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RPL30 AND HMGB1 ARE REQUIRED FOR NEURULATION IN GOLDEN HAMSTER

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Abstract

Neural tube defects (NTDs) are a group of severe congenital malformations resulting from the failure of neurulation. Genes influencing neurulation have been investigated for their contribution to neural tube defects. Ribosomal protein (Rp) is an abundant and high conservative gene family, which has the complex task of coordinating protein biosynthesis to maintain cell homeostasis and survival.

However, the mechanisms of ribosomal protein in the neural tube defects are unknown.

Understanding the mechanisms will lead to new insights into neural tube defects. In this report, we constructed a cDNA library from neural tube of golden hamster and screened the cDNA library by a subsection screening method (SSS). Our results demonstrate a possible essential role of the RPL30 cDNA gene during neurulation and in the risk of NTDs. Our study also suggests that another gene, HMGB1, may be significantly associated with neurulation and the risk of NTDs.

Keywords

cDNA library; Neural tube; RPL30; HMGB1; Subsection screening method

INTRODUCTION

Neurulation, from the appearance of the neural plate to the closure of the neural tube, is a fundamental event in embryogenesis and the first step in the development of the central nervous system. During this process, failure of the neural tube to close accurately and at the right time will result in neural tube defects (NTDs). The frequency of NTDs is about 1 per 1000 pregnancies, making NTDs the commonest type of congenital abnormality (Copp et al., 2003; Sadler, 2005). Although the morphological events comprising neurulation have been described in detail, the underlying molecular mechanism of neural tube closure remains poorly understood. Recent studies reported that the malformations of NTDs, rather than being caused by one major gene, are determined by combinations of different genes with additive effects (Kibar et al., 2007; Melton et al., 2004). Therefore, it is important to identify those genes from a library to enhance our understanding of the development of neurulation and the pathogenesis of NTDs. For this purpose, we constructed a neural tube cDNA library of the golden hamster at neurulation to identify relevant genes and then investigated their functions.

Ribosomal protein (Rp) is an abundant and highly conserved gene family with the complex task of coordinating protein biosynthesis to maintain cell homeostasis and survival. Recent evidence suggests that a number of ribosomal proteins have a secondary function independent of their involvement in protein biosynthesis. L30 is one of the most conserved ribosomal proteins and appears to play a central role in the ribonucleoprotein complex (Wiedemann & Perry, 1984; Wool et al., 1995). Thus, the *RPL30* gene may have an effect on neurulation. Until now, the link between neurulation and RP gene expression has been unclear.

HMGB1 (high-mobility group box 1 protein), a nonhistone protein component of chromatin, is an extremely well-conserved across species (Huttunen & Rauvala, 2004). In rat and mouse brains, the expression of *HMGB1* is high during the embryonic period, and *HMGB1* has been suggested to be involved in the outgrowth of neurites, the differentiation of neurons, and the migration of neural crest cell during development. Significantly, interactions between *HMGB1* and SGC are important for cell-cell recognition and cell migration (Chou et al., 2004; Zhao et al., 2000; Chou et al., 2001). However, *HMGB1* has not been to associate with the development of neurulation and the pathogenesis of NTDs.

In this study, we established a cDNA library and screened the cDNA library of the neural tube using the subsection screening method (SSS). We demonstrated, for the first time, the relationship between *RPL30* and *HMGB1* genes and neurulation and the risk of NTDs.

MATERIALS AND MATHODS

Tissue Collection

Mature golden hamster females were caged with breeding males, and the day of appearance of a vaginal plug was considered day 1 of embryonic development (E1d). Pregnant females of E8d, E8.5d, E9d, E9.5d, E10d, E11d, and E12d were sacrificed by cervical dislocation, and the fetuses were obtained from the pregnant females. Neural tube tissues were stripped under a dissection mirror and frozen immediately in liquid nitrogen. Some pregnant females of E8d were water bathed in 42°C for 20 min at 3:00 p.m. to make a hyperthermia animal model of NTDs (Feng et al., 1996); then 16h after treatment, the pregnant females were killed and the neural tube tissues of the embryos were stripped, labeled H8.5d group.

