MAN1 is a vertebrate nuclear inner membrane protein that inhibits Smad signaling downstream of transforming growth factor β. MAN1 has an exposed LEM domain-containing N-terminal region (“MAN1-N”), two transmembrane domains, and an exposed C-terminal domain (“MAN1-C”). Many regions of human MAN1 are homologous to emerin, a LEM domain nuclear protein, loss of which causes Emery-Dreifuss muscular dystrophy (EDMD). To test the hypothesis that MAN1 function might overlap with emerin, we tested different polypeptide fragments of MAN1 for binding to selected partners of emerin. Our findings support this hypothesis. Blot overlay assays and co-immunoprecipitation studies showed that MAN1-C binds the transcription regulators GCL, Baf, and barrier-to-autointegration factor (BAF). BAF binding to this region, which has no LEM domain, was notable. Sequence alignments identified a potential BAF-binding motif, characterized by the conserved residues Ser-Arg-Val, in MAN1-C and two other BAF-binding proteins. The other region, MAN1-N, bound directly to BAF, lamin A, and lamin B1, supporting functional overlap with emerin. Unexpectedly, three independent assays showed that MAN1-N also bound directly to emerin. Proposed MAN1-emerin complexes are discussed in the context of EDMD disease mechanisms and potential in vivo functions.

The “LEM domain” is a conserved ~40-residue folded motif that defines a family of nuclear proteins (1, 2). Vertebrate family members include LAP2β, emerin, and MAN1 at the nuclear inner membrane and LAP2α in the nuclear interior (3) plus several uncharacterized human proteins provisionally named LEM2, LEM3, LEM4, and LEM5 (2). The only known function of the LEM domain is to bind directly to a conserved chromatin protein, barrier-to-autointegration factor (BAF)1 (4). LEM domain proteins and BAF are conserved among multicellular animal eukaryotes but absent from yeast and plants. All characterized LEM domain proteins bind to nuclear intermediate filament proteins named lamins (5). In humans, loss of emerin or dominant mutations in A-type lamins cause Emery-Dreifuss muscular dystrophy (EDMD) (6, 7). EDMD is characterized by early contractures of the Achilles, elbow, and neck tendons, slow degeneration of skeletal muscles, and conduction system defects in the heart that can be fatal. Emerin itself is not essential for viability in either humans or Caenorhabditis elegans (8). However, in C. elegans the function of Ce-emerin overlaps with “Ce-MAN1” (9), the only other membrane-anchored LEM domain protein in C. elegans, which is homologous to human MAN1 but orthologous to human LEM2 (see below). Thus, an understanding of the EDMD disease mechanism will require knowing which functions of human emerin are unique and which overlap with MAN1 or other LEM domain proteins.

MAN1 was first identified by autoantibodies from a patient with collagen vascular disease (1). MAN1 localizes to the nuclear inner membrane and has two transmembrane domains, exposing both the N- and C-terminal domains (MAN1-N and MAN1-C, respectively) to the nucleoplasm (10). In addition to MAN1 (911 residues), the human genome also encodes an uncharacterized homologous protein named LEM2 (503 residues, Fig. 1). The LEM domain (Fig. 1, hatched) and three other regions (Fig. 1, shaded as A, C, and D) are conserved between human MAN1 and human LEM2. Domain D (105 residues) in the membrane-proximal half of MAN1-C is 54% identical between MAN1 and human LEM2. Domain A (170 residues) and luminal domain C (131 residues) are conserved to lesser extents. Because human LEM2 and the C. elegans lem-2 gene product (Ce-MAN1 protein) are similar in length (503 and 500 residues, respectively) and both lack domains B and E (apparently unique to human MAN1), we concluded that Ce-MAN1 is orthologous to human LEM2, not human MAN1 as originally thought. Thus, the functional overlap between Ce-emerin and Ce-MAN1 in C. elegans (shared binding to Ce-lamin and Ce-BAF and synthetic lethality in ~100-cell embryos) (9) predicts similar overlap between human emerin, LEM2, and possibly MAN1. However, the possibility of functional overlap between human emerin and human MAN1 had not been tested.

