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## **Plant-TFClass: a structural classification for plant transcription factors**

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## Highlights

Transcription factors (TF) are proteins that bind DNA specifically thanks to a DNA-binding domain (DBD).

TF DBDs fold into various 3D-structures that contact DNA through different elements (alpha helices, beta-strands and loops).

The vast diversity of DBD folds in mammalian TF families has been organized hierarchically, into superclasses and classes, based on their 3D-structure and their mode of interaction with DNA.

Such a classification has been missing for plants whose 56 recognized TF families harbor diverse types of DBD, many of which are absent in mammals.

The accumulated 3D-structures and recent availability of accurate models for plant TF DBDs open up the opportunity to organize plants TF families into a higher rank classification.

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# 1 **Plant-TFClass: a structural classification for plant transcription factors**

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## 17 **Keywords**

18 Transcription factors, classification, plants, 3D-structures, DNA-binding domain, TFClass

## 19 **Abstract**

20 Transcription factors (TFs) bind DNA at specific sequences to regulate gene expression. This  
21 universal process is achieved thanks to their DNA-binding domain (DBD). In mammals, the vast  
22 diversity of DBD structural conformations and the way they contact DNA has been used to  
23 organize TFs in the TFClass hierarchical classification. However, the numerous DBD types  
24 present in plants and absent from mammal genomes were missing from this classification. We  
25 reviewed DBD 3D-structures and models available for plant TFs to classify most of the 56  
26 recognized plant TF types within the TFClass framework. This extended classification adds  
27 **eight** new classes and **37** new families corresponding to DBD structures absent in mammals.  
28 Plant-TFClass provides a unique resource for TF comparison across families and organisms.

## 29 **Towards a universal classification for transcription factors**

30 Transcription factors (TFs) play essential roles in most processes occurring in living organisms.  
31 Thanks to a dedicated DNA binding domain (DBD), these proteins bind DNA at specific

32 sequences (called TF binding sites or TFBS) and regulate the expression of associated genes.  
33 Plant (Streptophyta: grouping the land plants – Embryophyta - and their closest algal relatives)  
34 genomes contain a high diversity of TF-encoding genes including a collection of plant-specific  
35 TFs [1–3] that evolved during their history. The initial analysis of *arabidopsis* (*Arabidopsis*  
36 *thaliana*) genome identified 1500 TFs corresponding to 28 gene families of which 45% (16  
37 families) were not found in mammals [4]. Since then, comparative genomic studies identified  
38 additional TFs and putative TF families in plant genomes [2] and various plant TFs  
39 classifications have been proposed, based on the nature of the DBD and other conserved  
40 protein domains [2,5–7]. While some of these classifications mention existing homology  
41 between different families, none of them proposes a hierarchical, higher-rank, organization.  
42 With the increasing number of 3D DBD structures being solved or reliably predicted, it now  
43 becomes possible to propose a hierarchical classification for plant TFs based on the 3D  
44 structure and the DNA binding mode of their DBD. Traditionally, TFs have been classified into  
45 superclasses based on broad structural similarities of their DBDs [8]. These superclasses  
46 should not be regarded as equivalent to phylogenetic classification since it remains uncertain  
47 whether all the diverse DBDs have independently emerged or if some have a shared ancestry.  
48 However, they are useful for grouping TFs based on distinct structural features. In mammals,  
49 the reference structural classification is TFClass [9,10]. This hierarchical classification starts  
50 with nine structurally defined superclasses, further decomposed into classes, families and  
51 subfamilies. It provides a framework for meta-analyses of TF properties and evolution [11], it  
52 facilitates comparisons between organisms and it is the reference classification used by  
53 JASPAR, the open access database for TFBS models [12].  
54 Here we built upon the TFClass framework to classify all plant TF types described to date. The  
55 new framework is referred to as “Plant-TFClass” (Figure 1, online supplemental information  
56 Table S1), illustrated with the DBD folds with or without DNA for each TF family (Figure 2). For  
57 this, we reviewed experimental and predicted data on DBD structures. For each TF type, we  
58 proposed a classification when sufficient evidence exist and we left the remaining ones in a  
59 tenth ‘Yet undefined DNA-binding domains’ superclass as in TFClass. In addition to this manual  
60 construction, we also used an automatic approach based on the pairwise structural alignment  
61 of TF DBDs: this returned a very similar grouping, but with some inconsistencies that highlight  
62 the merits of the manual approach (online supplemental information Figure S1). Compared to  
63 TFClass, Plant-TFClass has additional TFs of two natures: 1) TFs with a DBD type absent from



64 mammals. This is the case for **eight** new classes (LEAFY, **RWP-RK**, AP2/EREBP, EIL, **TCP**, **ALOG**,  
65 SBP and B3) and we explain on which basis these new classes were organized under a given  
66 superclass. Such TFs may have evolved (i) de novo, (ii) from existing DBD by rapid sequence  
67 evolution or (iii) from transposon proteins [13,14]. 2) Another type of plant-specific TFs  
68 corresponds to those possessing a DBD fold that exists in mammals but has diverged  
69 extensively, in particular with the acquisition of auxiliary domains. This usually defines new  
70 families within existing classes (e.g. HD-ZIP, MADS Type II, AHL).  
71 Among the 56 recognized plants TF types, **50** were classified in one of the nine superclasses  
72 from TFClass (Helix-turn-helix domains, Beta-hairpin exposed by an alpha/beta-scaffold, Other  
73 all-alpha-helical DNA-binding structures, Beta-sheet binding to DNA, Zinc-coordinating DNA-  
74 binding domains, Beta-barrel DNA-binding domains, Basic domains, Alpha-helices exposed by  
75 beta-structures, Immunoglobulin-fold) and the remaining **six** were added to the “Yet  
76 undefined DBD”.

#### 77 **Helix-turn-helix domains**

78 TFs of the helix-turn-helix (HTH) domains superclass bind specific DNA sequences by inserting  
79 a ‘recognition’ alpha-helix into the major groove. This recognition helix is followed by a turn  
80 and, in most cases, two additional alpha-helices packed nearly perpendicularly against it. HTH  
81 DBDs also often have an additional structure (usually a loop) that projects basic residues in  
82 the DNA minor groove, also contributing to the sequence specificity. TFClass divides the **HTH**  
83 **domains** superclass into seven classes, five of which are present in plants: **Heat shock factors**,  
84 **Fork head / winged helix factors**, **AT-rich interaction domain (ARID)**, **Homeo domain factors**  
85 **(HD)** and **Tryptophan cluster factors**.

86 The three first classes are represented by a single family in plants (**HSF**, **E2F** and **ARID**  
87 respectively). The ARID is sometimes associated with a HMG-box domain but models suggest  
88 that ARID-HMG proteins interact with DNA mainly thanks to their ARID domain [15]. Thus, we  
89 classified them within the ARID family.

