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Commonly asked questions about transcriptional activation domains

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Abstract

Eukaryotic transcription factors activate gene expression with their DNA binding domains and activation domains. DNA binding domains bind the genome by recognizing structurally related DNA sequences; they are structured, conserved, and predictable from protein sequence. Activation domains recruit chromatin modifiers, coactivator complexes, or basal transcriptional machinery via structurally diverse protein-protein interactions. Activation domains and DNA binding domains have been called independent, modular units, but there are many departures from modularity, including interactions between these regions and overlap in function. Compared to DNA binding domains, activation domains are poorly understood because they are poorly conserved, intrinsically disordered, and difficult to predict from protein sequence. This review, organized around commonly asked questions, describes recent progress that the field has made in understanding the sequence features that control activation domains and predicting them from sequence.

Keywords

Transcription; Transcription factor; Coactivator; RNA Polymerase II; Activation domain; Transactivation domain; Transcriptional activation domain; Protein-protein interactions (PPIs); intrinsically disordered protein; convolutional neural network; protein function prediction

What are transcriptional activation domains?

Transcriptional activation domains are the regions of transcription factors (TFs) that bind to coactivator complexes to activate transcription [1-3]. These regions are also called transactivation domains or activator domains, and all three terms have been applied both to minimized regions of high activity (10-80 AA) and the entirety of the TF outside the DNA binding domain (DBD; 100s of AA). We will use the term activation domain to refer to short regions that directly bind to coactivators. Activation domains are defined experimentally, most often in sufficiency assays, where candidate protein regions are fused to a heterologous DBD and activity is measured with a reporter gene. There are high-throughput sufficiency

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assays in yeast, fly, and human cells that use pooled oligo synthesis to study short TF fragments, ≤80AA [4-9], but some groups have queried longer regions [10,11]. The boundaries of nearly all annotated activation domains should be regarded as approximations because very little experimental effort has been devoted to defining boundaries precisely.

The primary known function of activation domains is to recruit coactivator complexes. These interactions are highly dynamic, with short dwell times that are hard to catch with pull-down assays *in vivo* [12-14]. There are now dozens of beautiful NMR structures of these interactions, as reviewed by Dyson and Wright [1]. It remains a goal in the field to map interactions between activation domains and coactivators. Genetically defining the coactivator dependence of activation domains yields complex results that have been hard to interpret [15]. Recent TURBO-ID experiments, which capture dynamic interactions *in vivo*, suggest that most activation domains preferentially bind to only 1-2 coactivators [16]. Importantly, different members of a single DBD family (e.g. FOXO) recruited different combinations of the TFIID, CBP/p300, NuA4, and BAF complexes [16]. Organizing TFs into families by DBD homology has been useful, but going forward we will require an orthogonal organization system for grouping TFs with functionally similar activation domains.

Are all activation domains acidic?

The first few dozen activation domains were all negatively charged [17,18], inspiring a seminal paper by Paul Sigler entitled, “Transcriptional activation. Acid blobs and negative noodles” [19]. Sigler leveraged his authority as a respected structural biologist to argue that activation domains did not need to fold in order to be functional. This seemingly simple idea was heretical at the time. It is now clear that transcription factors are highly enriched for intrinsically disordered protein regions (IDRs), which do not fold into a single 3D structure, comprise roughly a third of the residues in eukaryotic proteomes and are enriched for protein-protein interactions and post-translational modifications [20-22]. Sigler’s paper remains highly recommended reading.

Traditionally, activation domains are classified by their most common residue as acidic, glutamine-rich, proline-rich, or serine-rich. For this review, we collected lists of activation domains from recent surveys [9,16,23-25] (Table S1). After confirming the UniProt ID of each domain, we obtained the full-length sequences of all isoforms and used the published coordinates of the domain to find the sequence of each region. If the region matched the domain, we saved the UniProt ID of the isoform in the column of Table S1 titled “Matching Isoforms.” In cases where there were multiple matches, we selected one to record in the Uniprot ID column of Table S1, preferring the canonical isoform when it was among the matches. For a few members of the DelRosso library, we used the Ensembl ID to confirm which isoform to designate as the UniProt ID. Finally, we merged domains with the same UniProt ID with overlapping start and end coordinates, yielding the union of overlapping annotations. We used the updated start and end coordinates to find the region of the full-length sequence to use as the new domain sequence.

