

Staufen: from embryo polarity to cellular stress and neurodegeneration

Leandro Julian Martinez Tosar³, Maria Gabriela Thomas^{1,2}, Maria Veronica Baez⁴, Irene Ibanez^{1,3,5}, Ariel Chernomoretz^{1,3,5}, Graciela Lidia Boccaccio^{1,2,3}

¹Instituto Leloir Av. Patricias Argentinas 435 C1405BWE-Buenos Aires, Argentina, ²IIBBA-CONICET, C1405BWE-Buenos Aires, Argentina, ³Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Argentina, ⁴IBCN, CONICET and Facultad de Medicina, University of Buenos Aires, Argentina, ⁵IFIBA, CONICET, Argentina

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1. ABSTRACT

Staufen is a double-stranded RNA-binding protein that forms RNA granules by RNA-dependent and -independent interactions. Staufen was initially described in *Drosophila* as a key molecule for targeting maternal mRNAs. In vertebrates, two highly similar paralogs with several splicing variants mediate mRNA transport, thus affecting neuron plasticity, learning and memory. Staufen also regulates translation and mRNA decay. In recent years, Staufen was shown to be an important regulatory component of stress granules (SGs), which are large aggregates of silenced mRNPs specifically induced upon acute cellular stress. SGs contribute to cell survival by reprogramming translation and inhibiting pro-apoptotic pathways, and Staufen appears to negatively modulate SG formation by several mechanisms. More recently, mammalian Staufen was found in RNA granules and pathological cytoplasmic aggregates related to SGs containing huntingtin, TDP43, FUS/TLS or FMRP. In addition, Staufen binds CUG repeats present in mutant RNAs causative of degenerative conditions, thus ameliorating disease. Finally, Staufen affects HIV and influenza infection at several levels. Collectively, these observations unveil important roles for Staufen-mediated post-transcriptional regulation in a growing number of human diseases.

2. INTRODUCTION

Staufen is a double-stranded RNA-binding protein initially described in *Drosophila*. In this organism, Staufen mediates the localization of several maternal mRNAs encoding factors which ultimately control embryo polarity and cell fate. A similar role for vertebrate Staufen in defining polarity during early development was clearly shown in *Xenopus* oocytes by Mowry and co-workers. Here, Staufen 1 plays a critical role in the localization of maternal Vg1 and VegT mRNAs, two determinants of cell fate during embryo development (1, 2). Given the importance of Staufen in insect and amphibian early development, a similar role in mammalian early development was anticipated. Indeed, Staufen is required for the localization of maternal mRNAs during the maturation of pig oocytes (3), and Staufen orthologs are expressed in cow developing embryos from the germinal vesicle oocyte to the blastocyst stage (4). Staufen also plays a role in early development in zebrafish, where it is maternally and zygotically expressed (5). The expression of dominant negative constructs or the delivery of Staufen-directed antisense morpholinos into developing zebrafish embryos provoke an aberrant migration of the primordial germ cells –which eventually give rise to the gametes (6). This phenotype is rescued by co-injection of *Drosophila*'s Staufen mRNA, strongly supporting an evolutionarily

conserved function for this protein in developing embryos (6).

Apart from its established role in the localization of maternal mRNAs in *Drosophila* oocytes (7, 8), Staufen is required for the development of the fly central nervous system. Neuroblasts asymmetrically localize *prospero* mRNAs to the budding ganglion mother cell during cell division (9), a process dependent on Staufen and intact microfilaments. A related function seems to operate in vertebrates. Mills and co-workers reported a six-fold expression of Staufen in murine progenitor gastric epithelial cells with respect to their differentiated neighbors (10). This is likely related to the unequal partitioning of mRNAs during the asymmetric cell divisions which take place during gastric epithelium differentiation, thus resembling the Staufen-mediated localization of *prospero* in *Drosophila* (9).

In recent years, a number of research groups have focused on this highly conserved protein and its role on the regulation of cytoplasmic post-transcriptional processes in mammalian CNS cells (11-21). Two paralog genes are present in vertebrates, Staufen 1 and Staufen 2, and both proteins have been implicated in mRNA localization. In all cases, Staufen forms RNA granules which are thought as functional units for mRNA transport in polarized epithelial cells, in neuronal dendrites and axons, and at postsynaptic sites in both neurons and muscles (14, 15, 17, 22-27). Remarkably, in both, insects and mammals, defective Staufen function provokes alterations of neuron physiology, including impaired learning and memory, which frequently correlates with altered neuron morphology (26, 28-30). Also in zebrafish Staufen has a special relevance in the developing nervous system (5). Genetic interference of zebrafish Staufen provokes enhanced neuronal cell death, and this effect is reverted by fly Staufen (6).

