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STATE-OF-THE-ART REVIEW

Structure and function of p62/SQSTM1 in the emerging framework of phase separation

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p62/SQSTM1 is a multiprotein interaction hub forming cellular punctate structures known as p62 bodies. p62 is centrally involved in the degradation of ubiquitinated cargo through autophagy, as well as in a wide range of signaling activities as part of the cellular response to nutrient sensing, oxidative stress, infection, immunity, and inflammation. Structural work has shown that p62 forms flexible filamentous assemblies composed of an N-terminal PB1-domain scaffold and a C-terminal binding platform, including folded recognition domains and structurally disordered binding motifs. In the cell, these filaments are part of cellular p62 bodies that display properties of liquid–liquid-phase separation. Here, we review the accumulated structural and functional work of p62 and integrate them with the emerging framework of filamentous biomolecular condensates.

Introduction

p62/SQSTM1 (from here on p62) is a multidomain, multifunctional protein involved in autophagy and a series of signaling processes [1–3]. Approximately 25 years ago, p62 was identified as a novel interaction partner of the SH2 domain of tyrosine–protein kinase Lck and subsequently cloned [4]. Further interaction studies revealed that p62 bridges the interaction of atypical protein kinase C (PKC) with RIP1 to activate the NF-κB pathway [5]. The molecular link to autophagy was established by p62's involvement in the disposal of poly-ubiquitinated aggregates via the lysosome [6]. Due to its early discovery, p62 can be considered the archetypical autophagy receptor that is also involved in the targeted removal of other cargo types such as bacteria, viruses, and organelles [1]. In this process, a phagophore membrane is recruited through p62's interaction with LC3 and elongated until it encloses the cargo-p62 complex in a double-membrane

Abbreviations
FRAP, fluorescence recovery after photobleaching; IDR, intrinsically disordered region; LLPS, liquid–liquid-phase separation; p62, p62/SQSTM1; PAS, phagophore assembly site; PB1, Phox and Bem1; PKC, protein kinase C; TFG, TRK-fused gene; UPS, ubiquitin/proteasome system.

vesicle. This so-called autophagosome is directed to the lysosome where it fuses with the lysosomal membrane and its contents are degraded.

The p62 molecule is a 440 aa protein that contains three structurally folded domains: a PB1 domain (1–102), a ZZ-domain (122–167), and a UBA ubiquitin-binding domain (389–434). In addition, it contains a long intrinsically disordered region (IDR) (168–388), which provides binding sites to various interacting partners such as LC3, KEAP1, and FIP200 via short binding motifs [6–8]. p62's UBA domain is critical for the recognition of poly-ubiquitin and ubiquitinated cargo [6,9]. Due to the multitude of discovered interaction partners, the p62 interaction hub is able to integrate the signals of multiple pathways such as selective autophagy [6], MAP kinase [10], NF-κB [11], mTORC1 [12], Nrf2 [7,13,14], and N-end degradation [15], linking p62 to many essential biological processes, such as degradation, oxidative stress, nutrient sensing, and inflammation.

The complexity of the p62 interaction hub is remarkable and not easy to envision to function by simple one-to-one binding of protein interaction partners. More recently, p62 was shown to undergo liquid–liquid-phase separation (LLPS) *in vivo* and *in vitro* [16,17]. LLPS is a general cellular physico-chemical phenomenon involved in many biological processes and was characterized for cellular light-microscopic punctate structures such as Cajal bodies, stress granules, P bodies, and the nucleolus (recently reviewed in Ref. [18,19]). Cells make use of phase separation to sequester specific components into one cellular location and to modulate interaction kinetics by increasing the local concentration of proteins. Two principal constituents of liquid droplets are required: first scaffold proteins and second client proteins. Scaffold proteins are the drivers of the phase separation as they tend to self-associate or have repeating units in sequence, resulting in a relatively low mobility within the separated droplet. Client proteins are mobile with respect to the scaffold and not required to induce phase separation, but are capable of modulating the properties of the separated phase. Many scaffold proteins display high valency due to the presence of repeating interaction domains. Interactions between these domains are stronger than the interaction between the protein and the solvent and thereby drive the phase separation process. High-valency scaffolds tend to undergo phase separation more readily, because the entropic cost is less than for smaller proteins with fewer interaction domains [20]. Scaffolds can be repeating polymers, like RNA molecules, which further increase valency through oligomerization. Typical scaffold proteins also have IDRs with high-sequence repetition composed of polar and charged amino acids or of aromatic amino acids. Cells can tune the assembly of these droplets by changing the concentration of the scaffold or by modulating the solubility of the components. The water solubility depends mostly on charged residues that can be introduced by posttranslational modifications or by interaction with client proteins. This way, regulatory proteins can alter the dynamics of scaffold proteins inside biomolecular condensates.

As the structural and functional properties of phase-separated p62 bodies are emerging, several autophagy-related proteins have been identified to undergo LLPS on their own. In fertilized *Caenorhabditis elegans* embryos, P granules are critical compartments for further cell differentiation. Main P granule components PGL-1 and PGL-3 require degradation by selective autophagy via autophagy receptor SEPA-1 and the scaffold protein EPG-2. These proteins have been demonstrated to undergo LLPS *in vitro* and *in vivo* and their phase separation behavior was shown to be modulated by post-translation modifications in PGL-1 [21]. More recently, yeast hydrolase Ape1 was shown to form phase-separated droplets as part of the Cvt pathway *in vivo* as well as *in vitro* [22]. In this model system of selective autophagy, Atg19 corresponds to the autophagy receptor and due to the physico-chemical properties was found floating on the surface of the Ape1 droplets, which in turn recruits Atg8 and Atg5 of autophagy core machinery to the periphery of the droplet [22]. Fluorescence mobility measurements of the phagophore assembly site (PAS) in yeast revealed liquid-like phase properties of Atg1 kinase droplets [23]. The resulting liquidity is critical for the recruitment of PAS components and phosphorylation of Atg1 kinase component Atg13 can lead to the spatial exclusion from the PAS. The physico-chemical properties of interaction partners that are part of a LLPS system are important driving forces to organize and recruit the required molecules at specific cellular locations in addition to the complementary molecular interfaces within autophagy complexes [23].

