Plant-TFClass: a structural classification for plant transcription factors

Romain Blanc-Mathieu, Renaud Dumas, Laura Turchi, Jérémy Lucas, François Parcy

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.

Plant-TFClass: a structural classification for plant transcription factors

Romain Blanc-Mathieu[1], Renaud Dumas[1], Laura Turchi[1], Jérémy Lucas[1], François Parcy[1,*]

[1]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 (F. Parcy)

ORCID / twitter handles:

Romain BLANC-MATHIEU: 0000-0002-9485-6330 / @romain_bm

Renaud DUMAS: 0000-0003-1404-3274 / NA

Laura TURCHI: 0000-0002-2791-5505 / @turchi_l

Jérémy LUCAS: 0000-0002-2252-4732 / NA

François PARCY: 0000-0003-2191-500X / @Francois_Parcy

Keywords

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

Abstract

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

Towards a universal classification for transcription factors

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

sequences (called TF binding sites or TFBS) and regulate the expression of associated genes. Plant (Streptophyta: grouping the land plants – Embryophyta - and their closest algal relatives) genomes contain a high diversity of TF-encoding genes including a collection of plant-specific TFs [1–3] that evolved during their history. The initial analysis of arabidopsis (*Arabidopsis thaliana)* genome identified 1500 TFs corresponding to 28 gene families of which 45% (16 families) were not found in mammals [4]. Since then, comparative genomic studies identified additional TFs and putative TF families in plant genomes [2] and various plant TFs classifications have been proposed, based on the nature of the DBD and other conserved protein domains [2,5–7]. While some of these classifications mention existing homology between different families, none of them proposes a hierarchical, higher-rank, organization. With the increasing number of 3D DBD structures being solved or reliably predicted, it now becomes possible to propose a hierarchical classification for plant TFs based on the 3D structure and the DNA binding mode of their DBD. Traditionally, TFs have been classified into superclasses based on broad structural similarities of their DBDs [8]. These superclasses should not be regarded as equivalent to phylogenetic classification since it remains uncertain whether all the diverse DBDs have independently emerged or if some have a shared ancestry. However, they are useful for grouping TFs based on distinct structural features. In mammals, the reference structural classification is TFClass [9,10]. This hierarchical classification starts with nine structurally defined superclasses, further decomposed into classes, families and subfamilies. It provides a framework for meta-analyses of TF properties and evolution [11], it facilitates comparisons between organisms and it is the reference classification used by JASPAR, the open access database for TFBS models [12].

Here we built upon the TFClass framework to classify all plant TF types described to date. The new framework is referred to as “Plant-TFClass” (Figure 1, online supplemental information Table S1), illustrated with the DBD folds with or without DNA for each TF family (Figure 2). For this, we reviewed experimental and predicted data on DBD structures. For each TF type, we proposed a classification when sufficient evidence exist and we left the remaining ones in a tenth ‘Yet undefined DNA-binding domains’ superclass as in TFClass. In addition to this manual construction, we also used an automatic approach based on the pairwise structural alignment of TF DBDs: this returned a very similar grouping, but with some inconsistencies that highlight the merits of the manual approach (online supplemental information Figure S1). Compared to TFClass, Plant-TFClass has additional TFs of two natures: 1) TFs with a DBD type absent from

mammals. This is the case for eight new classes (LEAFY, RWP-RK, AP2/EREBP, EIL, TCP, ALOG, SBP and B3) and we explain on which basis these new classes were organized under a given superclass. Such TFs may have evolved (i) de novo, (ii) from existing DBD by rapid sequence evolution or (iii) from transposon proteins [13,14]. 2) Another type of plant-specific TFs corresponds to those possessing a DBD fold that exists in mammals but has diverged extensively, in particular with the acquisition of auxiliary domains. This usually defines new families within existing classes (e.g. HD-ZIP, MADS Type II, AHL).

Among the 56 recognized plants TF types, 50 were classified in one of the nine superclasses from TFClass (Helix-turn-helix domains, Beta-hairpin exposed by an alpha/beta-scaffold, Other all-alpha-helical DNA-binding structures, Beta-sheet binding to DNA, Zinc-coordinating DNA-binding domains, Beta-barrel DNA-binding domains, Basic domains, Alpha-helices exposed by beta-structures, Immunoglobulin-fold) and the remaining six were added to the "Yet undefined DBD".

