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Human EFO1p exhibits acetyltransferase activity and is a unique combination of linker histone and Ctf7p/Eco1p chromatid cohesion establishment domains

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ABSTRACT

Proper segregation of chromosomes during mitosis requires that the products of chromosome replication are paired together—termed sister chromatid cohesion. In budding yeast, Ctf7p/Eco1p is an essential protein that establishes cohesion between sister chromatids during S phase. In fission yeast, Eso1p also functions in cohesion establishment, but is comprised of a Ctf7p/Eco1p domain fused to a Rad30p domain (a DNA polymerase) both of which are independently expressed in budding yeast. In this report, we identify and characterize the first candidate human ortholog of Ctf7p/Eco1p, which we term hEFO1p (human Establishment Factor Ortholog). As in fission yeast Eso1p, the hEFO1p open reading frame extends well upstream of the C-terminal Ctf7p/Eco1p domain. However, this N-terminal extension in hEFO1p is unlike Rad30p, but instead exhibits significant homology to linker histone proteins. Thus, hEFO1p is a unique fusion of linker histone and cohesion establishment domains. hEFO1p is widely expressed among the tissues tested. Consistent with a role in chromosome segregation, hEFO1p localizes exclusively to the nucleus when expressed in HeLa tissue culture cells. Moreover, biochemical analyses reveal that hEFO1p exhibits acetyltransferase activity. These findings document the first characterization of a novel human acetyltransferase, hEFO1p, that is comprised of both linker histone and Ctf7p/Eco1p domains.

INTRODUCTION

Faithful transmission of chromosomes requires that chromosomes are replicated and that the resulting sister chromatids are paired together. This pairing, or sister chromatid cohesion, occurs concomitantly with chromosome replication during S phase (1). During mitosis, cohesion ensures that one chromatid associates with microtubules from the spindle pole opposite that of its sister chromatid. At anaphase onset, cohesion is inactivated, allowing each sister to move along the spindle apparatus into the newly forming daughter cells (2,3).

In budding yeast, several structural cohesion proteins (cohesins) have been identified: Smc1p, Smc3p, Mcd1p/Scc1p, Irr1p/Scc3p and Pds5p (4–10). One current model is that these cohesins form rings that encompass or tether together sister chromatids (11,12). Deposition cohesion factors Scc2p (Mis4p in fission yeast) and Scc4p are required for the proper association of structural cohesins with chromatin, with deposition occurring from G_1/S through most of mitosis (7,13,14). Ctf7p/Eco1p is an acetyltransferase that represents a third class of cohesion proteins, the establishment factors (7,15,16). Although loss of Ctf7p/Eco1p (herein termed Ctf7p) function leads to precocious sister chromatid separation and cell death, Ctf7p is not required to maintain cohesion nor deposit cohesins onto DNA. Instead, budding yeast Ctf7p appears to establish cohesion by coupling the cohesion machinery to DNA replication.

Several lines of evidence reveal that cohesion establishment is coupled to DNA replication. First, Ctf7p is required exclusively during S phase when DNA replication occurs (7,16). Second, cells defective for Ctf7p function are rescued by elevated levels of PCNA (proliferating cell nuclear antigen), a sliding clamp that promotes processive DNA replication (16,17). Third, Ctf7p physically associates with each of three independent replication factor C (RFC) complexes (18). RFC complexes load PCNA-like sliding clamps onto double-stranded DNA, at least a subset of which are known to function in sister chromatid cohesion (18–22). The characterization of Trf4p (Pol σ) provided the first evidence that a DNA polymerase could also function in sister chromatid cohesion (23,24). More recently, the large subunit of budding yeast DNA polymerase ε (Pol2) was found to associate with Pol σ and participate in cohesion (25).

Despite the essential roles of budding yeast Ctf7p in chromosome segregation and cell viability, nothing is known concerning the Ctf7p cohesion establishment pathway in higher eukaryotes. Recent findings from yeast reveal that Ctf7p function is conserved through evolution. For instance, in budding yeast, *CTF7* is located on chromosome VI and encodes 281 amino acids (7,16). *RAD30*, encoding a DNA

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repair polymerase (Pol η), is located on chromosome IV and encodes 632 amino acids (26–28). In fission yeast, *CTF7* and *RAD30* sequences are genetically fused to produce *ESO1*. Eso1p is comprised of 872 amino acids and functions in both cohesion establishment and DNA polymerase functions, indicating that both Ctf7p and Rad30p activities are preserved (29,30). In this report, we identify the first full-length human ortholog of Ctf7p and characterize its enzymatic activity, localization and novel genetic composition.

MATERIALS AND METHODS

Cell growth and database methods

Growth and sporulation media for yeast were described previously (31). Yeast and bacterial strains and transformations were performed as described, with minor modifications (32–36). HeLa cell cultures were maintained in MEM supplemented with 10% fetal bovine serum, Earle's BSS, L-glutamine and antibiotics.

Budding yeast Ctf7p and Rad30p sequences and fission yeast Eso1p sequence were used to perform PSI-BLAST searches of non-redundant nucleic acid and protein sequence databases (GenBank CDS, PDB, SWISS-PROT, PIR and PRF), expressed sequence tag (EST) databases and recent submissions to the human genonome project (http://www.ncbi.nlm.nih.gov/BLAST). Clustal X and Clustal W were used to connect overlapping sequences to produce extended contiguous (contig) coding sequences. A full-length cDNA containing human *EFO1* was identified from the HUGE (Human Unidentified Gene-Encoded Large Proteins) database and obtained from the Kazusa DNA Research Institute in Japan (37).

Cloning and molecular methods

The human *EFO1* opening reading frame (ORF) was independently derived by the HUGE database. *hEFO1* coding sequence, flanked by BamHI restriction sites, was generated by PCR using KIAA1911 as a template (obtained from Kazusa DNA Research Institute) and oligos 5′-CGCGGATCCATG-TCCATTCAGGAGAAATCAAAAGAG-3′ and 5′-CGCG-GATCCGGTTGTTGCCAGTCCTGAGTTCATTGT-3′. The resulting product contains hEFO1p in which the N-terminal methionine is deleted. The PCR product was then cloned into pCR 2.1 (Invitrogen) to generate pBS1088. The entire *EFO1* region was sequenced, and two errors, presumably generated during PCR, were identified (Y93F and L384S). We termed this allele *hEFO1-1*. These two alterations are located well outside of the Ctf7p functional core domain (762–831) and did not adversely affect either acetyltransferase activity or nuclear localization (see Results). To generate wild-type *hEFO1*, a BplI–BsgI fragment obtained from KIAA1911 cDNA was used to replace the short DNA region containing both PCR-based alterations. The resulting clone was confirmed as correct by sequence analysis and used for further analyses.

