Functional characterization of the cis-regulatory elements of the rat ribophorin I gene

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ABSTRACT

The ribophorin I gene encodes a rough endoplasmic reticulum (RER) specific membrane protein which is a subunit of the oligosaccharyltransferase. To establish the functional activity of its promoter region we have performed transient gene transcription experiments employing plasmid constructs that contain 5' flanking regions of the ribophorin I gene cloned upstream of the CAT reporter gene. Among the restriction fragments obtained from the 1.3-kilobase 5' flanking region, a proximal fragment (−42 to +24) containing two GC-rich elements was required for basic promoter activity, while a fragment (−364 to +24) encoding an additional GC-box and an octamer like motif at −233 conferred the maximal promoter activity. In order to investigate the functionality of an octamer-like sequence co-transfection experiments were performed with Oct-2 cDNA and the CAT reporter gene containing the ribophorin I fragment (−364 to +24). A 3–4-fold increase in the transcriptional activity was observed with this construct. In addition, gel shift experiments showed Oct-2 binding to this construct. These results indicate that Oct-2 is most likely involved in the regulation of the ribophorin I gene transcription. We suggest that the GC-rich elements are necessary for constitutive ribophorin I expression while octamer motif binding proteins function synergistically with the GC-rich element binding proteins to increase the expression of the ribophorin I gene during the proliferation of RER.

INTRODUCTION

Ribophorins I and II (RI and RII) have been previously identified as membrane proteins specific to the rough endoplasmic reticulum (RER) (1,2). Both ribophorins are rather stable (3) type I transmembrane glycoproteins (4,5) which are expressed in about equimolar amounts in the rough endoplasmic reticulum (6).

Together with a polypeptide of 48 kD (OST48) they form a heterotrimeric complex that carries the oligosaccharyl transferase activity (7) that affects certain asparagine residues in the nascent polypeptide chains made on membrane bound polysomes. In attempts to understand the domain structure of RI the intron, exon organization of the corresponding rat gene was determined (8). It was found that the rat RI gene contains 10 exons, seven of which encode the luminal domain of the polypeptide, exon 8 encodes the transmembrane domain and small portions of the flanking luminal and cytoplasmic domains, while exons 9 and 10 encode the remainder of the cytoplasmic domain. The latter includes the 3'-untranslated portion of the mRNA. The sequence of a 1.3 kb region upstream of the cap site was determined and found to contain three GC-rich, potential Sp1-binding sites beginning at −13, −24 and −91 base pairs (bp), two octamer-like sequences at −233 and −1250 bp and a CAAT-like box at −42 bp (Fig. 1). Furthermore, there is no typical TATA box, and multiple, closely spaced transcription start sites have been identified (8). GC-rich regions and the absence of a TATA box are characteristic of promoters of many other housekeeping genes (9). In order to further characterize and determine whether these putative regulatory elements are indeed functional several 5' end deletions at the RI gene were made, these fragments were then fused with the chloramphenicol acetyl transferase (CAT) reporter gene and the RI-CAT chimeras were tested in a functional assay in different cell lines.

Here we report on the functional analysis of RI gene promoter elements and show that the proximal GC boxes are necessary for the basic promoter activity. The octamer motif binding protein Oct-2, which is a POU homeodomain protein involved in transcriptional regulation of several genes (reviewed by Herr, 1992), increased the transcriptional activity of this promoter. The maximal promoter activity of RI gene appears to be dependent on the presence of both GC-boxes and octamer motif elements. These results for the first time suggest a role for octamer motif binding proteins in the regulation of genes encoding RER membrane proteins.

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TAAAAATTGAACATAATATTAGGGGAGAGACTTGAATCTTTGGAAATTTCCGGTTTTCTTTAAACATTTT - 331
 GCCCACTTAGTATTATCATATCAATGTAGAGAAGGCAAACTTATTTCTTAGACTAAGCTGTTCTTGAGCGG - 261
 AGAGGCCATTTTTTTTTTTTTTTTTTTT TTTTGCAT AAGCATGAAGGAAACAGGAGATAAGGAAGGACGAC - 191
 TACCACAGAAATCACTGGTGAGTCTAGCTAATAAGCGCTCAAAGACG TGTGCTGCGCTTTAAGATTTTT - 121
 CAATCAGGCGCCCATCGCGTTGCGCTTG GAGACGGGGT CCTTCCTGACCGGCGCCAGCCATCTTGTGGT - 51
 GCTGTGG/CAATCAACGGTTGCGCC GTGCGGGGGG GGCGGGGG GCCTGTGGTGGCGCTCAGCTCGC - 20
 GGTCTATGGAGGCGCCGATCGTCTTGCTGCTGCTATGGCTCGCCTTGCCGACGCTGGCAGCGCGCTC - 90
 TTGGAGGCTCCGCGCTGGTCAACGAGG

