Minireview

Structure and functional properties of the ubiquitin binding protein p62

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Abstract Several highly conserved p62 homologs have recently been isolated, e.g. the rat atypical protein kinase C-interacting protein (ZIP), the murine A170/signal transduction and adapter protein, and the human p62, a protein that binds the Src homology 2 domain of $p56^{lck}$. These proteins share striking similarity in amino acid sequence and structural motifs, thereby suggesting conserved functional properties. ZIP/p62 has been shown to play an important role as a scaffold leading to the activation of the transcription factor nuclear factor κB. In addition, a nuclear form of p62 has been characterized that can serve as a transcriptional co-activator. Moreover, p62 is capable of binding ubiquitin (Ub) non-covalently through its Ub-associated domain. In this review, we will focus on the structure and function of ZIP/p62. **© 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: p62; Atypical protein kinase C; Ubiquitin; Scaffold; Trafficking

1. Overview

In the past several years, several genes with considerable sequence homology have been cloned from rat, human and mouse libraries. Zeta protein kinase C (PKC)-interacting protein (ZIP) was isolated from a rat brain cDNA library [1], whereas a separate human p62 homolog, that binds the Src homology 2 (SH2) domain of $p56^{lck}$, was identified [2]. A170, a gene induced by oxidative stress in mouse macrophages [3], was cloned, as well as a cDNA for signal transduction and adapter protein (STAP) [4]. Comparison of the amino acid sequence between these proteins reveals that they are highly conserved (roughly 90%) to one another (Fig. 1). The structural motifs shared by these proteins include: an SH2 binding domain, an acidic interaction domain (AID) that binds the atypical PKC (aPKC) [5], a ZZ finger, a binding site for the ring-finger protein tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), two PEST sequences, and a ubiquitin (Ub)-associated (UBA) domain (Fig. 2). The high degree of sequence similarity between these two genes from different sources suggests conserved functionality.

2. A growing family

2.1. ZIP

The aPKC subfamily of kinases is composed of two members, ζPKC and λ/ιPKC. These proteins are related with a 72% overall amino acid identity [6]. When compared to the other isoforms, aPKCs lack the characteristic C2 domain and are insensitive to Ca^{2+}, diacylglycerol and phorbol esters [6]. A protein that specifically interacted with the regulatory domain of aPKCs, but not classical PKCs, was identified, and named ZIP [1]. ZIP was isolated from a λ-phage rat brain cDNA library and is encoded by a polypeptide of 439 amino acids. The structural motifs in ZIP include a ZZ zinc finger as a potential binding module, two PEST sequences, and a novel putative protein binding motif, consensus sequence YX-DEDX$_5$SDEE/D. ZIP binds to the regulatory domain of ζPKC, comprising the pseudosubstrate site and the module that drives interaction between ZIP and aPKC has been mapped to amino acids 41–105 of ZIP.

Two cDNA fragments, B20 and B24, were isolated from a rat hippocampal library screened against full length Kvβ2 subunits of the potassium channel [7]. B20 is identical to ZIP cloned by Puls et al. [1], whereas B24 is a 27-amino acid alternatively spliced form, ZIP2. Both ZIP1/ZP2 are ubiquitously expressed, with ZIP1 displaying significantly higher amounts than ZIP2 in non-excitable tissues, but the ZIP1/ZIP2 ratio is closer to 1 in the central nervous system [7]. ZIP1 interacts with both Kvβ2 and ζPKC, thereby resulting in the formation of a ζPKC–ZIP1–Kvβ2 complex. In this complex, ZIP1 serves as a link that targets the activity of ζPKC to the Kvβ2 subunit of the potassium channel (Fig. 3A). ZIP1 and ZIP2 possess distinct activities in stimulating ζPKC phosphorylation of Kvβ2, with maximum channel phosphorylation by ζPKC occurring in the presence of ZIP1.

ZIP3, a new splice variant of the ZIP, has recently been identified [8]. It contains a new C-terminal domain of 13 amino acids starting at S221. Bicuculline insensitive GABA receptors ($GABA_CR$) are composed of three subunits and ZIP3 selectively interacts with the large intracellular loop of the third subunit. The interaction of ZIP3 with $GABA_CR$ may form a ζPKC/ZIP3/$GABA_CR$ signaling complex [8], analogous to the complex formed by ζPKC/ZIP1/Kvβ2 [7].

