Tandem Organization of Medaka Fish Soluble Guanylyl Cyclase \( \alpha_1 \) and \( \beta_1 \) Subunit Genes

**IMPLICATIONS FOR COORDINATED TRANSCRIPTION OF TWO SUBUNIT GENES**

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We determined the complete nucleotide sequences of the \( \alpha_1 \) subunit gene (\( \text{OGICS-} \alpha_1 \)) and the \( \beta_1 \) subunit gene (\( \text{OGICS-} \beta_1 \)) of medaka fish soluble guanylyl cyclase. In the genome, \( \text{OGICS-} \alpha_1 \) and \( \text{OGICS-} \beta_1 \) are organized in tandem. The two genes are only 986 base pairs apart and span approximately 34 kilobase pairs in the order of \( \text{OGICS-} \alpha_1 \) and \( \text{OGICS-} \beta_1 \). The nucleotide sequence of a large part of the 5'-upstream region of \( \text{OGICS-} \alpha_1 \) is complementarily conserved in that of \( \text{OGICS-} \beta_1 \). To analyze the promoter activity of each gene, a fusion gene construct in which the 5'-upstream region was fused with the green fluorescent protein gene was injected into medaka fish 2-cell embryos. When the fusion gene containing the \( \text{OGICS-} \alpha_1 \) upstream region was injected, green fluorescent protein fluorescence was detected in the embryonic brain. The 5'-upstream region of \( \text{OGICS-} \beta_1 \) alone was insufficient for the reporter gene expression in the embryos. When the \( \text{OGICS-} \alpha_1 \) upstream region was located upstream of the \( \text{OGICS-} \beta_1 \), green fluorescence protein fusion gene, the reporter gene was expressed in the brain and trunk region of the embryos. These results suggest that the 5'-upstream region of \( \text{OGICS-} \alpha_1 \) can affect the expression of \( \text{OGICS-} \beta_1 \). It is therefore possible that the expression of \( \text{OGICS-} \alpha_1 \) and \( \text{OGICS-} \beta_1 \) is coordinated.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) AB022280 (\( \text{OGICS-} \alpha_1 \)) and AB022281 (\( \text{OGICS-} \beta_1 \)).

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† The abbreviations used are: NO, nitric oxide; GC, guanylyl cyclase; \( \text{OGICS-} \text{Oryza latipes} \) soluble GC; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; GFP, green fluorescent protein; bp, base pair(s); CAPS, 3-cyclohexylamino)propanesulfonic acid; BFP, blue fluorescent protein.

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Isolation of Genomic Clones for OlGCS-α1 and OlGCS-β1—A genomic library of medaka fish (white strain) constructed in the Lambda Fix II vector was purchased from Stratagene and used for the isolation of genomic clones for OlGCS-α1 and OlGCS-β1. The cDNA fragments (nucleotide positions 1290 to 2346 for OlGCS-α1 cDNA and nucleotide positions 241 to 1229 for OlGCS-β1 cDNA) amplified by polymerase chain reaction (PCR) were labeled with digoxigenin-dUTP using the digoxigenin-High Prime (Roche Molecular Biochemicals) and used for screening as probes. Finally, four positive clones were obtained from approximately 1.35 \times 10^6 recombinant phages. Phage DNA was purified by using the QIAGEN Lambda kit (QIAGEN), and the insert DNA was subcloned into pBluescript II KS(-) (Stratagene).

The sequence of the insert DNA was determined by the dideoxynucleotide chain termination procedure (30) with an Applied Biosystems 373A or PRISM 377 DNA sequencer and analyzed on DNASIS software.

FIG. 1. Genomic structure of OlGCS-α1 and OlGCS-β1. The exons are indicated by open boxes (noncoding regions) or solid boxes (coding region). The exons of OlGCS-α1 and OlGCS-β1 are indicated as a and b, respectively, followed by a number. Four isolated clones (A2, A11, B5, B16) and restriction enzyme sites are shown below the scheme of the genomic structure. E, EcoRI; H, HindIII; kbp, kilobase pairs.

FIG. 2. The nucleotide sequences of the 5'-upstream region of OlGCS-α1. The first nucleotide of exon 1 was determined by the primer extension method and numbered as +1. An open box indicates the exon, and the initiation and stop codons are shaded. A putative TATA box is double-underlined, and the consensus sequences of known cis-regulatory elements are underlined.