Helix-turn-helix domains

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

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

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

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

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

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

Beta-hairpin exposed by an alpha/beta-scaffold

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

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

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

Other all-alpha-helical DNA-binding domains

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

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

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

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

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

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

Beta-sheet binding to DNA

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

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

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

Zinc-coordinating DNA binding domains

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

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

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

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

recombinase of a retrotransposon in which a zinc ribbon was inserted [63] in the core tetrahelical structure. The structure of the DBD in complex with DNA revealed a bundle of alpha helices with a HC3-motif zinc-ribbon contributing to positioning the alpha-helices and the loop that directly contact DNA. As the zinc ribbon has a HC_3 motif, we created a new class (HC3 zinc ribbon factors) in the zinc-coordinating DNA-binding proteins superclass for the ALOG family.

Plants also encode zinc finger proteins containing a three cysteine and one histidine residues (C3H) motif, that defines the C3H zinc finger factors class in TFClass. Plant C3H are unrelated to those found in mammals and the majority of plant C3H motifs bind RNA. The only plant C3H TFs shown to bind DNA are the Growth-Regulating Factors (GRF family) : their putative DNA-binding domain WRC (tryptophan, arginine and cysteine) contains a conserved C3H ($CX_9CX_{10}CX_2H$) motif [64]. The WRC domain also plays a role in JMJ28, a negative regulator of immunity in arabidopsis, by providing target specificity for DNA binding of the RBL/ATX1/2-COMPASS complex [65]. Until more evidence is provided for the other groups of plant C3H proteins, only GRFs are considered as a TF, classified within the C3H zinc finger factors class of TFClass.

Beta-barrel DNA-binding domains

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

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

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

Basic domains

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

Alpha-helices exposed by beta-structures

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

Immunoglobulin-fold

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

Yet undefined DNA-binding domains

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

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

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

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

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

Concluding remarks and future perspectives

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

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

Supplemental information

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

Acknowledgments

We thank Edgar Wingender, Chloe Zubieta, Xuelei Lai and Gabrielle Tichtinsky for their advices on this work. We are grateful to the Rensing Lab for integrating plant-TFClass within the TAPscan database. We would like to thanks two anonymous referees for their constructive comments on the initial version of this manuscript. This work was supported by the GRAL Labex financed within the University Grenoble Alpes graduate school (Ecoles Universitaires de Recherche) CBH-EUR-GS (ANR-17-EURE-0003), by the French National Research Agency in the framework of the "Investissements d'avenir" program (ANR-15-IDEX-02) to RBM, by the ANR-18-CE12-0014 ChromAuxi project to RD and by the ANR-17-CE20-0014-01 Ubiflor and ANR-21-CE20-0024 Beflore projects to FP, and a PhD Fellowship from CNRS Prime80 to LT.

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

Competing interests

The authors declare no competing interests.

References

1. Shiu, S.-H. *et al.* (2005) Transcription Factor Families Have Much Higher Expansion Rates in Plants than in Animals. *Plant Physiol* 139, 18–26
2. Wilhelmsson, P.K.I. *et al.* (2017) Comprehensive Genome-Wide Classification Reveals That Many Plant-Specific Transcription Factors Evolved in Streptophyte Algae. *Genome Biology and Evolution* 9, 3384–3397
3. de Mendoza, A. *et al.* (2013) Transcription factor evolution in eukaryotes and the assembly of the regulatory toolkit in multicellular lineages. *Proceedings of the National Academy of Sciences* 110, E4858–E4866
4. Riechmann, J.L. *et al.* (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290, 2105–2110
5. Mukherjee, K. *et al.* (2009) A Comprehensive Classification and Evolutionary Analysis of Plant Homeobox Genes. *Mol Biol Evol* 26, 2775–2794
6. Jin, J. *et al.* (2017) PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Research* 45, D1040–D1045
7. Yilmaz, A. *et al.* (2011) AGRIS: the Arabidopsis Gene Regulatory Information Server, an update. *Nucleic Acids Research* 39, D1118–D1122

