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# Cyclic AMP-Dependent Modification of Gonad-Selective $TAF_{II}105$ in a Human Ovarian Granulosa Cell Line

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**Abstract** In response to gonadotropins, the elevated level of intracellular-cyclic AMP (cAMP) in ovarian granulosa cells triggers an ordered activation of multiple ovarian genes, which in turn promotes various ovarian functions including folliculogenesis and steroidogenesis. Identification and characterization of transcription factors that control ovarian gene expression are pivotal to the understanding of the molecular basis of the tissue-specific gene regulation programs. The recent discovery of the mouse TATA binding protein (TBP)-associated factor 105 ($TAF_{II}105$) as a gonad-selective transcriptional co-activator strongly suggests that general transcription factors such as TFIID may play a key role in regulating tissue-specific gene expression. Here we show that the human $TAF_{II}105$ protein is preferentially expressed in ovarian granulosa cells. We also identified a novel $TAF_{II}105$ mRNA isoform that results from alternative exon inclusion and is predicted to encode a dominant negative mutant of $TAF_{II}105$. Following stimulation by the adenylyl cyclase activator forskolin, $TAF_{II}105$ in granulosa cells undergoes rapid and transient phosphorylation that is dependent upon protein kinase A (PKA). Thus, our work suggests that pre-mRNA processing and post-translational modification represent two important regulatory steps for the gonad-specific functions of human $TAF_{II}105$. J. Cell. Biochem. 96: 751–759, 2005.


**Key words:** $TAF_{II}105$; cAMP; alternative splicing; phopshorylation; ovarian granulosa cells

The gonadotropin follicle-stimulating hormone (FSH) and leuteinizing hormone (LH) play pivotal role in dictating ovarian follicular development and function [Richards et al., 1998]. In response to the pituitary hormones, a plethora of ovarian genes are activated in an ordered fashion to promote follicular maturation and other ovarian functions such as steroidogenesis [Richards, 1994; Robker and Richards, 1998]. For example, FSH binds to its cognate membrane-associated receptor, which in turn triggers activation of the receptor-coupled adenylyl cyclase and elevation of the intracellular cAMP level. Increased cAMP level leads to activation of protein kinase A (PKA) and multiple downstream events in the signal transduction pathway [Tayor, 1989; Richards, 2001]. Notably, PKA-mediated phosphorylation of the site-specific transcription activator CREB is a key step in activation of multiple cAMP-responsive genes such as c-fos, serum glucocorticoid kinase (SGK), aromatase (Cyp19), and inhibin $\alpha$ [Woodruff et al., 1987; Delidow et al., 1992; Montminy, 1997; Gonzalez-Robayna et al., 1999; Mayr and Montminy, 2001; Quinn, 2002]. Therefore, cAMP acts as a "molecular switch" to turn on an entire program of pituitary hormone-responsive gene expression in the ovary. Elucidation of the underlying mechanism for the cAMP-stimulated gene expression will advance our understanding of the molecular events during the ovarian cycle as well as the development of hormone-related pathological conditions.

Intense work on eukaryotic gene regulation in the past several decades has provided a comprehensive picture of the initiation of RNA

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polymerase II (RNAPII)-directed transcription [Levine and Tjian, 2003; Boeger et al., 2005]. In particular, the general transcription factor TFIID serves as a nucleating factor in the assembly of the pre-initiation complex (PIC) at transcription promoters [Chen and Hampsey, 2002]. TFIID is composed of the TATA box-binding protein (TBP) and multiple TBP-associated factors ($TAF_{II}$s) [Verrijzer and Tjian, 1996; Albright and Tjian, 2000]. TAFIIs exert their functions at multiple aspects of transcriptional initiation including promoter recognition, bridging between enhancer-bound activators, and the basal transcription machinery, as well as modification of histones and other regulatory proteins [Berk, 1999; Pugh, 2000; Sterner and Berger, 2000].

