The Exon 6ABC Region of Amelogenin mRNA Contribute to Increased Levels of Amelogenin mRNA through Amelogenin Protein-enhanced mRNA Stabilization

Received for publication, June 6, 2006; in revised form, August 23, 2006 Published, JBC Papers in Press, September 5, 2006, DOI 10.1074/jbc.M605406200

Liming Xu, Hidemitsu Harada, and Akiyoshi Taniguchi

From the 4 Cell Engineering Technology Group, Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044 and the 5 Department of Oral Anatomy II, Iwate Medical University, School of Dentistry, 1-3-27 Chuodori, Morioka, Iwate 020-8505, Japan

We recently demonstrated that the reuptake of full-length amelogenin protein results in increased levels of amelogenin mRNA through enhanced mRNA stabilization (Xu, L., Harada, H., Tamaki, T. Y., Matsumoto, S., Tanaka, J., and Taniguchi, A. (2006) J. Biol. Chem. 281, 2257–2262). Here, we examined the molecular mechanism of enhanced amelogenin mRNA stabilization. To identify the cis-regulatory region within amelogenin mRNA, we tested various reporter systems using a deletion series of reporter plasmids. A deletion at exon 6ABC of amelogenin mRNA resulted in a 2.5-fold increase in the amelogenin mRNA expression level when compared with that of full-length mRNA, indicating that a cis-element exists in exon 6ABC of amelogenin mRNA. Furthermore, Northwestern analysis demonstrated that amelogenin protein binds directly to its mRNA in vitro, suggesting that amelogenin protein acts as a trans-acting protein that specifically binds to this cis-element. Moreover, recombinant mouse amelogenin protein extended the half-life of full-length amelogenin mRNA but did not significantly alter the half-life of exon 6ABC-deletion mutant mRNA. The splice products produced by deletion of exon 6ABC are known as leucine-rich amelogenin peptides and have signaling effects on cells. Our findings also suggest that the regulation of full-length amelogenin mRNA expression differs from the regulation of leucine-rich amelogenin peptide expression.

Amelogenin is a major component of enamel matrix. Amelogenin is unique in its localization on both X-chromosomes and Y-chromosomes in cows, pigs, and humans and on the X-chromosomes in mice (1–4) rather than on chromosome 4q like other enamel- and mineralization-associated proteins. Alternative splicing of amelogenin pre-mRNA leads to the production of many isoforms (5–11). The smaller splice products, produced upon the deletion of exon 6ABC, are known as the leucine-rich amelogenin peptides or LRAPs. Both full-length amelogenin and LRAP are capable of modulating expression of cementoblast-associated genes (12–16). In particular, LRAPs have been shown to act differently, as signaling molecules affecting odontogenic and other cell types (12–15). The larger forms, those that contain the intact proline-rich, hydrophobic exon 6 domains, are important for enamel mineralization (for review, see Ref. 17). Thus, the mRNA products of short and full-length amelogenin have different functions, and the regulation of full-length amelogenin protein expression appears to differ from the regulation of LRAP expression.

CCAAT/enhancer-binding protein α plays a key role in the developmentally regulated expression of the amelogenin gene at the transcription level (18), whereas Msx2 mediates interference with the binding of CCAAT/enhancer-binding protein α to its cognate site on the mouse amelogenin minimal promoter by protein-protein interaction (19). Our previous study indicated that the reuptake of full-length amelogenin protein results in increased levels of amelogenin mRNA through enhanced mRNA stabilization (20). Thus, amelogenin gene expression is regulated at both the transcription and the post-transcriptional level. However, still unclear is the difference between the molecular mechanisms of short and full-length amelogenin expression regulation.

The regulation of mRNA stability plays an important role in controlling gene expression in a broad range of contexts in eukaryotic cells. Differential mRNA turnover is determined by specific cis-acting elements within the mRNA and the trans-acting factors that bind them. The cis-elements are found in the 5’-untranslated region (UTR) (21, 22), the protein coding region (23–25), and the 3’-UTR (26, 27). Trans-acting proteins that specifically bind to certain of these elements have been identified, and most are mRNA-binding proteins (28–30).

