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Identification and characterization of the activation domain of Ifh1, an activator of model TATA-less genes

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ABSTRACT

In yeast, TATA box-binding protein TBP can be delivered to protein-coding genes by direct interactions with two different coactivators: TFIID, which delivers TBP preferentially to TATA-less promoters, and SAGA, which strongly favors TATA box-containing promoters. Transcriptional activators of SAGA-dependant genes are characterized by prototypic acidic activation domains (ADs) that efficiently recruit SAGA, but not TFIID, to UAS elements even in the absence of a core promoter. In contrast to the well-studied acidic activation domains, little is known about the activation domains of activators of TFIID-dependent genes, even though these genes constitute more than 80% of eukaryotic protein-coding genes. The paradigm for TATA-less genes are the ribosomal protein genes (RPGs). Here we have identified the AD of the RPG activator Ifh1p and demonstrate that a minimal Ifh1 AD represents a new class of AD that significantly differs from acidic ADs in amino acid signature, relative coactivator affinities, and core promoter selectivity.

TATA box-binding protein (TBP) is an essential component of the transcriptional machinery that in the yeast *Saccharomyces cerevisiae* can be delivered to core promoters by two different, partially redundant coactivators, TFIID and SAGA. Most protein-encoding genes in *S. cerevisiae* can be broadly divided into two major classes [1,2]. The class of TATA box-containing genes, exemplified by the well-studied *GAL* genes, is enriched for highly inducible genes that efficiently associate with SAGA to deliver TBP to the TATA box. The second class, exemplified by the ribosomal protein genes (RPGs), consists of predominantly 'housekeeping' genes that tend to lack TATA boxes and to associate with TFIID. TFIID delivers TBP presumably by its known interactions with initiator and downstream promoter elements [1–8]. Most yeast genes are not regulated exclusively by the TFIID pathway or the SAGA pathway [2,9], consistent with the partial functional redundancy of TFIID and SAGA. These and other observations suggest the presence of at least two types of transcriptional activators [2,3,10,11]. Both types are thought to be able to interact with either coactivator, although with different preferences, and it appears to be the type of activator, not the core promoter, which determines whether TFIID or SAGA is predominantly recruited [3–5]. The core promoter instead appears to determine coactivator compatibility for efficient TBP delivery [3,4]. As in yeast, the majority of human genes lack genuine TATA boxes [12], human TFIID is highly enriched at TATA-less genes [12], and human TATA-less genes are predominantly "housekeeping" genes [13].

Activators of TATA box-containing genes have been studied extensively. The most potent type of activation domain (AD) of these activators functions in all eukaryotes tested and depends on the presence of bulky hydrophobic amino acids interspersed by acidic amino acids [14]. These so-called "acidic ADs" lack clear sequence signatures [14,15], although relaxed homologies to a conserved nine amino acid motif have been described [16]. In addition to SAGA, these activators also recruit coactivators with functions different from TBP delivery, most notably the mediator and the Swi/Snf complexes, but not the histone H4-specific histone acetyltransferase complex NuA4 [5,6,8].

Unlike TFIID, SAGA by itself does not have any known promoter-recognition subunits. TBP delivery by SAGA therefore appears to be dependent upon a direct TBP–TATA box interaction, thus defining a pathway of transcriptional activation that relies on specific interactions between an acidic AD, SAGA–TBP and the TATA box. In contrast, TBP–TATA interactions are disfavored in the context of TFIID, possibly due to the TAND domain of its Taf1 subunit, which binds and masks the DNA-binding surface of TBP [17,18]. Although the majority of genes fall into the group of TFIID-dependent TATA-less housekeeping genes [2], very little is known about their activators. Since TFIID recruitment to TFIID-dependent genes is activator-dependent, but independent of core promoter and the transcriptional machinery [10,11], it is thought that activators of TFIID-dependent genes recruit TFIID by direct interaction. In support of this view, Rap1, a highly abundant multifunctional transcription factor that is required for transcription

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of most ribosomal protein genes (RPGs), the prototypical model for TATA-less genes, has been shown to physically and functionally interact with TFIID [19]. However, Rap1 also activates many TFIID-independent genes, functions in gene silencing, and activates genes indirectly by increasing DNA accessibility and by interacting with other DNA-bound regulatory factors. Rap1 may therefore not be a typical activator of TATA-less genes.