In Xenopus embryos, the proposed MAN1 alleles named XMAN1 and SANe (Smad1 antagonistic effector) regulate dorsal-ventral axis determination (3, 11, 12). The C-terminal domain of XMAN1 antagonizes signaling by bone morphogenetic proteins by binding directly to Smad1, Smad5, or Smad8, which are downstream effectors of signal transduction pathways triggered when BMP-4 binds its receptor at the plasma membrane (3). These results suggest XMAN1 inhibits Smad-dependent changes in gene expression (11). Similar roles are proposed for human MAN1, which binds Smads and regulates signaling downstream of the bone morphogenetic proteins and other members of the transforming growth factor β superfamily of proteins (13,14). Heterozygous loss-of-function mutations in MAN1 cause syndromes characterized by increased bone density in humans (13).
We tested five known partners of emerin for binding to the N- or C-terminal domains of MAN1. Our results show that human emerin and MAN1 have extensive functional overlap and unexpectedly revealed direct binding between emerin and MAN1, implicating MAN1 as functionally relevant to the EDMD disease mechanism.

**MATERIALS AND METHODS**

Expression and Purification of MAN1-C and Antibody Production—A cDNA encoding the MAN1-C polypeptide (residues 650–910) was PCR-amplified from a HeLa cell cDNA library using forward (5′-GCC TCG AGC GTT ACA TGA AAT ATC GAT GG-3′) and reverse (5′-GCC TCG AGT CAG GAA CTT CCT TGA GAA TT-3′) primers. The resulting 805-bp fragment was digested with XhoI and cloned into the XhoI sites in the pET3b vector (Novagen). After sequence verification, the construct was transformed into E. coli strain BLR(DE3)pLysS. Purified recombinant His-tagged prelamin A and MAN1-C were covalently linked to Affi-Gel-10 beads by human MAN1 (GenBank accession number AF112299), human LEM2 (GenBank accession number NM_181336), and C. elegans lem-2 (GenBank accession number NM_064543) is shown. The LEM motif (boxed), two transmembrane domains (TM, black bar), and predicted RNA recognition motif (RRM, ladder) are indicated along with the percent amino acid identity between corresponding regions in each pair of proteins. Regions designated BRB, dried, and exposed to Hyperfilm MP (Amersham Biosciences). (Promega, Madison, WI) as described (15). Blots were washed twice in 0.1% Tween 20) containing 5% nonfat dry milk. Blots were then washed twice (5 min each) in BRB at 22–24 °C and incubated overnight with 20 Ci of 35S-labeled probe protein diluted 1:200 into BRB containing 0.1% fetal calf serum (final volume, 3 ml).35S-Labeled probes were synthesized in eukaryotic transcription-translation extracts (see “Materials and Methods”) for the binding studies below.

**RESULTS**

The aligned amino acid sequences of human MAN1 and emerin revealed the conserved LEM domain as expected (Fig. 2A, boxed) plus a region (residues 185–385) homologous to the lamin-binding region in emerin (Fig. 2A, underlined) (16). Three regions appeared unique to MAN1: residues 50–184, residues 386–548, and the C-terminal domain (Fig. 2A and B). For further study, we focused on the full N terminus (designated MAN1-N), the N terminus minus the LEM domain (N-LEM), the LEM domain alone (LEM), the potential lamin-binding fragment (MAN1-mid), and the entire C-terminal domain (MAN1-C) as diagrammed in Fig. 2B. Each polypeptide was either expressed in bacteria (MAN1-C) or synthesized and 35S-labeled in eukaryotic transcription-translation extracts (see “Materials and Methods”) for the binding studies below.

**MAN1 Binds the Globular Tails of Lamins A and B†—** Lamins anchor and retain many proteins at the nuclear inner membrane (17). The MAN1-N polypeptide is both necessary and sufficient to localize MAN1 at the nuclear envelope in mammalian cells (10) and includes the region similar to the lamin-binding region in emerin (Fig. 2A, underlined) (16). MAN1-N is thought to bind lamins above the BSA background (Fig. 3A, B; C). Each polypeptide was either expressed in bacteria (MAN1-C) or synthesized and 35S-labeled in eukaryotic transcription-translation extracts (see “Materials and Methods”) for the binding studies below.

