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Structure Prediction in a Post-genomic Environment: A Secondary and Tertiary Structural Model for the Initiation Factor 5A Family

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Two predictions have been prepared for the fold of initiation factor 5A (IF5A) starting from a set of homologous sequences. In the first, a secondary structural model was predicted for the protein in 1994, when only eleven homologs (and no eubacterial homologs) had been sequenced. The second was made recently, after genome projects had generated a total of 33 sequences for the protein family from species of all three kingdoms of life. With the second set of sequences, but not with the first, it was possible to predict that the N-terminal domain of the protein folds in a possibly open beta-barrel/sandwich core structure, with a short helix capping one side of the barrel. We place the pair of predictions in the public domain before an experimental structure is known. This example illustrates the impact of genome sequencing projects on structure prediction from sequence alignments.

Key Words: **initiation factor 5A (IF5A); hypusine-containing protein; CASP3 prediction experiment; protein structure prediction; genome projects; beta-barrel fold.**

Predictions of protein structure made and announced before an experimental structure is available have shown that useful predictions of secondary structure can be obtained from a set of aligned sequences of homologous proteins diverging under functional constraints (1). Especially important in this context have been predictions made in the project known as "Critical Assessment of Techniques for Structure Prediction" (CASP), now in its third round (URL: http://PredictionCenter.llnl.gov/). In former rounds of this project, we used secondary structural predictions to identify the 8-fold alpha-beta barrel fold of phospho-beta-galactosidase (2), identify the beta-sandwich of synaptotagmin as one of three alternative folds (3), and build a tertiary structure model for the heat shock protein 90 family that predicted that it was a distant evolutionary relative of the DNA gyrases (4), *inter alia.* The quality of a prediction of a protein fold made from a set of aligned homologous protein sequences depends on the number of sequences in the alignment, the extent to which these have diverged, their relationship on an evolutionary tree, and the extent to which function has been conserved within the family (1). Such parameters are difficult to quantitate by automated tools for assessing the quality of a prediction. They have therefore been frequently overlooked in the process of evaluating prediction projects within the CASP framework (1). Understanding how the quality of the prediction "output" depends on the nature of the sequence "input" will nevertheless be central to efforts to rationally improve prediction methodology.

Initiation factor 5A (IF5A) is a widely conserved protein that is post-translationally modified on one lysine to incorporate the unusual amino acid hypusine (5, 6). Hypusinylation is known in archaea and eukaryotes, but not in eubacteria, where the two necessary enzymes, deoxyhypusine synthase (7) and hydrolase, appear to be missing. This may imply that the modification was either developed after eubacteria diverged from archaea and eukaryotes, or was present in the universal ancestor and lost selectively in eubacterial lineages (8).

Initiation factor 5A is best known as a monomer, although dimeric and higher oligomers might be formed as well (9). Deletion studies suggest that a core

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segment extending from F30 to D70 is required for human IF5A to be recognized by deoxyhypusine synthase; this may represent a core folding unit (10). While original assay of the protein was based on its ability to stimulate the formation of the first peptide bond in protein biosynthesis, its mechanism of action is now clearly more complicated (11, 12). For example, Ruhl et al. showed that IF5A binds to Rev, a nuclear phosphoprotein that accumulates in the nucleoli of cells that are expressing RNA molecules from HIV carrying the "Rev response element" (RRE). These messages are retained in the nucleus and appear in the cytoplasm only when Rev is present. IF5A appears to be necessary for Rev to function in mammalian cells (13, 14), and its expression is significantly increased in T-lymphocytes when they are activated (15). Liu et al. showed that IF5A binds to RRE in gel shift assays (16).

As a prediction target, IF5A was first encountered in 1994, when the protein was considered as a potential therapeutic target. At that time, only ten IF5A homologs with clearly alignable sequences were available (Figure 1), and these were not widely distributed on the evolutionary tree (Figure 2). Nevertheless, predictions were prepared using both transparent prediction tools (1) and the neural network program PHD (17) in the version then available by server. In the first prediction, a key element of secondary structure was ambiguously assigned as either a long strand or as an internal helix. Tertiary structure models were built using both alternatives. These predictions were published as supplementary information to the structural characterization of different isoforms of human IF5A (18). However, because no experimental structure has become available in the meantime, the accuracy of the predictions could not be assessed.

Very recently, a homolog to IF5A, translation factor 5A from the archaebacterium *Pyrobaculum aerophilum*, was announced as a target for the CASP3 project (19) by T. Peat (Los Alamos National Laboratory), implying that a crystal structure would shortly emerge. In the intervening time, genome sequencing projects have made available a total of 33 homologs in the superfamily. In particular, members of a protein family in the eubacterial kingdom, the eubacterial elongation factors P (EF-P), display significant sequence similarity with the N-terminal 90 residues of the hypusine-containing proteins (Figure 1). Thus, IF5A provides an opportunity to compare the impact of the systematic development of sequence databases on protein structure prediction in a *bona fide* prediction setting. This comparison makes evident how valuable genome projects, especially those selected from organisms widely dispersed on the universal tree of life, are in generating predictions.

MATERIALS AND METHODS

The multiple sequence alignment shown in Figure 1 was prepared using PileUp (Genetics Computer Group, (20)). Sequences marked with × were available in 1994. Positions in the multiple alignment predicted to lie on the surface, in the interior, in the active site and in parses in the protein fold were assigned (1) using the DARWIN tool available via server (21), using phylogenetic trees based on global pairwise alignments. Secondary structure predictions were made manually from these assignments following the procedures recently reviewed (1). In addition, PHD (17) neural network predictions were obtained via a server featuring the program in the version available at the time the predictions were obtained (22).