RNA Isolation

Total RNA of 100-mg neural tube samples taken from E8.5d embryos was isolated with Trizol reagent (Invitrogen). The quality of RNA was determined by measuring at OD₂₆₀, and the purity was calculated from the ratio OD₂₆₀/OD₂₈₀ by spectrophotometry. The integrity of the total RNA was analyzed by electrophoresis on a 1% denaturing agarose gel.

Construction of cDNA Library

According to the protocol of SMART cDNA Library Construction Kit (Clontech), first-strand cDNAs were synthesized with SMART Oligonucleotide (AAGCAGTGGTATCAACGCAG-AGTGGCCATTACGGCCGGG) and CDS 3' PCR Primer (ATTCTAGAGGCCGAGGCGG-CCGACATG-d (T)₃₀N₋₁N); double-strand cDNA was amplified with LD-PCR (long-distance) method. The dscDNAs were digested with proteinase K and *Sfi* I (IA & IB) restriction enzyme, fragments <500 bp were separated by CHROMA SPIN-400, and the cDNAs were ligated to the *Sfi* I-digested λTriplEx2 vector (Clontech). After packaging (MaxPlax Lambda Packaging Extracts, Epicentre Technologies), the neural tube cDNA library was constructed.

One microliter of the primary library was introduced into *E. coli* XL1-Blue (Clontech) competent cells, the plaques were counted, and the titer of unamplified library was calculated: pfu/ml = (Number of plaques × dilution factor × 10³ μl/mL)/μl of diluted phage plated.

Individual 40 white plaques were randomly picked from the plate as a template, using 5'-sequencing Primer (CTCCGAGATCTGGACGAGC), 3'-sequencing Primer (ATTATGCTGAGTGATATCCCCG) for PCR screening, following the thermal cycling parameters: 95°C for 2 min, 35 cycles of 95°C for 30s, 68°C for 3 min, and 68°C for 3 min.

To further test the cDNA library of golden hamster neural tube, we designed a fragment of a β -actin sequence, primers were designed according to golden hamster β -actin mRNA sequence (gi:14041682), P1: 5'-CGCGGGCGACGAT GCTC-3', P2: 5'-TTCACGGTTGGC-CTTGGGGTTTCAG-3'. PCR was performed following a hot start at 94°C for 2 min, samples were amplified for 35 cycles at 94°C for 30s, 55°C for 30 s and 72°C for 1 min, and additional 5 min at 72°C. PCR products were examined by electrophoresis on agarose gels.

Subsection Screening of RPL30 cDNA

RPL30 primers were designed according to mouse *RPL30* mRNA sequence (gi:42476346), P3: 5'-GCCGCAAAGAAGACGAAG-3', P4: 5'-GGGTCAA TGATAGCCAGT-3'. One microliter of the primary library was diluted by 10-fold step by step; then 1 μ l of the diluted library was detected by PCR to estimate the lowest limit. PCR was performed following the Touch-down PCR program consisting of a denaturation step of 5 min at 94°C followed by a Touch-down profile starting with 20 cycles of 1 min at 94°C, a pairing step of 1 min at 60°C, decreasing by 1°C per cycle until 51°C, and 1 min at 72°C. After Touch-down profiling, the mixture was subjected to 20 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. The program ended with one polymerization cycle at 72°C for 5 min. PCR products were checked by electrophoresis.

