Domain-specific Interactions of Human HP1-type Chromodomain Proteins and Inner Nuclear Membrane Protein LBR*

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HP1-type chromodomain proteins self-associate as well as interact with the inner nuclear membrane protein LBR (lamin B receptor) and transcriptional coactivators TIF1α and TIF1β. The domains of these proteins that mediate their various interactions have not been entirely defined. HP1-type proteins are predicted by hydrophobic cluster analysis to consist of two homologous but distinct globular domains, corresponding to the chromodomains and heterochromatin. We show here that the chromodomains mediate the self-associations of HP1-type proteins and is also necessary for binding to LBR both in vitro and in the yeast two-hybrid assay. Hydrophobic cluster analysis also predicts that the nucleoplasmic amino-terminal portion of LBR contains two globular domains separated by a hinge region. The interactions of the LBR domains with an HP1-type protein were also analyzed by the yeast two-hybrid and in vitro binding assays, which showed that a portion of the second globular domain is necessary for binding. The modular organization of HP1-type proteins and LBR can explain some of the diverse protein-protein interactions at the chromatin-lamina-membrane interface of the nuclear envelope.

HP1 is a heterochromatin protein originally identified in Drosophila melanogaster, where it functions as a suppressor of position effect variegation (1, 2). HP1 and homologous proteins in other species are included in the chromo superfamily of proteins (3, 4). The identifying feature of this superfamily is the chromodomain, which was first shown to be common to HP1 and Polycomb, a Drosophila protein involved in the downregulation of homeotic selector genes during development (5).

HP1-type proteins, which are included in Class A of the chromo superfamily, contain a second domain, homologous to but distinct from the chromodomain, which has been termed the chromo shadow domain (3).

In contrast to Drosophila, the functions of the highly conserved mammalian HP1-type proteins remain mostly unknown. In humans, three HP1-type proteins that arise from different genes, termed HP1α, HP1β, and HP1γ, have been described (6–8). In higher eukaryotic cells, a portion of the transcriptionally inactive heterochromatin is associated with the nuclear envelope (9, 10), and we have demonstrated that human HP1-type proteins bind to the inner nuclear membrane protein LBR (8). LBR (lamin B receptor), which also binds to B-type lamins, has a nucleoplasmic amino-terminal domain of ∼210 amino acids and a hydrophobic domain with eight putative transmembrane segments (11–14). Besides possibly playing a role in the attachment of the nuclear lamina and the heterochromatin to the nuclear envelope in interphase, LBR may also function in targeting inner nuclear membrane vesicles to chromatin at the end of mitosis (15, 16). Mouse HP1-type polypeptides have also been shown to bind to the transcriptional activators TIF1α and TIF1β (17). TIF transcriptional coactivators interact with the ligand-binding domains of retinoic acid and other nuclear receptors and may mediate their activation functions (18). In the yeast two-hybrid assay, mouse HP1-type proteins have also been shown to self-associate (17).

The structure of HP1-type proteins suggests that different domains may mediate their different interactions. In this study, we have used hydrophobic cluster analysis (HCA) to predict the secondary structures of human HP1-type proteins and LBR. We then used the structural information obtained by using HCA to identify the domains of these proteins that mediate some of their protein-protein interactions.

EXPERIMENTAL PROCEDURES

Protein Sequence Analysis Using HCA—Guidelines for the use of this method have been published previously (19–21). HCA allows comparisons not only of amino acid sequences, but also the protein secondary structures statistically centered on hydrophobic clusters and their distributions (20). The effectiveness of HCA in predicting protein secondary structure and identifying low levels of sequence homology has been widely demonstrated (for some examples, see Refs. 22–27).

For sequence alignments, the accuracy was assessed by computing identity, similarity, and HCA scores as well as the corresponding Z-scores (27). Z-scores represent the differences between the alignment score under consideration and the mean score of a distribution com-

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1 The abbreviations used are: HCA, hydrophobic cluster analysis; PAGE, polyacrylamide slab gel electrophoresis; GST, glutathione S-transferase.