To generate in-frame green fluorescent protein (GFP)–hEFO1p and GST–hEFO1p expression vectors, pCR 2.1-*hEFO1* was digested with BamHI and the resulting drop-out fragment ligated into either pGEX 4T-3 linearized with BamHI (Amersham/Pharmacia) or pEGFP C.1 linearized with BglII (Clontech). For both GST and GFP constructs, plasmids harboring hEFO1p in the reverse orientation were also identified. To generate a C-terminal truncation of hEFO1p, the *GST–hEFO1* construct was digested with BsmI to drop-out a 497 nucleotide fragment containing the *CTF7* core domain coding region. The remaining construct was ligated back together, termed *hEFO1-2*, and encodes a protein harboring a C-terminal truncation.

Expression and detection of recombinant proteins

pGST–hEFO1p and control constructs were transformed into BL-21 bacterial cells and selected on LB plates containing carbenicillin. Overnight liquid cultures were diluted 10-fold and incubated for 2 h at 37°C to obtain log phase growth. The cultures were then brought to a final concentration of 100 μM isopropyl-β-D-thiogalactopyranoside (IPTG; Fisher) and protein expression induced for 3–4 h at 23°C. Cells were then spun for 10 min at 9500 r.p.m. (Sorvall SS-34) and the pellet resuspended in SDS/Laemmli buffer and boiled prior to resolving on polyacrylamide gels. Proteins were visualized by transferring onto PVDF membrane (Amersham/Pharmacia) and detected with a combination of a mouse antibody directed against GST (Santa Cruz), goat anti-mouse horseradish peroxidase (HRP; Bio-Rad) and ECL$^+$ (Amersham/Pharmacia).

For acetylation assays, the bacterial cell pellet was suspended in 400 μl of 120 ELB buffer (38) supplemented with EDTA-free protease inhibitors (Roche) and phenylmethylsulfonyl fluoride (PMSF). De-acetylase inhibitors sodium butyrate (10 mM, Sigma) and nicotinamide (5 mM, Sigma) were added prior to cell lysis. The cell suspensions were lysed by sonication and brought to a final concentration of 50–100 μM acetyl-CoA (Sigma). After 5–10 min, the reaction was stopped by the addition of SDS/Laemmli buffer, boiled and resolved by polyacrylamide gel electrophoresis. Western blot detection of acetylated proteins was performed using a combination of mouse antibody directed against acetylated lysine residues (Cell Signaling Technology), goat anti-mouse HRP (Bio-Rad) and ECL$^+$ (Amersham/Pharmacia). An additional step to enrich for GST-coupled proteins was optionally performed using glutathione–Sepharose 4B beads (Amersham-Pharmacia) as previously described (18).

Transfection of pEGFP–hEFO1p into human tissue culture HeLa cells was performed according to the manufacturer's instructions (Roche). Briefly, cells were grown to ~30% confluence on coverslips housed in 6-well culture dishes. pEGFP–hEFO1p and control plasmids were diluted into serum-free medium supplemented with Fugene (Roche) and the resulting mixture added drop-wise into each of the wells before returning the cells to 37°C. Transfection and recovery were assessed 24–48 h later.

For visualization of GFP–hEFO1 protein *in vivo*, coverslips were dipped in warm PEM buffer and then fixed by plunging into –20°C methanol for 2 min. The cells were rehydrated in phosphate-buffered saline (PBS) and the coverslip mounted onto a glass slide using an antifade reagent to reduce photobleaching (Vectastain). Images were collected on a Nikon microscope (Eclipse E800) coupled to a Photometrics cooled CCD digital camera (CoolSnap *fx*).

For detection of GFP proteins by western blot analysis, coverslips were rinsed twice in cold PBS and the cells

immediately lysed in SDS/Laemmli buffer, boiled and resolved by acrylamide gel electrophoresis. GFP-tagged proteins were transferred onto PVDF membrane and detected with a combination of a mouse antibody directed against GFP (Cell Signaling Technology), goat anti-mouse HRP (Bio-Rad) and ECL$^+$ (Amersham/Pharmacia).

Northern blot analyses

Expression of hEFO1p was assessed using a multi-tissue human northern blot (Clontech) following the manufacturer's instructions. Two confirm expression, the membrane was independently probed twice using distinct probes: a 705 nt PCR product (oligos 5′-GACCCACCATTGGATAATCAG-3′ and 5′TGGATATGTTCCGCAATTAGG-3′) or a 690 bp fragment released via EcoRI–BamHI digestion. In both cases, the DNA probes were labeled with [^{32}P]dCTP by random priming and separated from unincorporated dNTPs prior to hybridization (39).

RESULTS

Ctf7p/Eco1p is highly conserved but genetically mobile through evolution

In budding yeast, Ctf7p is a 281 amino acid protein essential for sister chromatid cohesion (7,16). In fission yeast, Eso1p is an 872 amino acid protein that also functions in sister chromatin cohesion but is comprised of two distinct domains: a Rad30p domain as the N-terminus and a Ctf7p domain as the C-terminus (29,30). To date, it is unknown whether higher order eukaryotes display the stand-alone Ctf7p of budding yeast, the bi-functional Rad30p–Ctf7p fusion of fission yeast or a novel genetic fusion. Resolving this issue thus remains an important first step in identifying the factors and elucidating the molecular activities required for cohesion establishment in human cells.

We used computer-assisted database searches to assess which form of Ctf7p predominates in higher eukaryotes. First, we identified Rad30p-like proteins and then determined whether the Rad30p domain was coupled to a downstream Ctf7p domain. The Rad30p domain of fission yeast Eso1p was used to query non-redundant databases, including ESTs and newly released human genome sequences (Materials and Methods). We obtained multiple hits, consistent with previous studies documenting the high degree of conservation of Rad30p proteins through evolution (40–42). Sequence analyses revealed that these Rad30p-like proteins failed to contain domains that exhibited significant homology to Ctf7p (data not shown). The inability to identify Rad30p proteins that also contain a Ctf7p domain in higher eukaryotes suggests that the genetic fusion comprising Eso1p may be limited to close relatives of fission yeast.