Almost 25 years of research on p62 accumulated a comprehensive body of work detailing molecular interactions and involving p62 in a large number of biological pathways. Due to the emergence of p62 as a scaffold protein in phase-separated cellular compartments, we review and attempt to reconcile the existing structural and functional data with the growing number of reports of p62's involvement in LLPS.

The PB1 domain is the polymerization scaffold of p62

The primary structure of the PB1 domain reveals that the 102 N-terminal amino acids of p62 belong to a larger Phox and Bem1 (PB1) domain family conserved over the eukaryotic tree of life [24] (Fig. 1A). The PB1 domain contains secondary structures of 2 α-helices and one β-sheet and adopts the tertiary structure of a ubiquitin-like β-grasp fold resulting in two opposing surfaces. They can be both acidic (type A), both basic (type B), or acidic and basic each (type AB). These charged patches provide complementary interaction surfaces for either homo-multimerization or for hetero-dimerization with other PB1 domains [25,26]. In the case of p62, which contains an acidic and a basic surface (type AB), the PB1 domains are capable of homo-polymerization into the quaternary structure of flexible filaments [27], thereby forming the scaffold of the p62 interaction hub. Recently, the presence of a double-RR motif in p62-PB1 as well as the PB1 domain from TRK-fused gene (TFG) protein was demonstrated to be a requirement for polymer assembly in addition to the characterized AB-type interface [28]. Interestingly, filamentous structures of the PB1 domain were capable of forming different polymorphs; that is in addition to the canonical AB-type PB1-PB1 interaction forming a helical turn, the helical strands have been found to pair in different ways giving rise to two, three, and four-stranded assembly architectures of the PB1 scaffold [27,28]. Once homopolymers are formed, they are capable of interacting with other PB1 domain-containing proteins, such as PKCs, MEKKs, or NBR1 at their ends. Such interactions cap and thereby limit further assembly [28] (Table 1). C-terminally located to the PB1 domain is a filament-stabilizing stretch of 20 aa that has been termed the electrostatic bridge due to a high content of charged residues (> 40%) [27]. In support of this stabilizing effect, environmental changes in pH were shown to affect the PB1-mediated polymerization of p62 in solution [29]. Moreover, this region also has two critical cysteines that have been shown to promote higher-order assemblies upon changes in oxidative conditions [30].

The ZZ-domain is a multiprotein and RNA interaction hub

Adjacent to the PB1 scaffold is a 45-residue ZZ-domain (122–167) (Fig. 1B), which consists of an α/β-fold that binds two zinc atoms via cysteine and histidine residues within the ZZ-type zinc finger motif [29]. Originally, the ZZ-domain was identified to interact with RIP1, a kinase involved in NF-κB signaling [5] (Table 1). More recently, the ZZ-domain was successfully crystallized with the arginylated N-degrons of the BiP chaperon, which recognizes specific cargo destined for autophagosomal degradation via the N-degron pathway [29]. Interestingly, the binding affinity is increased by an order of magnitude in the oligomerized form of PB1-ZZ in comparison with the polymerization-deficient K7A/D69A mutant. In addition to p62's central role in linking the ubiquitin/proteasome system (UPS) and autophagy through the N-degron pathway, recently p62's ZZ-domain was also identified to interact with RNAs. Vault RNA-1 was shown to inhibit p62 polymerization and thereby regulating autophagy turnover [31]. The ZZ-domain of p62 hosts a series of interaction partners, and due to the spatial proximity to the PB1 scaffold, their binding can affect the assembly of p62 filaments.

The intrinsically disordered region contains critical interaction motifs

According to bioinformatic predictions and supported by visualization of purified full-length p62 [27], the region downstream to the ZZ-domain is an IDR, extending from residues 168 to 388. This IDR stretch has a high content of negatively charged and serine residues. Its total of 36 serine residues corresponds to approximately double the commonly observed frequency in vertebrates. Several important binding sites have been identified for the following interaction partners: TRAF6 [11], Raptor [32], LC3, and KEAP1 [7,13,33,34] (Table 1). The TRAF6 binding region consists of six amino acids and serves to recruit TRAF6 so that it can interact with various other proteins [11]. For instance, a ternary complex consisting of p62, TRAF6, and PKCζ leads to the activation of the NF-κB pathway. In addition, the p62-TRAF6 complex can interact with mTORC1 via Raptor and thereby guides mTORC1 to the lysosomal membrane, leading to its activation when nutrient levels are high [35]. The autophagy-related functions of p62 are dependent on its LC3 interacting region (336–341) (LIR) [34]. LC3 is a small protein that is covalently attached to the phospholipids and decorates the phagophore and the final autophagosome [36]. The core LIR motif, common to many autophagy-related proteins, is composed of the sequence W/F/YXXL/I/V [37] and the p62-LIR LC3B interaction is driven by packing of the W338 and L341 residues to two hydrophobic pockets in the LIR docking site of LC3B [33,38]. The core motif is flanked by N and C-terminal sequences that contribute both to

