The LIM-only Protein DRAL/FHL2 Interacts with and Is a Corepressor for the Promyelocytic Leukemia Zinc Finger Protein*

Members of the four-and-a-half-LIM domain (FHL) protein family, which are expressed in a tissue- and stage-specific manner, have been reported previously to function as transcriptional coactivators. One of these is the p53-inducible protein DRAL/FHL2 (where DRAL is down-regulated in rhabdomyosarcoma LIM domain protein). In this work, we identified potential binding partners for DRAL/FHL2 using an inducible yeast two-hybrid system. We present evidence of a functional interaction between the promyelocytic leukemia zinc finger protein (PLZF) and DRAL/FHL2. PLZF is a sequence-specific transcriptional repressor whose function relies on recruitment of corepressors that form part of the histone deacetylase complex involved in chromatin remodeling. DRAL/FHL2 interacts specifically with PLZF in vitro and in vivo and augments transcriptional repression mediated by PLZF. This is the first reported incidence of a bona fide FHL protein-mediated corepression and supports the notion of these proteins having a role as coregulators of tissue-specific gene expression.

Many cancers occur in association with chromosomal translocations that frequently involve genes coding for transcription factors. This structural interference results in a downstream disruption of crucial regulatory pathways such as may be involved in the control of growth, differentiation, and survival of normal cells. Regulation of such transcription factors often occurs via chromatin remodeling in that these proteins can themselves interact with components of the transcription complex such as the corepressors (NCoR/SMRT) or with histone deacetylases (HDACs). In a variant form of acute promyelocytic leukemia, the promyelocytic leukemia zinc finger protein (PLZF) and DRAL/FHL2. PLZF is fused to the retinoic acid receptor (23). Additionally, an isoform of FHL1 known as DRAL/FHL2 has been reported to enhance the transcriptional activity of the androgen receptor (24).

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Patricia McLoughlin, Elisabeth Ehler‡, Graeme Carlile$, Jonathan D. Licht‡, and Beat W. Schäfer‡

From the Division of Clinical Chemistry and Biochemistry, Department of Pediatrics, University of Zurich, CH-8032 Zurich, the Institute of Cell Biology, ETH Hönggerberg, 8093 Zurich, Switzerland, and the Department of Medicine, The Derald H. Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, New York 10029

‡To whom correspondence should be addressed. Tel.: 41-1-266-7553; Fax: 41-1-266-7169; E-mail: santer@kispi.unizh.ch.

†To whom correspondence should be addressed. Tel.: 41-1-266-7553; Fax: 41-1-266-7169; E-mail: santer@kispi.unizh.ch.

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The abbreviations used are: NCoR, nuclear receptor corepressor; HDAC, histone deacetylase; FHL, four-and-a-half-LIM domain protein; PLZF, promyelocytic leukemia zinc finger protein; DRAL, down-regulated in rhabdomyosarcoma LIM domain protein; ACT, activator of CREM in testis; GST, glutathione S-transferase; DNA-BD, DNA-binding domain; FCS, fetal calf serum; AD, activation domain; IVT, in vitro translated; CREM, cAMP-responsive element modulator.

A number of LIM-only proteins are clearly implicated in transcriptional regulation: LMO2, which interacts with GATA-1 and Tal1 and plays a definitive role in angiogenesis (17, 18); and MLP, which interacts with the myogenic transcription factor MyoD and is required for the correct development of cardiac cytoarchitecture (14, 19, 20).

Within this subgroup is a cohesive family of LIM-only proteins, which are highly homologous, are characterized by a specific arrangement of domains, bearing four complete and one N-terminal half LIM domain (FHL family), and are expressed tissue-specifically and in distinct cellular compartments. Recently, members of the FHL family were also shown to behave as transcriptional coactivators. ACT functions as a coactivator of CREM (21, 22), and DRAL/FHL2 has been reported to enhance the transcriptional activity of the androgen receptor (23). Additionally, an isoform of FHL1 known as KyoT2 interacts with RBP-J, a DNA-binding transcription factor, thus negatively regulating transcription (24). Accordingly, it is tenable that this family of FHL proteins may perform a function in transcriptional modulation.