Besides being an RNA granule-forming protein by means of RNA-protein and protein-protein interactions (21, 31, 32) Staufen associates to and regulates the translational apparatus (33-35). We and others have demonstrated that Staufen 1 and 2 associates to polysomes located at myelin processes and at post-synaptic densities (33, 34), and to polysomes and ribosomal subunits in primary neurons and fibroblast cells lines, all this supporting a regulatory role in translation (11, 16, 31, 35). More recently, we have found that Staufen is a conserved component of SGs, which are granular accretions of RNPs specifically assembled in a transient manner during the cellular response to stress. Staufen 1 is not an essential SG component and surprisingly, its overexpression promotes the disassembly of this *foci* (33, 36, 37), thus affecting cell survival under stress conditions. Remarkably, growing evidence from several research groups links stress granules with the abnormal protein aggregates that assemble in several neurodegenerative conditions. Moreover, Staufen was found to be present in these pathological aggregates. Thus, Staufen contributes to neuronal health by several mechanisms. Staufen regulates mRNA physiology by forming RNA granules which frequently include important

disease-related proteins, such as Fragile X Mental Retardation Protein (FMRP), TDP43 and huntingtin, thus suggesting that the normal functions of these molecules are linked to Staufen activity. In addition, in a number of pathological conditions involving nucleotide repeats, Staufen appears to have an intrinsic homeostatic role, neutralizing long, imperfectly matched double-stranded RNA structures that may interfere with normal RNA processing. Finally, Staufen controls SG assembly upon stress induction, likely negatively affecting the formation of cytoplasmic aggregates involved in neurodegeneration.

During the course of infection, viruses subvert important cellular functions to proliferate, thus interfering and adapting cellular processes to their own needs. As the multifaceted double-stranded RNA-binding protein that is, Staufen plays also an important role in several processes associated to viral infections. In some remarkable cases, Staufen has been evolutionarily exploited by viruses to help their own replication.

In this review, we will briefly describe basic concepts regarding Staufen-dependent mRNA transport, translation and decay, and we will focus on the relevance of these molecular functions to human diseases including neurodegeneration and viral infections.

3. STAUFEN'S DOMAINS AND FUNCTIONS

Staufen is a highly conserved double-stranded RNA-binding protein. A single gene has been described in invertebrates, including insects and worms, and two paralogs termed Staufen 1 and Staufen 2 were described in vertebrates, including mammals, amphibians, fish and birds (Table 1; (1, 11, 12, 38, 39)). Staufen 1 is expressed ubiquitously (11) whereas Staufen 2 expression is more restricted, the highest levels being found in brain and heart (16). An updated analysis of the Staufen 1 and Staufen 2 expression profile in a publically available gene expression data set derived from microarrays shows significant differences in the relative levels of the two paralogs in different human tissues (Figure 1). Staufen 1 levels are quite similar in almost all tissues, but are higher than average in thyroid gland, kidney cortex and liver, and lower in the pituitary gland. In the same data set, Staufen 2 mRNAs, specifically mRNA variants lacking amino acids 512 to 570, are the predominant isoforms in several brain regions, namely the cortex and the frontal, parietal, occipital and temporal lobes. In contrast, Staufen 2 variants with an extended C-terminus are expressed to higher levels in skeletal muscle, where the shorter Staufen 2 is less abundant. The biological relevance of this C-terminal extension remains to be determined.

In all species, Staufen molecules display four or five double-stranded RNA-binding domains (dsRBDs) of highly conserved structure and sequence. In this sense, Staufen differs from other dsRNA binding proteins, which usually contain one or two dsRBDs (38, 40). Among these domains, dsRBDs 1, 3, 4 and 5 show true RNA-binding activity *in vitro*, with varying affinity. The dsRBD2 is interrupted by a not-so-well conserved segment that

