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Review

The multifunctional roles of the four-and-a-half-LIM only protein FHL2

M. Johannessen[a, *], S. Møller[a, +], T. Hansen[a, ++], U. Moens[a], and M. Van Ghelue[b, *]

[a] Department of Microbiology and Virology, University of Tromsø, 9037 Tromsø (Norway), Fax: + 47 77645350, e-mail: monaj@fagmed.uit.no
[b] Department of Medical Genetics, University Hospital, 9038 Tromsø (Norway), Fax: + 47 77645430, e-mail: marijke.van.ghelue@unn.no

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Abstract. Numerous cellular processes require the concerted action of multiple proteins that assemble in functional complexes. Protein-protein interaction domains allow specific proteins to combine with certain partners. Specificity of protein-protein association can be obtained by an interaction code predicted by conserved amino acid sequences. One of the protein-protein interaction motifs is the LIM domain, a conserved cysteine-rich module present in more than 100 different human proteins. The human four-and-a-half-LIM-only protein family consists of the members FHL1, FHL2, FHL3, FHL4 and ACT. They are expressed in a cell- and tissue-specific manner and participate in various cellular processes, including regulation of cell survival, transcription and signal transduction. Here, we review the current knowledge of the best-studied member of this family, FHL2. We describe the transcription regulation, the expression profile, the interaction partners, the subcellular localization, the biological functions and discuss the possible involvement of FHL2 in human diseases.

Keywords. FHL2; interacting proteins; cancer; transcriptional regulation.

Introduction

In 1988, a novel *Caenorhabditis elegans* transcription factor, MEC-3, containing a homeodomain and a not previously described cysteine-rich domain was characterized. This cysteine-rich domain was shortly after also identified in the *C. elegans*-lineage protein LIN-11 and the rat insulin enhancer-binding protein ISL-1 and was therefore referred to as the LIM domain, an acronym derived from LIN-11, ISL-1 and MEC-3. LIM proteins have been found in all eukaryotes examined, but are absent in prokaryotes. The consensus amino acid sequence for the LIM domain based on 135 human LIM sequences is $CX_2CX_{16-23}C/HX_{2/4}C/H/EX_2CX_2CX_{14-21}C/HX_{1/2/3}C/H/D/EX$ (with X any amino acid) and consists of two zinc fingers that each coordinate the binding of one Zn^{2+} ion. There is a striking similarity between the second zinc finger and the DNA-binding zinc finger of the GATA and nuclear receptor transcription factors, suggesting that LIM domains may interact with DNA. However, there is currently no *in vivo* evidence for direct DNA binding with the LIM domain. Conversely, LIM domains provide protein-protein binding interfaces. The presence of a LIM domain is emerging as a hallmark of proteins that can associate with both the actin cytoskeleton and the transcriptional machinery. The LIM domains can function as adaptors or scaffolds to support the assembly of multimeric protein complexes and can operate as competitors, autoinhibitors and localizers (reviewed in [1, 2]).

* Corresponding authors.
+ Present address: Department of Medical Biochemistry, University of Tromsø, 9037 Tromsø (Norway).
++ Present address: Norwegian College of Fishery Science, Department of Marine Biology, 9037 Tromsø (Norway).

Two groups independently cloned the four-and-a-half LIM – only protein 2 (FHL2) complementary DNA (cDNA) [3, 4]. The protein consists of four complete and one half LIM domain only and belongs to a family that includes the members FHL1, FHL3, FHL4 and ACT in humans. FHL2 participates in cellular processes such as regulation of gene expression, cytoarchitecture, cell adhesion, cell survival, cell mobility and signal transduction. This review focuses on the transcriptional regulation and expression pattern of the *fhl2* gene and on the biological functions of the protein. Its role as transcriptional cofactor and its possible involvement in human diseases will be highlighted.

Genetic organization and transcriptional regulation of the FHL2 gene

The human *fhl2* gene maps to 2q12-q14 and consists of seven exons, of which the first three are non-coding. Computer analysis of the promoter reveals a plethora of putative transcription factor binding sites [5, 6]. The genomic organization and the promoter region of the *fhl2* gene with possible transcription factor binding motifs are depicted in figure 1. Although the actual contribution of particular transcription factors has not been scrutinized, some evidence exists that they may be involved in the transcription of the *fhl2* gene. For example, primary myoblasts A33GM, which express wild-type p53, possess clearly detectable levels of FHL2 messenger RNA (mRNA), while rhabdomyosarcoma cells with mutant p53 display very low levels of FHL2 transcripts. Moreover, increased FHL2 mRNA levels were monitored in rhabdomyosarcoma cells stably transfected with an expression vector for a temperature-sensitive p53 mutant at a permissive temperature, while no changes were measured in the control cells at both temperatures [7]. Furthermore, SaOS-2 osteoblasts that lack functional p53 have lower FHL2 mRNA levels compared with human osteoblast expressing wild-type p53 [8]. Finally, γ-irradiation, which activates p53, provoked elevated FHL2 mRNA levels in $p53^{+/+}$ but not in $p53^{-/-}$ mouse embryo fibroblasts [7] and irradiation induced FHL2 transcript levels up to ninefold in a dose-dependent manner in human peripheral blood lymphocytes [9]. We found that co-expression of FHL2 stimulated GAL4-p53-mediated transcription in NIH3T3 cells [our unpublished results], suggesting an autostimulatory regulation of the *fhl2* promoter involving both FHL2 and p53. In a yeast two-hybrid screen no interaction between FHL2 and the region encompassing amino acid residues 72–390 of p53 could be detected, but this does not exclude that FHL2 may bind full-length p53 in mammalian cells [10].

Overexpressing the serum response factor (SRF) in $SRF^{-/-}$ embryonic stem cells (ES) identified *fhl2* as an SRF-target gene. The *fhl2* promoter contains a full consensus CArG motif (fig. 1), and chromatin immunoprecipitation studies proved that SRF binds this promoter *in vivo*. Upon ES differentiation by either removal of leukaemia inhibitory factor or by adding retinoic acid, FHL2 protein levels increased. This effect was SRF dependent, as no such induction of FHL2 protein levels was observed in $SRF^{-/-}$ ES. As FHL2 is abundantly expressed in the heart (see further), it is not surprising that cardiac-specific transcription factors may contribute in regulating the promoter activity of this gene. Indeed, the *fhl2* promoter contains putative binding sites for the homeodomain transcription factor Nkx2.5, an SRF-interacting partner and one of the earliest markers of the cardiac lineage. Nkx2.5 expression is maintained throughout the developing and in adult heart. This suggests that Nkx2.5 and SRF can affect FHL2 expression in this tissue [7, 11]. Interestingly, transcripts of both FHL2 and Nkx2.5 coincide in the developing heart [12]. Another protein involved in cardiac development is the homeodomain containing Lbx1 transcription factor. In situ hybridization studies in wild-type and

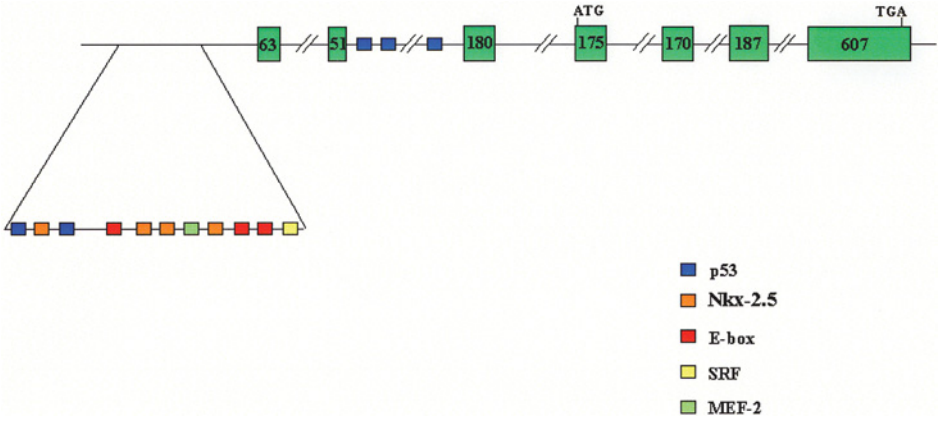

Figure 1. Schematic representation of the *fhl2*. Exons are depicted with boxes bound to each other by interrupted lines representing the introns. Numbers in the boxes indicate the size of the exons in bp. The smaller boxes in front of exon 1 and in intron 2 illustrate the presence of putative transcription factor binding sites.