90 As for the HD TF, their variety in plants comes from the diversity of adjacent domains including  
91 a leucine zipper in HD-Zip that affect DNA binding by allowing dimerization [16]. 3D structures  
92 of plant HD DBDs are available for WUSCHEL (WOX family) (6RYI) and revealed a canonical HD  
93 fold with loop regions connecting the three alpha-helices that are expanded compared to the  
94 drosophila Engrailed HD protein [17].

95 The **Tryptophan cluster factors class** contains **MYB** and **MYB-related** TFs families that are  
96 represented by few members in **mammals** but greatly expanded in land plants (as R2R3-MYB:  
97 MYB genes containing two repeats of the MYB-type HTH domain) to form one of the largest  
98 transcription factor family [18]. A structure of plant MYB DBD in complex with DNA is available  
99 for the R2R3-MYB WEREWOLF (6KKS) and revealed a similar fold as for **other eukaryotic** MYB  
100 [19]. We have added to the **Tryptophan cluster factors class**, three plant families **absent in**  
101 **mammals**: the **GARP**, the **Trihelix** and the **Storekeeper** (STK) TFs (also known as GeBP). **GARP**  
102 TFs have a DBD **sharing weak sequence similarity with MYB due to common ancestry**. These  
103 TFs come in two subfamilies, G2-like and ARR-B, with structures available in complex with DNA  
104 for the G2-like TFs PHR1 (6J4R) [20] and LUX (5LXU) [21] and without DNA for the ARR-B TF  
105 ARR10 (1IRZ) [22]. The Trihelix motif was initially proposed to be **evolutionary** related to the  
106 C-MYB DBD based on **weak sequence similarity** [23] and this hypothesis was later confirmed  
107 by a solution structure of arabidopsis GT-1 DBD (2JMW) [24]. **STK** TFs were included in this  
108 class based on the AlphaFold2 model of their DBD: this model shows similarity to HTH domains  
109 and 3D structure comparisons returns good alignments with members of the Trihelix family,  
110 MYB family and **GARP/G2-like** subfamily (with root mean square deviation (rmsd) of 3 Å on 80  
111 equivalent positions out of 90 residues). All these families harbor conserved tryptophan  
112 residues (three for R2R3-MYB WEREWOLF and STK down to one for GARP) within the HTH  
113 domain, justifying their inclusion in this class.

114 Finally, we added to this HTH domain superclass the plant specific **LEAFY** (LFY) class. Crystal  
115 structure of LFY DBD in complex with DNA have been solved for **arabidopsis** (2VY1, 2VY2) and  
116 *Physcomitrium patens* (4BHK) proteins, revealing the presence of an HTH motif [25]  
117 embedded within a 7-alpha-helices fold, plus a loop that adds base contacts in the DNA minor  
118 groove. LFY bind DNA as a dimer and possess a capacity to higher order assembly thanks to  
119 the presence of a SAM oligomerization domain [26].

120 **We also added RWP-RK or NIN-like Protein (NLP) factors as a new class. These TFs** possess  
121 RWP-RK DNA binding domain with a basic region [27] predicted to fold as three short and one  
122 long alpha-helix (AlphaFold2 model from Uniprot). They have been shown to bind DNA as  
123 dimers [28]. 3D-structures comparison revealed significant alignment with the helix-turn-helix  
124 motif **of bacterial DNA repair protein Ada (1U8B) (45 equivalent positions out of 132 residues**  
125 **with an rmsd of 2.1 Å).**

## 126 **Beta-hairpin exposed by an alpha/beta-scaffold**

127 TFs from this superclass have a DBD made of an alpha/beta-structured scaffold with a beta-  
128 hairpin inserting into the DNA major groove and acting as the main DNA contacting element.  
129 In TFClass, this superclass contains the **GCM** domain factors class represented by a single  
130 mammal-specific family (the GCM family) to which we have added five families found in  
131 plants: the **NAC**, **VOZ**, **CAMTA**, **WRKY** and **FRS/FRF**. The determination of DBD/DNA  
132 crystallographic structures of the **NAC** TF AtANAC019 (3SWP) and the **WRKY** TF AtWRKY4  
133 (2LEX) revealed that the DNA-interaction mode is analogous to that of GCM (1ODH) with good  
134 superimposition of the beta-strand's amino acids contacting the DNA [29]. We deliberately  
135 chose to include WRKY TFs here and no longer classify them as Zn-coordinating because the  
136 domain coordinated by Zn ions lies outside their DBD. We included the Vascular Plant One-  
137 Zinc-Finger (**VOZ**) and the calmodulin binding transcription activator (**CAMTA**) families  
138 because the AlphaFold2 structural predictions of their DBDs align well with NAC DBD (3SWP)  
139 (rmsd of 3 Å on 116 equivalent positions out of 171 residues for VOZ and 2.66 Å on 94  
140 equivalent positions out of 112 residues for CAMTA). These similarity levels are also found  
141 between WRKY (2LEX) and NAC (3SWP) (55 equivalent positions out of 63 residues with an  
142 rmsd of 3.06 Å).

143 **FRS/FRF** factors have been linked to WRKY factors based on an iterative PSI-blast search [30].  
144 AlphaFold2-predicted structure of the DBD of the FRS/FRF protein FAR1 revealed a fold similar  
145 to that of WRKY and 3D structures comparisons return significant alignment against AtWRKY4  
146 (2LEX) (60 equivalent positions out of 90 residues with an rmsd of 3.27 Å). FRS/FRF factors  
147 were thus added to the **GCM** class.

148

149 We made **one** more addition to this superclass by including the **AP2/EREBP** class  
150 corresponding to proteins with the AP2/ERF DBD, **absent in mammals** [14]. The **AP2/EREBP**  
151 class was divided into two families: the **AP2** and the **ERF/DREB**. In the **AP2** family, DBD/DNA  
152 structures are available for TEM1 (7ET4) and AtERF1 (1GCC). Both structures revealed a beta-  
153 hairpin overhanging the major groove and topped by an alpha-helix [31,32]. In the **ERF/DREB**  
154 family, the TF/DNA structure of AtERF96 (5WX9) additionally shows an N-terminal alpha-helix  
155 contributing to the DNA interaction by entering the minor groove [33].