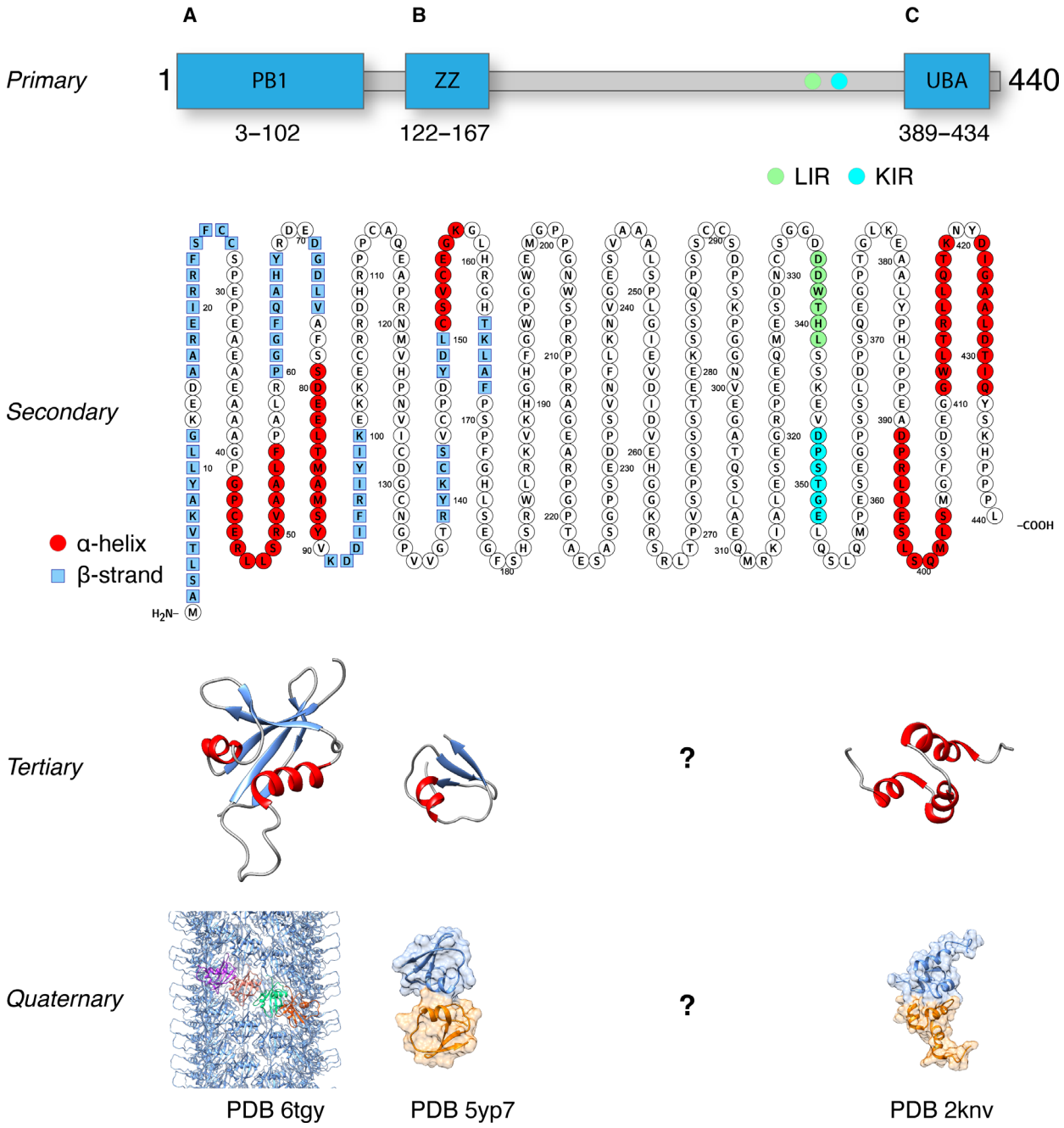

Fig. 1. Domain architecture of p62/SQSTM1 including a PB1, ZZ, and UBA domain. p62 is a 440 aa protein with (A) a PB1 (PDB ID 6TGY), (B) ZZ (PDB ID 5YP7), and (C) UBA domain (PDB ID 2KNV) (primary structure). The region between ZZ and UBA domain is an intrinsically disordered region with critical interaction motifs such as LIR interacting region (LIR) and KEAP1 interacting region (KIR). The PB1 and ZZ-domain are α/β-folds whereas the C-terminal UBA domain consists of 3 α-helices (secondary structure). Individual domain structures were determined by NMR and X-ray crystallography [26,29,91,92] (tertiary structure). PB1 domains assemble into filamentous assemblies determined by cryo-EM [28]. ZZ and UBA domains have been shown to form dimers (quaternary structure) [29,93].

binding affinity and specificity [39]. The KEAP1 interacting region (KIR) (347–352) of p62 binds to KEAP1 that causes the release of Nrf2, a transcription factor that promotes expression of antioxidant proteins [7,13]. The KIR contains S349 that can be phosphorylated by mTOR in response to oxidative stress, which

Table 1. Summary of binding partners of p62/SQSTM1 sorted by domain.

Domain p62/ cellular process	PB1(3–102)	ZZ (121 –167)	IDR (168– 388)	UBA (389– 434)
Autophagy	NBR1 [25,55]	Vault RNA [31]	LC3 [34]	Ubiquitin [9]
Nutrient sensing	MEKK3/ MEK5 [12,25]		Raptor [32]	
Inflammation Oxidative stress	Lck [4]	RIP1 [5]	TRAF6 [35] KEAP1/ Keap1 [7,13]	
Osteogenesis	PKCs, Par-4 [25,77]		FIP200 [8]	

has been shown to enhance the interaction between Keap1 and p62 [40]. According to the structural view of p62's assembled PB1-scaffold, the IDR is available as a binding platform for interacting client proteins taking advantage of high local concentration of p62's binding sites. In support of this view, it was shown that when the PB1-scaffold is prevented from forming p62 higher-order assemblies through TRIM21-mediated ubiquitylation of K7, p62 is not effective in sequestering Keap1 and fails to release Nrf2 for launching the oxidative stress response [41]. The solvent-accessible IDR of p62 hosts most of the post-translational modification sites within the protein (Table 2 and Fig. 2).

