We have recently cloned a cDNA encoding an embryonic stem cell transcriptional coactivator termed UTF1 from the mouse F9 teratocarcinoma cell line (Okuda, A., Fukushima, A., Nishimoto, M., Orimo, A., Yamagishi, T., Nabeshima, Y., Kuro-o, M., Nabeshima, Y., Boon, K., Keaveney, M., Stunnenberg, H.G., and Muramatsu, M. (1998) EMBO J., 17, 2019–2032). Here we have cloned a cDNA for human UTF1 and identified two highly conserved domains termed conserved domain (CD)1 and CD2. Human UTF1, like that of mouse, binds to ATF-2 and the mutagenesis analyses reveal that the leucine zipper motif within the CD2 of the UTF1 and metal binding motif of ATF-2 are involved in this interaction. The factor also binds to TATA-binding protein containing complex. By means of immunoprecipitation analysis, we mapped two domains which are independently able to bind to the complex. Importantly, both domains are located within the conserved domains (one in CD1 and the other in CD2). Furthermore, transient transfection analyses point out the importance of these domains for activating ATF-2. Thus, these results suggest that these two conserved domains identified here play important roles in activating specific transcription at least in part by supporting physical interaction between the upstream factor, ATF-2, and basal transcription machinery.

Most of organisms including mammals start their embryonic developments with fertilization. However, understanding of developmental stages of placental mammals is hampered due to a number of drawbacks such as their large genome size and extended gestation period (1, 2). In addition, the fact that mammalian embryogenesis proceeds within a protective environment, i.e. in the uterus, makes the experimental manipulation of embryos more difficult. Furthermore, there are two more additional major obstacles in elucidating the molecular basis of early embryogenesis (before gastrulation) of mammals.

First, it is usually very difficult to obtain enough materials to study the embryogenesis due to the extremely small size of embryos during these stages. More specifically, the early embryogenesis of mammals appears to be uniquely regulated (3–5) and, therefore, studies using Drosophila or other organisms do not seem to apply directly to understanding the gene regulation in mammals during these periods, although significant similarities in the way to proceed late developmental stages (post-gastrulation) are evident between these organisms (6). To circumvent these difficulties, tissue culture cell lines, i.e. embryonic carcinoma (EC) cell lines such as F9 and P19 cells, are widely used as a model for studying the molecular basis governing early developmental stages of mammals (7). EC cells indeed resemble early embryonic cells of mammals in many respects. These EC cells retain pluripotency and can be induced to differentiate into several types of cells such as muscle and nerve cells using retinoic acid and/or by aggregate formation (8). Furthermore, they have been shown to participate in regular embryonic development when injected into a normal blastocyst (9–11).

We have been interested in elucidating the molecular mechanism of maintenance of pluripotency of embryonic cells. Although numerous transcription factors including HOX family members have been shown to be up-regulated during differentiation (12, 13), to our knowledge, only five transcription factors, i.e. Oct-3/4 (14–16), Oct-6 (17–20), REX-1 (21), SOX-2 (22), and PEA3 (23) have been cloned whose expression is down-regulated during differentiation. Therefore, it appears that we need to identify more genes encoding transcription factors or cofactors which are specifically expressed in pluripotent (undifferentiated) states of EC cells and repressed when these cells are induced to differentiate.

Recently, we have isolated a transcriptional coactivator termed UTF1 from mouse F9 teratocarcinoma cell line which has properties satisfying the above criteria (24). In this context, we considered that it would be advantageous to obtain human UTF1 and compare the structure and functions of the protein with those of mouse UTF1 to assess the generalized roles of UTF1 during early embryonic development of mammals. Here we report the molecular cloning of the human UTF1 and demonstrate that unique expression profile and the ability to function as a coactivator of ATF-2 are both well conserved between these species. Sequence comparison between human and mouse UTF1s leads to the identification of two evolutionarily
conserved domains. Furthermore, our analyses reveal that these domains indeed play essential roles in potentiating ATF-2 activity by bridging between ATF-2 and basal transcription machinery.