DRAL/FHL2, the first FHL protein described, was isolated in our laboratory by virtue of its being down-regulated in rhabdomyosarcoma cells as compared with their non-malignant
Equivalent normal myoblasts (25). We have since shown that DRAL/FHL2 expression is regulated by p53 since mRNA levels are augmented by transient expression of functional p53 in rhabdomyosarcoma cells, as well as by endogenous p53 stimulated by ionizing radiation treatment. Moreover, overexpression of DRAL/FHL2 in both normal and tumor-derived cell lines efficiently induces an apoptotic program (26). Therefore, it is conceivable that DRAL/FHL2 has a role in tumor development.

In this work we present the results of a yeast two-hybrid screen with DRAL/FHL2, performed to further delineate the molecular purpose of this protein. It is noteworthy that DRAL/ FHL2 was isolated in several two-hybrid screens and is an interaction partner for the androgen receptor (23), presenilin-2 (27), CDC47 (28), several α- and β-integrin cytoplasmic tails (29), hNPP220 (30), and IGFBP5 (31). Here we demonstrate a physical and functional interaction between DRAL/FHL2 and the promyelocytic leukemia zinc finger protein (PLZF). DRAL/FHL2 acts as a corepressor for PLZF-mediated transcriptional repression, supporting the notion that DRAL/FHL2 is a tissue-specific transcriptional modulator.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—The full-length cDNA for human DRAL/FHL2 was cloned in-frame into the inducible yeast expression vector pGILDA (LexA Matchmaker Two-hybrid System, CLONTECH), as a fusion to the LexA DNA-binding domain (DNA-BD) by PCR using primers with a 5′ BamHI site and a 3′ NotI site. The following DRAL/FHL2 deletion constructs were generated by PCR also with a 5′ BamHI site and 3′ NotI site, as well as containing a Kozak consensus start and a C-terminal FLAG tag, and cloned into pDBN3.1 (Invitrogen): LIM(1–4) eliminating the N-terminal half LIM domain; LIM(0.5–2) and the half N-terminal LIM domains; LIM(3–4) the two C-terminal LIM domains of human DRAL/FHL2 and LIM(1–4) the complete single LIM domain numbered from the N to C terminus. A similar construct was designed using the full-length DRAL/FHL2 cDNA. The primers used to amplify the cDNA were confirmed by re-sequencing. To confirm specific two-hybrid interactions, transformed yeast were grown on SD/-Trp/-Leu without histidine, and incubated on ice for 30 min. Antibodies (rabbit anti-DRAL/FHL2 (25), anti-PLZF (Calbiochem)) were covalently coupled to protein A-Sepharose beads at a ratio of 1 mg/ml. Following 30 min of incubation, beads were washed three times with 50 m M Tris, pH 8.5. They were then re-suspended to 0.3 mg/ml of yeast lysates, and incubation was continued overnight at 4 °C. Beads were washed extensively (Wash 1: 50 m M Tris, pH 8.0, 0.2% Triton X-100, 500 m M NaCl; Wash 2: 50 m M Tris, pH 8.0, 0.1% Triton X-100, 150 m M NaCl, 0.1% SDS; Wash 3: 50 m M Tris, pH 8.0, 0.1% Triton X-100) prior to eluting the coupled proteins by boiling in 30 μl of SDS gel loading buffer, which were then resolved on either a 10 or 12.5% SDS-polyacrylamide gel. Resolved proteins were transferred to polyvinylidene difluoride membranes (PALL, Fluorotrans Transfer Membranes). Immunoblotting was carried out using the Tropix Western Light kit (Applied Biosystems) and anti-PLZF at 1 μg/ml or anti-DRAL/FHL2 at 1:1000, followed by 1:7500 anti-mouse or 1:10,000 anti-rabbit alkaline phosphatase-conjugated secondary antibodies (both Sigma), as appropriate.