Table 1. Similarities among Staufen domains throughout evolution

	Staufen 1					Staufen 2				
	RBD2	RBD3	RBD4	TBD	RBD5	RBD1	RBD2	RBD3	RBD4	RBD5
Primates	99	97,3	99	100	100	100	100	96,5	100	26,2
Rodents	92	95	98	97,5	99	96	100	100	100	24,6
Other mammals	98,5	100	98	99	100	100	100	100	100	100
Birds	96	96	95	95	100	95	99	99	99	-
Amphibia	94	91	96	91	99	93	92	96	95	91
Fish	79,5	92	89	81	99,5	89	97	99	98	87
Diptera ¹	73,8	72	71,5	-	68,5	74,3	61,6	71,4	69,3	59,75
Coleoptera ¹	55	73	77	-	69	78	23,1	77,7	75,7	53
Worms ¹	59	60	73	-	-	75	24,2	36	73,3	-

Domains of the two human Staufen isoforms were compared with the same domains in several species and the average percent similarities are shown. ‘Primates’ may include: *Pan troglodytes* (chimpanzee), *Pongo abelii* (gorilla), *Macacca mulata* (Rhesus monkey) and *Callithrix jacchus* (titi monkey). ‘Rodents’ include: *Mus musculus* (mouse) and *Rattus norvegicus* (rat). ‘Other mammals’ may include: *Ailuropoda melanoleuca* (giant panda), *Equus caballus* (horse) and *Bos taurus* (cow). ‘Birds’: *Gallus gallus* (chicken). ‘Amphibia’ may include: *Xenopus laevis* (african clawed frog) and *Xenopus tropicalis* (western clawed frog). ‘Fish’ may include: *Salmo salar* (salmon) and *Danio rerio* (zebrafish). ‘Diptera’ include: *Drosophila melanogaster*, *Drosophila yakuba*, *Drosophila viridis* (all fruit flies) and *Musca domestica* (domestic fly). ‘Coleoptera’: *Tribolium castaneum* (flour red beetle). ‘Worms’: *Caenorhabditis elegans*. (¹): given the absence of reported Staufen 2 orthologs in the indicated species, the only known Staufen molecule is compared to both human isoforms. In all cases, Staufen RBD domains are more similar to the corresponding domains in the different orthologs than to the other RBD domains in the same molecule (not shown). Staufen 1 orthologs bearing RBD1 (*Bos taurus*, avian, amphibian, and fish orthologs) were omitted for simplicity.