While TFIID was originally considered a general transcription factor (GTF) with a ubiquitous expression pattern and functions in RNAPII-mediated transcription initiation, recent findings indicate the existence of tissue and cell type-specific TFIID complexes that are directly responsible for gene expression of specialized developmental importance [Hochheimer and Tjian, 2003; Taatjes et al., 2004]. For example, the tissue-selective factor $TAF_{II}105$ was initially identified as a B-cell enriched component of TFIID [Dikstein et al., 1996] and subsequently shown to activate a subset of NF-κB-regulated genes via its interaction with the p65/RelA homodimer [Yamit-Hezi and Dikstein, 1998; Yamit-Hezi et al., 2000; Matza et al., 2001]. Although dominant-negative $TAF_{II}105$ transgenic mice displayed enhanced apoptosis of B- and T-lymphocytes that was linked to NF-κB [Silkov et al., 2002], $TAF_{II}105$ knockout mice showed no obvious defects in the immune system, leading to the notion that $TAF_{II}105$ and another $TAF_{II}$ may play a redundant role in B-lymphocytes [Freiman et al., 2002]. Interestingly, female mice lacking $TAF_{II}105$ were sterile due to defects in folliculogenesis [Freiman et al., 2001], and the male counterpart display impaired functions in spermatogenic maintenance [Falender et al., 2005]. Indeed, gene expression profiling studies strongly suggest a critical role of the mouse $TAF_{II}105$ in directing transcriptional activation of multiple ovarian genes that are involved in folliculogenesis and steroidogenesis in granulosa cells [Freiman et al., 2001]. Thus, murine $TAF_{II}105$ represents a tissue and cell type-specific transcriptional co-activator that may systematically control an entire gene expression program during ovarian follicular development. However, functions and regulation of the human counterpart of the mouse $TAF_{II}105$ in ovarian granulosa cells remain to be explored, partly due to the difficulty in obtaining the suitable materials required for the characterization.

In the current study, we showed that human $TAF_{II}105$ was preferentially expressed in an ovarian granulosa cell line that maintains the steroidogenic function. In addition, we identified a novel $TAF_{II}105$ mRNA isoform that resulted from alternative splicing. We also found that $TAF_{II}105$ was rapidly and transiently phosphorylated in response to an elevation of the intracellular cAMP level, the central driving force of follicular maturation. In contrast, $TAF_{II}130$, a $TAF_{II}105$-related but ubiquitously expressed $TAF_{II}$, did not display any detectable post-translational modifications following cAMP activation. Thus, alternative splicing and post-translational modification may represent two mechanisms by which $TAF_{II}105$ is regulated during folliculogenesis.

## MATERIALS AND METHODS

### Cell Lines and Plasmids

The culture conditions for the human ovarian granulosa-like tumor cell line KGN [Nishi et al., 2001] and immortalized breast-epithelial cell line MCF10A [Debnath et al., 2003] have been previously described. HEK293T, HeLa, T47D cells were cultured in DMEM, and ES-2 in McCoy's 5A medium, with 10% bovine fetal serum. To construct the expression vectors for human $TAF_{II}105$, cDNA that encodes the full-length protein was amplified by RT-PCR from RNA of KGN cells and subsequently cloned into the *Hind*III and *Eco*RI sites of pcDNA3-FLAG plasmid (Invitrogen). The expression vectors for the truncated $TAF_{II}105$ were derived from the full-length construct by standard PCR amplification and molecular cloning. The cDNA sequences were verified by DNA sequencing.

### Generation and Purification of Polyclonal Antibodies Against Human $TAF_{II}105$

The cDNA that encodes a central fragment of $TAF_{II}105$ (amino acids 275–535) was subcloned into the *Nde*I and *Bam*HI sites of pET19b (Novagen). The His-tagged $TAF_{II}105$ fragment was over-expressed by isopropyl-β-D-thiogalac-

topyranoside (IPTG) induction, purified using BD TALON metal affinity resins (BD Biosciences), and used to immunize New Zealand white rabbits (Covance). The same $TAF_{II}105$ cDNA was inserted into the *Bam*HI and *Eco*RI sites of the pGEX-KG vector (Pharmacia). The resulting GST-$TAF_{II}105$ (aa 275–535) fusion protein was purified with glutathione-Sepharose 4B beads (Amersham Biosciences), coupled to the beads with 20 mM of dimethylpimelimidate (Sigma), and used for the affinity purification of the $TAF_{II}105$ polyclonal antibody.

### Real-Time RT-PCR

Total RNA from various cell lines was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. RNA was reverse-transcribed using the oligo dT method of the ImPrompII kit (Promega). Real-time PCR was carried out using the fluorescent dye SYBR Green and an ABI 7300 Real-Time PCR System (Applied Biosystems). The following real-time primers were used for assessing the abundance of the two alternatively spliced isoforms of $TAF_{II}105$ mRNA.
$TAF_{II}105$FL-forward: TCCAGAACAGCTGAGATTAAAG;
$TAF_{II}105$FL-reverse: CCTTGGTCCAATAGCTGCAAG;
$TAF_{II}105$E9a-forward: AGAGAATGTAACATCATGCTTCCGG;
$TAF_{II}105$E9a-reverse: GCAGATGAACTTCATCCAACCAA.
β-actin was used as an internal control. (forward: CCAGATCATGTTTGAGACCTTCAAC; reverse: CCAGAGGCGTACAGGGATAGC).

