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# Characterization of a putative cis-regulatory element that controls transcriptional activity of the pig uroplakin II gene promoter

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ABSTRACT

Uroplakin II (UPII) is a one of the integral membrane proteins synthesized as a major differentiation product of mammalian urothelium. UPII gene expression is bladder specific and differentiation dependent, but little is known about its transcription response elements and molecular mechanism. To identify the cis-regulatory elements in the pig UPII (pUPII) gene promoter region, we constructed pUPII 5′ upstream region deletion mutants and demonstrated that each of the deletion mutants participates in controlling the expression of the pUPII gene in human bladder carcinoma RT4 cells. We also identified a new core promoter region and putative negative cis-regulatory element within a minimal promoter region. In addition, we showed that hepatocyte nuclear factor 4 (HNF4) can directly bind in the pUPII core promoter (5F-1) region, which plays a critical role in controlling promoter activity. Transient cotransfection experiments showed that HNF4 positively regulates pUPII gene promoter activity. Thus, the binding element and its binding protein, HNF4 transcription factor, may be involved in the mechanism that specifically regulates pUPII gene transcription.



## 1. Introduction

Uroplakins (UPs) are a group of integral membrane proteins that have been identified as the major proteins of urothelial plaque [1,2]. These plaques cover a large portion of the apical surface of mammalian urothelium and protect the urothelial luminal surface from rupturing during bladder distention [3,4]. Furthermore, it has been shown that mammalian urothelial plaques contain four major integral membrane proteins: UPIa (27 kDa), UPIb (28 kDa), UPII (15 kDa), and UPIII (47 kDa) [2,5,6]. The expression of UPs Ia, II, and III are specific to urothelium at both protein and RNA levels [1,2,5–9].

UP mRNA expression correlates with a differentiated phenotype in transitional cell carcinomas (TCCs). Human bladder carcinoma RT4 cells, associated with the most differentiated phenotype *in vitro*, expressed all four UP transcripts; UPIb and UPII showed high level expression, whereas UPIa and UPIII were expressed in lower abundance [10]. This suggests that the TCC cell lines, in particular RT4, will be invaluable as models for urothelial cytodifferentiation and for studying differential regulation and expression of the UP genes.

According to reports, human UPIa [11], UPIb [12], and UPII [13–15] gene promoters were highly active in human bladder cells, but not in human non-bladder cells. Among these promoters, that of the UPII gene has been used in constructing tissue-specific expression vectors for bladder cancer gene therapy or animal bioreactors. For instance, an adenoviral vector containing tumor necrosis factor alpha (TNFα) driven by the human UPII (hUPII) gene promoter showed high antitumor efficacy *in vitro* and *in vivo* and the combination of prostate stem cell antigen enhancer and UPII gene promoter improved target gene expression in bladder cancer cells but not in non-bladder cancer cells [16,17]. In addition, previous reports have detailed approaches using the mouse UPII (mUPII) gene promoter to evaluate the feasibility of using an animal bladder for production of biologically active foreign proteins such as human granulocyte macrophage colony stimulating factor (hGM-CSF) and human granulocyte colony stimulating factor (hG-CSF) [18,19]. However, these patterns of gene expression such as the cis-regulatory transcriptional control elements and the specific transcription factor which regulate or affect it are poorly defined.

Recently, we reported that a 2 kb region of the porcine UPII (pUPII) promoter contains multiple transcription factor binding

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**Table 1**
Primer sequences used for PCR cloning.

| Designation | Sequence |
|---|---|
| (−604) 0.6K | 5′-GGGGTACCCAGCATCAATTGGCT-3′ |
| −1* | 5′-CCGCTCGAGAGCTGGGCTGGTGCT-3′ |
| (−554) 0.6K-1 | 5′-GGGGTACCTGGGACTCACACCCCT-3′ |
| (−516) 0.6K-2 | 5′-GGGGTACCGACCTGGTCTTCAGGA-3′ |
| (−492) 1F | 5′-GGGGTACCGAACTGGCTCCCCCGGCT-3′ |
| (−442) 1F-1 | 5′-GGGGTACCTCCCATTCCTGCCCA-3′ |
| (−392) 2F | 5′-GGGGTACCACACCAGTGCAACCTCAG-3′ |
| (−365) 2F-1 | 5′-GGGGTACCCCTCCTGGGAACACCCA-3′ |
| (−347) 2F-2 | 5′-GGGGTACCCTACCACGTGGGAGA-3′ |
| (−323) 3F | 5′-GGGGTACCTAGGGGTTGGGCCCCAGA-3′ |
| (−282) 3F-1 | 5′-GGGGTACCCGAGCCCTTACATGT-3′ |
| (−232) 3F-2 | 5′-GGGGTACCCTTTAGTCACCCTGC-3′ |
| (−205) 3F-3 | 5′-GGGGTACCGCCTGAGCCACCAA-3′ |
| (−172) 4F | 5′-GGGGTACCGCCTTCTGGCCTGAGGG-3′ |
| (−134) 4F-1 | 5′-GGGGTACCTGTCTGGGGGCCT-3′ |
| (−110) 5F | 5′-GGGGTACCTGTAGGGCCCAAGGCCT-3′ |
| (−83) 5F-1 | 5′-GGGGTACCTGTAGGGCCCAAGG-3′ |
| (−42) 6F | 5′-GGGGTACCGCCCCTCCTACCCCCA-3′ |
| Δ28* | 5′-CCCTCGAGGCAAGCTGTGGTTTC-3′ |

The asterisks indicate reverse primers for PCR cloning and underlines identify restriction sites (KpnI or XhoI) introduced at the 5′ ends of primers for subcloning.

sites, including GC-boxes, SP1, AP2, and GATA-box sites, but no TATA or CAAT-box sequences, and also pUP0.6 as a minimal promoter may contain cis-regulatory elements which are likely to play an important role in the regulation of pUPII gene expression [20,21].