In the present study, we examined the molecular mechanism of the increased levels of amelogenin mRNA through enhanced mRNA stabilization. We found that the exon 6ABC region of amelogenin mRNA is involved in amelogenin mRNA instability and amelogenin protein-mediated stability and that full-length amelogenin mRNA specifically binds to amelogenin protein in vitro. These results suggest that the mRNA expression of LRAP, which results from the deletion of exon 6ABC, is not affected by amelogenin protein-mediated post-transcriptional regulation, which is different to full-length amelogenin mRNA. Furthermore, the regulation of full-length amelogenin protein expression differs from the regulation of LRAP expression.
Exon 6ABC of Amelogenin Contributes to Increased mRNA

MATERIALS AND METHODS

Construction of Reporter Plasmids—Reporter plasmid constructs were designed as described previously (31) with some modifications. The rat amelogenin sequence encoding the complete mRNA, comprising the 5′-UTR, coding region, and 3′-UTR (accession number U51195) or the alternative splicing product (exons 1, 2, 3, 5, 6D, and 8) were cloned into the pUC vector. The luciferase fragment taken from pGL3-Basic (1341–1649 nucleotides, Invitrogen) was inserted into the amelogenin sequence between the 5′-UTR and the coding region to serve as a reporter gene for real-time PCR determination using the fusion PCR method. In this step, one point mutation in the amelogenin translation start codon (ATG mutated to CTG) was made. These amelogenin/luciferase fragments were then inserted into an expression vector (pCR3.1, Invitrogen) driven by the CMV promoter using the BamHI/ApaI restriction enzyme sites. The promoter at the ApaI site downstream from the amelogenin sequence. This was followed by purification of the DNA by phenol/chloroform extraction and subsequent ethanol precipitation. Antisense neomycin cDNA was used as a control RNA. In vitro RNA transcription and DIG RNA labeling were performed using the DIG RNA labeling kit (Roche Applied Science, Rotkreuz, Switzerland) as follows: 1 μg of purified template DNA was added to diethyl pyrocarbonate-water to a volume of 13 μl and then added 2 μl of 10× NTP labeling mixture, 2 μl of 10× transcription buffer, 1 μl of protector RNase inhibitor, and 2 μl of RNA polymerase T7 with subsequent incubation for 2 h at 37°C. The reaction was stopped by the addition of 2 μl of 0.2 M EDTA (pH 8.0). The DIG-labeled amelogenin RNA was then purified using the High Pure PCR product purification kit (Roche Applied Science).

Northwestern analysis was carried out as reported previously (29, 30) with minor modifications. Briefly, proteins were separated on 8% non-denaturing polyacrylamide gel and electrophoretically transferred to polyvinylidene difluoride membrane in transfer buffer (25 mM Tris, 190 mM glycine, and 20% methanol) at 100 mA for 1 h. The membrane was rinsed in phosphate-buffered saline and then gently shaken for 2 h at room temperature in Northwestern buffer (10 mM Tris (pH 7.4), 50 mM NaCl, 1 mM EDTA, 1× Denhardt’s solution, and 1 mM dithiothreitol), after which it was incubated with the DIG-labeled RNA solution (5 μg/ml heparin, 5 μg/ml tRNA, and 60 ng/ml in vitro-transcribed DIG-labeled RNA) overnight at 4°C with shaking. The membrane was washed in Northwestern buffer, endogenous signals were blocked by incubating for 2 h in 1% bovine serum albumin-Tris-buffered saline, and signals were then detected by incubating with anti-DIG-peroxidase antibody (1:1000) for 1 h at room temperature followed by visualization with 3′,3′-diaminobenzidine.

