Molecular Determinants of the Interaction of Mad with the PAH2 Domain of mSin3*

Xavier Le Guenennec, Gert Vriend, and Hendrik G. Stunnenberg†‡

From the Department of Molecular Biology, Centre for Molecular and Biomolecular Informatics, University of Nijmegen, 6500 HB Nijmegen, The Netherlands

The Sin3 co-repressor acts as a protein scaffold to recruit transcription factors via its four highly homologous paired amphipathic helix (PAH) domains. This PAH2/Mad complex has been extensively characterized in NMR, but the molecular determinants that dictate the specificity of interaction remain to be elucidated. To uncover residues that convey the specificity of interaction between PAH2 and Mad, PAH2 residues contacted by the Mad-SID were introduced into the PAH1 domain of mSin3b and tested for gain-of-interaction in vivo and cell-free systems. This approach led to the identification of PAH2-Phe-7 as a critical residue. Stabilization of the interaction between PAH1-Phe-7 and the Mad-SID was achieved by introducing Val-14 and Gln-39 into PAH1. Substitution of PAH2 residues contacted by the Mad-SID with their respective residues in PAH1 corroborated and extended the critical role of Phe-7 and the stabilizing role of Val-14 and Gln-39. We conclude that Phe-7 is the critical determinant and provides the molecular specificity for the association between Sin3 and Mad in regulating cell growth and differentiation.

The ordered and specific/selective recruitment of multiprotein complexes with intrinsic chromatin remodelling or histone modifying activities to cis-acting DNA elements has emerged over the last decade as one of the major mechanisms to regulate transcription and cell fate determination (1). A plethora of studies have collectively shown that acetylation of specific N-terminal lysines of core histone tails by histone acetyl transferases (HATs)1 creates a chromatin state which results in transcription and cell fate determination (1). A plethora of studies have collectively shown that acetylation of specific N-terminal lysines of core histone tails by histone acetyl transferases (HATs) creates a chromatin state which results in transcription and cell fate determination (1).

1. The abbreviations used are: HAT, histone acetyl transferase; HDAC, histone deacetylase; PAH, paired amphipathic helix domain; GST, glutathione S-transferase; SID, Sin3 interacting domain; ONPG, O-nitrophenyl β-D-galactopyranoside; G4DBD, DNA-binding domain of Gal4; TRX, thioredoxin A; CSU, interatomic contacts of structural units; G4AD, Gal4 activation domain.

Received for publication, December 18, 2003, and in revised form, March 16, 2004
Published, JBC Papers in Press, March 26, 2004, DOI 10.1074/jbc.M313860200

‡ To whom correspondence should be addressed: Dept. of Molecular Biology, NCMLS 191, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-24361-0523; Fax: 31-24361-0520; E-mail: h.stunnenberg@ncmls.kun.nl

§ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. The abbreviations used are: HAT, histone acetyl transferase; HDAC, histone deacetylase; PAH, paired amphipathic helix domain; GST, glutathione S-transferase; SID, Sin3 interacting domain; ONPG, O-nitrophenyl β-D-galactopyranoside; G4DBD, DNA-binding domain of Gal4; TRX, thioredoxin A; CSU, interatomic contacts of structural units; G4AD, Gal4 activation domain.

To whom correspondence should be addressed: Dept. of Molecular Biology, NCMLS 191, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-24361-0523; Fax: 31-24361-0520; E-mail: h.stunnenberg@ncmls.kun.nl

† To whom correspondence should be addressed: Dept. of Molecular Biology, NCMLS 191, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-24361-0523; Fax: 31-24361-0520; E-mail: h.stunnenberg@ncmls.kun.nl

‡ To whom correspondence should be addressed: Dept. of Molecular Biology, NCMLS 191, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-24361-0523; Fax: 31-24361-0520; E-mail: h.stunnenberg@ncmls.kun.nl

§ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. The abbreviations used are: HAT, histone acetyl transferase; HDAC, histone deacetylase; PAH, paired amphipathic helix domain; GST, glutathione S-transferase; SID, Sin3 interacting domain; ONPG, O-nitrophenyl β-D-galactopyranoside; G4DBD, DNA-binding domain of Gal4; TRX, thioredoxin A; CSU, interatomic contacts of structural units; G4AD, Gal4 activation domain.
Gln-39. Strikingly, in PAH2, Phe-7 was further identified as a critical residue. Overall, we established Phe-7 as the most important molecular determinant of the interaction between PAH2 and Mad.