2.2. p62, a $p56^{lck}$ substrate

A human ZIP homolog encoding a cytosolic 62-kDa protein (p62), with the ability to bind to the SH2 domain of $p56^{lck}$ in a phosphotyrosine-independent manner, was identified and cloned [9]. Interestingly, p62 is capable of binding Ub

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ZIP   MASLTVKAYL LGKEEAAREI RRFSFCFSPE P--EAEAAAG PGPCERLLSR VAVLFPALRP GGFQAHYRDE
ZIP2  .......... .......... .......... .--..... .......... .......... ..........
P62   .......... ....D..... ......C... .EA..... .......... ..A....... ..........
A170  ...F...... ......T... .......... .EA..Q.... S......... ......T... ..........
STAP  ...F...... ......T... .......... .EA..Q.... .......... ......T... ..........

ZIP   DGDLVAFSSD EELTMAMSYV KDDIFRIYIK EKKECRREHR PPCAQEA-RS MVHPNVICDG CNGPVVGTRY
ZIP2  .......... .......... .......... .......... .......-.. .......... ..........
P62   .......... .......... .......... .......D.. .......P.N .......... ..........
A170  .......... .......... .......... .......... .......P.N .......... ..........
STAP  .......... .......... .......... .......... .......P.N .......... ..........

ZIP   KCSVCPDYDL CSVCEGKGLH REHSKLIFPN PFGHLSDSFS HSRWLRKLKH GHFGWPGWEM GPPGNWSPRP
ZIP2  .......... .......... .......... .......... .......... .......... ..........
P62   .......... .......... .G.T..A..S ......EG.. .......V.. .......... ..........
A170  .......... .......... .......... .......... .......... .......... ..........
STAP  .......... .......... .......... .......... .......... .......... ..........

ZIP   PRAGDGRPCP TAESASAPSE DPNVNFLKNV GESVAAALSP LGIEVDIDVE HGGKRSRLTP TSAESSSTGT
ZIP2  .......... ....------ ---------- ---------- -......... .......... ..........
P62   ....EA..G. ......G... ..S....... .......... .......... .......... V.P....---.
A170  .......... ........P. .......... .......... .......... .......... .TP.......
STAP  .......... ........P. .......... .......... .......... .......... .TP.......

ZIP   EDKSGTQPSS CSSEVSKPDG AGEGPAQSLT EQMKKIALES VGQPEELMES DNCSGGDDDW THLSSKEVDP
ZIP2  .......... .......... .......... .......... .......... .......... ..........
P62   ....SS.... .C.DP...G. NV..AT...A ...R...... E.A...Q... .......... ..........
A170  ....N..... .......... .......... .......... ......Q... G......... ..........
STAP  ....N..... .......... .......... .......... ......Q... G......... ..........

ZIP   STGELQSLQM PESEGPSSLD PSQEGPTGLK EAALYPHLPP EADPRLIESL SQMLSMGFSD EGGWLTRLLQ
ZIP2  .......... .......... .......... .......... .......... .......... ..........
P62   .......... .......... .......... .......... .......... .......... ..........
A170  .......... .......... .......... .......... .......... .......... ..........
STAP  .......... .......... .......... .......... .......... .......... ..........

ZIP   TKNYDIGAAL DTIQYSKHPP PL 439
ZIP2  .......... .......... .. 412
P62   .......... .......... .. 440
A170  .......... .......... .. 442
STAP  .......... .......... .. 442
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Fig. 1. Amino acid alignment of ZIP1, ZIP2, p62, A170 and STAP. Dots represent identical amino acids and dashes show gaps in the alignment.

non-covalently [10], via the presence of a UBA domain (Fig. 2, [11]).

EBIAP, a 60-kDa protein, has been found to associate with a novel hematopoietin receptor family member induced in B lymphocytes by Epstein–Barr virus (EBV) infection [12]. EBI3 exists on the plasma membrane of EBV-transformed B lymphocytes, and newly synthesized EBI3 is retained in the endoplasmic reticulum (ER) by association with calnexin, an integral ER membrane molecular chaperone that associates with the novel 60-kDa protein (EBIAP). Sequence alignment reveals that EBIAP is identical to p62 [12].