One microliter of the lowest library dilution was added into 1 ml of 1 $\times$ λ dilution buffer (λ means λ TriplEx2 vector, dilution buffer: 0.1 M NaCl, 0.01 M MgSO₄·7H₂O, 0.035 M Tris-HCl (pH 7.5), and 0.01% Gelatin), and the mixture was fractionated into 20 parts; each part was plated on LB agar plates and incubated overnight at 37°C. After the growth of plaques, the plate was washed with 2 ml of 1 $\times$ λ dilution buffer. Each 1 μ l of eluate was detected by Touch-down PCR to select the primary positive signal pool. One microliter of the primary positive signal pool was added into 1 ml of 1 $\times$ λ dilution buffer; each 10 μ l of the mixture was plated on LB agar plates and incubated overnight at 37°C. The plate was then divided into many groups each included ~50 plaques and were washed with 0.5 ml of 1 $\times$ λ dilution buffer. One microliter of this eluate of each group was detected by Touch-down PCR to select the secondary positive signal pool. The third positive signal pool, and then the fourth pool, was defined by the same method, until the single positive clones were found.

Positive phages were converted to plasmids by transfecting *E. coli* BM25.8 at 31°C. Transduction of λ pTriplEx2 lysate into BM25.8 promotes Cre recombinase-mediated release and circularization of plasmid pTriplEx2 at the LoxP sites. According to the protocol of the MiniBEST Plasmid Purification Kit Ver2.0 (TaKaRa), the target plasmid DNA was extracted by alkaline lysis, identified by Sfi I enzyme, and the insert was checked by electrophoresis.

Screening of *HMGB1* cDNA

During PCR screening of recombined cDNAs from the constructed library, we obtained a recombined plaque that appeared frequently, which we designated as No. 2. The No. 2 plaque was also converted to plasmids by transfected *E. coli* BM25.8 at 31°C, and the inserted DNA was extracted.

Sequence and Homology Analysis

The isolates selected for completion were entered into the cDNA processing pipeline for quality assurance, automated annotation, and sequence submission. Sequence database searches of

cDNA were performed with NCBI BLASTn sequence comparison programs (<http://www.ncbi.nlm.nih.gov>).

Northern Blot Analysis

For probe preparation and Northern blot analysis, two target cDNA fragments were purified according to QIA Quick Gel Extraction Kit (Qiagen) protocol. The dig-labeled probes were respectively prepared from the cDNA previously obtained by random priming, and the sensitivity of the probe was detected. Total RNA was isolated from neural tube tissues of each group. Thirty micrograms of each RNA sample was electrophoresed on a standard 1.2% formamide agarose gel, blotted onto a nylon membrane (Osmonies). cDNA hybridizations were performed according to DIG High Prime DNA Labeling and Detection Starter Kit (Roche) and stained with NBT/BCIP overnight.

RESULTS

Construction and Evaluation of the cDNA Library

The total RNA from neural tube tissues was not obviously degraded, and a ratio 28s/18s was equal to 1.8–2.0; clear bands of 18s and 28s were seen (Fig. 1A). The results show that the total RNA obtained from neural tube tissues was integrity and high purity. Double-strand cDNA was amplified by LD-PCR. The synthetic cDNA appeared as a smear of 0.1–5 kb, with some bright bands at ~2.0 kb that corresponded to abundant mRNA (Fig. 1B).

We then performed the titer of library and PCR insert screening of cDNA library. The double-strand cDNAs were ligated into the Sfi I-digested λ TriplEx2 vector, the 1.5:1 ligation ratio produced a high efficiency. After titration, the unamplified neural tube cDNA library consisted of 1.5×10^6 independent clones (Fig. 1C). The recombination efficiency was analyzed by X-Gal and IPTG. Counting the blue plaques and white plaques showed a recombination rate of 99%. The titer of the amplified cDNA library was 1×10^9 pfu/ml. Fig. 1D shows 11 cDNA inserts released by PCR amplification. The PCR products were larger than 0.5 kb, and the average inserts of the recombinants was 1.0 kb long.

After PCR amplification of β -actin from the constructed library, as expected, a clear band about 290 bp was observed (Fig. 1E). This sequence was confirmed to be β -actin of golden hamster by BLASTn sequence comparison programs.