**Affinity Bead Assays—** Purified recombinant human emerin (residues 1–222) and MAN1-C (residues 650–910) were covalently linked to Affi-Gel-10 beads and Affi-Gel-15 beads (Bio-Rad), respectively, per manufacturer’s instructions. Beads were washed three times in immunoprecipitation (IP) buffer (150 mM NaCl, 50 mM Tris, pH 8, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin). Emerin beads were incubated with HeLa cell lysates (1 μg of protein) overnight at 4 °C and washed three times with IP buffer. Bound proteins were eluted by boiling samples in 2× SDS sample buffer, resolved by SDS-PAGE, and immunoblotted using rat anti-MAN1 polyclonal serum 4279 at 1:1000 dilution. MAN1-C beads were incubated with 35S-labeled GCL (500,000 counts) overnight at 4 °C. Beads were washed three times with IP buffer, and bound proteins were eluted by boiling samples in 2× SDS sample buffer, resolved by SDS-PAGE, and exposed to x-ray film.

**Blot Overlay Assays—** His-tagged lamin, emerin, MAN1-L, and MAN1-C polypeptides were expressed in E. coli (strain BLR(DE3)pLysS). Samples were analyzed by SDS-PAGE; they were either stained with Coomassie Blue or transferred to membranes and probed with 35S-labeled polypeptides or BSA (as negative control) were immobilized in microtiter wells and probed with 25 pmol of 35S-labeled MAN1-N, 35S-labeled MAN1-mid, or 35S-labeled LEM domain (as a negative control). The LEM domain did not bind specifically to lamins as expected (Fig. 3A, LEM). MAN1-N bound both the prelamin A and lamin B1 tails at levels significantly above the BSA background (Fig. 3A, N). We concluded that human MAN1 can bind directly to both A- and B-type lamins in vitro via the lamin tail domain. The MAN1-mid fragment was insufficient to bind lamins above the BSA background (Fig. 3A, S), suggesting that the flanking regions in MAN1-N are required for this interaction. To test lamin binding by an independent method, we resolved the lamin tails by SDS-PAGE; they were either stained with Coomassie Blue or transferred to membranes and probed with 35S-labeled MAN1-C (Fig. 3B). As controls, each gel included lysates from bacteria expressing either wild-type (WT) emerin (residues 1–222; Fig. 3B) or a disease-causing emerin mutant (Δ595) that lacks residues 95–99 and fails to bind lamins (16). MAN1-C did not bind the emerin mutant Δ95 but recognized both lamin tails, particularly the B1 tail (Fig. 3B), independently confirming that MAN1 binds directly to both A- and B-type lamins. Surprisingly, MAN1-N also specifically recognized wild-type emerin (Fig. 3B); this interaction was investigated further below (see Fig. 6).
recombinant MAN1-C conjugated to Affi-Gel beads or BSA-conjugated beads as the negative control. The MAN1-C beads bound GCL significantly above the BSA background (Fig. 4A).

The equilibrium binding affinity between MAN1-C and GCL was measured in microtiter assays in which immobilized recombinant MAN1-C (3.18 pmol) was probed with increasing concentrations of 35S-labeled GCL (Fig. 4B, see “Materials and Methods”). Wells containing immobilized BSA served as nega-
B1 tail (residues 395–586) were immobilized in microtiter wells, incubated prelamin A tail (residues 94–664), or recombinant purified lamin B1.

Microtiter assays showed MAN1-C bound Btf with an equilibrium dissociation constant of 211 nM (range 124–312 nM, and N, respectively). The LEM domain was largely responsible for the BAF binding activity of MAN1-N because the N\(\alpha\)LEM signal was reduced to less than 2-fold above background (Fig. 5A, N\(\alpha\)LEM). Interestingly, MAN1-C bound BAF at levels ~4.8-fold above background (Fig. 5A, C), comparable with the LEM domain. Thus, BAF binds independently to two distinct functional domains of MAN1: the LEM domain and the “gene regulatory” C-terminal domain. We therefore considered the possibility that BAF might have two distinct interaction surfaces for MAN1.