Lbx1-deficient embryos at E10.5 revealed a strong decrease in FHL2 mRNA in the myocardium in Lbx1$^{-/-}$ embryos compared with wild-type embryos. These results suggest that Lbx1 may be involved in the transcriptional regulation of the *fhl2* gene [13]. However, a transcription factor database search failed to identify putative binding motifs for Lbx1 in the *fhl2* promoter [our unpublished results]. The transcription factor MEF-2 plays a pivotal role in determination and differentiation of skeletal and cardiac muscle cells. Abundant expression of *fhl2* in these tissues is in agreement with the presence of a MEF-2 binding site in the *fhl2* promoter.

Another family of transcription factors that could be involved in transcriptional regulation of *fhl2* are the E2F proteins. The *fhl2* promoter contains several E2F-binding sites and these proteins were identified as interaction partners in yeast two-hybrid screen, suggesting autoregulation of *fhl2* by its gene product through E2F [7, 14]. Autoregulation may also occur through putative AP1 sites in the *fhl2* promoter as FHL2 interacts with and stimulates AP1-dependent transcription [15]. Treatment of MCF7/BUS cells with 17β-oestradiol increased FHL2 transcript levels, despite the lack of putative oestrogen-response elements in the *fhl2* promoter [16]. Not yet identified long-distance oestrogen-response element-binding motifs may mediate 17β-oestradiol-induced *fhl*2 promoter activity. Alternatively, AP-1 sites may be involved in mediating oestrogen-induced expression of FHL2, as it is known that oestrogens may exert their effect through AP1 [17].

Whereas serum and ultraviolet (UV) radiation can induce transcription of the *fhl2* gene, neither the cyclic AMP (cAMP) elevating agent forskolin nor Ca^{2+} affected transcription levels [15]. The unresponsiveness of the *fhl2* promoter to increased cAMP levels was confirmed by another group who showed that the mRNA levels of FHL1 and FHL3, but not FHL2, were upregulated in dibutyryl cAMP exposed human A673 neuroepithelial cells [18]. Moreover, several studies have tried to identify cAMP-responsive genes, but none of these have identified *fhl2*, underscoring that cAMP does not influence the expression of this gene [19].

Other extracellular stimuli that induce *fhl2* expression included reoviral infection, hypertonicity, heat and ischaemia. Hyperosmolality or oxidative stress did not affect *fhl2* transcription levels [20–22]. The signalling pathways and *fhl2* promoter elements that mediate stimulus-induced elevation of FHL2 transcripts as well as the biological relevance remain elusive.

Studies aimed at understanding the regulation of FHL2 expression, including chromatin immunoprecipitation and promoter mutation studies, may provide further insight into and proof of the transcription factors and signalling pathways involved in transcription regulation of the *fhl2* gene.

The FHL2 protein

Structure and regulation

The *fhl2* gene is transcribed into an ~1.5-kb transcript that is translated into a protein of 279 amino acids. The FHL2 protein is evolutionally conserved and orthologs to human FHL2 are found in organisms others than mammalians such as *C. elegans*, *Drosophila* and amphioxis (fig. 2). Amino acid sequence comparison between human FHL2 and the other members of the FHL family reveals that hFHL2 is 47.9% identical with hFHL1, 51.8% with hFHL3, 47.1% with mFHL4, and

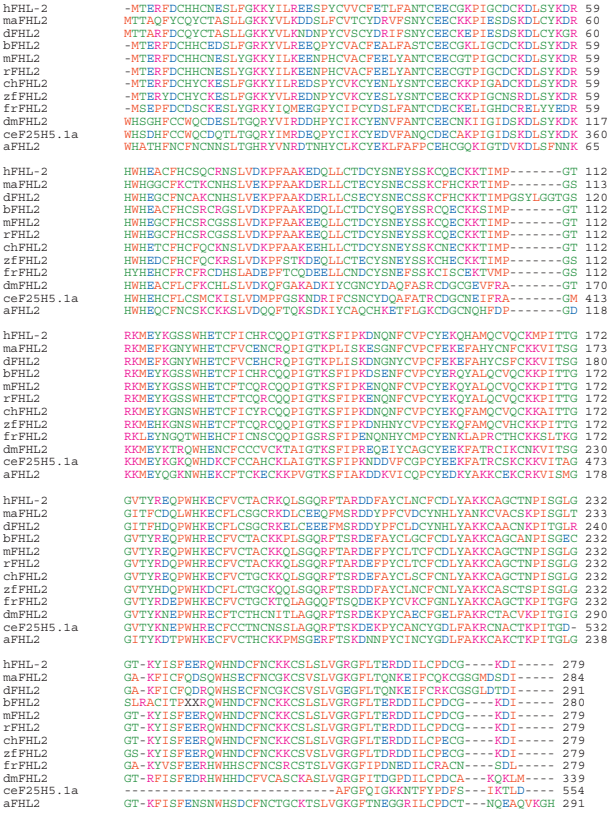

Figure 2. Sequence comparison of FHL2 proteins and homologues. Full-length protein sequences were used except for *C. elegans, Drosophila melanogaster and Branchiostoma floridae*. The alignment was produced using the CrustalW alignment program. Red colour represents small and hydrophobic amino acids (A, V, F, P, M, I, L, W), blue colour symbolizes acidic amino acids (D, E), magenta corresponds to basic amino acids (R, H, K), while green colour represents hydroxyl, amine and basic amino acids (S, T, Y, H, C, N, G, Q) [23]. The alignment was performed between the following sequences: hFHL2, human FHL2 (Q14192); maFHL2, macaque (*Macaca fascicularis*) BAE01018.1; mFHL2, mouse (*Mus musculus*) NP_034342.1; rFHL2, rat (*Rattus norvegicus*) NP_113865.1; bFHL2, bovine (*Bos Taurus*) XP_587087.1; dFHL2, dog (*Canis familiaris*) XP_532237.1; chFHL2, chicken (*Gallus gallus*) XP_416924.1; frFHL2, frog (*Xenopus laevis*) AAH73254.1; zfFHL2, Zebrafish (*Danio rerio*) AAH78393.1; aFHL2, amphioxis (*Branchiostoma floridae*) AAC69756.1; dmFHL2, *Drosophila melanogaster* AAF49396.2; ce F25H5.1a, *Caenorhabditis elegans* CAI46552.1.

Table 1. Expression pattern of FHL2 in adult human tissue as detected by different methods.

Tissue	Detection method					Reference
	Northern blot	RT-PCR	dot blot	in situ	Western blot	
Adrenal gland		+				7
Bladder		+				7
Brain	+					10
	–					7, 12
Cerebellum	–	–				10, 18
Colon		+			7	
Cortex		+				18
	–					7
Heart		+		+	+	7, 10, 12, 29–33
Kidney	+		+			7, 10
Liver	+					10
	–					12
Lung	+					10
	–				–	12
Mammary gland			+			7
Ovary	+		+		+	7, 34
Pancreas	+					10
Placenta	+		+			7, 10
Prostate			+			7
Skeletal muscle	+		+			4, 7, 10, 12
Small intestine			+			7
Stomach			+			7
Testis			+			7
Thyroid gland			+			7
Trachea			+			7

58.5% with ACT [24]. The calculated molecular mass for FHL2 is 32, 192 Da, which corresponds well with the estimated 32-kDA protein detected by FHL2 specific antibodies [4, 7, 25]. In addition, specific immunoreactivity was observed for a 25-kDA peptide, which could be a proteolytic fragment, because FHL2 contains the sequence YEKQHAMQ between the LIM2 and LIM3 domains with lysine as a potential residue for cleavage. The biological role of this cleavage remains unknown, but fragmentation of FHL2 may be a way to disconnect assemblies of specific FHL2-interacting proteins [26]. Indeed, some FHL2-interacting proteins require full-length FHL2 (see below) and proteolytic cleavage may facilitate loss of interaction. FHL2-specific antibodies have also been reported to recognize a 41-kDA protein. The increased molecular mass of FHL2 may be due to post-translational modifications, as FHL2 contains several potential phosphorylation sites and one O-glycosylation site [26]. However, Muller and co-authors failed to detect phosphorylation of FHL2 [27]. Despite the specific interaction between FHL2 and the mitogen-activated protein kinase ERK2 *in vitro* and *in vivo*, FHL2 is not phosphorylated by ERK2 [28]. Other protein modifications, including ubiquitination, sumoylation, methylation and acetylation are also possible, but have to date not been reported.