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Fig. 1. General organization of HP1<sup>hs</sup> as predicted from the analysis of its HCA plot. HCA readily detects two globular domains (boxed) of similar structure separated by a hinge region largely made up of hydrophilic amino acids. The first globular domain is the chromodomain (labeled A), and the second is the chromo shadow domain (labeled B). Protein sequences are shown on a duplicated α-helical net with amino acid positions indicated above. The contours of the hydrophobic residues are automatically drawn to form clusters that mainly correspond to the internal faces of regular secondary structures (20). Similarities between the distribution of clusters and their features (light shading) as well as sequence identities (dark shading) are readily observed between the chromodomain and chromo shadow domain, suggesting that they would adopt a similar fold. The symbols used in the plot are as follows: open square, threonine; square with dot inside, serine; diamond, glycine; and star, proline.
alignment of the tandemly duplicated domains with 10,000 alignments performed after randomization of one of the compared sequences. The Z-scores allow for evaluation of how the signal emerges from the background, with values exceeding 3.0 expected to represent an authentic relationship. The Z-identity value of 4.75, the Z-similarity value of 6.30, and the Z-HCA score of 4.72 confirm an authentic relationship between the chromodomain and the second globular domain in human HP1Hs. Similar values were obtained for HP1Hs and HP1Hs. While this work was in progress, Koonin et al. (4) and Aasland and Stewart (3), who termed the second globular domain the hinge region (Fig. 3a), showed that it contained the chromodomain plus the chromo shadow domain and its binding to HP1Hs can self-associate and bind to each other. 

**FIG. 2.** HP1Hs and HP1Hs self-associate and bind to each other. 35S-Labeled HP1Hs (lanes 1–4) and HP1Hs (lanes 5–8) were synthesized in reticulocyte lysates by in vitro translation and incubated with GST (lanes 2 and 6), GST-HP1Hs (lanes 3 and 7), or GST-HP1Hs (lanes 4 and 8) coupled to glutathione-Sepharose. Ten percent of the amount of incubated 35S-labeled protein used in each binding reaction is also shown (lanes 1 and 5). After washing, the proteins that remained bound to glutathione-Sepharose were eluted with SDS, separated by SDS-PAGE, and detected by autoradiography. Migration of molecular mass standards (in kilodaltons) is indicated on the left.

**FIG. 3.** The chromo shadow domain of HP1Hs mediates its binding to itself and to HP1Hs. A, shown is a schematic diagram of HP1Hs indicating the chromodomains and chromo shadow domain and the positions of amino acids 1, 69, 109, and 191. cDNAs for different domains of HP1Hs were cloned into pBT4 to express the polypeptides indicated by bars 1–4. The corresponding in vitro translated products of each are shown in the autoradiogram. Migration of molecular mass standards (in kilodaltons) is indicated on the left. B, 35S-labeled portions of HP1Hs shown in A were synthesized in reticulocyte lysates by in vitro translation and incubated with GST-HP1Hs (lanes 1–4) or GST-HP1Hs (lanes 5–8) coupled to glutathione-Sepharose. After washing, the proteins that remained bound to glutathione-Sepharose were eluted with SDS, separated by SDS-PAGE, and detected by autoradiography. Approximately 10–15% of the input material bound specifically to the proteins, which generated positive results. Migration of molecular mass standards (in kilodaltons) is indicated on the left.

To identify the domain of HP1-type proteins responsible for these associations, we expressed various portions of 35S-labeled HP1Hs by in vitro translation (Fig. 3A). GST-HP1Hs and GST-HP1Hs fusion proteins precipitated full-length HP1Hs (Fig. 3B, lanes 1 and 5) from reticulocyte lysates, but not the portion of HP1Hs that contained the chromodomain plus the hinge region (Fig. 3B, lanes 2 and 6). The GST fusion proteins also bound to the hinge region plus the chromo shadow domain of HP1Hs (Fig. 3B, lanes 3 and 7) and to the chromo shadow domain alone (Fig. 3B, lanes 4 and 8). Hence, the chromo shadow domain was necessary and sufficient for the self-association of HP1Hs and its binding to HP1Hs. These results are consistent with those previously reported using the yeast two-hybrid assay, which showed that the chromo shadow domain of the mouse homologue of HP1Hs interacted with mouse HP1Hs and a portion of MOD1, the mouse homologue of HP1Hs (17).