## 156 Other all-alpha-helical DNA-binding domains

157 This superclass gathers TFs possessing a DBD exclusively made of alpha-helices. **The structure**  
158 **of their DBDs and their mode of DNA-binding are different from other DBD made of alpha-**  
159 **helices (HTH domains and basic domains).** It contains the **heteromeric CCAAT-binding factors**  
160 (also known as nuclear factor Y or NF-Y) and the **High-mobility group (HMG) domain factors.**

161 The former class is represented by a single family, present in many eukaryotes including  
162 plants. **Heteromeric CCAAT-binding factors** bind DNA as trimers made of NF-YB and NF-YC  
163 subunits plus either NF-YA or the **viridiplantae**-specific CONSTANS (CO) protein. The NF-YB/C  
164 dimer binds to DNA in a non-specific manner acting as a scaffold for the binding of NF-YA or  
165 CO which provide DNA sequence specificity. Despite possessing **no high sequence similarity,**  
166 NF-YA6 and CO show similar 3D-structures in complex with NF-YB/C and DNA (6R2V, 7CVQ  
167 respectively) with one long helix interacting with the NF-YB/C dimer and one short helix  
168 entering the DNA minor groove [34,35]. We thus added CO to the **heteromeric CCAAT-binding**  
169 **factors** despite that it does not recognize a CCAAT motif.

170 **High-mobility group (HMG) domain factors** represent a broad group of proteins present in all  
171 eukaryotes that bind DNA thanks to an HMG-box to remodel chromatin and to regulate gene  
172 transcription. **The HMG-box domain forms an L-like structure made of three alpha helices.**  
173 **Unlike HTH domains factors, they bind DNA in the minor groove, widening the minor groove**  
174 **and bending the DNA at 90 degrees.** In TFClass, this class contains seven families. Plants HMG-  
175 box containing proteins are less diversified than **mammals'** and have a TF activity that is either  
176 not demonstrated or achieved via another DBD than the HMG-box (AT-hook or ARID domain)  
177 [36].

178 We classified the YABBY proteins, **absent in mammals but found in several other eukaryotes,**  
179 as a new family within the "**High-mobility group (HMG) domain factors**". YABBY TFs possess  
180 two highly conserved domains that have been proposed to contribute to DNA binding : the N-  
181 terminal zinc finger domain and the C-terminal YABBY domain [37,38]. **The predicted YABBY**  
182 **domain (39 amino acids long) has a helix-loop-helix domain that superimposes well (39**  
183 **equivalent positions, rmsd = 2.01 Å) with the two N-terminal helices of the HMG domain of**  
184 **the SRY protein (1HRY), with residues of similar nature potentially contacting DNA.** This  
185 superimposition does not exclude a direct DNA binding by the zinc finger domain but

186 experiments on the YABBY CRABS CLAW protein showed a more prominent role for the YABBY  
187 domain compared to the zinc finger domain for DNA binding [39].

188 To this superclass, we also added a new plant-specific class: **EIL** (standing for Ethylene-  
189 insensitive3 (EIN3)-like factors). The NMR solution structure of arabidopsis EIL3 DBD without  
190 DNA (1WIJ) revealed a novel fold made of five alpha-helices with candidate DNA-contacting  
191 residues that remain to be confirmed [40].

## 192 **Beta-sheet binding to DNA**

193 This superclass contains the **AT-hook factors** and TATA-binding proteins classes which bind  
194 DNA through single extended strands or beta-sheets, respectively, preferentially in its minor  
195 groove [10]. AT-hook motifs exist in a wide range of eukaryotic nuclear proteins [41]. In  
196 TFClass, the AT-hook factors class contains a single family, the **HMGA**, that bind DNA at AT-  
197 rich stretches thanks to a central Arg-Gly-Arg core that enters deep in the minor groove  
198 [42,43]. In plants, HMGA proteins contains four AT-hook DNA binding motifs with at least two  
199 required for efficient DNA binding [44]. The overall structure of plant and **metazoan** HMGA  
200 proteins is thus quite different.

201 To this class, we added the **land plant**-specific AT-hook motif nuclear-localized (AHL)  
202 transcription factors, not orthologous to the HMGA and possessing one or two AT-hook  
203 motifs. In addition AHL possess a plant and prokaryote conserved (PPC) domain, involved in  
204 protein-protein interactions [45,46].

205 **Finally, we also added the land plant-specific TCP factors. These TFs have been successively**  
206 **reported as similar to bHLH based on the presence of a putative bHLH motif [47] and to**  
207 **Ribbon-Helix-Helix (RHH) based on the crystallographic structure of the DBD of the rice (*Oryza***  
208 ***sativa*) PCF6 [48]. However, the recent determination of TCP-DNA structures revealed a new**  
209 **DBD fold (7VP2) where homodimers bind to DNA mainly through anti-parallel beta-strands at**  
210 **the dimer interface and two flexible loops at the N-terminal side of each monomer [49]. We**  
211 **have thus added the TCP class / TCP family to the “beta-sheet binding to DNA” superclass.**

## 212 **Zinc-coordinating DNA binding domains**

213 DBDs of TFs from this superclass have a fold organized by one or more zinc ions. The nature  
214 of the Zn coordination and the resulting DBD fold define nine classes in TFClass [10]. Within  
215 this superclass, plants possess two TFs families shared with animals: **C2H2** (from the “**C2H2**

216 Zinc finger factors” class) and the C4-GATA-related (from the “Other C4 zinc finger-type  
217 factors” class). In the C2H2 fold, the “zinc finger” is a loop formed between a beta-hairpin and  
218 an alpha-helix and the Zn atom is coordinated by two cysteine and two histidine residues.  
219 Several Zn fingers can wrap around the major groove of the DNA via interactions of the alpha-  
220 helices with the major groove, as shown in figure 2. In plants, the IDD TFs bind DNA thanks to  
221 two C2H2 and two C2CH zinc fingers, with the first C2H2 zinc finger being the most critical for  
222 specific binding [50]. We thus classified IDD TFs as a family within the C2H2 class. The C4-  
223 GATA-related fold is a variant of the C2H2 fold in which the coordination involves four cysteine  
224 residues.

225 We added five types of plant TFs within this superclass: the Squamosa promoter Binding  
226 Proteins (SBP), the DNA-binding One Zinc Finger (DOF), LATERAL ORGAN BOUNDARIES (LOB)  
227 DOMAIN (LBD), the Arabidopsis LSH1 and Oryza G1 (ALOG) and the Growth-Regulating Factors  
228 (GRF) proteins. SBP TFs possess two zinc-finger like structures where one zinc ion is  
229 coordinated by three cysteine and one histidine residues (or four cysteine residues in some  
230 cases) and the second one is coordinated by two cysteine and two histidine residues. The N-  
231 terminal Zn-finger like structure is the best candidate to contact DNA according to NMR  
232 experiments (performed in the presence or absence of DNA) and DNA docking on the NMR  
233 structure (1UL4) [51]. As the SBP DBD is dissimilar to other known zinc-finger structures it  
234 defines a new class “C3H(C),C2H2 zinc-fingers like factors” in Plant-TFClass. DOF TFs bind DNA  
235 via their highly conserved DOF domain. The DOF domain forms a single zinc-finger motif of  
236 C2C2-type and was thus added to the “Other C4 zinc finger-type factors” class. Their  
237 sequence-specific binding to DNA is supported by several *in vitro* functional studies [52–54]  
238 and DBD/DNA structure modelling studies [55,56]. The DOF domain is also able to form  
239 protein-protein interactions with other TFs [52,57–59]. LBD TFs bind DNA thanks to their  
240 dimeric and highly conserved LOB DBD. 3D structural analysis of the LOB domain from the  
241 wheat Ramosa2 protein (TtRa2LD, 5LY0) strongly supports that a C4 zinc finger motif is  
242 responsible for DNA binding while homodimerization is achieved via a C-terminal leucine  
243 zipper-like motif [60]. We thus classified LBD TFs as a new family in the existing Other C4 zinc  
244 finger-type factors class.