FHL2 expression pattern in human tissues

FHL2 expression is most abundant in the heart, but FHL2 is also observed in several other organs (table 1). FHL2 was not expressed in spleen, thymus, blood leukocytes or skin. Moreover, FHL2 mRNA was virtually not detected in skeletal muscle or in smooth muscle from different tissues including the stromal smooth muscle cells of the prostate [4, 25, 34]. While low levels of FHL2 transcripts could be detected by Northern blotting in brain, liver and lung [10], no FHL2 mRNA was monitored by slot blot hybridization in these tissues [7, 12]. The cortex was positive for FHL2 expression as assayed by reverse-transcription polymerase chain reaction (RT-PCR) and Northern blot [18], but slot blot hybridization could not confirm these findings [7]. Different detection methods may explain the discrepancies in the observed FHL2 expression in various tissues by the different research groups. FHL2 expression has also been examined in a wide variety of cell lines (see supplementary table).

Subcellular localization of FHL2

Subcellular localization studies with ectopic expressed FHL2 in a wide variety of cell lines revealed a cell-specific distribution. In some cells (A33 primary myoblasts, RD and HeLa) FHL2 was predominantly nuclear, while in others (Rh30, C2C12 cells, HepG2, H9C2, HEK293, D145) a more uniform distribution was observed [4, 7,

Table 2. FHL2-interacting proteins.

	Interacting domain		Detection method[1]	cDNA library	References
	FHL2	Partner			
Receptors					
integrin α_{3A}	LIM2–4[2]	NT[3]	Y2H, CoIP	human placenta	31, 43
integrin α_{3B}	LIM2–4	1022–1051[4]	Y2H	human placenta	31, 43
integrin α_{7A}	LIM2	1127–1138	Y2H, *in vitro*, CoIP	human placenta	31, 43
integrin $\alpha_{7\beta}$	LIM1–4	1104–1171	Y2H, *in vitro*, CoIP	human placenta	31
integrin β_{1A}	intact	C-terminal domain	Y2H, CoIP	human placenta	31, 43
integrin β_{1D}	LIM1–4	C-terminal domain	Y2H	human placenta	31, 43
integrin β_2	NT	C-terminal domain	Y2H	human placenta	43
integrin β_{3A}	NT	C-terminal domain	Y2H	human placenta	43
integrin β_6	NT	C-terminal domain	Y2H	human placenta	43
minK	LIM1–4	C-terminal 63 aa	Y2H,*in vitro*	mouse brain	44, 45
presenilin-2	intact	269–298	Y2H,*in vitro*, CoIP	human fetal brain	10
type IIa Na/P_i cotransporter	NT	563–637	Y2H[5]	adult mouse kidneys	46
Structural proteins					
α-actin ACTA1	LIM1+2 (weak) LIM3+4 (strong)	NT	Y2H[6]		47
titin	NT	N2B and is 2 regions	Y2H, *in vitro*, CoIP	human cardiac cells	48
Enzymes					
adenylate cyclase	NT	NT	*in vitro*		48
calpain	NT	large subunit (domain II)	Y2H	human heart	49
MM-creatine kinase	NT	NT	Y2H,*in vitro*	human cardiac cells	48
ERK2	>1 LIM domain	12–358	Y2H, *in vitro*, CoIP	adult mouse heart	28
phosphofructokinase	NT	NT	*in vitro*		48
pp125FAK	NT	NT	*in vitro*, CoIP		34
tumour necrosis factor-α converting enzyme (TACE)			Y2H		50
Transcription factors and cofactors					
ACT	NT	NT	Y2H[6]		51
androgen receptor	LIM1/2+LIM1–2	intact AR	Y2H, *in vitro*, CoIP	human prostate	29
	LIM3–4	NT	*in vitro*		38
β-catenin first armadillo repeat	LIM1–4	N-terminal 132 aa+ skeletal muscle cells	Y2H, *in vitro*, CoIP	differentiated	37
	intact FHL2	armadillo repeats 1–9	Y2H, *in vitro*, CoIP	HeLa	38
γ-catenin	LIM1–4	N-terminal 132 aa + first armadillo repeat	Y2H, *in vitro*, CoIP	differentiated skeletal muscle cells	37
CBP	LIM2–3	1–1099+1620–1877	*in vitro*, CoIP		39
E2F1–4	NT	N-terminal region	Y2H	human placenta	14
estrogen receptor	NT	NT	Y2H	fetal brain	52
estrogen receptor-α	NT	1–262	Y2H	human heart	53
FHL1[7]	NT	NT	*in vitro*, CoIP		31, 48
FHL2	LIM3 + LIM4	NT	Y2H, CoIP	human placenta	31, 48, 51
FHL3	intact	LIM2	Y2H[6], CoIP		31, 48, 51
c-FOS	NT	NT	*in vitro*, CoIP		15
FOXO1	LIM2	1–150	Y2H, *in vitro*, CoIP	human prostate cancer	54
Hand1	NT	BHLH domain	*in vitro*, CoIP		30
IGFBP-5	158–279	NT	Y2H, *in vitro*, CoIP	U2OS cells	8
c-JUN	NT	1–100	*in vitro*, CoIP		15
myocyte Nuclear factor (MNF)	NT	NT	Y2H	murine cardiac cells	12
NCoR	NT	NT	Y2H	human cardiac cells	55
p300	NT	1–670 are dispensable	*in vitro*, CoIP		39
proline-, glutamic acid-, and leucine-rich protein-1 (PELP1)	LIM3 + LIM4	1–400	Y2H, *in vitro*, CoIP	not mentioned	56
PLZF promyelocyt	LIM1–4	POZ plus RD2	Y2H, *in vitro*, CoIP	human cardiac cells	55

Table 2. FHL2-interacting proteins (Continued).

	Interacting domain		Detection method[1]	cDNA library	References
	FHL2	Partner			
Transcription factors and cofactors					
leukemia zinc finger protein					
Runx2/Cbfa1	LIM3–4 C-terminal region	RUNT domain +	*in vitro*, CoIP		57
RXR	NT	NT	Y2H	fetal brain	53
serum response factor (SRF)	LIM3–4 dimerization domain	DNA binding +	*in vitro*, CoIP		11
SKI	1/2LIM+LIM1	99–274	Y2H, CoIP	human melanoma	58
Thyroid hormone receptor (TRβ2)	NT	N-terminal is required but not sufficient	Y2H,*in vitro*	fetal brain	53
WT1	LIM1/2+LIM1–2	182–298	Y2H[6], *in vitro*, CoIP		36
Splicing factors					
hNP220	LIM2–4	1588–1978	Y2H	nasopharyngeal cancer cells	33
PTB-associated splicing factor (PSF)	LIM1/2	RRM2	Y2H	HeLa cells	35
DNA replication and repair					
BRCA1	3 LIM domains	BRCT2	Y2H, *in vitro*, CoIP	human ovary	59
hCDC47	LIM1/2+LIM2–4	592–719	Y2H, *in vitro*	HeLa cells	40, 60
TACC1	NT	NT	Y2H	mammary epithelium	59
Miscellaneous					
AIBP80	LIM1/2+LIM1–2	NT	Y2H, CoIP	human placenta	43
BIN1	NT	NT	Y2H	human placenta	43
clathrin assembly lymphoid myeloid leukemia protein (CALM)	NT	294–335	Y2H	not mentioned	62
TRAF2	NT	NT	Y2H, CoIP	mouse embryo	63
TRAF4	NT	NT	CoIP		63
TRAF6	intact FHL2	TRAF domain	CoIP		63
TUCAN/CARD8 CARDINAL	NT	179–341	Y2H, CoIP	HeLa cells	64

[1] Y2H, yeast two-hybrid; *in vitro*, GST-pull down or *in vitro*, translated proteins; CoIP, coimmunoprecipitation.
[2] Refers to the LIM domains: LIM1/2 is the half LIM domain, LIM1, LIM2, LIM3, LIM4, the first, second, third and fourth LIM domains respectively
[3] NT, not tested
[4] Numbers refer to amino acid residues in the protein.
[5] GST pull down failed to show interaction between FHL2 and type IIa Na/P_i cotransporter.
[6] Yeast two-hybrid screen with specific target protein instead of cDNA library.
[7] Samson et al. failed to find interaction between FHL1 and FHL2 in a yeast two-hybrid assay, but both proteins coimmunoprecipitated in extracts from HEK293 cells transfected with GST- and myc-tagged FHL proteins [30].