The UBA domain captures ubiquitinated cargo

The UBA domain of p62 extends from residues 389 to 434 (Fig. 1C) and binds to ubiquitinated cargo destined for lysosomal degradation by autophagy. The 3α-fold is made of approximately 45 amino acids and occurs in other ubiquitin-binding proteins involved in the UPS pathway, DNA excision-repair, and cell signaling via protein kinases. The UBA domain of p62 is capable of forming homodimers, which compete with ubiquitin binding [42]. Within the p62 filament, the UBA domain self-interactions appear to have a stabilizing role as competing poly-ubiquitin was shown to dissociate the assemblies [27] whereas ubiquitinated model cargo cross-linked shorter filaments into larger p62 condensates [17]. These *in vitro* results are in support of earlier studies that revealed p62's clustering activity of different cargos such as aggregates and mitochondria in cells [43–45] presumably mediated by the UBA ubiquitin interaction. Interestingly, the UBA domain can itself be ubiquitinated by the Keap1/Cullin3 ligase, which leads to reduced degradation of p62 [46]. In contrast, increased autophagy clearance was shown by phosphorylation of S403, which enhanced the affinity to poly-ubiquitin chain binding of the UBA domain [47]. Similarly, S403 phosphorylation by TBK1 was shown to modulate uptake of damaged mitochondria as well as the inflammatory response [48,49]. The UBA domain can also be phosphorylated by ULK1 at S409 in mouse p62, corresponding to human S407, in response to metabolic stress, which enhances the degradation of both p62 and any bound cargo [50]. Interestingly, the proteolysis of p62 at position D329 reveals an additional level of regulation and results in a trimmed protein that lacks LIR motif and UBA domain both of which critical for autophagy while keeping N-terminal interaction sites responsible for signaling [51].

p62 clusters in membrane-less structures inside the cell

In human cells, p62 is primarily located in the cytosol in light-microscopically observable punctate structures that have been termed p62 bodies. Smaller fractions of p62 can also be found in membrane-enclosed autophagosomes and lysosomes. A series of methods have been used to characterize p62 bodies and address different levels of structural organization. Co-immunoprecipitation in addition to biochemical studies with purified proteins revealed a number of p62 interacting proteins such as PKCs [25,28] (Fig. 3A). Cytosolic p62 bodies can be observed by fluorescence light microscopy showing that they are up to several μms in size and approximately circular [6,25] (Fig. 3B). Cellular electron microscopy studies revealed that p62 bodies do not have a membrane surrounding them and that they are composed of a meshwork of filamentous structures [6,28,52] (Fig. 3C). These filaments were found to have a diameter compatible with the cryo-EM structure that has been solved of the purified filamentous p62-PB1 assemblies [27]. The cryo-EM as well as the X-ray structures of p62-PB1 domain [26,28] (Fig. 3D) provided the basis for polymerization-deficient mutants such as K7A/D69A or p62ΔPB1 resulting in a diffuse cytosolic signal devoid of the typical p62 punctae when observed by fluorescence microscopy [6,33,53]. Cryo-EM also revealed that PB1 domain assemblies give rise to different polymorphs of strand organization mediated by residues outside the

Table 2. Summary of posttranslational modifications including references.

Domain (aa stretch)	Residue	Modification	Enzyme	Cellular process	Reference
PB1 (3–102)	K7	Ub	TRIM21	Oxidative stress	[41]
	S24	P	CK2, TAK1, PKA	Autophagy, signaling	[47,67,78]
	K91	Ub	RNF166	Autophagy	[79]
ZZ (122–167)	C105	SH	–	Oxidative stress	[30]
	C113	SH	–	Oxidative stress	[30]
	T138	P	LRRK2	Neuronal stress, autophagy	[80]
IDR (168–388)	Y148	P			[81][a]
	S170	P			[82][a]
	S176	P	LRRK2	Neuronal stress, autophagy	[80]
	K189	Ub	RNF166	Autophagy	[79]
	S207	P	CK2	Autophagy	[47]
	S226	P	TAK1	Autophagy, signaling	[67]
	S233	P			[81][a]
	S249	P			[82][a]
	S266	P	Unknown	Nuclear localization	[59]
	T269	P	CDK1, TAK1, CK2, p38δ	Cell cycle, autophagy, nutrient sensing	[12,47,59,67,83]
	S272	P	CDK1, TAK1, CK2, p38δ	Cell cycle, autophagy, nutrient sensing	[12,47,59,67,83]
	S275	P	TAK1	Autophagy	[67]
	S282	P	CK2	Autophagy	[47]
	S294	P	AMPK	Autophagy and cell death	[84]
	S306	P			[81][a]
	S328	P			[85][a]
	S332	P	TAK1, CK2	Autophagy	[47,67]
	S349	P	TAK1, PKC-δ, CK1	Autophagy	[67,86–88]
	S355	P			[89][a]
	S361	P			[81,85][a]
	S365	P	Unknown	Autophagy	[8]
	S366	P	Unknown, CK2	Autophagy	[8,47]
UBA (389–434)	S403	P	TAK1, TBK1, ULK1, CK2	Autophagy	[47,48,50,67]
	S407	P	ULK1	Autophagy	[50]
	K420	Ub	Keap/Cullin3	Oxidative stress	[46]
	K420	Ac	TIP60	Autophagy	[66]
	K435	SUMO			[90][a]
	K435	Ac	TIP60	Autophagy	[66]

[a]Reported by proteome-wide analysis.

canonical AB interface [28]. Nevertheless, despite the existence of these intriguing atomic models, the detailed fine structure of p62 in cells remains to be established.