EXPERIMENTAL PROCEDURES

PAH1/PAH2-Mad-SID Binding Assay—The Mad-SID(5–24) fused to the GB1 domain of streptococcal protein G was prepared as described earlier (37). GST-PAH1 (amino acids 34–108) and GST-PAH2 (amino acids 148–252) were expressed and purified as described previously (39).

G4DBD-PAH1 or PAH2 were incubated with ProtG-Mad and bound to IgG-Sepharose in the presence or absence of ProtG-Mad-SID(5–24) and washed extensively. Bound material was eluted, separated on SDS-PAGE, and Coomassie Blue-stained. Bottom panel, Mad preferentially binds to PAH2 upon reducing amounts of Mad. C, in vivo interaction in a yeast two-hybrid assay suggests an exclusive interaction of Mad with PAH2. G4DBD-PAH1 or PAH2 were transformed in yeast with a prey containing G4AD fused to the thioredoxin A protein (G4AD-TRX). Mad-SID(5–24) was inserted in the constrained active-site loop of thioredoxin to generate G4AD-TRX-Mad. Quantitative β-galactosidase assays were performed using five fresh transformants in triplicate.

G4DBD-PAH1 or PAH2 (10 μg) were incubated with ProtG-Mad and bound to IgG-Sepharose (Amersham Biosciences) in 150 mM NaCl, 20 mM Tris, pH 7.6. Beads were washed 3 times with 600 mM KCl, 0.2% Nonidet P-40 (v/v), 20 mM Tris, pH 7.6. GST-PAH1 and GST-PAH2 mutant fusion proteins were incubated with ProtG-Mad in 150 mM NaCl, 0.5% Nonidet P-40 (v/v), 0.01% SDS, 5 mM EDTA, 20 mM Tris, pH 7.6, and washed three times in the same buffer. Bound proteins were eluted with 0.1 M glycine, pH 2.6, separated by SDS gel electrophoresis, and Coomassie Blue-stained.

Cloning and Mutagenesis—Fragment containing the PAH1-PAH2 domain of pvzmsin3b was cloned into PBS-SK (Stratagene) as a SacI/
EcoRI fragment yielding PBS-PAH1-PAH2. Site-directed mutagenesis was performed on PBS-PAH1-PAH2 by using the QuikChange site-directed mutagenesis kit (Stratagene) following the manufacturer’s protocol. All mutations were confirmed by DNA sequencing.

Wild type or mutated PAH1 fragments of msin3b containing amino acids 25–107 were generated by PCR with the primer pair PAH1F97/PAH1R97m containing, respectively, SalI and NotI restriction site overhangs. PAH1F97 was composed of 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′, and PAH1R97m was composed of 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′. Wild type or mutated PAH2 fragments of msin3b containing amino acids 136–275 were generated by PCR using the PAH2F97/PAH2R97 primer pair containing SalI and NotI restriction sites. PAH2F97 was composed of 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′, and PAH2R97 was composed of 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′. In-frame cloning of the amplified fragments was performed by using Sall/NotI cleaved pCP97 bait vector containing the GAL4/DNA-binding domain (40). We generated PAH1 and PAH2 PCR fragments by using primer pair PGEXP AH1F/PGEXP AH1R or PGEXP AH2F/PGEXP AH2R, containing BamHI and EcoRI restriction sites. Amplified fragments were fused to GST using BamHI/EcoRI sites available in PGEX-2T (Amersham Biosciences). The amplified fragments were as follows: PGEXP AH1F, 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′; PGEXP AH1R, 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′; PGEXP AH2F, 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′; PGEXP AH2R, 5′-GTCAGAGTCCATGCGAGGCAGAAGCTG-3′. Completed constructs were generated with the G4DDBD and used as baits. As a prey, a constrained Mad-SID displayed in a yeast two-hybrid setting suggests that unique residues within PAH2 play a selective role in the binding of the Mad-SID, as TRX alone resulted in background levels. Thus, the PAH2 expression in combination with Mad resulted in a robust β-galactosidase level, whereas PAH1 yielded background levels (Fig. 1A). This activation mediated by G4DBD-PAH2 was strictly dependent upon the presence of the Mad-SID, as TRX alone resulted in background levels. Thus, the Mad-SID preferentially interacts with PAH2 in vitro and more selectively so in vivo.