TABLE I

| Exon no. | Exon size | Intron size | 5'-Splice site sequence | 3'-Splice site sequence |
|----------|-----------|-------------|------------------------|------------------------|
|          | bp        | bp          |                        |                        |
| OlGCS-α1 |           |             |                        |                        |
| 1        | 433       | 238         | GGG gtgagt . . . . . .  | gtacag TTT             |
| 2        | 68        | 635         | AAG gtaact . . . . . .  | tgacag CAC             |
| 3        | 89        | 142         | CAG gtagct . . . . . .  | tgacag CAA             |
| 4        | 716       | 900         | AAG gtagct . . . . . .  | ttccag CCC             |
| 5        | 486       | 3645        | AAG gtagct . . . . . .  | ttccag GTA             |
| 6        | 144       | 3391        | CAG gtagag . . . . . .  | cctcag ATG             |
| 7        | 155       | 283         | CAG gtagag . . . . . .  | tccccag ATT             |
| 8        | 469       | 497         | GGA gtaaag . . . . . .  | acaag TAA             |
| 9        | 295       |             |                        |                        |
| OlGCS-β1 |           |             |                        |                        |
| 1        | 252       | 269         | ATG gtaagt . . . . . .  | ttccag TAT             |
| 2        | 74        | 1834        | CAA gtaagt . . . . . .  | ttgac GAG             |
| 3        | 101       | 77          | TAA gtaagt . . . . . .  | ttcag AGA             |
| 4        | 119       | 2422        | CAG gtaagc . . . . . .  | ttcag GTA             |
| 5        | 198       | 998         | AAG gtcagc . . . . . .  | ttcag GTA             |
| 6        | 231       | 112         | CAG gttgagc . . . . . .  | cctcag AGA             |
| 7        | 117       | 2653        | AAG gtaagc . . . . . .  | ttccag GTA             |
| 8        | 134       | 742         | CAG gtagac . . . . . .  | gtttag TGT             |
| 9        | 198       | 1056        | TAG gtttaga . . . . . .  | cccacag ACT             |
| 10       | 238       | 554         | AAG gtaaat . . . . . .  | aaacag GTG             |
| 11       | 141       | 2291        | CAG gtagga . . . . . .  | ttgac GAG             |
| 12       | 155       | 1182        | CAG gtagga . . . . . .  | ttgac GAG             |

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FIG. 3. The nucleotide sequences of the 5′-upstream region of OlGCS-β. The first nucleotide of exon 1 was determined by the primer extension method and numbered as +1. An open box indicates the exon, and the initiation and stop codons are shaded. A putative TATA box is double-underlined, and the consensus sequences of known cis-regulatory elements are underlined.

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3′-rapid amplification of cDNA ends (3′-RACE)—Total RNA was extracted from Day 9 embryos according to the acid guanidinium thiocyanate-phenol-chloroform extraction method (31). The first strand synthesis and first PCR were performed using the 3′-Full RACE Core Set (Takara Shuzo Co., Ltd.). The specific primers for OlGCS-α, used for amplification as were as follows: LF-1 for first PCR, 5′-GTGCAACTCTTGTATGTTTC-3′ (identical to nucleotides 2522–2542). Second PCR was performed using a 1/50 volume of the first PCR products as a template. The PCR products were subcloned into pBluescript II KS(-) (Stratagene), and the sequence of the insert DNA was determined as described above.

Primer Extension Analysis—Total RNA was extracted from the adult medaka fish brain as described above. Poly(A)−RNA was isolated using Oligotex-dT30 Super (Roche) according to the manufacturer’s protocol. The oligonucleotides used for the primer extension experiments were as follows: PE-L1 for OlGCS-α, 5′-ACGACGATGCGCTCGAG-3′ (complementary to nucleotides 97–114) and PE-S3 for OlGCS-β, 5′-ATGCTGAGATTGCCGTTGAGT-3′ (complementary to nucleotides 2522–2542). The genomic DNA (10 μg) was digested overnight with HindIII and StuI and cloned into the corresponding site of pBluescript II KS(-) (Stratagene), and the sequence of the insert DNA was determined as described above.

