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PERSPECTIVE

The rapidly expanding role of LC3-interacting regions in autophagy

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LC3-interacting regions (LIRs), or Atg8-interacting motifs (AIMs), are short linear motifs found in unstructured loops or intrinsically disordered regions of many autophagy-related proteins. LIRs were initially identified for their role in binding to Atg8 family proteins on autophagosomal membranes. However, emerging evidence suggests that LIRs and their surrounding residues mediate interactions with a wide array of proteins beyond Atg8s. This broadens the biological significance of LIRs in autophagy, rendering them an organizing principle of the autophagy machinery. In this perspective, we explore recent advances highlighting the multifunctional roles of LIRs, including their capacity to mediate binding with diverse factors. We discuss insights into the mechanisms underlying LIR-mediated interactions and propose an updated model to explain Atg8 diversification in higher eukaryotes. We conclude by addressing key challenges and outlining future directions for understanding LIR biology and its broader implications for cellular homeostasis.

LC3-interacting regions/Atg8-interacting motifs in autophagy: Discovery and foundation

Macroautophagy (hereafter autophagy) is a highly conserved cellular process that degrades and recycles damaged organelles, misfolded proteins, and other cytosolic components (Mizushima, 2018; Lamark and Johansen, 2021; Dikic and Elazar, 2018; Kirkin, 2020). During autophagy, cells sequester cargo in a de novo–generated double-membrane vesicle—the autophagosome—which is then trafficked to the lysosome and degraded (Fig. 1 A). This dynamic process is orchestrated by a collection of >40 autophagy-related (ATG) genes involved in the initiation, expansion, closure, and trafficking of autophagosomes (Lamark and Johansen, 2021; Yamamoto et al., 2023).

The Atg8 protein family, a conserved group of ubiquitin-like modifiers, plays a central role in autophagy. In *Saccharomyces cerevisiae*, this family consists of a single member, Atg8p, while in humans, it comprises seven members spanning two subfamilies: MAP1LC3 (A, B, B2, and C) and GABARAP (GABARAP, GABARAPL1, and GABARAPL2) (Rogov et al., 2023) (hereafter collectively referred to as Atg8s). Atg8 proteins undergo lipidation during autophagy, forming an amide bond between their C-terminal glycine and phosphatidylethanolamine in the autophagosomal membrane (Ichimura et al., 2000). As a result, lipidated Atg8s are a key marker of autophagosomal membranes, although Atg8 lipidation also occurs in other cellular processes (Nieto-Torres et al., 2021; Deretic et al., 2024), and Atg8 proteins can also be conjugated to phosphatidylserine (Durgan et al., 2021; Hanada et al., 2007; Sou et al., 2006). The interaction between Atg8s and numerous proteins, including cargo-specific autophagy receptors, is mediated by short linear motifs known as Atg8-interacting motifs (AIMs) in yeast and LC3-interacting regions (LIRs) in mammals (hereafter collectively referred to as LIRs) (Rogov et al., 2014). The discovery of these motifs marks a pivotal advancement in our understanding of autophagy, particularly in the context of cargo specificity.

The canonical LIR motif, first observed in the autophagy receptors Atg19 (in yeast) and p62 (in mammals), was validated through a series of early foundational studies (Pankiv et al., 2007; Shintani et al., 2002; Noda et al., 2008). The elucidation of additional LIRs in rapid succession enabled the extraction of a core sequence motif ($[W/F/Y]_0$-X_1-X_2-$[L/I/V]_3$), where positions X_0 and X_3 anchor the motif in two hydrophobic pockets on Atg8 family proteins (Ichimura et al., 2008; Noda et al., 2008; Noda et al., 2010; Johansen and Lamark, 2011). This consensus motif provided a framework for identifying LIR motifs across a wide array of proteins (Chatzichristofi et al., 2023; Jacomin et al., 2016; Ibrahim et al., 2023), including autophagy receptor proteins (for a review on receptors and their interactions with Atg8s, see Kirkin and Rogov [2019]; Rogov et al. [2023]).

While this motif provides an important framework, it is now well recognized that many functional LIRs deviate from the canonical sequence and that binding specificity and affinity depend heavily on features beyond the core motif (for review, see Rogov et al. [2023]). Local structure also shapes LIR

