2 containing two copies of these sequence (5’-AAAAATGTTATCTGAAAAATGTTATCTGAAAAATGTT-3’), the Pgp1-226/218R1 containing only one copy (5’-AAAAATGTTATCTGAAAAATGTT-3’), and the Pgp1-234/197 oligonucleotides containing the wild type EhPgp1 promoter sequence from -234 to -197 bp (5’-TATCTGATAAAAATAGTTATCTGATTTAATCTGAAATGTT-3’).

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by a chromogenic method using anti-mouse and anti-rabbit peroxidase labeled secondary antibodies, respectively (Zymed laboratories) (1:3000) and revealed with H$_2$O$_2$ and 4-chloro-1-Naphtol.

**Supershift assays**

Supershift assays were performed as the EMSA described before, using the antibodies in the reaction mix. Briefly, we used as radio labeled probe 1 ng of the Pgp1-226/218R3 double stranded oligonucleotide, 20 µg of NE, mouse anti-EhEBP1, 1 µg of poly d(T-C) and DNA-protein binding buffer. As negative controls, anti-C/EBPβ human antibody was used (Santa Cruz Biotechnology).

**Results**

**Identification of consensus sequences in the region from -234 to -197 bp**

The structural analysis of the region from -234 to -197 bp in the EhPgp1 gene core promoter showed three repeated sequences of 7 bp each, located at the positions -203 to -197; -218 to -212; and -234 to -228 bp (Figure 1), we have named these sequences R7(1), R7(2), and R7(3), respectively. Also, we identified two repeats of 9 bp each located at the positions -203 to -197; -218 to -212; and -234 to -228 bp (Figure 1), we have named these sequences R7(1), R7(2), and R7(3), respectively. In silico analysis of the -234 to -197 bp region was performed to identify potential nuclear factor binding sites. Interestingly, in the R7 sequences we detected consensus sequences for GAL4, GATA-1, C/EBPβ and NIT-2 transcription factors, as was previously reported [23]. We also identified two new consensus sequences for the GATA-2 and NF-GMß transcription factors. Additionally we localized three consensus sequences for C/EBPβ and two sequences for EhEBP1 and EhEBP-2 transcription factors that overlap with the R9 motifs (Figure 1).

**The distal region of the EhPgp1 promoter, between -234 to -197 bp contains an activator sequence**

Using structural and in silico analysis of the promoter, we performed a series of deletions on the -234 to -207 bp promoter to locate cis-elements that could drive the EhPgp1 gene expression. Four different plasmids (p258Pgp1, p246Pgp1, p235Pgp1, and p231Pgp1) carrying -234 to +24, -222 to +24, -211 to +24 and -207 to +24 bp sequence of the E. histolytica EhPgp1 gene core promoter were constructed and transfected into C2 trophozoites. CAT activities were measured and compared to the p268Pgp1 plasmid (positive control). Results showed a marked reduction in CAT activity (58%) after truncation of -234 to -222 bp (p246Pgp1 plasmid), suggesting that the R7(3) and R9(2) sequences are required for the EhPgp1 gene expression (Figure 2).

As deletions progress toward the 3'-end of the promoter (p235Pgp1 and p231Pgp1 plasmids), in which almost all the repeated regions were eliminated, a decrease of 87 CAT activities were observed, with both constructions (Figure 2). These results provide evidence that the R7(3) and the R9(2) repeated sequences are involved in the EhPgp1 transcriptional activation, but also showed the presence of another positive regulatory sequence, between the position -218 to -211 bp that correspond to the R7(2) sequence (Figure 2).

Promoter activity comparison of p258Pgp1 (-234 to +24 bp) and p268Pgp1 (-266 to +24 bp) demonstrated that 5' deletion up to -234 bp increase the promoter activity in 27%, suggesting that the region from -259 to -234 bp could contain negative cis-regulatory elements.

**A 9 bp repeated sequence is critical for driving EhPgp1 gene expression**

To determine if R7(3) or R9(2) or both repeated sequences produced the major effects on promoter activity, we performed point mutations into the R7(3) and R9(2) core sequences. Mutations of one or two bases on different positions of the repeated sequences did not significantly modify the CAT reporter gene activity (data not shown). Thus, we carried out constructions containing more than three point mutations of the R7(3) and R9(2) repeats in different plasmids (pR7-2 and pR9-2) (Figure 3). These results demonstrated that the R7(3) and R9(2) repeated sequences are required for driving EhPgp1 gene expression.
R9 sequences DNA-protein interactions

Based on our observations that R9(2) could potentially up-regulate the transcriptional activation of the EhPgp1 promoter, we investigated the ability of this repeated sequence to bind nuclear proteins from E. histolytica. Moreover, R9 is present two times in this region, we also analyzed the relevance of the presence of one, two or three repeated sequences in the DNA-protein complexes formation. To perform these, we generated a set of double stranded oligonucleotides, the Pgp1-226/218R2 and the Pgp1-226/218R1, containing three, two, and one copy of the R9 repeated respectively (Figure 4, lanes 5 and 6). To further characterize if the EhPgp1 promoter wild type sequence (-234 to -197 bp) form the same DNA-protein complexes observed with Pgp1-226/218R3, we added as specific competitor the Pgp1-234/197 double stranded oligonucleotide. Interestingly, all the complexes competed. Together these results demonstrate that the R9 repeated could serve as a recognition sequence for a DNA binding protein in the parasite extract.