### Immunoprecipitation and Immunoblotting

HEK293T cells were transfected with various expression vectors using Lipofectamine 2000 (Invitrogen). Cells were harvested 24 h after transfection and resuspended in Lysis Buffer (50 mM Tris-Hcl pH7.9, 250 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonylfluoride (PMSF), 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A). The lysate was immunoprecipitated with different antibodies as indicated in the figure legend and protein A-Sepharose beads (Oncogene). After washing four times with Lysis Buffer, samples were subjected to SDS–PAGE and subsequently immunoblotting. Immunoprecipitation and immunoblotting were performed using the following antibodies: affinity-purified anti-$TAF_{II}105$, anti-$TAF_{II}130$ (BD PharMingen), anti-α-tubulin (Calbiochem), anti-FLAG (Sigma), anti-lamin A/C (Covance), anti-TBP (sc-421 and sc-204, Santa Cruz), and anti-mouse or rabbit IgG or IgM secondary antibodies (Pierce).

### Subcellular Fractionation

Harvested KGN cell pellet ($\sim 3 \times 10^7$ cells) was homogenized (30–40 strokes) using a Dounce homogenizer with a B-type pestle in 2 ml of Buffer A (20 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, 0.1 mM EGTA, with a cocktail of protease inhibitors as indicated above for the Lysis Buffer). The homogenate was centrifuged at 2,000 rpm for 10 min at 4°C. The pellet was washed once with Buffer A, resuspended in Laemmli buffer, and analyzed as the nuclear fraction. The supernatant was centrifuged again at 12,000 rpm for 10 min at 4°C, and the final supernatant analyzed as the cytoplasmic fraction. The total protein concentrations of different fractions were quantified by the BCA protein assay method (Pierce). An equal amount of total proteins was resolved by SDS–PAGE.

### Phosphorylation and Dephosphorylation Treatment

KGN cells were treated with 25 μM of forskolin (Sigma), 10 μM of the PKA inhibitor H89 (Upstate), and/or 25 μM of the ERK inhibitor PD98059 (Calbiochem) as indicated. For the assessment of in vivo phosphorylation, whole cell lysates were prepared by directly lysing the cells in 1 × SDS Laemmli buffer, and resolved by SDS–PAGE. The in vitro dephosphorylation assay was performed as previously described [Zhuo et al., 1993]. Briefly, KGN cells grown to 90% confluence were treated with forskolin for 30 min, and washed with PBS. The harvested KGN cell pellets were sonicated briefly in the Lambda protein phosphatase buffer (New England Biolab), and spun at 14,000 rpm for 1.5 min. The dephosphorylation reaction was then carried out by the addition of 40 U/ml of Lambda protein phosphatase at 28°C for 3 min with or without 50 mM of EDTA. The reaction was terminated by adding an equal volume of 2 × SDS Laemmli Buffer.

## RESULTS

### Human $TAF_{II}105$ Is Preferentially Expressed in Ovarian Granulosa Cells

To facilitate the characterization of human $TAF_{II}105$, we raised a rabbit polyclonal antibody against a central region of the protein that shares the least sequence similarity with the homologous $TAF_{II}130$. The affinity-purified antibody recognized ectopically expressed as well as endogenous $TAF_{II}105$ in both Western blotting and immunoprecipitation (IP) (Figs. 1A and 2). To further test the antibody specificity and the authenticity of the polypeptide that it recognizes, endogenous $TAF_{II}105$ was immunoprecipitated with the polyclonal anti-$TAF_{II}105$ antibody, followed by immunoblotting with an anti-TBP antibody (Fig. 1B). As expected, TBP was co-immunoprecipitated by the anti-$TAF_{II}105$ antibody, but not by the pre-immune serum.