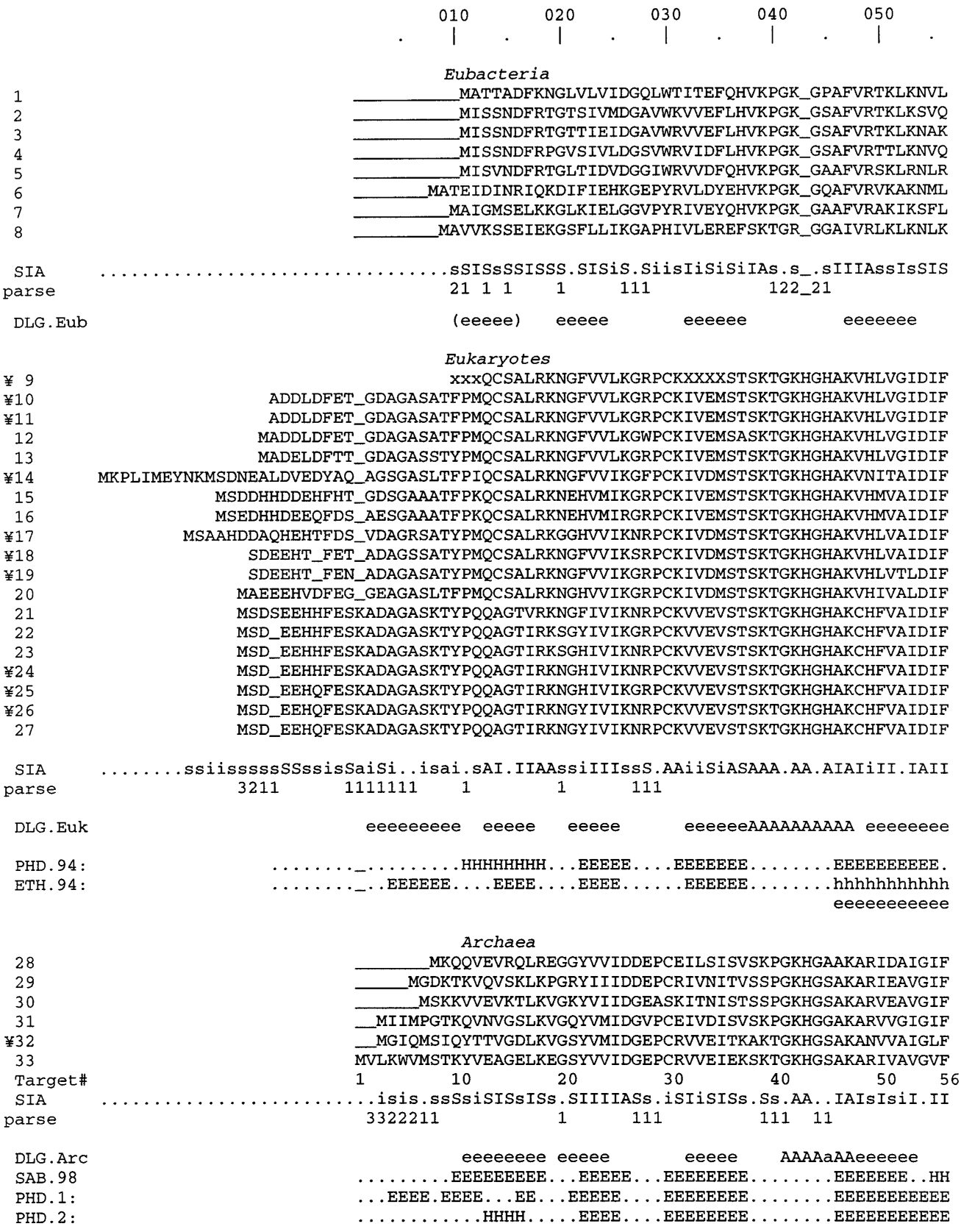
Maximum likelihood trees were prepared using the DARWIN server. To obtain reliable evolutionary distances (expressed as PAM units, the number of point accepted mutations per 100 amino acids), the IF5A family was first divided into subfamilies where each protein in a subfamily is essentially the same length. These subfamilies corresponded to the three major kingdoms of life. A maximum likelihood Darwin tree was built for each subfamily, comparing sequence fragments pairwise over the length of the corresponding multiple subalignments. This procedure yielded the PAM distances for each subtree. The sequences of all IF5A proteins were then truncated to a common core alignable over all kingdoms, and a second maximum likelihood tree was built. From this tree, PAM distances were extracted for the edges of the tree that join the subfamilies. The resulting tree is shown in Figure 2, with the sequences available in 1994 highlighted.

Tertiary structure modelling for the putative N-terminal domain followed analyses used for the prediction of the tertiary fold of protein kinase (23), synaptotagmin, and phospho-beta-galactosidase (24), aided by submissions to fold recognition servers on the World Wide Web (see below).

RESULTS AND DISCUSSION

A secondary structure model was generated in 1994 for IF5A from the 10 sequences available at the time