8. Luscombe, N.M. *et al.* (2000) An overview of the structures of protein-DNA complexes. *Genome Biology* 1, reviews001.1
9. Wingender, E. *et al.* (2018) TFClass: expanding the classification of human transcription factors to their mammalian orthologs. *Nucleic Acids Res* 46, D343–D347
10. Wingender, E. (2013) Criteria for an updated classification of human transcription factor DNA-binding domains. *J Bioinform Comput Biol* 11, 1340007
11. Ambrosini, G. *et al.* (2020) Insights gained from a comprehensive all-against-all transcription factor binding motif benchmarking study. *Genome Biology* 21, 114
12. Castro-Mondragon, J.A. *et al.* (2022) JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Research* 50, D165–D173
13. de Mendoza, A. and Sebé-Pedrós, A. (2019) Origin and evolution of eukaryotic transcription factors. *Current Opinion in Genetics & Development* 58–59, 25–32
14. Yamasaki, K. *et al.* (2013) DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. *Trends in Plant Science* 18, 267–276
15. Roy, A. *et al.* (2016) Deciphering the role of the AT-rich interaction domain and the HMG-box domain of ARID-HMG proteins of Arabidopsis thaliana. *Plant Mol Biol* 92, 371–388
16. Bürglin, T.R. and Affolter, M. (2016) Homeodomain proteins: an update. *Chromosoma* 125, 497–521
17. Sloan, J. *et al.* (2020) Structural basis for the complex DNA binding behavior of the plant stem cell regulator WUSCHEL. *Nat Commun* 11, 2223
18. Du, H. *et al.* (2015) The Evolutionary History of R2R3-MYB Proteins Across 50 Eukaryotes: New Insights Into Subfamily Classification and Expansion. *Sci Rep* 5, 11037
19. Wang, B. *et al.* (2020) Structural insights into target DNA recognition by R2R3-MYB transcription factors. *Nucleic Acids Research* 48, 460–471
20. Jiang, M. *et al.* (2019) Structural basis for the Target DNA recognition and binding by the MYB domain of phosphate starvation response 1. *FEBS J* 286, 2809–2821
21. Silva, C.S. *et al.* (2016) The Myb domain of LUX ARRHYTHMO in complex with DNA: expression, purification and crystallization. *Acta Crystallogr F Struct Biol Commun* 72, 356–361
22. Hosoda, K. *et al.* (2002) Molecular Structure of the GARP Family of Plant Myb-Related DNA Binding Motifs of the Arabidopsis Response Regulators. *Plant Cell* 14, 2015–2029
23. Nagano, Y. (2000) Several features of the GT-factor trihelix domain resemble those of the Myb DNA-binding domain. *Plant Physiol* 124, 491–494
24. Nagata, T. *et al.* (2010) Solution structures of the trihelix DNA-binding domains of the wild-type and a phosphomimetic mutant of Arabidopsis GT-1: Mechanism for an increase in DNA-binding affinity through phosphorylation. *Proteins: Structure, Function, and Bioinformatics* 78, 3033–3047
25. Hamès, C. *et al.* (2008) Structural basis for LEAFY floral switch function and similarity with helix-turn-helix proteins. *The EMBO Journal* 27, 2628–2637
26. Sayou, C. *et al.* (2016) A SAM oligomerization domain shapes the genomic binding landscape of the LEAFY transcription factor. *Nature Communications* 7, 11222
27. Schauser, L. *et al.* (1999) A plant regulator controlling development of symbiotic root nodules. *Nature* 402, 191–195
28. Nishida, H. *et al.* (2021) Different DNA-binding specificities of NLP and NIN transcription factors underlie nitrate-induced control of root nodulation. *Plant Cell* 33, 2340–2359

29. Welner, D.H. *et al.* (2012) DNA binding by the plant-specific NAC transcription factors in crystal and solution: a firm link to WRKY and GCM transcription factors. *Biochem J* 444, 395–404
30. Babu, M.M. *et al.* (2006) The natural history of the WRKY–GCM1 zinc fingers and the relationship between transcription factors and transposons. *Nucleic Acids Research* 34, 6505–6520
31. Allen, M.D. *et al.* (1998) A novel mode of DNA recognition by a beta-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA. *EMBO J* 17, 5484–5496
32. Hu, H. *et al.* (2021) TEM1 combinatorially binds to FLOWERING LOCUS T and recruits a Polycomb factor to repress the floral transition in Arabidopsis. *Proc Natl Acad Sci U S A* 118, e2103895118
33. Chen, C.-Y. *et al.* (2020) Structural insights into Arabidopsis ethylene response factor 96 with an extended N-terminal binding to GCC box. *Plant Mol Biol* 104, 483–498
34. Chaves-Sanjuan, A. *et al.* (2021) Structural determinants for NF-Y subunit organization and NF-Y/DNA association in plants. *The Plant Journal* 105, 49–61
35. Lv, X. *et al.* (2021) Structural insights into the multivalent binding of the Arabidopsis FLOWERING LOCUS T promoter by the CO–NF–Y master transcription factor complex. *The Plant Cell* 33, 1182–1195
36. Antosch, M. *et al.* (2012) Plant Proteins Containing High Mobility Group Box DNA-Binding Domains Modulate Different Nuclear Processes1[W]. *Plant Physiol* 159, 875–883
37. Bowman, J.L. and Smyth, D.R. (1999) CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126, 2387–2396
38. Sawa, S. *et al.* (1999) FILAMENTOUS FLOWER, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and HMG-related domains. *Genes Dev* 13, 1079–1088
39. Gross, T. *et al.* (2018) CRABS CLAW Acts as a Bifunctional Transcription Factor in Flower Development. *Frontiers in Plant Science* 9
40. Yamasaki, K. *et al.* (2005) Solution Structure of the Major DNA-binding Domain of Arabidopsis thaliana Ethylene-insensitive3-like3. *Journal of Molecular Biology* 348, 253–264
41. Aravind, L. and Landsman, D. (1998) AT-hook motifs identified in a wide variety of DNA-binding proteins. *Nucleic Acids Res* 26, 4413–4421
42. Fonfría-Subirós, E. *et al.* (2012) Crystal structure of a complex of DNA with one AT-hook of HMGA1. *PLoS One* 7, e37120
43. Huth, J.R. *et al.* (1997) The solution structure of an HMG-I(Y)–DNA complex defines a new architectural minor groove binding motif. *Nat Struct Mol Biol* 4, 657–665
44. Grasser, K.D. (2003) Chromatin-associated HMGA and HMGB proteins: versatile co-regulators of DNA-dependent processes. *Plant Mol Biol* 53, 281–295
45. Yun, J. *et al.* (2012) The AT-hook Motif-containing Protein AHL22 Regulates Flowering Initiation by Modifying FLOWERING LOCUS T Chromatin in Arabidopsis. *J Biol Chem* 287, 15307–15316
46. Meijer, A.H. *et al.* (1996) Novel members of a family of AT hook-containing DNA-binding proteins from rice are identified through their in vitro interaction with consensus target sites of plant and animal homeodomain proteins. *Plant Mol Biol* 31, 607–618