The TBP-$TAF_{II}105$ interaction was also demonstrated by IP with the anti-TBP antibody, followed by the detection of the ectopically expressed FLAG-$TAF_{II}105$ in the immunoprecipitate (Fig. 1C). The carboxyl-terminal region of $TAF_{II}105$ (solid box in Fig. 1C) shares a high degree of homology with the other two members of the same $TAF_{II}$ subfamily, namely, human $TAF_{II}130$ and *Drosophila* $TAF_{II}110$ termini of these $TAF_{II}$s are involved in multi-subunit interactions within the TFIID complex, whereas the more divergent N-termini are important

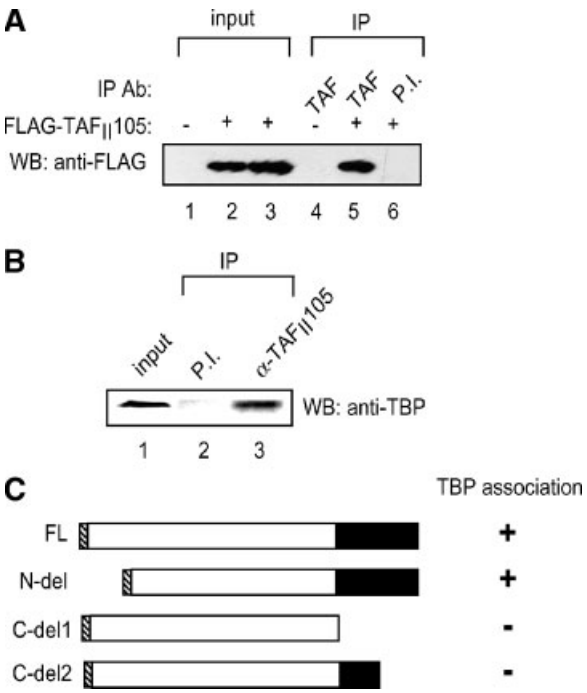


**Fig. 1.** Characterization of the interaction between $TAF_{II}105$ and TBP. **A**: Immunoprecipitation of ectopically expressed FLAG-$TAF_{II}105$. HEK293T cells were transfected with an expression vector for FLAG-tagged $TAF_{II}105$ or empty vector. Cell lysates were immunoprecipitated with either pre-immune serum (P.I.) or the polyclonal anti-$TAF_{II}105$ antibody, followed by immunoblotting with a monoclonal anti-FLAG antibody. **B**: Co-immunoprecipitation of the endogenous $TAF_{II}105$ and TBP. HEK293T-cell lysate was immunoprecipitated with either pre-immune serum or the anti-$TAF_{II}105$ antibody; and subsequently probed with a monoclonal anti-TBP antibody (sc-421). **C**: The highly conserved C-terminal region of $TAF_{II}105$ (solid box) is required for the TBP binding. Various expression vectors for the FLAG-tagged $TAF_{II}105$ proteins were transfected into HEK293T cells. Cell lysates were immunoprecipitated with a polyclonal anti-TBP antibody (sc-204), followed by immunoblotting with the anti-FLAG antibody. The hatched box indicates the FLAG tag. C-del1 and C-del2 lack the last 246 and 123 amino acids of $TAF_{II}105$, respectively.

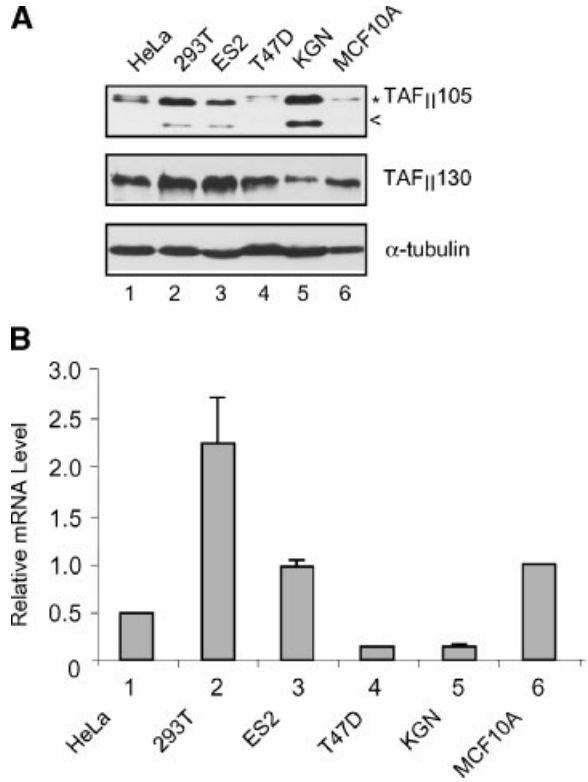


**Fig. 2.** $TAF_{II}105$ is preferentially expressed in an ovarian granulose cell line (KGN). **A**: An equal amount of whole cell lysates from various cell lines was analyzed by immunoblotting for $TAF_{II}105$ and $TAF_{II}130$. α-Tubulin was used as the loading control. The asterisk indicates the position of the full-length $TAF_{II}105$, whereas the arrow shows the band of approximate 70 kD. **B**: The abundance of $TAF_{II}105$ mRNA in various cell lines was assessed by real-time RT-PCR. The relative levels of the $TAF_{II}105$ mRNA were normalized against that of β-actin mRNA. The error bars stand for standard deviation.