Proteome analysis of high-density aggregates induced by proteasome inhibitors identified the presence of p62 and NBR1 in addition to ubiquitin and ubiquitin-binding proteins [54]. These content proteins are consistent with light-microscopic colocalization studies of p62 bodies [6,25,55]. NBR1 is approximately double in protein length and shares PB1, ZZ, and UBA domains including the LIR motif in primary structure with p62. p62 only exists in metazoans whereas NBR1 is found in plants, fungi, and in the closest living unicellular relatives of metazoan [56]. Nonmetazoan NBR1 like *Arabidopsis thaliana* NBR1 also has been shown to homo-oligomerize into filamentous polymers due to the AB-type PB1 domain [28]. It is thought that p62 arose from gene duplication of NBR1 in the early metazoan lineage while NBR1 later lost its ability to assemble into polymers [56]. Independently of p62, human NBR1 has been shown to be a *bona fide* autophagy receptor capable of bridging cargo and the LC3 protein [55,57]. NBR1 also forms a PB1-domain-mediated complex with p62 [25]. The PB1 domain of NBR1 is of the A type as opposed to p62's AB type and is, therefore, not capable of forming polymers on its own but was shown to cap the ends of PB1-p62 filaments *in vitro* [28]. p62 filaments mediated by PB1 domain interactions make up the principal framework of cellular p62 bodies.

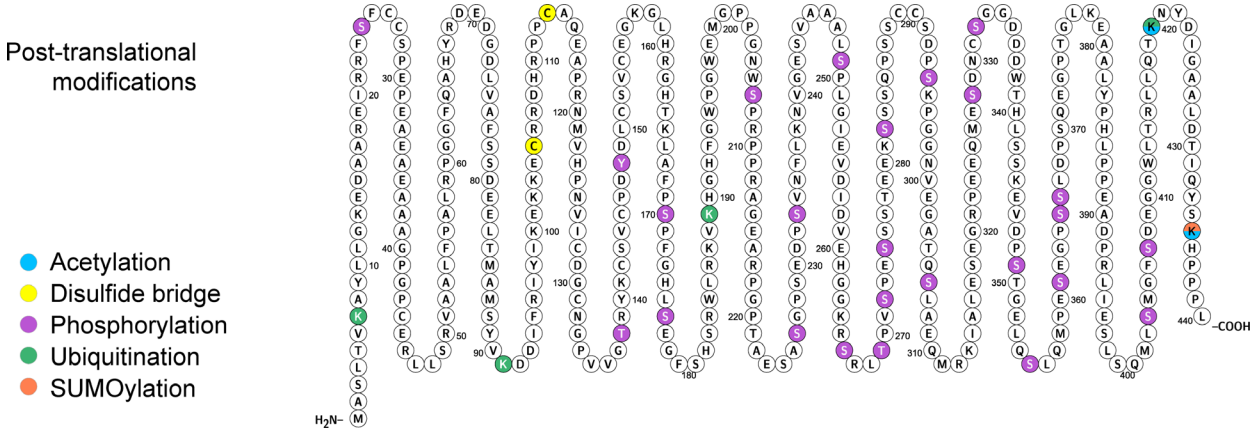

Fig. 2. Posttranslational modifications of p62/SQSTM1. A large series of posttranslational modifications have been reported for p62: acetylation, disulfide bridge, phosphorylation, SUMOylation as well as ubiquitination. The intrinsically disordered region (168–388) contains the majority of the modifications. The detailed list including references can be found in Table 2.

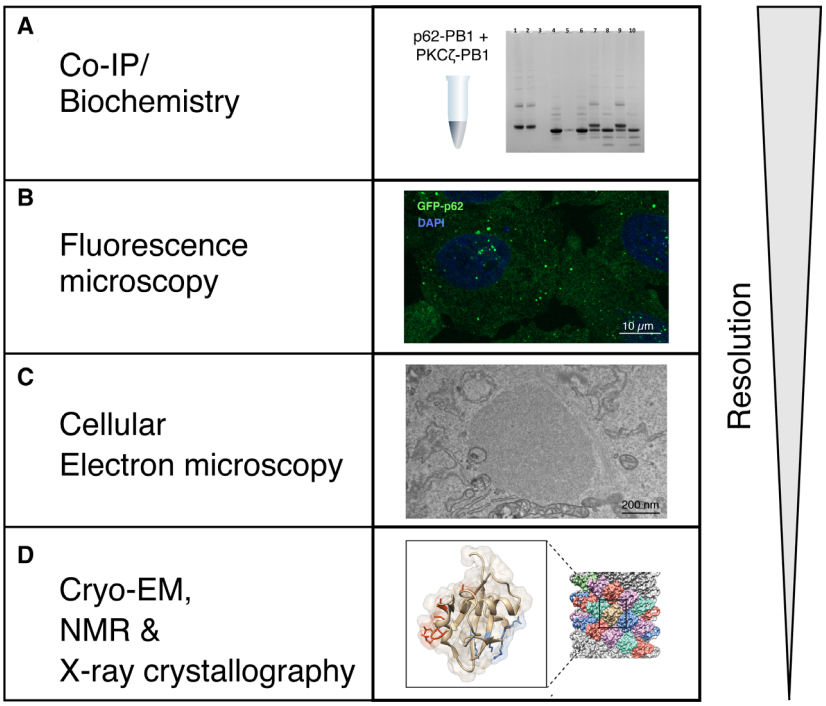

Fig. 3. Main methods of investigation on the structure and function of cellular p62 bodies. (A) Biochemistry using purified proteins or immuno-precipitation has been used to identify and study interaction partners of p62. (B) Fluorescence light microscopy can determine colocalization of labeled proteins with p62 bodies, in addition to dynamic exchange properties using FRAP and related fluorescence techniques. (C) Cellular electron microscopy (EM) provides the ultrastructural framework of cellular p62 bodies including immuno-labeling to identify specific components. (D) Cryo-EM, NMR, and X-ray crystallography provide atomic models of components of p62 bodies. The gel image, the fluorescence image, and the cellular electron micrograph were kindly provided by Simon Mortensen, Mireia Nàger/Terje Johansen, and Sebastian Schultz/Andreas Brech, respectively.