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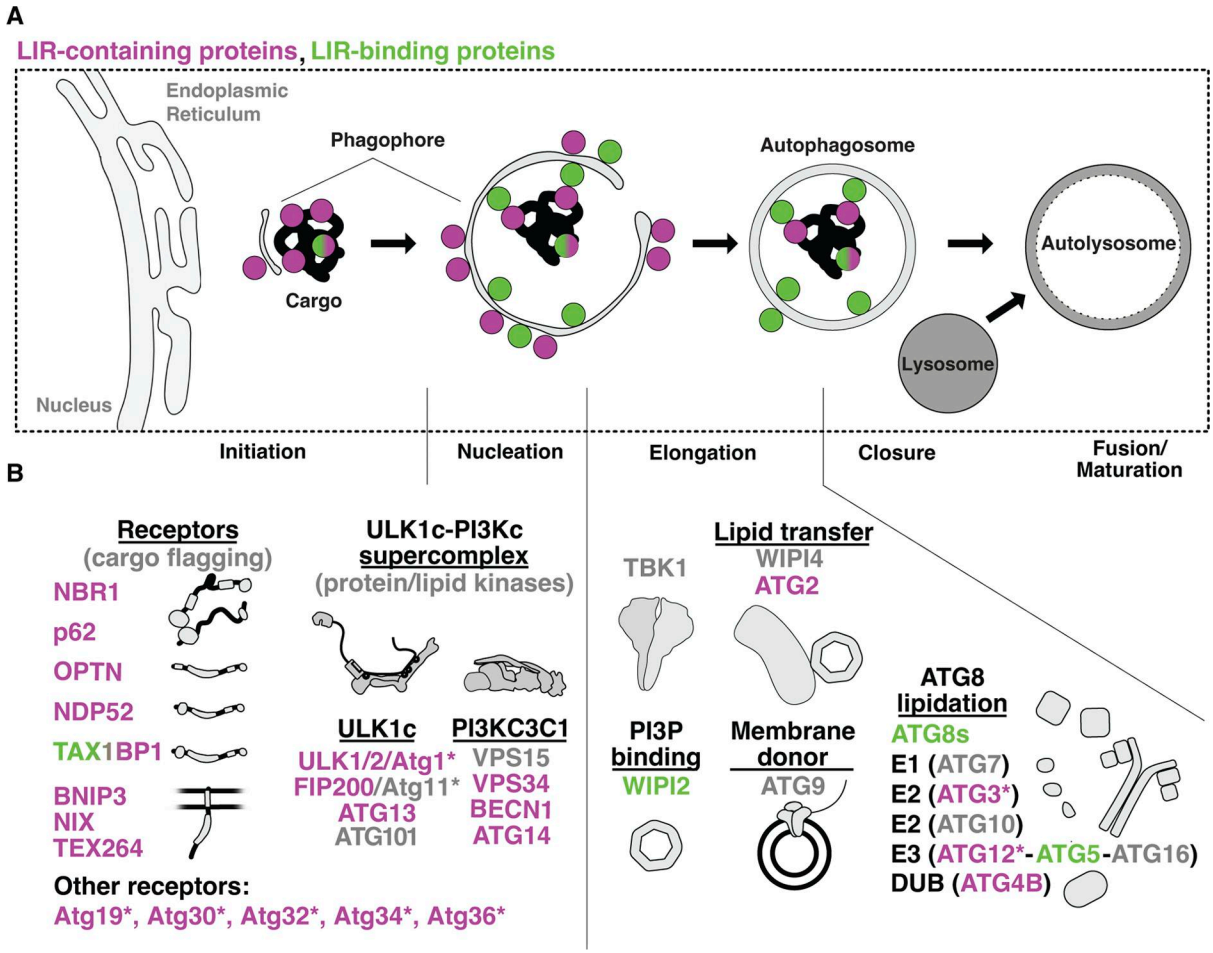

Figure 1. **LIR-containing and LIR-binding proteins in autophagy. (A)** Schematics of selective autophagy with LIR-containing (magenta) and LIR-binding (green) factors highlighted on the autophagosomal structure. Initiation cues—such as cargo recognition or nutrient signaling—recruit the autophagy initiation machinery to sites of autophagosome formation, where a de novo membrane structure called the phagophore is generated. The phagophore expands and closes, sequestering cytoplasmic components within a newly formed double-membrane vesicle known as the autophagosome. Autophagosomes ultimately fuse with lysosomes, where the inner membrane and enclosed cargo are degraded by lysosomal hydrolases. **(B)** LIR-containing (magenta) and LIR-binding (green) proteins are prevalent throughout the autophagy machinery in yeast and humans. Asterisks (*) mark findings from yeast. For details see Pankiv et al. (2007); Kirkin et al. (2009); von Muhlinen et al. (2012); Whang et al. (2017); Bauer et al. (2024); North et al. (2025); Wild et al. (2011); Rikka et al. (2011); Novak et al. (2010); Chino et al. (2019); Kraft et al. (2012); Alemu et al. (2012); Birgisdottir et al. (2019); Bozic et al. (2020); Yamaguchi et al. (2010); Noda et al. (2008); Satoo et al. (2009); An et al. (2019).

functionality: most LIRs are found in unstructured loops or intrinsically disordered regions, which allow flexible engagement with binding partners (Popelka and Klionsky, 2015; Ibrahim et al., 2023), although LIRs embedded in structured domains have also been reported (Keown et al., 2018). This sequence and contextual flexibility underlies the growing diversity of LIR-dependent interactions and reinforces the need to consider sequence and structural context when evaluating motif function.

