The promoter for Constitutive Expression of the Human ICln Gene CLNS1A*

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The ICln protein is expressed ubiquitously in mammals. Experiments designed to knock down the ICln protein in NIH 3T3 fibroblasts as well as in epithelial cells led to the conclusion that this protein is crucially involved in volume regulation after cytoplasmic swelling. Reconstitution of the ICln protein in lipid bilayers revealed the ion channel nature of ICln. Here we describe a new human promoter sequence, composed of 89 nucleotides, which is responsible for a highly constitutive expression of the ICln protein. The promoter sequence lacks a TATA box, and the transcription can be affected at multiple sites. In addition to the starting sites, upstream sequence elements are mandatory for an efficient transcription of the ICln gene (CLNS1A). These new nucleotide elements were defined by site-directed mutagenesis.

The increase of the cell volume results in the activation of pathways that effect the reduction of osmotically active molecules and ions in the cytoplasm, thus leading to compensatory cell shrinkage (1, 2). The loss of potassium and chloride caused by the activation of swelling-activated channels (SAC) plays a major role in regulatory volume decrease, evidenced by the consistent activation of SAC in mammalian cells after swelling (2). Using the expression cloning technique, we identified a protein (ICln) that, expressed in Xenopus laevis oocytes, leads to a current that is similar to SAC found in several cell types in terms of its kinetics, pharmacology, and relative selectivity for anions (4). The assumption that ICln is functionally tightly linked to SAC is supported further by the finding that the knock-down of the ICln protein in fibroblasts and epithelial cells by the use of antisense oligodeoxynucleotides specific to ICln mRNA can seriously hamper the activation of swelling-dependent anion channels (5, 6). Using fluorescence in situ hybridization, we identified two different human gene loci that carry the coding region for ICln. One locus at position 6p12, termed CLNS1B, contains an intronless gene (7), whereas the second gene locus at position 11q13.5–14.1, termed CLNS1A, is segmented by introns. The exon sequences of CLNS1A are identical with the cloned human cDNA, suggesting transcription of this gene (8). Because ICln is expressed ubiquitously and transcribed constitutively in mammalian cells (9), and volume regulation is an inherent regulatory entity of living cells, it seems likely that this protein is part of one of the housekeeping regulatory machineries of the cells. However, preliminary experiments indicate that this constitutive transcription of the CLNS1A gene is modulated by the volume stress placed upon the cells and, in addition, during their progression in the cell cycle. A prerequisite of the attempt to investigate these regulatory mechanisms for CLNS1A transcription is the identification of the promoter for the constitutive transcription of this gene. Furthermore, the constitutive expression of the ICln protein is remarkably high. Therefore, the identification of the minimal promoter region needed for its transcription should lead to the identification of a highly efficient human promoter that could also be used for an effective expression of other proteins in human cells. Such a promoter would be very instrumental in avoiding the use of viral promoters for the expression of proteins in human cells. To characterize the minimal promoter responsible for the constitutive expression of the ICln gene, we subcloned and analyzed the 5’-flanking region of the human CLNS1A gene.

EXPERIMENTAL PROCEDURES

Cloning of the ICln Promoter Region

By screening a human genomic P1 library (Ressourcen-Zentrum/Primär Datenbank RZPD library (10)) with a 700-bp cDNA probe specific to ICln, we isolated a clone termed P1R3 (the corresponding RZPD reference number is ICRFP700E1924Q). The 3’-end of this clone was sequenced and corresponded to the 5’-end of the human CLNS1A gene (7, 8). The P1R3 clone was digested with KpnI and SacI. The restriction enzyme products were separated on a 2% agarose gel and transferred to a nitrocellulose membrane for hybridization. They were subsequently probed using the 5’-end of the ICln open reading frame (ORF). This procedure led to the isolation of a ~2.3-kilobase fragment from the P1R3 clone, which was cloned into a pBluescript SK II+ vector thereafter. Sequencing analysis revealed that the clone carried 2151 nucleotides upstream from the first coding ATG and parts of exon 1 of the ICln gene.