Second, we asked the reciprocal question: is the Ctf7p domain coupled to an upstream Rad30p domain in higher eukaryotes? The Ctf7p domain of fission yeast Eso1p was used to query non-redundant and EST databases as described above. We then ascertained if any of the putative Ctf7p homologs contain a Rad30p domain. Database analyses revealed that conceptual translations of putative Ctf7p orthologs exist in several model organisms including *Drosophila*, mice and humans (Fig. 1A). An amino acid alignment of the Ctf7p core domain indicates the high degree of conservation (Fig. 1B). We found full-length cDNAs for both *Drosophila* and human candidate orthologs that contain coding regions that extend well upstream of the Ctf7p domain (N-terminal extensions of 854 residues in *Drosophila* and 641 residues in humans). Neither of these N-terminal upstream domains exhibit similarity to Rad30p. These results show that, in both *Drosophila* and humans, the highly conserved Ctf7p domain is fused to a domain other than Rad30p, indicating that Ctf7p is a modular element that has been genetically recombined to other elements through evolution.

Human EFO1p is one of four candidate proteins exhibiting homology to Ctf7p

A human EST that exhibits sequence homology to a single Ctf7p candidate ortholog in humans was previously reported (16). More recently, other short Ctf7p-like encoding sequences have been identified (http://mendel.imp.univie.ac.at). Here, we extend each of these sequences, all of which reside on different chromosomes and contain the highly conserved Ctf7p core domain (Fig. 2). At least one of these human ORFs contains an N-terminal extension that is absent from budding yeast Ctf7p and unique to the N-terminal region of fission yeast Eso1p (see below). To differentiate between these human sequences in an unbiased way (*Sc*Ctf7p/Eco1p versus *Sp*Eso1p) and to reflect that at least one of these human coding regions contains a domain unique to any Ctf7p protein thus far characterized, we name this family human Establishment Factor Orthologs (hEFO1s). In this report, we provide the full-length translation, expression pattern and characterization for one family member, hEFO1p (see below). *hEFO1* (KIAA1911, accession No. AB067498) resides on chromosome XVIII. *hEFO2–hEFO4* exhibit significant homology to the C-terminus of *hEFO1*, although their expression is currently unknown. *hEFO2* resides on chromosome VIII and represents the first human coding sequence identified that exhibited significant homology to Ctf7p (16). We used database search methods to extend the *hEFO2* coding region, identifying two new overlapping ESTs that produce a conceptual contiguous amino acid sequence of 334 residues (GenBank accession Nos N94144, BG288675 and BE247544). *hEFO3* (accession No. NT 005334.10) and *hEFO4* (accession No. NT 033232.2) reside on chromosomes II and XI, respectively. Both *hEFO3* and *hEFO4* coding sequences contain a 762 bp region that exhibits significant homology to positions 3139–3900 encoding hEFO1p.

Conceptual translation of *hEFO1* cDNA indicates that hEFO1p is comprised of 840 amino acid residues (Fig. 3A) encoded by a 4.6 kb message containing 5′- and 3′ untranslated regions (37). To both test for *hEFO1* expression and assess its relative abundance among various cell types, we probed a multi-tissue northern blot containing RNA isolated from heart, brain, placenta, lung, liver, muscle, kidney and pancreas tissues. ^{32}P-labeled probes from two regions of the *hEFO1* ORF were independently used and identified a single transcript (Fig. 3B). The results show that *hEFO1* is expressed in the majority of tissues tested, but elevated expression was detected in skeletal muscle.