47. Kosugi, S. and Ohashi, Y. (1997) PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. *Plant Cell* 9, 1607–1619
48. Sun, L. *et al.* (2020) The crystal structure of the TCP domain of PCF6 in Oryza sativa L. reveals an RHH-like fold. *FEBS Letters* 594, 1296–1306
49. Zhang, Y. *et al.* (2023) DNA–TCP complex structures reveal a unique recognition mechanism for TCP transcription factor families. *Nucleic Acids Research* 51, 434–448
50. Hirano, Y. *et al.* (2017) Structure of the SHR-SCR heterodimer bound to the BIRD/IDD transcriptional factor JKD. *Nat Plants* 3, 17010
51. Yamasaki, K. *et al.* (2004) A novel zinc-binding motif revealed by solution structures of DNA-binding domains of Arabidopsis SBP-family transcription factors. *J Mol Biol* 337, 49–63
52. Yanagisawa, S. (2004) Dof Domain Proteins: Plant-Specific Transcription Factors Associated with Diverse Phenomena Unique to Plants. *Plant and Cell Physiology* 45, 386–391
53. Umemura, Y. *et al.* (2004) The Dof domain, a zinc finger DNA-binding domain conserved only in higher plants, truly functions as a Cys2/Cys2 Zn finger domain. *The Plant Journal* 37, 741–749
54. O'Malley, R.C. *et al.* (2016) Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape. *Cell* 165, 1280–1292
55. Hamzeh-Mivehroud, M. *et al.* (2015) Identifying key interactions stabilizing DOF zinc finger–DNA complexes using in silico approaches. *Journal of Theoretical Biology* 382, 150–159
56. Pandey, B. *et al.* (2018) Dynamics of Dof domain-DNA interaction in wheat: Insights from atomistic simulations and free energy landscape. *Journal of Cellular Biochemistry* 119, 8818–8829
57. Zhang, B. *et al.* (1995) Interactions between distinct types of DNA binding proteins enhance binding to ocs element promoter sequences. *Plant Cell* 7, 2241–2252
58. Ruta, V. *et al.* (2020) The DOF Transcription Factors in Seed and Seedling Development. *Plants (Basel)* 9, 218
59. Gao, H. *et al.* (2021) PIF4 enhances DNA binding of CDF2 to co-regulate target gene expression and promote Arabidopsis hypocotyl cell elongation. *Nat. Plants* 8, 1082–1093
60. Chen, W.-F. *et al.* (2019) Structural analysis reveals a "molecular calipers" mechanism for a LATERAL ORGAN BOUNDARIES DOMAIN transcription factor protein from wheat. *J Biol Chem* 294, 142–156
61. Naramoto, S. *et al.* (2020) The origin and evolution of the ALOG proteins, members of a plant-specific transcription factor family, in land plants. *J Plant Res* 133, 323–329
62. Rieu, P. *et al.* (2023) The ALOG domain defines a new family of plant-specific Transcription Factors acting during Arabidopsis flower developmentbioRxiv, 2023.06.21.545689
63. Iyer, L.M. and Aravind, L. (2012) ALOG domains: provenance of plant homeotic and developmental regulators from the DNA-binding domain of a novel class of DIRS1-type retroposons. *Biology Direct* 7, 39
64. Omidbakhshfard, M.A. *et al.* (2015) Growth-Regulating Factors (GRFs): A Small Transcription Factor Family with Important Functions in Plant Biology. *Molecular Plant* 8, 998–1010
65. Xie, S.-S. *et al.* (2023) JMJ28 guides sequence-specific targeting of ATX1/2-containing COMPASS-like complex in Arabidopsis. *Cell Reports* 42, 112163