FIG. 1. Multiple sequence alignment and secondary structure predictions for the initiation factor 5A protein superfamily. Subalignments for each of the three primary kingdoms (eubacteria, eukaryotes, and archaea) are shown aligned in a master alignment, generated using PileUp (GCG Wisconsin Package) and edited manually. SIAP shows syrface, interior, active site assigments made separately foreach kingdom: S/s = strong/weak surface, I/i = strong/weak interior, A/a = strong/weak active site. Parse assignments indicate break in secondary structure, indicated by numbers 1-5 in order of decreasing reliability. Secondary structure assignments, H/h = strong/weak helix, E/e = strong/weak strand are made by experts (ETH.94 made by method in (1) with sequences marked with ×; DLG.Eub, DLG.Euk, and DLG.Arc, made by Gerloff on the eubacterial, eukaryotic, and archaeal sequences respectively; SAB.98 made by Benner for the superfamily as a whole) or the PHD neural network made through the PredictProtein-server (22) (PHD.94 in 1994 submitting sequence 11; PHD.1 in 1998 submitting sequence 33; PHD.2 in 1998 through the UCLA-DOE Fold Recognition-server (31) submitting sequence 33). ETH.94 had an ambiguous assignment around Ali# 050 (18). Sequences were extracted from publicly available databases. Sequences are: 1: efp_myctu, 2: efp_syny3, 3: efp_synp7, 4: efp_anasp, 5: efp_bacsu, 6: aquae950 *Aquifex aeolicus*, 7: efp_helpy, 8: bbur213 *Borrelia burgdorferi*, 9: if52_chick, 10: if5a_rabit, 11: if5a_human, 12: 2119941 *Homo sapiens*, 13: if51_chick, 14: if5a_dicdi, 15: if52_caeel, 16: if51_caeel, 17: if5a_neucr, 18: if52_yeast, 19: if51_yeast, 20: 3024014 *Solanum tuberosum*, 21: 3024018 *Zea mays*, 22: 3024019 *Solanum tuberosum*, 23: g3024022 *Solanum tuberosum*, 24: if51_nicpl, 25: if52_nicpl, 26: if5a_medsa, 27: 124230 *Nicotiana plumbaginifolia*, 28: aful634, *Archaeoglobus fulgidus*, 29: 2696455 *Pyrococcus horikoshii*, 30: mthe858 *Methanobacterium thermoautotrophicum*, 31: if5a_metja, 32: if5a_sulac, 33: CASP3-T0063 *Pyrobaculum aerophilum*.


010
020
030
040
050
Eubacteria
1 _____MATTADFKNGLVLVIDGQLWTITEFQHVKPGK_GPAFVRTKLKNVL
2 _____MISSNDFRTGTSIVMDGAVWKVVEFLHVKPGK_GSAFVRTKLKSVQ
3 _____MISSNDFRTGTTIEIDGAVWRVVEFLHVKPGK_GSAFVRTKLKNAK
4 _____MISSNDFRPGVSIVLDGSVWRVIDFLHVKPGK_GSAFVRTTLKNVQ
5 _____MISVNDFRTGLTIDVDGGIWRVVDFQHVKPGK_GAAFVRSKLRNLR
6 _____MATEIDINRIQKDIFIEHKGEPYRVLDYEHVKPGK_GQAFVRVKAKNML
7 _____MAIGMSELKKGLKIELGGVPYRIVEYQHVKPGK_GAAFVRAKIKSFL
8 _____MAVVKSSEIEKGSFLLIKGAPHIVLEREFSKTGR_GGAIVRLKLKNLK
SIA .....sSISsSSISSS.SISiS.SiisIiSiSiIAs.s_.sIIIAssIsSIS
parse 21 1 1 1 111 122_21
DLG.Eub (eeeee) eeeee eeeeee eeeeeee
Eukaryotes
¥ 9 xxxQCSALRKNGFVVLKGRPCKXXXXSTSKTGKHGHAKVHLVGIDIF
¥10 ADDLDFET_GDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLVGIDIF
¥11 ADDLDFET_GDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLVGIDIF
12 MADDLDFET_GDAGASATFPMQCSALRKNGFVVLKGWPCKIVEMSASKTGKHGHAKVHLVGIDIF
13 MADELDFTT_GDAGASSTYPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLVGIDIF
¥14 MKPLIMEYNKMSDNEALDVEDYAQ_AGSGASLTFPIQCSALRKNGFVVIKGFPCKIVDMSTSKTGKHGHAKVNITAIDIF
15 MSDDHHDDEHFHT_GDSGAAATFPKQCSALRKNEHVMIKGRPCKIVEMSTSKTGKHGHAKVHMVAIDIF
16 MSEDHHDEEQFDS_AESGAAATFPKQCSALRKNEHVMIRGRPCKIVEMSTSKTGKHGHAKVHMVAIDIF
¥17 MSAAHDDAQHEHTFDS_VDAGRSATYPMQCSALRKGGHVVIKNRPCKIVDMSTSKTGKHGHAKVHLVAIDIF
¥18 SDEEHT_FET_ADAGSSATYPMQCSALRKNGFVVIKSRPCKIVDMSTSKTGKHGHAKVHLVAIDIF
¥19 SDEEHT_FEN_ADAGASATYPMQCSALRKNGFVVIKGRPCKIVDMSTSKTGKHGHAKVHLVTLDIF
20 MAEEEHVDFEG_GEAGASLTFPMQCSALRKNGHVVIKGRPCKIVDMSTSKTGKHGHAKVHIVALDIF
21 MSDSEEHHFESKADAGASKTYPQQAGTVRKNGFIVIKNRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
22 MSD_EEHHFESKADAGASKTYPQQAGTIRKSGYIVIKGRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
23 MSD_EEHHFESKADAGASKTYPQQAGTIRKSGHIVIKNRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
¥24 MSD_EEHHFESKADAGASKTYPQQAGTIRKNGHIVIKNRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
¥25 MSD_EEHQFESKADAGASKTYPQQAGTIRKNGHIVIKGRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
¥26 MSD_EEHQFESKADAGASKTYPQQAGTIRKNGYIVIKNRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
27 MSD_EEHQFESKADAGASKTYPQQAGTIRKNGYIVIKNRPCKVVEVSTSKTGKHGHAKCHFVAIDIF
SIA .....ssiissssssSSssisSaiSi..isai.sAI.IIAAssiIIIssS.AAiiSiASAAA.AA.AIAIiII.IAII
parse 3211 1111111 1 1 111
DLG.Euk eeeeeeeee eeeee eeeee eeeeeeAAAAAAAAAA eeeeeeee
PHD.94: ....._.....HHHHHHHH...EEEEE....EEEEEEE.....EEEEEEEEE.
ETH.94: ....._..EEEEEE....EEEE....EEEE......EEEEEE.....hhhhhhhhhh
eeeeeeeeeee
Archaea
28 _____MKQQVEVRQLREGGYVVIDDEPCEILSISVSKPGKHGAAKARIDAIGIF
29 _____MGDKTKVQVSKLKPGRYIIIDDEPCRIVNITVSSPGKHGSAKARIEAVGIF
30 _____MSKKVVEVKTLKVGKYVIIDGEASKITNISTSSPGKHGSAKARVEAVGIF
31 __MIIMPGTKQVNVGSLKVGQYVMIDGVPCEIVDISVSKPGKHGGAKARVVGIGIF
¥32 __MGIQMSIQYTTVGDLKVGSYVMIDGEPCRVVEITKAKTGKHGSAKANVVAIGLF
33 MVLKWVMSTKYVEAGELKEGSYVVIDGEPCRVVEIEKSKTGKHGSAKARIVAVGVF
Target# 1 10 20 30 40 50 56
SIA .....isis.ssSsiSISsISs.SIIIIASs.iSIiSISs.Ss.AA..IAIsIsiI.II
parse 3322211 1 111 111 11
DLG.Arc eeeeeeee eeeee eeeee AAAAaAAeeeeee
SAB.98 .....EEEEEEEEE...EEEEE...EEEEEEEE.....EEEEEEE..HH
PHD.1: ...EEEE.EEEE...EE...EEEEE....EEEEEEEE.....EEEEEEEEEEE
PHD.2: .....HHHH.....EEEE....EEEEEEEE.....EEEEEEEEEEE