Figure 1. Nucleotide sequence of a portion of the 5' flanking DNA of the Rat RI gene. The protein coding sequence beginning at the initiator ATG (double underlined) is underscored with a dashed line. The six transcription start sites are indicated by downward arrows. The three potential Sp1-binding sites are framed by solid rectangular boxes. The potential CAAT box is shown in a broken ellipse and the octamer motif is shown in a broken rectangle. The numbers above the single vertical lines indicate the nucleotide at the 5' end and the number above double vertical lines represent the nucleotide at the 3' end of the constructs used in this study.

MATERIALS AND METHODS

RI-CAT plasmid constructs

The 1.3 kb *Pst*I, *Hinc*II fragment of the RI gene, which contains the entire 5' flanking region so far sequenced (8) and includes about 100 bp of the coding region, was subcloned into pUC18, cut with *Pst*I and subjected to *Bal*31 digestion to obtain several 5' end deletion fragments. The ends were filled in with Klenow and ligated to *Hind*III linkers. The fragments were released from pUC18 by *Hinc*II digestion and ligated with *Bam*HI linkers. After digestion with *Hind*III and *Bam*HI these fragments were subcloned into the *Hind*III and *Bam*HI cut pBLCAT3 vector (10). These constructs still contain 100 bp of coding sequence, which includes the signal sequence. In order to remove these 100 bp of the coding sequence, the 1.3 kb fragment contained in the pBLCAT3 vector was digested with *Esp*I and *Bam*HI. The larger fragment that lacks about 10 bp of the 5' untranslated region close to the translation start site was gel purified. Two complementary oligonucleotides that span the *Esp*I cleavage site and include the nucleotide sequence up to the translation start site were synthesized, annealed and ligated to the aforementioned *Esp*I-*Bam*HI fragment. The construct containing the 5' flanking DNA of the RI gene was cloned into the pBLCAT3 vector to generate pBLCAT3 (-1337 to +24). The first and second number in the parentheses represent the base pair at the 5' and 3' end, respectively of the 5' flanking DNA of the RI gene. All other deletion constructs were made by ligating the *Hind*III, *Esp*I fragments obtained from the above mentioned *Bal*31 digestion into pBLCAT3RI (-1337 to +24) *Hind*III and *Esp*I to generate the deletion constructs shown in Figure 2.

Constructs generated by PCR

Oligonucleotides were made on an Applied Biosystems model (380A DNA synthesizer). PCR reactions were carried out using an automated thermal cycler (Perkin-Elmer Cetus Corp. USA).

Twenty to thirty pmol of each primer and 2.5 U of *Taq* polymerase (Perkin-Elmer Cetus/Promega) were used for each reaction in a volume of 100 µl using the reaction buffer recommended by the supplier. The construct pBLCAT3RI (-4 to +24) was made using PCR with forward and reverse primers containing *Hind*III and *Bam*HI restriction sites, respectively. After 30 cycles of 1 min at 94°C, 30 s at 50°C and 2 min at 72°C the reaction product was digested with *Hind*III and *Bam*HI, separated on a 2% Nusieve GTG agarose gel (FMC Products, Rockland, MA), purified using DEAE paper and ligated into a *Hind*III and *Bam*HI cut pBLCAT3 vector.

DNA sequencing and plasmid preparation

For the 5' end deletion constructs the sequence close to the newly generated ends was verified by sequencing from both directions. When PCR was used to generate the constructs the entire sequence of the 5' flanking DNA in the respective constructs was verified applying dideoxy sequencing procedure (11). Plasmid DNA was made by alkaline lysis and purified by the CsCl method (12).