To map BAF residues important for binding to MAN1-C, we tested MAN1-C binding to 19 BAF missense mutants previously characterized for their effects on binding to emerin (the LEM domain), DNA (20, 21), and histones.\(^{2}\) MAN1-C or BSA was immobilized on microtiter wells and probed with each \(^{35}\)S-labeled BAF mutant or wild-type BAF. Ten BAF missense mutants (K6A, K6E, R8E, D9A, G25E, I26A, L46E, G47E, V51E, and W62A) had significantly reduced binding to MAN1-C (Fig. 5B). The remaining mutants bound MAN1-C at least 50% as well as wild-type BAF. The positions of wild-type BAF residues implicated in MAN1-C binding were mapped on the atomic structure of the BAF dimer (22) (Fig. 5C).

### Results

#### MAN1 Binds Emerin

We used microtiter assays to test MAN1 for binding to different gene regulatory proteins (GCL and Btf shown here strongly supporting the “overlapping functions” model. Both transcription regulators (GCL and Btf) can also bind MAN1, demonstrating its broad versatility as an interacting protein. Collectively, these findings showed that two emerin-binding BAF-binding proteins that lack LEM domains, such as Crx and H1.1.2 Pairwise alignments of MAN1-C with Crx and histone H1.1 revealed one region comprising residues 728–735 (S(R/K)V\(\times\)tetR) in MAN1 (Fig. 5D). This SRV motif was conserved in Crx and H1.1 as the consensus sequence S(R/K)V\(\times\)t, though the specificity of these interactions remains to be determined. Hence, we propose that MAN1 binds directly to emerin and not to BAF, which might recognize a novel motif conserved in other BAF-binding proteins that lack LEM domains, such as Crx and certain other homeodomain transcription activators (23, 24), and certain histones including linker histone H1.1.2 Pairwise alignments of MAN1-C with Crx and histone H1.1 revealed one region comprising residues 728–735 (S(R/K)V\(\times\)t, tetR) in MAN1 (Fig. 5D). This SRV motif was conserved in Crx and H1.1 as the consensus sequence S(R/K)V\(\times\)t, tetR. We propose that this motif might mediate binding to BAF (see "Discussion").

### MAN1 Binds Directly to Emerin

To explore possible direct binding between emerin and MAN1, we first used purified recombinant wild-type emerin (residues 1–222) covalently linked to Affi-Gel-10 beads or BSA-linked beads to affinity-purify proteins from HeLa cell lysates. Beads were incubated overnight with HeLa cell lysates and then washed and pelleted. Bound proteins were resolved by SDS-PAGE, transferred to membranes, and probed with immune serum 4279 against human MAN1 (Fig. 6A; see "Materials and Methods"). Endogenous MAN1 has a predicted mass of 100 kDa but migrates on gels at ~80 kDa (1, 10). Serum 4279 antibodies detected endogenous MAN1 bound specifically to emerin beads but not BSA beads (Fig. 6A), consistent with the direct binding of MAN1 to emerin seen in blot overlays (Fig. 3B). Direct binding
was further tested using emerin or BSA beads incubated with \(^{35}\text{S}\)-labeled MAN1-N or wild-type \(^{35}\text{S}\)-labeled BAF as the positive control. Both MAN1-N and BAF bound to emerin beads but not BSA beads (Fig. 6B). Based on these three independent assays (blot overlay in Fig. 3B, affinity purification from HeLa cell lysates in Fig. 6A, and radiolabeled proteins in Fig. 6B) we concluded that MAN1-N binds directly to emerin in vitro.