The combined list of activation domains revealed that glutamine-rich activation domains are rare (19/760, 2.50%) and that the three other classes overlap highly (Figure 1) [2,26,27]. Acidic activation domains were the first group described [17,18], remain the largest, and contain the strongest members [28]. In these domains, aromatic and leucine residues make the largest contributions to activation domain function [29]. Individual acidic residues are dispensable and poorly conserved but collectively essential for function [7]. Many acidic activation domains are well-described by our **acidic exposure model**, wherein the critical aromatic and leucine residues make contact with shallow hydrophobic grooves on coactivators (Figure 2) [4,30]. However, in the unbound state, the aromatic and leucine residues interact with each other and drive collapse into an inactive state. The acidic residues repel each other and favor solvation, keeping the hydrophobic residues exposed to solvent, where they are available to bind coactivators. Intermixing the positions and balancing the numbers of acidic and hydrophobic residues is important for full activity [4,5,7,26]. The exchange between collapsed and expanded states might be rapid, but the time scale has not yet been measured. The acidic exposure model is an instance of the stickers-and-spacers model with a very active role for the spaces [31]. This model is supported by work from many groups [5-8,27,32,33]. Surface plasmon resonance assays showed that acidic residues can also mediate fast, low-affinity electrostatic binding to coactivators and that hydrophobic residues mediate slow, high-affinity binding [7,34,35]. The overlap between acidic, serine-rich, proline-rich and glutamine (Q) rich activation domains have led Bintu et al. to playfully describe them all together as greasy acidic noodles sprinkled with salt (S), pepper (P), and queso (Q). Evidence that phosphorylation can modulate activation domain activity has led to speculation that some S-rich or P-rich activation domains are inducible acidic activation domains [36,37].

Within acidic activation domains, there is functional diversity. There are hints that L-rich activation domains bind to CBP/p300 and aromatic rich activation domains bind to Med25 [30,33,38]. Binding specificity arises from the structure of the coactivator binding interface imposing geometric constraints on the activation domain. For example, the deep hydrophobic canyon of Taz1 imposes more constraint than the shallow hydrophobic canyon of Med15 [9,39] A live-imaging study of transcriptional bursting found that 45/78 activation domains primarily regulate either transcriptional burst size or burst duration, but less often both (9/78) [40]. Activation domains that recruit Mediator or the general transcriptional machinery tended to modulate burst size, while activation domains that recruit SWI/SNF, histone acetyltransferases, or the super elongation complex tended to regulate burst intensity [16,40]

Why are so many active domains acidic?

In principle, the exposure of hydrophobic residues offered by acidic residues in the acidic exposure model could be achieved by basic residues, but acidic residues have several advantages. Most importantly, because DNA is acidic, it repels acidic activation domains, promotes exposure, and prevents non-specific DNA binding [41]. Acidic activation domains can have low-affinity, intramolecular, electrostatic interactions with DBD that can increase DNA specificity [42-45] and electrostatic interactions with basic coactivators [34,35]. When DNA repels activation domains, evolution can tune DNA affinity by acting only on the

DBD. A drawback of positively charged residues is that they can have cation-pi interactions with aromatic residues, which would increase collapse instead of exposure [46]. The electrostatic constraint posed by DNA can explain why many activation domains are acidic.

Acidic activation domain function is deeply conserved across eukaryotes. The Gal4/UAS system from yeast works beautifully in flies, mammals, and plants [47]. Acidic activation domains from animals, viruses, and plants work well in yeast [11,28,48]. This promiscuous species-crossing has fueled speculation that acidic activation domains existed in the ancestor of all eukaryotes.