Subsection Screening of RPL30 cDNA

We expect the fragment designed for *RPL30* sequence was 289 bp. When the library dilution was 10^{-2} , a clear band of ~289 bp was indeed seen (Fig. 2A), whereas at 10^{-3} dilution, the target gene could not be amplified. This suggested the limited library dilution that contained the target clone was defined at 10^{-2} , which included 10^4 independent clones.

After primary screening by PCR detection, we obtained one strong primary positive signal pool and three secondary positive signal pools. The strongest pool was selected and plated on single LB agar plates each containing ~300 independent clones. One microliter of eluate of each group was detected by PCR, and two positive signal pools were obtained by the third screening. The probability ratio of positive clones in this pool was above 1:10. By choosing one stronger positive signal pool to plate, we found the target clone by PCR detection. Positive phage was then converted to plasmids; the insert DNA was identified by enzymatic digestion with Sfi I. Four bands were seen before digestion; Those DNA represented different models of pTriplEx2 plasmid. After digestion, only one band of ~550 bp was seen in addition to the plasmid DNA (Fig. 2B). We suggest that it is corresponding to *RPL30* gene (also see Fig. 2D and Fig. 3A).

Further sequence analysis showed the 535 bp positive cDNA in Fig. 2B possessed a 348-bp ORF with polyA tail signal AATAA and a polyA tail, coding 115 aa (Fig. 3A). This gene had 100% identity to ribosomal protein L30 from mouse, rat, and human by amino acid sequence and an ~91% identity to ribosomal protein L30 from mouse (NM009083), rat (NM022699), and human (NM000989) by nucleotide sequence. The PI of this protein was 9.65, with a molecular weight of 12.8 kDa determined by ANTHERPROT software.

Screening of *HMGB1* cDNA

Our results showed that the No. 2 cDNA that appeared most frequently during PCR screening of recombined cDNAs from the constructed library was 1,242 bp (Fig. 2C). We suggest that it is corresponding to *HMGB1* gene (also see Fig. 2E and Fig. 3B).

Our analysis also showed the 1,242 bp cDNA that appeared most frequently during PCR screening (Fig. 2C) possessed a 648-bp ORF with polyA tail, coding 215 aa (Fig. 3B). This gene had 99% identity to *HMGB1* from mouse, rat, and human by amino acid sequence, and an ~91% identity to *HMGB1* from mouse (NM010439), rat (NM012963), and human (NM002128) by nucleotide sequence. The PI of this protein was 5.66, with a molecular weight of 30 kDa confirmed by ANTHERPROT software analysis.

Northern Blot Analysis

Northern blots probed with *RPL30* cDNA was performed for further confirmation. β -actin was as a control. The level of *RPL30* mRNA in the neural tube tissues was high from E8d~E12d and increased gradually during neurulation and significantly in the E8.5d and E12d groups, whereas the level decreased in the H8.5d NTD group (Fig. 2D)

Expression of *HMGB1* mRNA shown by Northern blot was high during neurulation, increased gradually and significantly at E10d, and was also high in the neural tube tissues at E11d, E12d, whereas the expression decreased in the H8.5d NTD group (Fig. 2E).

DISCUSSION

According to Clarke-Carbon's formula, a mammalian cDNA library should contain at least 1.7×10^5 independent clones (Clarke & Carbon, 1976; Joseph & Russell; 2001). These results confirm the construction of a successful cDNA library that could meet almost all requirements of finding a cDNA clone derived from limited mRNA. Using SMART (switching mechanism at 5' end of RNA transcription) technique we construct this cDNA library consisting of full-length transcripts (Fig. 1D shows). The fact that golden hamster β -actin segment can be obtained from this cDNA library confirms the library was really a cDNA library of golden hamster neural tube. These results, for the first time, identify that it was a successful cDNA library. This library may represent a valuable resource for gene cloning and the study of neurulation.

