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A Short History of LIM Domains (1993–2002)

From Protein Interaction to Degradation

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Abstract

The LIM domain is a cysteine-rich zinc-finger motif found in a large family of proteins. In LIM-homeodomain (LIM-hd) transcription factors and LIM-only (LMO) factors, the LIM domains are responsible for key interactions with co-activators, co-repressors, competitors, and other transcription factors, and are therefore of considerable importance for the regulation of associated transcriptional activity. In this review, the authors describe the progressive discoveries of NLI/Ldb/CLIM, LMO and RLIM, and discuss how the field was very recently updated by the finding that LIM-hd transcriptional activity is controlled by regulated degradation of cofactors and LIM-hd themselves.

Index Entries: LIM; LIM-homeodomain; LIM-only; NLI/Ldb/CLIM; RLIM; transcription; degradation; ubiquitination.

Introduction

This review is written with the deliberate intent to tell a story to the reader. For this reason, the results are presented in a chronological order, including that whose hypotheses proved false because they nevertheless brought insightful ideas to the field.

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LIM Domain Containing Proteins: A Family

The LIM domain is a specialized double zinc-finger motif found in a variety of proteins (reviewed in refs. *1*,*2*). The LIM domain is similar to the GATA-type zinc-finger domain,

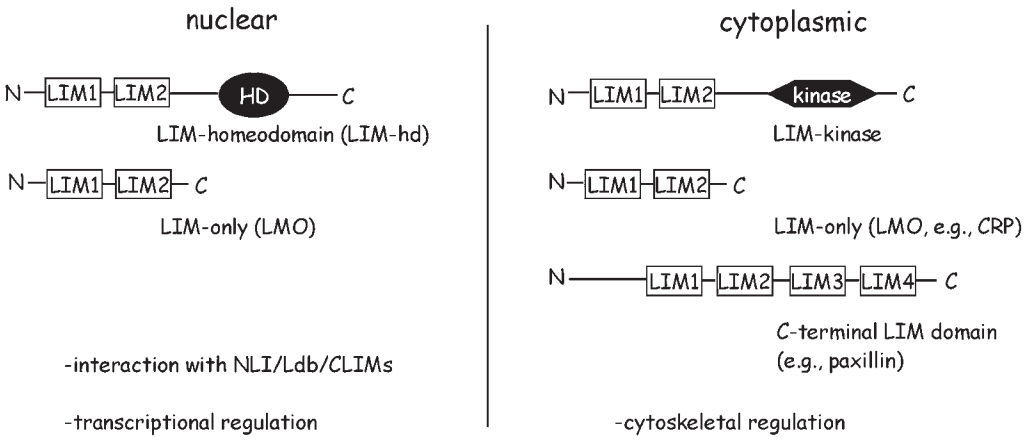


Fig. 1. LIM domain containing proteins. The classification and the general structure of LIM-containing proteins is shown. Two N-terminal LIM domains (LIM1 and LIM2, rectangles) can be associated to a homeodomain (HD, oval), a kinase domain, or little else (LMO). Among cytoplasmic LIM-containing proteins, the LIM domains can be either in N-terminus (example shown: LIM-kinase or CRP) or in C-terminus (example shown: paxillin). Major differences between nuclear and cytoplasmic LIM proteins include the interaction with cofactors and the type of biological function.

except LIM-type domains have a much shorter spacing between their two Zn^{2+}-fingers and, importantly, they don't bind DNA. The name LIM came into use in 1990 after the identification of the first three homeodomain-containing proteins, namely: *L*in11 *(3), I*sl1 *(4),* and *M*ec3 *(5).* Exemplified by the fact that *Lin11* and *Mec3* are *C. Elegans* genes, LIM-containing genes constitute a large gene super-family found from nematodes to mammals. They contain at least two N-terminal tandemly-organized LIM domains, and can be classified according to the additional domain they contain: a homeodomain (LIM-hd), a kinase domain (LIM-kinase), or little else (LMO for *LIM-o*nly, with up to five LIM domain repeats) (*see* Fig. 1). Alternatively, they can be classified after phylogenetic analysis of the LIM-domain sequences. Both types of classification allow for the recognition of two distinct groups of LIM-containing proteins: 1) nuclear (including LIM-hd and LMO), or 2) cytoplasmic (including LMO and LIM-kinase). While the twelve cytoplasmic LMO members (described in various species) and the two cytoplasmic LIM-kinases isolated are mainly associated with the cytoskeleton, nuclear LMO (four members, LMO-1 to LMO-4 in vertebrates) and LIM-hd transcription factors (thirteen members in mammals, *see* ref. *6*) are involved in a variety of developmental processes. (For a list of LIM-containing proteins, *see* ref. *7.*) LMO factors are recognized as powerful oncogenes when deregulated by chromosomal translocation or mutations (reviewed in ref. *8*). LIM-hd factors, on the other hand, have a prominent involvement in tissue patterning and differentiation, and their function in neuronal patterning is evident in all organisms studied to date (reviewed in ref. *9*).