The domains in emerin required to bind other partners (BAF, lamin A, transcription repressors, and actin) were previously mapped using a collection of Ala substitution mutations or selected EDMD disease-causing mutations in emerin (16, 18, 19, 25, 26). We used these mutants in blot overlay experiments to map the MAN1-binding region(s) in emerin. Lysates from bacteria expressing wild-type or mutant emerin (residues 19, 25, 26) were resolved in duplicate SDS-PAGE gels and either stained with Coomassie Blue to control for protein loading or probed with \(^{35}\text{S}\)-labeled Btf (upper panel). Arrows indicate the positions of each protein in the Coomassie Blue-stained gel. D, the affinity of MAN1-C for Btf was 211 nM (range 124–312 nM, \(n = 3\) sets of triplicates) determined as described in B. Bars in B and D indicate standard deviations.

**DISCUSSION**

The ability of BAF to bind LEM domains is well established, and we show here that MAN1 is no exception. We further report that BAF also binds the C-terminal domain of MAN1. C-terminal binding involves a number of surface residues in BAF not involved in binding the LEM domain (20). The wild-type BAF residues Lys-6, Arg-8, Asp-9, Gly-25, and Ile-26 (which cluster on the top and “shoulders” of the BAF dimer) and Val-51 and Trp-62 (on the bottom surface) appear critical to bind MAN1-C. In contrast, the BAF residues Lys-6 and Arg-8 are not essential to bind the LEM domain in emerin (20).

Similarly, Lys-53, which is critical to bind LEM domains, is not essential for MAN1-C. However, the D9A mutation reduces binding to both. Therefore BAF may see the LEM domain and C-terminal region in MAN1 in overlapping but distinct ways.

BAF was previously shown in blot overlay assays to bind the C-terminal domain of the *C. elegans* lem-2 gene product (Ce-MAN1) (9). Ce-MAN1 and its human ortholog, LEM2, both lack the MAN1-unique “domain E” (Fig. 1), suggesting BAF might recognize domain D, common to all three proteins.

Importantly, we identified a motif, S(R/K)Vx(t/v)(t/f)(R/K) that is conserved in MAN1-C and two unrelated proteins, Crx and histone H1.1, which also lack LEM domains but bind BAF. Crx is a homeodomain transcription activator; its SRV motif (residues 81–88) is located in the BAF-binding region of Crx (residues 34–107) (24). The E80A mutation in Crx, which alters a single residue flanking the SRV motif, disrupts Crx binding to BAF in vitro but does not disrupt Crx binding to DNA or Crx-activated gene expression (24). The SRV motif in histone H1.1 (residues 124–131) is located within the minimal region required to bind BAF (residues 108–215) (2). It is noteworthy that the SRV motif is also present in domain D, shared by Ce-MAN1 (data not shown) and human MAN1 (see Figs. 1 and 2A), which might account for the binding of Ce-MAN1 to BAF. We are currently mutating the SRV motif in MAN1-C to test its hypothetical role in binding to BAF. Regardless of motif, it is interesting to consider why BAF binds two different regions of MAN1. We

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3 Mutations that start with “m” designate a cluster of Ala substitution mutations; see Ref. 19.
speculate that BAF might either (a) bind each domain independently for different purposes or (b) bind simultaneously, bridging the two domains of MAN1 and possibly regulating MAN1 activity.

Functional Overlap and Implications of Emerin-MAN1 Complexes—Our results strongly support the hypothesis that human MAN1 and emerin have overlapping functions. MAN1 and emerin interact in vitro with many of the same binding part-

Figure 5. BAF binds two domains in MAN1; BAF residues implicated in binding to MAN1-C. A, microtiter assays show that BAF binds independently to the LEM domain and C-terminal domain of MAN1. BAF protein immobilized in microtiter wells was incubated with each 35S-labeled MAN1 polypeptide (named as shown in Fig. 2B), washed, counted, and graphed as the ratio (fold binding) of each MAN1 probe to BAF compared with BSA control. B, microtiter assay results for MAN1-C binding to WT BAF or each indicated BAF missense mutant. Purified recombinant MAN1-C protein or BSA (negative control) was immobilized in microtiter wells, incubated with 35S-labeled BAF (wild-type or mutant), washed, counted, and graphed relative to the binding of wild-type BAF to MAN1-C. Bars in A and B indicate standard deviations. C, atomic structure of BAF (22) showing BAF residues implicated in binding MAN1-C. Red, potentially enhanced binding; green, little/no binding; dark blue, ~50% reduced binding. D, partial amino acid sequences of BAF-binding regions of MAN1-C, Crx, and histone H1.1 reveal a conserved SR/K/Vx(t/v)x(t/f)(R/K) (SRV) motif, proposed to mediate binding to BAF.
ners, including BAF, A- and B-type lamins, and the transcription regulators GCL and Btf. It will be interesting in the future to determine whether emerin, like MAN1, also binds Smad proteins. Understanding the limits of functional overlap, specifically partners that bind emerin but not MAN1, will require further analysis of additional emerging partners for emerin such as splicing factor YT521-B, actin, and nesprins (25–27).