Functional flexibility: The versatile binding capabilities of LIRs

While the structural and functional relationship between Atg8s and LIRs has been extensively reviewed (Rogov et al., 2023; Wesch et al., 2020; Kirkin and Rogov, 2019; Johansen and Lamark, 2020), emerging evidence suggests a broader role for LIRs beyond Atg8 interactions (Fig. 1). Here, we examine growing evidence that LIRs contribute to interactions with a wide array of protein partners. Together with their flanking residues and accessory motifs—which contribute to the strength and specificity of these interactions and are often regulated by posttranslational modifications—LIRs emerge as one of the key organizing principles in the assembly of the autophagy machinery (Rogov et al., 2023). To structure this discussion, we categorize these interactions based on how each LIR, in conjunction with its flanking residues, engages non-Atg8–binding partner(s). We define three motif configurations—adjacent, bipartite, and hybrid—presented in order of increasing overlap (Fig. 2 and Table 1). We emphasize that this classification is intended as a flexible conceptual framework rather than a rigid or exhaustive taxonomy.

Adjacent

Adjacent motifs are neighboring sequence elements that bind distinct partners. Rather than defining adjacency by an arbitrary distance, we adopt a functional definition, emphasizing whether

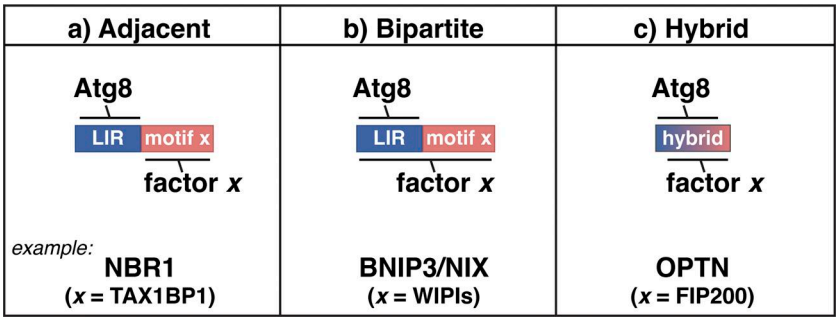

Figure 2. **Configurations of LIR motifs within protein interaction hotspots.** A single representative example of each configuration is shown below. Additional examples corresponding to each category are listed in Table 1. **(a)** Adjacent: Distinct motifs located in close proximity, allowing independently mediated interactions with two or more binding partners. Due to their proximity and/or conformational requirements, these interactions often exhibit cooperative or competitive binding. The NBR1 interaction with Atg8s and TAX1BP1 is an example of competitive binding. **(b)** Bipartite: Binding requires both the LIR motif and additional residues, either N- or C-terminal, surrounding the LIR. **(c)** Hybrid: Overlapping binding sites enable interactions with multiple partners. This configuration often involves a tradeoff in binding specificity or affinity.

spatially related motifs function as part of a coordinated mechanism rather than their exact physical distance. Accordingly, two motifs are adjacent if (1) they are nonoverlapping, (2) they are located within the same intrinsically disordered region, and (3) their proximity is likely to influence their function as determined through experimental evidence, such as competitive binding, or inferred from conserved spatial architecture.

A flagship example of adjacent motifs comes from early studies on the yeast autophagy receptor Atg19 (Shintani et al., 2002). Atg19 delivers the aminopeptidase ApeI to the yeast vacuole—the functional equivalent of the lysosome in mammalian cells—and requires a critical six-amino acid region (^{410}LTWEEL^{415}) that binds to yeast Atg8p (identified initially as Aut7). 14 amino acids upstream, an additional eight-amino acid sequence (^{389}DSSIISTS^{396}) is required for binding Atg11 (formerly Cvt9), a key scaffolding factor in the selective autophagy pathway. While neither motif was fully characterized at the time, both are individually required for the vacuolar delivery of Ape1. Thus, this study marked the first description of both a LIR and an Atg11-binding region, notably in close proximity. Two additional LIR motifs in Atg19, located immediately upstream of the Atg11-binding site, were later identified (Sawa-Makarska et al., 2014), revealing a total of four distinct but interrelated motifs within a ~40–amino acid region, each contributing to autophagic flux.