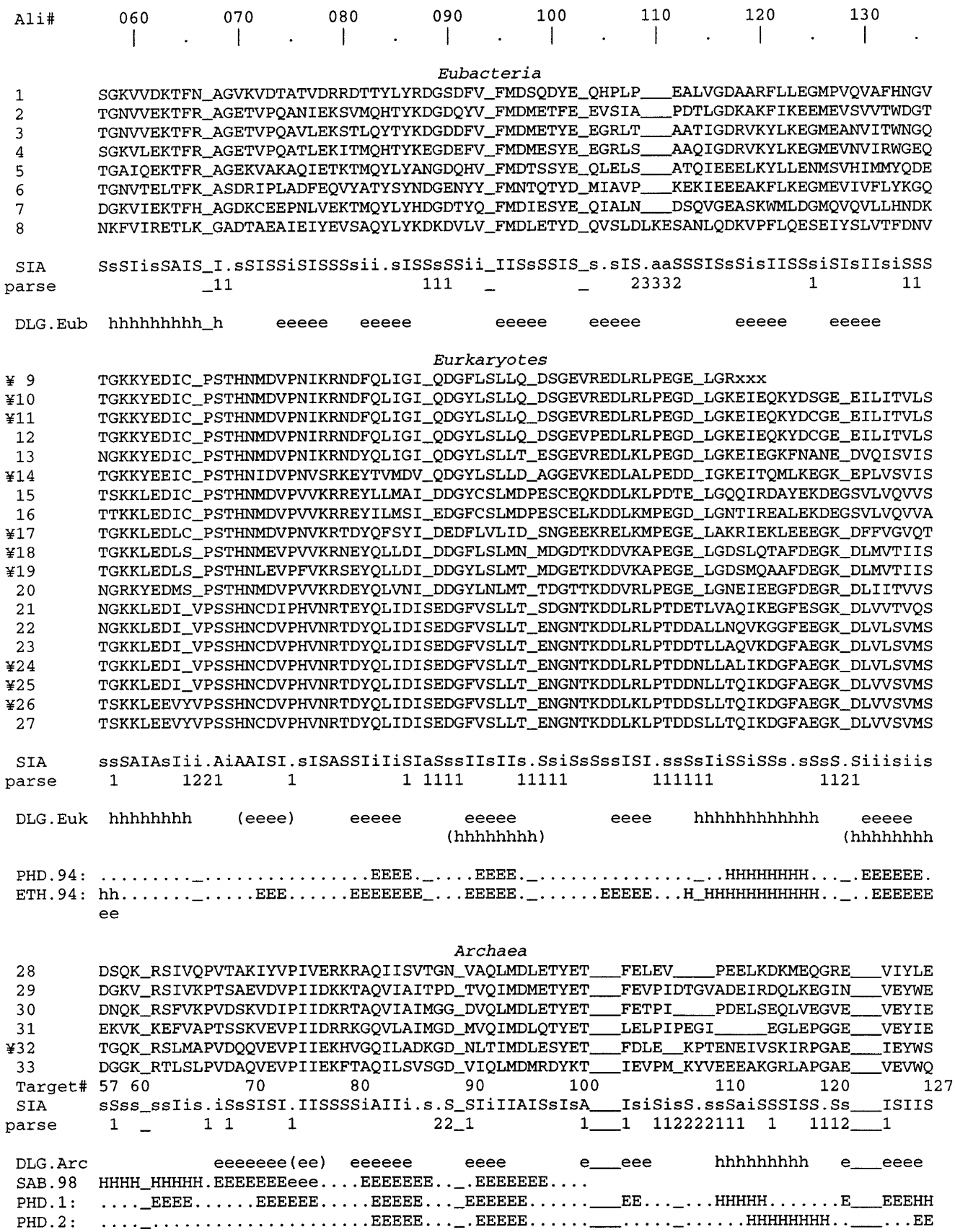


FIG. 1—*Continued*

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Ali#      140       150       160       170       180       190       200
          |    .    |    .    |    .    |    .    |    .    |    .    |    .