PCR Amplification

Standard PCR protocols with Taq or Pfu polymerase (Roche Molecular Biochemicals, Stratagene) were used for parts of the deletion mutations as well as for the site-directed mutations.
The 5′-flanking region of the 2151-bp fragment described above was cloned into the BamHI and HindIII restriction sites of the pGL3-basic vector (Promega) containing the gene for the firefly luciferase (isolated from Photinus pyralis). A size reduction of the 2151-bp fragment was obtained by using the restriction enzyme PvuII, the Erase-a-Base kit (Promega) according to the manufacturer’s instructions, or the PCR technique (see above). Site-directed mutagenesis was performed by introducing the respective mutations into the primer used for PCRs. All promoter fragments were sequenced to test for the correct sequence before applying them to the reporter gene assays. To normalize the changing transfection efficiency, the different promoter constructs were cotransfected in HEK 293 T cells with the pRL-TK vector (Promega), containing the gene for the Renilla luciferase (isolated from Renilla reniformis) controlled by the viral TK promoter. All values of the reporter assay are given as the ratio obtained from the firefly and Renilla luciferase readings (lux:ren).

**Transient Transfection**

The luciferase reporter gene constructs together with the pRL-TK plasmid were transfected into HEK 293 T cells by calcium phosphate precipitation (11, 12). For this reason, ~10⁴ HEK 293 T cells were spread on cell culture dishes with a diameter of 30 mm the day before transfection. For the transfection, 150 ng of promoter plasmid and 75 ng of pRL-TK vector were mixed with buffer A (0.5 M CaCl₂, 100 mM HEPES, pH 6.95; adjusted with NaOH) and incubated for 10 min at room temperature, before adding buffer B (0.28 M NaCl, 0.75 mM Na₂HPO₄, 0.75 mM Na₂HPO₄₉, 5 mM HEPES, pH 6.7; adjusted with NaOH). After a further incubation period of 10–20 min at room temperature, the transfection mix was spread over the cells. The next day the cells were washed twice with culture medium (see below). On the 2nd day in culture temperature, the transfection mix was spread over the cells. The next day the cells were harvested, and cell lysis was performed using 250 μl of lysis buffer (25 mM glycglycin, 15 mM MgSO₄, 8 mM EGTA, 2% Tween 20, 1 mM dithiothreitol), and the cells were scraped off the culture dishes. The lysates were analyzed in a luminometer (EG&G Berthold) for both firefly and Renilla luciferase by mixing 50 μl of lysate with 350 μl of firefly or Renilla assay buffer and 200 μl of substrate-buffer for the firefly or Renilla luciferase, respectively.

**Luciferase Reporter Gene Assays**

To remove traces of culture medium, cells were washed with ice-cold phosphate-buffered saline (PBS), then lysed by adding 250 μl of lysis buffer (25 mM glycglycin, 15 mM MgSO₄, 8 mM EGTA, 2% Tween 20, 1 mM dithiothreitol), and the cells were scraped off the culture dishes. The lysates were analyzed in a luminometer (EG&G Berthold) for both firefly and Renilla luciferase by mixing 50 μl of lysate with 350 μl of firefly or Renilla assay buffer and 200 μl of substrate-buffer for the firefly or Renilla luciferase, respectively.

**5′-RACE**

From the human mRNA, the first strand cDNA was made by using an ICln-specific primer (5′-TGT AGG AGG GTC CCC CTG TCG TTG-3′) and avian myeloblastosis virus reverse transcriptase (Promega). The reaction was heat inactivated and was then subjected to an RNase A treatment. Unincorporated nucleotides and enzymes were removed using a Qiagen PCR purification column (Qiagen). Polyadenosine tails were added to the 5′-end by using terminal deoxynucleotidyl transferase (MBI). Second strand synthesis was carried out by PCR using Taq polymerase (MBI) and the primer RACE-N (5′-GCA TCG ATC GCG CCA CTC TTT TTT TTT TTT TTT TTT-3′). For the first PCR, the gene-specific primer (5′-AGG TTC AAC ATC ATC ATC ACT GTC GTC-5′) and the RACE-N primer were added to the reaction above, and a standard PCR protocol was carried out. To enhance specificity further, the PCR products were subjected to a second nested PCR using the primer RACE-N2 (5′-GCC TCG ATC GCG CCA CTC-3′), corresponding to the 5′-end of the reporter gene (Promega). For this reason, ~10⁴ HEK 293 T cells were spread on cell culture dishes with a diameter of 90 mm the day before transfection. For the transfection, 7 μg of promoter plasmid was mixed with buffer A and incubated for 10 min at room temperature before adding buffer B. After a further incubation period of 10–20 min at room temperature, the transfection mix was spread over the cells. After 18 h the cells were washed twice with culture medium, and after an additional 6 h the cells were harvested, and cell lysis was performed using 200 μl of lysis buffer (1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml of each leupeptin, pepstatin A, antipain, and aprotinin, in phosphate-buffered saline at pH 8.00). A total volume of 200 μl of supernatant was used for the extraction of His-tag-fused ICln protein (Ni-NTA spin columns; Qiagen). Imidazole (400 mM imidazole, 50 mM potassium phosphate pH 7.4) was used for eluting the His-ICln. The extract was subjected to gel electrophoresis, blotted, and analyzed using antibodies specific for the ICln protein. The different Western blots were quantified using the ImageQuant (version 1.0) software (Molecular Dynamics), and the values were normalized according the total protein measurements (Bradford).