These findings were quickly extended to additional yeast cargo receptors, including Atg30 (Farré et al., 2008), Atg32 (Aoki et al., 2011; Okamoto et al., 2009; Kanki et al., 2009), Atg34 (Suzuki et al., 2010), and Atg36 (Farré et al., 2013; Motley et al., 2012). In each case, mutually exclusive binding of Atg11 occurs within 62 amino acids of a LIR and is critical for lysosomal delivery (Farré and Subramani, 2016). Moreover, many Atg8- and Atg11-binding motifs are phospho-regulated, suggesting a mechanism by which cells can temporally regulate these interactions (Pfaffenwimmer et al., 2014; Farré et al., 2013; Kanki et al., 2013; Tanaka et al., 2014; Aoki et al., 2011). Together, these findings establish a role for adjacent motifs in coordinating the function of yeast cargo receptors.

Adjacent motifs are also found in mammalian cargo receptors such as NBR1. Independent studies have shown that NBR1 interacts both with Atg8 family proteins and with an alternative cargo receptor, TAX1BP1 (Ohnstad et al., 2020; Turco et al., 2021). These interactions rely on the LIR motif of NBR1 to bind to Atg8s and a second motif immediately downstream of the LIR (^{736}LPECF^{740}) to bind to TAX1BP1 (North et al., 2025; Bauer et al., 2024). Despite nonoverlapping motifs, the binding of Atg8s and TAX1BP1 is mutually exclusive, likely due to steric constraints (Bauer et al., 2024). Separation-of-function mutations—including NBR1^{F740A}, which impairs TAX1BP1 binding, and NBR1^{Y732A}, which disrupts Atg8 binding—reveal distinct roles for these motifs in NBR1 turnover. TAX1BP1 binding promotes NBR1 turnover by facilitating the recruitment of a TAX1BP1:NAP1:FIP200 ternary complex (Zhang et al., 2024a), which initiates autophagosome formation around cargo condensates (Bauer et al., 2024; Turco et al., 2021; Ohnstad et al., 2020; North et al., 2025). As a result, failure to recruit TAX1BP1 impedes the transition from cargo collection to autophagosome biogenesis. In contrast, LIR mutants (e.g., NBR1^{Y732A}) arrest autophagy at a later stage (Kirkin et al., 2009), likely during membrane expansion. These findings suggest that adjacent motifs within NBR1 independently contribute to its autophagic function. The spatial proximity of these motifs likely enables coordinated molecular interactions to fine-tune autophagic progression, ensuring efficient turnover of cellular components.

A final example of adjacent motifs comes from the autophagy receptor p62, which harbors a KEAP1-interacting region (KIR; ^{347}DPSTGE^{352}) immediately downstream of its LIR (Komatsu et al., 2010; Jain et al., 2010). Under basal conditions, KEAP1 targets the transcription factor NRF2 for proteasomal degradation. However, binding of the p62 KIR to KEAP1 displaces NRF2, preventing its degradation and enabling the activation of NRF2-driven genes. At the same time, p62 also targets KEAP1 for autophagy, further stabilizing NRF2. As with other adjacent motifs, interactions involving the p62 LIR and KIR are mutually exclusive and coordinated by phosphorylation (Ichimura et al., 2013). This example further illustrates that adjacent motifs can function as coordinated modules—in this case, coupling selective autophagy with broader stress-responsive signaling pathways.

Bipartite

Unlike adjacent motifs—which are spatially close but functionally distinct—bipartite motifs integrate a LIR with an additional N- or C-terminal element. These bimodular motifs thereby enhance the specificity and versatility of LIR-mediated interactions through coordinated, dual-site recognition.

Table 1. **Multifunctional LIRs and their non-Atg8–binding partners**

Model	LIR	non-Atg8 partner	Sequence
Adjacent	Atg19 (3x)	Atg11	371 ASQEPFYSFQIDTLPELDD**SSIISTS**ISLSYDGDDNEKALTWEEL 415
	Atg30	Atg11	68 TDNSEWILFSPENA..[24]..YNE**DDILSSS**RRSSEDVY 123
	Atg32	Atg11	81 SISGSWQAIQPLDL..[12]..TTNG**SILSSS**DTSEEEQE 124
	Atg36	Atg11	28 DEESLFEVLELSEE..[48]..SDEIA**ILS**I**S**SDSNKNSP 107
	NBR1	TAX1BP1	727 ASSEDYIII**LPECF**DTSRP 745
	CCPG1	FIP200	9 DSSCGWTVISHEGSD**IEML**NSVTP 32
	p62	KEAP1	333 GGDDDWTHLSSKEV**DPSTGE**LQSLQ 357
Bipartite	BNIP3	WIPIs	13 SLQGS**WVEL**HFSNN..[14]..G**DMEKILLDAQHE**SGRSS 58
	NIX	WIPIs	31 GLNSS**WVEL**PMNSS..[24]..G**DMEKILLDAQHE**SGQSS 86
Hybrid	OPTN	FIP200	173 SSE**DSFVEI**RMAEG 186
	p62	FIP200	333 GGD**DDWTHL**SSKEV 346
	TNIP1	FIP200	120 GTS**SEFEVV**TPEEQ 133
	NDP52	FIP200	129 NE**EDILVV**TTQGEV 142
	NAP1	FIP200	4 LVE**DDICIL**NHEKA 17
	STBD1	FIP200	198 VDH**EEWEMV**PRHSS 211
	BCL2L13	FIP200	271 LGP**ESWQQI**AMDPE 284
	FUNDC1	FIP200	13 SDD**DSYEVL**DLTEY 26
	CCPG1	FIP200	101 SDD**SDIVTL**EPPKL 114
	NBR1	FIP200	727 ASS**EDYIII**LPECF 740
	C53 (4x)	UFM1 (3x)	269 AAADS**IDWD**ITVETPE**IDWD**VS..[11]..GSYEIVNA..[21]..SE**ISWD**VSVET 341
	Uba5	UFM1	335 IHEDN**EWGIELVSE**VSEEELKN 356
	Atg19 (3x)	Atg5 (3x)	371 ASQEP**FYSF**QIDT**LPEL**DDSSIISTSISLSYDGDDNEKALT**WEEL** 415