Due to the existence of a nuclear localization signal and nuclear export signal, p62 is capable of shuttling between the cytosol and the nucleus. Therefore, p62 has also been found in the nucleus in speckles or PML bodies [6,58,59]. In these nuclear bodies, p62 colocalizes with another autophagy scaffold protein called ALFY [60]. Upon oxidative stress, nuclear p62 bodies were found to selectively sequester K48-linked poly-ubiquitinated proteins and be involved in programmed cell death [61]. In the nucleus, p62 has also been shown to contribute to the assembly of proteasome-containing degradative compartments to dispose nuclear aggregates [59] and prevent histone ubiquitination to trigger DNA repair [62]. Although cytosolic and nuclear p62 bodies are similar in principal appearance and properties [63], further research will be

needed to clarify how p62 bodies differ in content and structure in different cellular locations.

p62 bodies are tunable, phase-separated cellular structures

Early characterization of p62 bodies by confocal fluorescence microscopy indicated that p62 bodies can be found in different populations with regard to size, intensity, and mobility [6]. Fluorescence recovery after photobleaching (FRAP) experiments characterized the dynamic properties of LC3 in cellular p62 bodies [64]. They revealed that LC3 is mobile within p62 bodies while p62 is engaged in a slowly diffusing polymeric complex. Further characterization of *in vitro* p62 structures confirmed this principal mobile behavior: While purified p62 on its own did not form comparable body structures, ubiquitinated model cargo promoted p62 clustering and coalescence into p62 punctate structures [16,17]. These two independent studies established that p62 structures undergo LLPS and belong to the group of biomolecular condensates.

Many p62 binding partners have been described to alter this LLPS behavior of p62 bodies (Fig. 4). The overexpression of NBR1 increased the size and mobility of p62 bodies [65]. Interestingly, even the D50R NBR1 mutant incapable of interacting with p62's PB1 domain promoted faster signal recovery in FRAP experiments, suggesting direct or indirect interactions apart from the PB1-PB1 domain association. Autophagy scaffold ALFY was shown to be critical for the formation of p62 bodies in the cell as ALFY siRNA knockdown gave rise to very few p62 bodies per cell [60]. By abolishing interaction with LC3 through a LIR mutant, p62 bodies were significantly reduced, demonstrating the contribution to p62 clustering [17]. While LC3B was readily incorporated in p62 droplets, ubiquitin-coupled cargo as well as longer poly-ubiquitin chains also promoted cluster formation in addition to the uptake [16,17]. FIP200, a component of the ULK1 kinase complex, inhibits formation of p62 bodies as its binding site overlaps with the LIR and thereby competes with LC3 binding [8]. In contrast, ubiquitination of p62 at K420 by the Keap1/Cullin3 ubiquitin ligase has the opposite effect, LC3 association is enhanced and more p62 bodies were found inside the cell [46]. As a result of the K420 ubiquitination, p62 signal intensity after FRAP recovers more slowly than with wild-type p62 and thus reduces liquidity of p62 bodies. In support, many reported post-translational modifications (Table 2) have been shown to affect the size and liquidity of p62 bodies. For instance, after K420 and K435 acetylation by TIP60, p62 was found to have a higher affinity for poly-ubiquitinated proteins and forms larger and more p62 bodies inside the cell [66]. Phosphorylation of p62 also affects p62 body structure. Examples include S409 and S405 phosphorylation by ULK1, which enhances the affinity of p62 for poly-ubiquitin [50], in addition to increased phosphorylation levels at S24, S226, T269, S272, S275, and S332 by TAK1, which reduce p62 presence in autophagosomes while increasing the number of p62 bodies [67]. p62 was found to be ubiquitinated by TRIM21 at residue K7 in the canonical AB-type PB1 interface, which reduces p62's propensity to oligomerize and form p62 bodies [41]. Other proteins, like the death-associated protein DAXX and TRIM17, an autophagy regulator, both colocalize with p62 in p62 bodies and increase the number of p62 droplets per cell [68,69]. In the presence of DAXX, fluorescent p62 recovers more slowly after photobleaching, also indicating a more rigid gel-like structure [69]. Oxidation of p62 after induction of cellular redox stress by H_2O_2 has also been described to enhance oligomerization and higher-molecular weight species through intermolecular disulfide bridges between residues C105 and C113 [30]. Interestingly, several p62 mutants in the UBA domain associated with Paget's disease of bone and with amyotrophic lateral sclerosis with dementia, such as P392L, M404V, G411S, and G425R, showed increased signal recovery in FRAP experiments in addition to reduced ubiquitination of the UBA domain [46]. Although it is unclear whether these changes in p62 body properties are causative of the disease, they affect the dynamic equilibrium inherent to p62 bodies with consequences for their respective functions. Unrestrained growth of p62 bodies is expected to be harmful as it may lead to an overwhelming load of the autophagy degradation system, while diffuse monomeric p62 molecules cannot assist in the autophagy disposal of aggregates [53]. Therefore, regulated p62 body removal is critical to maintain cellular homoeostasis. In addition to p62 self-degradation by selective autophagy, two additional cellular pathways have been identified that are capable of degrading p62. First, under starvation conditions, p62 can be taken up into multivesicular bodies or late endosomes via endosomal microautophagy [70]. Second, the E3 ubiquitin ligase parkin has been shown to ubiquitinate p62, initiating p62 degradation by the proteasome [71]. p62 degradation as a self-cargo by selective autophagy in the lysosome, however, is thought to be the predominant way of removing p62 bodies from the cell and thereby contributes critically to cellular homeostasis. In conclusion, the frequency, size, and dynamics of p62 bodies are the result of a