for the $TAF_{II}$ interactions with various DNA-binding transcriptional activators [Saluja et al., 1998; Yamit-Hezi and Dikstein, 1998; Furukawa and Tanese, 2000; Wolstein et al., 2000; Silkov et al., 2002]. Indeed, a truncated FLAG-$TAF_{II}105$ that lacks either the entire or part of the conserved C-terminal domain failed to interact with the endogenous TBP in the co-IP experiment (C-del1 and C-del2 in Fig. 1C).

Next, expression of the endogenous $TAF_{II}105$ protein in multiple human cell lines was compared by Western analysis (Fig. 2). The cell lines examined include HeLa (cervical cancer), HEK293T (embryonic kidney), ES-2 (ovarian clear cell carcinoma), T47D (ERα positive breast cancer), KGN (ovarian granulosa cancer), and MCF10A (immortalized breast epithelial cells). The immunoblot contained a predominant band at the expected molecular weight position (asterisk in the top panel of Fig. 2A). Among all the cell lines of different tissue origins surveyed, the ovarian granulosa cell line KGN expressed the highest amount of $TAF_{II}105$ (lane 5, Fig. 2A). Interestingly, the same KGN cells contained the lowest level of $TAF_{II}130$ (middle panel in Fig. 2A), a $TAF_{II}105$ homolog with a broader cell type and tissue expression spectrum. The relative abundance of $TAF_{II}105$ in KGN cells is consistent with the previous finding that knockout of $TAF_{II}105$ in mice profoundly affects ovarian granulosa cell functions [Freiman et al., 2001]. The full-length $TAF_{II}105$ bands in the HeLa and T47D cell lysates migrated as doublets on the SDS–PAGE (lane 1 and 4 in the top panel of Fig. 2A), which might represent post-translational modification of the protein (see below). The same $TAF_{II}105$ immunoblot also revealed a 70 kD band in some cell lysates (arrow in the top panel of Fig. 2A), with the most prominent signal in the ovarian granulosa cells (lane 5).

To explore the possible basis for the cell type-dependent regulation of the $TAF_{II}105$ protein, we compared the relative abundance of the $TAF_{II}105$ transcript in the various cell lines by real-time RT-PCR (Fig. 2B). Intriguingly, the protein and mRNA levels of $TAF_{II}105$ did not correlate with each other. For example, the ovarian granulosa cells contain the most abundant $TAF_{II}105$ protein, yet a relatively small amount of the transcript (compare lane 5 in Figs. 2A and 2B). On the other hand, ES-2 cells express less $TAF_{II}105$ protein but a larger amount of the transcript than KGN cells. This result supports the notion that the cell type-selective expression of $TAF_{II}105$ protein in ovarian granulosa cells may be controlled predominantly at the post-transcriptional level. For example, it is possible that the 5′ untranslated region (5′UTR) of the $TAF_{II}105$ transcript may confer high translation efficiency in a cell type-specific manner. Alternatively, the $TAF_{II}105$ protein in ovarian granulosa cells may be stabilized via a post-translational mechanism.

### Identification of an Alternatively Spliced Isoform of $TAF_{II}105$ Transcript

In isolating the cDNA clones of $TAF_{II}105$ from mRNA of KGN cells, we found that 3 out of 97 full-length $TAF_{II}105$ cDNA clones contained an extra 120-bp sequence inserted between exon 9 and 10. The isoform appears to result from an exon inclusion event of alternative splicing, as the predicted extra exon (designated as exon 9a) contains the consensus splicing acceptor and donor sequences at the intron/exon junctions (underlined in Fig. 3A). The predicted translation product of the $TAF_{II}105$ mRNA isoform (approximately 70 kD) lacks the entire TFIID-associating domain at the C-terminus (Fig. 3B). Instead, it contains 7 amino acids that are encoded by the inserted exon 9a (Fig. 3B). Real-time PCR analysis using an exon 9a-specific primer set indicated that the exon 9a-containing transcript was present in multiple cell lines examined (Fig. 3C). Furthermore, the relative abundance of the E9a isoform mirrors that of the major isoform of the $TAF_{II}105$ transcript (compare Fig. 3C with 2B).