33, 35–40]. Coexpression studies with FHL2-GFP and FHL3-BFP in C2C12 cells demonstrated that both proteins localized in the mitochondria [41]. However, studies with ectopic expressed FHL2 may have some pitfalls. The FHL2 concentrations in transfected cells may be too high for limiting concentrations of interacting proteins that direct the subcellular localization of FHL2 under normal conditions, or the fused tag may affect the subcellular localization pattern of FHL2. Nevertheless, studies with specific antibodies in C2C12, H9C2, HeLa S3, HepG2 and human fibroblast Wi26 cells confirmed that endogenous FHL2 resides in both the cytoplasm and the nucleus [30, 36, 39, 41, 42].

The molecular mass of FHL2 (32-kDA), which is well below the cut-off (50-kDA) for active transport through the nuclear pores, may explain the lack of an apparent nuclear localization signal and/or nuclear export signal and could lead to passive shuttling between the cytoplasm and the nucleus. Several groups addressed the possibility that subcellular distribution of FHL2 is stimulus-regulated. Serum and the serum components lysophosphatidic acid and sphingosine-1-phosphate stimulated nuclear translo-

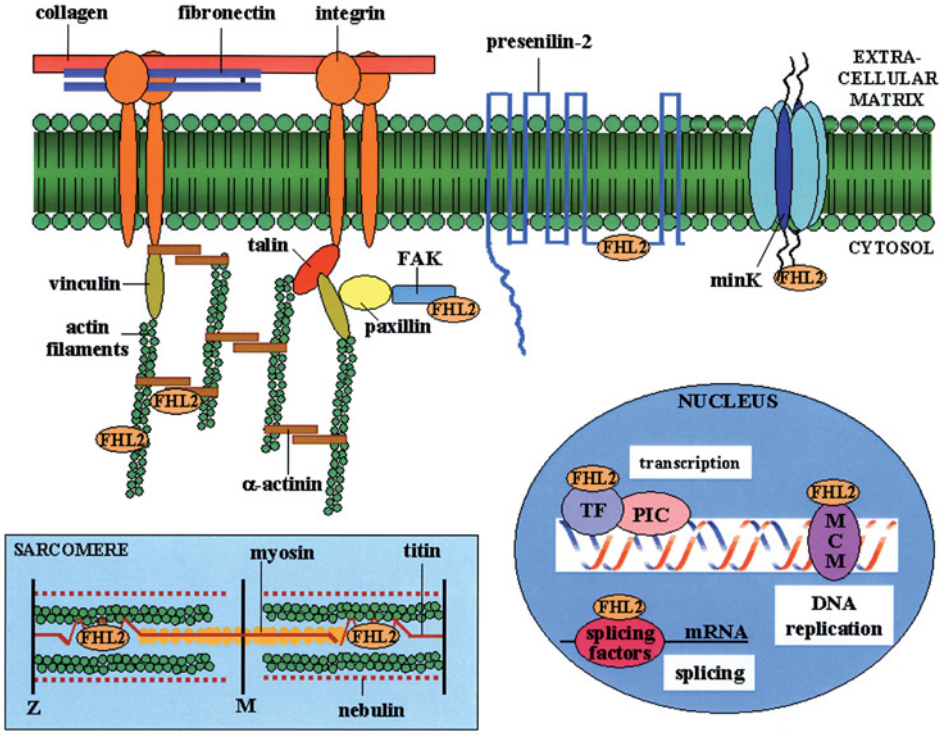

Figure 3. FHL2 is a multifunctional protein that resides in different subcellular compartments. A schematic presentation of an idealized cell depicts the localization and some of the biological processes in which FHL2 seems to be involved. FHL2 can interact with the integrin, presenilin-2 and voltage-gated K+-channel receptors. Through its association with signal transducers, including ERK2, FAPp175 and adenylate cyclase, FHL2 can influence the transmission of signals to transcription factors and cofactors, thereby affecting gene expression. FHL2 may also modulate splicing, DNA replication and DNA repair through its physical interaction with proteins participating in these processes. Moreover, FHL2 can bind structural proteins such as β-actinin, actin and titin. See text for further details.

cation of FHL2 in a ROCK/Rho GTPases-dependent way [15, 27, 40, 41]. UV treatment of NIH3T3 cells also induced nuclear translocation of ectopic Myc-FHL2, but the mechanism was not investigated [15]. Stimulus-regulated subcellular localization of FHL2 suggests a role for FHL2 in signalling transduction, a role that is further supported by the variety of proteins that can interact with FHL2 (see next section).

FHL2 interaction partners

FHL2 can interact with more than 50 different proteins, which belong to different functional classes, including receptors, structural proteins, signal transducers, transcription factors and cofactors, splicing factors, DNA replication and repair enzymes, and metabolic enzymes (table 2). The functional relevance for most of these interactions remains unsolved. In the next section some of the cellular functions of FHL2 and the participation of FHL2-interacting partners will be discussed. Figure 3 summarizes the subcellular localization of FHL2, some of the functional classes of FHL2-interacting partners and the cellular processes in which FHL2 is implicated.

Functions of FHL2

FHL2 and heart physiology

To exploit the expression of FHL2 in developing mice, Chu and co-workers and Kong and colleagues introduced the *lacZ* gene in the *fhl2* locus by homologous recombination. Whole-mount β-galactosidase assays are a sensitive method that allows monitoring FHL2 expression at a single-cell level. These expression assays were supplemented with in situ hybridization on endogenous FHL2 mRNA. The results showed high expression in embryonic heart compared with other organs. Expression of FHL2 in embryonic heart was highest in the ventricular septum and areas adjacent to the atrio-ventricular ring. FHL2 was also detected in developing vasculature, including the aortic arch arteries, dorsal aorta, and intersegmental arteries and in all compartments of the heart. [12, 65]. FHL2 mRNA and protein are also abundantly expressed in the adult heart (table 1 and references therein). These expression patterns suggest that FHL2 may play a role in the development of the cardiac septa and the cardiac circulatory system, and point to a crucial role for this protein in cardiac physiology. It was also tested whether the expression of the *fhl*1, *fhl*2, and *fhl*3 genes was altered

during hypertrophy and diluted cardiomyopathy. While *fhl*1 mRNA expression was significantly upregulated compared with control normal hearts, no changes were detected for FHL2 and FHL3 [65]. Both groups pursued their studies on a role of FHL2 in cardiac development and function by generating a *fhl*2 null mouse. Surprisingly, these mice were viable and had no detectable abnormal cardiac phenotype. The knockout mice had normal heart functions, and the heart and blood vesicles developed normally. There were no differences in the heart-weight/body-weight ratio compared with heterozygous and wild-type groups of mice. The *fhl2*$^{-/-}$ mice responded as homozygous wild-type to acute pressure overload induced by transverse aortic constriction. Moreover, the frequency of homozygous *fhl2* null mice born was consistent with a predicted Mendelian distribution. In addition, FHL1 and FHL3 mRNA levels were not upregulated in *fhl2*$^{-/-}$ mice compared with wild-type mice, suggesting that FHL1 and/or FHL3 do not compensate for the loss of FHL2 [42]. Kong and co-workers showed that sustained β-adrenergic stimulation, which is known to provoke hypertrophy in normal mice, resulted in an exaggerated hypertrophic response in the *fhl*2 null mice. The exaggerated hypertrophic reaction in knockout mice was associated with a greater activation in expression of atrial natriuretic factor, a well-characterized molecular marker of cardiac hypertrophy. Moreover, after treatment with the β-adrenergic agonist isoproterenol, heart-weight/body weight ratios were significantly increased in the mutant mice compared with wild-type mice [12].