There are many approaches to library screening, including nucleic acid hybridization and immune screening (Sioud et al., 2000; Appenzeller et al., 2001; Campbell & Choy, 2002). SSS is characterized by greater simplicity, rapidity, lower cost, higher efficiency, and higher information harvest compared with traditional methods (Qu et al., 2003). With SSS, the cDNA phage plate was divided into several blocks and target genes were available screened from the library by PCR. PCR-based strategies for pooling schemes make the tedious task of library screening less labor-intensive and more cost-efficient. Hence this novel method of obtaining the target genes may provide a complement to the large EST full length clone libraries and whole genome sequencing.

Neurulation results in the formation of neural tube, which occurs in two phases in vertebrate embryos, primary and secondary neurulation. Primary neurulation includes the formation of neural plate and subsequent morphogenetic movements that transform it into a neural tube and the closure of tube from the head to the level of future somite 31. This stage is the most significant phase of neurulation from a clinical perspective because abnormalities in this phase results in open neural tube defects. In the golden hamster, neurulation occurs from E8d to E10d; E8.5d is the crucial stage for primary neurulation. Factors affecting the neurulation process come from inside and outside the neural tube; many are some conserved genes regulating morphogenesis or some oncogenes (Schoenwolf & Smith, 2000).

RPL30 is one of the abundant RP genes, as the highly conserved gene family, it is composed of 60S ribosomal subunits and maintains cell growth and survival. *RPL30* gene is similar to *RPL32*, which has secondary functions for morphogenesis. Recent research showed that *RPL30* may be oncogenes, it represents a novel group involved in translational regulation and is associated with outcome in medulloblastoma (De et al., 2006). Furthermore, a temperature shift can cause immediate inhibition of transcription and translation of all ribosomal proteins (Wool et al., 1995), but there are no reports about the role of the *RPL30* gene with temperature shift in the development of neural tube and NTDs caused by hyperthermia.

Our findings suggest that, in response to neurulation, the transcription of *RPL30* mRNA is enhanced. As a result, all genes relevant to the *RPL30* were also upregulated. In contrast, the transcription of *RPL30* mRNA is severely inhibited in response to hyperthermia, and as a consequence, the biosynthesis of several proteins, some of which were involved in neural tube development, was reduced or stopped, inhibiting normal closure of the neural tube and possibly causing neural tube defects. Hence *RPL30* may play an essential role during neurulation and the risk of NTDs.

During PCR screening of recombined cDNAs from the constructed library, a recombined plaque appeared frequently. The cDNA insert was found to be a full-length cDNA of *HMGB1*. However, there was no information about *HMGB1* mRNA of the golden hamster in Genebank; our current study firstly obtains this information. *HMGB1* has been suggested to be involved in neurite outgrowth, differentiation of neurons, and migration of neural crest cells during development (Fossati & Chiarugi, 2007; Chou et al., 2001). Significantly, *HMGB1*-*SGC* interaction is important for cell-cell recognition and cell migration. *SGC* (sulfoglucuronyl sargbohydrate) has been implicated in cell-cell recognition and migration of neural crest cell (Chou et al., 2004; Zhao et al., 2000). Neural crest cells begin migration before neural tube closure. In the cranial region, neural crest cells migrate from the neural folds early in the process of elevation, which is necessary for neural tube closure (Schoenwolf & Smith, 2000). However, the *HMGB1* gene has not previously been associated with neurulation and NTD pathogenesis.

Our Northern blot analysis suggested that *HMGB1* mRNA was increased during neurulation, this may enhance *HMGB1* protein biosynthesis and improve *HMGB1*-*SGC* interaction, which is very important in the migration of neural crest cells. In H8.5d NTD group, hyperthermia decreased the transcription of *HMGB1* mRNA, induced the biosynthesis of *HMGB1* protein, affected *HMGB1*-*SGC* interaction, and inhibited migration of neural crest cells, resulting in an inability of the neural folds to fuse in the midline and a failure of the neural tube to close, producing NTDs.