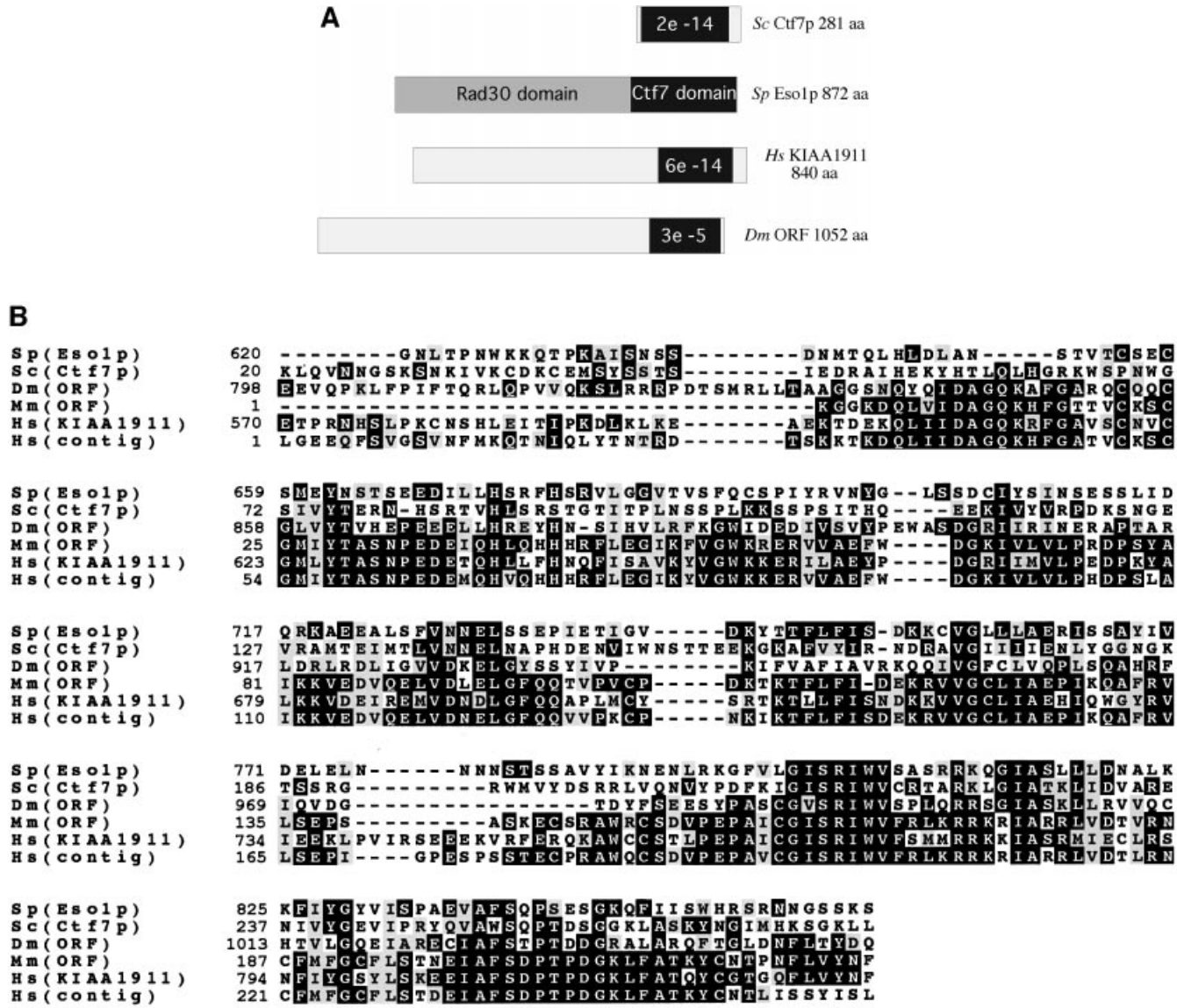

Figure 1. Identification of Ctf7p-like proteins in higher eukaryotes. (**A**) Schematic illustrating Eso1p and conceptual translations of cDNAs that encode Ctf7p-like proteins. E values (a measurement of similarity) for Ctf7p domains are shown in black. The N-terminal region of Eso1p that exhibits homology to Rad30p is shown in dark gray. Regions that extend upstream of the Ctf7p region in human and *Drosophila* are shown in light gray. (**B**) Amino acid alignments reveal the high degree of conservation of the Ctf7p domain for a selection of coding sequences. Residues are numbered according to conceptual translations of cDNA sequences, except for contig sequences which were assigned a starting residue number of 1. Accession numbers are as follows: *Schizosaccharomyces pombe* Eso1p (BAA95122), *Saccharomyces cerevisiae* Ctf7p/Eco1p (NP 116683), *Drosophila melanogaster* (AAF50579.1), *Mus musculus* ORF (MGC:30637), *Homo sapiens* KIAA1191 (AB067498) and *Homo sapiens* contig (comprised of N94144, BG288675 and BE247544). Other mouse (AAH08220, BAB26905 and AA881675) and human orthologs were also identified.

Human EFO1p is comprised of linker histone protein and Ctf7p domains

In both *hEFO1* and in the *Drosophila* cDNA containing the Ctf7p core domain that we identified by computer-assisted database searches (Fig. 1), the C-terminal Ctf7p domain is fused to an N-terminal extension other than Rad30p. We next determined whether these N-terminal extensions are similar to each other or represent yet further novel genetic fusions. Database search methods reveal that both N-terminal extensions are conserved. Importantly, the N-terminal extensions of both hEFO1p and *Drosophila* cDNA translations exhibit significant homology to micronuclear linker histone proteins identified in *Tetrahymena* (Fig. 4). Based on similarities between these unique genetic fusions, we term the *Drosophila* homolog *dEFO1*. Micronuclear linker histone proteins are unique from macronulcear histone H1 and are critical for normal chromosome compaction (43,44). In *Tetrahymena*, the 71 kDa linker histone polyprotein is processed to produce independent α, β, γ and δ linker histone proteins (α linker histone is an early cleavage product, but later processed to generate γ and δ linker histone proteins). Sequence alignments

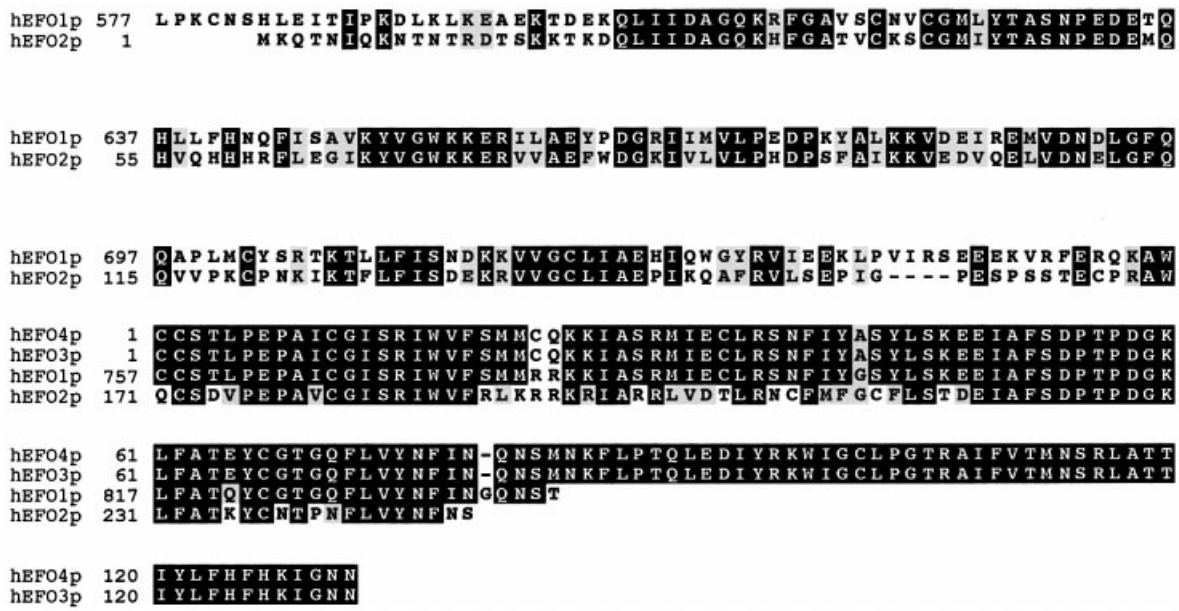

Figure 2. Amino acid alignments for conceptual translations of candidate human Ctf7p family members. See text for accession numbers and details.

reveal that the N-terminal extension in hEFO1p is comprised of δ and β linker histone proteins (Fig. 4). These findings document a unique genetic fusion between linker histone protein and a Ctf7p domain.