Molecular Mechanisms Associated with LIM Domains: A Short History

LIM Domains Are "Inhibitory"

From the very beginning of the LIM story, the LIM domains, presenting a zinc-finger-like structure, were proposed to be involved in protein–protein interactions *(10);* also reviewed in

refs. *(1,11).* They were initially shown in vitro to engage in low affinity homodimer and heterodimer formation with LIM domains of other LIM-containing proteins *(12,13),* and to bind to other protein motifs including basic helix-loop-helix (bHLH) motifs *(14–16).* The first evidence that LIM domains were inhibitory to LIM-hd function emerged in 1993 from studies on *Mec-3* (a *C. Elegans* gene) and *Isl1* (a mammalian gene). In these two factors, the LIM domains inhibit both the DNA binding of their associated homeodomain assayed in gel shift experiments, and the transactivation of transcription assayed on a reporter construct *(17,18).* It is noteworthy that these properties do not remain valid for all subsequently tested LIM-hd proteins. Using mRNA injection in *Xenopus,* Taira et al. *(19)* showed that the LIM domains are inhibitory in vivo, since injection of a LIM domain-deleted version of *x-lim 1* was capable of inducing a partial secondary axis formation, whereas wild type *x-lim 1* was not. This led to the useful first model (now modified) proposed by Dawid and coworkers, where an intra-molecular folding of the LIM domains onto the homeodomain prevented its binding to DNA, and subsequent transcriptional activation. This also implied, as in the case of *x-lim 1,* that the largely inactive *x-lim 1* protein had to be activated in vivo, probably through interactions (via the LIM domains) with co-activators or cofactors.

NLI/Ldb/CLIM: LIM Cofactors

The search for such cofactors proved successful in 1996–1997, when three independent groups reported the cloning of factors that specifically associate to the LIM domains of all nuclear LIM proteins tested (but not cytoplasmic LIM domains such as those of enigma, paxillin, zyxin or CRP, and LIM-kinase). These factors, diversely called NLI *(20),* Ldb1 *(21),* or CLIM1/CLIM2 *(22)* (NLI, Ldb1, and CLIM2 are the same molecule; the NLI nomenclature will be used for clarity in the ensuing text) were shown to be necessary for LIM-hd function in vivo. Indeed, the co-injection of *x-lim1* and NLI (but, again, not *x-lim1* alone) into *Xenopus* embryos was able to induce a dorsalized phenotype and a secondary axis formation *(21).* The NLI/LIM-hd interaction was dissected, and shown to occur through a 63 a.a. region in the C-terminus of NLI, called LID (*L*IM *I*nteraction *D*omain), and to involve at least one of the two LIM domains (with intact cysteine residues) of LMO and LIM-hd proteins *(20,22).* As expected, the LIM domains of LIM-hd proteins were required for the cofactor function of NLI on Lhx3/P-lim in transactivation assays *(22),* or on *x-lim1* function in *Xenopus* in vivo *(21).* Moreover, the formation of DNA-bound complexes consisting of at least Lhx3/NLI were shown in electrophoretic mobility shift assay (EMSA) experiments *(22).* Collectively, these findings created a molecular basis for the "inhibitory" nature of LIM domains.

Interestingly, Bach et al. *(22)* also showed that NLI was able to confer transcriptional synergism between two distinct transcription factors, including non LIM-containing factors: although P-Otx is a homeodomain protein without LIM domain, a NLI-dependent synergy between P-Otx and Lhx3 (a LIM-hd protein) was observed on the α-GSU promoter, and the formation of DNA-bound complexes of P-Otx/NLI was described (Fig. 2A). Similarly and more recently, NLI was shown to confer synergism between Otx2 (a homeodomain protein) and *x-lim1* on the goosecoid promoter in *Xenopus* in vivo *(23).* These reports, together with other findings of LIM-hd proteins interacting with bHLH and POU families of transcription factors, suggest that the LIM domains and NLI act as adapter elements able to promote the assembly of multimeric protein complexes, in this case for the purpose of transcriptional regulation. It is quite remarkable that Arber and Caroni *(24)* came to the same conclusion for the LIM domains of cytoplasmic proteins by using totally different approaches of cell culture system to study protein targeting to the actin cytoskeleton. This crucial role of LIM domains, which can be regarded as scaffolds for the formation of higher order complexes, has been reviewed by Bach *(25).*