The Chromo Shadow Domain of HP1Hs Is Necessary for Binding to LBR—We used the yeast two-hybrid assay to identify the domain of HP1Hs that binds to the nucleoplasmic amino-terminal domain of LBR (Fig. 4). Full-length HP1Hs and the hinge region plus the chromo shadow domain interacted with LBR in the two-hybrid assay. The chromo shadow domain alone also gave a positive result. Neither the portion of HP1Hs that contained the chromodomain plus the hinge region nor the hinge region alone interacted with the amino-terminal domain of LBR. The chromo shadow domain was therefore necessary and sufficient for the interaction of HP1Hs with LBR in the yeast two-hybrid assay.

We also examined the binding of various domains of HP1Hs synthesized by in vitro translation, to a GST fusion protein of the amino-terminal domain of LBR (Fig. 5). 35S-Labeled full-length HP1Hs and polypeptides containing the chromodomain plus the hinge region, the chromo shadow domain plus the hinge region, and the chromo shadow domain alone were synthesized by in vitro translation in reticulocyte lysates. Full-length HP1Hs was precipitated by the GST-LBR fusion protein (Fig. 5, lane 1), as was the protein that contained the chromo shadow domain.
HP1 and LBR Interactions

The chromo shadow domain of HP1Hs is necessary and sufficient for binding to the amino-terminal domain of LBR in the yeast two-hybrid assay. Schematic diagrams of the various HP1Hs polypeptides (shaded rectangles) fused to the transcription activation domain of GAL4 (white rectangles) used in the two-hybrid assay are shown. The names of the plasmids that encoded each construct are given to the right of each schematic diagram. Plasmid pACT2 expresses the GAL4 transcription activation domain alone and was used as a negative control. These plasmids were cotransformed with pGB79-LBRAT, which expresses the amino-terminal domain of LBR fused to the DNA-binding domain of GAL4.

The panel on the right shows the results of filter assays for β-galactosidase (β-Gal) activity, in which the dark rectangles indicate detectable β-galactosidase activity. The results of a liquid culture assay to measure β-galactosidase activities are also shown, with the values given as means ± S.E. in arbitrary units (n = 3).

To confirm the results obtained in the yeast two-hybrid assay, we examined the binding of domains of LBR synthesized by in vitro translation to a GST-HP1Hs fusion protein (Fig. 8). The 35S-labeled LBR amino-terminal domain and several portions were synthesized by in vitro translation in reticulocyte lysates (Fig. 8A). The LBR amino-terminal domain was precipitated by the full-length GST-HP1Hs fusion protein (Fig. 8B, lane 1), as was the portion from amino acids 97 to 208 that contained the second globular domain, the hinge region between amino acids 124 and 208 that contained the second globular domain, and the chromo shadow domain in HP1-type proteins (8). The sequence of this segment of LBR was analyzed by HCA (Fig. 6). This analysis suggested that the amino-terminal domain of LBR contained two globular domains separated by a hinge region of ~40 amino acids. The first globular domain of LBR is located between amino acids 1 and 60. The second globular domain is roughly located between amino acids 105 and 210. The hinge region between these two globular domains is highly charged between amino acids 70 and 100. The globular domains of LBR are distinct from the chromodomain and chromo shadow domains in HP1-type proteins.

Identification of the Domain of LBR That Interacts with HP1Hs—We examined the interactions of various portions of the amino-terminal domain of LBR with HP1Hs in the yeast two-hybrid assay (Fig. 7). Full-length LBR interacted with full-length HP1Hs in this assay. The first 100 amino acids of LBR that contained the first globular domain detected by HCA plus the hinge region did not interact with HP1Hs. The portion of LBR from amino acids 97 to 208 that contained the second globular domain interacted with HP1Hs. LBR from amino acids 1 to 174 also interacted with HP1Hs, as did the stretch between amino acids 97 and 174, which contained only the first portion of the second globular domain. The region of LBR from amino acids 124 to 208 did not interact with HP1Hs. These results show that the first portion of the second globular domain identified by HCA in the amino-terminal domain of LBR is responsible for its binding to HP1Hs in the yeast two-hybrid assay.