**Cell Culture**

HEK 293 T cells were grown in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 44 mM NaHCO₃, 280 μM penicillin, 114 μM streptomycin, and 10% fetal calf serum.

**DNA Sequencing**

All plasmids used were sequenced using an automatic sequencer (LiCor Gene ReadIR 4200) with the protocols suggested by the manufacturer.

**Chemicals**

All chemicals used were of pro analysis grade.

**Statistical Analysis**

Where applicable, data are expressed as arithmetic means ± S.E. Statistical analysis was made by t test, where appropriate. Significant difference was assumed at p < 0.05.

**RESULTS**

**Cloning of the 5′-Untranslated Region of CLNS1A and Transcription Start Estimation**

By screening a human genomic P1 library as described under “Experimental Procedures” with a 700-bp cDNA probe specific for ICln, we isolated a clone termed PRI3 (the corresponding RZPD reference number is
ICln so far.

Several human ICln cDNA clone sequences have been reported so far. For the different clones, 5'-untranslated regions of varying lengths have been described, i.e. –88 (accession number 4502890 (13)). –84 this sequence was submitted by Lamb et al. (13) or Lamb et al.2 At present we cannot clearly determine whether the initial 32 nucleotides of the sequence published by Anguita are related to the ICln mRNA or whether they are the result of the cloning procedure used.

The different lengths of the 5'-untranslated regions of the reported ICln cDNAs could indicate that multiple starting sites are used for the ICln gene transcription. To test this possibility, we performed several tests including primer extension, nuclelease protection, and 5'-RACE using poly(A)+ RNA from HEK 293 T cells as a template. For the primer extension, the mRNA was annealed with a 5'-end 32P-labeled primer, designed to anneal within exon 2 of ICln, and the primer was extended using a Moloney murine leukemia virus reverse transcriptase. Autoradiographic analysis of the primer extension products separated by polyacrylamide gel electrophoresis revealed products with a transcription start at positions –78 and –49. Using a primer starting at position +87, labeled with IRD-800, a predominant start for transcription at position –48 was measured. By using the 5'-RACE, we obtained three major products, 163, and 48 clusters were also verified by using the nuclease protection assay. From these experiments we conclude that there are several starting points for human ICln gene transcription, thus providing an explanation for the varying 5'-untranslated sequences published for ICln so far.

**Determination of the Promoter Sequences Responsible for Base-line ICln Expression**—The ICln protein is expressed ubiquitously in mammals (4, 9, 13). To identify the promoter re-

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2 F. S. Lamb, T. Barna, and B. C. Schutte, GenBank™ accession number HSU53454.
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sponsible for this base-line expression, we used the luciferase reporter system (Promega) by transfecting HEK 293 T cells with promoter-luciferase vector constructs together with the reporter system (Promega) by transfecting HEK 293 T cells responsible for this base-line expression, we used the luciferase reporter vector pGL3-basic. As shown in Fig. 1, this basic description of the figure is given in the legend of Fig. 1. An asterisk indicates the values taken from Fig. 2.

Fig. 3. Single nucleotide mutations made in the region between positions −159 and −150. Mutation of the thymidine triplet −159TTT−157/−159GGG−157 leads to a reduction of the activity similar to that effected by a proximal deletion mutation to position −154. A basic description of the figure is given in the legend of Fig. 1. An asterisk indicates the values taken from Fig. 2.