Fig. 2. Interactions between LIM-hd, NLI, and LMO. (A–C) Recapitulate the progressive understanding of the LIM-hd mechanisms of transcriptional control described in text. The broken arrow depicts a generic promoter. The homeodomain of LIM-hd is a black oval, whereas the LIM domains are white rectangles. NLI is represented by light gray rectangles (DD: dimerization domain; LID: LIM-interacting domain). LMO LIM domains are dark in gray. Drawings and sketches are adapted from illustrations in articles from authors cited in text. **(A)** Illustrates the model of Jurata and coworkers where the active from of the complex is an heterotetramer. It is represented in the cases of Lhx3/Lhx3, Lhx3/Isl1, and P-Otx/Lhx3 complexes which have been shown molecularly to occur. The interrupted line on the promoter is to illustrate long distance or local interactions between transcription factors rendered possible through complex formation. In the case of the αGSU promoter, the LIM-hd binding site, and the P-Otx binding site are located –342/–329 and –390/–383 bp upstream of the transcriptional start, respectively. **(B)** Sketches *Drosophila* genetic experiments. Two examples are represented: the first line shows that an excess of Chip can be compensated by Ap overexpression, whereas the second line shows that an excess of ChipΔLID cannot. This leads to the proposal in *Drosophila* that the active form of the complex is an heterotetramer. **(D)** Illustrates the mechanisms of LMO/LIM-hd competition, where the LIM domains of the two proteins compete for NLI/Chip binding. The second line shows the elegant experiments where the ChipDD-ApHD chimera escapes from dLMO competition, and where swapping the LIM domains of LIM-hd for those of LMO allows the chimeric protein to compete more effectively with dLMO.