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RESULTS

Isolation and Characterization of Medaka Fish OIGCS-α1 and OIGCS-β1 Genes—A medaka fish genomic library was screened with cDNA fragments of OIGCS-α1 or OIGCS-β1. After repeated screening, four different clones (A2, A11, B5, B16) were obtained. Restriction enzyme mapping of these clones demonstrated that they overlap each other (Fig. 1). The nucleotide sequence of the insert DNA of each clone was determined and compared with that of the respective cDNA. As shown in Fig. 1 and Table I, OIGCS-α1 consists of 9 exons and OIGCS-β1 consists of 13 exons. The GT-AG rule was conserved for all splice sites except exon/intron 8 in OIGCS-α1 (Table I). In the medaka fish genome, OIGCS-α1 and OIGCS-β1 are 986 bp apart and organized in tandem. The two genes span approximately 34 kilobase pairs in the order of OIGCS-α1 and OIGCS-β1.

In a previous study, the nucleotide sequence of a part of the 3'-noncoding region of the cDNA for OIGCS-α1, which should contain a polyadenylation signal sequence, has not been determined (28). To determine the nucleotide sequence of the 3'-end of OIGCS-α1, 3'-RACE was performed using total RNA from the Day 9 embryos. The 3'-RACE product with LF-2 primer contained the nucleotide sequence corresponding to that of intron 8 in OIGCS-α1. The 3'-RACE with LF-4 primer, which was designed to cross the insert site of the intron 8, produced a 344-bp cDNA fragment. Each 3'-RACE product contained a putative polyadenylation signal sequence, AATAAA, 24–18 bp upstream of poly(A).

Analysis of the 5'-Upstream Regions of OIGCS-α1 and OIGCS-β1—To determine the transcription initiation sites of OIGCS-α1 and OIGCS-β1, primer extension experiments were performed using medaka fish brain poly(A)+ RNA (2 μg). Using a specific primer for OIGCS-α1, one major and two minor bands were detected. The nucleotide corresponding to the major band, which is located most upstream of the three bands, was assigned to the transcription initiation site for OIGCS-α1 (Fig. 2). In the same way, the nucleotide corresponding to one detected band was assigned to the transcription initiation site for OIGCS-β1. A putative TATA box, TATAGAA, is present 30–25 bp upstream of the transcription initiation site of OIGCS-β1 (Fig. 3). There is no TATA box in the corresponding region of OIGCS-α1, although a TATA box consensus sequence, TATAGATA, is present at 141–136 bp upstream of the transcription initiation site for OIGCS-α1 (Fig. 2). A number of E-boxes are present in the 5'-upstream regions of each gene (Fig. 4). Other known cis-regulatory elements, AP1, MEF-2, GATA, Sp1, CREB, and C/EBP binding sequences, are found in the 5'-upstream region of OIGCS-α1. On the other hand, there is one GATA, AP1, and two C/EBP binding sequences in the 5'-upstream region of OIGCS-β1.

Comparison of the nucleotide sequence of the 5'-upstream region of OIGCS-α1 with that of the intervening region between OIGCS-α1 and OIGCS-β1 demonstrated that the nucleotide sequences of about 330 bp (–1061 to –733 for OIGCS-α1, –370 to –701 for OIGCS-β1) are complimentarily conserved (Fig. 5). The conserved sequences are divided into six highly conserved regions (I, –1046 to –977 for OIGCS-α1; II, –971 to –953 for OIGCS-α1; III, –942 to –888 for OIGCS-α1; IV, –850 to –840 for OIGCS-α1; V, –824 to –791 for OIGCS-α1; VI, –753 to –742 for OIGCS-α1). Some of these regions contain several
consensus sequences of cis-regulatory elements, such as an E-box and C/EBP, which could be used for the transcription of both \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \).

**Genomic Southern Hybridization**—In a previous study we performed a Southern hybridization analysis using medaka fish genomic DNA to examine whether \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \) exist as a single copy (28). However, the results were not clear because the cDNA fragments used as probes crossed over many introns. Therefore, in this study we performed a genomic Southern analysis using a different probe containing a single exon (exon 5 of \( \text{OlGCS-} \alpha_1 \)). As shown in Fig. 6, only one major band was detected in each of the three lanes. The size of the band in each lane is consistent with that of the DNA fragments obtained from the digestion of genomic clones by the respective restriction enzymes. These results suggest that \( \text{OlGCS-} \alpha_1 \) is a single copy gene.