After the restriction of the −167/−35 fragment to −163/−56, there was no significant loss of activity compared with the former level (Fig. 2). However, further reduction of the 5’-end to position −154, −148, −135, or −126 decreased the activity by half. Therefore, the region between −163 and −150 was examined by site-directed mutagenesis to detect the nucleotides responsible for the ~50% reduction of the promoter activity. As shown in Fig. 3, the mutants A−150G, T−154G, and −156CC−154/−156TTT−154 show no reduction of the signal, whereas the mutant −159TTT−157/−159GGG−157 is associated with an activity not significantly different from that of the −154/−56 fragment (0.69 ± 0.1 lucifer, n = 15 for the mutant and 0.60 ± 0.1 lucifer, n = 26 for the −154/−56 fragment). These experiments indicate that the thymines at positions −157, −158, and −159 are most likely essential for half of the transcriptional capacity observed. The remaining half can be annihilated by further reduction of the 5'-end of the −126/−56 clone.

As shown in Fig. 4, the reduction of the 5’-end to the positions −113, −99, or −81 leads to values not significantly different from those of the empty pGL3 vector. Therefore, the thymidine triplet together with nucleotides comprising the string from −126 to −113 is necessary for the activity observed. By mutating the sequence between −126 and −113, further reduction of the essential sequence to single nucleotides was not possible. As shown in Fig. 5, the mutant A−119G/T−117G was not followed by a decrease in activity. However, the activity of the mutant −122CA−121/−122GG−121was significantly impaired. Accordingly, the reduction of the 5’-end to position −120 (fragment −120/−56) leads to an activity that does not deviate significantly from the value obtained by the transfect-
tion of the −122CA−121/−122GG−121 mutant. The activity produced by the transfection of the −120/−56 fragment can be annihilated by further reduction at the 5′-end to position −117 (Fig. 5).

The experiments show that the base-line activity measured after the transfection of fragment −2151/−1 can be obtained when the thymidine triplet (−159TTT−157), the cytosine C−122 and adenosine A−121, and the guanine G−120 and thymidine T−118 are present. In addition to these nucleotides mandatory for the promoter activity, the presence of starting sites is also necessary because the fragment embraced by the positions −163 and −113 shows no activity compared with the empty pGL3 vector (0.0137 ± 0.00056 luc/ren, n = 6). As mentioned above, major transcription starts were identified at positions −95, −78/−77, and −49/−48. Surprisingly, the restriction of the 3′-end of the tested sequences to position −74, which therefore excludes a transcription start at position −49/−48, has a promoter activity similar to that of the −163/−1 fragment (Fig. 6), indicating that the critical sites for the transcription start are located upstream from position −74. This is also evidenced by the finding that four out of the five clones we have identified start at the −95 cluster (two clones) and the −78 cluster (two clones). Accordingly, the fragment starting at position −163 and ending at position −95, i.e. exactly at the most proximal end of the cDNA identified by 5′-RACE, leads to a signal not different from that of the empty pGL3 vector (Fig. 6). This indicates that the critical sequence for the transcription start is between positions −95 and −74. This stretch of nucleotides contains two pyrimidine-rich clusters with the CT-CC sequence. As shown in Fig. 7, these pyrimidine-rich clusters are perfectly conserved from humans to monkeys and mice (16). If the −78 cluster is omitted (fragment −163/−88), the transcription efficacy is half of that of the −163/−74 fragment (Fig. 6). The reduction of the same amount can be observed when the −78 cluster is present, and a mutation was introduced in the −95 cluster (−96CTTC−93/−96GGGG−93), thus suggesting that both pyrimidine-rich clusters are equally important for an effective transcription.

**Efficacy of the −163/−74 Promoter Compared with the Viral Promoters RSV and CMV**—To estimate the efficacy of expression driven by the −163/−74 ICln promoter fragment, we compared the ICln fragment with the expression governed by the viral promoters RSV and CMV. Because the ICln protein is endogenously expressed in HEK 293 T cells, we made promoter constructs harboring the coding region of an ICln protein fused to a string of five histidines, allowing enrichment of the protein extract for this newly expressed protein. We quantified the expressed ICln protein using a polyclonal antibody made against a peptide composed of the C-terminal amino acids of the ICln protein. The −163/−74 ICln promoter fragment leads to an expression of the histidine-tagged ICln protein, which is only four times or three times lower compared with the CMV or RSV promoter, respectively (−163/−74 ICln promoter fragment expression = 0.69 ± 0.21, n = 4, RSV = 2.22 ± 0.31, n = 3 and CMV = 2.76 ± 0.26, n = 4).