EFO1p expressed in human cells is a nuclear protein

In budding yeast, Ctf7p is a nuclear protein required for proper chromosome segregation (7,16). It thus became important to test whether hEFO1p localizes to a specific region in the vertebrate cell. The *hEFO1* coding cDNA was obtained from the Kazusa DNA Research Institute in Japan (37) and inserted in-frame behind GFP in a mammalian expression vector containing the cytomegalovirus (CMV) promotor (Materials and Methods). The resulting GFP–hEFO1p construct and GFP control plasmids were transfected into HeLa tissue culture cells. Within 24 h, GFP–hEFO1p expression in HeLa cell extracts was readily detected by western blot at the appropriate molecular weight. A protein band of similar molecular weight was absent in HeLa extracts expressing GFP alone (data not shown).

We then determined the localization of GFP–hEFO1p in human tissue culture cells. Transfected HeLa cells were grown on coverslips for 24–48 h. Images were then collected using both phase contrast microscopy to visualize cell morphology and epi-illumination to detect GFP-tagged proteins. GFP visualization revealed that hEFO1p localizes specifically to the human cell nucleus (Fig. 5). In contrast, GFP was predominantly cytoplasmic. Untransfected cells were devoid of GFP signal. These results reveal that hEFO1p localizes to the nucleus of human cells, consistent with a role in chromosome segregation.

Human EFO1p exhibits acetyltransferase activity

Bacterial expressed budding yeast Ctf7p was recently shown to possess acetyltransferase activity *in vitro* (15). Although physiologically relevant substrates have yet to be identified, Ctf7p is known to serve as its own substrate. We exploited this autoacetylation activity to determine whether hEFO1p also exhibits acetyltransferase activity. The *hEFO1* coding sequence was inserted in-frame behind that of *GST* in an inducible bacterial expression vector. The resulting *GST–hEFO1* construct was transformed into bacterial cells and protein expression induced using IPTG (Materials and Methods). As a positive control, *GST–CTF7* was independently transformed and expressed in bacteria. Post-induction, the bacterial strains were lysed and assessed for protein expression. An optional protein enrichment step via glutathione–Sepharose bead pull-down was also used (Materials and Methods). Both GST–hEFO1p and GST–Ctf7p protein bands of the appropriate molecular weights were readily detectable by western blot analyses (Fig. 6).

We then tested whether bacterially expressed hEFO1p was capable of acetyltransferase activity. As before, GST–EFO1p and GST–Ctf7p expression was induced, the bacterial cells lysed and GST-containing proteins enriched by glutathione–Sepharose bead pull-downs. The resulting protein fractions were incubated for 10 min in buffer containing a final concentration of 50 μM acetyl-CoA. The addition of acetyl moieties onto GST–hEFO1p or GST–Ctf7p was then visualized by western blot analysis using an acetylated lysine-specific antibody previously documented to detect Ctf7p acetylation reactions (15). As expected, GST–Ctf7p exhibited a robust autoacetylation activity. GST–hEFO1p also exhibited a similar autoacetylation activity (Fig. 6). No antibody cross-reactivity was observed for GST alone. These findings reveal that the acetyltransferase activity of Ctf7p-like proteins is conserved through evolution. We next tested for the specificity of the hEFO1p acetylation reaction by generating a loss-of-function allele in which the C-terminal region containing the Ctf7p core domain was deleted (hEFO1-2p). The resulting construct was expressed at levels similar to hEFO1p but was unable to catalyze the autoacetylation reaction (data not shown).

