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Crystal Structure of a Subcomplex of Human Transcription Factor TFIID Formed by TATA Binding Protein-associated Factors hTAF4 (hTAF_{II135}) and hTAF12 (hTAF_{II20})

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The crystal structure is presented of a complex formed by the interacting domains from two subunits of the general transcription factor TFIID, the human TATA binding protein-associated factors hTAF4 (hTAF_{II135}) and hTAF12 (hTAF_{II20}). In agreement with predictions, hTAF12 forms a histone fold that is very similar to that of histone H2B, yet unexpected differences are observed between the structures of the hTAF12 interaction domain and the hTAF4 and histone H2A. Most importantly, the hTAF4 fragment forms only the first two helices of a classical histone fold, which are followed by a 26-residue disordered region. This indicates that either full-length TAF4 contains an unusually long connecting loop between its second and third helix, and this helix is not required for stable interaction with TAF12, or that TAF4 represents a novel class of partial histone fold motifs.

Structural models and structure-based sequence alignments support a role for TAF4b and hTAF42/yADA1 as alternative partners for TAF12 and are consistent with the formation of nucleosome-like histone-fold octamers through interaction of TAF12 with a TAF6–TAF9 tetramer, yet argue against involvement of TAF12-containing histone-fold pairs in DNA binding.

TFIID is one of the central factors that control transcription initiation by RNA polymerase II (for recent reviews, see Gangloff et al. (1), Bell and Tora (2)). The complex is composed of the TATA-binding protein (TBP) and at least fourteen TBP-associated factors (TAFs). Via its TBP subunit, TFIID binds to the TATA element that is present in the majority of core promoters, an event thought to constitute the first step in RNA polymerase II preinitiation complex assembly (3). On a subset of promoters, DNA binding may be enhanced by some of the TAFs, through specific recognition of additional sequence elements. For instance, TAF6 (human (h) TAF_{II80}) and TAF9 (hTAF_{II31}) are thought to interact with the downstream promoter element (4), whereas TAF1 (hTAF_{II250}) and TAF2 (hTAF_{II150}) may bind to the initiator element (5, 6, 7). Furthermore, a double bromodomain enables TAF1 to interact with acetylated histone tails in chromatin (8). The latter TAF in addition has multiple enzymatic activities (kinase, acetyl transferase, and ubiquitination activity; for review see Wasserman and Sauer (9)), allowing it to covalently modify various proteins, including histones and other transcription (co)factors. Although the precise roles played by the remaining TAFs are considerably more elusive, many of them have been reported to interact with transcription activation domains of gene-specific activators. This suggests that through these TAFs, the TFIID complex acts as an integration point for regulatory cues. It is likely that TAF-activator contacts either stabilize the preinitiation complex or induce essential structural changes in it.

Most of the TAF components of TFIID engage in alternative interactions, giving rise to transcription complexes of varying functionality. On the one hand, tissue-specific homologs of Drosophila (d) TAF4 (10) and dTAF5 (dTAF_{II80}) (11) have been found that can replace these TAFs in TFIID to produce specialized versions of the complex. Both of the alternative TAFs have been shown to be required for normal development of tissues in which they occur (11, 12). On the other hand, many TAFs are not only present in TFIID, but also in one or more other global regulators of transcription by RNA polymerase II that lack TBP, such as the yeast SAGA and human STAGA, PCAF, and TFTC histone-acetylating complexes (2, 13, 14) and the Polycomb group repressor complex PRC1 (15). Interestingly, yeast (y) TFIID may be replaced by TBP alone on some promoters, according to recent studies (16, 17). Thus, a cofactor role for TAFs is emerging, a notion supported by numerous inactivation studies that indicate that various TAFs influence transcription of large, non-identical but overlapping subsets of genes (18, 19, 20). However, in what fashion the intricate cross-talk through exchange of oligomerization partners between the TAFs within TFIID and other transcription complexes takes place at the structural level is largely unclear.