**EXPERIMENTAL PROCEDURES**

Cloning of Human UTF1 and Its Sequence Analysis—A cDNA library was constructed according to the standard procedure (25) using poly(A){superscript +} RNA from undifferentiated human teratocarcinoma cell line, NEC14 (26) and screened with the entire mouse UTF1 cDNA (24) as a probe. Sequencing of the obtained clones was done by the dideoxynucleotide chain reaction method as BamHI/EcoRI fragment and subcloned into Bluescript KSII{superscript +} vector. Construction of human UTF1 expression vector under the control of β-actin promoter is as follows. Single-stranded DNA was recovered from the cDNA covering entire human UTF1 cDNA including 5{superscript ’} and 3{superscript ’} noncoding region subcloned into Bluescript KSII{superscript +} vector and then Asp718 site was created at the position of 13 (immediate upstream of initiating ATG codon). Subsequently, Asp718/BamHI fragment was recovered and subcloned into pUC18 vector (27) with the aid of a linker. For constructing the ΔCD1 expression vector as well as internal deletion mutant vectors shown in Fig. 1A, two XhoI sites were created at the indicated positions using the above single-stranded DNA and the internal XhoI fragment was removed. The remaining vector fragment including the rest portion of the cDNA was self-ligated and Asp718/BamHI fragment was recovered from the resulting plasmid. These fragments were attached with Flag-tag sequence and subcloned into pHSApr-1 vector (27) with the aid of a linker. For constructing the ΔCD1 expression vector as well as internal deletion mutant vectors shown in Fig. 1A, two XhoI sites were created at the indicated positions using the above single-stranded DNA and the internal XhoI fragment was removed. The remaining vector fragment including the rest portion of the cDNA was self-ligated and Asp718/BamHI fragment was recovered from the resulting plasmid. These fragments were attached with Flag-tag sequence and subcloned into pHSApr-1 as above. For constructing internal deletion mutants shown in Fig. 1A, the BamHI site was created at the amino acid position of 208 and then single-stranded DNA was prepared with the aid of the helper phages. The same mutagenesis procedure was performed to create the same sets of the above XhoI sites. After removal of the internal XhoI fragments, Asp718/BamHI fragments were recovered and subcloned in the expression vector as above. Deletion mutants shown in Fig. 1A were also constructed by creating XhoI or BamHI sites at the appropriate positions by the site-directed mutagenesis. DNA fragments used in Fig. 6 were also subcloned into pTM1 vector (28) for producing in vitro translated proteins. This analysis further revealed that the amino-terminal half and carboxyl-terminal end of the protein indicated by boxes are significantly conserved between these two species (more than 85% amino acid identity) and we designated these regions as conserved domain (CD1 and CD2, respectively). However, the rest of portions are found to be highly diverged. Interestingly, all of hydrophobic residues at the first and fourth positions within the seven-amino acid repeat of the leucine zipper in the CD2 are 100% identical, implying that this motif is functionally important for the UTF1 activity.

**RESULTS**

Molecular Cloning of Human UTF1—We have recently reported the molecular cloning of a transcriptional cofactor expressed in pluripotent embryonic stem cells (24). We have further shown that UTF1 boosts the level of specific transcription by functioning as a coactivator of ATF-2. The amino acid sequence of UTF1 is very unique. Indeed, comparison with the available sequence data reveal that UTF1 is not significantly homologous to any previously reported protein. UTF1 does not even have any obvious structural motifs other than a leucine zipper. In this context, we considered that we should know which region(s) of UTF1 is evolutionarily conserved to deduce the functionally important domain(s) of the protein. Therefore, we have searched for human UTF1 by screening a cDNA library derived from a human teratocarcinoma cell line, NEC14 (26) using mouse UTF1 cDNA as a probe. Screening of 10{superscript 6} colonies from a plasmid cDNA library yielded five independent clones. Subsequent partial sequence analyses revealed that all of them were originated from the same mRNA. Hence, we have sequenced a cDNA clone bearing the longest insert and found that, as shown in Fig. 1A, this clone encodes a protein composed of 341 amino acids covering the entire human UTF1 protein. Fig. 1B shows the comparison between human and mouse UTF1 sequences. This process revealed that there are 64% amino acid identity and 87% similarity between these two proteins. This analysis further revealed that the amino-terminal half and carboxyl-terminal end of the protein indicated by boxes are significantly conserved between these two species (more than 85% amino acid identity) and we designated these regions as conserved domain (CD1 and CD2, respectively). However, the rest of portions are found to be highly diverged. Interestingly, all of hydrophobic residues at the first and fourth positions within the seven-amino acid repeat of the leucine zipper in the CD2 are 100% identical, implying that this motif is functionally important for the UTF1 activity.