245 With functional roles established in several angiosperms and *Marchantia polymorpha*, the  
246 ALOG family of proteins, proposed as a novel plant-specific TF family [61], was recently  
247 characterized at the structural level (8P5Q) [62]. These proteins likely originated from a



248 recombinase of a retrotransposon in which a zinc ribbon was inserted [63] in the core  
249 tetrahelical structure. The structure of the DBD in complex with DNA revealed a bundle of  
250 alpha helices with a HC<sub>3</sub>-motif zinc-ribbon contributing to positioning the alpha-helices and  
251 the loop that directly contact DNA. As the zinc ribbon has a HC<sub>3</sub> motif, we created a new class  
252 (HC<sub>3</sub> zinc ribbon factors) in the zinc-coordinating DNA-binding proteins superclass for the  
253 ALOG family.

254 Plants also encode zinc finger proteins containing a three cysteine and one histidine residues  
255 (C3H) motif, that defines the C3H zinc finger factors class in TFClass. Plant C3H are unrelated  
256 to those found in mammals and the majority of plant C3H motifs bind RNA. The only plant C3H  
257 TFs shown to bind DNA are the Growth-Regulating Factors (GRF family) : their putative DNA-  
258 binding domain WRC (tryptophan, arginine and cysteine) contains a conserved C3H  
259 (CX<sub>9</sub>CX<sub>10</sub>CX<sub>2</sub>H) motif [64]. The WRC domain also plays a role in JM28, a negative regulator of  
260 immunity in arabidopsis, by providing target specificity for DNA binding of the RBL/ATX1/2-  
261 COMPASS complex [65]. Until more evidence is provided for the other groups of plant C3H  
262 proteins, only GRFs are considered as a TF, classified within the C3H zinc finger factors class  
263 of TFClass.

#### 264 **Beta-barrel DNA-binding domains**

265 TF from this superclass bind DNA thanks to a beta-barrel of variable number of beta-strands.  
266 It is represented by a single family in mammals (The Dbp family of the Cold-shock domain  
267 factors class). Distant homologs to this family in arabidopsis (CSP1 and CSP3) bind RNA to act  
268 as chaperone but have no known DNA binding activities.

269 On the other hand and as noted by Wingender et al. [10], the plant-specific B3 domain TFs  
270 have sequence specific DNA binding and belong to this superclass. The B3 domain contains  
271 approximately 110 residues with seven beta-strands and two alpha-helices folded into a  
272 pseudo-barrel. The residues belonging to the loops between the beta-strands 1-2 and 4-5  
273 contact bases in the DNA major groove allowing each B3 family to recognize a specific  
274 sequence [66–68]. B3 domains TFs are present in four families identified as LAV (LEAFY  
275 COTYLEDON2 (LEC2)-ABSCISIC ACID INSENSITIVE-3 (ABI3)-VAL), ABI/VP1-related protein  
276 (RAV), REPRODUCTIVE MERISTEM (REM), and AUXIN RESPONSE FACTOR (ARF) [69]. ARFs and  
277 LAVs have a single B3 domain, whereas REM family proteins have from one to eleven B3  
278 domains [70]. RAVs have an AP2/ERF DNA binding domain in addition to the B3 domain. Most

279 ARF TFs possess a PB1 oligomerization domain [71–73] and often bind DNA as dimers with  
280 preference regarding the orientation and the spacing of ARF binding sites [54,74–76]. In  
281 contrast to the other families, studies performed on VRN1 [77] and REM16 [78] belonging to  
282 the REM family suggest that these TFs do not show sequence-specific DNA binding.

### 283 **Basic domains**

284 This superclass includes TFs that bind DNA with a basic alpha-helix usually inserted in the DNA  
285 major groove. It contains two classes: the basic leucine zipper (**bZIP**) and basic helix-loop-helix  
286 (**bHLH**) TFs that are present in most eukaryotes. Both types are dimeric TFs that pinch DNA  
287 between two basic helices. We have divided the **bHLH** class into two families: the classical  
288 **bHLH** and the **BES/BZR** plant-specific family. **BES/BZR** TFs slightly differs from classical bHLH.  
289 Indeed, instead of having only the long basic helix interacting with DNA as in classical bHLH,  
290 in **BES/BZR** DBDs, the loop between the two helices adds contacts with the minor groove and  
291 the second helix involved in dimerization is much shorter.

### 292 **Alpha-helices exposed by beta-structures**

293 Members of this superclass have an all-alpha-helically folded DBD but contrary to the HTH  
294 domains, the basic domains and the other all-alpha-helical DNA-binding domains  
295 superclasses, the DNA-binding helices are exposed by a scaffold of beta-strands and do not  
296 insert in either DNA groove but are packed against the DNA double helix. This superclass  
297 contains two classes: the SAND domain factors and the MADS box factors. SAND domain  
298 factors are represented by ULTRAPETALA genes in plants and by VARL genes in green algae  
299 but a sequence-specific DNA binding activity has never been demonstrated [79,80]. The **MADS**  
300 **box factors class** contains two families in plants (**Type I** and **Type II**) with a DBD/DNA complex  
301 structure only described in animals. The DBD structure of arabidopsis floral organ identity  
302 MADS TF SEPALLATA3 was recently obtained without DNA (7NB0) and did not reveal any  
303 major differences with metazoan proteins [81].

### 304 **Immunoglobulin-fold**

305 In TFClass, this superclass contains 16 families grouped into six classes that have no homologs  
306 with demonstrated TF activity in plants.



### 307 **Yet undefined DNA-binding domains**

308 This superclass gathers TFs with functionally well-characterized DBD but awaiting structural  
309 data for a definitive classification. In TFClass, it includes four classes, plus an “uncharacterized”  
310 class gathering six families. Among those families, plants possess NFX1-like proteins whose TF  
311 activity remains to be firmly established [82].