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Several TAFs have been reported to interact via histone-fold motifs (for review, see Gangloff et al. (1)). The observation that TAF6, TAF9, and TAF12 show homology with histones H4, H3, and H2B, respectively, led to the hypothesis that TFIIID contains a core structure resembling the histone octamer that organizes genomic DNA into nucleosomes (21). Subsequent studies even hinted at DNA binding by these TAFs in a nucleosome-like manner (22). The core TFIIID structure was believed to be composed of a H3/H4-like tetramer formed by TAF6 and TAF9 (23) plus two putative homodimers of the H2B-like subunit, TAF12. Because all of these histone-like TAFs either belong to the family of “shared” TAFs or are replaced by highly homologous proteins in other global transcription regulators, a similar structure would be likely to exist in the SAGA/TAGA, TPTC, PCAF, and PRC1.

However, several recent observations suggest that the network of histone-fold proteins within TFIIID is more complicated than initially anticipated. First, a crystallographic study revealed that hTAF11 (hTAF928) and hTAF13 (hTAF18) also contain histone folds, although this feature had gone unnoticed in the initial sequence comparisons of TAF-proteins (24). Furthermore, instead of forming homodimers, the putative hTAF12 histone-fold domain interacts strongly with a conserved 70-amino acid region of hTAF4, likely constituting a novel histone-like heterodimer within TFIIID (25).

Here, we describe the crystal structure of the complex formed by the interacting regions of hTAF4 and hTAF12, confirming their specific association into heterodimers. The structure reveals that hTAF12 indeed forms the anticipated H2B-like histone fold, but surprisingly, hTAF4 interacts with this motif via a partial histone fold consisting of two helices only. The region expected to form the C-terminal portion of the hTAF4 histone fold on the basis of alignments is found to be disordered. This suggests that a third helix is located downstream from the region included in our crystallographic study, and connected to the histone fold by an atypically long linker, or, alternatively, that hTAF4 represents a novel variant of the histone fold that lacks a third helix altogether. Functional and biochemical analysis of yTAF4 (45) provides evidence supporting the idea that an α3 helix is present in a strongly conserved domain at the C terminus of TAF4 proteins. Conservation of core residues of TAFs in yTAF4(hTAF11105) and hSTAT4/hADA1 agrees with the proposed roles for the latter two proteins as alternative histone-like interaction partners for TAF12 in a specialized TFIIID complex and the S/TAGA coactivator complex, respectively. Model building supports the hypothesis that two copies of the TAF4-TAF12 complex interact through four-helix bundles with a heterotetramer consisting of TAF6 and TAF9 to form an octameric complex analogous to the histone core of the nucleosome. Because the surface of the TAF4-TAF12 complex is mostly negatively charged, and side chains that mediate contacts between the H2A-H2B complex and DNA have not been conserved in the TAFs, involvement of the histone fold domains in nucleosome-like DNA wrapping is nonetheless unlikely.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The predicted histone-fold region of hTAF12 (amino acids 57–129) and the region of hTAF4 that interacts with it (amino acids 870–1280) were expressed in Escherichia coli using pET-15b and pACYC11b constructs as described (25, 26). Bacteria were resuspended in buffer A (25 mM Bis-Tris/Tris pH 6.0, 400 mM NaCl) and lysed by sonication. The protein complex, present in the soluble fraction, was purified by means of covalent-Sepharose affinity chromatography (Talon Affinity Resin, Clontech) in buffer A and gel filtration (Superdex 75, Amersham Biosciences) in buffer B (25 mM Tris/Tris pH 8.0, 400 mM NaCl). The histidine tag at the N terminus of hTAF12 was removed by incubation with thrombin (Sigma) in buffer B, followed by an additional, identical, gel filtration step. Prior to crystallization, dithiothreitol was added to 5 mM, and the complex was concentrated to 10–15 mg/ml by means of Microspin size-exclusion dialysis tubes (molecular mass cutoff 3 kDa, Pall Filtration Corporate Bank) (46). A derivative containing selenomethionine (Se-Met) was prepared in similar fashion, starting from a culture of B834 (DE3) bacteria grown on M9 minimal medium (27) supplemented with 0.3 mM seleno-methionine (ICN Biochemicals). Both the native and the Se-containing complex were verified by mass spectrometry.