Cell culture and DNA transfection

AR42J, a rat pancreatic acinar tumor cell line; HepG2, a liver cell line derived from a human hepato blastoma and Rat2, a rat fibroblast cell line were selected in order to investigate the regulation of the RI gene in different cell types. Cells were maintained in DMEM containing 10% FCS and supplemented with glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 mg/ml). AR42J cells also contained polymyxin B (50 µg/ml) and fungisone (2 µg/ml) in their medium. Transfections were done by the Ca-phosphate precipitation method (13) using 7 µg DNA of the test plasmid and 2-3 µg DNA of pRSV β-gal. Expression of the β-gal was used to normalize transfection efficiency per 100 mm dish. AR42J cells were transfected according to the protocol described by Cockell *et al.* (14). Forty-eight hours after transfection the cells were washed with PBS and suspended in 0.25 M Tris-HCl pH 7.8 and sonicated. After centrifugation in a microfuge for 15 min the supernatant was analyzed for protein content using the Biorad reagent (Bio Rad, Richmond, CA) and used for further analysis.

CAT and β-galactosidase activity

CAT activity was measured according to standard protocols (13). To estimate transfection efficiency HepG2 and Rat2 cells were co-transfected with pRSV β-gal and the β-gal activity was measured according to Herbomel *et al.* (15). Transfections were done in single or duplicate plates and repeated at least two to three times.

Gel retardation assay

Oct-2 binding to the RI promoter was analysed by gel retardation assays essentially as described by Calzone *et al.* (16). Nuclear extract from Oct-2 transfected A293 cells was prepared according to Das and Herr (17). The labeled probes were incubated with nuclear extract for 15 min at room temperature and were loaded onto 5% polyacrylamide gels. Complexes and unbound DNA were resolved by electrophoresis at 15-20 mA for 5-6 h. Gels were dried prior to autoradiography.

Figure 2. The relative CAT activities of RI-CAT chimeras expressed in AR42J, Rat2 and HepG2 cell lines. The ribophorin I deletion constructs shown on the left hand side were made as described in Materials and Methods. The designated name for each plasmid indicating the 5' and 3' end of the ribophorin I gene are given next to the schematic representation of the construct. The major transcription start site (-1/+1) and also the other transcription start sites used in this gene are those represented by downward arrows in Figure 1. For the position of the GC boxes, as well as CAAT and the octamer motif see also Figure 3. The relative CAT activities shown on the right hand side were obtained by comparing the normalized CAT activity (as described in Material and Methods) of cells transfected with each of the constructs with the activity seen in cells transfected with the construct pBLCAT3RI(-364 to +24). Cells were transfected with 7 μ g of test plasmid and 2 μ g of the reporter plasmid RSV β -gal. Cell lysates were prepared after 48 h and the CAT activities were normalized with respect to the β -gal activities. The data represent an average of five independent determinations for each cell type. Bars represent the standard error.

Northern blot analysis

Actively growing rat hepatoma HTC or mouse myeloma MPC-11 cells were washed once with PBS and total RNA was isolated using the acid phenol-guanidinium thiocyanate procedure (18). RNA was dissolved in autoclaved ddH₂O and the concentration was determined by measuring the OD at 260 nm. After electrophoretic separation on a 1.2% formaldehyde gel the RNA was transferred to a Gene Screen hybridization membrane (NEN, Boston, MA, USA) according to the procedure specified by the manufacturer. The filters were baked at 80°C for 2 h and hybridized with the full length RI cDNA labelled with ³²P-dCTP (specific activity 3000 Ci/mmol) using the BRL nick translation kit (BRL, Gaithersburg, MD, USA). The filters were washed four times for 10 min each at room temperature with 2 $\times$ SSC containing 0.1% SDS and once for 15 min at 60°C with 0.1 $\times$ SSC containing 0.1 $\times$ SDS and exposed at -70°C using Kodak X-Omat AR film.

RESULTS AND DISCUSSION

To establish the functional activity of the putative RI promoter region, we have performed transfection experiments employing plasmid constructs that contain portions of the 5' flanking region of the RI gene cloned upstream of the CAT reporter gene. In order to correct for the differences in DNA uptake by the cells, they were cotransfected with a plasmid containing β -galactosidase under the control of the RSV promoter and the β -galactosidase

activities in the cell lysates were used to normalize the CAT activities. In the case of AR42J cells the transfection efficiency was low compared to those in HepG2 and Rat2 cells, and the β -galactosidase activity was not measurable. Therefore, we normalized the CAT activity of transfected AR42J cells with respect to the protein content in the lysate. The finding that after transfection of HepG2, a human cell line, with the various constructs showed similar increases in the levels of transcriptional activity as rat cells (AR42J and Rat2) suggests that the regulation of the ribophorin I gene expression in human and rat are conserved.