312 To this superclass, we added nine classes corresponding to plant factor families with well  
313 characterized TF activity but lacking crucial structural clues on the DBD/DNA interaction. For  
314 three classes (the cysteine-rich polycomb-like protein (**CPP**) [83], the DNA-binding protein  
315 phosphatase (**DBP**) [84] and the **S1Fa-like** factors [85]), there is no structural data and  
316 AlphaFold2 predictions of the 3D structure for their DBD were uninformative (low confidence  
317 model or no hit in 3D-structure comparison). Putative classification is discussed below for **3**  
318 classes (**GRAS**, **PLATZ** and **BCP**) based on experimental or modeled structures and was  
319 mentioned before for GRF TFs.

320

321 The **GRAS** domain of SCARECROW-LIKE7 from rice was crystallized as a dimer (5HYZ) with each  
322 monomer consisting of a core region made of alpha and beta-structures, topped with an  
323 alpha-helical cap structure. The two cap regions form a candidate DNA-binding groove  
324 containing positives residues and DNA docking in this groove predicted tight protein/DNA  
325 interactions that were validated experimentally [86]. Once validated at the structural level,  
326 such DNA binding mode would likely define a new superclass.

327

328 The **Archaeplastida**-specific **PLATZ** TFs are zinc-dependent DNA binding proteins. The zinc  
329 finger coordination motif has, in its N-terminal part, a consensus signature C-x2-H-x11-C-x2-  
330 C-x(4–5)-C-x2-C-x(3–7)-H-x2-H which is different from other characterized zinc-binding motifs.  
331 It also has four conserved cysteine residues in its central region [87]. Structural models show  
332 various folds (alpha-helices, a beta-hairpin and a beta-sheet). In the absence of experimental  
333 structure in complex with DNA or extensive modeling, it is not possible to firmly place this  
334 family within the Zinc-coordinating DNA-binding domains superclass.

335

336 The **BBR/BPC** DBD has a conserved WAR/KHGTV motif required for DNA binding, reminiscent  
337 of the WRKYCGK consensus of WRKY proteins suggesting that it may bind DNA in similar way  
338 [88].

### 339 **Concluding remarks and future perspectives**

340 TFClass organizes mammalian transcription factors in a hierarchical classification  
341 (Superclasses / Classes / Families) based on the 3D-structure of their DNA-binding domain and  
342 the way it interacts with DNA. Historically, the determination of plant TF DBD structures have  
343 lagged behind that of mammals preventing such classification for plants. The relatively recent  
344 resolution of plant DBD-DNA structures along with breakthrough and massive prediction of  
345 protein structures now enable classifying plant TF families based on the 3D-structures of their  
346 DBD. In this opinion paper, we proposed a classification for plant TFs using the TFClass  
347 framework. Most plant TFs were classified: either assigned to existing mammalian classes or  
348 families or by defining new families or new classes under existing classes and superclasses,  
349 respectively. **Until experimental data exist for all types of DBD in complex with DNA, Plant-**  
350 **TFClass can be subject to updates.**

351 **To our knowledge, this is the first comprehensive hierarchical classification of plant TF**  
352 **families. Other plant TF databases have listed TF families and grouped only those with high**  
353 **sequence similarity of their DBD. The classification of biological entities is an important step**  
354 **in understanding how the diversity of life is organized and, the merit of a particular**  
355 **classification scheme depends on the perspective of the user (see “Outstanding questions”).**  
356 **Our hierarchical classification is an ideal basis for evolutionary studies. Based on existing**  
357 **sequence homology for portions of DBD containing the residues contacting the DNA, it is**  
358 **indeed likely that TF families within a given class derive from a common ancestor. The only**  
359 **exceptions are families within the “Other C4 zinc finger-type factors” and within the “bHLH”**  
360 **classes. This work offers the necessary framework to study in details other evolutionary**  
361 **relationships, for example between the different classes of a given superclass. Plant-TFClass**  
362 **will also structure meta-analyses of TFs aiming for instance at evaluating whether TF**  
363 **properties depend on their structural features.** Finally, it is the first classification that brings  
364 mammals and plants TFs together under a same umbrella. As new TFs DBD types are reported  
365 in other kingdoms of life, our work is another step towards a universal classification of  
366 transcription factors.

367

368 **Supplemental information**

369

370 Supplemental information associated with this article can be found at doi:XXXXXXX'

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381 **Author contributions:** FP conceived the study. RBM and RD defined the plant structural  
382 classes. All authors performed the classification. RBM, FP and RD wrote the manuscript.

383 **Competing interests**

384 The authors declare no competing interests.

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592

### 593 **Figure legend**

594

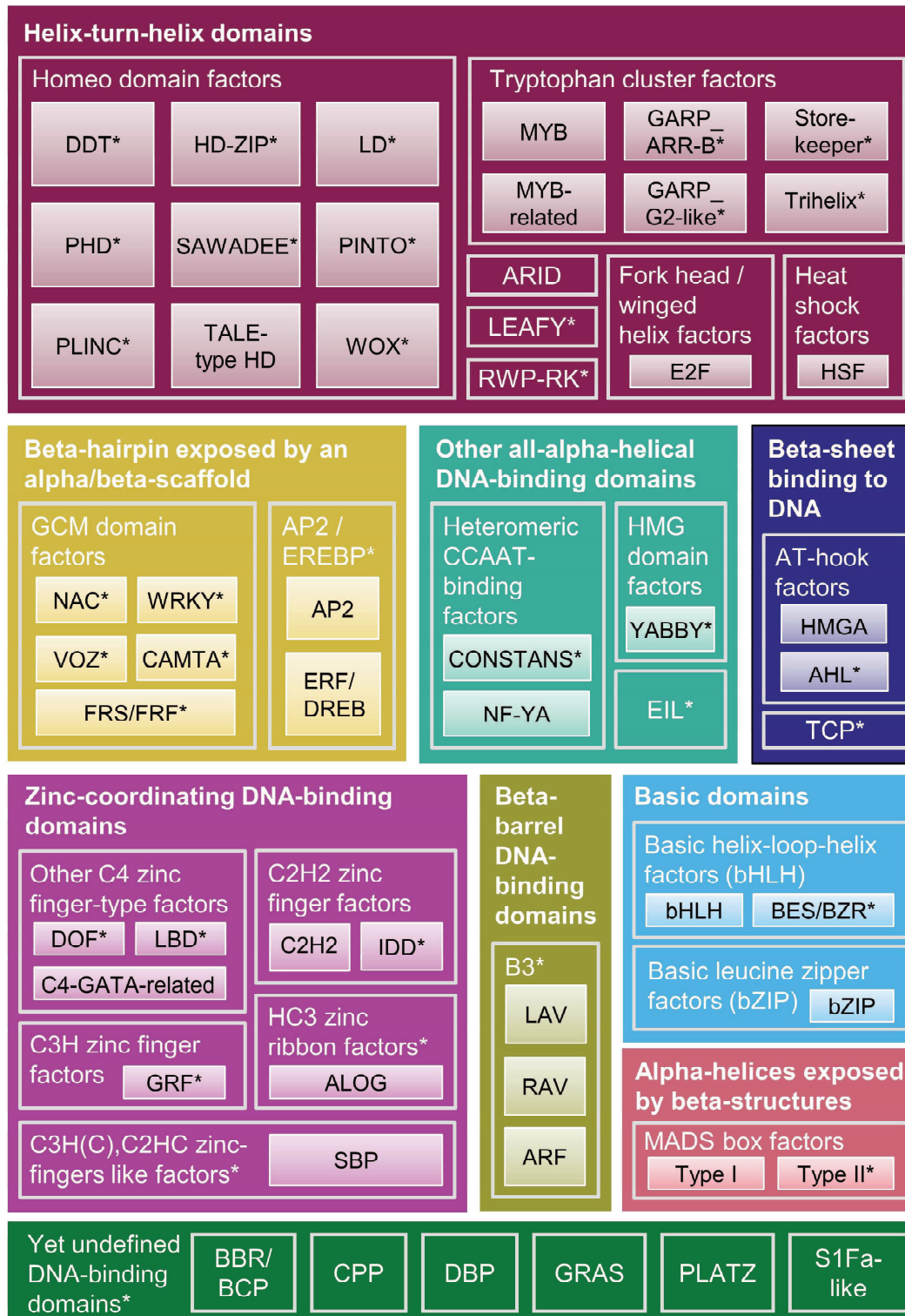
595 **Figure 1:** Color map illustrating the Plant-TFClass structural classification. Colors separate the  
596 **eight** structurally defined superclasses of transcription factors DNA binding domain **with**  
597 **homologs in plants**, plus the “Yet undefined DNA-binding domains”. Gray frame delimits  
598 classes, while the families are framed with a gradient-colored background. Asterisks indicate  
599 TF families and classes absent from TFClass. Asterisks in classes correspond to DBD fold absent  
600 in mammals. TF families within a given class are evolutionary related, at least based on remote  
601 sequence homology for a portion of DBD containing the residues contacting the DNA. The only  
602 exception are families within the “Other C4 zinc finger-type factors” and within the “bHLH”  
603 classes. No evolutionary links are established outside classes.