**Crystallization and Data Collection**—Monoclonal crystals of native and Se-Met hTAF4-hTAF12 complexes were obtained via the hanging-drop method at 24 °C, using a reservoir buffer containing 100 mM sodium acetate pH 5.25 and 2.2 mM (NH4)2SO4. Crystallization droplets were produced by mixing equal volumes of the concentrated protein and the reservoir buffer. Thin platelets with typical dimensions around 0.5 × 0.5 × 0.1 mm grew within 1 week. Prior to flash-freezing in liquid ethane, these crystals were equilibrated in stepwise fashion in a cryoprotectant buffer containing 100 mM sodium acetate pH 5.25, 2.4 mM (NH4)2SO4, 0.4 mM NaCl, and increasing concentrations (0, 5, 10, 15, 20, and 25%) of glycerol (10 s for each incubation step). Although most of these crystals diffracted to ~2.8 Å, diffraction patterns typically contained mostly elongated, smeary spots that could not be integrated accurately. Adapting the crystallization conditions, changing the cryoprotection procedure, crystal annealing, or measuring crystals in capillaries at 4 °C did not alter this. However, of a large number of frozen crystals (around 50), a few could be identified that produced a better spot shape and in addition, diffracted to higher resolution. Using one of these, multiple anomalous-diffraction data up to 2.2 Å were collected at the BMBW (Deutsches Elektronen Synchrotron, Hamburg) (wavelengths: 0.97740 and 0.90730 Å). Crystals belong to space group C2, with a = 112.9 Å, b = 36.8 Å, c = 73.9 Å, and β = 97.9°. The asymmetric unit contains two heterodimers of hTAF12 and hTAF4, resulting in a Matthews coefficient of 2.2 and an estimated solvent content of 42%.

Tetragonal crystals with dimensions up to 0.2 × 0.2 × 0.2 mm grew between several weeks and several months, when using 100 mM (carbamoylmethyl)amino)acetic acid (ADA) pH 6.5, 2.0 mM (NH4)2SO4, and 10 mM MgSO4 as the reservoir buffer (again, crystallization droplets were produced by mixing equal volumes of the concentrated protein and the reservoir buffer). During the long crystallization period, the (NH4)2SO4 concentration increased to values up to 3 M due to evaporation of water from the wells. Before flash-freezing in liquid ethane, crystals were equilibrated in stepwise fashion in a cryoprotectant buffer containing 100 mM ADA pH 6.5, 3.0 mM (NH4)2SO4, 10 mM MgSO4, and increasing concentrations (0, 5, 10, 15, 20, and 25%) of glycerol (10 s for each incubation step). The tetragonal crystals did not produce the smeary diffraction spots typical of the C2 form but diffracted more weakly. Also, Se-Met crystals for this form could not be obtained. Native data to 3.1 Å were measured at 100 K on beamline ID14–3 at European Synchrotron Radiation Facility, Grenoble, France. The crystals belong to space group P42,2,2, with a = b = 67.5 Å and c = 127.3 Å. The asymmetric unit contains 2 heterodimers of hTAF4 and hTAF12, resulting in a Matthews coefficient of 2.1 and an estimated solvent content of 40%.

All data sets were processed and scaled using the programs Denzo and Scalepack, versions 1.96.7 (28).

**Structure Determination**—The structure of the complex was solved by multiple anomalous diffraction of the Se-Met containing C2 crystals using Solve 1.17 (29) for initial phase determination. Eight Se sites could be determined automatically by the program. After density modification of the resulting map in CNS 1.1 (30), electron density was observed for the entire complex, except for a small number of residues in loop regions. Model building and refinement were carried out using the program O (31), version 6.2, in combination with CNS. Structure determination statistics are listed in Table I. Atomic coordinates and structure factor data have been deposited in the Protein Data Bank (ID codes 1hs0 and 1hs3f, respectively). The structure of the tetragonal crystal form was solved by molecular replacement, using EPRM 2.4 (32) with the refined hTAF4-hTAF12 heterotetramer from the C2 form as a search model. Ribbon representations of protein models were prepared using Molscript 1.4.3 (33) in combination with Raster3D 2.0 (34, 35), except for the model generated with O (31), which was superimposed on the crystal structure of the HeLa cell particle (36) so as to optimize the fit between the Cα atoms of residues 53–77 of hTAF6 and the corresponding residues (68–92) of H4. This region in H4 corresponds to the part of the protein that contacts H2B. Next, a hTAF4-hTAF12 heterodimer was docked onto the tet-
rimer by fitting residues 102–123 of hTAF12 to residues 77–98 of H2B, the region that contacts H4. Side-chain conformations in the interface area were modified manually to mimic the residues in the histones as closely as possible. The second half of the octamer was produced through application of the symmetry operator that generates the tetramer closely as possible. The second half of the octamer was produced through application of the symmetry operator that generates the tetramer in the Xie et al. crystal structure.