**Promoter Analysis of \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \)—**To examine whether the 5'-upstream region of \( \text{OlGCS-} \alpha_1 \) and the intervening region between \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \) have promoter activity, a fusion gene containing the 5'-upstream region of each gene and the GFP gene was constructed and named \( \text{AG-} \alpha_1 \text{ or } \text{BG-}1 \), respectively (Fig. 4). The fusion gene was injected into the cytoplasm of both blastomeres of medaka fish 2-cell stage embryos. At 7 days after fertilization, the number of live embryos and embryos with GFP fluorescence was counted (Table II). GFP fluorescence was detected in the brain and somite of embryos injected with \( \text{ABBG-}1 \) (Table II). On the other hand, the reporter gene expression was not observed when a short upstream region (from -226 to +154) of \( \text{OlGCS-} \beta_1 \) containing a putative TATA box and a transcription initiation site was removed from \( \text{ABBG-}1 \) (Table II, \( \text{ABBG-2} \)). These results suggest that the 5'-upstream region of \( \text{OlGCS-} \alpha_1 \) can activate transcription of \( \text{OlGCS-} \beta_1 \) in conjunction with the intervening region between \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \).

Although \( \text{ABBG-1} \) and \( \text{ABBG-2} \) contain the GFP gene driven by the 5'-upstream region of \( \text{OlGCS-} \alpha_1 \), GFP fluorescence was not observed in embryos injected with these constructs (data not shown). This might be because of much weaker fluorescence intensity of GFP than that of the enhanced variant of GFP used in this study.

**DISCUSSION**

In this study we demonstrated that the 5'-upstream region of \( \text{OlGCS-} \alpha_1 \) is essential for the expression of \( \text{OlGCS-} \alpha_1 \). On the other hand, the intervening sequence between \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \) seemed not to be sufficient for \( \text{OlGCS-} \beta_1 \) expression. Considering the tandem organization of \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \), both genes might be cotranscribed as a single polycistrionic mRNA as in the mouse and human upstream of the GDF gene (\( \text{UOG-}1 \)) and the growth/differentiation factor-1 gene (\( \text{GDF-}1 \)) (33). Alternatively, other regions such as the 5'-upstream region and/or intron of \( \text{OlGCS-} \alpha_1 \) would affect the transcription of \( \text{OlGCS-} \beta_1 \). Our results support the latter possibility. First, GFP fluorescence was observed in embryos injected with the \( \text{ABBG-2} \) construct in which the 5'-upstream region of \( \text{OlGCS-} \alpha_1 \) was located upstream of the \( \text{OlGCS-} \alpha_1 / \text{OlGCS-} \beta_1 \) intervening sequence followed by the GFP gene. Second, there is a transcription initiation site for \( \text{OlGCS-} \beta_1 \) and a TATA box consensus sequence, TATAGAA, 30–25 bp upstream of the transcription initiation site for \( \text{OlGCS-} \beta_1 \). The reporter gene expression was abolished when the TATA box and the transcription initiation site were removed from \( \text{ABBG-1} \). Therefore, basal transcription factors probably act on the intervening region between \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \), and an enhancer in the \( \text{OlGCS-} \alpha_1 \) upstream region can affect the promoter activity.

The above could make it possible to temporally and spatially coordinate the transcription of \( \text{OlGCS-} \alpha_1 \) and \( \text{OlGCS-} \beta_1 \) during development.
the embryogenesis of the medaka fish.

Sequence analysis revealed that a number of E-boxes, which are known to play a critical role in nerve and muscle differentiation (34), are present in the 5'-upstream region of each gene. This is consistent with our detection of GFP fluorescence in the brain of Day 4 embryos injected with the 5'-upstream region of the OIGCS-α1-GFP construct. Scholz et al. (35) have demonstrated that NO synthase and NO-sensitive guanylyl cyclase are broadly distributed in the central nervous system of lobsters at hatching. The participation of the NO/cGMP signaling pathway in synaptogenesis has also been reported (22). Detection of GFP fluorescence in the brain of Day 4 embryos suggests a relation between soluble GC and neuronal development during the embryogenesis of the medaka fish. The ratio of embryos with GFP fluorescence to live embryos 7 days after fertilization was relatively low (14.8%). Although a higher concentration of DNA solution was able to increase the number of embryos with GFP fluorescence, it tended to cause morphological abnormalities and/or death (data not shown).