Multifunctional LIR motif sequences are shown, with the core LIR (underlined), non-Atg8 motifs (bold), and overlapping motifs (underlined and bold) indicated. Only the core LIR residues are marked, although flanking residues often contribute critically to LIR function. Motifs are categorized by how each non-Atg8 motif is integrated with its corresponding LIR—adjacent, bipartite, and hybrid—in order of increasing overlap.

A notable bipartite motif is found in BNIP3 and BNIP3L/NIX, mitophagy receptors that bind both Atg8s and WIPI family proteins (Adriaenssens et al., 2024, *Preprint*; Bunker et al., 2023). Through their association with WIPIs, BNIP3 and NIX indirectly recruit the FIP200 initiation complex and induce selective mitophagy (Adriaenssens et al., 2024, *Preprint*). Structural predictions reveal that BNIP3 binding to WIPIs involves two distinct binding interactions: one mediated by the LIR and another by a sequence ∼20–30 residues downstream, termed the minimal essential region (MER) (Adriaenssens et al., 2024, *Preprint*). Consistent with this model, mutations in either the LIR or MER disrupt WIPI binding, highlighting that the LIR motif is necessary, but not sufficient, for WIPI recruitment in this context (Adriaenssens et al., 2024, *Preprint*; Bunker et al., 2023). Correspondingly, mutation of either motif significantly impairs WIPI clustering and BNIP3/NIX-mediated mitophagy in vivo, highlighting the physiological relevance of this bipartite interaction. Intriguingly, additional autophagy receptors, including FKBP8 and TEX264, also recruit WIPI proteins (Adriaenssens et al., 2024, *Preprint*). Whether these receptors similarly rely on bipartite LIR motifs or engage through alternative mechanisms remains an open question.

A second instance of bipartite motifs is found in extended LIR motifs, which play a critical role in determining the specificity of LIRs for LC3 or GABARAP subfamilies. In such cases, extended contacts (that is, residues N- or C-terminal to the core LIR) alter binding affinity and dictate specificity by providing secondary interactions that complement the primary LIR-docking site. For example, the LIR of FYCO1 contains a short C-terminal helix that confers specificity for LC3A and LC3B (Olsvik et al., 2015; Cheng et al., 2016), while proteins such as AnkG, AnkB, and FAM134B utilize an extended helix to enhance binding to all Atg8s (Li et al., 2018). C-terminal residues similarly contribute to the binding preferences of PCM1, ULK1, and ATG13, even without a helical structure (Wirth et al., 2019). These examples further illustrate how bipartite motifs can modulate the interaction landscape of LIRs, enhancing their functional versatility.

Hybrid

A hybrid motif integrates the binding preferences of two or more motifs within a single locus, enabling interactions with multiple partners. Such overlap generally necessitates a tradeoff in binding affinity, as enhancing interaction with one partner often reduces binding to the other. Nevertheless, both interactions are

necessary for function, highlighting the delicate balance these motifs must achieve.