**DISCUSSION**

The cytoplasmic volume of cells is tightly regulated, and the mechanisms involved comprise ion transporters and/or channels (2) as well as cellular osmolyte production, uptake, or exit (17, 18). The transcriptional regulation of transporters for compatible osmolytes activated after increasing the extracellular osmolarity is well understood (17, 18). The control of rENaC sodium channel expression, again in response to hypertonicity, is in the process of investigation (19). However, little is known regarding the transcription of genes involved in the ion conductive pathways implicated in volume regulation after swelling.

When whole-cell patch-clamp experiments are made using symmetrical CsCl concentrations, a chloride current can be measured after the reduction of the extracellular osmolarity (5, 20, 21). Similar findings have been reported for a variety of different cells (2). The channels activated under hypotonic conditions are, however, more permeable to other anions than to
chloride \textit{i.e.} SCN\(^-\), Br\(^-\), and I\(^-\). They are also permeable, even though to a lower degree, to cations and, in addition, to osmolytes (22–25). Therefore the habit to use terms such as “swelling-induced,” “volume-regulated,” or “volume-sensitive outwardly rectifying” chloride \textit{channels} seems inappropriate. It is probably more suitable to name these ionic pores swelling-activated channels. Several proteins are thought to be molecular targets for SAC. The list of the candidates comprises CIC-2 (26), mdr-1 (27), phospholemman (28, 29), CIC-3 (30), and ICln (4, 31). Because the expression of these different proteins was done in cells bearing endogenous SAC, the experiments cannot unambiguously clarify whether these proteins are the channels responsible for the currents observed after cytoplasmic swelling. When using antisense oligodeoxynucleotides that specifically impair the ICln production in NIH 3T3 fibroblasts (5) or epithelial cells (32), the activation of SAC is reduced dramatically after the decrease of extracellular osmolality. This indicates that the ICln protein is critical to the appearance of the SAC current under hypotonic conditions. Based on experiments using immunohistochemistry and Western blots, we developed the hypothesis that ICln is a constitutively expressed protein that can be transposed from a water-soluble form in the cytosol into the membrane, thus leading to an ionic current (31). The fact that ICln can indeed act as an ion channel was confirmed by functionally reconstituting the protein into lipid bilayers (31, 33). In the absence of calcium, the current obtained in bilayers is more selective for cations than for anions; however, the addition of calcium shifts the selectivity toward chloride. The reconstituted ICln current is, just like the current effected by native SAC, rectifying and can be blocked by nucleotides. Therefore, we conclude that ICln is the molecular entity of SAC or a substantial part thereof (31).

As mentioned above, little is known regarding the transcriptional regulation of SAC. It has been shown that the transcription of the CIC-2 protein in rat lung is modulated by Sp1 and Sp3 (34); however, nothing is known about the transcriptional regulation of potential SAC candidates in human cells. This has prompted us to study the transcription regulation of human ICln, for which an important role in regulatory volume decrease (35, 36) and seems to be dependent on cell cycle progression. A number of different consensus regions for the binding of a variety of transcription factors can be identified 5’ of the first coding ATG of the CLNS1A gene. Among them also a Sp1 site identical to the one found in the rat CIC-2 promoter was identified; however, the role of these sites needs to be scrutinized before a definitive function can be assigned. The characterization of the nucleotide elements needed for the constitutive expression of ICln is a prerequisite for the in-depth analysis of these regulatory mechanisms. Furthermore, it may also facilitate the definition of a human promoter sequence that could allow a high copy expression of foreign proteins in human cells without the need for viral promoters. The experiments summarized in this paper were therefore made to define the nucleotides necessary for the constitutive expression of human ICln.