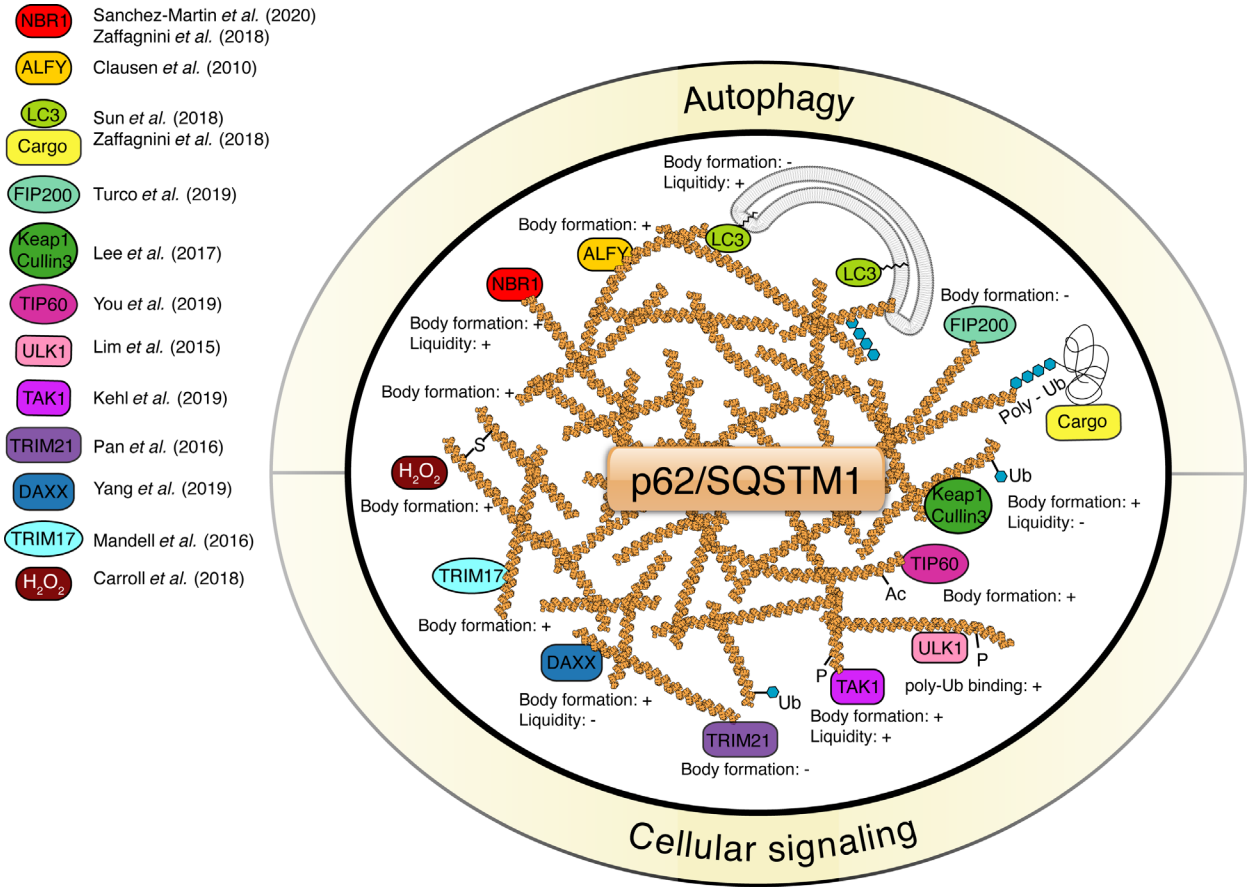

Fig. 4. p62/SQSTM1 makes up the framework of cellular p62 bodies that are tunable protein interaction hubs. p62 is organized in flexible 15-nm-wide filaments forming the framework of cellular p62 bodies. Many interaction partners of p62 have been found to colocalize with p62 bodies and shown to affect the size and liquidity of p62 bodies, for example, NBR1, ALFY, LC3, poly-ubiquitinated cargo, FIP200, KEAP1, TIP60, ULK1, TAK1, TRIM21, DAXX, and TRIM17. Oxidative stress induced by H_2O_2 has also been shown to lead to increased body formation. The respective references are listed in the legend.

fine-tuned equilibrium between p62's tendency to self-assemble, interactions with multiple proteins and different chemical protein modification states, all of which are integrated to determine the downstream functions of p62 in autophagic degradation and signaling.

The physico-chemical basis of filamentous biomolecular condensates

Scaffold proteins and client proteins are the minimal components of phase-separated systems [72]. One organization type that readily fulfills the requirements of scaffolds are head-to-tail protein filaments such as p62 [73]. Protein polymers or filaments assemble spontaneously in a reversible manner conferring dynamics and fluidity to the phase-separated system (Fig. 5A). The high valency of the repeating scaffold domains in filaments offer high local concentration of binding sites to the clients or interacting partners. Even at low affinities, this multivalent configuration will result in apparent high avidity interactions. Filamentous p62 assemblies consist of the N-terminal PB1-scaffold and large C-terminal binding platform formed by folded recognition domains and a long IDR stretch. The long regions of intrinsic disorder contribute entropically to phase separation within the cell. Upon successful binding of client proteins, they can decorate the scaffold protein to prime it for further downstream interactions (Fig. 5B). The binding of decorating interactors to p62 filaments may favor certain polymorphic assembly types due to steric hindrance, which have been observed to exist in equilibrium when isolated [28]. When client proteins bind directly to the PB1 scaffold, they compete with the homo-polymerization of the PB1 domain and lead to end capping of the filaments