A U937 monocytic cell line expressing PLZF under the control of the tetracycline repressor (34) was grown in the presence or absence of tetracycline. These cells were harvested with phosphate-buffered saline at 4 °C and exposed to lysis buffer (150 m M NaCl, 20 m M Tris-Cl, pH 7.4, Tween 20, plus protease inhibitors) on ice for 15 min. This suspension was centrifuged at 6000 rpm for 10 min at 4 °C. The supernatant was pre-cleared by exposure to beads bound to a nonspecific rabbit IgG (Zymed Laboratories Inc., South San Francisco) and mixed for approximately 1 h at 4 °C. The beads were then pelleted and exposed to PLZF antibodies (IgG1, isotype) (35) covalently bound to protein A-Sepharose and mixed on a rotator overnight. The pellets were washed in fresh cold lysis buffer six times, the last three times using Nonidet P-40 instead of Tween 20 in the lysis buffer. The beads were then placed in Laemmli buffer and the precipitated proteins released by boiling. This was followed by centrifugation and the phosphorylated proteins were separated by SDS-PAGE and transfer to an Immobilon-P membrane (Millipore, Bedford, MA). The blot was incubated with a 1:1000 dilution of rabbit anti-DRAL/FHL2 antibody followed by a 1:3000 dilution of horseradish peroxidase-conjugated anti-rabbit secondary antibody (Roche Molecular Biochemicals). Autoradiography was performed using the ECL chemiluminescence kit (Amersham Biosciences).

**Northern Analysis**—Northern blotting and hybridization were carried out as described previously (26) on total RNA isolated from normal human tissues from autopsy material. cDNAs of DRAL/FHL2, PLZF, and β-actin were used as probes.

**Immunohistochemistry**—Adult rat cardiomyocytes were isolated and immunostained as described previously (36). Endogenous proteins were localized with the monoclonal antibody to PLZF (Calbiochem) at 1:10 dilution and the polyclonal antibody to DRAL/FHL2 (25) at 1:100. NIH3T3 cells were fixed and permeabilized as described (26), prior to staining with anti-DRAL/FHL2 at 1:100 dilution, followed by Cy3-conjugated goat anti-rabbit (Jackson ImmunoResearch) at 1:400 dilution. Nuclei were visualized by Hoechst staining.

**Mammalian Two-hybrid Assays**—The full-length cDNA for PLZF was cut into the BamHI or EcoRI-Ac8 plasmids and inserted into a mammalian two-hybrid system VP16 activation domain expression vector (Promega Checkmate System). 293T cells were plated at a density of 3 × 10⁵ cells/60-mm dish. The following day 1 μg each of pBIND-DRAL and pACT-PLZF were transfected, together with 1 μg of the GAL4-responsive reporter plSG5. pBSV-β-Gal was included in all transfections at a concentration of 100 ng/dish to correct for transfection efficiency. After 48 h of induc-
**TABLE I** Yeast two-hybrid interactions

| LexA DNA-BD construct | LexA AD construct | Relative β-galactosidase activity |
|-----------------------|------------------|----------------------------------|
| DRAL                  | PLZF             | 690                              |
| DRAL                  | SV40 large T antigen | 0.2                   |
| p53                   | PLZF             | 0.3                              |
| p53                   | SV40 large T antigen | 1040                        |

*β-Galactosidase activity was measured in liquid assay using O-nitrophenyl β-D-galactopyranoside and is representative of three independent experiments.*

**RESULTS**

Identification of Putative Protein Interaction Partners for DRAL/FHL2—in order to identify potential binding partners for DRAL/FHL2 as well as to further delineate its molecular role, we have exploited the yeast two-hybrid system. A human adult cardiac cDNA library was selected for screening since we and others have shown previously (23, 25, 26, 37–40) that expression of DRAL/FHL2 is highest in heart muscle, and specifically the protein localizes to diverse structures such as the nucleus, focal contacts, and the Z-discs and M-bands of cardiac myofibrils (26). An inducible LexA-based two-hybrid system was used, and yeast clones were identified in the presence of the POZ and RD2 domains of PLZF interact with DRAL/FHL2, and deletion of both abrogates this interaction.

The association of DRAL/FHL2 with PLZF was further corroborated using a monoblastoid cell line, U937, expressing PLZF in a tetracycline-repressible manner (34); only when PLZF expression is induced can DRAL/FHL2 also be precipitated by the anti-PLZF antibody (Fig. 1, **IP**, −tet), suggesting an interaction between DRAL/FHL2 and PLZF both in the yeast two-hybrid system as well as in mammalian cells.