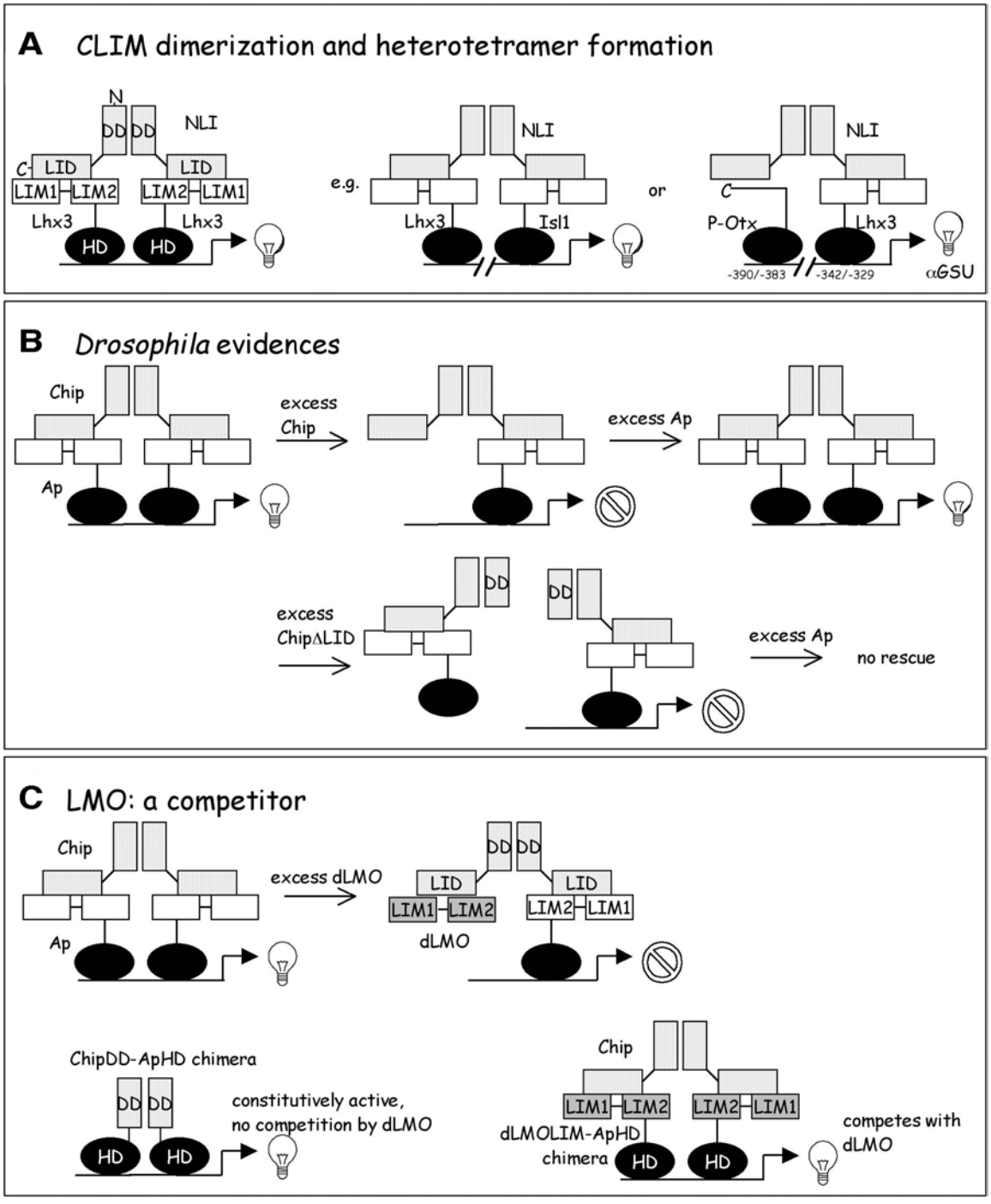
NLI/Ldb/CLIMs: Bridging Molecules

The ability of NLI to confer synergism between transcription factors from two distinct families suggested that they contained several interaction motifs. Further molecular dissection of their functional domains yielded an unexpected result. Besides restricting the LID to 38 residues in the C-terminus of the protein, Jurata and Gill *(26)* found in 1997 that NLI was able to dimerize through a dimerization domain (DD) located within the 200 N-terminal residues of the protein. The presence of two functionally independent domains in NLI molecules opened up the possibility of specific-partner interactions for transcriptional regulation. Jurata and Gill *(26)* also showed that NLI mediates homo- and heteromeric complex formation between LIM-hd proteins including Lhx3, IsI1 and IsI2 *(27).* Therefore, NLI emerged as a bridging molecule able to mediate combinatorial interactions between LIM-hd transcription factors whose DNA-binding sites may be very distant (*see* Fig. 2A). Breen et al. *(28)* supplemented in vivo arguments to the biochemical evidences of Jurata and coworkers, by showing in *Xenopus* animal caps experiments that NLI-dependent activation of downstream genes by *x-lim1* required both the LID and the DD of NLI. These findings, and the deduced model which is still very accurate *(27)* (Fig. 2A), gave a molecular foundation to the LIM-hd combinatorial code that was shown to be involved in axonal pathfinding of spinal cord motorneurons *(29–32)* and suggested in the forebrain during development *(6, 33–35).* Such direct interactions mediated by NLI dimerisation could also explain transcriptional synergy at a local DNA level, such as those expressed between Lhx3 and Pit1 *(36),* or Lhx3 and P-Otx *(22).*

Ap and Chip: The Drosophila Players

Until this point in LIM domain history, *Drosophila* provided an "abnormally" low contribution to the field, but was about to catch up very rapidly, to opening a new era in LIM-dependent regulation of transcription.

Two of the most studied key players of the LIM "system" in the fly are essentially apterous (Ap) and Chip. Ap is a LIM-hd transcription factor (ortholog of vertebrate Lhx2/Lhx9), and was isolated independently in 1992 for its role in wing-disk development *(37)* and neuronal and muscle development *(38).* Interestingly, like its vertebrates counterparts, Ap regulates axonal pathway selection in the embryonic ventral nerve cord *(39),* and a con-


A CLIM dimerization and heterotetramer formation
N
DD DD
NLI
C
LID
LID
LIM1 LIM2
LIM2 LIM1
Lhx3
Lhx3
HD
HD
e.g.
NLI
Lhx3
Isl1
or
NLI
C
P-Otx
Lhx3
-390/-383
-342/-329
αGSU
B Drosophila evidences
Chip
Ap
excess Chip
excess Ap
excess ChipΔLID
DD
DD
excess Ap
no rescue
C LMO: a competitor
Chip
Ap
excess dLMO
DD DD
LID
LID
LIM1 LIM2
LIM2 LIM1
dLMO
ChipDD-ApHD chimera
DD DD
HD
HD
constitutively active,
no competition by dLMO
Chip
LIM1 LIM2
LIM2 LIM1
dLMOLIM-ApHD
chimera
HD
HD
competes with
dLMO

servation of the expression and function of Ap and its mammalian orthologs has been reported *(40,41).* Thus, the study of LIM-hd regulatory mechanisms in the fly is relevant at both levels of mechanism and function. The topic of LIM-hd across evolution has been discussed elsewhere (reviewed in refs. *6,42*).