### PKA-Dependent Phosphorylation of $TAF_{II}105$ in Ovarian Granulosa Cells

The finding that the human $TAF_{II}105$ protein is highly abundant in ovarian granulosa cells is consistent with the previously reported function of mouse $TAF_{II}105$ in ovarian folliculogenesis. In light of the paramount importance of cyclic AMP (cAMP) to the functions of ovarian granulosa cells, we examined the potential impact of cAMP elevation on $TAF_{II}105$ in KGN cells. Previous characterization of this cell line indicates that it maintains many of the physiological functions of granulosa cells in vivo such as steroidogenesis, cell growth, and apoptosis [Nishi et al., 2001; Mukasa et al., 2003; Chu et al., 2004; Morinaga et al., 2004]. When KGN cells were treated with the adenylyl cyclase

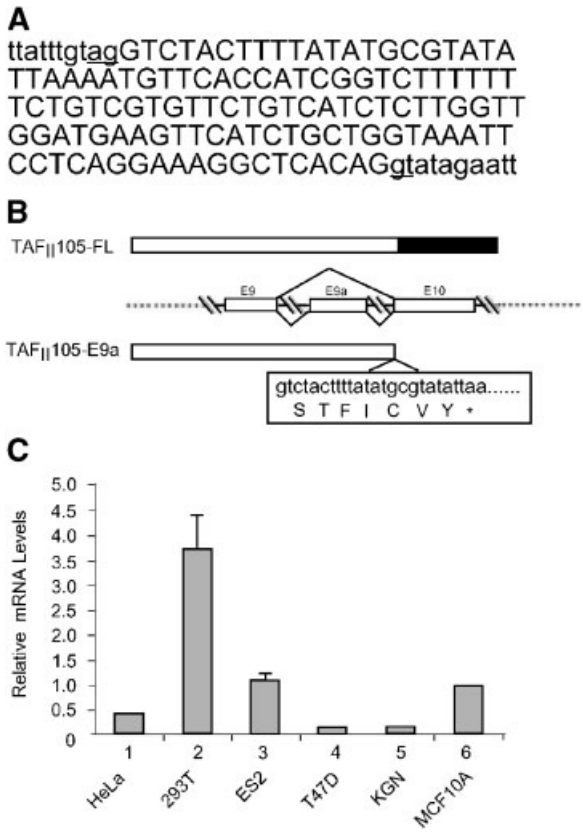


**Fig. 3.** Identification of a novel isoform of $TAF_{II}105$ transcript. **A**: The nucleotide sequence of the inserted exon (in upper case) and the flanking introns (in lower case). The splicing acceptor and donor sequences are underlined. **B**: Diagram of the full-length and isoform $TAF_{II}105$ as a result of alternative splicing. The additional amino acid sequence encoded by the inserted exon (E9a) is shown in the box. **C**: Real-time RT-PCR analysis of the novel isoform in various human cell lines. The levels of $TAF_{II}105$ mRNA are normalized with those of β-actin.

activator forskolin, the majority of $TAF_{II}105$ was rapidly shifted to a higher position (indicated by the bar in Fig. 4), whereas the total amount of $TAF_{II}105$ did not change significantly. This presumed modified $TAF_{II}105$ appeared within 15 min post the forskolin treatment (lane 2), and slowly diminished over a period of 60 h in the presence of forskolin (lane 8). When forskolin was removed from the culture medium after the initial treatment, the presumed modified form of $TAF_{II}105$ disappeared accordingly (lane 9 and 10). Of note, the 70 kD band in the same blot did not show any signs of post-translational modification (indicated by the arrow in Fig. 4). Unlike forskolin, neither 12-o-tetradecanoylphorbol-13-acetate (TPA) nor tumor necrosis factor α (TNFα) caused any detectable changes of $TAF_{II}105$ in KGN cells (data not shown).

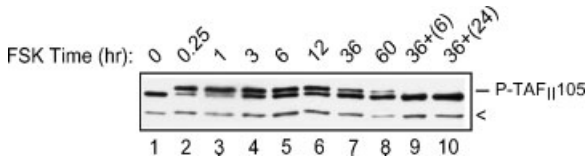


**Fig. 4.** The effect of adenylyl cyclase activator forskolin on $TAF_{II}105$ in KGN cells. KGN cells were treated with 25-μM forskolin and harvested at various time points as indicated. For the samples shown in lane 9 and 10, cells were treated with forskolin for 36 h, followed by the removal of forskolin from the medium and incubation for additional 6 or 24 h before harvest. The bar indicates the position of the shifted full-length $TAF_{II}105$ ($P\text{-}TAF_{II}105$). The arrow points to the 70 kD protein species.