Purcell and co-workers found that FHL2 interacts with the mitogen-activated protein (MAP) kinase ERK2 and negatively interferes with signal-induced nuclear accumulation of ERK2. The MAP kinase MEK1/2-ERK1/2 signalling pathway is implicated as an important signal transducer in hypertrophic growth response within cardiomyocytes and the intact heart. Therefore, FHL2 may antagonize the cardiac hypertrophic response induced by activated ERK2, its transcription factor substrates and their target genes in wild-type mice, while mice lacking FHL2 will display higher ERK2 activity following stimulation and hence perturbed hypertrophy responses [28]. It has also been proposed that FHL2 may modulate ventricular functions via structural remodelling and/or alternations in energy consumption in response to hypertrophic stimuli [32].

FHL2 may also participate in repolarization in cardiac cells through its interaction with minK, a β subunit of voltage-gated K^+ channel. Indeed, expression of the a subunit KvLQT1 and the β subunit minK of K^+ channels in cells that lack endogenous FHL2 protein generated I_{Ks} only when FHL2 was coexpressed, while antisense FHL2 suppressed I_{Ks} in cells that contain endogenous FHL2. A possible task for FHL2 is to link minK to the cytoskeleton [45].

Another indication for a role of FHL2 in cardiac functions derives from a clinical study examining FHL2 protein levels in biopsy samples of the right atrium of 33 patients before and after cardiopulmonary bypass. In 17 patients, positive FHL2 expression was detected, and 11 of them had elevated FHL2 protein levels after cardiopulmonary bypass. The mechanisms involved in the induction of FHL2 as well as the biological consequences are not understood, but suggest a correlation of FHL2 with myocardial injury [32].

FHL2 and bone formation

FHL2 was found to be strongly expressed in human osteoblasts, and FHL2 transcript levels increased threefold during differentiation of mouse bone marrow cells into osteoblasts, while the synthetic glucocorticoid dexamethasone, an inhibitor of bone formation, decreased FHL2 levels [8, 65]. These indications that FHL2 may play a role in osteogenesis are underscored by a recent study with mice deficient in FHL2. A closer examination of the *fhl2* null mice generated by Kong and colleagues [12] revealed that such mice developed osteopenia due to decreased activity of the osteoblasts, while the number of osteoblast and osteoclasts remained unchanged compared with wild-type littermates. Transgenic mice with enforced overexpression of FHL2 in osteoblasts had increased bone mass compared with wild-type mice, while the osteoblast and osteoclast numbers again remained unchanged in these FHL2 knock-in mice [57]. Participation of FHL2 in bone morphogenesis was recently confirmed by the work of Bai and colleagues. RANKL, ligand for receptor activator of NFκB (RANK), is the key osteoclastogenic cytokine. RANK mediates signalling through different tumour necrosis factor (TNF) receptor-associated factors (TRAF). The authors demonstrated that FHL2 acts as a suppressor of TRAF6-mediated RANK signalling by binding TRAF6 and displacing it from RANK. By ablating TRAF6/RANK/NFκB signalling, FHL2 attenuated osteoclast formation and cytoskeletal organization. As a consequence, osteoclastogenesis and bone resorption were accelerated in FHL2$^{-/-}$ mice [63]. The molecular mechanism underlying FHL2 participation in bone formation is discussed below.

FHL2 and muscular function

Several experimental results point to a role for FHL2 in muscular processes. Stable expression of FHL2 in mouse myoblasts induced differentiation of myoblasts into myotubes [37]. Moreover, FHL2 interacts with titin, a protein that plays a crucial role as organizer of the sarcomere (fig. 3), and functions as an adaptor molecule that links the metabolic enzymes MM-creatine kinase, adenylate cyclase and phosphofructokinase to titin, thereby helping

to recruit metabolic enzymes needed for energy provision during muscle contraction. [48]. A major task of integrins is adhesion of cells to the extracellular matrix and supporting cell migration. In cardiac muscle, FHL2 has been shown to interact specifically with certain integrin receptors at cell adhesion sites at the periphery of Z-discs, but cells stably overexpressing FHL2 had no changes in migration velocity or cell attachment compared with control cells. So far, the biological relevance of this interaction remains unsolved, but FHL2 may play a role in mechanical stabilization of muscle cells, as binding of FHL2 may provide a stronger connection of the receptor to the cell interior [31].

FHL2 and placental development

In order to isolate differentially expressed genes during placental development, divergence of gene expression profiles between embryonic and mouse extraembryonal tissue and trophoblast cells was studied. Among others, FHL2, c-Fos and JunB transcripts were detected in placental tissue [66]. The biological implication was not addressed, but FHL2 is a coactivator for AP1 and has been suggested to act in a time-coordinated manner to stimulate AP1-mediated transcription [15]. This may suggest that FHL2 is a coregulator for AP1 target genes whose gene products may be candidate proteins involved in placental development.

FHL2 as a transcriptional cofactor

Intrinsic transcriptional activity of FHL2

The structural similarity between the N-terminal half LIM domain of FHL2 and the DNA-binding domain of the transcription factor GATA-1 suggests that FHL2 may act as a transcription factor [3]. Direct binding of FHL2 to DNA has not been shown, but several studies have demonstrated that GAL4-FHL2 fusion proteins stimulated transcription of reporter genes containing GAL4 binding motifs and a minimal promoter. The individual LIM domains seem to contribute in a cell-specific way to the intrinsic transcriptional activity of FHL2. GAL4-FHL2 was transcriptionally inactive in HT1080 and C2C12A cells, but active in CV-1, 293T, HL-1, MCF-7 and NIH3T3 cells [29, 37, 51, 59, 67, 68]. Deletion of the N-terminal half LIM domain severely impaired FHL2's transactivation potential in MCF-7 and 293T cells, but enhanced GAL4-FHL2-dependent transcription in NIH3T3 cells [27, 59]. Deletion of LIM3 alone did not affect the transcriptional activity of FHL2 in 293T cells, while a mutant lacking LIM4 domain was totally inactive. Additional deletion of LIM3 partially restored the transcriptional activity of this ΔLIM4 mutant, indicating that LIM4 is critical for the transcription activation function, while LIM3 may be a negative regulation region for LIM4 [67]. Cell-specific coactivators or corepressors that interact with the distinct LIM domains may account for the cell-specific contribution of the individual LIM domains to the intrinsic transcriptional activity of FHL2. A likely cofactor candidate is proline-, glutamic acid-, and leucine-rich protein-1 (PELP1/MNAR). A recent meeting abstract reported that PELP1/MNAR modulated the transactivation functions of FHL2 [56]. PELP1/MNAR has a cell-specific expression, with highest expression in testis, mammary gland, brain, skeletal muscle and lungs, and can act as both a coactivator through recruitment of p300/CBP and a corepressor through recruitment of HDAC2 [69].

Serum and the serum components lysophosphatidic acid and sphingosine-1-phosphate induced GAL4-FHL2-dependent transcription through activation of Rho-kinase ROCK [27]. We found that specific inhibition of PP2A in NIH3T3 cells resulted in increased GAL4-FHL2 transactivity, but the exact mechanism for this was not addressed [67].

Nuclear receptor-mediated transcription

The androgen receptor (AR) was the first transcription factor described whose transcriptional activity was stimulated by FHL2 [29]. FHL2, CBP/p300 and β-catenin synergistically enhanced ligand-activated AR-mediated transcription when AR was expressed at relatively low levels [39]. On the other hand, the orphan nuclear receptor Short Heterodimer Partner (SHP) counteracted activation of AR by FHL2. SHP did not interact with FHL2, suggesting a competition between FHL2 and SHP in binding to AR [70].