A

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    atg atg tcc att cag gag aaa tca aaa gag aat tcc tcc aaa gtt act aaa aaa agt gac gat aag aat tca gaa aca gaa
1   M   M   S   I   Q   E   K   S   K   E   N   S   S   K   V   T   K   K   S   D   D   K   N   S   E   T   E
    att cag gat tct caa aag aat cta gca aaa aaa tca ggt cca aag gag act ata aaa tca cag gct aaa tct tcc agt gaa
28  I   Q   D   S   Q   K   N   L   A   K   K   S   G   P   K   E   T   I   K   S   Q   A   K   S   S   S   E
    agt aaa ata aat cag cca gaa ttg gaa aca cgc atg agt aca agg tca tca aag gca gca tct aat gat aaa gct act aaa
55  S   K   I   N   Q   P   E   L   E   T   R   M   S   T   R   S   S   K   A   A   S   N   D   K   A   T   K
    tcc att aat aaa aat acg gtg act gtg agg gga tat tca caa gaa tct aca aaa aag aaa tta tct cag aaa aaa tta gta
82  S   I   N   K   N   T   V   T   V   R   G   Y   S   Q   E   S   T   K   K   K   L   S   Q   K   K   L   V
    cat gaa aac cct aaa gca aat gaa cag ctt aac cgg aga tca caa agg cta caa caa tta aca gag gtt tca aga agg tcg
109 H   E   N   P   K   A   N   E   Q   L   N   R   R   S   Q   R   L   Q   Q   L   T   E   V   S   R   R   S
    tta cgc agt aga gaa att cag ggt caa gtt caa gca gtt aaa cag agt ttg cca cca act aaa aaa gag cag tgt agc agt
136 L   R   S   R   E   I   Q   G   Q   V   Q   A   V   K   Q   S   L   P   P   T   K   K   E   Q   C   S   S
    act cag agt aaa tct aat aaa aca agt caa aaa cat gtg aag aga aaa gta ctg gaa gta aag tct gac tct aaa gaa gat
163 T   Q   S   K   S   N   K   T   S   Q   K   H   V   K   R   K   V   L   E   V   K   S   D   S   K   E   D
    gaa aat cta gta att aat gaa gta ata aat tct ccc aaa ggg aaa aaa cgc aag gta gaa cat cag aca gct tgt gct tgt
190 E   N   L   V   I   N   E   V   I   N   S   P   K   G   K   K   R   K   V   E   H   Q   T   A   C   A   C
    agt tct caa tgc acg caa gga tct gaa aag tgt cct cag aag act act aga aga gac gaa acg aaa cct gtg cct gta act
217 S   S   Q   C   T   Q   G   S   E   K   C   P   Q   K   T   T   R   R   D   E   T   K   P   V   P   V   T
    tct gag gtg aaa aga tca aaa atg gct act tca gtg gtc ccg aaa aag aat gag atg aag aag tcg gtt cat aca caa gtg
244 S   E   V   K   R   S   K   M   A   T   S   V   V   P   K   K   N   E   M   K   K   S   V   H   T   Q   V
    aat act aac aca aca ctc cca aaa agt cca cag cca tca gtg cct gaa caa agt gat aat gag ctg gag caa gca gga aag
271 N   T   N   T   T   L   P   K   S   P   Q   P   S   V   P   E   Q   S   D   N   E   L   E   Q   A   G   K
    agc aaa cga ggt agt att ctc cag ctc tgt gaa gaa att gct ggt gaa att gag tca gat aat gta gag gta aaa aag gaa
298 S   K   R   G   S   I   L   Q   L   C   E   E   I   A   G   E   I   E   S   D   N   V   E   V   K   K   E
    tct tca caa atg gaa agt gta aag gaa gaa aag ccc aca gaa ata aaa ttg gaa gag acc agt gtt gaa aga caa ata ctt
325 S   S   Q   M   E   S   V   K   E   E   K   P   T   E   I   K   L   E   E   T   S   V   E   R   Q   I   L
    cat cag aag gaa aca aat cag gat gtg caa tgt aat cgt ttt ttc cca agt aga aaa aca aag cct gtg aaa tgt ata cta
352 H   Q   K   E   T   N   Q   D   V   Q   C   N   R   F   F   P   S   R   K   T   K   P   V   K   C   I   L
    aat gga ata aac agc tta gcc aag aag aac tcc aac tgg act aaa att aaa ctc tca aaa ttt aac tct gtg cag cac aat
379 N   G   I   N   S   L   A   K   K   N   S   N   W   T   K   I   K   L   S   K   F   N   S   V   Q   H   N
    aag ttg gac tct caa gtt tcc cct aaa tta ggc tta tta cga acc agt ttt tca cca cca gct tta gaa atg cat cat cca
406 K   L   D   S   Q   V   S   P   K   L   G   L   L   R   T   S   F   S   P   P   A   L   E   M   H   H   P
    gtg act caa agt aca ttt tta ggg aca aag cta cat gat aga aat ata act tgc cag cag gaa aaa atg aaa gaa att aat
433 V   T   Q   S   T   F   L   G   T   K   L   H   D   R   N   I   T   C   Q   Q   E   K   M   K   E   I   N
    tct gaa gaa gtg aaa att aat gat att aca gta gaa att aat aaa acc aca gaa agg gct cct gaa aat tgt cat ttg gcc
460 S   E   E   V   K   I   N   D   I   T   V   E   I   N   K   T   T   E   R   A   P   E   N   C   H   L   A
    aat gag ata aaa cct tct gac cca cca ttg gat aat cag atg aaa cat tct ttt gat tca gca tca aat aag aat ttc agc
487 N   E   I   K   P   S   D   P   P   L   D   N   Q   M   K   H   S   F   D   S   A   S   N   K   N   F   S
    caa tgt ttg gaa tcc aag cta gaa aac agt cca gtg gaa aat gtt act gct gct tcg act ctg ctc agt caa gca aaa att
514 Q   C   L   E   S   K   L   E   N   S   P   V   E   N   V   T   A   A   S   T   L   L   S   Q   A   K   I
    gat aca gga gag aat aaa ttt cca ggt tca gct ccc caa cag cat agt att ctc agt aac cag aca tct aaa agc agt gat
541 D   T   G   E   N   K   F   P   G   S   A   P   Q   Q   H   S   I   L   S   N   Q   T   S   K   S   S   D
    aac agg gag aca cca cga aat cat tct ttg cct aag tgt aat tcc cat ttg gag ata aca att cca aag gac ttg aaa cta
568 N   R   E   T   P   R   N   H   S   L   P   K   C   N   S   H   L   E   I   T   I   P   K   D   L   K   L
    aaa gaa gca gag aaa act gat gaa aaa cag ttg att ata gat gca gga caa aaa aga ttt gga gca gtt tct tgt aat gtt
595 K   E   A   E   K   T   D   E   K   Q   L   I   I   D   A   G   Q   K   R   F   G   A   V   S   C   N   V
    tgt gga atg ctg tat aca gct tca aat cca gaa gat gaa aca cag cat ctg ctt ttc cac aac cag ttt ata agt gct gtt
622 C   G   M   L   Y   T   A   S   N   P   E   D   E   T   Q   H   L   L   F   H   N   Q   F   I   S   A   V
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649 K   Y   V   G   W   K   K   E   R   I   L   A   E   Y   P   D   G   R   I   I   M   V   L   P   E   D   P
    aag tat gcc ctg aaa aag gtt gac gag att aga gag atg gtt gac aat gat tta ggt ttt caa cag gct cca cta atg tgc
676 K   Y   A   L   K   K   V   D   E   I   R   E   M   V   D   N   D   L   G   F   Q   Q   A   P   L   M   C
    tat tcc aga act aaa aca ctt ctc ttc att tcc aat gac aaa aaa gta gtt ggc tgc cta att gcg gaa cat atc caa tgg
703 Y   S   R   T   K   T   L   L   F   I   S   N   D   K   K   V   V   G   C   L   I   A   E   H   I   Q   W
    ggc tac aga gtt ata gaa gag aaa ctt cca gtt atc agg tca gaa gaa gaa aaa gtc aga ttt gaa agg caa aaa gcc tgg
730 G   Y   R   V   I   E   E   K   L   P   V   I   R   S   E   E   E   K   V   R   F   E   R   Q   K   A   W
    tgc tgc tca aca tta cca gag cct gca atc tgc ggg atc agt cga ata tgg gta ttc agc atg atg cgt cgg aag aaa att
757 C   C   S   T   L   P   E   P   A   I   C   G   I   S   R   I   W   V   F   S   M   M   R   R   K   K   I
    gct tct cgc atg att gaa tgc cta agg agt aac ttt ata tat ggc tca tat ttg agc aaa gaa gaa att gct ttc tca gat
784 A   S   R   M   I   E   C   L   R   S   N   F   I   Y   G   S   Y   L   S   K   E   E   I   A   F   S   D
    ccc act cct gat gga aag ctg ttt gca aca cag tac tgt ggc act ggt caa ttt ctg gta tat aat ttt att aat gga cag
811 P   T   P   D   G   K   L   F   A   T   Q   Y   C   G   T   G   Q   F   L   V   Y   N   F   I   N   G   Q
    aat agc acg
838 N   S   T
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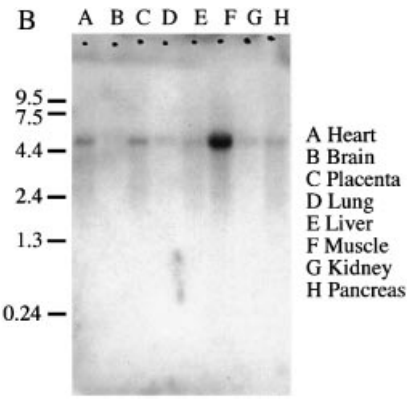
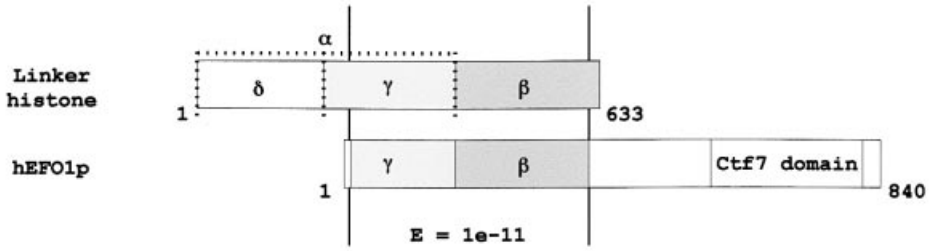