HCA of the Sequence of the Nucleoplasmic Domain of LBR—We have previously demonstrated that the nucleoplasmic amino-terminal domain of LBR binds to human HP1-type proteins (8). The sequence of this segment of LBR was analyzed by HCA (Fig. 6). This analysis suggested that the amino-terminal domain of LBR contained two globular domains separated by a hinge region of ~40 amino acids. The first globular domain of LBR is located between amino acids 1 and 60. The second globular domain is roughly located between amino acids 105 and 210. The hinge region between these two globular domains is highly charged between amino acids 70 and 100. The globular domains of LBR are distinct from the chromodomain and chromo shadow domains in HP1-type proteins.

Identification of the Domain of LBR That Interacts with HP1Hs—We examined the interactions of various portions of the amino-terminal domain of LBR with HP1Hs in the yeast two-hybrid assay (Fig. 7). Full-length LBR interacted with full-length HP1Hs in this assay. The first 100 amino acids of LBR that contained the first globular domain detected by HCA plus the hinge region did not interact with HP1Hs. The portion of LBR from amino acids 97 to 208 that contained the second globular domain interacted with HP1Hs. LBR from amino acids 1 to 174 also interacted with HP1Hs, as did the stretch between amino acids 97 and 174, which contained only the first portion of the second globular domain. The region of LBR from amino acids 124 to 208 did not interact with HP1Hs. These results show that the first portion of the second globular domain identified by HCA in the amino-terminal domain of LBR is responsible for its binding to HP1Hs in the yeast two-hybrid assay.

To confirm the results obtained in the yeast two-hybrid assay, we examined the binding of domains of LBR synthesized by in vitro translation to a GST-HP1Hs fusion protein (Fig. 8). The 35S-labeled LBR amino-terminal domain and several portions were synthesized by in vitro translation in reticulocyte lysates (Fig. 8A). The LBR amino-terminal domain was precipitated by the full-length GST-HP1Hs fusion protein (Fig. 8B, lane 1), as was the portion from amino acids 97 to 208 that contained the second globular domain (Fig. 8B, lane 2). A polypeptide that contained the first globular domain plus the hinge region from amino acids 1 to 100 of LBR was not precipitated from reticulocyte lysates by the HP1Hs fusion protein (Fig. 8B, lane 3). LBR from amino acids 1 to 174 and from amino acids 97 to 174 (Fig. 8B, lanes 4 and 5) were also precipitated from lysates by the HP1Hs fusion protein, but the region of LBR between amino acids 124 and 208 was not pre-
precipitated (Fig. 8B, lane 6). These findings are consistent with those obtained in the yeast two-hybrid assay and show that the first portion of the second globular domain identified by HCA in the nucleoplasmic domain of LBR mediates its binding to human HP1-type proteins.

**DISCUSSION**

**Domain-specific Interactions of HP1-type Chromodomain Proteins**—We have used HCA to analyze the structures of human HP1-type proteins and have shown that the chromo shadow domain mediates the self-associations of these proteins as well as their binding to the inner nuclear membrane protein LBR. In both the yeast two-hybrid and *in vitro* binding assays, the chromo shadow domain was necessary and sufficient for the self-association of HP1\(^{H\alpha}\) and for its binding to HP1\(^{H\alpha}\). In the yeast two-hybrid assay, the chromo shadow domain of HP1\(^{H\alpha}\) was also necessary and sufficient for its interaction with LBR. However, in the *in vitro* binding assay, ~30 additional amino acids in the hinge region at the amino-terminal side were required for the chromo shadow domain to bind to LBR. The portion of HP1\(^{H\alpha}\) containing the chromodomain and these same 30 amino acids of the hinge region did not bind to LBR in either the yeast two-hybrid or *in vitro* binding assay. These additional amino acids may have been necessary for the initial portion of the chromo shadow domain to achieve a proper conformation. In the yeast two-hybrid assay, the chromodomain also contained a portion of yeast GAL4 at its amino-terminal side.