FHL2 has been shown to act as an activator for the nuclear receptors orphan nuclear receptor LRH-1, the peroxisome proliferator activated receptor α/retinoid X receptor α (PPARα/RXRα) and the thyroid hormone receptor (TR). A direct interaction between FHL2 and different TR isoforms has been confirmed, but FHL2 has not been shown to interact with PPARα [52, 71, 72]. FHL2 interacts also with the oestrogen receptor (ER) and with RXR in a yeast two-hybrid screen, but the effect on transcription mediated by these nuclear receptors was not investigated [52, 71]. However, Muller and colleagues found that the ligand-dependent transcriptional activity of the nuclear receptors GR, PR, MR, ER, RXR, RAR, TR, VDR, GCNF and ROR was not enhanced by FHL2 [29]. The reason for these discrepancies may be due to different cells used. Pyrimidine tract-binding protein (PTB)-associated splicing factor PSF can act as a repressor for AR-, PR-, GR- and TR-, but not ERα- and ERβ-driven transcription. Inhibition of TR-dependent transcription is achieved by PSF-mediated recruitment of histone deacetylase, while PSF induces proteosomal degradation of PR and prevents PR from binding DNA

through interaction with the DNA-binding domain [73]. The mechanism for repression of AR-mediated transcription is not known, but PSF may usurp FHL2, thereby depriving AR of this coactivator.

Runt-related transcription factor 2 (Runx2 or Cbfa1)

Runx2 is essential for normal function of osteoblasts because Runx2 null mice completely lack mineralized tissue and die soon after birth [74]. Recently, it was shown that FHL2 and Runx2 interact *in vivo* and that FHL2 stimulated Runx2-dependent transcription. Fhl2 null mice developed osteopenia, while transgenic mice overexpressing FHL2 in osteoblasts had increased bone mass compared with wild-type littermates. The authors therefore postulated that the property of FHL2 to enhance Runx2-mediated transcription could be a molecular mechanism involved in the process of normal bone formation [57].

cAMP response element binding protein (CREB)-mediated transcription

It is generally accepted that CREB-dependent transcription requires phosphoserine-133 mediated recruitment of the coactivator CBP/p300 [75]. Others and we have shown that GAL4-CREB- and GAL4-CREBS133A-mediated transcription was similarly stimulated by FHL2 [51, 68]. FHL2 can interact with the kinase-inducible domain (KID) of CREB (spanning amino acids 101–160), but the exact region was not mapped [51]. Both CBP and FHL2 bind KID, but FHL2 can interact with CREB independent of the phosphorylation status of serine-133, while CBP only associates with the phosphoSer-133 form of CREB. It is not known whether FHL2 and CBP bind phosphoCREB in a mutually exclusive way or whether they can bind simultaneously. If these proteins bind CREB simultaneously, does this result in a stronger CREB/CBP/FHL2 transcription complex compared with CREB/CBP or CREB/FHL2? This is an important question as it has been shown that an activated CREB is not always sufficient to transcribe cAMP-responsive genes, but that CREB-mediated transcription also depends on the amplitude and kinetics of CREB activation (reviewed in [19, 75]).

WT1-dependent transcription

Müllerian inhibiting substance (MIS), a transforming growth factor β-like hormone, regulates the regression of the Müllerian ducts in males. This is a crucial event during sex determination, as those ducts differentiate into oviduct, uterus and upper end of the vagina. The Wilms' tumour suppressor (WT1) and the orphan nuclear receptor SF1 cooperatively induce the MIS promoter, and FHL2 further activated this promoter. FHL2 was shown to interact with WT1, but not with SF1 *in vitro*. FHL2 also potentiated WT1-induced activation of the WT1 target promoter DAX1 [36]. These results illustrate that FHL2 functions as a bona fide coactivator in WT1-dependent gene regulation.

Effect of FHL2 on β-catenin/TCF-mediated transcription

The Wnt signalling pathway regulates morphology, proliferation, motility and cell fate, and deregulation of this pathway is implicated in several human cancers. An important component of the Wnt pathway is β-catenin, which resides in the cytoplasm in a multiprotein complex. One component of this complex, glycogen synthase kinase 3β, phosphorylates β-catenin in the absence of Wnt signalling. Phosphorylated β-catenin is subsequently ubiquitinated and degraded via proteasomes. Upon Wnt activation, cytosolic β-catenin is stabilized and translocates to the nucleus, where it associates with and activates the transcription factors of the T-cell factor/lymphoid enhancer factor family (TCF/LEF). FHL2, but not FHL1 or FHL3 was found to interact with β-catenin and to affect β-catenin-mediated transcription in a cell- and promoter-specific manner. FHL2 activated β-catenin-mediated transcription of TCF/LEF-dependent target genes in HEK293, SW480, A375, UDC-Mel-N and 293 cells, while a repression was observed in I28, CHO and C2C12 myoblasts [37–39, 58]. FHL2 and β-catenin enhanced AR-mediated transcription in CV-1 cells and stimulated SKI-induced activity of a synthetic β-catenin responsive promoter in 293T, UCD-Mel-N and A375 cells [38, 58]. However, FHL2 plus β-catenin repressed the activity of this promoter in HeLa and CHO cells. FHL2 enforced β-catenin/SKI-induced activity of MITF (microphthalmia-associated transcription factor) promoter, but repressed β-catenin/SKI stimulated activity of the Nr-CAM (neuronal cell adhesion molecule) promoter in 293T, UCD-Mel-N and A375 cells [58]. The cell- and promoter-specific effect of FHL2 on β-catenin-mediated transcription may be explained by FHL2-mediated recruitment of cell- and promoter-specific coactivators or corepressors. Alternatively, FHL2 may compete for or mask binding sites for cell-specific β-catenin-interacting proteins. Several proteins, including α-catenin, β-TrCP, IQGAP, NEMO-like kinase, CBP/p300, TATA-binding protein and Pontin 52, positively and negatively regulate β-catenin. Hence, FHL2 may interfere with the binding of such proteins, thereby inhibiting or stimulating β-catenin/TCF-mediated transcription. Interestingly, FHL2 increased acetylation of β-catenin by p300 *in vivo* [37, 39, 76].

AP1-mediated transcription

The AP-1 transcription factor is composed of members of the Jun and Fos protein family. FHL2 stimulated AP1-mediated transcription and was also a strong coactivator for GAL4-Jun- and GAL4-Fos-dependent transcription [15]. Phosphorylation of c-Jun Ser-63 and Ser-73 stimu-

lates the transcriptional activity of c-Jun, and two mechanisms have been proposed for phosphorylation-induced augmentation of c-Jun transcriptional activity. Phosphorylation of these two residues prevents ubiquitination and degradation of c-Jun, leading to accumulation and increased c-Jun activity in the cell. Furthermore, phosphorylation induces a conformation change, allowing the interaction of the KIX domain of CBP with the NH_2-terminal transactivation domain of c-Jun and subsequent augmentation of the transcriptional activity of c-Jun [79]. This phosphorylation-induced activation of c-Jun strongly resembles phosphorylation-dependent activation of CREB. In its non-phosphorylated state, CREB possesses a very low basal transcription activity that is mediated through its Q-rich Q2 domain. Stimulus-provoked phosphorylation of Ser-133 induces a conformational change of the KID region of CREB, which allows the recruitment of CBP via its KIX domain and a potent increase in CREB activity [19]. Substitution of Ser-63/Ser-73 (respectively Ser-133) into alanine renders c-Jun (respectively CREB) transcriptionally inactive [19, 77]. However, while FHL2 was able to stimulate transcription mediated by the CREBAla-133 mutant, FHL2 could not restore the transcription potentials of c-Jun Ala-63/Ala-73 mutant [15]. This indicates differential mechanisms of FHL2-induced activation of these two transcription factors.