Chip, on the other hand, is the homolog of NLI, and was isolated in 1997 as a facilitator of enhancer-promoter communication in the regulation of *cut* (cut wing) genes *(43).* Chip protein interacts with the LIM domains of Ap. Importantly, *Chip* interacts genetically with *Ap,* showing that the Ap/Chip interaction is crucial for Ap function in vivo. Interestingly, Morcillo and coworkers *(43)* previously noted that Chip was a ubiquitous protein, and that embryos without Chip activity lack segments and have gap and pair-rule gene-expression defects, even though there are no LIM-hd proteins involved in *Drosophila* segmentation. Hence, they suggested that Chip cooperates with various proteins (*see also* ref. *44*), including LIM-domain containing proteins, to structurally support remote enhancer-promoter interactions—a hypothesis which fits well with the model of Jurata et al. *(27)* (Fig. 2A). Finally, Ap activity strictly depends on the availability and the correct amount of its cofactor, Chip for dorso-ventral compartimentalization of the wing *(45).* Indeed, *Chip* excess-of-function wing phenotypes can be rescued by *ap* overexpression (*see* Fig. 2B, first line). This finding introduced, for the first time in LIM-hd field, the idea of stoïchiometry, which would prove to be critical in the Chip/NLI- and Ap/LIM-hd-dependent regulation of transcription.

dLMO: A Competitor

In 1998, three groups independently found that the *Beadex* locus (*Bx* is a dominant mutation affecting wing development) encodes a *Drosophila* LIM-only protein, dLMO *(46–48).* The *Bx* mutation caused a disruption to the 3′UTR of the dLMO transcript, abrogating putative negative control elements, resulting in dLMO overexpression and in the wing scalloping phenotype that is typical of *Bx* mutants *(46–48).* The findings that: there are genetic interactions between *dLMO/Bx, Ap,* and *Chip (46);* that overexpression of dLMO in *Bx* mutant interferes with Ap function (but not Ap expression); and that the dLMO protein competes in a concentration-dependent manner with Ap for binding to the cofactor Chip *(47),* collectively suggests that the level of Ap activity is regulated by dLMO through a competition of the two LIM-containing proteins for Chip-binding (Fig. 2C, first line). These results were not only exciting for the understanding of fly-wing development (*see* comment by Dawid *[49],* and below), but also because they uncovered a possible molecular mechanism for the oncogenic activity of vertebrate LMO genes. Indeed, misexpression of LMO proteins after chromosomal translocations/gain of function mutations has been implicated in cancers of the lymphoid system (for a review, *see* ref. *8*). In *Drosophila,* overexpression of dLMO causes a dominant-interfering reduction of the activity of the LIM-hd protein Ap. As suggested by Milan and coworkers, it is possible in human cancer that the de-regulation of a LIM-hd activity by LMO overexpression alters the proper maintenance of the differentiated state in T cells, and leads to a failure in control of cell proliferation.

Importantly, the functional significance of the cross-talk between Ap, dLMO, and Chip for wing development is becoming clear. *apterous* is the selector gene responsible for the establishment of dorsal and ventral compartments in the developing-wing disk: it is expressed in dorsal cells, where it is responsible for boundary formation, establishment of the signaling center, and specification of dorsal cell-identity. An important finding was the discovery that Ap initiates dLMO expression in dorsal cells *(47).* Thus, Ap induces its own inhibitor, restricting the time-window of its own action, and allowing the down-regulation of its downstream genes, *Serrate* and *fringe,* and the induction of *Delta* in dorsal cells. This temporal regulation of Ap activity appears

necessary for a correct induction of the dorsoventral organizer and a normal wing development *(50).*

The Active Complex: An Heterotetramer

Although they clarified important aspects of LIM-associated regulations, these *Drosophila* findings did not deal with the mechanisms by which NLI/Chip cofactors activate LIM-hd proteins in vivo. It was nevertheless clear that the model where cofactor-binding to LIM domains alleviates repression on homeodomain activity and/or binding, was too simple (*see also* next section): among other inconsistencies, the fact that a mutant form of Ap lacking LIM domains has no biological activity in vivo *(51)* (note that deleting LIM domains renders *x-lim1* active in *Xenopus* in vivo *[19]*); and that overexpression of Chip causes a reduction of Ap activity in vivo *(45)* suggests that the regulation was more complex.