604

605 **Figure 2:** Illustration of plant TFs DNA binding domains with or without DNA. TFs are classified  
606 in their respective superclasses as in figure 1 (frames follow the superclasses color codes from  
607 Figure 1). Experimentally determined structures are colored in pale cyan (first monomer) and  
608 green cyan (second monomer). The side chains of DNA interacting residues are shown as  
609 sticks. DNA is colored orange when it was crystalized with the TF DBD and grey when it comes  
610 from another experimentally derived TF/DBD structure used in superimposition (example: the  
611 Trihelix superimposed on a MYB crystalized with DNA). When no experimental structure  
612 exists, the superimposed AlphaFold2 computed structure is shown in yellow. In  
613 superimposition the structure that was used as a template is hidden. For the two heteromeric  
614 CCAAT-binding factors, NF-YB and C are colored in deep teal. PyMOL session files are available  
615 at <https://github.com/Bioinfo-LPCV-RDF/Plant-TFClass>. For source of structures see online  
616 supplemental information Method.

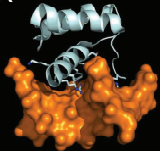


Figure1



### Helix-turn-helix domains

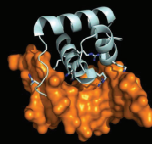
WOX



MYB



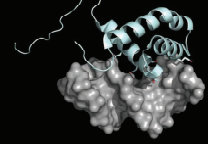
GARP



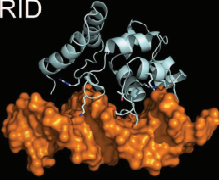
Storekeeper



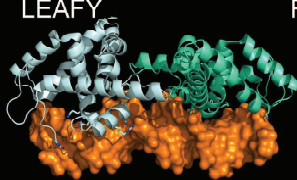
Trihelix



ARID



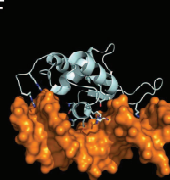
LEAFY



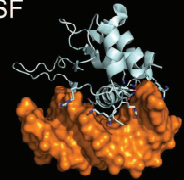
RWP-RK



E2F

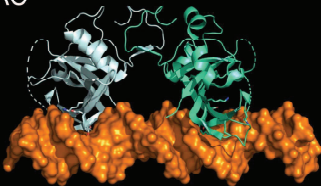


HSF



### Beta-hairpin exposed by an alpha/beta-scaffold

NAC



AP2



VOZ



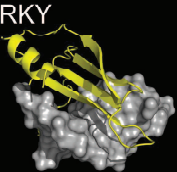
CAMTA



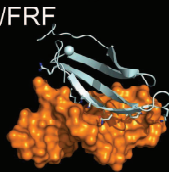
ERF/DREB



WRKY

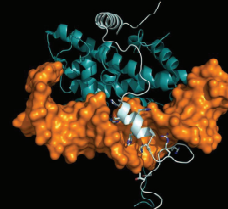


FRS/FRF

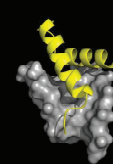


### Other all-alpha-helical DNA-binding domains

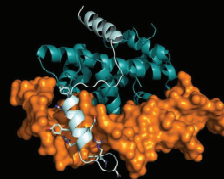
CONSTANS



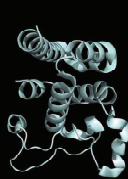
YABBY



NF-YA

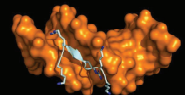


EIL

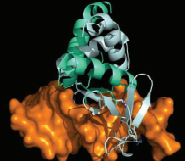


### Beta-sheet binding to DNA

AT-hook factors

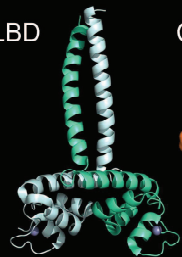


TCP

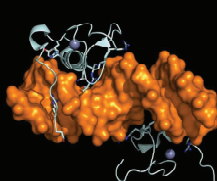


### Zinc-coordinating DNA-binding domains

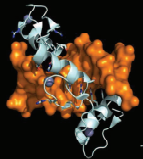
LBD



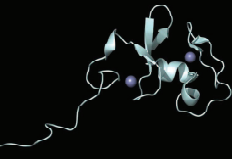
C4-GATA-related



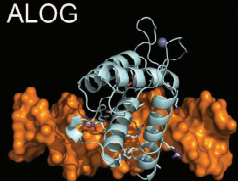
C2H2



SBP



ALOG

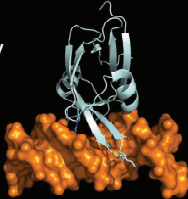


C3H