FHL2 as a repressor for ERK2-mediated induction of transcription

FHL2 was shown to physically interact with ERK2, and phosphorylation of ERK2 increased the affinity for FHL2. Overexpression of FHL2 ablated signal-induced nuclear accumulation of ERK2 in cardiomyocytes, suggesting that FHL2 prevents nuclear import or/and stimulates nuclear export of activated ERK2 [28]. FHL2 can negatively interfere with transcription by preventing ERK2-mediated phosphorylation of transcription factors (e.g. Ets family, GATA4, c-Myc and STAT) and cofactors (e.g. CBP and p300) for numerous transcription factors that are not direct substrates of ERK2 [28, 78].

Hand1/E12-mediated transcription

The basic helix-loop-helix transcription factor Hand1 plays an essential role in cardiac morphogenesis. This protein is coexpressed with FHL2 in the developing mouse heart, and FHL2 directly interacts with Hand1, but not with E12 and E47, two other interaction partners of Hand1 [30]. FHL2 was shown to repress Hand1/E12 heterodimer-induced transcription, but had no effect on Hand1/Hand1 homodimer-mediated transcription. The mechanism whereby FHL2 represses Hand1/E12-dependent transcription is not completely understood, but FHL2 does not interfere with Hand1-E12 dimerization, nor does it prevent Hand1-E12 DNA binding *in vitro*. It has therefore been proposed that FHL2 inhibits Hand1/E12-dependent transcription through recruitment of a corepressor or by disrupting the interaction of Hand1 with the coactivator p300 [30].

Serum response factor (SRF)-dependent transcription

FHL2 inhibits SRF-dependent transcription in a promoter-specific way. FHL2 was shown to bind SRF directly, which prevents the recruitment of SRF coactivator MRTF-A/MAL. The molecular basis for the promoter selectivity of FHL2-mediated repression is unknown, but it may involve additional DNA sequence elements to which proteins bind that stimulate or prevent the recruitment of FHL2, SRF-coactivators (e.g. MRTF-A/MAL, ternary complex factors) and corepressor (GATA transcription factors, HOP, Elk-1) [11].

FOXO1-dependent transcription

FHL2 directly interacts with the Forkhead transcription factor FOXO1, thereby suppressing the transcriptional activity of FOXO1. Inhibition is achieved by FHL2-induced interaction between FOXO1 and histone deacetylase SIRT1. This enhances deacetylation of FOXO1, which in turn suppresses FOXO1 activity [54].

PLZF-regulation of transcription

Promyelocytic leukaemia zinc finger protein (PLZF) is a sequence-specific transcriptional repressor that exerts its inhibiting effect by recruiting corepressors that form part of the histone deacetylase complex. FHL2 interacts with PLZF and further increases PLZF-mediated transcriptional repression. FHL3, but not FHL1 or ACT also enhanced PLZF-mediated repression of transcription [55].

NFκB

TNF signalling occurs through binding of TNF to TNF receptors and subsequent activation of signalling transducing molecules. One pathway engages TRAF, which can induce phosphorylation and subsequent degradation of IκB. As a result NFκB is released and translocated to the nucleus, where it affects transcription of several target genes. FHL2 stimulated TRAF-induced expression of NFκB-responsive promoters in transient transfection studies in HEK293 cells, but FHL2 alone was not sufficient to induce the NFκB responsive promoter [64]. Bai and co-workers observed a more rapid and profound p65 nuclear translocation and enhanced expression of NFκB target genes in RANKL-treated FHL2$^{-/-}$ cells compared with wild-type cells. These results indicate that FHL2 attenuates NFκB activity [63]. Whether FHL2 is a direct cofactor for NFκB remains elusive, but despite the numerous yeast two-hybrid screens (table 2), a direct interaction between these two proteins has never been reported.

FHL2 involved in splicing

The RNA- and DNA-binding protein NP220 resides in the interchromatin space. This nuclear subcompartment is rich in small nuclear ribonucleoproteins (snRNPs), which form part of the spliceosome. NP220 contains an arginine/serine-rich motif found in non-small nuclear RNP splicing factors and polypyrimidine tract-binding RNA recognition motifs found in heterogeneous nuclear RNPs. These structural properties suggest that NP220 is a splicing factor [79]. The observation that FHL2 interacts with human NP220 may imply that FHL2 is involved in splicing, which is further underscored by the observation that FHL2 interacts with the pyrimidine tract-binding protein-associated splicing factor PSF [33, 35]. The role of FHL2 in splicing remains speculative because targeted studies to investigate the implication of FHL2 in splicing and reports of aberrant splicing in cells with dysfunctional FHL2 are currently lacking. The tissue-restricted expression pattern of FHL2 and the viability of *fhl2* null mice strongly argue against an essential role for FHL2 in splicing.

FHL2 and signalling transduction

Focal contacts are implicated in a number of signalling pathways. Some of the proteins at these specialized sites serve as scaffolding proteins and can provide docking sites for other proteins. Several of the scaffolding molecules are characterized LIM proteins (e.g. Hic-5, paxillin, PINCH and zyxin). FHL2 may therefore function as a scaffolding protein in signalling pathways [7]. Twenty-two proteins were retained when cytosolic fractions of human fibroblast Wi26 cells were separated over a column to which FHL2 was coupled. These proteins included metabolic enzymes, chaperones, signalling pathway components, and molecules involved in ubiquitination and in the organisation and dynamics of the cytoskeleton [80]. The identification of these proteins suggests that FHL2 may be involved in the nucleation of nascent cell adhesion structures by bringing key effector proteins in close proximity and in the control of actin bundling [80]. Moreover, the presence of FHL2 in different subcellular localizations and the physical association of FHL2 with proteins such as receptors, receptor-linked proteins, signal transmitter proteins and transcription factors (table 2) further support a role for FHL2 as a signal transducer [34, 43].

FHL2 in cell cycle regulation

Expression of the c-myc and cyclin D1 genes was drastically reduced in fhl2$^{-/-}$ mouse embryonic fibroblasts, and these cells grew much slower than normal embryonic fibroblasts when immortalized spontaneously. Furthermore, stable ectopic expression of FHL2 potentiated SKI-induced proliferation of UCD-Mel-N melanoma cells [58]. These results point to a positive effect of FHL2 on cell cycle-regulated processes. FHL2 can affect cell proliferation by modulating the expression of proteins involved in cell cycle regulation. For example, FHL2 may stimulate β-catenin-induced cyclin D1 promoter activity and ablate the inhibitory effect of FOXO1 on the cyclin D1 promoter [38, 54]. The recent finding that the common FHL2-interacting partners FOXO1 and β-catenin also interact with each other suggests that both mechanisms may be operational on the cyclin D promoter [81]. FHL2 may also antagonize cell proliferation, as FHL2 co-repressed PLZF-mediated transcriptional inhibition of cyclin A and downregulated cyclin D1 expression in differentiating myoblasts [37, 55].

FHL2 and apoptosis

Overexpression of FHL2 can induce apoptosis in a variety of cells, but the mechanism for FHL2-induced apoptosis is unknown [7, 39, 57]. It has been suggested that FHL2 may act as a transducer of apoptotic signals from the extracellular matrix at the focal adhesion plaques to the mitochondria. Interestingly, FHL2-GFP fusion protein was detected in the mitochondria [41]. FHL2 may also be implicated in radiation-induced apoptosis, as irradiation increases expression of p53. Enhanced p53 levels can in turn stimulate expression of FHL2 [7]. The FHL2-interacting protein SKI could counteract FHL2-induced apoptosis in UCD-Mel-N cells [40]. The SKI protein functions as a corepressor and may antagonize FHL2-induced expression of apoptotic genes.