To determine whether the cAMP-induced $TAF_{II}105$ band shift was due to phosphorylation, lysate of the forskolin-treated KGN cells was incubated with either buffer alone or Lambda protein phosphatase (lane 4 and 2 of Fig. 5A, respectively). The forskolin-induced $TAF_{II}105$ band was completely obliterated by the phosphatase treatment (lane 2). Inclusion of EDTA in the reaction mixture inhibited the phosphatase effect (lane 3), indicating that the disappearance of the shifted $TAF_{II}105$ band was indeed due to dephosphorylation of the protein.

Given that protein kinase A (PKA) is the primary kinase activated by the elevated levels

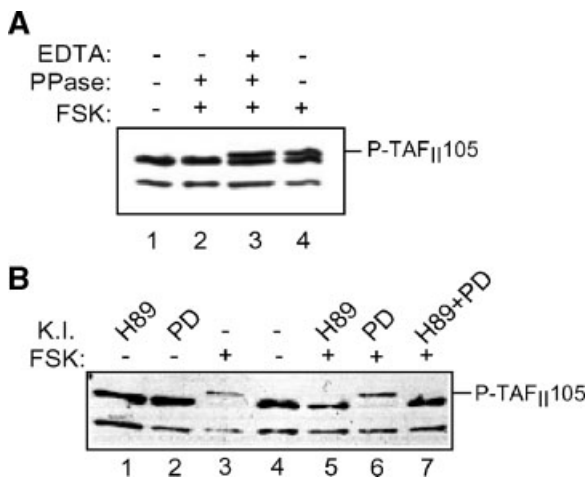


**Fig. 5.** PKA-dependent phosphorylation of $TAF_{II}105$. **A**: The shifted protein band represents phosphorylated $TAF_{II}105$. Lysate from forskolin-treated KGN cells was incubated with either buffer alone (lane 4) or Lambda protein phosphatase (lane 2). For lane 3, EDTA was included in the in vitro reaction mixture to inhibit the phosphatase activity. The lysates were then resolved by SDS–PAGE and probed with the anti-$TAF_{II}105$ antibody. **B**: KGN cells were treated with forskolin in the presence or absence of the kinase inhibitors (K.I.): PKA inhibitor H89 or ERK inhibitor PD98059 (PD). The bars indicate the position of the phosphorylated $TAF_{II}105$ ($P\text{-}TAF_{II}105$).

of intracellular cAMP [Tayor, 1989; Montminy, 1997], we sought to ascertain that PKA activity was required for the cAMP-triggered phosphorylation of $TAF_{II}105$. Addition of the PKA inhibitor H89 to KGN cells along with forskolin prevented phosphorylation of $TAF_{II}105$ (compare lane 3 and 5 in Fig. 5B). On the other hand, treatment with an ERK inhibitor (PD98059) did not affect the forskolin-induced phosphorylation of $TAF_{II}105$ (compare lane 3 and 6). Neither kinase inhibitors affected $TAF_{II}105$ in the absence of the forskolin treatment (lane 1 and 2).

It has been previously reported that subcellular distribution of $TAF_{II}105$ in B-lymphocytes is regulated in response to B-cell-specific stimuli [Rashevsky-Finkel et al., 2001]. To test whether TAFII105 in forskolin-treated ovarian granulosa cells could be regulated in a similar manner, lysates of forskolin-treated and untreated KGN cells were fractionated; the cytoplasmic and nuclear fractions were probed for the presence of $TAF_{II}105$ and $TAF_{II}130$ (Fig. 6). Compared with $TAF_{II}130$, which was predominantly nuclear, a significant proportion of $TAF_{II}105$ was detected in the cytoplasmic fraction of the forskolin-untreated cells (compare lane 1 and 5 in Fig. 6). A significant level of $TAF_{II}105$ in the cytoplasm was also detected by immunostaining (data not shown). Upon forskolin treatment, the cAMP-induced phosphorylated $TAF_{II}105$ appeared in both nuclear and cytoplasmic fractions (compare lane 2–4 with 6–8 in Fig. 6). Therefore, activation of the cAMP-dependent pathway in ovarian granulosa cells does not appear to dramatically affect the sub-cellular localization of the $TAF_{II}105$ protein.