The sequence of 400 bp of the 5' flanking region of the RI gene, which contains several putative cis-acting elements involved in the transcriptional regulation of the RI gene is shown in Figure 1. The relative CAT activity of RI-CAT chimeras containing different portions of the 5' flanking region of the RI gene are presented in Figure 2. Although the transfection efficiencies varied in different cell lines, the CAT activities of different constructs, when normalized with respect to the maximal expression observed after transfection with the construct pBLCAT3 RI(-346 to +24), were similar. Transfection of cells with the promoter-less plasmid pBLCAT3 alone did not show any detectable CAT activity in any of these cell lines. Also the construct pBLCAT3RI(-4 to +24) did not have significant (<2%) CAT activity, indicating that no regulatory elements necessary for the transcriptional initiation are present in this construct.

The promoter regions of so-called housekeeping genes are frequently characterized by segments with a high content of G +

C residue (GC boxes), especially if they are located within the first 100 bp of the 5' flanking region (9,19). In the 5' flanking region of the RI gene there are three GC boxes, two of which are present in tandem beginning at -13 and -24, and the third starting at position -91 (Figs 1 and 2). While the most proximal of these boxes corresponds exactly to the consensus sequence for Sp1 binding (20) the other two at -24, and -91 have one or two mismatches. pBLCAT3RI(-42 to +24) containing the two proximal GC-boxes and the CAAT-like elements shows ~20% CAT activity, suggesting that these sequences are necessary for the basic promoter activity of the RI gene. The finding that transfection with pBLCAT3RI(-142 to +24) resulted in a 2-3-fold higher CAT activity than pBLCAT3RI(-42 to +24) indicates that also the third GC box at -91 can function synergistically, presumably with the other two GC boxes, to increase the transcriptional efficiency of this promoter. Similar conclusions with respect to the synergistic functioning of GC boxes have been drawn for the transcriptional regulation of several genes (21,22). Furthermore, it has been shown that GC boxes with one or two mismatches are still functional (23). From the existing data it is not clear whether the CAAT-like element at -41 is functional and necessary for the transcriptional activity of this promoter.

The constructs pBLCAT3RI(-364 to +24) and pBLCAT3RI(-598 to +24) showed the highest transcriptional activity of all the constructs tested (Fig. 2), suggesting that they have the necessary regulatory elements for the maximal transcriptional activity of the promoter. Apart from the three SP1 binding sites, these constructs also contain an octamer motif at -226 to -233 (see also Fig. 3) which shows 90% homology to the consensus octamer sequence (24,25). The octamer motif mediates transcriptional regulation by several octamer motif-binding proteins and is found in a variety of ubiquitously and cell-specifically active cellular promoters as well as viral promoters (26). This motif is present either near the start site of transcription or in distal enhancer sequences (27-29). Among the octamer motif-containing genes that are cell-specifically active, the B cell-specific immunoglobulin genes are the best characterized (24,25).

The octamer sequence can be recognized by two mammalian transcription factors. One is expressed in almost all cell types and is referred to as Oct-1 (30-32), while another, Oct-2 is predominantly expressed in lymphoid tissues (33-37). These two transcription factors are members of the POU family of homeodomain proteins (38-42) and are known to activate several promoters (26). To elucidate the role of the octamer motif in the regulation of the RI gene we co-transfected pBLCAT3RI(-364 to +24) or pBLCAT3RI(-142 to +24) with Oct-2 cDNA in an eukaryotic expression vector under the control of CMV promoter (43) into Rat2 cells. It was found that co-transfection of pBLCAT3RI(-364 to +24) with Oct-2 resulted in a 3-4-fold increase in transcriptional activity (Fig. 3). On the other hand a similar analysis of pBLCAT3RI(-142 to +24) did not show any increase in its CAT activity. These results indicate that Oct-2 can increase the transcriptional activity of the RI promoter.