Rap1 binds the UASs of RPGs constitutively and sequence-specifically and at many RPGs is required for the constitutive association with the DNA-binding protein Fhl1. Favorable growth conditions and the absence of stress then trigger the inducible interaction of the RPG-specific activator Ifh1 with the FHA domain of Fhl1 [20–23]. Here we have identified and analyzed a minimal AD of the exclusively TFIID-dependent activator Ifh1p. We demonstrate that this AD exhibits a pattern of relative coactivator affinities and core promoter specificities that are distinctly different from that of prototypic acidic ADs, implying the existence of at least two types of ADs in yeast.

Materials and methods

Plasmid construction and reporter gene assays. Ifh1 and Ifh1 fragments were amplified by PCR and cloned into pGBKT7 (Clontech) for expression as Gal4 DBD fusion proteins. The reporters XCZ171 and XCZ174 were described previously [3]. Two consensus Gal4 binding sites spaced 10 bp apart were PCR-amplified and inserted into *CYC1*ΔUAS-*lacZ* reporter vector pJS205-XXB [33]. The 153 bp *RPS5* core promoter was PCR-amplified and inserted into recombinant vector pJS205-2xUAS$_C$ downstream of the two Gal4 binding sites to create 2xGal4-*RPS5* prom-*lacZ*. β-Galactosidase assays were performed as described [34]. Expression of DBD-Ifh1 AD and DBD-Gal4 AD clones from pGBKT7 yielded very similar expression levels as confirmed by immunoblotting using anti-myc antibody.

Recombinant proteins. Ifh1 AD1 and Ifh1 AD2 open reading frames were amplified by PCR and cloned into pGexCS [35] for expression. Recombinant GST fusion proteins were expressed in *Escherichia coli* Rosetta2 (Novagen) and bound and purified on glutathione Sepharose (Pharmacia) using standard protocols.

Genomic tagging and preparation of whole cell extracts. Yeast strains with genomically triple-HA-tagged *TAF4*, *ADA2*, *MED6*, and *SNF12* were described previously [36]. *EPL1* and *EAF6* were genomically triple-HA-tagged for this work using standard procedures. Transcriptionally competent whole cell extracts were prepared from 800 ml cultures of each genomically tagged yeast strain. Cells were grown to a density of $OD_{600} \sim 1$ and extracts were prepared according to Wootner et al. [37].

Pulldowns. 0.1, 0.3, and 1 μM of each GST fusion protein were incubated with 40 μl whole cell extract mix (equal amounts of HA3-Ada2 and HA3-Med6, each, or of HA3-Snf12 and HA-Epll1, each) in 140 μl of Buffer A (25 mM Tris/HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.1% Triton X-100, 2 mM DTT, with freshly added protease inhibitors) for 70 min at 4 °C on a tiltboard. Beads were then washed 4x with 1 ml Buffer A, each. 100 μl aliquots were removed for SDS PAGE/Coomassie staining, the rest pelleted, eluted with SDS sample buffer and analyzed by anti-HA immunoblotting. Input was 10 μl of each whole cell extract mix. For detection of Taf4, blots were stripped and reprobed with anti-Taf4 antibody.

Results and discussion

Ifh1 contains a bipartite C-terminal AD

To map potential activating function(s) within Ifh1 we generated fusions between Gal4 DNA-binding domain (DBD) and the open reading frames of full length Ifh1p, overlapping fragments spanning the open reading frame of Ifh1p, and the Ifh1-interacting FHA domain of Fhl1. We determined the abilities of these fusion proteins to activate expression from an integrated *GAL1* promoter-*lacZ* reporter, as well as from a set of two integrated reporters consisting of *GAL1* promoter-*lacZ* (XCZ171) and a variant of this reporter, in which the TATA-containing *GAL1* core promoter was exchanged against the TATA-less core promoter from the RPG *RPS5* (XCZ174) [3]. As shown in Fig. 1A, the C-terminus of Ifh1 fused to DBD activated expression of each of the three reporters, demonstrating that it contains an activation function. Activation of the two TATA box-containing reporters is consistent with previous reports, in which FHA-interacting factor(s) activate *GAL1* promoter reporters [20,22,23]. Surprisingly, full length Ifh1 fused to DBD was incapable of activating transcription in this system (Fig. 1A). Preliminary studies indicate that the C-terminus in the context of full length Ifh1 is masked by intramolecular interactions that become dissociated upon Fhl1 binding, thus limiting AD exposure to promoter-bound Ifh1 (P.Z. and K.M., unpublished).