Hybrid motifs are well illustrated by mammalian FIP200-interacting regions (FIRs). FIRs are highly prevalent in mammalian autophagy receptors, where they recruit the CLAW domain of FIP200 to initiate autophagosomes around cargo (Turco et al., 2019). The FIR consensus consists of an acidic sequence followed by a hydrophobic motif resembling a LIR: (I/V/L/W/F/Y)-X_1-X_2-(I/L/V) (Zhou et al., 2021). Consequently, many—but not all—LIRs overlap with FIRs. Examples of proteins with overlapping LIRs and FIRs include OPTN (Zhou et al., 2021), p62 (Turco et al., 2019), TNIP1 (Le Guerroué et al., 2023; Wu et al., 2024), NDP52 (Fu et al., 2021), NAP1/SINTBAD (Ravenhill et al., 2019), STBD1 (Zhang et al., 2024b), BCL2L13 (Adriaenssens et al., 2024, *Preprint*), FUNDC1 (Adriaenssens et al., 2024, *Preprint*), CCPG1 (Zhou et al., 2021), NBR1 (North et al., 2025), and ATG16L1 (Pan et al., 2024, *Preprint*).

Recently, a comparative analysis of 100 LIR motifs revealed an unexpectedly modest correlation between FIP200 and Atg8 binding, reaffirming that not all LIRs are FIRs despite their overlapping motifs (North et al., 2025). This discordance suggests that the preferred motifs for FIP200 and Atg8s are not identical; instead, many LIRs are likely hybrid motifs that balance affinities between the two. To this end, mammalian LIRs and FIRs are also modulated through phosphorylation, providing cells with an additional mechanism to control these interactions and ensure precise directionality in autophagic processes (Wild et al., 2011; Zhou et al., 2021; Rogov et al., 2023).

Another example of a hybrid motif is found in *Arabidopsis thaliana* C53, an ER-phagy receptor that interacts with both Atg8s and another ubiquitin-like modifier, UFM1, involved in a posttranslational modification pathway known as UFMylation (Stephani et al., 2020). C53 binds to these partners through one canonical LIR and three noncanonical motifs, called "shuffled AIMs" (sAIMs) (Picchianti et al., 2023; Stephani et al., 2020). While the canonical LIR is selective for Atg8, sAIMs bind both Atg8s and UFM1. Moreover, sAIMs exhibit a direct tradeoff in binding between Atg8s and UFM1, with stronger binding of one coming at the expense of the other (Picchianti et al., 2023). Critically, both interactions are important for C53 function, suggesting that the tradeoff in binding enables C53 to integrate multiple cues to fine-tune ER-phagy in vivo (Picchianti et al., 2023).

Similar findings have been reported for UBA5, another component of the UFMylation machinery. UBA5 contains a noncanonical LIR motif (EWGIELV) that enables binding to both Atg8s and UFM1 (Habisov et al., 2016; Huber et al., 2020; Padala et al., 2017). In this context, Atg8 binding localizes UBA5 to the ER (Huber et al., 2020), while interactions with UFM1 enable UFMylation (Habisov et al., 2016; Padala et al., 2017). These findings further underscore the dual-binding capability of hybrid motifs and indicate a role in coordinating autophagy and UFMylation pathways.

A final example of hybrid motifs is found in the yeast-selective autophagy receptor, Atg19. Atg19 employs its three LIR motifs to bind both Atg8 and Atg5, a core autophagy factor required for Atg8 lipidation (Fracchiolla et al., 2016). By recruiting Atg5, Atg19 couples the lipidation of Atg8 to the presence of cargo. As Atg8 accumulates, it displaces Atg5, keeping the autophagosomal membrane close to the cargo. Notably, the interaction between Atg19 and Atg5 was among the earliest documented examples of LIR interactions with non-Atg8 proteins. This observation led to early speculation that such dual-binding capabilities of LIRs could represent a broader phenomenon (Fracchiolla et al., 2017)—a prediction substantiated by the many above examples.

Section summary

What were once scattered observations of "promiscuous" LIR interactions are coalescing into a broader understanding: LIRs—and their surrounding sequences—are far more than simple one-to-one binders of Atg8 family proteins. Rather, LIRs serve as versatile platforms that coordinate multiple, functionally significant interactions (Table 1). As research continues, additional interactors and layers of complexity will undoubtedly emerge, further expanding the central role of LIRs in autophagy.

A potential role for non-Atg8 binding in Atg8 diversification

The role of Atg8 is evolutionarily conserved, but the number of Atg8 orthologs varies widely between species. Yeast has a single homolog (Atg8p), *Caenorhabditis elegans* and *Drosophila* have two (LGG-1/LGG-2 and Atg8a/Atg8b, respectively), humans have seven, and some plants up to 22 (Rogov et al., 2023; Kellner et al., 2017). A straightforward hypothesis for this expansion is that diversification provides redundancy, thereby increasing pathway resiliency. However, unique functions among certain Atg8 homologs suggest functional specialization as an additional driving force behind Atg8 expansion (Fig. 3).