Determination of mRNA Expression Level and Half-life Analysis—Total RNA was extracted at each time point using the RV total RNA isolation system, which includes DNase I treatment (Promega, Madison, WI). A 4-μg amount of total RNA was reverse-transcribed into cDNA using the SuperScript first-strand synthesis system (Invitrogen) according to the supplier’s protocol. The expression levels of mRNA or remaining mRNA after actinomycin D treatment were determined using the real-time PCR SYBR Green method, as described previously (33–35). In the case of quantitative analysis of mRNA expression levels, similar transfection/expression efficiency was produced by normalizing the levels obtained against the pRL expression level. The analysis of mRNA half-life determined from actinomycin D treatment was as described in our previous study (20). The primers used for real-time PCR were designed by PrimerExpress software (Applied Biosystems, Foster City, CA) and are as follows: luciferase reporter gene, forward, 5′-TGGGAGCGGAAGACACACTTC-3′, and reverse, 5′-GCCACCTGATTGCGCTTTTGTACTTAA-3′, and Renilla luciferase gene, forward, 5′-GAAATTTCGACGATATCTTGAACCA-3′, and reverse, 5′-GGATTTTCAGGAGCCATG-3′.

RNA Synthesis and Northwestern Blot Analysis—The DNA template used for amelogenin RNA synthesis was linearized by cutting the pCMV-amelogenin plasmid containing the T7 promoter at the Apal site downstream from the amelogenin sequence. This was followed by purification of the DNA by phenol/chloroform extraction and subsequent ethanol precipitation. Antisense neomycin cDNA was used as a control RNA. In vitro RNA transcription and DIG RNA labeling were performed using the DIG RNA labeling kit (SP6/T7) (Roche Applied Science, Rotkreuz, Switzerland) as follows: 1 μg of purified template DNA was added to diethyl pyrocarbonate-water to a volume of 13 μl and then added 2 μl of 10× NTP labeling mixture, 2 μl of 10× transcription buffer, 1 μl of protector RNase inhibitor, and 2 μl of RNA polymerase T7 with subsequent incubation for 2 h at 37°C. The reaction was stopped by the addition of 2 μl of 0.2 M EDTA (pH 8.0). The DIG-labeled amelogenin RNA was then purified using the High Pure PCR product purification kit (Roche Applied Science).

Northwestern analysis was carried out as reported previously (29, 30) with minor modifications. Briefly, proteins were separated on 8% non-denaturing polyacrylamide gel and electrophoretically transferred to polyvinylidene difluoride membrane in transfer buffer (25 mM Tris, 190 mM glycine, and 20% methanol) at 100 mA for 1 h. The membrane was rinsed in phosphate-buffered saline and then gently shaken for 2 h at room temperature in Northwestern buffer (10 mM Tris (pH 7.4), 50 mM NaCl, 1 mM EDTA, 1× Denhardt’s solution, and 1 mM dithiothreitol), after which it was incubated with the DIG-labeled RNA solution (5 μg/ml heparin, 5 μg/ml tRNA, and 60 ng/ml in vitro-transcribed DIG-labeled RNA) overnight at 4°C with shaking. The membrane was washed in Northwestern buffer, endogenous signals were blocked by incubating for 2 h in 1% bovine serum albumin-Tris-buffered saline, and signals were then detected by incubating with anti-DIG-peroxidase antibody (1:1000) for 1 h at room temperature followed by visualization with 3′,3′-diaminobenzidine.

Transferrin and human IgG were used as the control proteins (WAKO, Osaka, Japan), as was cell lysate protein extracted from HAT-7 or NIH 3T3 cells using M-PER mammalian protein extraction reagent (Pierce).

Statistical Analysis—Data is presented as means ± S.D. Single group comparisons were evaluated by Student’s t test. Statistical significance was set at * p < 0.01, ** p < 0.001.
RESULTS

Presence of a Cis-acting Element in Exon 6ABC of Amelogenin mRNA—We used HAT-7 cells, a dental epithelial cell line originating from the apical bud of a rat incisor (32). HAT-7 cells constitutively express amelogenin when cultured at high confluence (32). Expression levels of amelogenin protein in HAT-7 cells were very low when compared with exogenous amelogenin concentration. Thus, we expect that the effect of endogenous amelogenin on mRNA stabilization is very low.