As already mentioned earlier, overexpression of Chip had a dominant-negative effect on wing development, but could be suppressed by overexpression of Ap *(45)* (Fig. 2B). This led the authors to propose that the active form of Ap was an Ap dimer bridged by a Chip dimer. This was elegantly presented in 1999 by Milan and Cohen *(52)* and Van Meyel et al. *(53)* who designed a chimeric molecule consisting of the DD of Chip fused to the homeodomain of Ap. It was shown that this chimeric protein can completely replace Ap function in vivo (Fig. 2C, second line). Milan and Cohen *(52)* further revealed that this chimeric ChipDD-ApHD molecule was not susceptible to dLMO regulation, and was constitutively active. Consequently, Ap activity was not down-regulated and a wing phenotype comparable to the dLMO loss-of-function phenotype was observed. These findings (and others not detailed here; *see also* ref. *54*) are therefore in full agreement with the hypothesis of a dimer of Ap bridged by Chip being the tetrameric active form of the complex. Interestingly, Van Meyel et al. *(55)* demonstrated that this heterotetramer is also the active form of Ap for the regulation of axonal guidance in the ventral nerve cord. However, in this case, since there is no dLMO expression (and therefore no dLMO-dependent regulation of Ap activity in Ap-expressing neurons) the ChipDD-ApHD chimeric molecule rescued the axon pathfinding defects of *Ap* mutants, *Chip* mutants, and *Ap/Chip* double mutants *(55).*

RLIM: A Co-Repressor

Combining *Drosophila* insights and classical molecular approaches was extremely useful in trying to resolve the mechanisms of LIM-hd activation in vivo: a solid convergence of results confirmed the original model proposed by Jurata and coworkers. However, some questions remain unanswered from vertebrate/molecular studies. In the case of Lhx3, the release of the inhibitory effect of LIM domains by NLI was not mediated through an effect on Lhx3 DNA binding *(22),* as could have been expected from the earlier Isl1 data *(17).* Indeed, Lhx3-ΔLIM (LIM domains deleted) binds DNA better than Lhx3, but NLI does not improve Lhx3 DNA binding. Moreover, NLI does not modify the DNA footprint of Lhx3 on the α-GSU promoter *(22).* If the NLI co-activator effect is not due to an effect on DNA binding, Bach and coworkers reasoned that its biological effect is rather to promote synergism, or/and to relieve the action of a LIM-associated inhibitor.

Such a co-repressor was isolated in 1999 by virtue of its ability to bind LIM domains, and was called RLIM *(56,57).* RLIM is a RING-H2 zinc-finger protein which interacts with nuclear LIM domains and LIM-kinase, but not with cytoplasmic LIM domains. Interestingly, RLIM is also able to interact with NLI (at the level of its DD) via a basic domain (BD) located in the middle of the molecule *(56,58).* In EMSA experiments, RLIM prevented Lhx3/NLI/DNA ternary complex formation, suggesting that RLIM modulates NLI action on LIM-hd at the level of NLI-binding or NLI-function *(56).* In transient transfection experiments, RLIM inhib-

ited the transcriptional activity of Lhx3 and the transcriptional synergism between Lhx3/Pit1 or Lhx3/NLI/P-Otx, and this repressor effect proved to be mediated through the recruitment of the Sin3A histone deacetylase complex *(56).* Finally using retroviral infection of chick limb buds, in vivo, Bach and coworkers found similar phenotypes of perturbation of limb-outgrowth when they overexpressed either RLIM, a dominant-negative version of NLI (DD deleted), or a version of the LIM-hd protein Lhx2 fused to the engrailed repressor domain (i.e., repressing Lhx2 function). These findings suggest that the alternate or combinational interaction of NLI and RLIM with LIM domains of LIM-hd control the transcriptional activity of the LIM-hd factor (in addition to, or in combination with, the regulation exerted by the competition between LMO and LIM-hd for NLI-binding). However, another small inconsistency arose from the following findings: RLIM was "stronger" than NLI in transactivation experiments (i.e., RLIM prevents NLI-dependent activation of transcription by Lhx3), whereas NLI affinity for LIM domains is much higher. This contradiction was soon to be resolved by looking at post-translational control of protein level.

RLIM Is a Ubiquitin-Ligase

As mentioned earlier, RLIM is a RING-H2 zinc finger protein. The authors reasoned that this kind of motif was often found in ubiquitin-protein ligases *(59)* and that ubiquitination-dependent cofactor exchange would be an attractive mechanism to explain LIM-hd transcriptional regulation mediated by NLI and RLIM (and LMO) cofactors (Fig. 3A). Indeed, RLIM is specifically able to ubiquitinate NLI, LMO2, and itself, but not LIM-hd proteins in vitro; and RLIM induces NLI and LMO2 degradation in a RING-finger dependent manner in vivo *(58).* In transactivation assays, RLIM exerts its repressive function through two distinct mechanisms: 1) through a recruitment of Sin3A histone deacetylase complex (in a RING finger-independent manner), or 2) through NLI-degradation (in a RING finger-dependent manner). The fact that RLIM targets NLI for degradation explains, fittingly, why RLIM is able to inhibit NLI-dependent LIM-hd activation of transcription, though its affinity for LIM-hd is lower. Finally, Ostendorff and coworkers elegantly show in a combined EMSA/ubiquitination assay the existence of a RLIM- and ubiquitination-dependent displacement of DNA-bound NLI/Lhx3 complex *(58)*. Therefore, a regulated cofactor exchange occurs on DNA-bound Lhx3 (Fig. 3A).