                                        Eubacteria
1         PLYIELPVTVELEVTHTEPGLQGDRSSAGTKPATLQTGAQINVPLFINTGDKLKVDSRDGSYLGRVNA
2         ILDVELPTSVVLEIVETDPGVKGDTATGGTKPAILETGAQVMVPLFIAQGERIKVDTRDGSYLGRDNA
3         VIEVELPNSVVLEVIETDPGVKGDTATGGTKPAKVETGAQVMVPLFISVGERIKIDTRNDSYLGRE
4         VLEVELANSVVLEVIQTDPGVKGDTATGGTKPAIVETGATVMVPLFISQGERIKIDTRDDKYLGRE
5         TLGIELPNTVELKVVETEPGIKGDTASGGTKPAKTETGLVVNVPFFVNEGDTLVVNTSDGSYVSRA
6         PIGIELPKHVELQVVETEPAFKGDTQAGGTKPAKLETGAVIQVPFFVKEGDIVKVDTRTGSYVERVKEAK
7         AISVDVPQVVALKIVETAPNFKGDTSSASKKPATLETGAVVQVPFHVLEGEIIKVNTETEEYLEKVK
8         VIDIKLAPKIAFEVVEVEAAVKGDTVTNAMKNITLNTGLVVKAPLFINVGDKVLINSETKEYAERIKN

SIA       sIsISI.SsISISIiSiS.SIs.Assss.SAsISisA.I.IsI.IiISS.SsIsISsSSSSIIsSsss.a
               11          11  1    11  1           1     1        11      1221

DLG.Eub  eeeee  eeeeeeeeee       eeeee  eeee  eeeee(eeee)   eeeeee

            Eukaryotes
¥ 9
¥10       AMTEEAAVAIKAMAK
¥11       AMTEEAAVAIKAMAK
 12       AMTEEAAVAIKAMAK
 13       AMNEECAVAIKPCK
¥14       ALGKEGVVSVKVSNN
 15       AIGEEAILGWKVSTKE
 16       ACGEEAILGYKISTKE
¥17       AMGEEAAIDVKEASNKD
¥18       AMGEEAAISFKEAARTD
¥19       AMGEEAAISFKEAPRSD
 20       AMGEETALACRDAPSS
 21       AMGEEQICALKDVGPK
 22       AMGEEQICAVKDIGTKS
 23       AMGEEQICGIKDIGPK
¥24       AMGEEQICGIKDVGPK
¥25       AMGEEQICALKDIGPK
¥26       AMGEEQICALKDIGGKN
 27       AMGEEQICALKDIGGKN

SIA       iissasiisiSSisSss.
            1          12234

DLG.Euk       eeeeee
          hh)

PHD.94: ...HHHHHHH.....
ETH.94: EE....EEEE.....


            Archaea
 28       SLGKRKIERMA
 29       TLGRIKIMRIKGEGE
 30       ALGQRKLMRTKG
 31       AVGQYKITRVIGGK
¥32       VMGRRKIVRVK
 33       ILDRYKIIRVK
Target# 128       138
SIA       .IsSSAIsAiS.ssa
            1       12344

DLG.Arc       (eeeee)

PHD.1:  H..EEEEEEE.
PHD.2:  EEEEEEE....
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FIG. 1—*Continued*