**Evidence for the Human UTF1 as the Ortholog of Mouse UTF1**—We have screened the cDNA library for the ortholog of mouse UTF1 and obtained a good candidate, as described above. However, because of significant divergence in amino acid sequence between human and mouse UTF1s (only 64% identity as a whole), it is possible that these two proteins represent distinct factors sharing only similar structural motifs. To determine which is the case, we have done two different types of experiments as follows. First, we used genomic Southern blot analyses. As shown in Fig. 2A, lane 1, hybridization with the human UTF1 cDNA probe yielded a strong single band on the filter. Furthermore, hybridization at lower temperature (60 °C) or washing conditions (3 × SSC, 55 °C) did not give rise to any additional bands in the filter (data not shown), indicating that UTF1-related gene exists in human genome. When the filter was dehybridized and rebritized with mouse UTF1 cDNA probe, we obtained the same sized band as that obtained with the human cDNA, albeit the signal was much weaker (lane 2). We have also isolated a genomic clone carrying human UTF1 gene from bacteriophage library and determined the restriction map of the clone (Fig. 2B). From these analyses,
we find that the size of the observed band in the blot (Fig. 2A) is indeed equivalent to the one expected from the restriction map (corresponding DNA fragment was indicated by an asterisk). Therefore, these results strongly suggest that the former possibility is the case. To further confirm the above idea, we determined the chromosomal localization of the human UTF1 gene by the FISH technique, as we already know that mouse UTF1 is located at 7F5 by the analysis. 2 Using the whole genomic clone DNA shown in Fig. 2B, the human UTF1 gene has been located at 10q26, which is the syntenic region of the murine chromosome (7F5) where mouse UTF1 is located (Fig. 3). Based on these results, we conclude that human UTF1 reported here is the ortholog of mouse UTF1.

**UTF1 Transcriptional Coactivator**

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Fig. 2. Southern blot analysis of human genomic DNA with human or mouse UTF1 cDNA. A, human genomic DNA was subject to double digestion with EcoRI and BamHI. The digest (15 μg/lane) was electrophoresed on a 1.0% agarose gel and then transferred to a nylon filter. Hybridization and washing were done as described under "Experimental Procedures." B, the restriction map of a recombinant phage DNA carrying human UTF1 gene. The solid boxes indicate the region complementary to human UTF1 mRNA sequence. An asterisk represents the DNA fragment detected by Southern blot analysis. Restriction enzymes are abbreviated as follows: S, SalI; E, EcoRI; N, NotI; X, XhoI; B, BamHI; H, HindIII. The wavy lines indicate vector portions.
cell lines. To determine whether the teratocarcinoma cell-specific expression is regulated during differentiation of these cells, we induced NEC 14 cells to differentiate by using N,N\textsubscript{9}-hexamethylene bisacetamide according to Hasegawa et al. (26).

As shown in Fig. 4B, this differentiation process was indeed accompanied by the extinction of UTF1 expression. NEC14 cells were induced to differentiate with 10 mM N,N\textsubscript{9}-hexamethylene bisacetamide for 4 days as described by Hasegawa et al. (26). RNA was prepared before or after induction. RNAse mapping analysis was done as in “A.” Figure 4C, the human UTF1 protein disappears during differentiation of NEC 14 cells. The NEC14 cells are induced to differentiate as in B and then whole cell extract were prepared. Subsequently, the level of human UTF1 protein was examined by Western blot analysis. D, the human UTF1 is a phosphorylated protein. The hUTF1 protein was immunopurified and subsequently mixed with manganese-containing buffer in the absence (lane 1) or presence (lane 2) of \( \lambda \)-protein phosphatase (PP) as described under “Experimental Procedures.”