Why are activation domains disordered?

Virtually all activation domains are intrinsically disordered, but many undergo coupled binding and folding, often into short alpha helices [1]. The first explanation for intrinsic disorder is that it allows activation domains to fold differently with each interaction partner [1,49]. In p53, varying the helical propensity trades off affinity for two partners, drastically modulating protein function [50]. Known activation domains are enriched for low-confidence secondary structural predictions in AlphaFold models [16]. So far, there are few clear examples where the activation domain remains disordered while bound to the coactivator, but we suspect this type of interaction is underreported due to ascertainment bias [51]. The second explanation comes from the acidic exposure model, where disorder reduces the entropic cost of keeping W,F,Y,L residues exposed to solvent because they need to be exposed for only a fraction of the time to allow coactivator binding. The third explanation is that intrinsically disordered sequences can use long–range, low–affinity electrostatic interactions to achieve diffusion-limited binding [52-56].

A controversial idea is the detergent model, which argues that activation domains loosen the interactions between nucleosomes and DNA to help create nucleosome-free regions for TF binding [8,57]. This idea contrasts with the standard model where activation domains recruit chromatin remodeling enzymes, including ISWI, SWI/SNF, CHD, and INO80, which use ATP-hydrolysis to slide or evict nucleosomes [58]. We do not endorse this model.

What is the molecular grammar in activation domains?

Molecular grammar describes how the arrangement or order of amino acids contributes to function. There is a spectrum ranging from an extreme of “no grammar,” where only the composition matters, to an extreme of “strict grammar,” where the exact order of residues is essential for function. The dominant model for activation domains is that they are short linear motifs (SLiMs) of hydrophobic residues surrounded by a permissive context [59]. For example, the ΦxxΦΦ motif, where Φ is a hydrophobic residue, is surrounded by acidic residues on many activation domains, often forming an amphipathic alpha helix that presents a continuous hydrophobic surface to the coactivator [1,60]. There are two problems with motif-centered models. First, individual motifs are conserved within a family of orthologs, but each motif is rarely present in many families, making each one too specific to be a useful predictor of activation domains [4,37,60]. Second, our mutagenesis has revealed that multiple motifs are necessary for full activity [4,30]. The reliance on motifs has served as

a robust set of training wheels for the field, but as our understanding of activation domain function matures, the fixation on motifs is holding us back because motifs imply a strict molecular grammar. We believe that it is time to focus on **clusters** of hydrophobic residues embedded in a permissive context, emphasizing a much more flexible grammar.

There is strong evidence against strict grammar: random peptides with activation domain activity do not have enriched motifs [6], shuffling activation domain sequence can increase activity in a third of examples [4,7], and searching for clusters of W,F,Y,L residues in acidic regions is a good predictor of activation domains [26,30]. Interesting work on Abf1 in yeast completely blurs the line between motifs and context [61]. There is also strong evidence against no grammar: shuffling sequence often has profound effects on activity, especially in helices [4,7,30], and interchanging aromatic residues can disrupt activity [30]. We are left with weak grammar, which we still do not fully understand and is at times disconcerting. Shuffling sequences can disrupt activity, or it can have little effect; breaking helices can disrupt activity or have little effect; interchanging similar residues (D>E or F>W) can disrupt activity or have little effect [7,29,30,39]. We refer the reader to Kotha and Staller 2023 for an extended discussion of the role of motifs and grammar in activation domains [26].

Can we predict activation domains from protein sequence?