Exchange of Contact Residues between PAH2 and PAH1—The observed selective interaction between Mad and PAH2 in vivo in a yeast two-hybrid setting suggests that unique residues within PAH2 play a selective role in the binding of the Mad-SID. We used molecular modeling of PAH1 with WHAT IF (41) and superposition of the PAH1 model with the NMR structure of the PAH2 domain of mSin3b with the Mad-SID revealed a number of PAH2-specific residues. Some contact residues are conserved between PAH1 and PAH2 whereas others are distinct and may provide specificity to the interaction. Interestingly, they are exclusively located in helices 1 and 2 (Fig. 1A, boxed).

To assess the ability of PAH1 and PAH2 to interact with the Mad-SID, the domains were fused to GST, purified, and incubated with immobilized ProtG-Mad bound to IgG-Sepharose beads. When ProtG-Mad was in excess, both GST-PAH2 and GST-PAH1 were able to bind the Mad-SID (Fig. 1B). Incubating equal amounts of GST-PAH1 and GST-PAH2 with decreasing amounts of ProtG-Mad, i.e. creating a competitive setting, indicated that Mad-SID binds preferentially to PAH2 in vitro. To ascertain this differential interaction in vitro, we performed yeast two-hybrid experiments. The PAH1 and PAH2 domains of msin3b were fused to the DNA-binding domain of Gal4 (G4DBD) and used as baits. As a prey, a constrained Mad-SID displayed in the active-site loop of E. coli thioredoxin (TRX) molecule was used, fused to the gal4 activation domain (G4AD-TRX-Mad). PAH2 expression in combination with Mad resulted in a robust β-galactosidase level, whereas PAH1 yielded background levels (Fig. 1C). This activation mediated by G4DBD-PAH2 was strictly dependent upon the presence of the Mad-SID, as TRX alone resulted in background levels. Thus, the Mad-SID preferentially interacts with PAH2 in vitro and more selectively so in vivo.

RESULTS

PAH1-PAH2

| PAH1          | PAH2          |
|---------------|---------------|
| His6          | Glu6          |
| Val7          | Phe7          |
| Leu11         | Ile11         |
| Leu14         | Val14         |
| Asp15         | Asn15         |
| Met35         | Leu35         |
| Lys36         | His36         |
| Lys39         | Gln39         |

Exchange of Contact Residues between PAH2 and PAH1—The observed selective interaction between Mad and PAH2 in vivo in a yeast two-hybrid setting suggests that unique residues within PAH2 play a selective role in the binding of the Mad-SID. We used molecular modeling of PAH1 with WHAT IF (41) and superposition of the PAH1 model with the NMR structure of the PAH2 domain of mSin3b with the Mad-SID revealed a number of PAH2-specific residues. Some contact residues are conserved between PAH1 and PAH2 whereas others are distinct and may provide specificity to the interaction. Interestingly, they are exclusively located in helices 1 and 2 (Fig. 1A, boxed).