MAN1 did not have the same affinity as emerin, for all transcription factors tested. Specifically, MAN1-C binds GCL more weakly than emerin (affinities of 364 and 30 nM, respectively). This difference might be meaningful in vivo, given the presence of competing partners with higher affinity for either emerin or MAN1 (see below). However, MAN1 and emerin have similar strong affinities for Btf (211 and 100 nM, respectively), a transcription regulator. Binding between Btf and emerin appears to be relevant to EDMD disease because the EDMD-causing missense mutation in emerin, S54F, selectively disrupts emerin binding to Btf but not GCL or other tested partners (18). We are intrigued by the possibility that Btf might have even stronger affinity for joint emerin-MAN1 complexes.

Our results predict that emerin and MAN1 can form functional complexes in vivo. Consistent with this idea, MAN1 and emerin are both expressed (detectable at the level of mRNA or protein) in essentially all tissues tested (1, 28). More specifically, mRNAs for both emerin and MAN1 are present in skeletal muscle and heart (1, 28), the tissues primarily affected in EDMD. Direct support for the existence of emerin-MAN1 complexes comes from a recent analysis of native emerin-containing complexes purified from HeLa cells.4 One 500-kDa complex includes emerin, MAN1, lamin A, GCL/Btf, actin, and MAN1).

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The proposed co-binding of MAN1 and emerin has interesting implications for their in vivo functions. Their interac-

4 J. M. Holaska and K. L. Wilson, unpublished observations.
tion may positively or negatively regulate binding to shared or unique partners in vivo and thus regulate multiple downstream pathways. For example, transcription regulators (GCL, Btf) might bind more tightly to emergin-MAN1 complexes than to either protein alone. Alternatively, transcription regulators such as GCL, which binds preferentially to emergin, might disassemble the complex and liberate MAN1 for other functions. A related possibility is that emergin might positively or negatively regulate MAN1 binding to Smads. In this case, loss of emergin might disrupt MAN1-dependent Smad signaling in EDMD patients. Notably, our work showed that the EDMD disease-causing mutations Δ95 and Q133H in emergin each abolished binding to MAN1. Although the Δ95 mutation disrupts binding to all of the partners of emergin (6), the Q133H mutation is noteworthy because it selectively abolishes binding to MAN1 (our work) and actin (26) but no other tested partners (a) how interactions between MAN1 and emergin affect their in vivo functions and (b) whether MAN1-emerin interactions regulate the EDMD phenotype.