Furthermore, as shown in Fig. 4D, when the immuno-purified UTF1 protein was treated with \( \lambda \)-protein phosphatase (for details see “Experimental Procedures”), the protein gave a single band in the gel whose mobility was almost identical to the fastest migrating polypeptide obtained with the control protein which was not subject to the phosphatase treatment (compare lanes 1 and 2), indicating that the abnormal pattern of UTF1 in Western blot is due to phosphorylation of the protein by certain kinases.

**Functional Conservation of Human UTF1**—To explore the functional conservation between human and mouse UTF1s, we have examined whether human UTF1 is also able to boost the level of specific transcription through association with ATF-2 using ATF-2 fused to DNA-binding domain of c-Myb as described previously (24). Constant amounts of the reporter plas-
UTF1 Transcriptional Coactivator

mid, Collagen CAT-Myb bearing 6 copies of Myb-binding site and Myb/DNA-binding protein/ATF-2 fusion protein expression vector were introduced into Chinese hamster ovary cells together with either mouse or human UTF1 expression vector. Consistent with previous results, mouse UTF1 augmented the promoter activity about 20-fold (Fig. 5). Interestingly, the human UTF1 stimulated this specific transcription even more potently than the mouse counterpart. In both cases, too large amounts of UTF1 protein in the cell resulted in attenuation of the magnitude of the induction probably by a squelching phenomenon. Immunoblots confirmed that both mouse and human UTF1s were expressed in parallel with increasing amounts of the expression vectors transfected. Multiple bands were again observed and we also confirmed that these were due to phosphorylation of UTF1 proteins (data not shown). The activations observed are ATF-2 portion-specific because neither mouse nor human UTF1 could cooperate with Myb/DNA-binding protein alone (bottom half of Fig. 5).

Functional Relevance of the Evolutionarily Conserved Domains—To address the functional importance of two evolutionarily conserved domains, we have examined whether the UTF1 mutants lacking CD1 or CD2 shown in Fig. 6A are able to augment the ATF-2-dependent transcription as above. A leucine zipper mutant was also generated and subject to the same analysis. We found that neither mouse nor human UTF1s were able to elevate the level of transcription in an ATF-2 dependent manner. Immunoblot analyses confirmed that comparable amounts of these UTF1 mutants as well as wild-type protein were produced in the cell, eliminating the possibility that apparent failure of UTF1 mutants in stimulating ATF-2 dependent transcription was due to the insufficient production of these proteins. These results clearly indicate that both CD1 and CD2 bearing the leucine zipper motif of the protein are essential for the UTF1 activity in terms of stimulating transcription via ATF-2.

We have previously proposed that UTF1 activates ATF-2 by connecting physically between the ATF-2 activation domain and basal transcriptional machinery. We therefore examined whether two evolutionarily conserved domains are involved in the interaction between ATF-2 and UTF1 by the GST pull-down analysis. [35S]Methionine-labeled UTF1 protein or its derivatives were produced in rabbit reticulocyte lysates and then mixed with bacterially produced activation domain of ATF-2(1–109) fused to GST in the presence of glutathione-Sepharose beads. After extensive washing, protein bound to beads was quantified. As shown in Fig. 7A, lanes 3 and 6, ΔCD1 as well as wild-type protein could bind to GST-ATF2(1–109), whereas neither ΔCD2 nor the leucine zipper mutant bound to the fusion protein (lanes 9 and 12), indicating that the leucine zipper motif resided in CD2 is crucial for the interaction between these two proteins. At this stage, it occurred to us that the metal binding motif present in the activation domain of ATF-2 may be involved in the interaction with UTF1 through its leucine zipper motif, as there are a number of examples in which these two motifs are involved in protein-protein interactions. Therefore, we have examined whether two cysteine residues in the metal binding motif of ATF-2 are required for the association with UTF1. As shown in Fig. 7B, we found that both metal-binding motif mutants failed to interact with UTF1, indicating that the above possibility is the case.