FHL2 and diseases

FHL2 and cancer

In an effort to investigate the implication of FHL2 in cancer, transcript levels of FHL2 have been examined in different cancer cell lines. Transcript levels were high in patient samples derived from squamous carcinoma, glioblastoma, melanoma, chronic myelogenous leukaemia and cancers of the cervix, colon, lungs and kidneys. Low or no transcript levels were observed in lymphoblastic leukaemia, promyelocytic leukaemia and Burkitt's lymphoma cells [24, 59]. All these results are difficult to interpret, as information on the expression levels in matching normal tissue samples of the patients is scanty. Some studies have monitored FHL2 expression both in corresponding normal and tumour tissue to obtain an indication whether FHL2 may be implicated in oncogenesis. FHL2 mRNA levels were increased in patients with the poor risk FAB-M7 subtype of acute myeloid leukaemia compared with the other classes of acute myeloid leuk-

aemia [83]. Strong nuclear immunoreactivity was observed in lung tumour biopsies, while no FHL2 was detected in healthy lung tissue [12, 82]. Patients with high FHL2 protein levels had a significantly higher probability ($p=0.0001$) of dying within 12 months than patients with low FHL2 levels, indicating a contributing role of FHL2 in lung cancer [82]. Elevated expression of FHL2 was monitored in human epithelial ovarian cancer specimens compared with normal tissue. The FHL2-interacting tyrosine kinase pp125FAK is not detectable in normal ovarian tissue, but was highly expressed in the cancer samples [34]. These observations and the fact that a role for pp125FAK in tumorigenesis of cervical cancer has been proposed underscores a supporting role of FHL2 in pp155FAK-mediated transformation of ovarian cells [34]. FHL2 mRNA levels were also markedly upregulated in 8 out of 10 liver tumours compared with matched healthy tissues. β-catenin is frequently (>50%) mutated in these tumours, and FHL2, as a coactivator for β-catenin/TCF/LEF-mediated transcription, may play a role in hepatoblastoma cells by enhancing the transactivation functions of β-catenin [38].

Other tumour samples possess no or low FHL2 expression levels. FHL2 expression was reduced in rhabdomyosarcoma cells relative to normal myoblasts. Depletion of the protein was not the result of absence of the *fhl2* gene, as it was still present [4]. Many aggressive breast cancer cells have reduced FHL2 mRNA levels and/or contain BRCA1 mutants that have partially lost their ability to potentiate FHL2-induced transcription [4, 59, 67]. Abrogation of a functional FHL2-BRCA1 transcription coactivator complex, either by reducing FHL2 protein levels or by impairing BRCA1-mediated activation of FHL2, may be implicated in the development of cancer. FHL2 mRNA was present in the prostate cancer cell lines PC-3, Du-145 and in two primary prostate stromal cell lines, but not in LNCaP cells and three derivatives [84]. These findings argue against a role of FHL2 in prostate carcinoma. In another study, significant reduction of FHL2 transcripts in prostate cancer tissues (n=15) compared with normal tissues (n=15) was observed. Another group reported a close association of the amounts of AR and FHL2, as the expression levels of AR and FHL2 were low at an early stage of cancer, but enhanced in advanced cancer [85].

Other mechanisms by which FHL2 may contribute to malignancy may be a result of mutations in the *fhl2* gene or abnormal subcellular localization of the protein. Reports of mutation analyses of the *fhl2* gene in tumours are lacking in the literature. We sequenced the four coding exons of this gene in samples derived from breast cancer (n=9) and prostate cancer (n=14) patients, but could not detect mutations [our unpublished results]. However, based on the limited number of samples and the restricted types of tumours, it is too preliminary to exclude that mutation in the *fhl2* gene may be involved in malignant transformation of particular cell types. Increased accumulation of nuclear FHL2 was observed in human prostate cancers compared with normal tissue and enhanced nuclear localization of FHL2 correlated with progression to a highly malignant phenotype of prostate carcinoma. FHL2 was predominantly redistributed from the cytoplasm in healthy epithelial ovarian cells to the cell membrane in malignant cells [27, 34].

FHL2 and other diseases

Despite the numerous biological functions of FHL2 that have been revealed during the past years, clinical proof that perturbed FHL2 expression and/or activity contributes to human diseases is lacking. A recent study with *fhl2* null mouse model has provided the first possible link between anomalous FHL2 activity and a human disease. Mice deficient in FHL2 expression developed osteopenia, suggesting a role of FHL2 in human osteoporosis [57]. Comparing FHL2 expression and activity in osteoblasts from osteoporosis patients with healthy individuals may provide further proof that deficient FHL2 function contributes to osteoporosis.

Altered FHL2 expression has been detected in other clinical conditions. For example, FHL2 expression was increased in colonic tissue samples obtained from patients with Crohn's disease and in endometrial cyst samples from patients suffering from endometriosis [86, 87]. On the other hand, reduced FHL2 expression was measured in dilated cardiomyopathy patients compared with healthy individuals [32]. These observations indicate that anomalous FHL2 expression may be associated with all these pathophysiological conditions, but a causative involvement of FHL2 remains to be established.

Another mechanism by which FHL2 could contribute to a disease is by modifying the normal function of an interacting protein. Aberrant calpain activity has been measured in hypertrophy, heart failure, myocardial ischaemia and platelet aggregation, and activation of the calpain system has been suggested to contribute to the impairment of synaptic transmission in Alzheimer's disease. FHL2 can interact with the Ca^{2+}-dependent cysteine protease calpain, but whether FHL2 modulates the enzymatic activity has not been investigated [49]. FHL2 can also associate with presenilin-2, a protein associated with inherited risk for Alzheimer's disease [10]. A recent study demonstrated that wild-type presenilin-2, but not the mutant M238V that is associated with Alzheimer's disease, facilitated nuclear translocation of FHL2 [88]. The altered subcellular distribution pattern in cells with mutated presenilin-2 may therefore contribute to this disease. The FHL2-interacting PSF protein was shown to bind the DJ-1 protein. Mutations in the *DJ-1* gene cause

early-onset autosomal recessive Parkinson's disease [33, 89]. It is not known whether FHL2 interacts directly with DJ-1, participates in a ternary complex with PSF and DJ-1, or interferes with PSF-DJ-1 interaction, but it could be worth testing whether the interaction between FHL2 and PSF affects the normal functions of DJ-1. FHL2 stimulates AR-mediated transcription in a ligand-dependent fashion. Androgens can mediate cardiac hypertrophy; therefore, FHL2 may fulfil a role in the pathogenesis of human heart disease as a coactivator for the AR [29]. The identification of FHL2 as an I_{Ks} modifier makes it a candidate modulator for arrhythmia syndromes such as congenital long QT syndrome [44].

Conclusions and future directions

Multiple functions, mainly based on observations in cell cultures, have been ascribed to FHL2, but the molecular mechanisms by which this protein exerts its roles are incompletely understood. It is puzzling how a protein that consists of LIM domains only and that lacks any obvious enzymatic activity can exert such a diversity of functions. The LIM domains are protein-interaction domain and approximately 50 different proteins have been described so far that specifically interact with FHL2. The majority of these proteins show no or little sequence or structural homology and participate in a wide variety of cellular processes. Many of these proteins are components of signalling pathways (receptors, receptor-interacting proteins, protein kinases, transcription factors and cofactors; see table 2) that regulate e.g. cell proliferation, apoptosis and gene expression. The plethora of functionally diverse interacting proteins probably explains the multifunction properties of FHL2. The biological importance of many of these protein-protein interactions has not been determined, but such information will contribute to further clarifying the roles of FHL2. The mechanisms that allow FHL2 to discriminate between its different interacting partners remains unsolved, especially in cells where expression of FHL2 is limited. Cell-specific expression and cell-specific subcellular distribution of FHL2, as well as the available concentrations of the interacting partners may partially favour certain interactions, but fine-tuned regulation allowing selective interaction of FHL2 with a particular target is required.

Furthermore, studies examining the possible implications of FHL2 in human diseases should be pursued. Results from studies with mouse models deficient in FHL2 strongly indicate that FHL2 may be involved in the development of osteoporosis due to abnormal function of osteoblasts. Viral-mediated delivery of the *fhl2* gene under control of an osteoblast-specific promoter (e.g. the promoter of the osteocalcin gene) could be used to rescue FHL2 levels in osteoblasts of osteoporosis patients. Upregulated FHL2 expression has also been observed in several cancer cells and other human diseases. RNA interference-based therapy offers a possibility to reduce the FHL2 levels in diseased cells and restore normal cellular functions. Unravelling the functions of this protein and the design of therapy may help to improve the conditions of patients in whom FHL2 plays an injurious role.

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