Differential mRNA turnover is determined by specific cis-acting elements within the mRNA. These cis-elements are found in the 5′-UTR, the protein coding region, and the 3′-UTR. To identify the cis-regulatory region within amelogenin mRNA, we constructed and tested reporter plasmids using partial luciferase sequences (Fig. 2A). In the case of rat amelogenin cDNA, R1 isoforms, which have a deletion exon 7 and encode exon 8, are major transcripts (10). Thus, we used R1 from amelogenin cDNA in this study. To avoid exogenous translated amelogenin proteins directly feeding back to stabilize its mRNA after transient transfection, the translation start codon was mutated. HAT-7 cells were transiently transfected separately with each reporter plasmid, and the levels of luciferase mRNA were then determined by real-time PCR (Fig. 1B). Deletion of the ORF region (pL-dORF) resulted in a 2.5-fold increase in the mRNA level when compared with the full-length amelogenin (pL-FL) level (Fig. 1B). Deletion of the ORF and 5′-UTR (pL-d(5′-UTR+ORF)), the ORF and 3′-UTR (pL-d(ORF+3′-UTR)), or the 5′-UTR, ORF, and 3′-UTR (pL) also resulted in increased mRNA levels when compared with the full-length amelogenin (pL-FL) level (Fig. 1B). However, deletion of the 5′-UTR (pL-d5′-UTR), the 3′-UTR (pL-d3′-UTR), or the 5′-UTR and 3′-UTR (pL-d(5′-UTR+3′-UTR)) did not increase the mRNA level when compared with the full-length amelogenin (pL-FL) level. These results indicated that a cis-element was present in the ORF of amelogenin mRNA.

To further characterize the cis-region of destabilizing sequence in the ORF, we constructed a deletion series of reporter plasmids with deletion of the ORF (Fig. 2A). HAT-7 cells were transiently transfected separately with each reporter plasmid, and the levels of luciferase mRNA were then determined by real-time PCR. Deletion of the ORF region (pL-dORF), exons 2, 3, 5, and 6ABC (pL-d2356ABC), and exon 6ABC (pL-d6ABC) resulted in a 2.5-fold increase in the mRNA level when compared with the full-length amelogenin (pL-FL) level (Fig. 2B). However, deletion of exons 6D and 8 (pL-d6D8) did not increase the mRNA level when compared with the full-length amelogenin (pL-FL) level. These results indicate that the cis-acting element is present in the exon 6ABC sequence of amelogenin mRNA.

Amelogenin Protein Extends the Life Span of Amelogenin mRNA via the Exon 6ABC Region—Next, we analyzed the half-life of the pL-d6ABC transcript when compared with the pL-FL transcript with or without the addition of recombinant amelogenin protein. Recombinant amelogenin protein extended the half-life of pL-FL-driven mRNA in transient transfected cells when compared with cells without amelogenin protein (Fig. 3A). In contrast, the half-life of pL-d6ABC-driven chimeric mRNA was 2-fold longer than that of pL-FL (Fig. 3B), and no changes were observed in pL-d6ABC-driven mRNA in transient transfected cells with or without amelogenin protein (Fig. 3B). These results indicate that amelogenin protein extends the life span of amelogenin mRNA via the exon 6ABC region. The splice products produced by deletion of exon 6ABC are known as LRAP and have signaling effects on cells. These findings suggest that the full-length amelogenin protein do not regulate the LRAP mRNA level through mRNA stabilization.

Amelogenin RNA Specifically Binds to Recombinant Amelogenin Protein in Vitro—We previously showed that extracellular amelogenin protein undergoes reuptake into the cytoplasm of...
Exon 6ABC of Amelogenin Contributes to Increased mRNA

**FIGURE 2.** The destabilizing sequence located in exon 6ABC. A, schematic representation of reporter plasmids. ORF (pL-ORF), exons 2, 3, 5, and 6ABC (pL-d2356ABC), exon 6ABC (pL-d6ABC), or exons 6D and 8 (pL-d6D8) were deleted. All plasmids included the amelogenin polyadenylation signal (788–825 bp) and point mutation in the amelogenin translation start codon (ATG to CTG). Transcription was driven by the constitutive CMV promoter upstream of the amelogenin cDNA. The upper panel shows the schematic representation of rat amelogenin full-length cDNA. Luc, Partial luciferase sequence; B, luciferase reporter mRNA levels in each reporter plasmid. HAT-7 cells were transiently transfected with each reporter plasmid separately, co-transfected with the Renilla luciferase reporter plasmid (pRL-CMV), and cultured for 24 h. The levels of luciferase reporter mRNAs were determined by quantitative real-time PCR and normalized against the pRL-CMV level. Results are representative of six experiments. Error bars indicate the standard deviation of mean changes. ***, p < 0.001 versus pL-FL.