RESULTS

Crystallization and Structure Determination—Initial crystallization trials were undertaken with the predicted hTAF12 histone-fold region (residues 57–128) carrying an N-terminal histidine tag and an untagged fragment (residues 870–952) of hTAF4, comprising the presumed histone fold domain and C-terminal flanking region (37). Large single crystals of this complex were obtained that did not diffract beyond 8 Å. When the protein content of some of the crystals was analyzed by mass spectrometry, a small fraction (roughly 10%) of hTAF4 was found to be truncated by 9 amino acids at its C terminus. This A construct corresponding to this breakdown product (residues 870–943) was coexpressed with the histidine-tagged hTAF12 construct as before. The new complex gave rise to morphologically identical crystals in similar conditions, but this now required proteolytic removal of the N-terminal histidine tag from hTAF12 (whereas the initial complex had required its presence). Using these crystals, belonging to space group P4_2_1_2, data could be collected to 3.1 Å. At lower pH, a second crystal form was obtained that belongs to space group C2. These crystals diffracted to around 2.5 Å.

We first tried to solve the structure of the complex by molecular replacement, using either unmodified models or alanine models constructed from known histone-fold structures, as well as averages or truncated versions of such models. Because neither P4_2_1_2 nor C2 data yielded correct solutions, we proceeded to solve the structure by multiple anomalous diffraction, using a selenomethionine-containing complex crystallized in the C2 space group. Table I lists statistics concerning data collection and structure refinement for this crystal form. The lower resolution structure of the P4_2_1_2 form was subsequently solved by molecular replacement, using the C2 multiple anomalous diffraction structure as a search model. Details of protein production, crystallization and structure determination are given in “Experimental Procedures.”

Structure of the hTAF4-hTAF12 Heterodimer—The structure of the hTAF4-hTAF12 complex as determined from the C2 crystal form is depicted in Fig. 1A. As expected, hTAF12 forms a regular histone fold, consistent with the high degree of homology between this protein and histone H2B. The helical regions and intervening loops of hTAF12 and H2B can be superimposed very well (root mean square deviation = 1.1 Å for Cn atoms). The most significant differences between hTAF12 and H2B (see Fig. 2A) are a change in orientation of helix α1 and the conformation of the C-terminal extension in hTAF12 (residues 126–128), which folds back onto a surface that in H2B accommodates an additional C-terminal helix (αC, not shown). Density for this extension is only seen in the C2 crystal structure, where it is stabilized by crystal packing contacts.

Surprisingly, hTAF4 forms only a partial histone fold, because the C-terminal two turns of the α2 helix, the loop L2, and helix α3 are absent from the structure. The C-terminal 26 residues of the polypeptide (residues 918–943) do not appear in the electron density maps, yet mass spectroscopy of redissolved crystals shows that these residues are present and have not been removed by proteolysis (data not shown). Thus, this region must be disordered within the crystal lattice. The presence of a flexible tail of such substantial length (the 26 residues make up 35% of the total chain length of the hTAF4 fragment and account for 18% of the mass of the entire complex) may explain the difficulties in obtaining well diffracting single crystals (see “Experimental Procedures”). Several additional differences can be observed between the structures of hTAF4 and H2A (see Fig. 2A). First, helix α1 of hTAF4 contains an additional helical turn at its C terminus, and as a consequence the loop between helices α1 and α2 is longer and adopts a slightly different conformation. Furthermore, the orientation of both of the helices of hTAF4 with respect to hTAF12 is significantly different from the orientation of the corresponding helices in H2A with respect to H2B.