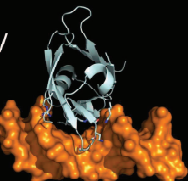


### Beta-barrel DNA-binding domains

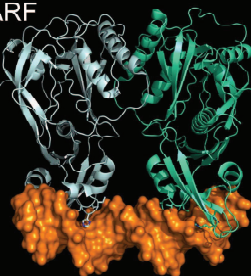
LAV



RAV



ARF

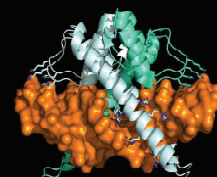


### Basic domains

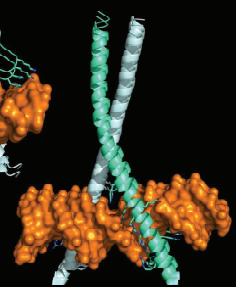
bHLH



BES/BZR



bZIP

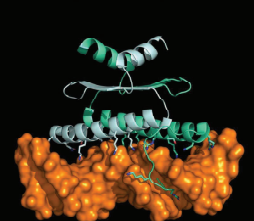


### Alpha helices exposed by beta-structures

MADS (Type I)



MADS (Type II)



*Supplemental information*

## **Plant-TFClass: a structural classification for plant transcription factors**

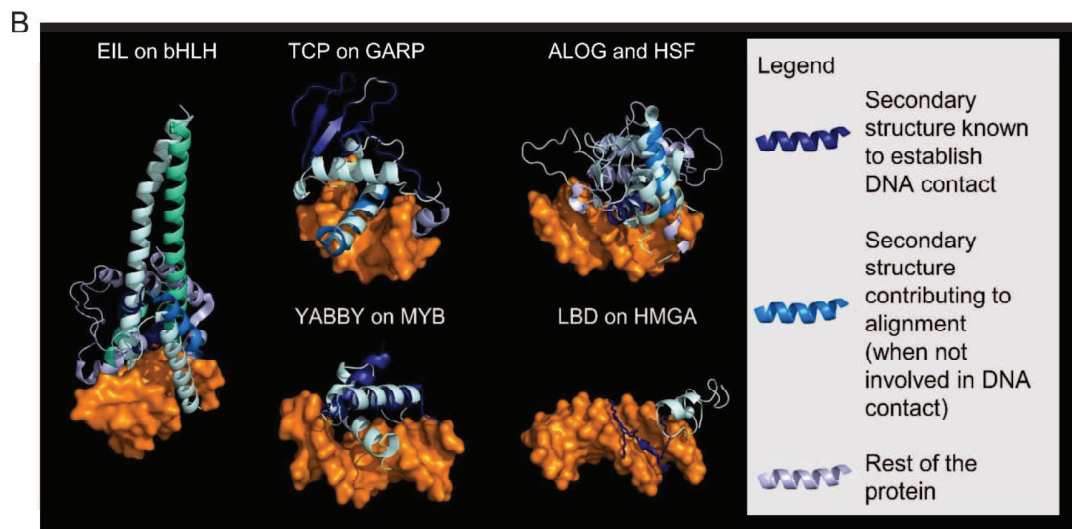
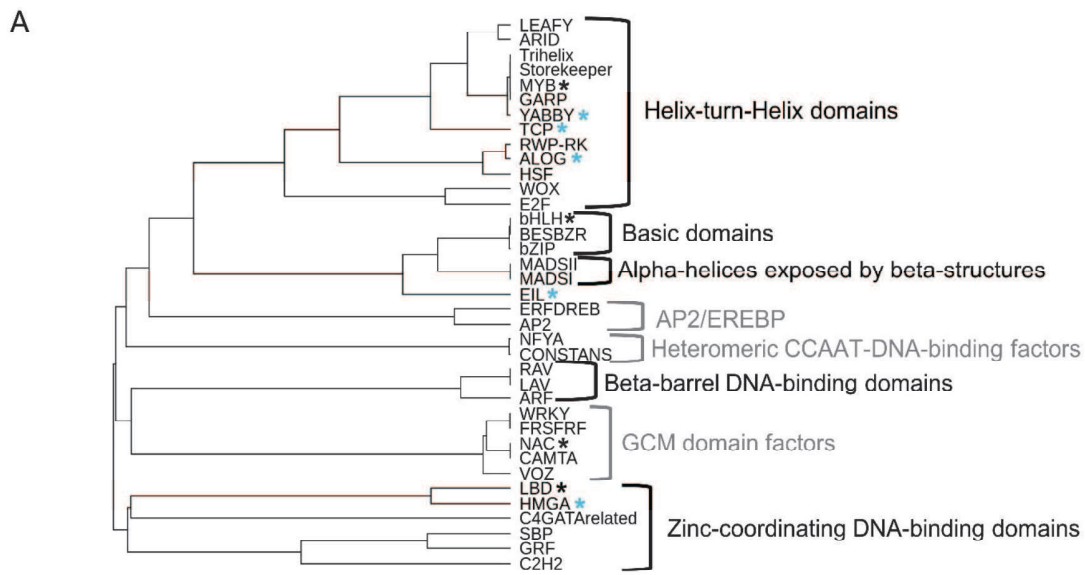
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<sup>1</sup>Laboratoire Physiologie Cellulaire et Végétale, Université Grenoble Alpes, CNRS, CEA, INRAE, IRIG-DBSCI-LPCV, 17 avenue des martyrs, F-38054, Grenoble, France

\* Correspondence: [francois.parcy@cnrs.fr](mailto:francois.parcy@cnrs.fr) (F. Parcy)

### **Method**

Experimentally-determined 3D structures were downloaded from the Protein Data Bank. AlphaFold2 [86] computed structure models were downloaded from UniProt. For each model, position of the DBD was obtained directly from the UniProt “Family and Domains” annotation or identified using CD-search against the CDD database [87]. Structure predictions at the DBD positions were extracted from the PDB files using the Gemmi library (<https://gemmi.readthedocs.io/en/latest/>). Protein 3D structure comparisons and representation were performed using DALI [88], FATCAT [89] and PyMOL. In the main article and for the sake of clarity, we colored names of TF classes and families in blue and orange, respectively.



**Figure S1: Automatic grouping of transcription factor (TF) families based on similarity of their DNA binding domains (DBDs).** A: Dendrogram illustrating the structural distance of DBDs. The dendrogram is generated through hierarchical clustering using P-values from FATCAT structural alignments. Superclasses are labeled in black if they are correctly identified, while split superclass classes are in grey. Blue asterisks indicate placements that are incompatible with our classification in Figure 1. Black asterisks represent PDBs (Protein Data Bank entries) where protein segments not directly involved in DNA binding have been removed. B: Superimposition of DBD structural alignments between families that do not cluster as per our classification (colored as indicated in the legend) and one of the families with the best alignment in A (using the same color code as in Figure 2).