Atg8 specialization is best illustrated in the differences between the LC3 and GABARAP subfamilies. Early RNAi studies demonstrated that GABARAPs and LC3s perform nonoverlapping functions, with GABARAPs functioning downstream of LC3s (Weidberg et al., 2010). Subsequent studies corroborated this model and highlighted a more critical role for GABARAPs, particularly in autophagosome-lysosome fusion (Nguyen et al., 2016; Vaites et al., 2018). These functional differences can be attributed partially to distinct LIR preferences for LC3s and GABARAPs (Wirth et al., 2019; Rogov et al., 2017; Wu et al., 2015), which enable differential recruitment of effector proteins (Wang et al., 2016; Wang et al., 2015; Nguyen et al., 2016). Additionally, LC3 and GABARAP homologs contain intrinsic differences in their ability to facilitate membrane tethering and hemifusion due to distinct N-terminal helical regions (Weidberg et al., 2011; Iriondo et al., 2023). Similar distinctions in binding and membrane fusion have also been reported for *C. elegans* LGG-1 and LGG-2, reinforcing that Atg8 diversification enabled functional specialization across evolution (Wu et al., 2015).

Although less well characterized, there is also evidence for specialization within subfamilies, such as differences between LC3A and LC3B (Nguyen et al., 2016), as well as LC3C (von Muhlinen et al., 2012). Accordingly, the interactomes of human Atg8 homologs are strikingly divergent (Le Guerroué et al., 2017), suggesting further specialization remains to be uncovered. Collectively, these findings challenge the redundancy only

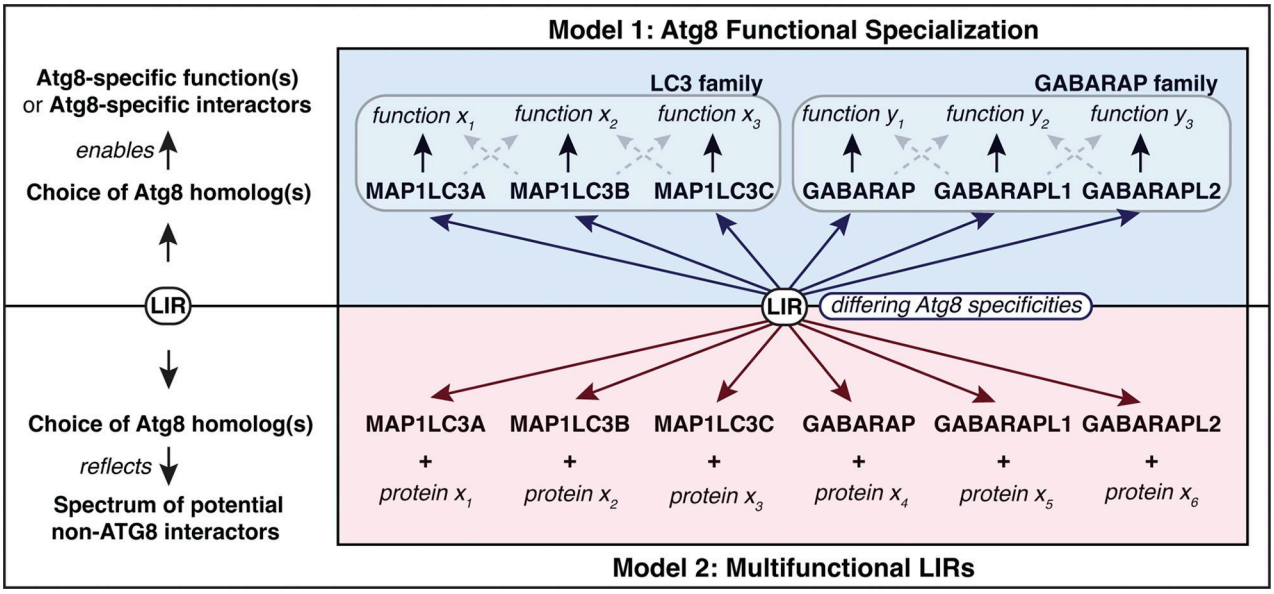

Figure 3. **Models of Atg8 functional diversification beyond redundancy.** Atg8 functional specialization (*top*). LIRs dictate which Atg8 homolog(s) are recruited, which in turn drives specific autophagic functions. Different functions for different Atg8s enable diverse autophagic processes to be invoked in different instances. Multifunctional LIRs (*bottom*). The evolution of Atg8 homologs expanded the diversity of the LIR sequence space, enabling the emergence of multifunctional LIRs.

model and support an alternative explanation for Atg8 expansion: functional specialization (Fig. 3, top). However, additional studies are needed to fully refine our understanding of the functional differences between closely related orthologs.

As an alternative, another driving force behind Atg8 expansion may have been the increasing need for LIRs to facilitate interactions with non-Atg8 proteins (Fig. 3, bottom). To this end, Atg8 diversification likely enabled a parallel expansion of the LIR sequence space, increasing the range of possible LIR sequences. A broader LIR repertoire could allow LIRs to engage non-Atg8 partners while retaining interactions with at least one Atg8 member. While speculative, this model suggests that the evolutionary expansion of the Atg8 family could have been at least in part driven by the need for a more diverse LIR sequence space that enables multifunctional LIRs.