Notwithstanding the considerable structural differences between hTAF4 and H2A, the interaction between hTAF4 and hTAF12 monomers to form heterodimers resembles that observed in H2A-H2B and other histone-fold pairs. Hydrophobic residues on the inside of the crossed-over α2 helices of hTAF4 and hTAF12 form an elongated hydrophobic core, to which a number of residues from the shorter helices and inward-pointing residues from the connecting loops also contribute. The hydrophobic character of each of these residues is conserved in all known homologous TAFs (Fig. 1, B and C). In addition to these interactions, loop L1 of hTAF4 aligns in antiparallel fashion with loop L2 of hTAF12 to form several β-sheet type backbone-backbone hydrogen bonds. Two highly conserved intramolecular networks of multiple salt bridge interactions (Fig. 1B) stabilize the positioning of the helices and the conformation.
of the loops in hTAF12. A single intermolecular salt bridge is observed, between residue Asp-89 in the \( \alpha_9 \) helix of hTAF12 and Arg-879 in the \( \alpha_1 \) helix of hTAF4, but this contact is not conserved in all species.

Crystal Contacts between hTAF4-hTAF12 Heterodimers—In our crystals, two hTAF4-hTAF12 heterodimers interact to form a heterotetramer making up the asymmetric unit (see Fig. 3A). Intriguingly, a virtually identical tetrameric arrangement is found in the monoclinic and tetragonal crystal structures (the Ca atoms of the two structures can be superimposed with a RMSD of 1.2 Å), although the packing of the tetramers into the lattice is very different in the two crystal forms (data not shown). The interaction between two heterodimers involves the N-terminal part of the \( \alpha_2 \) helix of hTAF12 and the C-terminal region of the \( \alpha_2 \) helix of hTAF4. Hence, the tetrameric arrangement observed does not resemble any of the heterodimer-heterodimer interactions within the nucleosome core particle (see Fig. 4 for comparisons). Details of the interaction are shown in Fig 3B. Three conserved hydrophobic residues of hTAF12 (Val-80, Met-83, and Ile-87) and two from hTAF4 (Leu-911 and Ile-915) associate across the approximate twofold non-crystallographic symmetry axis with the corresponding residues from the second heterodimer to form a hydrophobic interface of considerable dimensions (buried surface area: 500 Å\(^2\) per heterodimer). In addition, salt bridges (although not conserved in all species) exist between the two heterodimers, as Asp-77 and Asp-79 of hTAF12 are in contact with Arg-114 of hTAF4. An interesting contribution to the interaction comes from a tight carboxyl-carboxyl contact between the conserved residue Asp-90 of hTAF12 and its non-crystallographic symmetry-related residue. Very clear density is seen for this contact in both crystal structures, even though the tetragonal crystals were grown at pH 6.5. As is often seen for this type of contact (38), the interaction between the two acidic groups is stabilized by a neighboring residue (Gln-86).

Like the interaction between hTAF4-hTAF12 heterodimers...
within the asymmetric unit, the crystal packing interactions observed between tetramers do not resemble the interaction between H2A-H2B heterodimers in the nucleosome core particle. However, in the tetragonal crystal form, heterotetramers join to form a spiral along the crystallographic 43 axis by means of a four-helix bundle. This interaction is very similar to the interactions between H3-H4 heterodimers and that between H2A-H2B and H3-H4 heterodimers in the nucleosome (see Fig. 4B).

**DISCUSSION**

**TAF12 Forms a Histone-like Heterodimer with TAF4**—The present crystal structure confirms recent results from yeast two-hybrid experiments (25) and bacterial coexpression analysis (26) indicating that hTAF12 forms histone-like heterodimers with hTAF4, rather than homodimerizing as suggested by an earlier study (39). Indeed, in view of our results it seems unlikely that hTAF12 would be capable of forming a stable homodimer even in the absence of hTAF4, since the hydrophobic interface between the two proteins shows considerable asymmetry. A second copy of hTAF12 therefore could not readily mimic the interaction surface provided by hTAF4.