Association of UTF1 with TBP-containing Complex Does Not Require the Presence of ATF-2—The above analyses have indicated that the leucine zipper motif within the CD2 of UTF1 plays an essential role in association with ATF-2. The next obvious question is which domain(s) are required for another
important properties of a coactivator, i.e., functional association with the basal transcription machinery. Before addressing this question, we considered that it is important to know whether the association of UTF1 with the basal transcription complex requires the presence of ATF-2. To this end, we have expressed wild-type UTF1 in HeLa cells which do not express endogenous UTF1 and prepared nuclear extract. Subsequently, ATF-2 present in the extract was immuno-depleted using anti-ATF-2 antibody-bound Sepharose beads (see “Experimental Procedures” for details). As shown in Fig. 8A, the antibody-bound, but not control protein A-Sepharose beads, completely removed the ATF-2 from the extract. Using these extracts, interaction between UTF1 and TBP, a central component of THIID complex in vivo was examined by coimmunoprecipitation analysis using anti-TBP antibody-bound Sepharose beads. As shown in Fig. 8B, anti-TBP antibody coimmunoprecipitated UTF1 irrespective of the presence of ATF-2 in the extract. The specificity of the interaction was confirmed by the fact that Sepharose resins which were not coupled to the anti-TBP antibody did not pull-down the UTF1 protein (lanes 2 and 5). Therefore, these results indicate that ATF-2 are not required for the interaction of UTF1 with the TBP-containing complex. Thus, it is assumed that the domain(s) of UTF1 required for the interaction of UTF1 and TBP-containing complex can be delineated independent of its interaction with ATF-2.

Localization of UTF1 Domains Involved in Interaction with TBP-Containing Complex in Vivo—To roughly map the region(s) required for the interaction of UTF1 with TBP-containing complex, we have expressed ΔCD1 and ΔCD2 in HeLa cells and examined whether either one of these proteins has lost the ability to associate with the complex. However, these experiments revealed that both ΔCD1 and ΔCD2 were able to associate with TBP-containing complex in vivo (Fig. 9B, lanes 6 and 9). These results indicate that the non-conserved domain(s) of UTF1 may be responsible for the interaction between UTF1 and TBP-containing complex. Alternatively, it is possible to assume that each conserved domain can independently associate with the complex. To determine which is the case, we expressed four additional UTF1 mutants depicted in Fig. 9A in cells and examined the interaction between these mutants and TBP-containing complex in vivo as above. These analyses revealed that mutant α, β, and δ which contained neither intact CD1 nor CD2 could not interact with TBP-containing complex, whereas mutant γ carrying complete CD1 interacted with the
as mutant dependent interaction of UTF1 with TBP-containing complex, sustained by the leucine zipper motif is involved in the CD2-region is not necessary for the interaction, ever, analyses with three deletion mutants revealed that this involved in the interaction with TBP-containing complex. How-

involved in the interaction with TBP-containing complex. Although these results may indicate that the unusual feature of this portion is functionally impor-

complex. In summary, these results revealed that any mutants having either of the two conserved domains could bind to TBP-containing complex in vivo, indicating that the latter is the case. These results also revealed that the α-helical structure sustained by the leucine zipper motif is involved in the CD2-dependent interaction of UTF1 with TBP-containing complex, as mutant β carrying the mutated motif failed to interact with the complex.