It has previously been demonstrated that the mRNA level of each subunit gene is decreased by cAMP (24, 25). In this regard, it should be noted that a cAMP-response element is present in the 5'-upstream region of OIGCS-α1. This element may participate in the regulation of transcription with a cAMP-response element modulator, which is known to inhibit transcription by binding to the cAMP-response element (36). There is one GATA and one MEF-2 binding sequence in the 5'-upstream region of OIGCS-α1, and it has been suggested that these sequences may participate in the differentiation of vascular smooth muscle cells (37–39). Considering that soluble GC induces the relaxation of vascular smooth muscle in mammals (1), these elements may regulate the expression of both genes in the medaka fish. In addition, others including Sp1, C/EBP, and AP1 in the 5'-upstream region of OIGCS-α1 and OIGCS-β1 may also be involved in the regulation of expression of both genes for soluble GC subunits in the medaka fish (40).

The nucleotide sequences of the 5'-upstream region of OIGCS-α1 are highly conserved in relation to that of the intervening region between OIGCS-α1 and OIGCS-β1 in six different regions. Some of these regions have cis-regulatory elements in common with each other, suggesting that the highly conserved regions also participate in coordinated transcription.

Adenylyl cyclase, which synthesizes another second messenger, cAMP, also has two catalytic domains punctuated by a

![Fig. 7. Promoter analysis and expression of GFP in medaka fish embryos injected with fusion genes.](image)
membrane-spanning domain (41). The primary structure of each catalytic domain of adenylyl cyclase is conserved in those of the soluble and membrane forms of GCs (5). It has been demonstrated that adenylyl cyclase as well as soluble GC require the two catalytic domains for cyclase activity (41) and that changes in a couple of the amino acids in the catalytic domain of adenylyl cyclase cause a functional change in adenylyl cyclase from cAMP production to cGMP production (42). This suggests that soluble GC is evolutionarily related to adenylyl cyclase, although their forms are quite different. In this study, we determined the complete structure of \textit{OlGCS-a1} and \textit{OlGCS-b1}, demonstrating that the two genes are tandemly organized like a single gene. A comparison of the genomic structure between soluble GC and adenylyl cyclase may clarify the evolutionary relationship between both enzymes, although the genomic structure of the latter enzyme has not yet been reported.

The soluble form of GC is present as a heterodimer, and the coexpression of both subunits is required for generating enzyme activity (13, 14). It has been reported that two functionally related genes such as collagen IV α1 and α2 chain genes are coordinately expressed by a bidirectional promoter (43). In this regard, our results presented here suggest the possibility of temporally and spatially coordinated transcription of both subunit genes for soluble GC during embryogenesis.