66. Ulmasov, T. *et al.* (1997) ARF1, a transcription factor that binds to auxin response elements. *Science* 276, 1865–1868
67. Kagaya, Y. *et al.* (1999) RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Research* 27, 470–478
68. Mönke, G. *et al.* (2004) Seed-specific transcription factors ABI3 and FUS3: molecular interaction with DNA. *Planta* 219, 158–166
69. Swaminathan, K. *et al.* (2008) The plant B3 superfamily. *Trends in Plant Science* 13, 647–655
70. Romanel, E.A.C. *et al.* (2009) Evolution of the B3 DNA Binding Superfamily: New Insights into REM Family Gene Diversification. *PLOS ONE* 4, e5791
71. Korasick, D.A. *et al.* (2014) Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. *Proceedings of the National Academy of Sciences* 111, 5427–5432
72. Nanao, M.H. *et al.* (2014) Structural basis for oligomerization of auxin transcriptional regulators. *Nat Commun* 5, 3617
73. Han, M. *et al.* (2014) Structural basis for the auxin-induced transcriptional regulation by Aux/IAA17. *Proc Natl Acad Sci U S A* 111, 18613–18618
74. Boer, D.R. *et al.* (2014) Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. *Cell* 156, 577–589
75. Stigliani, A. *et al.* (2019) Capturing Auxin Response Factors Syntax Using DNA Binding Models. *Molecular Plant* 12, 822–832
76. Cancé, C. *et al.* (2022) Auxin response factors are keys to the many auxin doors. *New Phytologist* 235, 402–419
77. Levy, Y.Y. *et al.* (2002) Multiple Roles of Arabidopsis VRN1 in Vernalization and Flowering Time Control. *Science* 297, 243–246
78. Yu, Y. *et al.* (2020) Arabidopsis REM16 acts as a B3 domain transcription factor to promote flowering time via directly binding to the promoters of SOC1 and FT. *The Plant Journal* 103, 1386–1398
79. Carles, C.C. and Fletcher, J.C. (2009) The SAND domain protein ULTRAPETALA1 acts as a trithorax group factor to regulate cell fate in plants. *Genes Dev.* 23, 2723–2728
80. Duncan, L. *et al.* (2006) Orthologs and paralogs of regA, a master cell-type regulatory gene in Volvox carteri. *Curr Genet* 50, 61–72
81. Lai, X. *et al.* (2021) The intervening domain is required for DNA-binding and functional identity of plant MADS transcription factors. *Nature Communications* 12
82. Lisso, J. *et al.* (2012) NFXL2 modifies cuticle properties in Arabidopsis. *Plant Signal Behav* 7, 551–555
83. Cvitanich, C. *et al.* (2000) CPP1, a DNA-binding protein involved in the expression of a soybean leghemoglobin c3 gene. *Proc Natl Acad Sci U S A* 97, 8163–8168
84. Carrasco, J.L. *et al.* (2005) A novel DNA-binding motif, hallmark of a new family of plant transcription factors. *Plant Physiol* 137, 602–606
85. Santi, L. *et al.* (2003) The GA octodinucleotide repeat binding factor BBR participates in the transcriptional regulation of the homeobox gene Bkn3. *Plant J* 34, 813–826
86. Li, S. *et al.* (2016) Crystal Structure of the GRAS Domain of SCARECROW-LIKE7 in Oryza sativa. *The Plant Cell* 28, 1025–1034
87. Nagano, Y. *et al.* (2001) A novel class of plant-specific zinc-dependent DNA-binding protein that binds to A/T-rich DNA sequences. *Nucleic Acids Res* 29, 4097–4105