The chromo superfamily of proteins is generally divided into several classes with distinct structural features (3, 4). Members of all classes in this superfamily contain at least one classical chromodomain, which was first shown to be common to the *Drosophila* proteins HP1 and Polycomb (5). The HP1-type proteins compose one class (Class A) of the chromo superfamily. Our finding that the HP1-type proteins contain a second globular domain that is similar to the classical chromodomain was also reported by others while this work was in progress (3, 4). This second globular domain, called the chromo shadow domain, is not present in other classes of the chromo superfamily. The Polycomb-type proteins contain only one classical chromodomain, and some other superfamily members, such as CHD-1, contain two classical chromodomains. Proteins with one or two classical chromodomains and no chromo shadow domain can be considered as members of separate classes (Classes B and C, respectively). Using HCA, we have recently demonstrated that *Tetrahymena* Pbd1p contains three chromo-like domains and that it may represent a fourth class in the chromo superfamily.\(^2\)

The chromodomain and chromo shadow domain demonstrate their greatest sequence divergence in their carboxyl-terminal regions (3, 4).\(^3\) As aromatic amino acids play important roles in protein-protein interactions (34), differences in such residues

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\(^2\) I. Callebaut, J.-C. Courvalin, H. J. Worman, and J.-P. Mornon, submitted for publication.

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**FIG. 6.** General organization of the nucleoplasmic amino-terminal domain of LBR as predicted from the analysis of its HCA plot. HCA detects two globular domains (boxed) separated by a hinge region that is highly charged between amino acids 70 and 100. The first putative transmembrane segment of LBR that follows the nucleoplasmic domain stretches from approximately amino acids 210 to 230 (shaded). Details on the presentation of the data and the symbols used can be found in the legend to Fig. 1.

**FIG. 7.** The first part of the second globular domain of LBR binds to HP1\(^{H\alpha}\) in the yeast two-hybrid assay. Schematic diagrams of the various LBR polypeptides (hatched rectangles) fused to the DNA-binding domain of GAL4 (white rectangles) used in the two-hybrid assay are shown. The names of the plasmids that encoded each construct are given to the right of each schematic diagram. Plasmid pGBT9, which expresses the GAL4 DNA-binding domain alone, was used as a negative control. These plasmids were cotransformed with pACT2-HP1\(^{H\alpha}\) expressing amino acids 2–191 of HP1\(^{H\alpha}\) fused to the transcription activation domain of GAL4. The panel on the right shows the results of filter assays for β-galactosidase activity; Blue indicates detectable β-galactosidase activity, and White indicates no detectable enzyme activity. The results of a liquid culture assay to measure β-galactosidase activities are also shown, with the values given as means ± S.E. in arbitrary units (\(n = 3\)).
HP1 and LBR Interactions

The first part of the second globular domain of LBR binds to HP1<sup>His</sup><sup>14</sup> in vitro. A, CDNs for different domains of LBR were cloned into pBFT4, and the corresponding in vitro translated products of each are shown in the autoradiogram. The <sup>35</sup>S-labeled polypeptides synthesized were the full-length nucleoplasmic amino-terminal domain (lane 1) and the portions from amino acids 1 to 100 (lane 2), from amino acids 97 to 208 (lane 3), from amino acids 1 to 174 (lane 4), from amino acids 97 to 174 (lane 5), and from amino acids 124 to 208 (lane 6). Migration of molecular mass standards (in kilodaltons) is indicated on the left. B, <sup>35</sup>S-labeled portions of LBR shown in A were synthesized in reticulocyte lysates by in vitro translation and incubated with GST-HP1<sup>His</sup><sup>14</sup> coupled to glutathione-Sepharose. After washing, the proteins that remained bound to glutathione-Sepharose were eluted with SDS, separated by SDS-PAGE, and detected by autoradiography. Approximately 10–15% of the input material bound specifically to the proteins, which generated positive results. Migration of molecular mass standards (in kilodaltons) is indicated on the left.