In summary, the expansion of Atg8 homologs likely reflects multiple concurrent pressures: (1) the need for pathway redundancy, (2) functional specialization among Atg8s, and (3) the capacity to accommodate multifunctional LIRs. These models are not mutually exclusive. Quite possibly, Atg8 expansion reflects a balance between all three. Future studies will be needed to unravel how these mechanisms interact and further identify the evolutionary pressures that have shaped the Atg8 system.

Implications and emerging directions in LIR biology

Once thought to function solely by binding Atg8 family proteins, LIR motifs are emerging as general organizing elements within the autophagy network. One key practical implication of this is that LIR mutations may disrupt more than just LIR:Atg8 interactions. For example, mutating the LIR motif in BNIP3 (BNIP3$^{W18A/L21A}$) impairs the interactions of both BNIP3:Atg8 and BNIP3:WIPI2/3 (Adriaenssens et al., 2024, *Preprint*; Bunker et al., 2023). Even in proteins whose LIR motifs are thought to bind only Atg8s, it remains possible that such motifs also engage additional, unidentified binding partners. Thus, while canonical Atg8 interactions remain a central function for many LIRs, studies should also consider that LIR mutations may disrupt other critical interactions.

The remaining work to fully elucidate which LIRs are multifunctional and which are physiologically relevant is extensive. While significant progress has been made in identifying and characterizing canonical LIR motifs (e.g., studies separating the binding preference of LC3s and GABARAPs [Wirth et al., 2019; Rogov et al., 2017]), most studies have relied on low- to medium-throughput approaches (e.g., peptide arrays, co-immunoprecipitations, etc.). There is likely still much to learn about the subtle sequence variations that drive specificity for different Atg8 family members and the motifs that enable interaction with additional binding partners. High-throughput approaches, such as proteomics or deep mutational scanning, could help uncover previously unappreciated determinants of LIR selectivity. Additionally, comprehensively mapping how these preferences change in response to cellular cues, such as phosphorylation or oxidative stress, will provide a more dynamic view of LIR functionality.

The overlap of LIRs, FIRs, and other autophagy motifs remains an active area of investigation, raising important questions about multifunctional LIRs' evolutionary and functional advantages. One potential benefit is that by centering autophagy interactions around LIR motifs, autophagy gains directionality: during autophagy, Atg8 proteins locally accumulate, displacing upstream machinery. This coupling is thought to drive several key transitions in autophagy (Turco et al., 2019; Zhou et al., 2021; Fracchiolla et al., 2016; Bauer et al., 2024). Another possible

advantage is that by serving as a central interaction hub, LIRs offer an opportunity to dynamically cluster the autophagy machinery—bringing multiple components together in a flexible, reversible manner that facilitates efficient assembly and function. One may further hypothesize that by using a limited number of motifs, the same kinases could simultaneously increase the interaction between multiple autophagy factors during autophagy initiation. While this is presently speculative, the regulation of complex cellular pathways by a limited number of motifs is not without precedent (e.g., see Schmid and McMahon [2007]). Finally, computational simulations suggest that heterotypic (one-to-many) interactions enhance the efficiency of phase transitions within cellular condensates (Riback et al., 2020; Krishnan et al., 2022). With the growing recognition of liquid condensates in autophagy regulation (Fujioka and Noda, 2021), multifunctional LIRs may enable the formation of higher-order condensates critical for autophagy.

Another intriguing possibility—though largely unexplored—is that LIR motifs may reside within structured regions of folded proteins, buried in the hydrophobic core and normally inaccessible to the cellular environment. These domains are often rich in aromatic and hydrophobic residues, the same chemical features that define canonical LIR motifs. Upon partial unfolding or misfolding, such buried motifs could become exposed, acting as "eat me" signals for the autophagy machinery. These cases may be underexplored due to the well-established role of the proteasome in degrading misfolded proteins, but a similar mechanism could plausibly contribute to aggrephagy. It is tempting to speculate that LIR motifs may have originally evolved from such generic damage-associated signatures, later acquiring adjacent polar residues or posttranslational modifications to increase specificity and regulatory potential.

In sum, recognizing that LIR motifs anchor protein interaction hotspots opens new avenues of investigation into autophagy regulation. Given that LIR-mediated interactions are typically low affinity, it is likely that even more interactors have been overlooked. As new interactors are identified, it will be necessary to dissect these interactions systematically. The future of LIR biology lies in unraveling these complex interaction networks and translating this knowledge into mechanistic insights and therapeutic strategies targeting autophagic processes.

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