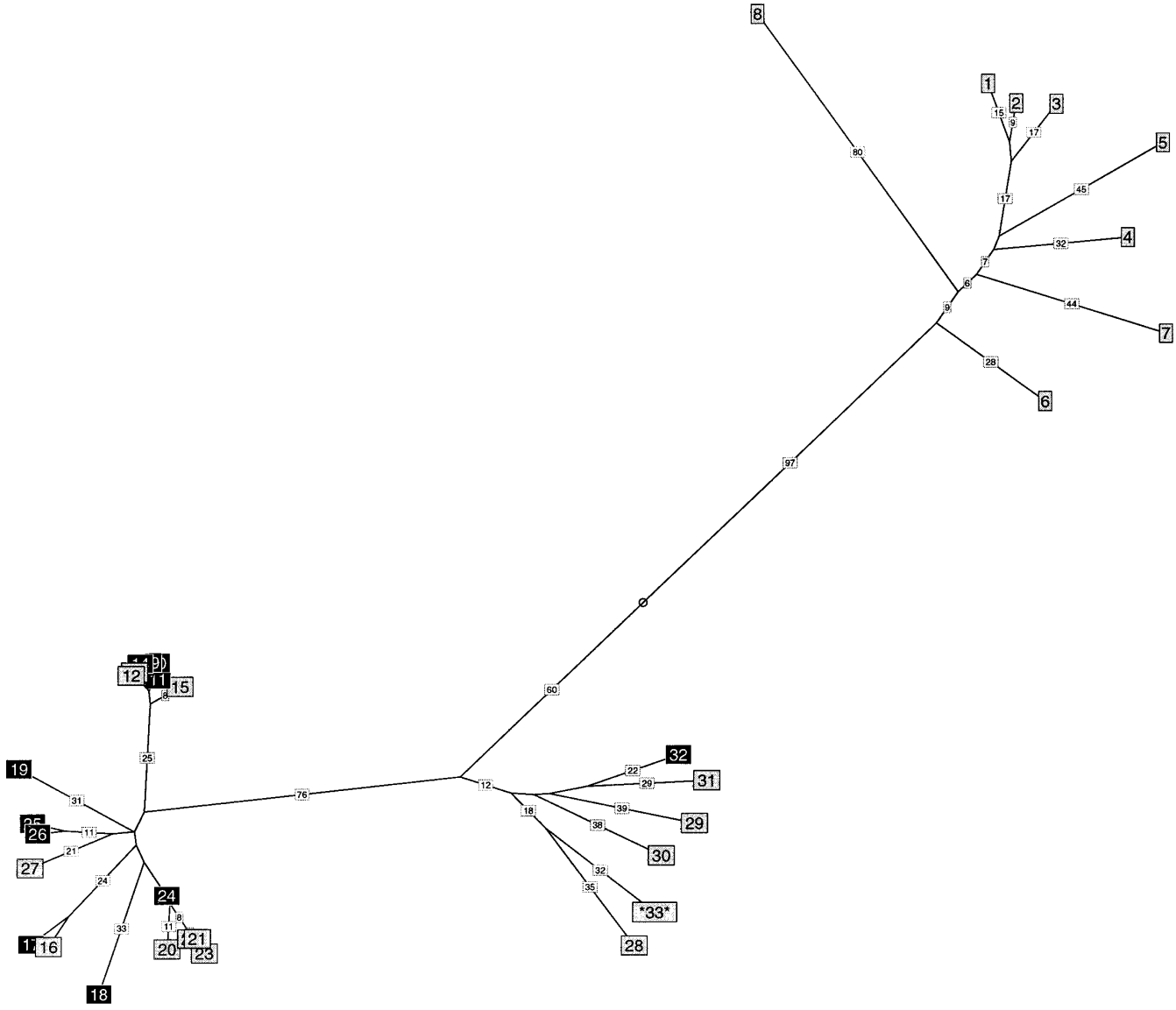

FIG 2. Maximum likelihood tree for the initiation factor 5A superfamily. Derived from the sequences aligned in Figure 1. Numbers in gray boxes denote sequences as listed in Figure legend 1, small boxed numbers are evolutionary distances in PAM units, see Methods for details. Sequences available for the 1994 prediction are marked black, the target sequence for the CASP3 prediction experiment, IF-5A from *Pyrobaculum aerophilus*, is marked with asterisks.

(Figure 1), using an archaebacterial sequence as an outgroup (18). Several ambiguities characteristic of predictions made from multiple sequence alignments were present in this prediction, including an uncertain internal helix (frequently confused with an internal strand) and several possible edge strands (frequently confused with coils). These ambiguities were manifest when transparent prediction tools (1) were used, and tertiary structure modelling based on the predicted secondary structure therefore was recognized as being unreliabile. Figure 3 allows the comparison of secondary structure predictions made in 1994 (ETH.94,

FIG. 3. Summary of secondary structure predictions and assignment of core tertiary structure elements. Secondary structure predictions are labeled and aligned as in Figure 1 and shown with CASP3-target sequence 33. DLG.98 is a consensus prediction for all three subfamilies over the N-terminal approx. 100 positions, and over the eukaryotic and archaeal subfamilies in the C-terminal part, where structures may have diverged in the eubacteria. Elements predicted to form the N-terminal tertiary structural core are numbered corresponding to the OB-fold description by Murzin (25), although the tertiary structure may adopt a more open, twisted Greek-key beta-barrel/sandwich topology than found in the “classic” OB-fold topology, see Discussion.

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==========================================================================================

Ali#                              001       010       020       030       040       050
                                   |   .     |   .     |   .     |   .     |   .     |   .
DLG.Eub                                     (eeeee)   eeeee       eeeeee          eeeeeee
DLG.Arc                                      eeeeeeee eeeee       eeeee    AAAAaAAeeeeeee
DLG.Euk                            eeeeeeeee  eeeee   eeeee      eeeeeeAAAAAAAAAA eeeeeeee

PHD.94(Euk)               ........_.........HHHHHHHH...EEEEE....EEEEEEE........EEEEEEEEEE.
ETH.94(Euk)               ........_..EEEEEE....EEEE....EEEE......EEEEEE........hhhhhhhhhh
                                                                                eeeeeeeeeee

PHD.1 (Arc)                        ...EEEE.EEEE...EE...EEEEE....EEEEEEEE........EEEEEEEEEE
PHD.2 (Arc)                        ............HHHH.....EEEE....EEEEEEEE........EEEEEEEEEE
SAB.98(Arc)                        .........EEEEEEEEE...EEEEE...EEEEEEEE........EEEEEEE..HH
DLG.consensus.98                   ............EEEEEE..EEEEE.......EEEEEE..........EEEEEE...
3D-CORE ELEMENTS                                    S1a    S1b          S2 ActSite-loop S3
33 (Target)                        MVLKWVMSTKYVEAGELKEGSYVVIDGEPCRVVEIEKSKTGKHGSAKARIVAVGVF
Target#                            1         10        20        30        40        50    56

==========================================================================================

Ali#       060       070       080       090       100       110       120       130
            |   .     |   .     |   .     |   .     |   .     |   .     |   .     |   .
DLG.Eub   hhhhhhhhh_h     eeeee   eeeee         eeeee    eeeee          eeeee    eeeee
DLG.Arc             eeeeeee(ee)  eeeeee     eeee        e___eee       hhhhhhhhh   e___eeee
DLG.Euk   hhhhhhhh    (eeee)     eeeee      eeeee          eeee     hhhhhhhhhhhh    eeeee
                                            (hhhhhhhh)                           (hhhhhhhh

PHD.94    ........_.............EEEE._....EEEE._.............._..HHHHHHHH..._.EEEEEE.
ETH.94    hh........_.....EEE......EEEEEEE_...EEEEE._......EEEEE...H_HHHHHHHHHHH..._..EEEEEE
          ee

PHD.1     ...._EEEE......EEEEEE.....EEEEE..._EEEEEE......___EE..._...HHHHH.......E___EEEHH
PHD.2     ....._.....................EEEEE..._.EEEEE......___.....__......HHHHHHHH..___...EE
SAB.98    HHHH_HHHHH.EEEEEEEeee....EEEEEEE.._.EEEEEEE....
DLG.98    .hhhhhhhhh....eeeeeeee..EEEEEE......EEEE.........EEEE......HHHHHHHHH......EEEE..
3D-CORE      H1          S4          S5        (S6?)
33        DGGK_RTLSLPVDAQVEVPIIEKFTAQILSVSGD_VIQLMDMRDYKT___IEVPM_KYVEEEAKGRLAPGAE___VEVWQ
Target#   57 60          70         80          90         100            110        120        127

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Ali#       140       150       160       170       180       190       200
            |   .     |   .     |   .     |   .     |   .     |   .     |   .
DLG.Eub   eeeee  eeeeeeeeee        eeeee  eeee  eeeee(eeee)   eeeeee
DLG.Arc       (eeeee)
DLG.Euk       eeeeee
          hh)


PHD.94:  ...HHHHHHH.....
ETH.94:  EE....EEEE.....