To assess the ability of PAH1 and PAH2 to interact with the Mad-SID, the domains were fused to GST, purified, and incubated with immobilized ProtG-Mad bound to IgG-Sepharose beads. When ProtG-Mad was in excess, both GST-PAH2 and GST-PAH1 were able to bind the Mad-SID (Fig. 1B). Incubating equal amounts of GST-PAH1 and GST-PAH2 with decreasing amounts of ProtG-Mad, i.e. creating a competitive setting, indicated that Mad-SID binds preferentially to PAH2 in vitro. To ascertain this differential interaction in vitro, we performed yeast two-hybrid experiments. The PAH1 and PAH2 domains of msin3b were fused to the DNA-binding domain of Gal4 (G4DBD) and used as baits. As a prey, a constrained Mad-SID displayed in the active-site loop of E. coli thioredoxin (TRX) molecule was used, fused to the gal4 activation domain (G4AD-TRX-Mad). PAH2 expression in combination with Mad resulted in a robust β-galactosidase level, whereas PAH1 yielded background levels (Fig. 1C). This activation mediated by G4DBD-PAH2 was strictly dependent upon the presence of the Mad-SID, as TRX alone resulted in background levels. Thus, the Mad-SID preferentially interacts with PAH2 in vitro and more selectively so in vivo.
Single amino acid replacement in PAH1 revealed only one mutation, M35L, that yielded elevated levels of β-galactosidase expression, i.e. 5% of the level obtained with PAH2 and Mad (Fig. 3A). To identify additional residues, a second cycle of single amino acid substitutions was performed this time in the context of M35L. Some combinations, such as K36H with M35L, yielded reduced levels, whereas others seemed neutral. A significant enhancement of the interaction was observed with the double mutant containing V7F (Fig. 3B). Unexpectedly, two-hybrid assays performed in the absence of Mad or in the absence of a prey showed that PAH1-M35L had gained intrinsic transcriptional activity. On top of this, intrinsic transcriptional activity was displayed relative to G4DBD-PAH2/G4AD-TRX-Mad set at 100. A, single amino acid replacement in PAH1 reveals M35L above background. B, M35L converts PAH1 into a weak transcription activator and allows detection of V7F. Control experiment with G4AD or without a prey was performed only for G4DBD-PAH1-M35L and G4DBD-PAH1-V7F-M35L. C, in the context of PAH1-V7F, L14V and K39Q enable a specific interaction with Mad-SID. D, in the context of PAH1-V7F/L14V, K39Q augments interaction with Mad-SID. E, PAH1-V7F/L14V/K39Q restores a specific interaction with Mad-SID at 30% of the level of PAH2. Indicated mutants were transformed in yeast strain Y190 with G4AD-TRX-Mad or as a control G4AD-TRX. β-Galactosidase units activity and standard error are indicated. Results are the average of at least five assays.

Single amino acid replacement in PAH1 revealed only one mutation, M35L, that yielded elevated levels of β-galactosidase expression, i.e. 5% of the level obtained with PAH2 and Mad (Fig. 3A). To identify additional residues, a second cycle of single amino acid substitutions was performed this time in the context of M35L. Some combinations, such as K36H with M35L, yielded reduced levels, whereas others seemed neutral. A significant enhancement of the interaction was observed with the double mutant containing V7F (Fig. 3B). Unexpectedly, two-hybrid assays performed in the absence of Mad or in the absence of a prey showed that PAH1-M35L had gained intrinsic transcriptional activity. On top of this, intrinsic transcriptional activity displayed by PAH1-M35L, the double-mutant PAH1-V7F/M35L yielded a significantly higher β-galactosidase level that was dependent upon the presence of the Mad-SID. Apparently, the intrinsic activity of PAH1-M35L had elevated the overall activity into a measurable range, thereby facilitating detection of the V7F effect in the yeast two-hybrid assays. As observed in Fig. 3A, the interaction of PAH1-V7F with Mad-SID was not sufficient to boost β-galactosidase levels above background.

Given the “disturbing” intrinsic transcriptional activity of PAH1-M35L, we decided to re-screen for second-site mutations, this time in the context of V7F. Combining PAH1-V7F with L14V and, to a lesser extent, K39Q, significantly boosted β-galactosidase levels, whereas all other combinations remained at background levels (Fig. 3, C and E). Control experiments showed that the β-galactosidase level was strictly dependent upon the presence of Mad-SID. To test whether the affinity of the mutated PAH1 for the Mad-SID could be further improved, third-site mutations were introduced, this time in the context of PAH1-V7F/L14V (Fig. 3, D and E). As expected from the second screen, K39Q increased the β-galactosidase level (L11I and K36H showed modest effects). Interaction of PAH1-V7F/L14V/K39Q with Mad yielded β-galactosidase activity up to ~30% of that obtained with PAH2 and Mad in this yeast
two-hybrid setting. Remarkably, PAH1-L14V/K39Q yielded background levels of β-galactosidase, thus reinforcing the critical role of Phe-7 and suggesting that Val-14 and Gln-39 contribute to the strength of the interaction, but do not represent the strongest determinants.

**Phe-7 Is Critical in PAH2/Mad Interaction**—To corroborate and extend the importance of PAH2 contact residues 7, 14, and 39 in the interaction with Mad-SID, these residues in PAH2 were mutated into the amino acid present at the respective positions in PAH1 and screened for their ability to interact with the Mad-SID. Strikingly, PAH2-F7V completely abolished the interaction with Mad-SID, further substantiating its critical role (Fig. 4). Single mutations of V14L and Q39K displayed only a reduced but appreciable level of interaction between PAH2 and Mad (17.49 and 8.71%, respectively, of PAH2/Mad -galactosidase, as compared with the constrained version. These results show that free helicity plays an important role in establishing an interaction with Mad, but without a phenylalanine at position 7, unleashing of free helical properties is insufficient to support an interaction.