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REFERENCES

1. James, T. C., and Elgin, S. C. R. (1986) Mol. Cell. Biol. 6, 3862–3872
2. Eissenberg, J. C., James, T. C., Foster-Hartnett, D. M., Hartnett, T., Nguyen, V., and Elgin, S. C. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9923–9927
3. Aasland, R., and Stewart A. F. (1995) Nucleic Acids Res. 23, 3168–3173
4. Koonin, E. V., Zhou, S., and Lucchesi, J. C. (1995) Nucleic Acids Res. 23, 4229–4233
5. Paro, R., and Hogness, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 263–267
6. Singh, P. B., Miller, J. R., Pearce, J., Kohthary, R., Burton, R. D., Paro, R., James, T. C., and Gauntt, S. J. (1991) Nucleic Acids Res. 19, 789–794
7. Saunders, W. S., Chue, C., Ischeb, M., Craig, C., Clark, R. F., Powers, J. A., Eissenberg, J. C., Elgin, S. C. R., and Earnshaw, W. C. (1995) J. Cell Sci. 104, 573–582
8. Ye, Q., and Worman, H. J. (1996) J. Biol. Chem. 271, 14653–14656
9. Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber, H., and Sedat, J. (1984) Nature 308, 414–421
10. Blobel, G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8527–8529
11. Worman, H. J., Yuan, J., Blobel, G., and Georgatos, S. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8531–8534
12. Worman, H. J., Evans, C. D., and Blobel, G. (1990) J. Cell Biol. 111, 1535–1542
13. Ye, Q., and Worman, H. J. (1994) J. Biol. Chem. 269, 11312–11317
14. Schulter, E., Lin, F., and Worman, H. J. (1994) J. Biol. Chem. 269, 295–306

Domain-specific Interactions of LBR—LBR was originally identified by its in vitro binding to B-type nuclear lamins (11), which has been confirmed in several subsequent studies (13, 35–37). The amino-terminal domain of LBR has also been shown to bind to double-stranded DNA in vitro (13) and more recently to HP1-type proteins (8). Using HCA, we have predicted that the amino-terminal domain of LBR can be divided into distinct subdomains. This suggests that distinct domains may mediate different protein-protein and possibly protein-nucleic acid interactions.

Indirect evidence suggests that the first 60 amino acids of LBR, which correspond to the first globular domain identified by HCA, may play an important role in its association with B-type lamins. LBR autoantibodies present in occasional patients with primary biliary cirrhosis have been shown to be anti-idiotypic to some B-type lamin autoantibodies, suggesting that they recognize a region of LBR involved in its binding to B-type lamins (35). We have mapped the minimal epitope of LBR recognized by these autoantibodies to the region of the protein between amino acids 1 and 60 (38), a region that corresponds to the first globular domain identified by HCA.

Although the entire amino-terminal domain of LBR is necessary for binding to B-type lamins in vitro (13), the data obtained on the anti-idiotypic autoantibodies suggest that its first globular domain may be critical for this interaction.

We have also shown that the stretch of LBR from amino acids 70 to 100 can bind to double-stranded DNA in vitro. Sequence-specific DNA binding has not been identified, and the physiological significance of the in vitro protein-DNA interaction is not yet known. HCA shows that the DNA-binding domain of LBR is the hinge region of the amino-terminal domain. This region does not have predicted secondary structure, is highly basic, and contains many proline residues, features that would be expected in polypeptides that can bind to DNA.