The TAF12-TAF4 heterodimerization has important implications for the higher order structure of the histone-like TFIIID subunits. On the basis of biochemically observed interaction patterns, Hoffmann et al. (21) proposed a nucleosome-like substructure within TFIIID, consisting of a tetramer of TAF6 and TAF9 plus two homodimers of TAF12. This model has been the framework for many subsequent biochemical and genetic investigations into TFIIID function. It now seems more likely that if such a nucleosome-like octamer occurs, it would rather contain two TAF4-TAF12 heterodimers. Consistent with this idea, an in vitro-reconstituted octameric complex has recently been described that consists of yTAF6, yTAF9, yTAF4, and yTAF12 (40).

**Lack of an α3 Helix in TAF4 Leads to Dimerization of the TAF4-TAF12 Heterodimer within the Crystals**—Heterodimers in both of our crystal structures arrange themselves into tight dimers of dimers within the asymmetric unit. This dimerization is very different from the interaction between H2A-H2B heterodimers as seen in the nucleosome (Fig. 4) and, importantly, would preclude the formation of nucleosome-like octamers. The amount of buried surface area involved in the interaction (500 Å²) and the fact that essentially the same heterotetramer is found in two otherwise very different crystal forms indicate a tight interaction that in principle could correspond to a biologically relevant substructure of TFIIID. However, the interface between the two heterodimers consists of hydrophobic residues that in a canonical histone octamer would not be surface-exposed, but instead covered by the α3 helix of TAF4.

The surprising inability of hTAF4 residues 918–943 to form an α3 helix suggests one of several possibilities. A spurious explanation would be that the lack of an α3 helix represents a crystallization artifact. Alternatively, the TAF4 histone-fold...
domain may represent a novel class lacking an α3 helix or containing an α3 helix that only forms under specific conditions (for example, on interaction with additional interaction partners). If this were case, the tetramers observed in our crystals may be physiologically relevant. The most likely explanation, however, is that an α3 helix is located downstream from the region of hTAF4 coexpressed with hTAF12. A potentially helical region that is conserved in all TAF4 orthologs does exist at the very C terminus of the protein, between residues 1055 and 1083, designated the conserved C-terminal domain (CCTD). The amino acid composition of the CCTD is compatible with the formation of an α3 helix, since the sequence would give rise to a hydrophobic face on one side of the helix that can be matched onto the appropriate area of the hTAF4-hTAF12 structure without any obvious steric hindrance occurring (data not shown). Recent experiments with yTAF4 indicate that the CCTD is essential for yeast viability and interacts genetically with yTAF12, whereas deletion or mutation of the presumed α3 helix located immediately downstream of the α2 helix has no effect on yTAF4 function (45). Furthermore, in coexpression experiments, the presence of the CCTD in yTAF4 significantly enhances the formation of a soluble heterodimer. It therefore seems likely that an α3 helix is indeed located within the CCTD and that the tetramer we observe does not reflect the situation in TFIID. This implies that TAF4 contains an exceptionally long linker of more than 100 residues within its histone-fold motif. The remarkable length of this linker is conserved in all TAF4 orthologs. Although its deletion in yTAF4 leads to a loss of complementation, its function at the molecular level remains to be determined.

**Formation of a Nucleosome-like Octamer**—In gel filtration and analytical ultracentrifugation experiments, Selleck et al. (40) observed association of two yTAF4-yTAF12 heterodimers with a (yTAF6-yTAF9)2 heterotetramer to form an octameric complex. Assembly of this octamer was shown to be abrogated by mutation of residue Leu-464 in the α2 helix of yTAF12.
Crystal Structure of the hTAF4-hTAF12 Complex

![Diagram](image)