**FIGURE 3.** The half-lives of chimeric mRNA with or without amelogenin protein. A, half-lives of pL-FL with (3 μg/ml) or without amelogenin protein. Act D, actinomycin D. B, half-lives of pL-d6ABC with or without amelogenin protein. After 24 h of transfection, cells were treated with actinomycin D (0.5 μg/ml) for various periods. The levels of the remaining mRNAs were determined by real-time PCR. The plots represent the mean values from five separate experiments. The horizontal dotted line indicates the value of the mRNA half-life. *, p < 0.001 versus without amelogenin.

**FIGURE 4.** Amelogenin mRNA binds to recombinant amelogenin protein in vitro. Lane M, protein molecular marker; lane 1, transferring (2 μg/lane); lane 2, recombinant mouse amelogenin (2 μg/lane); lane 3, HAT-7 cell lysate (2 μg/lane); lane 4, NIH3T3 cell lysate (2 μg/lane); and lane 5, human IgG (2 μg/lane). A, silver staining of proteins separated by 8% native polyacrylamide. B, Northwestern blotting probed with in vitro transcribed DIG-labeled amelogenin RNA. C, Northwestern blotting reacted with in vitro transcribed DIG-labeled control RNA. The signals were detected by incubating the membrane with anti-DIG-POD antibody and visualization using DAB.
DISCUSSION

Reuptake of amelogenin protein results in increased levels of amelogenin mRNA through enhanced mRNA stabilization (20), suggesting that in vivo, ameloblasts are able to dramatically increase the production of amelogenin in an autocrine fashion. Indeed, ameloblasts secrete a large amount of amelogenin for enamel formation during the short periods of tooth development. When dentin matrix is formed between the inner enamel epithelium and mesenchymal cells, amelogenin accumulates at the proximal side of the inner enamel epithelium. The deposition of amelogenin helps the inner enamel epithelium cells reuptake amelogenin into the cytoplasm. It could be speculated that amelogenin proteins, which are incorporated into enamel matrix or proteolytically degraded amelogenin, could not be reuptaken by cells. We speculate that the shutdown of amelogenin production in the maturing ameloblast occurs because of its transition from secretory to maturation phase by the shutdown of reuptaken. Taken together, we propose a unique biological function of amelogenin in regulating the expression of amelogenin through stabilizing amelogenin mRNA.

The regulation of mRNA stability is an important process in controlling gene expression. Differential mRNA stability is determined by the specific cis-acting elements within mRNA and the trans-acting factors that bind to them. Here, we have demonstrated that a cis-acting element is present in exon 6ABC of amelogenin mRNA. We have also demonstrated that amelogenin protein binds to amelogenin mRNA in vitro, suggesting that amelogenin protein acts as a trans-acting factor that binds to the exon 6ABC region of amelogenin mRNA.

Multitype alternative splicing mRNAs generated by the amelogenin gene have been identified in various species. In mice, nine gene products of amelogenin have been identified and detected as protein, all of which contain exon 7 (5, 6, 14) in addition to the recently identified exon 8/9 (36). In rat, the amelogenin mRNAs that have been identified, with the exception of the exon 7 types, are R195 (contains exons 2, 3, 4, 5, 6ABCD, and 7), R181 (exons 2, 3, 5, 6ABCD, and 7), R73 (exons 2, 3, 4, 5, 6D, and 7), R59 (exons 2, 3, 5, 6D, and 7) (13), and R2–156 (exons 2, 3, 5 6BCD, and 7) (10). In addition, the amelogenin mRNAs that contain exon 8 are R1–203 (contains exons 2, 3, 5, 6ABCD, and 8), R1–179 (exons 2, 3, 5, 6BCD, and 8), R1–82 (abc) (exons 2, 3, 5, 6D, and 8), and R1–57 (exons 2, 3, 5, and 8) (10). The smaller splice products with deletion of exon 6ABC are known as LRAP and have been shown to act differently as signaling molecules affecting ameloblasts and other cell types (12–15). These findings indicate that the mRNA products of short and full-length amelogenin have different functions.