LIM-hd Factor Degradation

Interestingly, the degradation of LIM-hd factors themselves has also been recently suggested in *Drosophila* with the observation that the complex formation between Ap, Chip, and DNA stabilizes Ap protein in vivo *(60).* When competition between Ap and dLMO for Chip binding occurs, Ap is destabilized because it is no longer bound to DNA as part of an active complex with its Chip cofactor. Importantly, Weihe et al. *(60)* showed that this competition is rendered possible in vivo because the LIM domains of dLMO bind Chip more effectively than the LIM domains of Ap. These results offer an explanation to long-standing question of why Ap overexpression does not rescue dLMO overexpression, and is not able to increase Ap activity in vivo.

Collectively, these recent data (2001–2002) on NLI, LMO2, and LIM-hd degradation suggest two things. Firstly, a subtle equilibrium between nuclear concentrations and activity levels of LIM-hd, LMO, NLI, RLIM, and other LIM-interacting partners must be at work to control the selective interactions between the members of the "LIM system" and their regulated exchange with the final aim of transcriptional regulation. Secondly, although a number of questions remain, the emerging picture suggests that LIM-hd activity is "simply" regulated by two waves of degradation (Fig. 3B). The first wave, involving RLIM and ubiquitination/degradation of NLI removes NLI from the active complex of NLI/LIM-hd bound to