Next we determined the transcriptional activation potential of segments from the C-terminal Ifh1 AD region. Since the *GAL1* upstream region employed in all three reporters contains a binding site for the repressor Mig1, which significantly reduces reporter sensitivity (and possibly contains other elements that might interfere with TATA-less regulation by Ifh1 AD), we generated a construct that contains just a tandem copy of the minimal 17 bp Gal4 consensus binding site upstream of the *RPS5* core promoter. Using this reporter, we identified at least two regions within the C-terminus of Ifh1, spanning amino acids 716–822 (AD1) and 911–1030 (AD2) (Fig. 1B), that were capable of activating transcription when fused to Gal4 DBD.

Amino acid signatures and coactivator interactions of Ifh1 AD1 and AD2

The hallmark of acidic ADs is the interspersion of bulky hydrophobic amino acids with acidic amino acids. While several regions within Ifh1 are highly enriched for both acidic and bulky hydrophobic amino acids, Ifh1 AD1 is not (Fig. 2A). AD1 consists of an N-terminal part that has an average content of bulky hydrophobic amino acids (25% F, W, L, I, V relative to 27.2% in microbial [24] and 23.5% in vertebrate [38] proteins) and lacks negative net charge, and a C-terminal part that is enriched in acidic amino acids, yet has an under-representation of bulky hydrophobic amino acids. AD1 therefore lacks the direct interspersion of acidic and bulky hydrophobic amino acids that is the hallmark of acidic ADs. Neither the acidic C-terminal part nor the more hydrophobic N-terminal part of AD1 is capable of activating transcription individually (Fig. 2B). Although AD2 bears more resemblance with acidic ADs, it fails to interact as recombinant, *E. coli*-produced protein with any of several coactivators tested (SAGA, TFIID, mediator, NuA4, and Swi/Snf; data not shown), in clear contrast to AD1 (Fig. 3). Moreover, both AD1 and AD2 lack similarity to a nine amino acid motif found in a large number of acidic ADs [16,39]. Since Ifh1 function is controlled by phosphorylation [25], we speculate that AD2 may require posttranscriptional modification for *in vivo* activity. We therefore focused on AD1 as a minimal Ifh1 AD that is sufficient for transcriptional activation *in vivo* and for coactivator interaction *in vitro*.

Ifh1 AD1 exhibits a distinct pattern of relative coactivator affinities

Chromatin immunoprecipitation (ChIP) experiments have demonstrated that the UAS-binding transcription factors of TFIID-dependent and TFIID-independent (SAGA-dependent) genes are, directly or indirectly, associated with different sets of coactivator complexes [5,6,10,11,26]. To test whether differential coactivator