An EhEBP1 recognized the R9 repeated sequences

As was observed before in Figure 1, the sequences R9 contain almost the complete EhEBP1 recognition sequence (URE4). To identify whether this protein was a component of the gel shift complexes of R9 DNA with NE from the trophozoites, we partially-purified the proteins interacting with it by DNA affinity chromatography.

Thus, we performed a Western blot assay using NE from trophozoites, the partially-purified fraction and the antibody against the EhEBP1 protein (kindly supplied by Dr. Carol A. Gilchrist). The results revealed the presence of a specific band of 28 kDa in NE and in the protein fraction (Figure 5A, lanes 1 and 2) indicating that one of the proteins that bind to R9 is an EhEBP1-like protein. In contrast, unrelated antibodies against to human C/EBPβ did not produce any detection (Figure 5A, lane 3).

To confirm the observation that R9 repeated sequence is recognized by an EhEBP1, we performed supershift assay using anti-EhEBP1 antibody. The results did not show a supershifted band, but caused the

complexes formation, we performed competition assays using the Pgp1-226/218R2 and the Pgp1-226/218R1 double stranded oligonucleotides, both completely competed the formation of the complex a and produced the formation of a new complex with a minor electrophoretic mobility (complex d) (Figure 4, lanes 5 and 6). While the intensity of the complex b was reduced by 52% when two R9 repeats are present and a reduction of only 4% was detected when only one repeat was used in the competitor (Figure 4, lanes 5 and 6). Similarly, the intensity of complex c was reduced by 71 and 10% with two and one R9 motifs respectively (Figure 4, lanes 5 and 6). To further characterize if the EhPgp1 promoter wild type sequence (-234 to -197 bp) form the same DNA-protein complexes observed with Pgp1-226/218R3, we added as specific competitor the Pgp1-234/197 double stranded oligonucleotide.

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sequences did not produce any CAT activity modification, indicating that the DNA-protein interactions were not modified by these point changes. Four or more mutations into R7(3) did not affect promoter activity, suggesting that R7(3) is not important for the EhPgp1 gene expression; however, we could not ignore the possible participation of the R7(2) and R7(1) sequences in the transcriptional regulation of the EhPgp1 gene. On the other hand, we found that the R9(2) repeat was important for promoter activity, because its deletion or mutations in three bases at the 3' side (AAAAAAATG) at five mutations at the middle of the sequence (TTTCTAGT) or eight mutations of the nine bases (TTTCTAATG) produced a 70% reduction in CAT activity. These results clearly demonstrate that the R9 repeated sequences are necessary for the EhPgp1 gene expression. Interestingly, similar results were observed when the URE4 sequence was identified and characterized in the E. histolytica hgl5 promoter. Four mutations of the middle residues (AATCTAGAA) or in the 3' side (AAAAAAATG) within the upstream repeat or mutations into the two repeated sequences produced an 85% and 93% reduction in luciferase activity in the last two conditions, respectively [22]. Additionally, they found that the upstream repeat is more relevant for the promoter activity than the downstream one, because mutations into the upstream repeat diminish 85% luciferase reporter gene of wild type levels, while mutations into the downstream repeat only decreased 39% reporter gene. They suggested that the downstream repeat may play a role of supporting binding of factors to the upstream repeat, as evidenced by the fact that separating the repeats by seven base pairs decreased reporter gene activity to 22% [22]. These results are consistent with our findings, because the deletion of the R9(2) repeat drastically diminish CAT reporter gene activity (58%), while the elimination of both R9 repeats produced an additional activity reduction of 29%. The existence of a synergistic and accumulative R9 effect involved in the EhPgp1 gene control may be possible.

Interestingly, the appearance of R9 repeat sequences in URE4 motif is not only important for their functional role, but also by the times

**Discussion**

In this study, we have identified a cis-acting element that controls the EhPgp1 gene expression in drug resistant trophozoites (clone C2) of *E. histolytica*. Since EhPgp1 gene is implicated in the multidrug resistance of this parasite, the study of its transcriptional control in the clone C2 trophozoites provide an excellent *in vitro* system for studying molecular basis of EhPgp1 gene regulation. Our previous work has defined that transcriptional regulation of the EhPgp1 gene promoter depends on two CCAAT/enhancer binding sites (-54 to -43 and -198 to -186 bp) and other motifs present in the 53 bp upstream of the C/EBPβ site [9]. Moreover into the 53 bp of this promoter we delimited a functional region of 38 bp (-234 to -196 bp) that interact with nuclear proteins from *E. histolytica* and by *in silico* analysis showed the presence of GATA-2 and NF-GMb sequences as well as the GAL4, NIT2, GATA-1, and C/EBP binding sites [23]. However, although we identified several consensus sequences in this region, in the present work two interesting types of repeat sequences were also located. One of them are the R7 repeats which are present three times (-203 to -197, -218 to -212 and -228 to -234 bp) and the others are the R9 repeats located in two positions (-203 to -211 and -218 to -226 bp), at the EhPgp1 promoter.