**Specific Domains of PLZF Are Required for the DRAL/FHL2 Interaction**—At this juncture, having demonstrated an interaction between the two proteins, it was instructive to attempt to define the domains required for the interaction. Therefore we first performed an in vitro protein binding assay. **35S-Labeled in vitro translated PLZF or deletion constructs thereof (Fig. 2A)** were incubated with GST-DRAL/FHL2 recombinant protein (25) immobilized on glutathione-Sepharose beads. Bound complexes were washed extensively, prior to elution and resolution by SDS-PAGE. This GST-pulldown assay provided further credence to the interaction between the full-length proteins (Fig. 2B, **lane 5**). Deleting the POZ domain of PLZF does not rescind the interaction with GST-DRAL/FHL2 (Fig. 2B, **lane 7**), and this is also true when the RD2 domain is deleted (Fig. 2B, **lane 8**); however, deletion of both of these domains drastically reduces the ability of the protein to complex with GST-DRAL/FHL2 on the glutathione beads (Fig. 2B, **lane 6**). This would imply that either the POZ or RD2 domains of PLZF interact with DRAL/FHL2, and deletion of both abrogates this interaction.

**Association of the two proteins in vivo** was further supported by immunoprecipitation experiments. Human kidney carcinoma cells were transfected with full-length PLZF and DRAL/FHL2 expression constructs; the cell lysates were then immunoprecipitated with a monoclonal PLZF antibody and immunoblotted with a polyclonal anti-DRAL/FHL2 antibody. DRAL/FHL2 was immunoprecipitated by the full-length PLZF protein only when both specific antibodies were used (Fig. 2C, **lane 1**) but not with preimmune serum (Fig. 2C, **lane 2**). We then performed additional coimmunoprecipitation experiments...
with [35S]methionine-labeled conjugated to glutathione-agarose beads. Beads were then incubated

**E. coli** schematic representation of PLZF and deletion constructs.

as well as deletion constructs of PLZF. Bound complexes were washed,

lanes 6

IVT delPOZ;

lane 5

lane 8

IVT delRD2;

lane 4

20% input IVT delPOZ;

lane 3

20% input IVT PLZF;

lane 2

20% input IVT delRD2; lane 1). The autoactivation control for DRAL/FHL2 shows a negligible level of reporter activation at 1.4-fold (Fig. 3B, lane 3), whereas PLZF does not show any intrinsic activity (Fig. 3B, lane 2). The same results were also obtained in a different cellular background with HT1080 cells suggesting that the interaction observed in this assay is independent of other tissue-specific cofactors (data not shown).

In order to reveal the functional domains of DRAL/FHL2 that mediate the interaction with PLZF, the same mammalian two-hybrid assay was used with various deletion constructs of DRAL/FHL2 (Fig. 3A). As shown in Fig. 3C, it is apparent that removing the N-terminal half LIM domain of DRAL/FHL2 has minimal effect on the avidity of the binding between DRAL/FHL2 and PLZF, reducing the activity from 14- to 9.7-fold (Fig. 3C, lanes 2 versus 3). However, the constructs LIM(0.5–2) and LIM(3–4) show a very reduced activity when cotransfected with full-length PLZF, namely only 3.6- and 1.1-fold, respectively (Fig. 3C, lanes 4 and 5), which is not related to differences in expression levels (Fig. 3E, upper panel). This would suggest that either the relevant LIM domain or domains required to mediate the interaction are internal or, alternatively, that any manipulation of the protein in any permutation eliminates the possibility of a full interaction. To examine these hypotheses, we explored the effect of single LIM domain constructs transfected together with the full-length PLZF fusion protein. No activity is observed with these single LIM constructs (Fig. 3D, lanes 3–6), despite their being expressed at similar levels (Fig. 3E, lower panel), suggesting that no single LIM domain can mediate the interaction, but instead the integrity of the complete protein seems to be required.

**PLZF-mediated Transcriptional Repression Is Enhanced by DRAL/FHL2**—Next we wanted to examine the possible functional significance of the observed interaction. As PLZF is a known transcriptional repressor (3, 33, 42), and since other proteins that interact with PLZF have proven to function in the manner of a corepressor (4), we determined if DRAL/FHL2 might have an analogous function. A reporter system composed of four high affinity PLZF-binding sites from the IL3R chain promoter upstream of a minimal promoter and a luciferase cassette has been described previously (4) (see Fig. 4A); when this construct is expressed in 293T cells, a particular level of luciferase activity is observed, which can be transcriptionally repressed by inclusion of a PLZF expression construct in the system, as shown in Fig. 4B, lane 3 (−2.9-fold repression). Addition of DRAL/FHL2 expressed from a cytomegalovirus promoter results in an enhancement of this repression up to 5.6-fold (Fig. 4B, lane 4), whereas use of DRAL/FHL2 alone has no effect on reporter activity (data not shown). This potentiation of repression was not observed when a reporter lacking the PLZF-specific binding sites was used (Fig. 4C), indicating that the effect is specific and dependent on PLZF binding. However, titration experiments revealed that a higher concentration of