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REFERENCES

1. Drewett, J. G., and Garbers, D. L. (1994) \textit{Endocrinol. Res.} 15, 135–162
2. Holscher, C. (1997) \textit{Trends Neurosci.} 20, 298–303
3. Garbers, D. L., Koesling, D., and Schultz, G. (1994) \textit{Mol. Biol. Cell} 5, 1–5
4. Kamisaki, Y., Saheki, S., Nakane, M., Palmieri, J. A., Kuno, T., Chang, B. Y., Waldman, S. A., and Murad, F. (1986) \textit{J. Biol. Chem.} 261, 7236–7241
5. Nakane, M., Arai, K., Saheki, S., Kuno, T., Buechler, W., and Murad, F. (1990) \textit{J. Biol. Chem.} 265, 16841–16845
6. Wedel, B. J., and Garbers, D. L. (1998) \textit{Trends Endocrinol. Metab.} 9, 213–219
7. Koelsing, D., Herz, J., Gausepohl, H., Nieromand, F., Hinsch, K.-D., Mulsch, A., Böhm, E., Schultz, G., and Frank, R. (1988) \textit{FEBS Lett.} 239, 29–34
8. Koelsing, D., Harteneck, C., Humbert, P., Bosserhoff, A., Frank, R., Schultz, G., and Böhm, E. (1999) \textit{FEBS Lett.} 266, 128–132
9. Nakane, M., Saheki, S., Kuno, T., Ishii, K., and Murad, F. (1988) \textit{Biochem. Biophys. Res. Commun.} 157, 1139–1147
10. Giuili, G., Scholl, U., Bulle, F., and Guelläen, G. (1992) \textit{FEBS Lett.} 304, 83–88
11. Harteneck, C., Wedel, B., Koelsing, D., Malkevitch, J., Böhm, E., and Schultz, G. (1991) \textit{FEBS Lett.} 292, 217–222
12. Yuen, P. S. T., Potter, L. R., and Garbers, D. L. (1990) \textit{Biochemistry} 29, 10872–10878
13. Harteneck, C., Koelsing, D., Soling, A., Schultz, G., and Böhm, E. (1990) \textit{FEBS Lett.} 272, 221–223
14. Buechler, W. A., Nakane, M., and Murad, F. (1991) \textit{Biochem. Biophys. Res. Commun.} 174, 351–357
15. Gupta, G., Azam, M., Yang, L., and Danziger, R. S. (1997) \textit{J. Clin. Invest.} 100, 1488–1492
16. Pfeifer, A., Klatt, P., Massberg, S., Ny, L., Saubier, M., Hirinejí, C., Wang, G.-X., Korth, M., Azzid, A., Anderson, K.-E., Kronbach, F., Mayerhofer, A., Ruth, P., Fassler, R., and Hofmann, F. (1998) \textit{EMBO J.} 17, 3045–3051
17. Haley, J. E., Wilcox, G. L., and Chapman, P. F. (1992) \textit{Neuron} 8, 211–216
18. Zhuo, M., Hu, Y., Schultz, C., Kandel, E. R., and Hawkins, R. D. (1994) \textit{Nature} 368, 635–639
19. Kendrick, K. M., Guevara-Guzman, R., Zorrilla, J., Hinton, M. R., Broad, K. D., Mimmack, M., and Ohkura, S. (1997) \textit{Nature} 386, 670–674
20. Koch, K.-W., Lambrecht, H.-G., Haberecht, M., Redburn, D., and Schmidt, H. H. W. (1994) \textit{EMBO J.} 13, 3312–3320
21. Savchenko, A., Barnes, S., and Kramer, R. H. (1997) \textit{Nature} 390, 694–698
22. Truman, J. W., De Vente, J., and Ball, E. E. (1996) \textit{Development} 122, 3949–3958
23. Wang, T., Xie, Z., and Lu, B. (1995) \textit{Nature} 374, 262–266
24. Shimouchi, A., Janssens, S. F., Bloch, D. B., Zapol, W. M., and Bloch, K. D. (1993) \textit{Am. J. Physiol.} 265, L456–L461
25. Papapetropoulos, A., Marezin, N., Mora, G., Mili, A., Murad, F., and Catravas, J. D. (1995) \textit{Hypertension} 26, 696–704
26. Giuili, G., Boccelli, N., Scholl, U., Mattei, M.-G., and Guelläen, G. (1993) \textit{Hum. Genet.} 91, 257–260
27. Azam, M., Gupta, G., Chen, W., Wellington, S., Warburton, D., and Danziger, R. S. (1998) \textit{Hypertension} 32, 149–154
28. Miki, T., Kusakabe, T., and Suzuki, N. (1998) \textit{Eur. J. Biochem.} 253, 42–48
29. Seimiya, M., Kusakabe, T., and Suzuki, N. (1997) \textit{J. Biol. Chem.} 272, 23407–23417
30. Saper, F., Nieken, S., and Coulson, A. R. (1977) \textit{Proc. Natl. Acad. Sci. U. S. A.} 74, 5463–5467
31. Chomczynski, P., and Sacchi, N. (1987) \textit{Anal. Biochem.} 162, 156–159
32. Iwamatsu, T. (1983) \textit{J. Exp. Zool.} 228, 83–89
33. Blumenthal, T. (1998) \textit{Bioessays} 20, 480–487
34. Jan, Y.-N., and Jan, L. Y. (1993) \textit{Cell} 75, 827–830
35. Scholz, N. L., Chang, E. S., Graubard, K., and Truman, J. W. (1998) \textit{J. Neurosci.} 18, 208–226
36. Foulkes, N. S., Borrelli, E., and Sassone-Corsi, P. (1991) \textit{Cell} 64, 739–749
37. Firulli, A. B., Miano, J. M., Bi, W., Johnson, A. D., Cassells, W., Olson, E. N., and Schwarz, J. J. (1996) \textit{Circ. Res.} 78, 196–204
38. Morrisey, E. E., Ip, H. S., Lu, M. M., and Parmacek, M. S. (1998) \textit{Proc. Natl. Acad. Sci. U. S. A.} 95, 5993–5997
39. Tucker, C. L., Hurley, J. H., Miller, T. R., and Hurley, J. B. (1998) \textit{Proc. Natl. Acad. Sci. U. S. A.} 95, 5993–5997
40. Burbelo, P. D., Martin, G. R., and Yamada, Y. (1988) \textit{Proc. Natl. Acad. Sci. U. S. A.} 85, 9679–9682