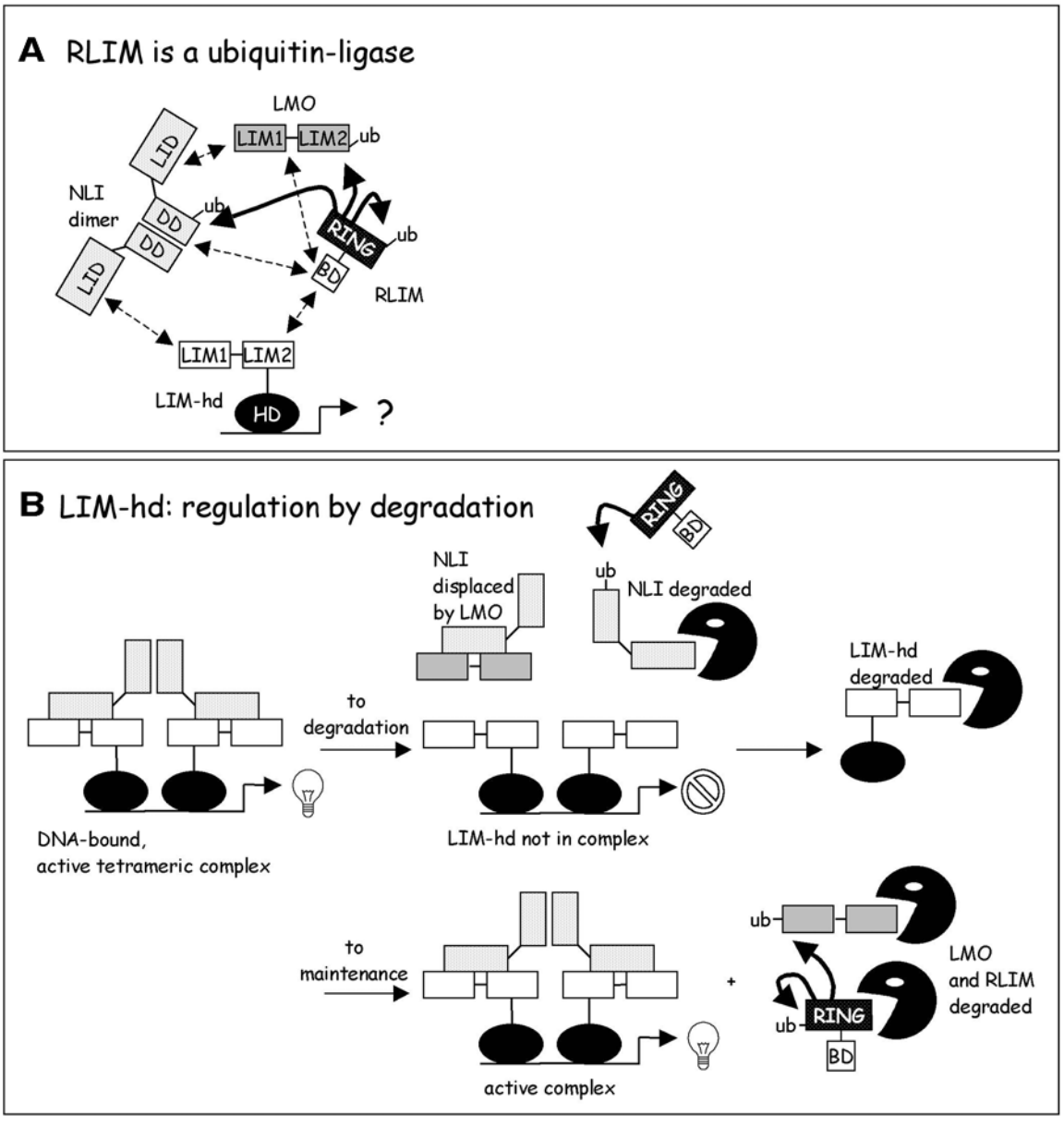


Fig. 3. Regulation by degradation. **(A)** Illustrates the various interactions between the players of the LIM system, and is redrawn with slight modifications from Ostendorff et al. *(58)*. RLIM is drawn with a black rectangle (RING domain and its associated ubiquitin-ligase activity, noted ub) and a white square (BD, basic domain for interactions with LMO, NLI, and LIM-hd). The dotted lines with double-arrows depict known molecular interactions between identified domains or functional motifs. The question mark for transcriptional activity illustrates the idea that transcriptional control will depend on the protein level and activity level of each player. **(B)** Proposes a model summary.

The first line illustrates the possible two "waves" of degradation involved in regulation of LIM-hd activity. To stop LIM-hd action (first line), NLI would be displaced from the active complex either by LMO competition or/and by RLIM ubiquitination and subsequent degradation. The un-complexed LIM-hd transcription factor would then be degraded by an, as yet unknown, mechanism.

Alternatively (second line), to maintain LIM-hd action, the ubiquitin-ligase activity of RLIM would target LMO and itself, thereby leading to degradation of the negative regulators of LIM-hd activity. In this case, NLI is not displaced from its complex with LIM-hd transcription factor, which stays in an active form.

DNA. The same effect can similarly be achieved by LMO through competition for NLI, in a RLIM-regulated manner (since LMO2 is a target of RLIM ubiquitination). The second wave, involving an unknown proteasome-mediated mechanism, would target LIM-hd itself (no longer protected after NLI removal) for degradation. Alternatively, if RLIM activity is directed towards LMO or itself (Fig. 3B, second line), the active tetrameric complex would be maintained for a sustained control of transcriptional activity by LIM-hd factors.

Conclusions

Some important questions remain, however. Among them and pell-mell, what regulates the ubiquitin-ligase activity of RLIM towards itself, NLI, or LMO? Is the *Drosophila* RLIM homolog (identified on sequence basis) involved in the same function within the LIM system as in mammals? Are there more LIM-interacting factors to be discovered? What is the mechanism of LIM-hd degradation? Are there distinct modes of LIM-hd regulation in cells expressing LMO (e.g., the wing disk) and cells that do not (e.g., Ap-expressing neurons)? What is the mechanism of NLI action? How does activation of LIM-hd occur? More generally speaking, could this type of tightly regulated, combinatorial, and cofactor-based mode of transcriptional regulation be extrapolated for other (homeodomain-) transcription factors?

In conclusion, spatial and temporal regulation of LIM-hd transcription factor activity appears mediated through the interaction of their LIM domains with cofactors, co-repressors, competitors, and other transcription factors. Significantly, it seems that LIM domains are also responsible for mediating target gene specificity, depending on the surrounding molecular context and the developmental process considered *(51).* Moreover, LIM-hd-specific cofactors have been described recently, such as SLB which specifically binds to Lhx3 and Lhx4, but to no other LIM-containing factor, and which negatively regulates their transcriptional activity *(61).* Thus, the authors foresee that an additional level of regulation, which would be specific for a given LIM-domain sequence, will soon be added to the complex control of LIM-hd activity. These distinctive features of the LIM system allow its prototypical combinatorial mode of action, which depends primarily on the spatial and temporal regulation of the expression for various players of the system in a given tissue, or a given cell.

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