PHD.1:   H..EEEEEEE.
PHD.2:   EEEEEEE....
SAB.98
DLG.98   ......eeeee

33        ILDRYKIIRVK*
Target#   128       138

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PHD.94) with 1998 predictions based on better populated sequence alignments and more balanced trees. While the exact sequence submitted to the PHD-servers differed (see Figure 1), we find that the 1998 predictions by the different methods seem to converge better than those generated in 1994, with the exception of PHD.2, returned by the UCLA-DOE server, which seems to be based on a preformatted multiple sequence alignment. As the differences in the PHD outputs could reflect differences in the alignment used, and the UCLA-DOE prediction was discarded.

Two points become apparent when the secondary structure predictions for the three kingdoms are compared. First, the correspondence between the prediction for the eubacterial proteins and the prediction for the other two kingdoms becomes doubtful in the C-terminal part of the alignment (Figure 3, after alignment position # 110, approximately). This suggested an end of the core domain and/or significant structural divergence between the eubacterial EF-P structure and the IF5A structure in eukaryotes and archaea. Second, we can identify seven, or possibly eight, elements of secondary structure which are likely to form the core of the N-terminal part of the protein. These elements are marked in Figure 3 and include (a) three consecutive beta strands, with a possible bend in the first of the three, (b) an alpha helix, which may be replaced by a surface loop in the archaeal structures, and (c) two (or possibly three) beta strands. Together, the predicted core elements can be described as S1a-S1b-S2-S3-H1-S4-S5-(S6?) (Figure 3). While secondary structure content based on circular dichroism spectroscopy is rarely reliable quantitavely, it is interesting to note that the reported, high, beta-sheet content (18) agrees well with the prediction, and supports it qualitatively.

Next, an analysis of highly conserved, functional and/or aromatic amino acid residues indicative of functionally important sites (where sequence divergence has been constrained during divergent evolution) was used to orient the predicted secondary structural elements (1, 23). Position 042 in the alignment holds the hypusinylated Lys; residues in the surrounding positions are well conserved in the eukaryotic and archaeal proteins. This indicates the presence of either a single, bipartite, or two separate functional sites in the three-dimensional structure. Finally, while some of the strands appear to be bent, or bulged, none of them could be identified with certainty to be an edge strand, as it had been the case for the combinatorial tertiary structure prediction of synaptotagmin (3).

These observations indicate that the folded structure of the first domain is composed of highly twisted, antiparallel beta-sheet and a single alpha-helix, and that the residues involved in functional interactions are located at both "ends" of the resulting barrel/sandwich-structure. With respect to the strand order in the sheet, our preference is for a "Greek-Key" topology, or similar, with the predicted helix in the long connection. For core strand segments and a closed barrel structure, this topological arrangement is exemplified in the superfold described by Murzin as the "OB-fold" (25). While we cannot exclude with certainty the possibility that the core is made of more than five strands, or that the chain arrangement follows a different topology (due to possible mispredictions in both the exact location and the core assignment of the strands), one of our preferred tertiary structural models would bear resemblance to the OB-fold topology, but with some noticeable deviations from the structural properties typically conserved in the "classic" members of this fold family (26), which has been found in many variations (see the SCOP database, (27)).

One of the deviations would be the non-classic location of active site residues in the connecting loop between strands 2 and 3 in our model (25). While unusual, this location is not without precedent in the structures listed with OB-fold topology in SCOP. Namely, the NMR structure of the ribosomal protein S17 from Bacillus stearothermophilus (PDB:1rip) has an insertion of functional residues at what would be the equivalent location (28). The observation that the RPS17 structure would be a somewhat unusual member of the OB-fold family in that the barrel is opened, and maybe better described as a strongly twisted beta-sheet, is further compatible with a second deviation from the OB-fold characteristics, the exclusion of aromatic side-chains from the packed core (26). Finally, the reported minimum human substrate for deoxyhypusine synthase (10) corresponds to positions 022-073 in our alignment (Figure 1). This would be too short to include the entire open barrel domain proposed in Figure 3. Reasons for this inconsistency could be a misassignment and/or misalignment of any core secondary structure, supersecondary structure formation in the fragment, or misprediction of the tertiary structure altogether.

The proposed tertiary structural features were compared them with the output of the automated fold recognition methods by Fischer (29) and Rice (30), accessible through the UCLA-DOE server (31), and the ProCyon method by Sippl (King's Beech, H. Floeckner & M. Sippl, URL:http://www.horus.com/sippl/download.html) (data not shown). The top 15 ranked folds by all methods were a mix of beta sheet-rich folds, dominated by several examples of the immunoglobulin-like beta sandwich and OB-superfolds. However, all returned matches had sub-significant scores. Nevertheless, the structure of ribosomal protein S17 (which bears a functional site between strands 2 and 3), was ranked 11 by one of the UCLA-methods (gonnet+predss, (29)). The structure ranked among the top five by most of the UCLA-methods, Rous Sarcoma Viral Protease (PDB: 2rsp),

forms a 6-stranded barrel of unusual topology, but would lead to a remotely similar structure to the open barrel discussed above, if the assignment of core strands and active site locations, with an alternative position for strand S3, were used.