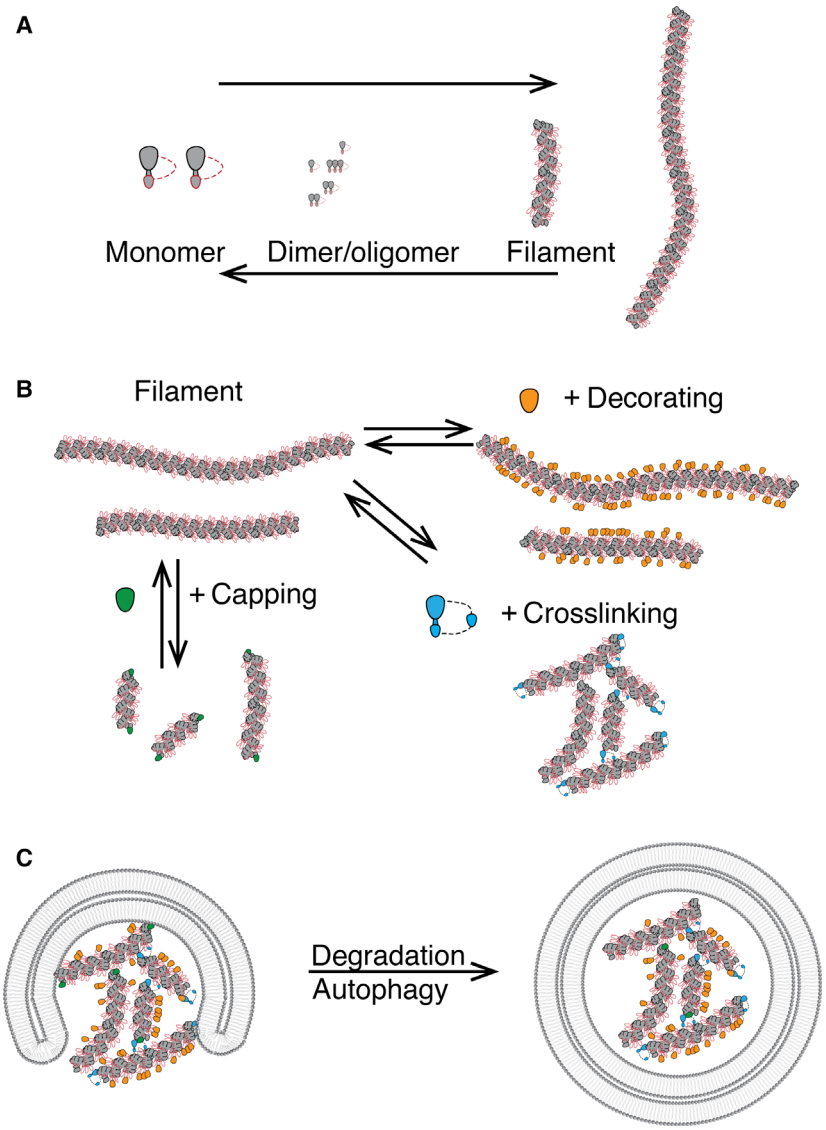

Fig. 5. The life cycle of p62 filamentous biomolecular condensates: from polymerization to degradation. (A) The formation of filamentous p62 is a reversible homo-polymerization reaction via dimers and oligomers driven by the N-terminal PB1 scaffold (gray). The intrinsically disordered region of p62 forms the flexible binding platform (red). (B) Different client proteins bind to filamentous p62. Interaction with the binding platform leads to decoration of filaments. Interaction with the PB1 scaffold competes with homo-polymerization and caps the filament ends preventing further growth. Multivalent interactions with p62 lead to cross-linking of filaments. (C) p62 bodies are predominantly degraded by selective autophagy as p62 self-cargo.

preventing further growth in filament length [28]. Clients that possess multiple binding sites to the scaffold domain as well as to the binding platform will enable cross-linking of one-dimensional filamentous structures into three-dimensional clusters. When multiple clients with competing interactions are considered, a comprehensive interaction network is formed within the structural entity of the biomolecular condensate. The degree of phase separation and client accessibility can be modulated and regulated by changing the charge distribution along the scaffold's disordered protein stretches through posttranslational modifications. More drastically, by modifying the scaffold core and disrupting the contacts between repeating units, LLPS will be reduced as multivalency is given up. To the contrary, when modifications and clients stimulate

excessive growth of biomolecular condensates, they need to be removed by autophagy in order to maintain cellular homeostasis (Fig. 5C).

Conclusion

While this review summarized the accumulating evidence and state of the art of the p62's emerging role in phase separation, there are related filament systems that share many of the described fundamental properties and give rise to phase-separated droplets. Among them are head-to-tail polymers of the DIX domain that is structurally closely related to p62's PB1 domain [74]. The DIX domain forms the repeating scaffold of the disheveled protein as part of the Wnt signaling pathway promoting cell differentiation [75]. Moreover, TFG involved in COP-II transport shares basic structural features with p62 including the N-terminal PB1 domain and TFG-PB1 has been shown to assemble into filamentous polymers [28]. Moreover, phase-separated TFG has been observed in the cell and is thought to concentrate COP-II transport carriers at the ER/ERGIC interface [76]. Due to the sheer number of studies available for p62, it may become one of the reference systems for liquid filamentous scaffolds forming biomolecular condensates. Given the remarkable complexity of p62 as a scaffold protein involved in autophagy and many signaling pathways, the emergence of p62's participation in LLPS now offers the unique opportunity for future studies to merge the existing structural and functional data into a coherent picture of a liquid interaction hub.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SB, SM, and CS designed the review. SB, SM, and CS wrote the manuscript.

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