In this study, we have shown that part of the second globular domain of LBR mediates its binding to HP1-type proteins. A threonine residue in this second globular domain of LBR is phosphorylated by p<sup>34</sup>cdc<sup>2</sup>-type protein kinase during mitosis (33). Although this residue is outside of the minimal binding domain, it is located within the globular domain, and phosphorylation may alter the structure of the entire domain, including the HP1-binding region. Hence, mitotic phosphorylation of LBR on a threonine residue in the second globular domain may disrupt its binding to HP1-type proteins at the start of mitosis when the inner nuclear membrane dissociates from chromatin. Dephosphorylation at the mitosis to G<sub>i</sub> interphase (33) may activate the binding capacity, allowing for the targeting of membrane vesicles to chromatin early in nuclear envelope re-assembly (15, 16).

may explain the specific interactions of the chromo shadow domains with themselves and with proteins such as LBR (8) and the TIF transcriptional coactivators (17). In particular, Phe-167 and Tyr-168 are highly conserved in chromo shadow domains, but not in chromodomains of different proteins (3, 4). These residues may therefore be critical in the specificity of protein-protein interactions of the chromo shadow domain. On the other hand, Trp-41 of the chromodomain, which corresponds to Trp-142 of the chromo shadow domain, is conserved in almost all chromo-type domains and could be significant in their general interacting properties. The chromodomain, and various other domains in different members of the chromo superfamily of proteins, may mediate protein-protein (and possibly protein-nucleic acid) interactions different from those mediated by the chromo shadow domain. Hence, chromodomain-containing proteins may function in determining overall chromatin organization by mediating a series of hierarchical modular interactions with many other proteins and possibly DNA.
16. Collas, P., Courvalin, J.-C., and Poccia, D. (1996) J. Cell Biol. 135, 1715–1725
17. Le Douarin, B., Nielsen A. L., Garnier, J.-M., Ichinose, H., Jeanmougin, F., Losson, R., and Chambon, P. (1996) EMBO J. 15, 6701–6715
18. Chambon, P. (1996) FASEB J. 10, 940–954
19. Gaboriaud, C., Bissery, V., Benchetrit, T., and Mornon, J.-P. (1987) FEBS Lett. 234, 149–155
20. Woodcock, S., Mornon, J.-P., and Henrissat, B. (1992) Protein Eng. 5, 629–635
21. Lemesle-Varloot, L., Henrissat, B., Gaboriaud, C., Bissery, V., Morgat, A., and Mornon, J.-P. (1990) Biochimie (Paris) 72, 555–574
22. Thoreau, E., Petridou, B., Kelly, P. A., Kjiane, J., and Mornon, J.-P. (1991) FEBS Lett. 292, 26–31
23. Callebaut, I., and Mornon, J.-P. (1995) FEBS Lett. 374, 211–215
24. Henrissat, B., Callebaut, I., Fabrega, S., Lehn, P., Mornon, J.-P., and Davies, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7090–7094
25. Moriuchi, H., Moriuchi, M., Pichyangkura, R., Triezenberg, S. J., Strauss, S. E., and Cohen, J. I. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9333–9337
26. Callebaut, I., and Mornon, J.-P. (1997) Biochem. J. 321, 125–132
27. Callebaut, I., and Mornon, J.-P. (1997) FEBS Lett. 400, 25–30
28. Sakai, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1987) Science 238, 487–491
29. Laemmli, U. K. (1970) Nature 227, 680–685
30. Smith, D. B., and Johnson, K. S. (1988) Gene (Amst.) 67, 31–40
31. Fields, S., and Song, O. (1989) Nature 340, 245–246
32. Ye, Q., and Worman, H. J. (1995) Exp. Cell Res. 210, 292–298
33. Courvalin, J.-C., Segil, N., Blobel, G., and Worman, H. J. (1992) J. Biol. Chem. 267, 19035–19038
34. Clackson, T., and Wells, J. A. (1995) Science 267, 383–386
35. Lassoued, K., Danon, F., and Brouet, J.-C. (1991) Eur. J. Immunol. 21, 1959–1962
36. Simos, G., and Georgatos, S. D. (1992) EMBO J. 11, 4027–4036
37. Smith, S., and Blobel, G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10124–10128
38. Lin, F., Noyer, C. M., Ye, Q., Courvalin, J.-C., and Worman, H. J. (1996) Hepatology 23, 57–61
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