Recently, amelogenin-binding proteins have been cloned (37, 38). Wang et al. (37) have identified an integral membrane protein, CD63, that interacts with amelogenin. Tompkins et al. (38) also have shown that LAMP-1 interacts with LRAP. These results suggested that the amelogenin proteins are taken up by endocytic pathway of amelogenin into the HAT-7 cells via these binding molecules. The means by which amelogenin exerts its biological function could be viewed as either an extracellular signaling event or an intracellular events. Further, findings showing the localization of exogenous recombinant amelogenin in the cytoplasm suggest that amelogenin acts intracellularly on some kind of target to regulate amelogenin mRNA.

Our results showed that the stability of LRAP mRNAs is higher than that of full-length amelogenin mRNA. If the transcription ratio of full-length amelogenin and LRAP is the same, the level of LRAP expression should be higher than that of full-length amelogenin. However, the LRAP expressions are present at very low levels in the enamel when compared with the full-length amelogenin form. Thus, it is very difficult to explain how LRAP expression is in fact regulated without the use of the alternative splicing mechanism of amelogenin mRNA.

In conclusion, we have identified a cis-element in exon 6ABC of the coding region of amelogenin mRNA. Furthermore, our findings suggest that LRAP mRNA expression is not affected by amelogenin protein through this enhanced mRNA stabilization. Therefore, we speculate that mechanisms of full-length amelogenin expression are different from that of LRAPs.

Acknowledgment—We thank Dr Ken Matsumoto (RIKEN, Japan) for valuable comments on this paper.

REFERENCES

1. Lai, E. C., Mohandas, T. K., Shapiro, L. J., Slavkin, H. C., and Sneed, M. L. (1989) Genomics 4, 162–168
2. Gibson, C. W., Golub, E. E., Abrams, W. R., Shen, G., Ding, W., and Rosenbloom, J. (1992) Biochemistry 31, 8384–8388
3. Ikawa, T., Kakegawa, A., Nagano, T., Ando, H., Yamakoshi, Y., Tanabe, T., Simmer, J. P., Hu, C. C., Fukae, M., and Oida, S. (2005) J. Dent. Res. 84, 144–148
4. Nakahori, Y., Takenaka, O., and Nakagome, Y. (1991) Genomics 9, 264–269
5. Lai, E. C., Simmer, J. P., Bringas, P., Hsu, D. D., Hu, C. C., Zeichner-David, M., Thiemann, F., Sneed, M. L., Slavkin, H. C., and Fincham, A. G. (1992) Biochem. Biophys. Res. Commun. 188, 1253–1260
6. Simmer, J. P., Hu, C. C., Lai, E. C., Sarte, P., Slavkin, H. C., and Fincham, A. G. (1994) Calcif. Tissue Int. 55, 302–310
7. Brookes, S. J., Robinson, C., Kirkham, J., and Bonass, W. A. (1995) Arch. Oral Biol. 40, 1–14
8. Simmer, J. P. (1995) Connect. Tissue Res. 32, 131–136
9. Bonass, W. A., Kirkham, J., Brookes, S. J., Shore, R. C., and Robinson, C. (1994) Biochim. Biophys. Acta. 1219, 690–692
10. Li, R., Li, W., and DenBesten, P. K. (1995) J. Dent. Res. 74, 1880–1885
11. Hu, C. C., Bartlett, J. D., Zhang, C. H., Qian, Q., Ryu, O. H., and Simmer, J. P. (1996). J. Dent. Res. 75, 1735–1741
12. Boabaid, F., Gibson, C. W., Kuehl, M. A., Berry, J. E., Sneed, M. L., Nociti, F. H., Jr., Katchburian, E., and Somerman, M. J. (2004) J. Periodontol. 75, 1126–1136
13. Veis, A., Tompkins, K., Alvare, K., Wei, K., Wang, L., Wang, X. S., Brownell, A. G., Jeng, S. M., and Healy, K. E. (2000) J. Biol. Chem. 275, 41263–41272
14. Veis, A. (2003) CMLS Cell. Mol. Life Sci. 60, 38–55
15. Tompkins, K., Alvare, K., George, A., and Veis, A. (2005) J. Bone Miner. Res. 20, 341–349
16. Viswanathan, H. L., Berry, J. E., Foster, B. L., Gibson, C. W., Li, Y., Kulkarni, A. B., Sneed, M. L., and Somerman, M. J. (2003) J. Periodontol. 74, 1423–1431
17. Moradian-Oldak, J., Simmer, J. P., Lai, E. C., Diekwisch, T., Slavkin, H. C., and Fincham, A. G. (1995) Connect. Tissue Res. 32, 125–130
18. Zhou, Y. L., and Snead, M. L. (2000) J. Biol. Chem. 275, 12273–12280
19. Zhou, Y. L., Lei, Y., and Sneed, M. L. (2000) J. Biol. Chem. 275, 29066–29075
20. Xu, L., Harada, H., Tamaki, T. Y., Matsusho, S., Tanaka, J., and Tanigui,
Exon 6ABC of Amelogenin Contributes to Increased mRNA