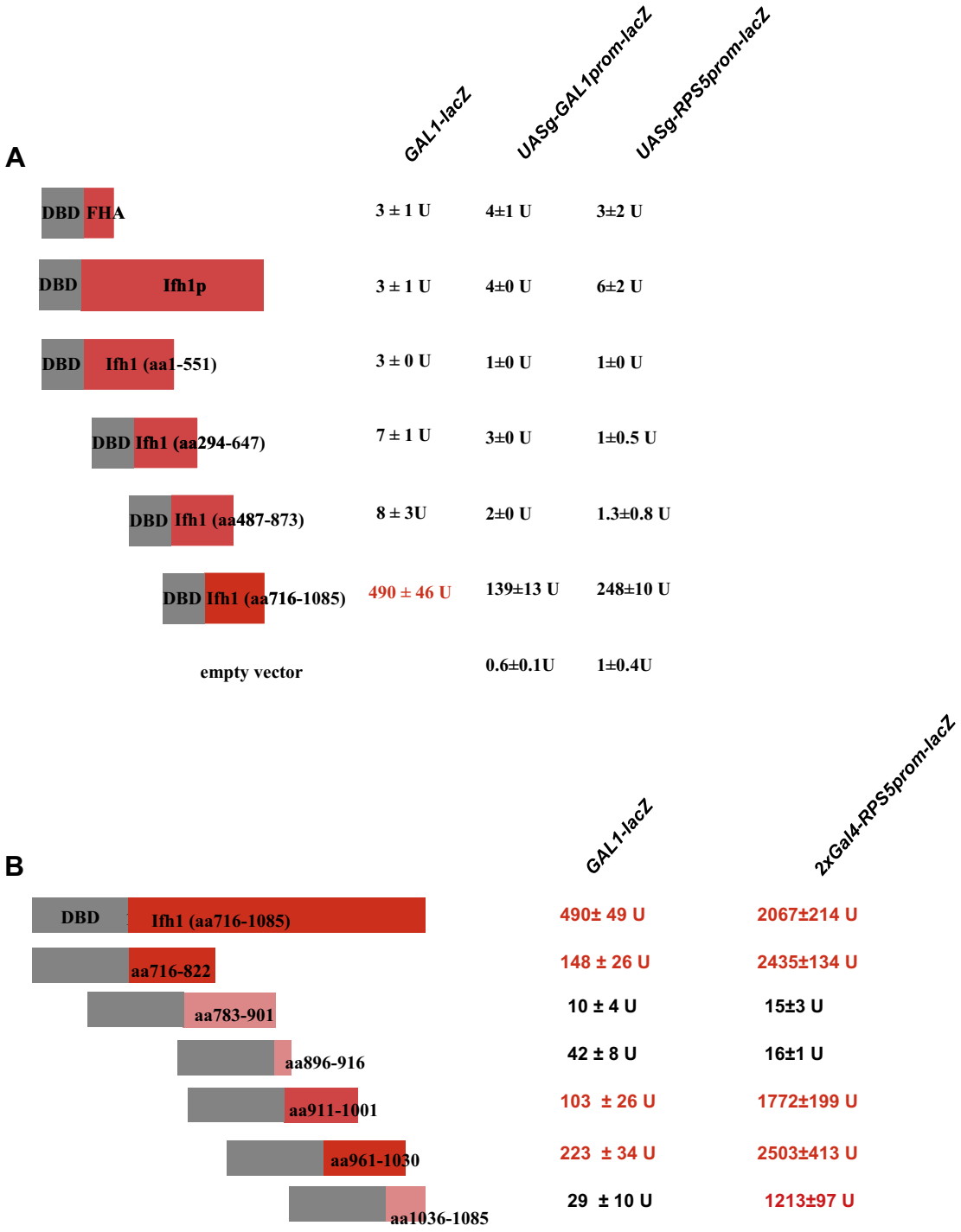

Fig. 1. The C-terminus of Ifh1 contains a potent activation function. (A) β-Galactosidase reporter gene activities of Gal4 DBD hybrid proteins. (B) Mapping of the C-terminal Ifh1 activation domain. *GAL1* contains the entire *GAL1* upstream region, UASg contains a truncated version of the *GAL1* upstream region that lacks the core promoter, but does contain the Mig1 repressor site, 2xGal4 consists just of two 17 bp consensus Gal4 binding sites. DBD: Gal4 DNA-binding domain. Note that the 2xGal4-*RPS5*prom-*lacZ* reporter is much more sensitive due to the lack of Mig1 sites.

associations *in vivo* reflect relative AD-coactivator affinities, we performed semi-quantitative GST pulldown experiments. We prepared transcriptionally competent whole cell extracts from 5 yeast strains, each of which expressed a specific genomically HA-tagged coactivator subunit: SAGA (Ada2), NuA4 (Epl1 and Eaf6), mediator (Med6), or Swi/Snf (Snf12). Extracts were combined and loaded directly onto a SDS–PAGE gel (input) or incubated with increasing concentrations of either GST-Ifh1 AD1 or GST fusions to the prototypic acidic ADs of yeast Gal4 or Herpes simplex virus VP16. After extensive washing, coactivators bound to the AD fusion proteins were eluted with SDS sample buffer and visualized by anti-HA and anti-Taf4 (detection of TFIID) immunoblotting (Fig. 3 and Supplemental Figure). Eaf6 retention relative to input is lower than Epl1 retention since Eaf6 is also a subunit of the NuA3 complex [27]. Determination of relative binding of multiple coactivators to input within the same reaction allowed an internally controlled comparison for relative coactivator binding, making this approach highly accurate for determination of coactivator binding prefer-