2.3. Mouse STAP/A170 gene

A 60-kDa stress-induced protein was cloned from macrophages called A170 [3,13,14]. This protein is 97% identical to ZIP1 and roughly 90% related to the human protein that binds the SH2 domain of $p56^{lck}$ [9]. The C-terminal domain of p62, which is completely conserved in A170, also has affinity for Ub [10]. The expression of A170 is regulated at both transcriptional and post-transcriptional levels [13]. A170 is phosphorylated by several kinases [15]. In brain, A170 can be induced by kainate-mediated excitotoxicity [16].

A cDNA encoding a 442-amino acid protein was isolated from a library of mouse osteoblastic cells, MC3T3-E1 [4], and called STAP. STAP is homologous to A170 [13] and shares 97% homology with rat ZIP [1] and 90% with human p62 [9]. The STAP gene consists of eight exons and seven introns, spanning a region of 11 kb [4]. Sequencing of the 5′ flanking region of the STAP gene revealed multiple consensus motifs for binding of several transcription factors: two potential Sp1 binding sites, AP-1, NF-E2, MyoD, and nuclear factor κB (NF-κB).

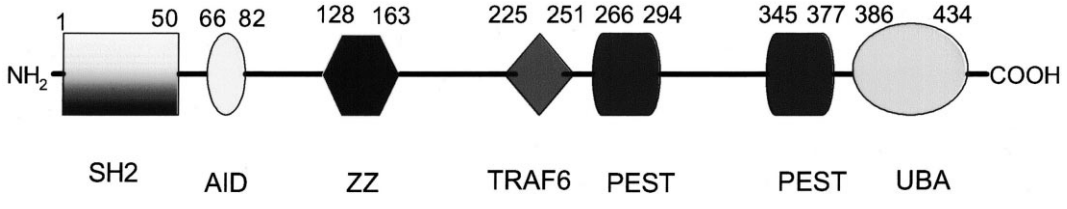

Fig. 2. A schematic diagram showing the domain organization of the p62 protein. The general structure is common to all p62 homologs except for ZIP2, which lacks the TRAF6 binding domain.