21. Dibbens, J. A., Miller, D. L., Damert, A., Risau, W., Vadas, M. A., and Goodall, G. J. (1999) Mol. Biol. Cell 10, 907–919
22. Cannons, A. C., and Cannon, J. (2002) Planta 214, 488 – 491
23. Wellington, C. L., Greenberg, M. E., and Belasco, J. G. (1993) Mol. Cell Biol. 13, 5034–5042
24. Schiavi, S. C., Wellington, C. L., Shyu, A.-B., Chen, C.-Y. A., Greenberg, M. E., and Belasco, J. G. (1994) J. Biol. Chem. 269, 3441–3448
25. Wisdom, R., and Lee, W. (1991) Genes Dev. 5, 232–243
26. Winstall, E., Gamache, M., and Raymond, V. (1995) Mol. Cell Biol. 15, 3796–3804
27. Shaw, G., and Kamen, R. (1986) Cell 46, 659 – 667
28. Sachs, A. B. Cell 74, 413–421
29. Wang, X., Kiledjian, M., Weiss, I. M., and Liebhaber, S. A. (1995) Mol. Cell Biol. 15, 1769–1777
30. Yu, J., and Russell, J. E. (2001) Mol. Cell Biol. 21, 5879–5888
31. Koga, T., Sardina, E., Tidwell, R. M., Pelletier, M., Look, D. C., and Holtzman, M. J. (1999) Proc. Natl. Acad. Sci. 96, 5680–5685
32. Kawano, S., Morotomi, T., Toyono, T., Nakamura, N., Uchida, T., Ohishi, M., Toyoshima, K., and Harada, H. (2002) Connect. Tissue Res. 43, 409 – 412
33. Taguchi, T., Xu, L., Kobayashi, H., Taniguchi, A., Kataoka, K., and Tanaka, J. (2005) Biomaterials 26, 1247–1252
34. Ishibashi, Y., Inouye, Y., Okano, T., and Taniguchi, A. (2005) Glycoconj. J. 22, 53–62
35. Kurosawa, Y., Taniguchi, A., and Okano, T. (2005) Tissue Eng. 11, 1650–1657
36. Papagerakis, P., Ibarra, J. M., Inozentseva, N., DenBesten, P., and MacDougall, M. (2005) J. Dent. Res. 84, 613–617
37. Wang, H., Tannukit, S., Zhu, D., Snead, M. L., and Paine, M. L. (2005) J. Bone Miner. Res. 20, 1032–1040
38. Tompkins, K., George, A., and Veis, A. (2006) Bone (NY) 38, 172–180