![Diagram](image-url)
DRAL/FHL2 with a constant amount of PLZF results in a lower level of repression, with a peak repression level of 5.7-fold using 400 ng of DRAL/FHL2 DNA (Fig. 4D, lanes 4–7). Expression of DRAL/FHL2 in the absence of PLZF has no effect on reporter activity (Fig. 4D, lane 10).

It has been established that PLZF mediates transcriptional repression through its ability to recruit corepressors such as the NCoR, which in turn can then recruit the HDAC complex; furthermore, PLZF itself can interact with HDAC1 and -2 (42, 43). To test for the specificity of the observed repression, we included an HDAC inhibitor, namely sodium butyrate, throughout the transfection period. As expected, this eliminated PLZF-mediated repression regardless of the addition of DRAL/FHL2 (Fig. 4D, lanes 8 and 9), suggesting that augmented repression mediated by DRAL/FHL2 is also depending on the formation of a HDAC complex. To exclude any potential effects of cell background, all results have been duplicated in human fibrosarcoma cells, HT1080 (data not shown).

DRAL/FHL2 can be localized to various structures in the cytoplasm such as focal adhesions as well as the nucleus (26).
Recently it has been shown that nuclear accumulation can be enhanced through serum stimulation (47). Therefore, we wanted to investigate whether transcriptional corepression by DRAL/FHL2 can be further enhanced when DRAL/FHL2 is predominantly localized in the nucleus. Accordingly, we transfected NIH3T3 cells with the IL3R-tk-luc reporter, full-length PLZF, and full-length DRAL. After transfection and serum starvation, cells were treated for 3 h with serum and transcriptional repression analyzed. While no repression was observed without PLZF (Fig. 5A, lanes 1 and 2), serum stimulation also had no effect on PLZF repression (Fig. 5A, lanes 3 and 4). In contrast, a marked increase in corepression by DRAL/FHL2 (Fig. 5A, lanes 5 and 6; 7.7- versus 3.3-fold) was observed which was indeed paralleled by nuclear accumulation of DRAL/FHL2 (Fig. 5B). These experiments further support the corepressor activity of nuclear DRAL/FHL2.

Specificity of DRAL/FHL2 as a Cofactor for PLZF-mediated Repression—DRAL/FHL2 belongs to a subset of the family of LIM-only proteins, which are structurally related in that they possess four complete and one N-terminal half LIM domain (FHL proteins). Within this family of five proteins there exists a high degree of homology that is particularly evident within...
individual LIM domains (22). Thus we examined other FHL proteins, namely FHL1, FHL3, and ACT, for their ability to act as cofactors in PLZF-mediated repression. Cotransfection of FHL3 with PLZF also stimulates repression of the reporter to a level comparable with that obtained with DRAL/FHL2 (Fig. 6A, lane 3 versus 2), increasing repression from 1.7-fold with PLZF alone to 2.6-fold with the combination. No corepression was seen when ACT was cotransfected with PLZF (Fig. 6A, lane 4) nor when FHL1 was included in the assay (Fig. 6A, lane 5). Consequently, this suggests that corepression is specific to some members of the FHL family, namely DRAL/FHL2 and FHL3.

Having established that only FHL3 may substitute for the corepressor function of the DRAL/FHL2, we wanted to test other LIM-only proteins in order to further demarcate specificity. Three LMOs were chosen: MLP, which is involved in myogenesis (14, 19, 20); CRP2, which is related to MLP but expressed in smooth muscle (45); and PINCH, which interacts with integrin-linked kinase (12). When each of these were cotransfected with PLZF, no additional repression of the reporter was detected (Fig. 6B, lanes 3–5, respectively). Accordingly, we concluded that significant functional repression of PLZF targets is restricted to DRAL/FHL2 and FHL3 only.