In order to determine whether Oct-2 interacts directly with RI promoter elements gel retardation assays were performed. For these experiments nuclear extracts enriched with Oct-2, or control extracts were prepared as described earlier (17) and incubated with the RI promoter sequences excised from pBLCAT3RI(-364 to +24) or pBLCAT3RI(-142 to +24). The presence of Oct-2 in the nuclear extract was verified by using a wild type or mutant

Figure 3. Effect of Oct-2 on the transcriptional activity of the ribophorin I promoter. Rat2 cells were cotransfected with Oct-2 and a RI/CAT chimera containing various portions of the RI promoter. CAT activities measured after 48 h were normalized with respect to β -gal activities as described in Materials and Methods. The autoradiographs of the thin layer plate is shown below the respective column in the histogram. Quantitation was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) of two independent experiments. Values are expressed as percent of that obtained for pBLCAT3RI(-364 to +24) in the absence of transfection with Oct-2 cDNA (lane a).

Oct-2 probe (Fig. 4) as described earlier (17). The Oct-2 probe showed a striking shift in the presence of Oct-2 enriched nuclear extract (compare lanes a and b), while the mutant probe did not show such a shift (lanes f and g). Furthermore, cold octamer binding site oligonucleotide completely competed for Oct-2 binding to the wild type probe (lane d), but did not show any effect in the case of the mutant probe (lane i). Control extract prepared from untransfected cells did not show any shifted bands (lanes c, e, h and j).

The RI promoter sequences excised from pBLCAT3RI(-364 to +24) and pBLCAT3RI(-142 to +24) were tested for Oct-2 binding to the promoter elements. As shown in Figure 4B, fragment (-364 to +24) showed a clear shift in the presence of Oct-2 enriched nuclear extract (compare lanes a and b) and the shifted band was effectively competed with cold oct-oligomer (lane d). The control extract did not reveal any bands (lanes c and

Figure 4. Gelshift analysis of Oct-2 binding to the ribophorin I promoter. The [32 P]-labeled probes as indicated on top of the autoradiogram were incubated for 15 min at room temperature with 0.25 μ l of Oct-2 enriched nuclear extract [(A) lanes b, d, g, i and (B) lanes b, d, g, i] obtained from A293 cells transiently transfected with Oct-2 cDNA (18). Controls shown in lanes c, e, h and j in (A) and (B), respectively, were incubated with a nuclear extract from untransfected A293 cells. Reactions analyzed in lanes d, e, i and j in (A) and (B) received in addition an excess of cold Oct-binding site oligonucleotide (37). The reactions were analyzed on a 5% polyacrylamide gel using Tris-Borate-EDTA buffer (0.5 $\times$). The dried gels were autoradiographed at -70°C for 24 h.

e). Fragment (-142 to +24) did not show any specific Oct-2 binding. The bands observed in the presence of Oct-2 enriched extracts are nonspecific since cold octamer binding site oligonucleotide did not show any effect on these bands (lanes f, g and i). As revealed by other probes the control extract did not show any shift (lanes h and j). These results demonstrate that octamer binding sites between -142 and -364, including most likely the octamer motif at -233 of the RI gene promoter are functional and that Oct-2 binds to the promoter elements and increases the transcription of the RI gene. Although our results demonstrate the involvement of octamer motif binding protein Oct-2 in the transcriptional regulation of RI gene, the precise location of the octamer binding site in the promoter region of this gene needs further experiments. Furthermore, we cannot rule out from these experiments the involvement of similar elements upstream of -364 to +24 in the regulation of RI gene.

The involvement of Oct-2 in the transcriptional regulation of a RER-specific protein has important biological implications. During differentiation of pre B cells to immunoglobulin secreting plasma cells a several fold increase in the RER has been reported (44,45). This proliferation of the RER is accompanied by a corresponding increase in the levels of RI, RII and of OST48 (45), all of which are components of the oligosaccharyltransferase. Oct-2 expression is predominantly B cell lineage specific (36,46)

Figure 5. Northern blot analysis of rat ribophorin I mRNA in a hepatoma and in myeloma cell lines. Total RNA was isolated by the guanidinium thiocyanate procedure from actively growing rat hepatoma (HTC), myeloma (MPC-11) or pre B (70Z/3.12) cells. Northern blot analysis was carried out on 5 μ g of total RNA out as described in Materials and Methods. The top panel shows the RI mRNA levels in HTC (lane a), MPC-11 (lane b), 70Z/3.12 (lane c) cells, while the bottom panel allows us to determine the position and the amount of 28S and 18S RNA (indicated by arrows) transferred to the filter. After autoradiography the signal intensity was quantitated by densitometric scanning.