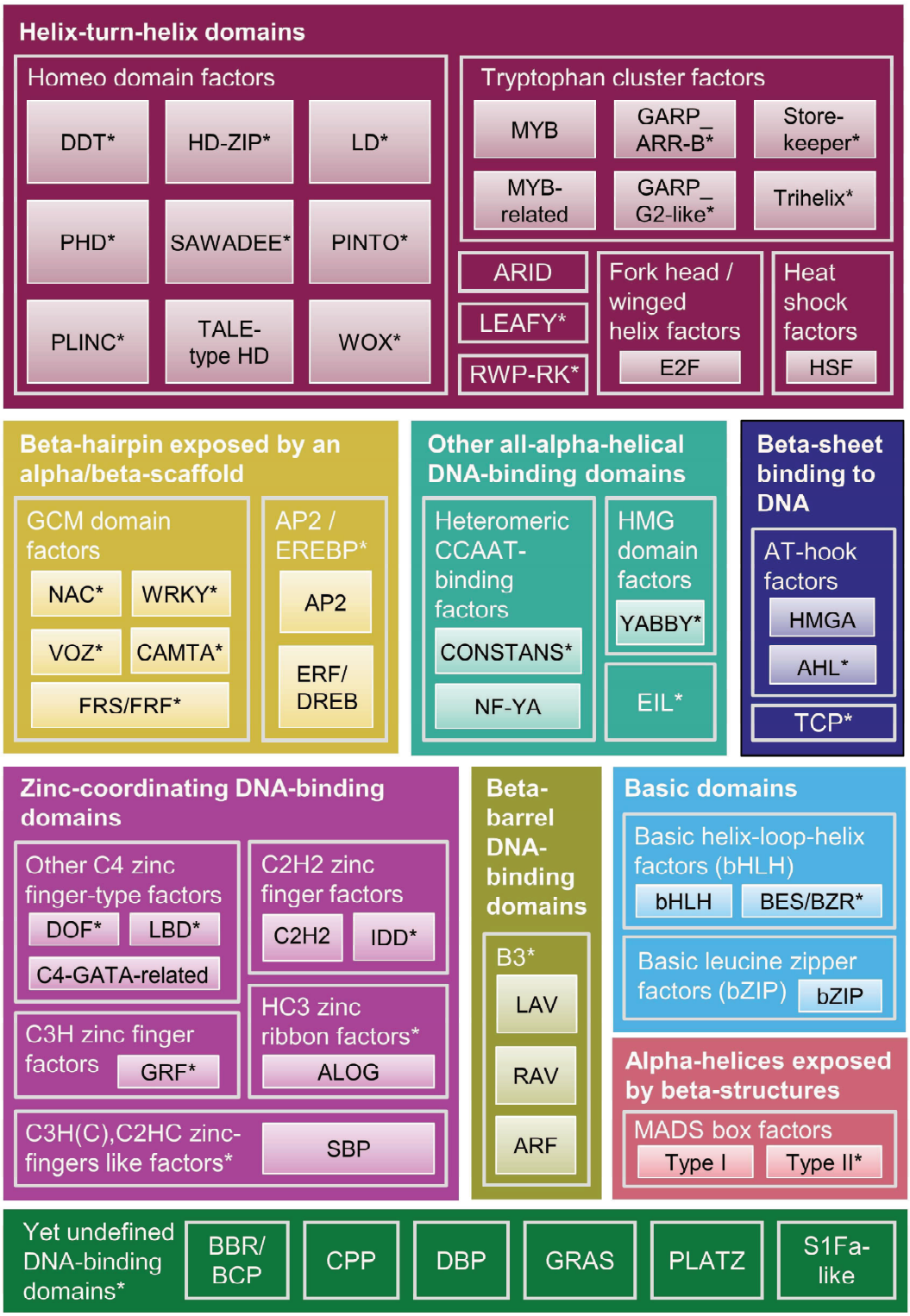
88. Theune, M.L. *et al.* (2019) Phylogenetic Analyses and GAGA-Motif Binding Studies of BBR/BPC Proteins Lend to Clues in GAGA-Motif Recognition and a Regulatory Role in Brassinosteroid Signaling. *Frontiers in Plant Science* 10