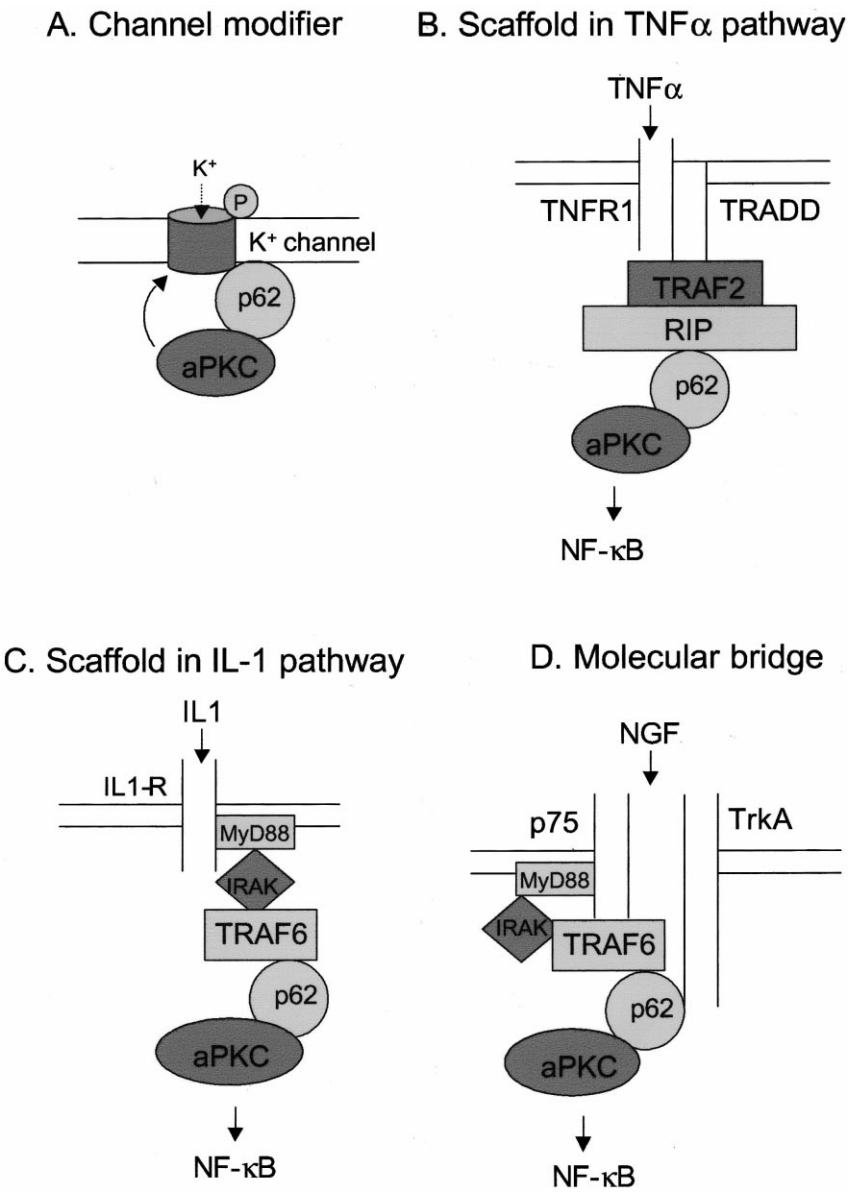

Fig. 3. p62 functional role in various signal cascades. A: p62 plays a role in recruitment of aPKC to the Kvβ2 subunit of the K^+ channel thereby leading to phosphorylation and modification of the channel [7]. B: p62 links RIP and aPKC thereby leading to phosphorylation of IKK and activation of NF-κB upon stimulation with TNF-α. C: p62 forms a ternary complex with TRAF6 and IRAK leading to recruitment of aPKC with subsequent phosphorylation of IKK and activation of NF-κB upon stimulation with IL-1 [22]. D: p62 serves as a bridge between p75 and TrkA neurotrophin receptors by binding TrkA and connecting p75 through interaction with TRAF6 [27]. aPKC is recruited to the complex followed by phosphorylation and activation of IKKβ.

3. p62's ability to bind Ub

The ability of p62 to bind Ub non-covalently was first described in 1996 [10]; amino acids 386–434 in the C-terminus are required for the Ub binding property of p62. Further studies have revealed that p62 has affinity for multi-Ub chains and may serve as a receptor to bind and store ubiquitinated proteins [17]. A novel sequence motif of 45 amino acids is conserved among proteins that can bind Ub, and it is referred to as a UBA domain (Fig. 4) [11,18]. A conserved domain alignment of amino acids in p62 which have been shown to bind Ub [10] reveals homology to other proteins that contain a UBA domain (Fig. 4). The structure of the UBA domain has recently been determined by nuclear magnetic resonance [19]. The domain forms a compact three-helix bundle, with a hydrophobic surface on one side that is likely the target interface for protein–protein interactions [19,20]. Moreover, proteins which contain the UBA domain are more likely to bind multi-Ub chains over mono-Ub [21,22]. This observation correlates nicely with the presence of a UBA domain within p62 and its ability to bind multi-Ub chains [17].

4. p62 serves as a scaffold for the NF-κB pathway

The most well-described and extensively studied function of p62 is its role as a scaffold for selective activation of transcription factor NF-κB. The most classical form of NF-κB is a heterodimer of p50 and p65 (Rel A), which is sequestered in the cytosol by IκB that prevents its nuclear translocation and activity [23]. Two IκB kinases (IKKα and IKKβ) responsible