and is necessary for the activation of B cells (47). If in fact Oct-2 increases the transcription of the RI gene in a plasma cell the steady state level of RI mRNA is expected to be much higher than in pre B cells or in non-lymphoid cells. We tested this proposition by a Northern blot analysis using RNA isolated from a rat hepatoma cell line (HTC), an IgG secreting mouse plasma cell line (MPC-11) or the pre B cell line 70Z/3.12 which does not secrete IgG. We found that the levels of RI mRNA in MPC-11 was at least 5-fold higher than in HTC or pre B cells (Fig. 5). Therefore, it is likely that the octamer-like sequences in the RI gene of lymphoid cells bind Oct-2 and enhance the expression of this gene during differentiation of B cells to plasma cells, a process which is accompanied by an enormous proliferation of the RER supporting the massive secretion of immunoglobulins. Furthermore, recent experiments with Oct-2 knock-out mice (47) have shown that Oct-2 is crucial for the maturation of B cells to immunoglobulin-secreting cells. That a deficiency in Oct-2 can lead to a defect in the same stage of B cell development where a considerable increase in RER has been reported (44,45) strongly supports our suggestion that Oct-2 is involved in upregulating the transcription of the RI gene which encodes an important component of the RER. Intriguing is also the finding, that although lipopolysaccharide (LPS) induces normal B cells to develop into immunoglobulin-secreting cells, the Oct-2 $^{-/-}$ cells responded poorly (47). Since LPS can also be a potent inducer of the RER (44,45) it is possible that the similar response with regard to LPS induction reflects an important role for Oct-2 in regulating the biogenesis of RER, at least in B cell related lines.

Ribophorin I appears to be ubiquitously present in the microsomal membranes of all mammalian tissues and cells so far analyzed (5,48). It is likely that the GC elements in the RI gene are an important part of its constitutive expression. Other

elements like CAAT or OCT or other unidentified elements might be involved in further strengthening the promoter at times when RI expression is increased, as is the case during the differentiation of B cells to become plasma cells which is accompanied by the proliferation of the RER.

The construct pBLCAT3RI(-1337 to +24) and pBLCAT3RI(-1337 to +14) gave only 40% of the transcriptional activity when compared with that of pBLCAT3RI(-364 to +24) or pBLCAT3RI(-598 to +24) (Fig. 2). These results suggest the presence of a negative regulatory element between -598 and -1337 of the 5' flanking DNA of RI gene. Such a negative element in the RI gene may have a role in the transient repression or fine tuning of the gene under circumstances where the newly synthesized RER is being degraded as is the case during crinophagy, a process in which the RER is degraded in the lysosomes. In order to generate the construct pBLCAT3RI(-1337 to +14) 10 bp were removed at the 3' end of pBLCAT3RI(-1337 to +24), thus bringing the promoter region of the gene closer to the translation start site, preserving three minor transcription start sites. Since the transcriptional activity of this construct did not change it may be concluded that at least one of the remaining three transcriptional start sites is actually used.

The observation that most TATA-less promoters harbor several SP1 binding sites and have multiple transcription initiation sites suggest that the transcription initiation in these genes differ mechanistically from genes containing a TATA element. In the dihydrofolate reductase gene (DHFR) GC boxes are involved not only in the efficiency of transcription, but also in the transcriptional initiation (49). The transcriptional activity of the RI gene as shown by these constructs suggests an interaction between the different transcription factors especially the SP1 and OCT binding proteins, and possibly with other unidentified factors, which may be involved in the transcriptional regulation of genes coding for RER specific membrane proteins. It is possible that a combination of interactions between various trans-acting factors dictate the transcriptional initiation and hence the regulation of this gene. RI has been shown to be a component of the oligosaccharide transferase activity (7) which is in close proximity to other constituents of the translocation machinery (2,50-52). We have recently cloned the RII gene and found a similar organization of its promoter as in the RI gene (53). It is possible that similar cis-elements as those found in the RI gene are also present in the genes coding for other polypeptides that constitute the translocation machinery, and that all these components are regulated by similar trans-acting factors. This would explain the coordinate regulation of different components of the translocation apparatus.

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