The interaction of the flexible Mad-SID with single PAH2 mutants V14L and K39Q or their combination yielded a 5-fold higher level of β-galactosidase, as compared with the constrained setting (Fig. 5C). Mutation of F7V again abolished the interaction, as observed in constrained conditions. Noticeably, the gain of free movement by Mad-SID tolerated and partially compensated for the loss of interaction because of V14L and Q39K, but not when the mutation F7V was present.

To extend and validate in a cell-free system the observed role of PAH residues 7, 14, and 39 in the interaction with Mad, we used the IgG pull-down assay. PAH1 and PAH2 mutants were fused to GST, purified, and incubated with fixed amounts of immobilized ProtG-Mad bound to IgG-Sepharose beads (Fig. 5D). In the context of PAH1, Phe-7 partially facilitated binding to Mad-SID, which was further enhanced with the inclusion of Val-14 and/or Gln-39. Val-14 in the context of PAH1 displayed some binding to the Mad-SID, whereas Gln-39 was unable to support an interaction. Overall, the in vitro data show that Phe-7 is the residue that contributes most for binding, followed by Val-14 and Gln-39. In the reverse situation, mutation in PAH2 of Phe-7 abolished the interaction in vitro, as observed in the yeast two-hybrid setting. Mutating Gln-39 in PAH2 slightly affected the interaction, whereas no effect could be observed when Val-14 was mutated. Combining Phe-7 and Gln-39 mutations yielded background levels as observed in vivo. Mutations combining Phe-7 and Val-14 in PAH2 resulted in a substantial interaction, whereas no effect could be observed from combining Val-14 and Gln-39. We conclude that the phenylalanine at position 7 is the specific, critical molecular determinant of complex formation between PAH2 and Mad-SID in vivo.

**DISCUSSION**

In this study, we employed NMR structural information and homology modeling to direct a mutagenesis screen and identify amino acids conveying specificity of PAH2/Mad-SID interaction. Introduction of PAH2 contact residues Phe-7, Val-14, and Gln-39 at their corresponding position in PAH1 enabled a specific interaction with the Mad-SID in vivo and in vitro. Phe-7 at the PAH1 domain restores on its own an interaction with the Mad-SID in a free helical environment, whereas Val-14 and Gln-39 stabilize this interaction. Furthermore, we showed that mutation of Val-14 and Gln-39 in PAH2 were not critical for the interaction with Mad-SID, as opposed to the change of Phe-7 into a Val, which abolished the interaction. Overall, these results demonstrate that, to a large extent, Phe-7 conveys the specificity of the interaction between PAH2 and Mad-SID in vivo in a yeast two-hybrid assay, as well as in vitro in a direct interaction assay.
drophilic-hydrophilic contacts with other PAH2 residues, such as His-36, Thr-37, Tyr-38, and Gln-42. Therefore, Gln-39 could have an important role in charge maintenance. A lysine mutation would lead to a unilateral positive charge and, as a consequence, these hydrophilic-hydrophilic interactions could be affected and disturb the folding of PAH2.

Careful inspection of the NMR structure with respect to Val-14 residue as well as CSU analysis show intermolecular contacts with conserved residues Leu-12, Ala-15, Ala-16, and Leu-19 of the Mad-SID. It has already been remarked that the short side-chain of Ala-15 and Ala-16 of the Mad-SID might play a crucial role in allowing the maintenance of the PAH2/Mad-SID interface. Val-14 fits in a hole formed by Ala-15, Ala-16, and Leu-19 of the Mad-SID (38). Replacement of Val-14 with a leucine would increase the side-chain length. This increase could generate a distance as close as 2.4 Å between Leu-14 in PAH2 and Ala-15 in the Mad-SID. It suggests that Leu-14 bumps into the Mad-SID, and thus explains our results with the PAH2-V14L mutation. The PAH1 molecular model depicts a distance as close as 2.2 Å between Leu-14 and Ala-15 and 2.6 Å between Leu-14 and Ala-15. Proper contact distances can be restored when introducing the PAH1-L14V mutation. Distance can be measured as close as 3.4 Å between Val-14 and Ala-15 and 4.4 Å between Val-14 and Ala-15 (Fig. 6), overall strengthening the stabilizing role of Val-14.