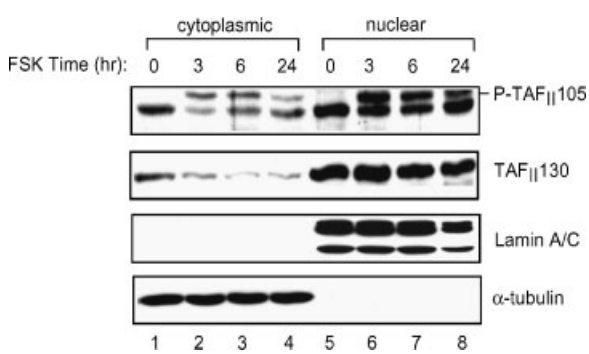


**Fig. 6.** Subcellular fractionation of $TAF_{II}105$ in KGN cells. Cells were fractionated into cytoplasmic and nuclear fractions. The lysates were then probed with either anti-$TAF_{II}105$ or $TAF_{II}130$ antibodies. Lamin A/C and α-tubulin were used as the nuclear and cytoplasmic marker, respectively.

## DISCUSSION

The cAMP-responsive signal transduction pathways are paramount to ordered gene expression during ovarian follicular development [Richards, 2001]. Research in the past two decades has uncovered multiple site-specific transcription factors that play critical roles in cell type- and developmental stage-specific ovarian gene activation in response to pituitary gonadotropins [Richards, 1994; Richards et al., 1995, 1998]. For example, CREB binds to the promoters of multiple steroidogenic genes and stimulates gene expression in a cAMP-responsive manner [Montminy, 1997; Mayr and Montminy, 2001; Quinn, 2002]. In addition, other transcription activators with a more tissue-restricted expression spectrum such as steroidogenic factor 1 (SF-1) confer the tissue and cell type-specificity of ovarian gene expression [Hanley et al., 2000]. On the other hand, general transcription factors including TFIID were thought to act in a universal fashion to select core promoter sequence and mediate the interactions between upstream activators and the basal transcription machinery. This classical view of gene activation was challenged by the finding that mouse $TAF_{II}105$, a subunit of TFIID, directs cell type-selective gene activation in gonadal cells [Freiman et al., 2001; Falender et al., 2005]. By using a steroidogenic ovarian granulosa cell line, our study indicates that human $TAF_{II}105$ protein is preferentially expressed in ovarian granulosa cells, thus extending the previous observation of the mouse counterpart. In addition, the current work leads to the identification of a novel isoform of $TAF_{II}105$ transcript that results from alternative splicing. Furthermore, we show that $TAF_{II}105$ in granulosa cells is rapidly phosphorylated in response to an elevation of the intracellular cAMP level, and that this post-translational event occurs in a PKA-dependent manner. Thus, regulation of $TAF_{II}105$ at the levels of both post-translational modification and mRNA processing in ovarian granulosa cells may contribute to the role of this cell type-selective $TAF_{II}$ in follicular development.

The functional significance of the cAMP-stimulated phosphorylation of $TAF_{II}105$ remains to be explored. The fast kinetics of the

modification upon the forskolin treatment is reminiscent of that of CREB phosphorylation, which is critical for the recruitment of the CREB coactivator CBP to the cAMP-responsive promoters [Lamph et al., 1990; Mayr and Montminy, 2001]. Thus, it is tempting to speculate that phosphorylation of $TAF_{II}105$ may contribute to the cAMP-responsive transcription activation by enhancing the interactions between the cell type-selective TFIID complex with the upstream activators. Alternatively, the phosphorylated $TAF_{II}105$ may facilitate subsequent loading of other components of the preinitiation complex (PIC). Future functional analysis in the human tissue culture system should shed light on the functional relationship between $TAF_{II}105$ and multiple cAMP-responsive genes in ovarian granulosa cells.

The identification of the alternatively spliced isoform of the $TAF_{II}105$ transcript raises an intriguing question concerning its physiological significance. The predicted translation product from the novel mRNA isoform has the molecular weight of 70 kD, which may correspond to the prominent band of the same size in the $TAF_{II}105$ immunoblot of the KGN cell lysate (Fig. 2A). The predicted protein would also share almost the same amino acid sequence with a laboratory-generated, carboxy-terminal truncated mutant ($TAF_{II}105\Delta C$), which can function as a dominant negative mutant to interfere with the activity of endogenous full-length $TAF_{II}105$ [Yamit-Hezi and Dikstein, 1998; Yamit-Hezi et al., 2000]. The existence of a $TAF_{II}105$ mRNA isoform that encodes a dominant negative mutant suggests that the biological activity of $TAF_{II}105$ in ovarian granulosa cells may be dictated by the relative abundance of the full-length and the alternatively spliced transcripts. In this regard, it would be interesting to examine the expression levels of the two forms at different stages of ovarian folliculogenesis.

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