The important stage for primary neurulation is at E8.5d, which is the sensitive period in golden hamster. After a temperature shock (42°C for 20 min), transcription of *HMGB1* mRNA was decreased, biosynthesis of *HMGB1* protein was induced, and the *HMGB1*-*SGC* interaction was decreased; these changes might inhibit migration of neural crest cells and closure of the neural tube. *HMGB1* protein, which plays a key role in the assembly of transcription initiation

and enhanceosome complexes, might act as a compensatory modulator of transcription in response to temperature and thus as a global gene expression temperature sensor (Podrabsky & Somero, 2004). Our research confirmed this view. It appears likely that the variation of HMGB1 expression plays a crucial role in the pathogenesis of NTDs caused by hyperthermia. Our data indicated that *RPL30* and *HMGB1* genes are required for neurulation.

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Fig. 1.

The construction of a cDNA library from neural tube taken from golden hamster embryos. (A, left) An example of E8.5d golden hamster embryo. (A, right) Total RNAs isolated from the neural tube of a E8.5d golden hamster embryo. The dscDNA (B) was amplified by LD-PCR, appearing as a smear of 0.1–5 kb with some bright bands at ~2.0 kb corresponding to abundant mRNA. The titer of primary cDNA library was 1.5×10^6 pfu/ml by calculation of the plaques (C). Eleven cDNA inserts were released by PCR amplification to detect the quantity of the library. The PCR products were larger than 0.5 kb, and the average insert of the recombinants was 1.0 kb long. Marker DL2000 was used (D); β -actin was amplified from the constructed library, and the PCR product was seen as a clear band of about 290 bp (E).

Fig. 2.

Expression analysis of RPL30 and HMGB1. The PCR product of *RPL30* from the diluted library; a clear band of about 289 bp can be seen (A). Results of enzyme digestion of the positive plasmid are shown (B). Four bands were seen before enzyme digestion; those DNA represented different models of pTriplEx2 plasmid. After enzyme digestion, one band of about 550 bp is seen in addition to the plasmid DNA (B). The results of enzyme digestion of No. 2 plasmid are shown (C). Four bands were seen before enzyme digestion; those DNA represented different models of pTriplEx2 plasmid; After enzyme digestion, one band about 1300 bp is seen in addition to the plasmid DNA (C). Expression of *RPL30* mRNA in neural tube during neurulation (D). Northern blot was performed with RNA from neural tube tissues. The blot

was probed with inserts from the *RPL30* cDNA and β -actin (control) as indicated. Each lane contains 30 μ g of total RNA. The level of *RPL30* mRNA in the neural tube tissues was high from E8d~E12d and increased gradually during neurulation and significantly in the E8.5d and E12d groups, whereas the level decreased in the H8.5d NTD group. Expression of *HMGB1* mRNA in neural tube during neurulation (E). Northern blot was performed with RNA from neural tube tissues. Blot was probed with inserts from *HMGB1* cDNA and β -actin (control) as indicated. Each lane contains 30 μ g of total RNA, Expression of *HMGB1* mRNA shown by Northern blot was high during neurulation from E8d-E12d, increased gradually and significantly at E10d, and was also high in the neural tube tissues at E11d, E12d, whereas the expression decreased in the H8.5d NTD group (E represents Embryos, and H represents Embryos with Hyperthermia treatment in D and E).

A.