Unlike CD2, CD1 has no obvious structural motif. Therefore, we could not deduce which portion(s) of CD1 is critical for the interaction with TBP-containing complex in vivo. In this context, we made a series of internal deletion mutants using mutant γ as a starting material as depicted in Fig. 10A and examined their ability to associate with the complex. As shown in Fig. 10B, it was found that mutant γ-4 was not able to interact with the TBP-containing complex, while all other mutants retained their ability. The amino acid sequence deleted in mutant γ-4 is double-underlined in Fig. 1A. The amino acid composition reveals an extraordinarily high level of basic amino acids (6 residues out of 14 amino acids) in this portion. Interestingly, we noted that this 14-amino acid sequence is 100% identical between mouse and human UTF1s, implying that the unusual feature of this portion is functionally important. However, we noted some discrepancy between the data shown in Fig. 9 and those in Fig. 10. That is, mutant δ which carries the above domain failed to interact in the complex. Although these results may indicate that additional region(s) are also required to interact with TBP-containing complex, we consider that this is not likely because of the following reason. From the analyses shown in Fig. 9, the region encompassing from 135 to 208 amino acids appears to be involved in the interaction with TBP-containing complex. However, analyses with three deletion mutants revealed that this region is not necessary for the interaction, i.e. mutant γ-5, γ-6, and the mutant carrying only the first 166 amino acids were all found to be able to interact with TBP-containing complex (Fig. 10 and data not shown), although these mutants together cover the entire portion of the region (135–208). Therefore, we assume that mutant δ may have abnormal conformation and the region needed for the interaction with TBP-containing complex is not accessible.

Identification of Domains within CD1 Required for Potentiating ATF-2 Activity—To assess the functional importance of the highly basic domain identified in Fig. 10 and, at the same time, search for other domain(s) involved in ATF-2 activation, we made a series of internal deletion mutants, depicted in Fig. 11A, and examined their abilities in terms of activating ATF-2-dependent transcription. Fig. 11B shows the results of the analysis. It was found that some of mutants (mutants Δ49–73, Δ73–87, and Δ122–143) retained their ability to activate the specific transcription fairly well. Especially, Δ49–73 appears to act as a more potent activator than the wild-type protein. However, Δ107–122, which lacks the aforementioned highly basic region, displays much weaker activity than the wild-type protein, implicating that this domain is indeed involved in activating ATF-2. In addition to this mutant, Δ87–107 and Δ143–166 also show relatively weaker activities than the wild-type protein, indicating that the region deleted or mutated in these mutants are also involved in the activation in a way which is currently unknown. We will discuss these data in more detail below (see “Discussion”).

DISCUSSION

In this paper, we described the molecular cloning and characterization of a cDNA encoding a human transcriptional coactivator UTF1. By genomic Southern blot and FISH analyses, we have confirmed that molecularly cloned human protein corresponds to the ortholog of mouse UTF1. However, sequence comparison between human and mouse UTF1s reveals that these two proteins are rather highly diverged (only 64% identity and 87% similarity as a whole) in view of the high degree
Ten percent input sample was applied to the blot analysis using anti-Flag antibody. UTF1 mutants were detected by Western Procedures. Coimmunoprecipitated analysis as described under “Experimental Procedures.”

However, we cannot eliminate the possibility that other types of UTF1 may be a mammal-specific transcriptional coactivator. The genome of cow (data not shown). Therefore, we suspect that UTF1 is originally cloned from mammals (24). Therefore, it is not known at present whether other classes of organisms such as Drosophila and Xenopus possess a protein similar or equivalent to UTF1. To address this question, we have compared the UTF1 sequence with available sequence data of yeast, Drosophila, Caenorhabditis elegans, and Xenopus genomes. However, these analyses did not yield any single gene having significant similarity in nucleotide sequence to UTF1 cDNA. Moreover, we obtained no evidence of UTF1-related gene in Drosophila nor in Xenopus genome by Southern blot analyses with a variety of hybridization and washing conditions. On the other hand, the same approaches led to the identification of the UTF1 gene in the genome of cow (data not shown). Therefore, we suspect that UTF1 may be a mammal-specific transcriptional coactivator. However, we cannot eliminate the possibility that other types of organisms also have UTF1-related gene(s) in their genomes whose similarities are not high enough to be detected by regular hybridization techniques.