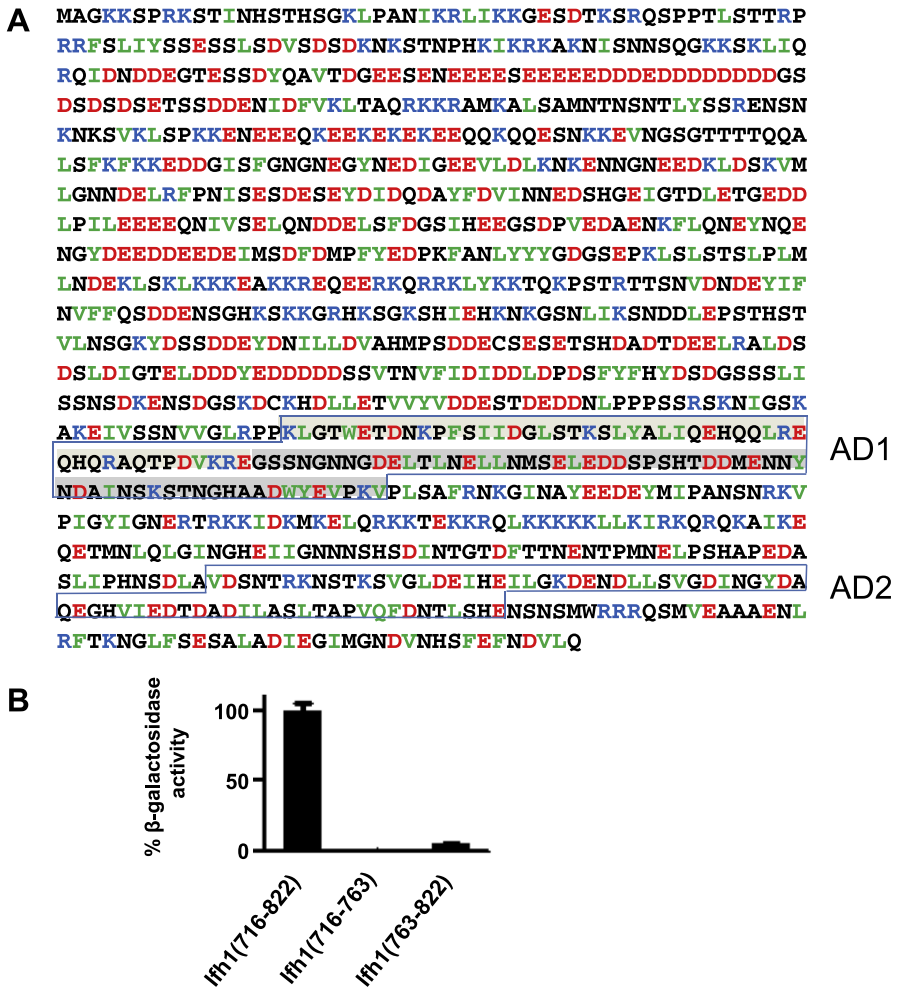

Fig. 2. Ifh1 AD1 and AD2. (A) Sequence of Ifh1. Ifh1 AD1 and AD2 are boxed and the two subregions of AD1 indicated by different shading. Amino acids are color-coded as acidic (red), basic (blue), and bulky hydrophobic (green). (B) Neither the more hydrophobic (aa 716–763) nor the acidic (aa763–822) subregion of AD1 can independently support transcriptional activation. Subregions were fused to Gal4 DBD and β-galactosidase activity from the 2xGal4-*RPS5*prom-*lacZ* reporter determined. (For interpretation of color mentioned in this figure, the reader is referred to the web version of this article.)