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1  ggggggtgtagaagagcctggcgtgdtgogcgctcctcttctctgctgcccggccatc
61  ttggcggctgogcttggtttgggggcccgcgcgcgcaocggaggcaggaagatggtggcc
                                     M V A
121 gcaaaagaagcgaagaagtccttgagtcacatcaggtccagctggtgatgaag
   A K K T K K S L E S I N S R L Q L V M K
181 agcggcaagtagctgctgggtacaagcagacgctcaagatgatcggcagggcaagcc
   S G K Y V L G Y K Q T L K M I R Q G K A
241 aagdtggtatcctcgccaaactgcccggccttgaggaaatctgaatcgagtactat
   K L V I L A N N C P A L R K S E I E Y Y
301 gccatgttggctaagactggtgtccatcactacagtggcaataacatogagctggcaca
   A M L A K T G V H H Y S G N N I E L G T
361 gogtgggaaatadacagagtgtgcacatggctatcattgacccaggtgactctgat
   A C G K Y Y R V C T L A I I D P G D S D
421 attattagaagatgccagaacagactggtgagaagtagtaaacagaaaagcctgcctt
   I I R S M P E Q T G E K *
481 taataaaaccccccagctctcttcaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

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B.

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1  gcagtagcattgagctccatagagacagcgcgggggcaagcgcgagccggagcggcactgg
61  gcgactctgtgcctcgaggacgaaatcatctaaacatgggcgaagagatcctaagaag
                                     M G K G D P K K
121 ccgagaggcaaaatgtcctcatatgcattctttgtgcaaaacttgccgggaggaacgcaag
   P R G K M S S Y A F F V Q T C R E E R K
181 aagaagcaccggatgcttctgtcaacttctcagagtctcgaagaagtgtctcagaagg
   K K H P D A S V N F S E F S K K C S E R
241 tggaaagaccatgtctgctaaagaaaaggggaaatttgaagacatggcaaggctgacaag
   W K T M S A K E K G K F E D M A K A D K
301 gctcgttatgaaagagaaatgaaaacctacatccccccaaaggggagacccaaaagaag
   A R Y E R E M K T Y I P P K G E T K K K
361 ttcaaggaccccaatgcacccaagaggcctccttcggctttcttctgttctgttctgag
   F K D P N A P K R P P S A F F L F C S E
421 tatcgccccaaaatcaaggagaaacacctggtttgttcattggtgatgttgcaagaaa
   Y R P K I K G E H P G L S I G D V A K K
481 ctgggagagatgtggacaacactgctgcagatgacaagcagccctatgaaaagaaggct
   L G E M W N N T A A D D K Q P Y E K K A
541 gccaaagctgaaggagaagtacgaaaggatattgctgttacagagctaaaggaaaaccc
   A K L K E K Y E K D I A A Y R A K G K P
601 gatgcagcgaaaaaggggtggtcaaggcagaaaagagcaagaaaaggaaggaaagaa
   D A A K K G V V K A E K S K K K K E E E
661 gatgatgaggaagatgaaggagtagggaaggaggaggaagaggaagacgaagatgaa
   D D E E D E E D E E E E E E E D E D E
721 gaagaagatgatgatgatgaattaagttggttctagcgcagtttttttgtctataaagca
   E E D D D D E *
781 ttttaacccccctgtacacaactcactccttttaagaaaaaaattgaaatgtaaggctg
841 tgtgaagatttgttttaactgtacagtgtcttttttgtatagttaacacactaccgaa
901 tgtgtcttttagatagccctgtcctggttggtattttcagtagccactaaccttgccctgta
961 cagctcgggggttgtaattggcatggaatttaagcaggttcttggttggtgcacagca
1021 caaattagttatatatgggacggtagttttctttttttcttttttttccactcttcag
1081 ttgtctctgatgcagcttatcgaagataattgttctgttaactgaataccactctgtaa
1141 ttgcaaaaaaaattgcagctgtttgttgacattctgaatgcttctaagtaaatacaat
1201 ttttttatcaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

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M means MET * means terminator

Fig. 3.

Nucleotide and predicated amino acid sequence of the RPL30 and HMGB1 genes of golden hamster. Amino acid sequence of the gene *RPL30* inferred from the nucleotide sequence is represented below the DNA sequence with one-letter amino acid abbreviation (A). Amino acid sequence of the gene *HMGB1* inferred from the nucleotide sequence is represented below the DNA sequence with the one-letter amino acid abbreviations (B).