Recently, high-throughput assays for measuring activation domain activity have powered convolutional neural network (CNN) models for predicting activation domains from protein sequence. The first computational model for activation domains, the 9aaTAD model, used regular expressions to find matches to a highly degenerate motif and context pattern [62]. However, in two high-throughput screens in yeast, this pattern was not enriched in activation domains and was not a useful predictor [6,7]. Rational mutagenesis of one activation domain and lasso regression models on random peptides revealed key amino acids [4,5,8]. The first CNN activation domain predictor was developed with a dataset of 3.6 million 30-AA random peptides tested in yeast [6]. The second CNN was developed with a dataset of 53-AA peptides from 180 *Saccharomyces cerevisiae* transcription factors (n = 7460 tiles) [7]. In our experience [63] and the work of others [11,26,27,63], both of these models do a good job predicting the general location of activation domains on human and plant TFs. We have found they do an excellent job of prioritizing a few regions of a TF that are likely to be activation domains. Both models struggle to find activation domain boundaries accurately, but these boundaries are poorly defined. To our surprise, scanning for clusters of W,F,L residues in acidic regions performs nearly as well as CNN models for human TFs, implying both that prediction is simpler than anticipated and that the grammar is highly degenerate [26]. Second-generation CNN models with more sophisticated architectures are already more accurate [64]. As more data becomes available, we anticipate the activation domain predictors will improve.

What is the link between activation domains and phase separation?

It is now clear that transcription occurs in dynamic clusters [65]. These clusters are non-stoichiometric assemblies with dozens of copies of each TF and coactivator complexes that

together recruit dozens of Pol II molecules, some of which successfully transcribe mRNAs [66-71]. It remains deeply contested whether these clusters of active transcription are phase-separated biomolecular condensates [72,73]. There is evidence that the same protein-protein interactions that enable activation domain function *in vivo* enable phase separation *in vitro* [72]. Modulation of phase separation *in vitro* can identify interaction binding partners or drugs [74]. There are examples from plants where a TF becomes inactive as it enters the condensate for long-term storage [75,76]. Careful studies in a synthetic system showed that phase separation can be completely separated from activation domain strength [77].

We speculate that the reason there has been so much confusion between transcriptional activation and phase separation is that both processes rely on multivalency. Multivalency is essential in many phase-separated systems and dynamic protein-protein interactions (See companion review by Berlow and colleagues). TFs and activation domains engage in multivalent binding with coactivators. Activation domains show multivalency on two length scales. First, many TFs have multiple activation domains that can bind the same coactivator: there are five patches of Gcn4 that each contact many (but not all) of the four activation domain-binding domains of Med15 [13]. For p53, the active form is a tetramer [78], and it can form four contacts with CBP/p300 [49]. Second, within activation domains, adding aromatic or leucine residues near key hydrophobic motifs boosts activity by lengthening the interaction surface [4,60,79].

Concluding remarks

Over the past 6 years, new methods have clarified the sequence determinants that control acidic activation domain function. We anticipate the next few years will expand these approaches to activation domains from other classes and investigate post-translational modifications. The major open questions are to define functional classes of activation domains, map interactions with coactivators, and build improved predictors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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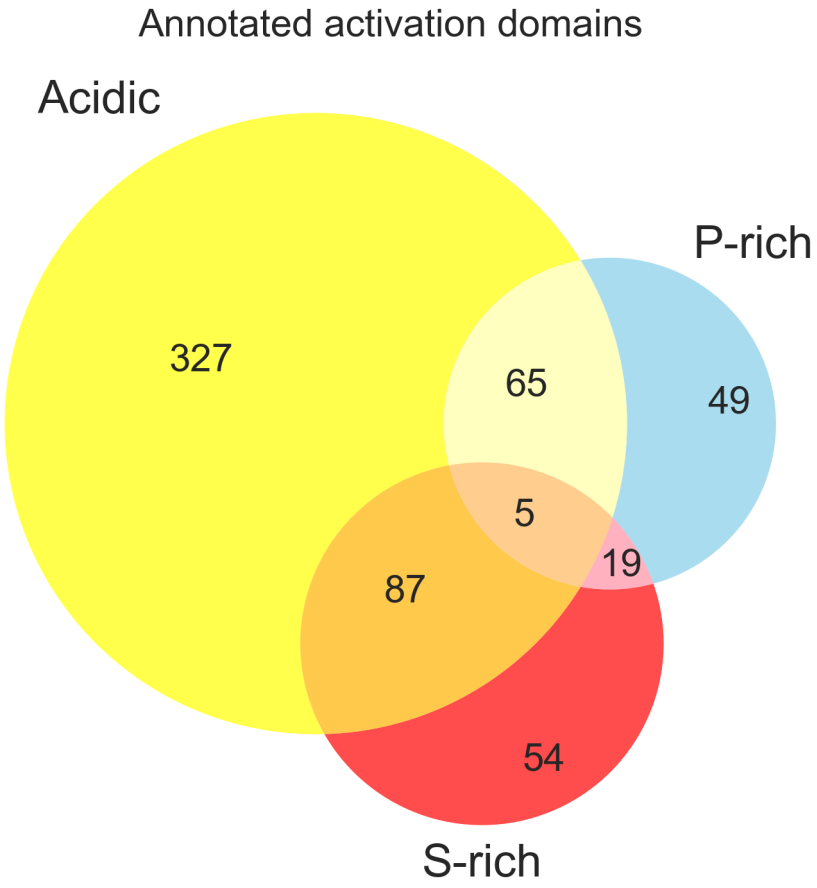