ences (see Supplemental Figure for uncropped immunoblots). Many, if not all, acidic ADs have essentially superimposable binding preferences [28]. As therefore expected, Gal4 and VP16 ADs exhibited the same rank order of coactivator binding (SAGA > Swi/Snf > mediator > NuA4 > TFIID) with VP16 being the stronger binder of each coactivator complex. In contrast, Ifh1 AD1 displays a weak interaction with SAGA in comparison to both Gal4 and VP16, but a relatively stronger interaction with the two NuA4 subunits tested, particularly in comparison to Gal4. For example, Ifh1 AD1 at the highest concentration clearly retains more NuA4 than the 25% input (compare lanes 1 and 7) while Gal4 AD at the highest concentrations retains only a small fraction compared to input (compare lanes 8 and 14), yet binding of SAGA relative to input is much higher for Gal4 AD (compare lanes 25 and 31) than for Ifh1 AD1 (compare lanes 18 and 24). Ifh1 AD1 therefore exhibits a pattern of relative coactivator affinities that is distinct from that of the acidic ADs of Gal4 and VP16. We note that this is the first demonstration that, *in vitro*, an activator of TATA-less genes can bind Swi/Snf, mediator, and SAGA.

The binding preference of Ifh1 AD1 for NuA4 and of the acidic ADs for SAGA is consistent with ChIP experiments which imply that SAGA is the primary *in vivo* target of acidic ADs [5,8] and NuA4 the primary target of the activators of RPGs [26]. However, TFIID bound only weakly to either Ifh1 AD1 or the acidic ADs, despite the fact that it is associated with RPG, but not Gal4-bound promoters *in vivo*. Moreover, the TFIID-dependent, SAGA-independent Ifh1 AD1 bound TFIID less efficiently than it did SAGA, suggesting that *in vivo* TFIID recruitment is not simply determined by AD-TFIID affinity. These results are consistent with the *in vivo* dependence of the TFIID-RPG association on the prior recruitment of NuA4 and its histone H4-specific histone acetyltransferase subunit Esa1 [7,29] as well as the double-bromodomain protein Bdf1 [29]. Bdf1 is a substoichiometric TFIID subunit that is thought to link H4 acetylation to TFIID recruitment via its preferential association with hyperacetylated histone H4 [29–32].

Ifh1 AD and Gal4 AD differ in promoter specificities

It had been shown that both *GAL1* UAS and *RPS5* UAS cooperate efficiently with the strong *GAL1* core promoter, while *GAL1* UAS functions relatively inefficiently in combination with *RPS5* core promoter [3,4]. These experiments employed complex UAS elements that include binding sites for multiple transcription factors (Fhl1–Ifh1, Rap1 and Hmo1 for *RPS5* UAS, Gal4, Reb1, and Mig1 for *GAL1* UAS). To test whether the minimal Ifh1 AD1 and the minimal 34 amino acid Gal4 AD would be sufficient to reproduce promoter selectivity, we expressed both ADs as Gal4 DBD fusions in combination with *GAL1* and *RPS5* core promoter reporters driven