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REFERENCES
1. Lin, F., Blake, D. L., Callebaut, I., Skerjanc, I. S., Holmer, L., McBurney, M. W., Paulin-Levasseur, M., and Worman, H. J. (2000) J. Biol. Chem. 275, 4849–4847
2. Lee, K. K., and Wilson, K. L. (2004) Symp. Soc. Exp. Biol. 56, 329–339
3. Gruenbaum, Y., Margalit, A., Goldman, R. D., Shumaker, D. K., and Wilson, K. L. (2005) Nat. Rev. Mol. Cell Biol. 6, 21–31
4. Segura-Totten, M., and Wilson, K. L. (2004) Trends Cell Biol. 14, 261–266
5. Goldman, R. D., Gruenbaum, Y., Moir, R. D., Shumaker, D. K., and Spann, T. P. (2002) Genes Dev. 16, 533–547
6. Bengtsson, L., and Wilson, K. L. (2004) Curr. Opin. Cell Biol. 16, 73–79
7. Mounkes, L., Kozlov, S., Burke, B., and Stewart, C. L. (2003) Curr. Opin. Genet. Dev. 13, 223–229
8. Gruenbaum, Y., Lee, K. K., Liu, J., Cohen, M., and Wilson, K. L. (2002) J. Cell Sci. 115, 923–929
9. Liu, J., Lee, K. K., Segura-Totten, M., Neufeld, E., Wilson, K. L., and Gruenbaum, Y. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4508–4603
10. Wu, W., Lin, F., and Worman, H. J. (2002) J. Cell Sci. 115, 1361–1371
11. Osada, S., Ohmori, S. Y., and Taira, M. (2003) Development (Camb.) 130, 1783–1794
12. Raja, G. P., Dimova, N., Klein, P. S., and Huang, H. C. (2003) J. Biol. Chem. 278, 428–437
13. Hellemans, J., Prebrahnenska, O., Willsart, A., Debeer, P., Verduyn, P. C., Costa, T., Jansens, K., Menten, B., Van Roy, N., Vermeulen, S. J., Savarirayan, R., Van Hul, W., Vanhoenacker, F., Huylebroeck, D., De Paepe, A., Naeve, J. M., Vandesompele, J., Slepeman, F., Verschooren, K., Coucke, P. J., and Mortier, G. R. (2004) Nat. Genet. 36, 1213–1218
14. Lin, F., Morrison, J. M., Wu, W., and Worman, H. J. (2005) Hum. Mol. Genet. 14, 437–445
15. Mansharamani, M., Graham, D. R., Monie, D., Lee, K. K., Hildreth, J. E., Siliciano, R. F., and Wilson, K. L. (2003) J. Virol. 77, 13084–13092
16. Lee, K. K., Haraguchi, T., Lee, R. S., Koujin, T., Hirakoa, Y., and Wilson, K. L. (2001) J. Cell Sci. 114, 4567–4573
17. Zastrow, M. S., Vlcek, S., and Wilson, K. L. (2004) J. Cell Sci. 117, 979–987
18. Haraguchi, T., Holaska, J. M., Yamane, M., Koujin, T., Hashiguchi, N., Mori, C., Wilson, K. L., and Hirakoa, Y. (2004) Eur. J. Biochem. 271, 1035–1045
19. Holaska, J. M., Lee, K. K., Kowalski, A. K., and Wilson, K. L. (2003) J. Biol. Chem. 278, 6969–6975
20. Segura-Totten, M., Kowalski, A. K., Craige, R., and Wilson, K. L. (2002) J. Cell Biol. 158, 475–485
21. Harris, D., and Engelmann, A. (2000) J. Biol. Chem. 275, 39671–39677
22. Umland, T. C., Wei, S.-Q., Craige, R., and Davies, D. R. (2000) Biochemistry 39, 9130–9138
23. Chen, S., Wang, Q. L., Nie, Z., Sun, H., Lenngen, G., Copeland, N. G., Gilbert, D. J., Jenkison, N. A., and Zack, D. J. (1997) Neuron 18, 1017–1030
24. Wang, X., Xu, S., Rivolta, C., Li, L. Y., Peng, G. H., Swain, P. K., Sung, C. H., Swaaroop, A., Berson, E. L., Dryja, T. P., and Chen, S. (2002) J. Biol. Chem. 277, 43288–43300
25. Wilkinson, F. L., Holaska, J. M., Zhang, Z., Sharma, A., Manilal, S., Holt, I., Stamm, S., Wilson, K. L., and Morris, G. E. (2003) Eur. J. Biochem. 270, 2459–2466
26. Holaska, J. M., Kowalski, A. K., and Wilson, K. L. (2004) PLoS Biol. 2, E251
27. Mislow, J. M., Holaska, J. M., Kim, M. S., Lee, K. K., Segura-Totten, M., Wilson, K. L., and McNally, E. M. (1992) FEBS Lett. 325, 135–140
28. Manilal, S., Nguyen, T. M., Sewry, C. A., and Morris, G. E. (1996) Hum. Mol. Genet. 5, 801–808