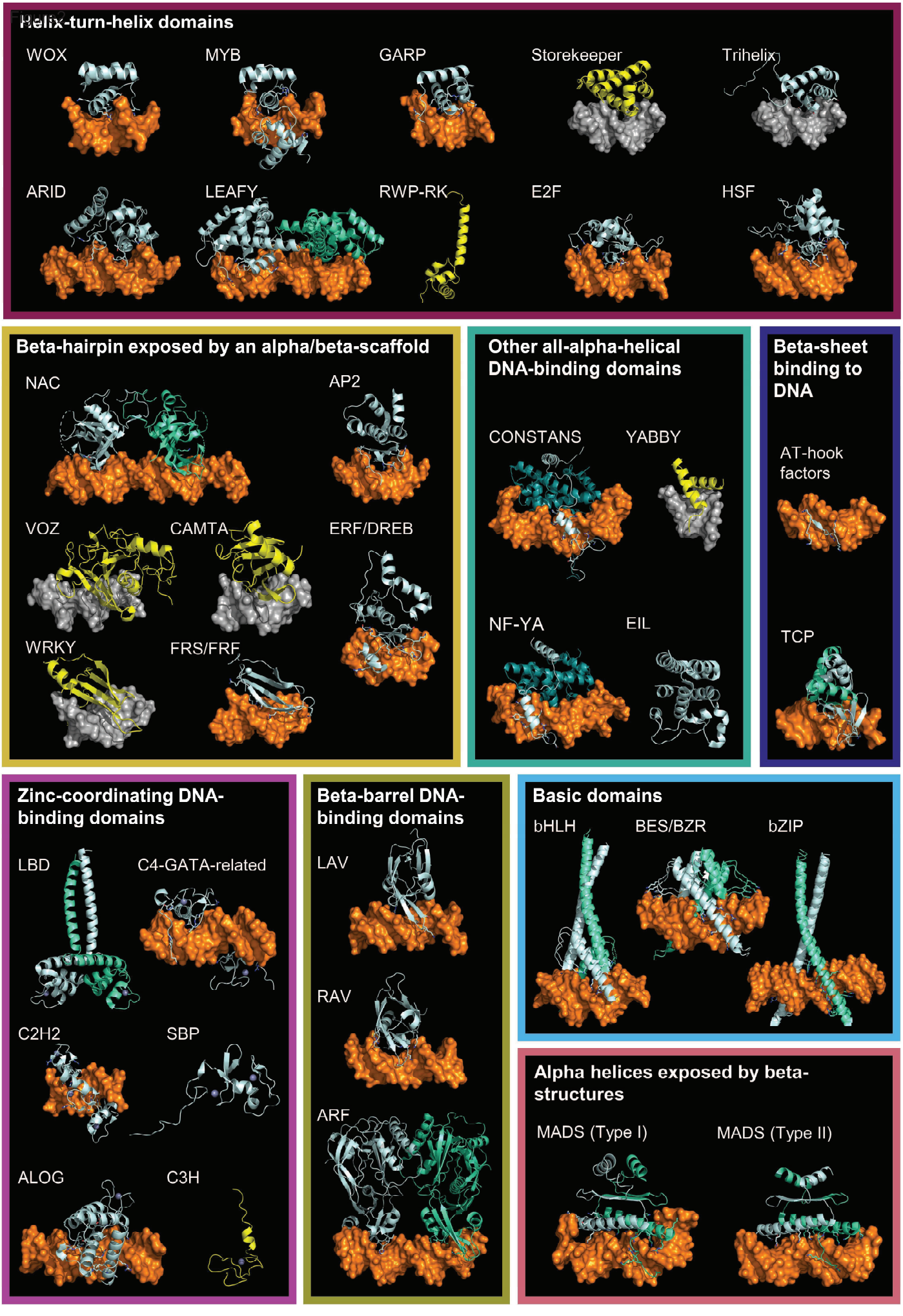
Figure legend

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

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

Figure1


Helix-turn-helix domains
Homeo domain factors
DDT*
HD-ZIP*
LD*
PHD*
SAWADEE*
PINTO*
PLINC*
TALE-type HD
WOX*
Tryptophan cluster factors
MYB
GARP_ ARR-B*
Store-keeper*
MYB-related
GARP_ G2-like*
Trihelix*
ARID
LEAFY*
RWP-RK*
Fork head / winged helix factors
E2F
Heat shock factors
HSF
Beta-hairpin exposed by an alpha/beta-scaffold
GCM domain factors
NAC*
WRKY*
VOZ*
CAMTA*
FRS/FRF*
AP2 / EREBP*
AP2
ERF/ DREB
Other all-alpha-helical DNA-binding domains
Heteromeric CCAAT-binding factors
CONSTANS*
NF-YA
HMG domain factors
YABBY*
EIL*
Beta-sheet binding to DNA
AT-hook factors
HMGA
AHL*
TCP*
Zinc-coordinating DNA-binding domains
Other C4 zinc finger-type factors
DOF*
LBD*
C4-GATA-related
C2H2 zinc finger factors
C2H2
IDD*
C3H zinc finger factors
GRF*
HC3 zinc ribbon factors*
ALOG
C3H(C),C2HC zinc-fingers like factors*
SBP
Beta-barrel DNA-binding domains
B3*
LAV
RAV
ARF
Basic domains
Basic helix-loop-helix factors (bHLH)
bHLH
BES/BZR*
Basic leucine zipper factors (bZIP)
bZIP
Alpha-helices exposed by beta-structures
MADS box factors
Type I
Type II*
Yet undefined DNA-binding domains*
BBR/ BCP
CPP
DBP
GRAS
PLATZ
S1Fa-like


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

Romain Blanc-Mathieu[1], Renaud Dumas[1], Laura Turchi[1], Jérémy Lucas, François Parcy[1,*]

[1]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 (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.