Figure 1:
Among annotated activation domains, the traditional classes are highly overlapping. Acidic activation domains have a net charge < −3, P-rich have >15% proline, and S-rich have >15% serine.

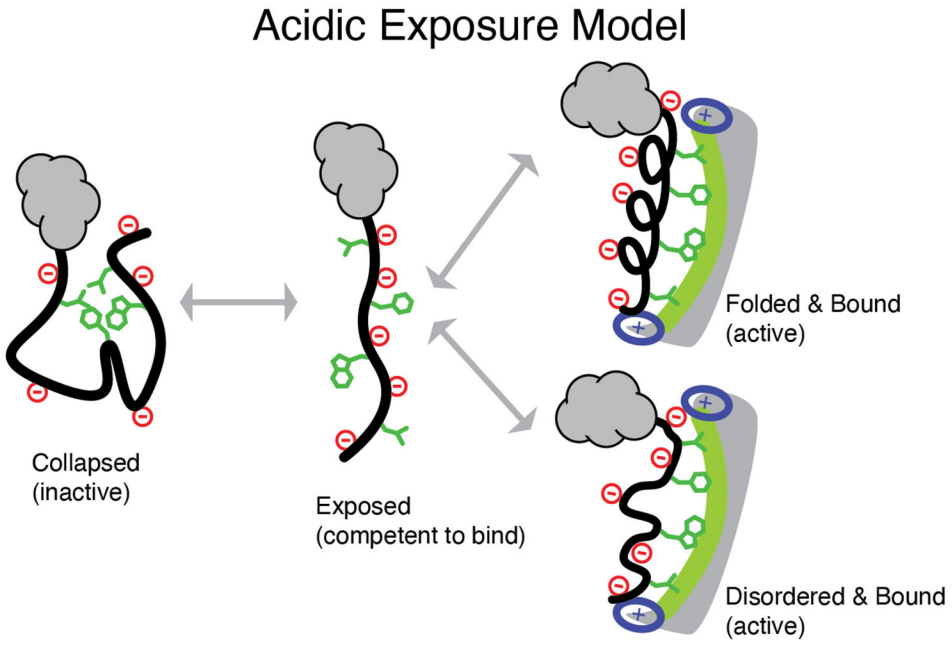

Figure 2:

In our acidic exposure model, disordered activation domains rapidly transition between collapsed and expanded states. The collapsed state is inactive. The expanded state is competent to bind coactivators because the W,F,Y,L residues are exposed to solvent. The W,F,Y,L residues make critical contacts with hydrophobic surfaces on the coactivator. Many activation domains experience coupled folding and binding, but folding is not essential. Electrostatic interactions between the activation domain and coactivator can contribute to binding or steering, but these interactions are of low affinity and not always necessary.