p62-human	gi 1184951	374	LIESLSQMLSMGFSDEggWLTRLLQTKNYD-IGAALDTIQY 413
RAD23A-human	gi 7245807	4	EKEAIERLKALGFPES--LVIQAYFACEKN-ENLAANFLLS 41
RAD23A-mouse	gi 1709984	163	YETMLTEIMSMGYERE--RVVAALRASYNN-PHRAVEYLLT 200
RAD23A-yeast	gi 418413	148	RNETIERIMEMGYQRE--EVERALRAAFNN-PDRAVEYLLM 185
RAD23A-*O.sativa*	gi 7446462	344	EDEAILRLEPMGFDRA--LVLDVFFACNKD-EQLAANYLLD 381
C-Cbl-mouse	gi 115857	847	LSSEIERLMSQGYSYQ--DIQKALVIAHNN-IEMAKNILRE 884
Cbl-b-human	gi 8928017	932	VDAKIAKLMGEGYAFE--EVKRALEIAQNN-VEVARSILRE 969
UBC1-human	gi 2507504	162	YTKKIENLCAMGFDRN--AVIVALSSKSWD-VETATELLLS 199
UBC4-drome	gi 1717856	162	CDSKIQRLRDMGIDEH--EARAVLSKENWN-LEKATEGLFS 199
UBPD-human	gi 2501459	654	DESSVMQLAEMGFPLE--ACRKAVYFTGNMgAEVAFNWIIV 692
UBPD-human	gi 2501459	729	PEEIVAIITSMGFQRN--QAIQALRATNNN-LERALDWIFS 766
UBPA-DICDI	gi 1718037	630	NQEVLDTLLSMDFPLV--RCKKALLATGGKdAELAMNWIFE 668
UBPA-DICDI	gi 1718037	702	NSQDVDNIIGMGFTDS--QAKLALKNTKGN-LERAADWLFS 739
Ser/thr kinase-human	gi 7446398	292	DPRRTELMVSMGYTRE--EIQDSLVGQRYN-EVMATYLLLG 329
Ser/thr kinase-*R.norvegicus*	gi 2052189	332	DAKRIDIMVTMGFARD--EINDALVSQKYD-EVMATYILLG 369
Ser/thr kinase-*C.elegans*	gi 733123	445	RIEKLIQIFQLGFNKA--AILESVEKEKFE-DIHATYLLLG 482
Ser/thr kinase-*S.tuberosum*	gi 7434355	292	DEEILQQVSRMGLDRD--QLLDSLQKRIQD-DATVAYYLLY 329
p78-human	gi 125529	328	DQKRIDIMVGMGYSQE--EIQESLSKMKYD--EITATYLLL 364
REF2P-drome	gi 132465	555	INKSIHAMMAMGFSNEgaWLTQLLESVQGN-ISAALDVMNV 594
DDI1-yeast	gi 731521	391	PEQTIKQLMDLGFPRD--AVVKALKQTNGN-AEFAASLLFQ 428
GTS1-yeast	gi 1708067	195	YSRQLAELKDMGFGDTn-KNLDALSSAHGN-INRAIDYLEK 233
SNF1-AKIN10	gi 6166239	294	DEEILQEVINMGFDRN--HLIESLRNRTQN-DGTVTYYLIL 331

Fig. 4. Alignment of the UBA domain of p62 with proteins containing known UBA domains [18]. The UBA domain (amino acids 374–413) of p62 was subjected to a conserved domain sequence alignment (NCBI-CD search). gi = GenInfo Identifier.

for the signal-induced phosphorylation and degradation of IκB. The IKKs bind the NF-κB inducing kinase [24], a member of the mitogen-activated protein kinase kinase family that interacts with TRAF2 [25], linking IκB degradation and NF-κB activation to the TNF receptor complex. aPKCs bind to the IKKs in vivo and in vitro [26], whereby ζPKC selectively phosphorylates and activates IKKβ.

The association of p62 with aPKC provides a scaffold for the NF-κB pathway in both the TNF-α and interleukin-1 (IL-1) receptor signaling pathway [27–29]. In the TNF signaling cascade, TRAF2 and RIP simultaneously bind to TRADD, and the coiled-coil region of RIP interacts with the zinc finger (ZZ) of p62. The aPKC interaction domain (AID) of p62 interacts with the V1 domain of the aPKCs and serves to recruit ζPKC to the receptor signaling complex (Fig. 3B). The mechanism by which the aPKCs are activated in these complexes remains unknown. On stimulation of the IL-1 receptor the intracellular domain interacts with TIR domain of MyD88, a functional analog of TRADD [30,31]. MyD88 then binds IRAK which recruits TRAF6, followed by recruitment of p62. The interaction of TRAF6 with p62 in either the TNF-α (Fig. 3B) or IL-1 (Fig. 3C) signaling cascades is required for NF-κB activation [27,28].

Recently a requirement for multi-Ub chains in the activation of the NF-κB pathway by TRAF6 [32], a recently described E3 Ub ligase [33], has been documented. Since p62 possesses a UBA domain (Fig. 4), which displays a preference for multi-Ub chains [17,21], it is possible that p62 may regulate activation of NF-κB through recognition of TRAF6-catalyzed multi-Ub chains [34].

Nerve growth factor (NGF) binds to both p75 and TrkA neurotrophin receptors leading to activation of the transcription factor NF-κB [35]. Previous work in our lab has shown that p62 binds TrkA selectively, and interacts with p75 through TRAF6 [36]. Upon NGF stimulation, the interaction between TRAF6 and p62 occurs before p62 binds the TrkA receptor, suggesting that p62 interacts with TRAF6 to then link p75 and TrkA receptors (Fig. 3D). p62 then recruits aPKC, which phosphorylates IKKβ, leading to the activation of NF-κB. In PC12 cells expressing both receptors, transfection of antisense p62 inhibits NGF-induced NF-κB activation [36]. Therefore, p62 serves as a bridge that scaffolds together both p75 and TrkA receptors for NF-κB activation and survival signaling [36]. ZIP2 fails to activate NF-κB [36], since it lacks the TRAF6 binding domain [28]. In this regard, ZIP2 serves as an endogenous dominant negative regulator of NF-κB activation.