A striking resemblance between mammals and Xenopus is evident in the way to proceed late developmental stages (after gastrulation). However, it is also known that early embryogenesis (before streak stage) proceeds in quite a different manner between these organisms in many respects. For example, Xenopus early embryogenesis (before stage of 12 divisions) is largely sustained by factors generated from maternally expressed genes play major roles throughout mammalian development including early stages (2). Therefore, it is intriguing to speculate that, given that the UTF1 is a mammal-specific transcriptional coactivator, the factor is involved in unique regulation of transcription governing early developmental stages of mammals.

Relatively lower conservation of the amino acid sequence of UTF1 allowed us to identify evolutionarily conserved domains of the protein simply by comparing the sequence of mouse and human UTF1s. This process indeed led to the identification of two evolutionarily conserved domains termed CD1 and CD2. The CD2 contains the leucine zipper motif. The sequence comparison also reveals that the first and fourth positions within the 7-amino acid repeat of this coiled-coil motif are 100% identical between human and mouse UTF1s, implicating the functional importance of the motif. Biochemical analyses revealed that this motif is involved in the interaction with the activation domain of ATF-2. Mutagenesis analyses further defined the way of UTF1 to interact with ATF-2, i.e., these analyses have revealed that this interaction is sustained by the metal binding motif of ATF-2 and leucine zipper motif of UTF1. It is interesting to note that adenovirus E1A gene product, another ATF-2 interacting protein, also use these two motifs for their interaction, although UTF1 and E1A bind to distinct portions of ATF-2 (24, 43). UTF1 is also able to interact with TBP-containing complex. It is known that TBP is not a specific component of TFIIID complex, but is also present in other protein complexes such as SL1 and TFIIIB involved in pol I and pol III-specific transcription, respectively (44, 45). However, interaction between UTF1 and TBP in vivo appears to reflect the association of UTF1 with the TFIIID complex, since UTF1 is specifically involved in RNA polymerase II-specific transcriptions as described in the text, but not in RNA polymerase I- nor III-dependent transcription (data not shown). Furthermore, previous studies unequivocally established the physical interaction of UTF1 with TFIIID complex (24). Coimmunoprecipitation analysis with a series of truncated forms of UTF1 protein revealed that both CD1 and CD2 could bind to TBP-containing complex independently. Detailed domain mapping analyses further revealed that the highly basic region and the leucine zipper motifs in CD1 and CD2, respectively, are involved in the interaction with TBP-containing complex. Functional relevance of the former interaction is supported by the fact that deletion of the basic domain attenuates the transcriptional stimulating activity of UTF1 significantly. It is also noteworthy that the
additional domains (87–107 and 144–166) which appear to be involved in UTF1 activity in terms of activating ATF-2. As these regions are not involved in the interaction with either ATF-2 or TBP-containing complex, it is not known at present how these domains are involved in UTF1 activity. One possibility is that Δ87–107 and Δ144–166 may have abnormal conformations which attenuate the UTF1 activity, although the domains directly involved in functioning as a bridging factor are intact in these mutants. Alternatively, it is possible that these regions may be involved in bridging between ATF-2 and basal transcriptional machinery by interacting with other members of general transcription factors such as TFIIIB and RNA polymerase II. In the latter case, it is conceivable that the mutant, Δ107–122, which lacks the aforementioned highly basic region may exert its weak activity by communicating with the basal transcriptional machinery through either one or both of these two domains.

What roles does UTF1 play in early embryogenesis? From its unique expression profile, we speculate that UTF1 may play an important role in maintaining the undifferentiated or pluripotent states of embryonic cells. So far, we demonstrate that UTF1 functions as a coactivator of ATF-2. However, more systematic screening may lead to the identification of other transcriptional factors which cooperate with UTF1. It is probable that application of the gene targeting technique would provide important information to assess the biological role of UTF1 during early developmental stages of mammals. In any event, characterization of UTF1 appears to provide an additional unique approach toward understanding of elusive regulation of transcription in pluripotent and omnipotent cells of mammals.

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