Strikingly CSU analysis of PAH2 residue Phe-7 depicts a contact with Mad-SID residue Tyr-18. Careful inspection of the surrounding of PAH1-Phe-7 in our molecular model suggests contact with the Mad-SID aromatic residue Tyr-18. A distance as close as 3.7 Å can be measured between those residues (Fig. 6). Based upon the results from our experiments, structural
information, and modeling, a stacking interaction between the aromatic rings of Phe-7 and Tyr-18 is most likely the key to the complex formation between PAH2 and Mad.

In conclusion, interaction between the Sin3 co-repressor, the tumor suppressor Mad, and other PAH interactors seems to be characterized by conserved properties such as amphipathicity and α-helicity and reach their specificity at a molecular level through residues such as Phe-7 that is present only at the PAH2 domain of Sin3. Open questions remain on understanding the specificity of PAH1, PAH3, and PAH4 and their interacting partners. Consensus sequences for PAH-interacting protein are still unsolved and subject to many questions. Ultimately, a definition of the specificity of every PAH domain can provide a new way to identify the residues required and needed in amphipathic α-helix motifs of Sin3-interacting members, enlarging our molecular view of the role of the Sin3 co-repressor complex in transcriptional repression and cell growth.

Acknowledgments—We thank Edwin Lasonder, Micha Willems, and Michel Vermeulen for GB1-MAD, GST-PAH1, and GST-PAH2 setup of the binding assay. PAH-TRX and pCP97 were generously given by Felix Hoppe-Seyler and Karin Butz. Pictures of molecular models were generated with the help of Elmar Krieger and the great graphics of Felix Hoppe-Seyler and Karin Butz. We also thank members of the Stunnenberg lab, Rein Ausland and Chris Sprong, for critical reading of the manuscript.