Deletions of the EhPgp1 region (-234 to -197 bp) showed that the R7(3) and/or R9(2) repeated sequences could be necessary for the EhPgp1 gene expression. One or two point mutations into these

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**Figure 5:** EhEBP1 binds to R9 motifs. (A) Western blot analysis of the partially purified R9(2) binding proteins. Lane 1, NE; lanes 2 and 3, partially purified fraction. Polypeptides were revealed using anti-C/EBPβ (lanes 1 and 2), and anti-C/EBPβ antibodies (lane 3). Arrowhead, polypeptides detected by the anti-C/EBP1 antibody. (B) Supershift assay show binding of the EhEBP1 protein to labeled Pgp1-226/218R3 that specifically competed with an anti-EhEBP1 antibody (lane 3), but no by another heterologous antibodies against C/EBPβ transcription factor, (lane 4). Arrowhead, specific DNA-protein complex. (C) Comparative analysis of the URE4 sequences and the R9 motifs. Graphic representation of the URE4 and R9 nucleic acid sequences of the hgl5 and EhPgp1 gene promoters. Boxes sequences indicate the URE4 and R9 motifs and bold letters marked the differential bases in each sequence. Bases between each R9 and URE4 motifs are underlined.

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**Figure 6:** A model for coordinated transcriptional regulation of the EhPgp1 gene. This model represents our understanding of the EhPgp1 gene expression in the drug resistant clone C2 from *E. histolytica*. Our data demonstrate that the DNA sequences contained within the -226 to +24 bp of the EhPgp1 gene promoter are sufficient to direct EhPgp1 gene expression. We speculate that the C/EBP sequences are recognized by a C/EBP-like transcription factor and the R9(2) and may be the R9(1) have been recognized by an ENBP1 protein. We do not know yet the order in which the proteins specifically recognize each sequence, neither how these proteins interact with the basal machinery, but we go on with the study of the EhPgp1 gene control. TPC, transcriptional preinitiation complex. Arrow, transcription initiation site.
that are present in the EhPgp1 promoter and by its sequence. Each R9 repeat contains 9 bp (AAAAATGTT) and is separated from the other by 6 bp. These elements present a similar arrangement to the URE4 motif identified in the *E. histolytica* Gal/GalNac lectin heavy subunit hgl5 gene promoter [22]. The URE4 sequence is also composed of two 9 bp repeats (AAAAATGAA) but separated by only 3 bp. Two main differences between URE4 and R9 sequences are the last two bases in the sequences (AA/TT) and the distance between them, nevertheless they are very similar, suggesting that the R9 repeats form an URE4 element (Figure 5C).

Moreover, our DNA-protein interaction assays strongly suggest that R9 motifs have been specifically recognized by nuclear proteins from amoeba and that these proteins required at least the presence of two R9 sequences. Thus because in competition assays the DNA-protein complexes disappeared or their intensity was strongly diminished (more than 70%) when three or two R9 motifs were used as competitors. Whereas one copy of this motif did not modify the DNA-protein complexes formation except the complex a, indicating that one R9 is able to interact with amoeba proteins. However, this may not happen with the same affinity and may diminish the stability of the DNA-protein interaction. In addition we observed the formation of a new low mobility complex showing that DNA-protein interactions with one, two or three R9 motifs generate different mobility complexes. Our results provide evidence that probably, the transcription factors bind in an independent fashion on each R9 sequence or at the same time to allow protein-protein interactions for the formation of a stable dimeric complex. A similar point of view was suggested by Shaeman et al. [13] because they observed a decrease in the hgl5 promoter activity when they modified the spacing between the URE4 repeats. In other systems this kind of interactions has been also reported like the protein binding to the retinoic acid response element or the participation of the C/EBP between others have been reported to act both as a positive or negative regulator of transcription depending on promoter and cell type [28-30]. A similar role seems to be occurring with the EhEBP1 in the amoeba; however, more in depth investigation will be required to know more about this protein and its functional role in the transcriptional control of the EhPgp1 and other genes.

Taking these results together and in concordance with our previous reports [9,23], we proposed a model to address molecular insights into regulation of the EhPgp1 gene. In this model, we showed that transcriptional control of the EhPgp1 gene is coordinated by C/EBP1, C/EBPII and R9 motifs that have been recognized by C/EBP-like and EhEBP1 transcription factors respectively. Then, these complexes may interact with basal machinery of transcription and activate the EhPgp1 gene (Figure 6). However, the chronological events that drive the binding of each transcription factor and how these interact to enhance the EhPgp1 transcriptional activation, poses an open question to be solved.

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