5. Subcellular localization of p62

5.1. A role for p62 in the nucleus

Chicken ovalbumin upstream promoter transcription factor (COUP-TF), an orphan member of the nuclear hormone receptor subfamily, includes COUP-TFI and COUP-TFII [37]. The DNA binding domain of COUP-TF contains two Sp1 binding sites, which have been proposed to serve as docking sites [38]. Interestingly, ζPKC has been shown to bind and phosphorylate Sp1 [39–41]. The orphan receptor co-activator (ORCA) binds COUP-TF in vitro and allows COUP-TF to act as a transcriptional activator in mammalian cells [42]. ORCA is identical to the 62-kDa protein that binds to tyrosine kinase signaling molecule $p56^{lck}$ [2]. Given the interactions between aPKCs/p62 [1], COUP-TFII–p62 [42], and aPKC–Sp1 [39], we speculate that a complex may be formed, in which aPKC regulates transcription directly or indirectly.

Additionally, p62 has been shown to stimulate transcription

of reporter genes linked to the enhancer of simian virus 40 (SV40) [43]. p62 does not bind to the SV40 enhancer on its own, but appears to act in conjunction with cellular factors to indirectly modulate transcription. Recent findings suggest that the localization of p62 to the nucleus may be signal-regulated. For example, stimulation of HeLa cells with sorbitol results in localization of p62 into the nucleus [44]. However, p62 does not appear to possess any of the classical nuclear localization signal (NLS) [45]. Treatment of cells with leptomycin B, an inhibitor of nuclear export, drives accumulation of p62 within the nucleus, suggesting the presence of a nuclear export signal. We speculate that p62 shuttles into the nucleus bound to aPKC, which is known to possess a functional NLS [46]. This would be in keeping with the ability of aPKCs to regulate the localization of ZIP/p62 [1]. Once localized into the nucleus, p62 directly co-associates with chromatin [45], further suggesting a role in regulating gene transcription [42,43].

5.2. Vesicle and inclusion body localization

In the lysosome-targeted endosome, endogenous and ectopically expressed p62 co-localizes with both endogenous λ/ιPKC and ζPKC [47,48]. Additionally, p62 co-localizes with the receptor for epidermal growth factor (EGF) in activated cells, whereas impaired activation of aPKC enzyme activity has been observed to severely impair the endocytic membrane trafficking of the EGF receptor and transport of internalized EGF receptors to the endosome [47]. p62 also binds the NGF receptor, TrkA [48], which localizes to the endosomal–lysosomal network. Inhibition of p62 expression has been shown to block NGF-induced neurite outgrowth [48]. Since delivery of TrkA–NGF receptor is required for neurite outgrowth, it is possible that p62, via its interaction with TrkA as well as other receptors, may be capable of directing their localization to the endosome.

Recently, p62 immunoreactivity has been observed in neuronal and glial Ub-containing inclusions in Alzheimer's, Pick's, Parkinson's, dementia with Lewy bodies, and multiple system atrophy [49,50]. Also, p62 has been identified as a major constituent of intracytoplasmic hyaline bodies [51]. Accumulation of p62 in these cells may be due to recognition of multi-ubiquitinated proteins by p62. Ub-dependent and -independent mechanisms could contribute to the deposition of p62 in neuronal and glial inclusions [50–52]. The presence of p62 in protein aggregates in vivo, and the induction of p62 expression in neuronal cells in vitro suggest that p62 may play a role in the pathogenesis of a number of neurodegenerative diseases, where faulty destruction of ubiquitinated proteins leads to the formation of cellular inclusions symptomatic of the pathology of the disease (reviewed in [53]).

6. Summary

The amino acid sequences and the structural motifs shared by all of the p62 genes suggest a high degree of conserved function among members of this family (Figs. 1–3). The ability of p62 to bind Ub through its UBA domain (Fig. 4), and to reside in both the nucleus as well as to traffic receptors, suggests that p62 serves a complex role in uniting receptor-mediated signaling events to ubiquitination.

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