**Table S1. List of plant TFs as organized in Plant-TFClass framework**

Common names	SuperClass	Class	Family	PDB ID (used in fig 2)
ARID/HMG, ARID/Bright	Helix-turn-helix domains	ARID		1KQQ
E2F, E2F-DP, E2F/DP	Helix-turn-helix domains	Fork head / winged helix factors	E2F	2C6Y
HSF	Helix-turn-helix domains	Heat shock factors	HSF	5D8K
HD-DTT	Helix-turn-helix domains	Homeo domain factors	DDT	NA
HD-Zip	Helix-turn-helix domains	Homeo domain factors	HD-ZIP	NA
LD	Helix-turn-helix domains	Homeo domain factors	LD	NA
HD_PHD	Helix-turn-helix domains	Homeo domain factors	PHD	NA
HD_PINTOX	Helix-turn-helix domains	Homeo domain factors	PINTOX	NA
HD_PLINC	Helix-turn-helix domains	Homeo domain factors	PLINC	NA
HD SAWADEE	Helix-turn-helix domains	Homeo domain factors	SAWADEE	NA
HD TALE, HD_BEL, HD_KNOX	Helix-turn-helix domains	Homeo domain factors	TALE-type HD	NA
HD_WOX	Helix-turn-helix domains	Homeo domain factors	WOX	6RY1
LEAFY, LFY	Helix-turn-helix domains	LEAFY		2VY1
RWP-RK, NIN like, AtRKD, RWPRK	Helix-turn-helix domains	RWP-RK		AF2 model
GARP, ARR-related, ARR-B, G2like, G2-like	Helix-turn-helix domains	Tryptophan cluster factors	GARP_ARR-B	Not shown
GARP, ARR-related, ARR-B, G2like, G2-like	Helix-turn-helix domains	Tryptophan cluster factors	GARP_G2-like	5LXU
MYB	Helix-turn-helix domains	Tryptophan cluster factors	MYB	6KKS
MYB, MYB-related	Helix-turn-helix domains	Tryptophan cluster factors	MYB-related	NA
GeBP, Storekeeper	Helix-turn-helix domains	Tryptophan cluster factors	Storekeeper	AF2 model
Trihelix	Helix-turn-helix domains	Tryptophan cluster factors	Trihelix	2JMW
AP2	Beta-hairpin exposed by an alpha/beta-scaffold	AP2/EREBP	AP2	7ET4
AP2, EREBP, ERF	Beta-hairpin exposed by an alpha/beta-scaffold	AP2/EREBP	ERF/DREB	5WX9
CAMTA, CAMTA-like	Beta-hairpin exposed by an alpha/beta-scaffold	GCM domain factors	CAMTA	AF2 model
FAR1, FHY3	Beta-hairpin exposed by an alpha/beta-scaffold	GCM domain factors	FRS/FRF	AF2 model
NAC, NAC/NAM	Beta-hairpin exposed by an alpha/beta-scaffold	GCM domain factors	NAC	3SWP
VOZ	Beta-hairpin exposed by an alpha/beta-scaffold	GCM domain factors	VOZ	AF2 model
WRKY	Beta-hairpin exposed by an alpha/beta-scaffold	GCM domain factors	WRKY	2LEX
EIL	Other all-alpha-helical DNA-binding domains	EIL		4ZDS
CONSTANS,CO	Other all-alpha-helical DNA-binding domains	Heteromeric CCAAT-binding factors	CONSTANS	7CVQ
CCAAT, HAP3, NF-YB	Other all-alpha-helical DNA-binding domains	Heteromeric CCAAT-binding factors	NF-YA	6R2V
YABBY, C2C2-YABBY, C2C2_YABBY	Other all-alpha-helical DNA-binding domains	High-mobility group (HMG) domain factors	YABBY	AF2 model
A.T hook, AT hook	Beta-sheet binding to DNA	A.T hook factors	AHL	NA

<b>HMGA</b>	Beta-sheet binding to DNA	A.T hook factors	HMGA	3UXW
<b>TCP, bHLH_TCP</b>	Beta-sheet binding to DNA	TCP	TCP	7VP2
<b>C2H2, C2H2 ZF</b>	Zinc-coordinating DNA-binding domains	C2H2 zinc finger factors	C2H2	1A1F
<b>IDD</b>	Zinc-coordinating DNA-binding domains	C2H2 zinc finger factors	IDD	NA
<b>GRF</b>	Zinc-coordinating DNA-binding domains	C3H zinc finger factors	GRF	AF2 model
<b>SBP, SBP-type zinc finger</b>	Zinc-coordinating DNA-binding domains	C3H(C),C2HC zinc fingers-like factors	SBP	1UL5
<b>ALOG</b>	Zinc-coordinating DNA-binding domains	HC3 zinc ribbon factors	ALOG	NA
<b>GATA, Tify</b>	Zinc-coordinating DNA-binding domains	Other C4 zinc finger-type factors	C4-GATA-related	4HC7
<b>DOF, C2C2-Dof, C2C2_Dof</b>	Zinc-coordinating DNA-binding domains	Other C4 zinc finger-type factors	DOF	NA
<b>AS2/LOB, LOBAS2</b>	Zinc-coordinating DNA-binding domains	Other C4 zinc finger-type factors	LBD	5LY0
<b>ARF</b>	Beta-barrel DNA-binding domains	B3	ARF	6YCQ
<b>ABI3VP1, ABI3/VP1, B3</b>	Beta-barrel DNA-binding domains	B3	LAV	6J9C
<b>RAV</b>	Beta-barrel DNA-binding domains	B3	RAV	7RT6
<b>BES/BZR, BES1</b>	Basic domains	Basic helix-loop-helix factors (bHLH)	BES/BZR	5ZD4
<b>bHLH</b>	Basic domains	Basic helix-loop-helix factors (bHLH)	bHLH	5GNJ
<b>bZIP</b>	Basic domains	Basic leucine zipper factors (bZIP)	bZIP	1NWQ
<b>MADS type I, MADS-Box</b>	Alpha-helices exposed by beta-structures	MADS box factors	Type I	1SRS
<b>MADS type II, MADS-Box</b>	Alpha-helices exposed by beta-structures	MADS box factors	Type II	1EGW
<b>BBR/BPC, BBR-BPC</b>	Yet undefined DNA-binding domains	BBR/BPC		NA
<b>CPP Cysteine-rich polycomb-like protein</b>	Yet undefined DNA-binding domains	CPP		NA
<b>DBP</b>	Yet undefined DNA-binding domains	DBP		NA
<b>GRAS</b>	Yet undefined DNA-binding domains	GRAS		NA
<b>PLATZ</b>	Yet undefined DNA-binding domains	PLATZ		NA
<b>S1Fa1, S1Fa-like</b>	Yet undefined DNA-binding domains	S1Fa-like		NA