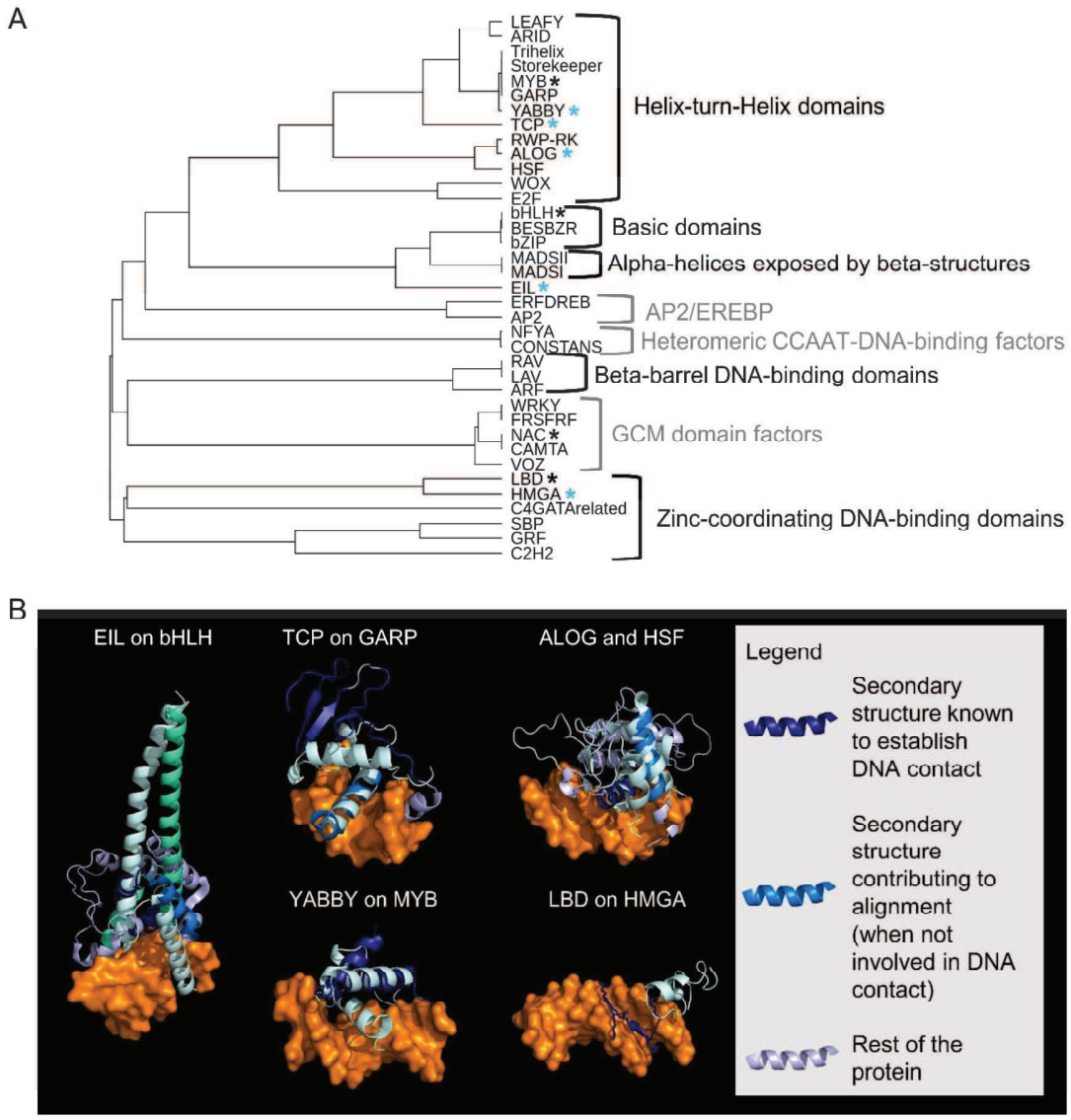


Figure S1: Automatic grouping of transcription factor (TF) families based on similarity of their DNA binding domains (DBDs). A: Dendrogram illustrating the structural distance of DBDs. The dendrogram is generated through hierarchical clustering using P-values from FATCAT structural alignments. Superclasses 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	6RYI
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

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