**Fig. 5. Hypothetical model for an octamer formed by two hTAF4-hTAF12 heterodimers and the hTAF6-hTAF9 heterotetramer.** Dark red, hTAF6; dark green, hTAF9; pink, hTAF4; blue, hTAF12. A, reconstruction of the interface between the hTAF4-hTAF12 and hTAF6-hTAF9 complexes, modeled so as to maximize resemblance to the corresponding region in the structure of the nucleosome core particle (36). For clarity, only hTAF12 and hTAF6 are shown. Hydrophobic residues contributing to the interface are shown in dark red. The yTAF9 complex in the B lobe of native yeast TFIID (41). However, the additional observation in the latter experiments of TAF6-TAF9 in lobe A and TAF4-TAF12 in lobe CII indicates that alternative dimer-dimer contacts (and alternative octamers) are likely to exist. This raises interesting questions about the functional differences between the various combinations of histone-like heterodimers and the way these complexes are targeted to the appropriate lobes of TFIID. A model of the entire (TAF6-TAF9)₂-2(TAF4-TAF12) octamer (obtained by modeling two copies of hTAF4-hTAF12 onto the hTAF6-hTAF9 tetramer in the manner described above) is shown in Fig. 5B. Despite the similarity of the interface regions of the TAFs and the histones, the overall shape of the reconstructed TAF octamer differs from the histone octamer in that it is not a closed, compact particle, but rather an open spiral. Unlike the H2A-H2B pairs in the nucleosome, the two hTAF4-hTAF12 heterodimers are not in contact with one another through the C-terminal end of the α1 helices and the L1 loops, but are instead separated by a gap of about 20 Å (Fig. 5B). This lack of closure of the octameric ring ultimately results from the difference in “twist” within the dTAF6-dTAF9 tetramer as compared with the H3-H4 tetramer: the two dTAF6-dTAF9 heterodimers in the crystal structure by (23) are rotated apart by about 30° as compared with the relative orientation of the corresponding histones (see Ref. 42). The exceptional twist angle in the dTAF structure may reflect the actual situation in TFIID, but could also result from a distortion of the dimer-dimer interface in the dTAF6-dTAF9 crystal structure due to crystal-packing effects. However, if the twist difference is corrected for, for instance by fitting two halves of the TAF octamer independently to the nucleosome structure so as to reconstruct the TAF6-TAF9 interface, a collision occurs between the α1 helices of the two copies of hTAF4, which contain one more turn than the corresponding helix in H2A. Therefore, some qualitative differences must exist between the quaternary structures...
of a TAF octamer and the nucleosomal histone octamer. Such differences could account for specificity, preventing formation of mixed octamers of TAFs and histones despite strong conservation of the interfaces involved. The TAF4-TAF12 Histone Fold Domains Are Unlikely To Contribute to DNA Binding by TFIIID—Irrespective of whether histone-like TAFs associate into nucleosome-like octamers or a different quaternary structure, the question remains whether such a complex can bind directly to DNA as proposed by Hoffman et al. (21). As noted before, most of the surface residues in the H3-H4 heterotetramer that make critical DNA contacts in the nucleosome have not been conserved in the TAF6-TAF9 complex. In fact, many are replaced by negatively charged residues (42). A similar observation can be made for the TAF4-TAF12 complex: of the eight residues of H2A and H2B that are involved in direct side chain interactions with DNA in the nucleosome core particle (42), two are present in the hTAF4-hTAF12 complex (Fig. 2, B and C), and of these only one (Lys-107) is conserved among the TAFs. Moreover, the surface-exposed area of the hTAF complex is essentially negatively charged, and contains only a very limited number of positively charged residues (Fig. 3C). It is interesting to note that almost all of the negative charges are highly conserved throughout evolution (Fig. 1, B and C). Hence, it seems highly improbable that the function of these histone-fold domains would be to directly bind to DNA. Alternative Dimerization of TAF12—An intriguing property of TAF12 is that it has several alternative heterodimerization partners. Within TFIIID, hTAF4 can be replaced by hTAF4b, a tissue-specific TAF12 complex: of the eight residues of H2A and H2B that are involved in direct side chain interactions with DNA in the nucleosome core particle (42), two are present in the hTAF4-hTAF12 complex (Fig. 2, B and C), and of these only one (Lys-107) is conserved among the TAFs. Moreover, the surface-exposed area of the hTAF complex is essentially negatively charged, and contains only a very limited number of positively charged residues (Fig. 3C). It is interesting to note that almost all of the negative charges are highly conserved throughout evolution (Fig. 1, B and C). Hence, it seems highly improbable that the function of these histone-fold domains would be to directly bind to DNA. Alternative Dimerization of TAF12—An intriguing property of TAF12 is that it has several alternative heterodimerization partners. 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