Four different cDNAs coding for the human ICln have been reported so far, each having a 5’-untranslated region of varying length (−88, −84, −73, and −30) and indicating that the transcription of the ICln gene starts at multiple sites. With the aid of primer extension, nuclease protection, and 5’-RACE, we were able to define three different initiator regions (Inr) in close proximity to the 5’-untranslated sequences published. The Inr closest to the ORF of the ICln gene is similar to the canonical Inr sequence described as Py\(_2\)ANT/APy\(_2\) (the underlined \text{A} corresponds to position +1 of the transcript (37)). In the human CLNS1A gene this site is located at position −50 and comprises the nucleotides CGCAATTCGT. The identical sequence is also found in the ICln gene of monkeys (Fig. 7). In mice this sequence is slightly changed to GCGATTGCG but reveals a thymidine at the crucial position of +3 (A being at position +1 and therefore confining the predicted transcription start (37)). Testing the efficacy of this Inr revealed that this starting point is probably not mandatory for base-line ICln gene transcription because the deletion of this nucleotide cluster does not reduce the constitutive expression of the reporter system we used. In contrast, two clusters at position −95 and −78 seem to be critical for the transcription of the human ICln gene. The nucleotide sequences of both clusters are identical, reading CTTCC, which are in addition completely conserved in mouse, monkey, and humans. Despite the fact that the first base of the mRNA tends to be adenosine, weight-matrix analysis of an extensive number of Inr sequences revealed that the transcription start can be efficiently effected at a cytosine embraced by pyrimidines. An effective Inr tested was built by the sequence GTTCTTCC (the underlined \text{C} would be the predicted start of the transcription (38)). This consensus region for Inr is
ATG sites for additional factor(s) needed for ICln transcription. The minimal nucleotide sequence necessary for constitutive promoter activity is located between positions -163 and -74.

Sequence analysis of the fragments used in this study failed to identify any obvious TATA box. Similar results were obtained when analyzing the promoter of the CIC-2 chloride channel (34), which is also a possible candidate for SAC (26). The products of genes lacking a TATA box are often involved in housekeeping functions. However, TATA-less genes could also be identified when tissue-specific expression is observed, i.e. the CIC-K1 chloride channel (39), CFTR (40, 41), or the betaine transporter (34), which is also a possible candidate for SAC (26). The ClC-K1 chloride channel (39), CFTR (40, 41), or the betaine transporter (42). The lack of a TATA box usually results in multiple starting sites, as also shown in this study, and does not rule out that sequences upstream from the starting sites are necessary for the binding of proteins needed for effective transcription. It was shown for the TATA-less simian virus 40 major late promoter that effective transcription is only possible when the cloned human TATA box-binding protein hTFIID functionally binds to an upstream sequence element reading 5'-TACCT-3'.

The mutation of both thymidines reduced transcription more than 50%, whereas the exchange of the two cytosines in thymidines enhanced transcription 8-fold (43). We performed similar mutation experiments in a region of the ICln promoter (5'-TTCCT-3'), which is closely related to the 5'-TACCT-3' region of the simian promoter, and we found that the mutation of the first thymidine was also followed by a reduction of the transcription. The mutation of the second thymidine, however, showed no effect. Moreover, mutating the two cytosines in thymidines was not followed by an increased transcription. Therefore it is not certain at the moment whether the binding of hTFIID is obligatory for the ICln gene transcription. Beside the described thymidine, which is part of the -159 triplet, a second nucleotide triplet at position -122 was identified being crucial for ICln transcription. These triplets have identical sequences in humans and monkeys and are similar in mice (Figs. 7 and 8). Interestingly enough, these nucleotide triplets are in close proximity to a sequence stretch with a length of 11 nucleotides, which is identical in all three organisms. Further experiments are needed to elucidate whether this region of high homology is functionally important for transcription.

The expression driven by the -163/-74 ICln promoter fragment is highly efficient because the viral RSV or CMV promoters lead to an expression efficacy, which is only three or four times higher compared with the ICln fragment.

In conclusion, we identified promoter elements and Inr sites, which are essential for the constitutive expression of the ICln protein (Fig. 8). The minimal sequence stretch showing full efficacy compared with the -2151/-1 fragment has a length of 89 nucleotides and is limited by the positions -163 and -74, related to the human CLNS1A. The efficacy of this fragment to express the reporter system is high; and given the fact that no cell type has been identified so far which would lack ICln expression, it might appear possible to use this 89-nucleotide minimal promoter sequence for the expression of other proteins in human cells, when the use of viral promoters is not desired.

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Note Added in Proof—Characterization of reconstituted ICln in lipid bilayers is summarized in Functional Reconstitution of ICln in Lipid Bilayer by Puerst et al. (Puerst, J., Bazzini, C., Jakab, M., Meyer, G.)
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