Figure 3. Expression and conceptual translation of hEFO1p. (**A**) Nucleotide and corresponding amino acid sequence for hEFO1p. (**B**) Northern blot analysis reveals that hEFO1p is expressed in most of the human tissues tested.




```
Linker histone  232 SKDQKGTRSSSRKASNSKGRKNSTSNKRNSSSSSKRSSSSKNKKSSSSKN
hEFO1p            3 SIQEKSKENSSKVTKKSDDKNSETEIQDSQKNLAKKSGPKETIKSQAKSS

Linker histone  282 KKSSSSKGRKSSSSRGRKASSSKNRKSSKSKDRKSSSSKGRKSSSSKSN
hEFO1p           53 SESKINQPELETRMSTRSSKAASNDKATKSINKNTVTVRGYSQESTKKKL

Linker histone  332 KRKASSSRGRKSSSSKGRKSSKSQERKNSHADTSKQMEDEGQKR--RQSS
hEFO1p          103 SQKKLVHENPKANEQLNRRSQRLQQLTEVSRRSLRSREIQGQVQAVKQSL

Linker histone  380 SSAKRDESS---KKSRRNSMKEARTKKANNKSASKASKSGSKSKGKSASK
hEFO1p          153 PPTKKEQCSSTQSKSNKTSQKHVKRKVLEVKSDSK--------EDENLVI

Linker histone  427 SKGKSSSKGKNSK--SRSASKPKSNAAQNSNNTHQTADSSENASSTTQTR
hEFO1p          195 NEVINSPKGKKRKVEHQTACACSSQCTQGSEKCPQKTTRRDETKPVPVTS

Linker histone  475 TRGRQREQKDMVNEKSNSKSSSKGKKNSKSNTRSKSKSKSASKSRKN---
hEFO1p          245 EVKRSKMATSVVPKKNEMKKSVHTQVNT-NTTLPKSPQPSVPEQSDNELE

Linker histone  522 -ASKSKKDTTNHGRQTRSKSRSESKSKSEAPNKPSNKMEVI--EQPKEES
hEFO1p          294 QAGKSKRGSI---LQLCEEIAGEIESDNVEVKKESSQMESVKEEKPTEIK

Linker histone  569 SDRKRRESRSQSAKKTSDKKSKNRSDSKKMTAEDPKKNNAEDSKGKKKS
hEFO1p          341 LEETSVERQILHQKETNQDVQCNRFFPSRKTKPVKCILNGINSLAKKNS
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Figure 4. hEFO1p and *Tetrahymena* linker histone alignments. Top: the schematic depicts the region of conservation between *Tetrahymena* linker histone proteins and hEFO1p. The MLH polyprotein cleavage products α, δ, γ and β linker histones are shaded (AAC18874). Bottom: amino acid alignment reveals the high degree of conservation (E of 1×10^{-11}) between hEFO1p and *Tetrahymena* γ and β linker histone proteins. Identical residues are boxed in black; conserved residues are boxed in gray.

DISCUSSION

Sister chromatid cohesion plays a central role in chromosome transmission, cell cycle progression and genome maintenance (45,46). To date, Ctf7p-like proteins identified in yeast remain the only essential establishment factors identified: factors that couple sister chromatid cohesion to DNA replication without functioning in DNA replication *per se* (7,16,29). In this report, we identify and characterize the first candidate Ctf7p ortholog in humans, hEFO1p. hEFO1p is present in almost all tissues tested. It is unknown whether the inability to detect *hEFO1* in brain tissue is due to detection limitations or to the terminally differentiated nature of this tissue, but BLAST searches of EST databases identify *CTF7*-like cDNAs from brain libraries (A.Bellows and R.V.Skibbens, unpublished observations). We also identified three other chromosomal loci within the human genome that encode Ctf7p-like proteins (hEFO2p–hEFO4p). One of the probes used to identify the *hEFO1* transcript is co-incident with *hEFO2–hEFO4* sequences. However, we did not detect novel sized transcripts, suggesting that either the reaction temperature precluded binding of similar but not identical sequences, that the other transcripts occur at very low

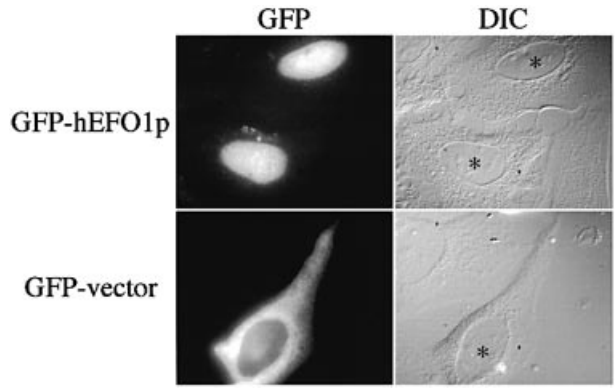

Figure 5. Localization of hEFO1p in mammalian cells. GFP-tagged hEFO1p versus GFP vector alone constructs were transfected into HeLa cells and localization assessed 24 h later. Cell images obtained using the GFP channel reveal that hEFO1p specifically localizes to the nucleus while GFP is uniformly distributed throughout the cell. Cell images obtained by differential interference contrast microscopy (DIC) are also shown (* denotes nuclei in transfected cells). GFP expression was not observed in untransfected cells.

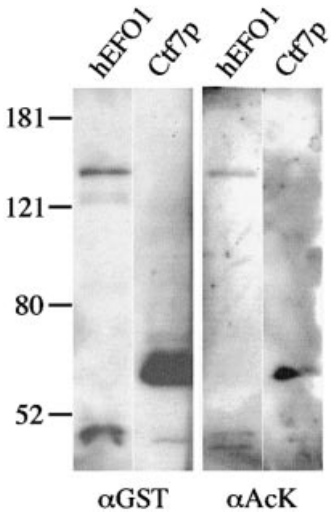

Figure 6. Expression and acetyltransferase activity of human EFO1p and budding yeast Ctf7p. (**A**) GST–EFO1p and GST–Ctf7p expressed in bacterial cells and visualized using an antibody directed against GST (α GST). Acetyltransferase activity of human EFO1p and budding yeast Ctf7p using an antibody directed against acetylated lysines (α Ac-K) previously described to detect Ctf7p acetylation reactions (15).

levels, or that they are not expressed. Thus, while our analyses of *hEFO2–hEFO4* greatly extend the limited sequences previously described (15,16), the full-length conceptual translations and expression patterns remain unknown. Given the essential function of Ctf7p in both budding and fission yeast and the apparent frequency through which *CTF7* recombines through evolution, it would not be surprising that multiple editions exist within the human genome.