REFERENCES
1. Cosma, M. P. (2002) Mol. Cell 10, 227–236
2. Grunstein, M. (1997) Nature 389, 349–352
3. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) Cell 88, 341–347
4. Laherty, C. D., Yang, W. M., Sun, J. M., Davie, J. R., Seto, E., and Eisenman, R. N. (1997) Cell 89, 349–356
5. Zhang, Y., Iritani, R., Ejdremont-Bromage, H., Tempst, P., and Reinberg, D. (1997) Cell 88, 357–364
6. Kadosh, D., and Struhl, K. (1997) Cell 88, 365–371
7. Kadosh, D., and Struhl, K. (1998) Mol. Cell. Biol. 18, 5121–5127
8. Kadosh, D., and Struhl, K. (1998) Genes Dev. 12, 797–805
9. Zhang, Y., Sun, Z. W., Iritani, R., Ejdremont-Bromage, H., Tempst, P., Hampsey, M., and Reinberg, D. (1998) Mol. Cell 1, 1021–1031
10. Lai, A., Kennedy, B. K., Barbie, D. A., Bertos, N. R., Yang, X. J., Theberge, M. C., Tsai, S. C., Seto, E., Zhang, Y., Kozuchew, A., Lane, W. S., Reinberg, D., Harlow, E., and Branton, P. E. (2001) Mol. Cell. Biol. 21, 2918–2922
11. Alland, L., David, G., Shen-Li, H., Potes, J., Muhle, R., Lee, H. C., Hou, H., Jr., Chen, K., and DePinho, R. A. (2002) Mol. Cell. Biol. 22, 2743–2750
12. Fleischer, T. C., Yun, U. J., and Ayer, D. E. (2003) Mol. Cell. Biol. 23, 3456–3467
13. Vermeulen, M., Carroza, M. J., Lasonder, E., Workman, J. L., Logie, C., and Stunnenberg, H. G. (2004) Mol. Cell. Biol. 24, 2364–2372
14. Yochum, G. S., and Ayer, D. E. (2001) Mol. Cell. Biol. 21, 4110–4118
15. Zhang, J. S., Moncrieffe, M. C., Kaczynski, J., Ellenrieder, V., Prendergast, F. G., and Urrutia, R. (2001) Mol. Cell. Biol. 21, 5041–5049
16. Naruse, Y., Aoki, T., Kojima, T., and Mori, N. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13691–13696
17. Wagner, C., Dietz, M., Wittmann, J., Albrecht, A., and Schualler, H. J. (2001) Mol. Microbiol. 41, 155–166
18. Yang, S. H., Vickers, E., Brehm, A., Kouzarides, T., and Sharrocks, A. D. (2001) Mol. Cell. Biol. 21, 2802–2814
19. Yang, Q., Gong, Y., Rothermel, B., Garry, D. J., Bassel-Duby, R., and Williams, R. S. (2000) Biochem. J. 345, Pt 2, 335–343
20. Wotton, D., Knoepfler, P. S., Laherty, C. D., Eisenman, R. N., and Massague, J. (2001) Cell Growth & Differ. 12, 457–463
21. Hurlin, F. J., Queva, C., and Eisenman, R. N. (1997) Genes Dev. 11, 44–58
22. Ayer, D. E., Lawrence, Q. A., and Eisenman, R. N. (1995) Cell 80, 767–776
23. Schreiber-Agus, N., Chin, L., Chen, K., Torres, R., Rao, G., Guida, P., Skoultchi, A. I., and DePinho, R. A. (1995) Cell 80, 777–786
24. Blackwood, E. M., and Eisenman, R. N. (1991) Science 251, 1211–1217
25. Ayer, D. E., Kretzner, L., and Eisenman, R. N. (1993) Cell 72, 211–222
26. Bouchard, C., Dittrich, O., Kiermaier, A., Dohrmann, M., Gruber, A., Benkel, A., Eilers, M., and Luscher, B. (2001) Genes Dev. 15, 2042–2047
27. Xu, D., Popov, N., Hou, M., Wang, Q., Bjorkholm, M., Gruber, A., Benkel, A. R., and Henriksen, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3826–3831
28. Nair, S. K., and Burley, S. K. (2003) Cell 112, 193–205
29. Siegel, P. M., Shu, W., and Massague, J. (2003) J. Biol. Chem. 278, 35444–35450
30. McMahon, S. B., Wood, M. A., and Cole, M. D. (2000) Mol. Cell. Biol. 20, 556–562
31. Ayer, D. E., and Eisenman, R. N. (1993) Genes Dev. 7, 2110–2119
32. Larsson, L. G., Pettersson, M., Oberg, F., Nilsson, K., and Luscher, B. (1994) Oncogene 9, 1247–1252
33. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) Cell 89, 373–380
34. Alland, L., Muhle, R., Hou, H., Jr., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) Nature 387, 49–55
35. Yang, X., Zhang, F., and Kodlow, J. E. (2002) Cell 110, 69–80
36. Eilers, A. L., Billin, A. N., Liu, J., and Ayer, D. E. (1999) J. Biol. Chem. 274, 32755–32764
37. Sprong, C. A., Tessari, M., Kanaan, A. M., Janssen, J. F., Vermeulen, M., Stunnenberg, H. G., and Vuister, G. W. (2000) Nat. Struct. Biol. 7, 1100–1104
38. Brubaker, K., Cowley, S. M., Huang, K., Loo, L., Yochum, G. S., Ayer, D. E., Eisenman, R. N., and Radhakrishnan, I. (2000) Cell 100, 655–665
39. Sprong, C. A., Janssen, J. F., Tessari, M., Kanaan, A. M., Aelen, J., Lasonder, E., Stunnenberg, H. G., and Vuister, G. W. (2001) J. Biomol. NMR 19, 377–378
40. Butz, K., Denk, C., Ullmann, A., Schaeffer, M., and Hoppe-Seyler, F. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6693–6697
41. Friend, G. (1990) J. Mol. Graphics 8, 52–56
42. Krieger, E., and Friend, G. (2002) Bioinformatics 18, 315–318
Molecular Determinants of the Interaction of Mad with the PAH2 Domain of mSin3
Xavier Le Guezennec, Gert Vriend and Hendrik G. Stunnenberg

J. Biol. Chem. 2004, 279:25823-25829.
doi: 10.1074/jbc.M313860200 originally published online March 26, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M313860200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 20 of which can be accessed free at http://www.jbc.org/content/279/24/25823.full.html#ref-list-1