This communication shows the impact of genomic sequences on the quality of secondary and tertiary structural models derived from analyses of multiple sequence alignments. As is evident from a comparison of the tree in Figure 2 with the universal tree of life, the set of sequences available for this target is now as widely dispersed as possible. It will, of course, be necessary to wait for the experimental structure to emerge to learn whether prediction methods were successful in this case even with a set of sequences as effectively distributed as possible on the universal tree. When the experimental secondary structure of the protein becomes known, and our prediction can be evaluated in its individual elements, this comparison will be useful for estimating the reliability that can be expected from contemporary prediction methods using multiple sequence analysis, and for guiding the design of additional genome projects to support a balanced distribution of organisms for best results in protein structure prediction.

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REFERENCES

1. Benner, S. A., Cannarozzi, G., Gerloff, D., Chelvanayagam, G., & Turcotte, M. (1997) *Chem. Rev.* **97,** 2725–2843.
2. Gerloff, D. L., & Benner, S. A. (1995) *Proteins: Struct. Funct. Genet.* **21,** 273–281.
3. Gerloff, D. L., Chelvanayagam, G., & Benner, S. A. (1995) *Proteins: Struct. Funct. Genet.* **21,** 299–310.
4. Gerloff, D. L., Cohen, F. E., Korostensky, C., Turcotte, M., Gonnet, G. H., & Benner, S. A. (1997) *Proteins: Struct. Funct. Genet.* **27,** 450–458.
5. Chen, K. Y., & Liu, A. Y.-C. (1997) *Biol. Signals* **6,** 105–9.
6. Park, M. H., Lee, Y. B., & Joe, Y. A. (1997) *Biol. Signals* **6,** 115–123.
7. Liao, D.-I., Wolff, E. C., Park, M. H., & Davies, D. R. (1998) *Structure* **6,** 23–32.
8. Bartig, D., Lemkemeier, K., Frank, J., Lottspeich, F., & Klink, F. (1992) *Eur. J. Biochem.* **204,** 751–758.
9. Chung, S. I., Park, M. H., Folk, J. E., & Lewis, M. S. (1991) *Biochim. Biophys. Acta* **1076,** 448–451.
10. Joe, Y. A., & Park, M. H. (1994) *J. Biol. Chem.* **269,** 25916–25921.
11. Park, M. H., Wolff, E. C., & Folk, J. E. (1993) *BioFactors* **4,** 95–104.
12. Kang, H. A., & Hershey, J. W. B. (1994) *J. Biol. Chem.* **269,** 3934–3940.
13. Ruhl, M., Himmelspach, M., Bahr, G. M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G. K., Probst, H., Bevec, D., & Hauber, J. (1993) *J. Cell Biol.* **123,** 1309–1320.
14. Bevec, D., & Hauber, J. (1997) *Biol. Signals* **6,** 124–133.
15. Bevec, D., Klier, H., Holter, W., Tschachler, E., Valent, P., Lottspeich, F., Baumruker, T., & Hauber, J. (1994) *Proc. Nat. Acad. Sci.* **91,** 10829–10833.
16. Liu, Y. P., Nemeroff, M., Yan, Y. P., & Chen, K. Y. (1997) *Biol. Signals* **6,** 166–174.
17. Rost, B., & Sander, C. (1993) *J. Mol. Biol.* **232,** 584–599.
18. Klier, H., Csonga, R., Joao, H. C., Eckerskorn, C., Auer, M., Lottspeich, F., & Eder, J. (1995) *Biochemistry* **34,** 14693–14702.
19. CASP3-Organizers (1998) WWW-site: 3rd community wide experiment on the critical assessment of techniques for protein structure prediction (CASP3), (URL: http://PredictionCenter.llnl.gov/).
20. Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucl. Acids Res.* **12,** 387–395.
21. Hallett, M., Korostensky, C., Knecht, L., & Gonnet, G. H. (1992) WWW: Computational Biochemistry Research Group (CBRG) Server (ETH Zürich, Switzerland, URL: http://cbrg.inf.ethz.ch).
22. Rost, B., & Sander, C. (1993) WWW: The PredictProtein Server (EMBL Heidelberg, Germany, URL: http://www.embl-heidelberg.de/predictprotein/).
23. Benner, S. A., & Gerloff, D. (1991) *Adv. Enz. Regul.* **31,** 121–181.
24. Benner, S. A., Gerloff, D. L., & Chelvanayagam, C. (1995) *Proteins: Struct. Funct. Genet.* **23,** 446–453.
25. Murzin, A. G. (1993) *EMBO J.* **12,** 861–867.
26. Bycroft, M., Hubbard, T. J. P., Proctor, M., Freund, S. M. V., & Murzin, A. G. (1997) *Cell* **88,** 235–242.
27. Murzin, A. G., Brenner, S. E., Hubbard, T., & Chothia, C. (1995) *J. Mol. Biol.* **247,** 536–540.
28. Jaishree, T. N., Ramakrishnan, V., & White, S. W. (1996) *Biochemistry* **35,** 2845–2853.
29. Fischer, D., & Eisenberg, D. (1996) *Protein Sci.* **5,** 947–955.
30. Rice, D., & Eisenberg, D. (1996) *J. Mol. Biol.* **267,** 1026–1038.
31. Fischer, D., Rice, D., & Eisenberg, D. (1995) WWW: UCLA-DOE Structure Prediction Server (UCLA-DOE Laboratory, Los Angeles, U. S. A., URL: http://www.doe-mbi.ucla.edu/people/frsvr/frsvr.html).
32. Rost, B. (1996) *Methods Enzymol.* **266,** 525–539.