Coexpression of DRAL and PLZF—Specific expression of DRAL/FHL2 has been assigned previously to cardiac muscle in terms of both mRNA and protein (23, 26, 39), and PLZF also presents expression in mouse heart (5). To corroborate these findings as well as demonstrate endogenous coexpression of DRAL/FHL2 and PLZF, we analyzed expression of these proteins in a small panel of human tissues with a Northern blot (Fig. 7A) and immunostaining of adult rat cardiomyocytes (Fig. 7B). Northern blotting indeed shows expression of both DRAL and PLZF in both skeletal muscle and heart (Fig. 7A). Immunohistochemical analysis of adult rat cardiomyocytes shows nuclear expression of PLZF (Fig. 7B, panel a), whereas DRAL is found both in the same nuclei, as well as at the Z-discs and M-bands of the cardiomyofibrils (26) (Fig. 7B, panel b), suggesting that coexpression does in fact occur, at least in cardiomyocytes.

**DISCUSSION**

While many of the primary components involved in chromatin remodeling have been recognized, there remain additional cofactors that must work in concert with transcription factors to achieve spatio-temporal variations in and control of gene expression. Here we present evidence of a functional interaction between DRAL/FHL2 and PLZF, a transcriptional repressor with a role in the control of cellular proliferation and Hox gene regulation (41, 42, 46). The interaction has been validated by a number of in vitro and in vivo assays, including yeast two-hybrid, mammalian two-hybrid, in vitro protein binding, and coimmunoprecipitation. More importantly, we can demonstrate that DRAL/FHL2 functionally enhances PLZF-mediated repression, thus propounding the notion that it may represent a novel class of tissue-specific corepressor.

From the yeast two-hybrid screen using DRAL/FHL2 as bait, one clone was selected that upon partial sequencing was found
to correspond to PLZF. Analysis of the incomplete cDNA clone revealed that it began at amino acid 100, which eliminates the POZ/BTB domain of this protein. More detailed analyses suggest that either the POZ and/or RD2 domain of PLZF is important for this interaction, since deletion of either domain alone does not affect the interaction with DRAL/FHL2 (Fig. 2, B and C), whereas deletion of both domains simultaneously abrogates binding of recombinant DRAL/FHL2 in vitro (Fig. 2B) as well as preventing precipitation of DRAL/FHL2 by the PLZF antibody in vivo (Fig. 2C). On the other hand, it appears that the full complement of LIM domains of DRAL/FHL2 is necessary for an interaction, as implied by the mammalian two-hybrid experiments (Fig. 3). The only small domain that could be deleted without affecting the interaction was the N-terminal half LIM domain. This domain is also not required for the assayed DRAL/FHL2 function and therefore remains uncharacterized. It has, however, recently been shown that the N-terminal half LIM domain is involved in Rho signaling (47).

PLZF is a known transcriptional repressor by reason of its ability to recruit corepressors such as NCoR/SMRT, Sin3A, and histone deacetylases that together form part of the Sin3A/B complex involved in chromatin rearrangement. It binds to target gene promoters, such as that of the IL3Ra chain (33) and cyclin A (42) by means of its C-terminal zinc fingers. Here we have shown that DRAL/FHL2 can enhance transcriptional repression mediated by PLZF (Fig. 4) and thus can also be considered as a corepressor.

Recently, it was shown that transcriptional activation by DRAL/FHL2 occurs concomitantly with its translocation to the nucleus as a result of stimulation of the Rho signaling pathway (47). Serum stimulation of NIH3T3 cells results in an augmentation of DRAL/FHL2-enhanced corepression of PLZF target genes (Fig. 5A), which is associated with nuclear translocation (Fig. 5B), and thus may also involve a similar signaling mechanism.