hEFO1p exhibits several characteristics consistent with a role in chromosome segregation and cohesion establishment. First, hEFO1p contains the highly conserved Ctf7p core domain found in budding yeast Ctf7p and fission yeast Eso1p (this study) (7,16,29). Secondly, hEFO1p localizes specifically to the nucleus. We were unable to identify mitotic cells in which hEFO1p associated with condensed chromosomes (M.Kenna and R.V.Skibbens, personal observation). Similarly, budding yeast Ctf7p is a nuclear protein that functions during S phase. Ctf7p is not required during mitosis, nor is Ctf7p detectable within the bulk of the chromatin mass (7,16) (R.V.Skibbens and D.Koshland, unpublished results). Instead, Ctf7p is thought to transiently associate with the DNA replication fork during cohesion establishment (18). In combination, these findings indicate that the cell cycle activity and chromatin association of Ctf7p-like factors are probably conserved through evolution. Thirdly, hEFO1p exhibits acetyltransferase activity and is capable of acting as its own substrate. A comparable activity has been reported for budding yeast Ctf7p (15). Currently, physiologically relevant substrates of budding yeast Ctf7p acetylation have yet to be identified (15); thus, characterization of hEFO1p acetylation targets in human cells is beyond the scope of this report. However, the finding that acetyltransferase activities are conserved from yeast to humans strongly suggests that this enzymatic activity will play a central role in establishing cohesion. Future endeavors in solving the mystery of sister chromatid pairing for both yeast and humans will probably entail identifying physiologically relevant substrates for Ctf7p acetylation.

Our findings are novel in that human EFO1p is comprised of a Ctf7p core domain genetically fused to a domain that exhibits significant homology to structural nucleosome compaction factors—the linker histones. In *Tetrahymena*, the micronuclear linker histone gene (*MLH*) is translated into a single 71 kDa polypeptide which is then cleaved to produce four distinct linker histones: α and β in which α is further processed to produce δ and γ linker histone proteins. These four linker histone proteins are distinct from one another and unique from macronuclear histone H1 protein (43). In humans, EFO1p is comprised of a C-terminal Ctf7p domain fused to an N-terminal domain comprised of contiguous γ and β linker histone proteins in which the predominant form appears uncleaved (Fig. 6). This genetic fusion is conserved through evolution in that a similar linker histone protein–Ctf7p chimera exists in *Drosophila* (this study). At present, efforts to complement yeast *ctf7* mutant phenotypes with hEFO1p have been unsuccessful (A.Bellows, M.Kenna and R.V.Skibbens, personal observation), although even close orthologs often fail to rescue essential functions across model organisms.

Linker histones have been implicated in transcription regulation and chromosome compaction (47). For instance, in *Tetrahymena MLH* knockouts, loss of α, β, δ and γ linker histone proteins resulted in micronuclei that were over twice the size of wild-type cell micronuclei (44). Similarly, in budding yeast, mutations in many cohesion factors, including Mcd1p, Pds5p and Ctf7p, also exhibit defects in chromosome condensation (4,9,16) (R.V.Skibbens and D.Koshland, unpublished results). It is worth speculating that linker histone modifications may help target the acetyltransferase activity of hEFO1p to chromatin in a cell cycle-dependent manner. Previous studies revealed that *Tetrahymena* linker histones contain cAMP-dependent protein kinase (PKA) target sequences, with evidence that at least δ linker histone protein is a PKA substrate. Thus, the phosphorylation state of δ linker histone may regulate chromosome condensation/decondensation (48). Future directions will relate to the role of post-translational hEFO1p modifications in chromosome condensation and phenotypic analyses of cells lacking hEFO1p. These endeavors may be difficult given the possible functional redundancy of the hEFO family and the recent observations that a separate class of proteins, called the condensins, appears to perform the majority of chromosome condensation in humans (49,50). Similarly, there is an ever-growing number of cohesion proteins that, in addition to the structural and deposition cohesion factors, include RFC factors, DNA polymerases and an acetyltransferase (15,18–21,23–25). Deciphering the extent to which these players promote sister chromatid cohesion and chromosome condensation will continue to be an intriguing endeavor for cell biology.

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