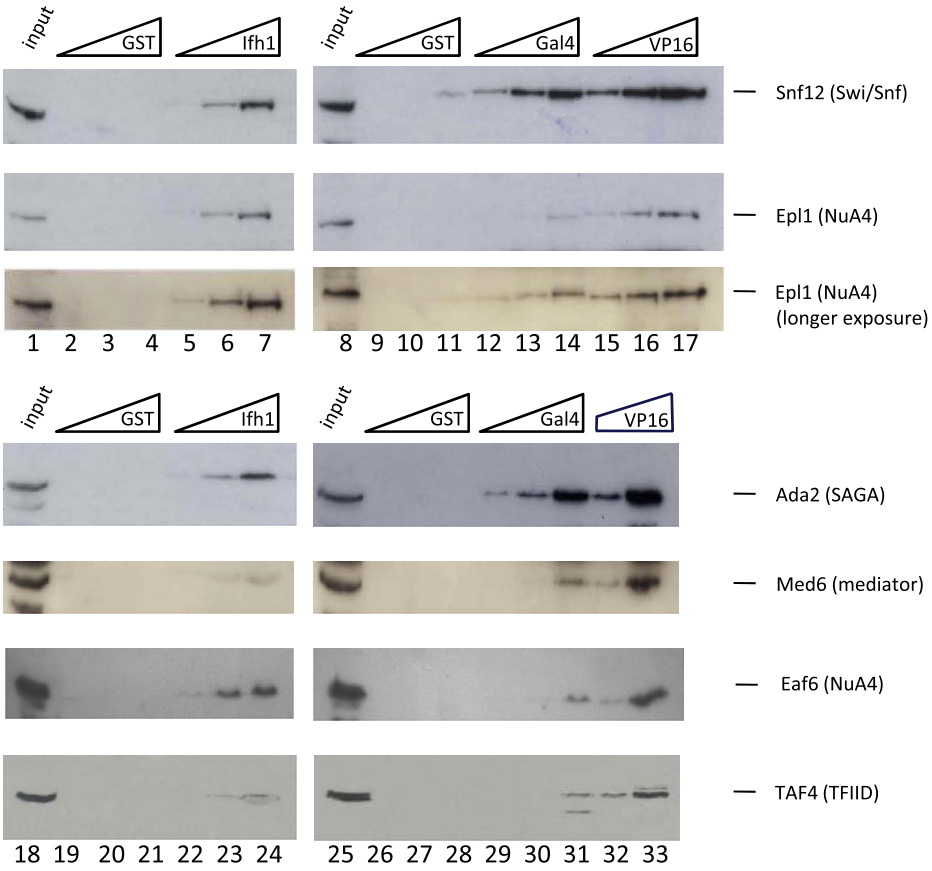

Fig. 3. Coactivator binding preferences of Ifh1, Gal4, and VP16 ADs. Extracts from isogenic strains with genomically HA3-tagged subunits of Swi/Snf and NuA4 (top) or SAGA, NuA4, and mediator (below) were mixed and incubated with increasing concentrations (0.1, 0.3, and 1.0 μM, each) of GST-AD proteins. Retained proteins were eluted with SDS sample buffer and separated by SDS–PAGE. Coactivator retentions were determined relative to input (25% binding reactions) by immunoblotting with anti-HA antibody or, for TFIID detection, anti-Taf4 antibody (bottom panels).

by two synthetic 17 bp Gal4 binding sites (no additional *GAL1* or *RPS5* upstream sequences). As shown in Fig. 4, both minimal ADs efficiently activate the *GAL1* core promoter reporter while Ifh1 AD1 activates the *RPS5* reporter three times more efficiently than Gal4 AD. Thus Ifh1 AD1 functions *in vivo* efficiently with the TATA-less *RPS5* promoter and interacts *in vitro* preferentially with NuA4. In contrast, Gal4 AD poorly activates the *RPS5* reporter and interacts preferentially with SAGA.

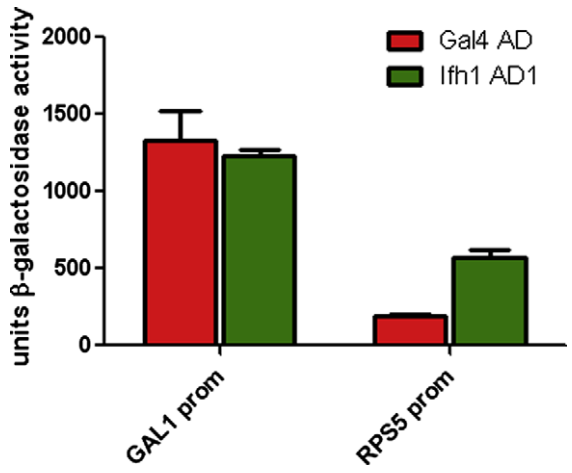

Fig. 4. Core promoter selectivities of Ifh1 and Gal4 ADs. Ifh1 AD1 and Gal4 AD were expressed as Gal4 DBD fusion proteins either in combination with 2xGal4-*GAL1*prom-*lacZ* or 2xGal4-*RPS5*prom-*lacZ* reporters.

In summary, we have identified a minimal Ifh1 AD that differs from acidic ADs in its sequence signature, its relative coactivator affinities, and its core promoter preferences. This study thus confirms that an activator of TFIID-dependent, TATA-less genes is distinct from activators of SAGA-dependent, TATA-containing genes, and that this difference can be explained by AD-specific coactivator preferences reflected by distinct amino acid signatures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.12.172.

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