To identify the cis-regulatory elements in the pUPII promoter region, we constructed pUPII 5′ upstream region deletion mutants and investigated the promoter activities of these deletion mutants. In this study, we demonstrated that each of the deletion mutants participates in controlling the expression of the pUPII gene in RT4 cells. We also newly identify a core promoter region and putative negative cis-regulatory element within a minimal promoter region. In addition, we showed that hepatocyte nuclear factor 4 (HNF4) can directly bind in the 5F-1 region, which plays a critical role in controlling promoter activity. Transient co-transfection experiments show that HNF4 positively regulates pUPII promoter activity. Thus, the binding element and its binding protein, HNF4 transcription factor, may be involved in the mechanism that specifically regulates pUPII transcription. Therefore, our results provide evidence of a newly identified core promoter region which regulates pUPII transcription. A promoter that directs the expression of pig UP genes may be useful in constructing efficient tissue-specific vectors for bladder cancer gene therapy and animal bioreactors.

## 2. Materials and methods

### 2.1. DNA sequence analysis

Promoter sequence analysis was performed using TFsearch (http://www.cbrc.jp/research/db/TFSEARCH.html) and rVista 2.0 (http://rvista.dcode.org/).

### 2.2. Construction of plasmids

Luciferase report constructs were prepared by polymerase chain reaction (PCR)-based methods from the clone pUPII-0.6 [21]. The putative promoter segment for −604 to +59 (0.6K–6F, 0.6K-1–0.6K-2, 1F-1, 2F-1–2F-2, 3F-1–3F-3, 4F-1 and 5F-1) was PCR-amplified with forward and reverse primers (Table 1) flanked by KpnI and XhoI sites at the 5′ end. Following enzymatic digestion, the PCR products were ligated between KpnI and XhoI sites of the

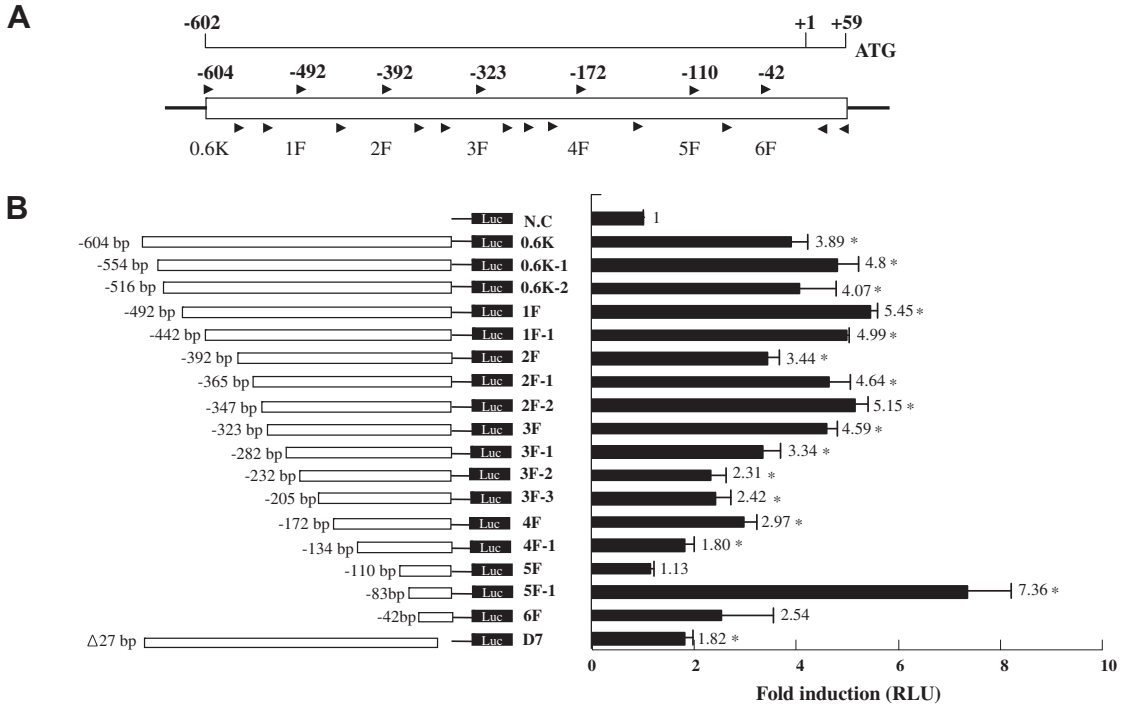


**Fig. 1.** Effect of deleting parts of the pig uroplakin II (pUPII) promoter on its transcriptional activity. (A) Schematic representation of the pUPII 5′ upstream region. The nucleotide numbers represent the distances from the transcription start site (+1). The locations of the primers used to construct the deletion mutants are depicted by arrowheads. (B) The plasmids containing the luciferase gene flanked with various lengths of the pUPII upstream region were transfected into human bladder cancer (RT4) cells, as described in the "Section 2". The transfection efficiency was normalized relative to Renilla luciferase (pRL-SV40). The luciferase activity of the 5′ upstream regions is shown as fold induction relative to that of the promoter-less pGL3-basic plasmid. *$P < 0.01$.