In order to examine the specificity of DRAL/FHL2-governed corepression of PLZF target genes, we employed other members of the FHL family of LIM-only proteins in the IL3Ra functional assay. To our surprise, corepression was not entirely specific to DRAL/FHL2 but was in fact shared by FHL3, which displayed a similar level of activity (Fig. 6A). Other FHL proteins assayed, ACT and FHL1, did not manifest any corepressor activity (Fig. 6A) nor did other LIM-only proteins such as MLP, CRP2, and PINCH (Fig. 6B). Thus, the ability to corepress transcription is exclusive to a tissue-specifically expressed subset of FHL proteins. Interestingly, FHL3, apart from being structurally related to DRAL/FHL2, also shares a similar pattern of expression, being expressed predominantly in skeletal muscle and in heart (48), tissues which both also express PLZF (Fig. 7). Functional redundancy might therefore explain the lack of an obvious phenotype in DRAL/FHL2 knockout mice (49), a notion that is supported by the fact that DRAL/FHL2 and FHL3 are able to interact with each other (40); DRAL/FHL2 can also interact with ACT at lower affinity (22). However, cotransfection of combinations of FHL3, ACT, and DRAL/FHL2 together with PLZF did not result in a cooperative augmentation of repression (data not shown).

Previous work suggests that FHL proteins can act as activators or coactivators of transcription: ACT, which is expressed specifically in testis (21), serves as a coactivator of CREM, and DRAL/FHL2 is a known coactivator of the androgen receptor (23). Here we observed an additional repressive function of DRAL/FHL2, suggesting that these proteins can have a dual role depending on the promoter context. There are other examples of transcription factors possessing a dual activator-repressor function, like PML which associates with CREB-binding protein to activate transcription (50) and also interacts with several corepressors and HDAC1 (51). Recently FKH1, a member of the hepatocyte nuclear factor 3/forkhead homologous gene family (HNF3/FKH), has been described to interact with nuclear receptors, exhibiting corepressor activity on steroid receptors and coactivator activity on non-steroid receptors (52). Moreover, a splicing isofrom of FHL1 bearing only the first two and a half LIM domains can interact with and negatively regulate the activity of RBP-J, a transcription factor involved in the Notch signaling pathway (24). One possible explanation for the dual role of FHL proteins in transcription might be that they act to stabilize the transcriptional complexes as a type of bridging factor. This interpretation is supported by the fact that, in addition to PLZF, we found an interaction between DRAL/FHL2 and the corepressor NCoR in our two-hybrid screen (data not shown). Three clones were isolated, all of which corresponded to the region upstream of the second receptor interaction domain (IDII) of NCoR. The POZ domain of PLZF is known to be the interface mediating the interaction with NCoR (53, 54), whereas the putative DRAL/FHL2 interacting domain would appear to be downstream of this domain (Fig. 2, B and C).

It has been reported that DRAL/FHL2 might be an interaction partner of myocyte nuclear factor, a winged-helix/forkhead protein that forms a corepressor complex with Sin3B (49, 55) which in turn can also recruit NCoR (56). Hence, it remains to be seen if DRAL/FHL2 might also take part in this transcriptional complex, making it likely that additional repressing or activating complexes containing FHL proteins will be identified in the future.

Expression of both DRAL/FHL2 and PLZF has been described previously (5, 23, 25, 26, 37–39) in cardiac tissue. This has again been substantiated by examination of mRNA expression from various tissues (Fig. 7A, lane 4). Furthermore, immunohistochemical analysis of adult rat cardiomyocytes finds both proteins endogenously coexpressed, PLZF presenting its typical nuclear-speckled pattern (Fig. 7B, panel a), corresponding to nuclear bodies where it colocalizes with LAZ3/BCL6 (57), and DRAL/FHL2 with expression both in nucleus and along the Z-discs and M-bands of the myofibrils (Fig. 7B, panel b) (26), suggesting endogenous colocalization of these proteins in the nucleus.

Up to now, a possible implication for PLZF in heart function has not been discussed. However, PLZF is also known to effect an increase in growth arrest and/or apoptosis upon overexpression in myeloid cells (41). Because another putative interaction partner of DRAL/FHL2 in myocyte nuclei, myocyte nuclear factor, can also negatively regulate cell growth (55) and suppress oncogenic transformation, one might speculate that DRAL/FHL2 participates in cellular growth control. This is supported by the initial observation that DRAL/FHL2 is downregulated in myogenic tumor cells compared with their normal counterparts (25) and that its expression can be stimulated by p53 (26). However, definitive assessment of such a function will require analysis of mutant animals that lack expression of all FHL proteins within a given expression domain.

FHL proteins appear to participate in transcriptional complexes where they can modulate tissue-specific activity of activators or repressors. It remains to be seen whether their presence is also required for the regulation of specific downstream target genes.

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