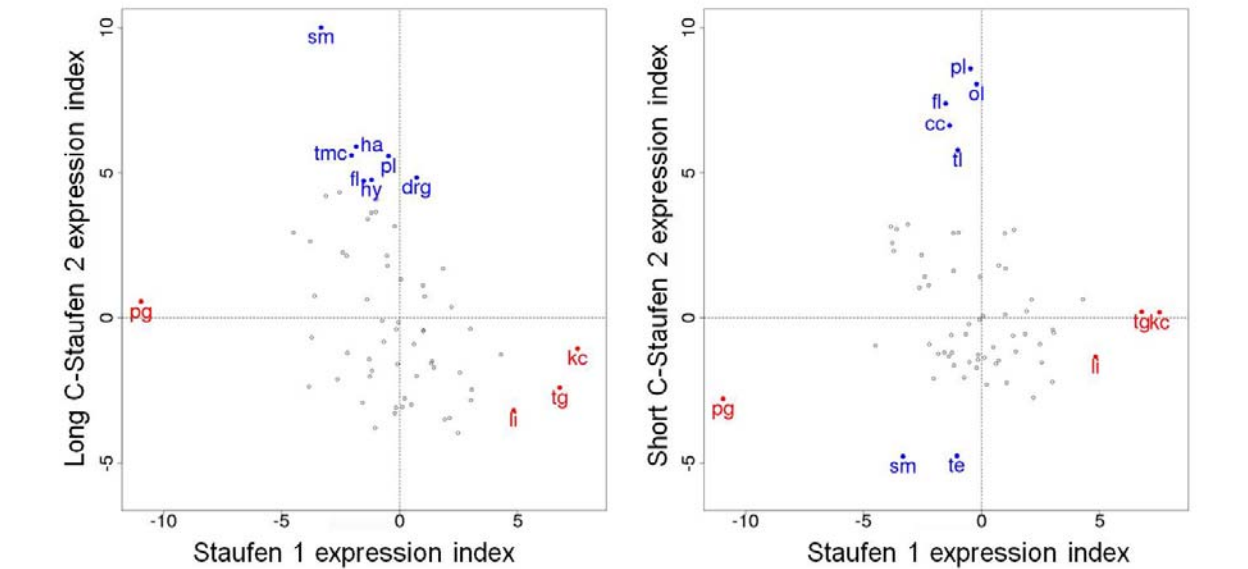


Figure 1. Differential expression of Staufen 1 and Staufen 2 variants in human tissues.. Student’s statistics for Staufen 1 and two Staufen 2 variant were calculated using data from 353 Affymetrix U133 plus 2.0 arrays (see (177, 178) for further details) in 65 tissues, which include 20 distinct regions of the CNS, taken from NCBI GEO accession GSE3526. To assess deviations from the global mean expression of each Staufen isoform in each tissue, linear models were used to fit the signal of the three probesets for Staufen 1 and two for the Staufen 2 variants using the Bioconductor package limma (179, 180). Statistical significance was tested by the Bonferroni multiple comparisons test (p=0.001). The expression index (Student’s “t”) of C-terminally extended isoforms of Staufen 2 (Long C-Staufen 2) and Staufen 1 are compared in the left panel. The right panel compares Staufen 2 C-terminal truncated isoforms (Short C-Staufen 2) with Staufen 1 expression indexes. Statistically significant deviations in expression are shown in red (Staufen 1) or blue (Staufen 2) filled circles. Abbreviations are as follows: cc, cerebral cortex; drg, dorsal root ganglia; fl, frontal lobe; ha, heart atrium; hy, hypothalamus; kc, kidney cortex; li, liver; ol, occipital lobe; pg, pituitary gland; sm, skeletal muscle; te, testes; tg, thyroid gland; tl, temporal lobe; tmc, tongue main corpus.

negatively affects RNA-binding. Presumably, the rise in local RNA concentration achieved by neighboring dsRBDs *in vivo* would force dsRBD2 to assume a canonical dsRBD structure, thus extruding the hydrophobic loop away from the folded domain. In this conformation, the hydrophobic

loop would recruit protein partners required for the active localization of target RNAs (41). Besides binding RNA, dsRBDs have been proposed to establish homotypical and heterotypical associations with domains of similar nature (40, 42, 43), and thus, their presence is usually associated

with RNA-protein and protein-protein interactions, suggesting a potential structural role for Staufen in the assembly of ribonucleoprotein complexes (44).

The structural organization of vertebrate Staufen 1 is characterized by the generalized lack of dsRBD1 and the presence of a domain able to bind tubulin –termed *tubulin binding domain*, TBD– in the region connecting dsRBD4 and dsRBD5 (12). The structure of vertebrate Staufen 2 seems to complement that of Staufen 1, since the dsRBD1 is present in this paralog, but the TBD and the dsRBD5 are degenerated. This suggests that after the gene duplication event giving rise to the two variants, selective pressure favored the conservation of some functions in one of the paralogs, and others in its counterpart.

Both Staufen 1 and 2 are mainly cytoplasmic proteins. However, the two molecules show also nuclear localization, preferentially nucleolar. Staufen subcellular distribution depends on a bipartite nuclear localization signal located between the dsRBDs 3 and 4, and on a cytoplasmic anchoring mechanism relying in the binding activities of the dsRBD2 and dsRBD3 (21).

In both insects and vertebrates, the dsRBD3 binds to RNA with the highest affinity. The structure of this domain and its RNA-contacting residues are well characterized (45). Recently, this domain has been successfully used to protect a short RNA hairpin against the action of RNases, which has proven useful in improving the efficiency of siRNA-mediated knockdown strategies (46). In addition, the dsRBD3 from vertebrate Staufen 1 seems to mediate the protein's association to the nucleolus in a manner that is independent of its RNA-binding activity (21). This association is modulated positively by the dsRBD4 and negatively by the spacer between the dsRBD2 and the dsRBD3. This is in accordance with the reported affinity of Staufen for ribosomes (31). In this case, the association is mediated by an RNA-protein interaction region located within the dsRBD3, and by a protein-protein interaction region spanning the dsRBD4 and TBD domains. Thus, Staufen would be able to bind ribosomes as well as free ribosomal subunits.

In vertebrates, Staufen mRNAs are subject to alternative splicing processes that affect the protein and/or the stability of the messenger (33-35). An extended N-terminus of unknown function was described. Another Staufen 1 isoform bears a 6 amino acids-long insertion in the middle of dsRBD3. This insertion impairs the domain's affinity for RNA, and putatively modulates Staufen's physiological role. Finally, a rare Staufen 1 variant, termed deltaE9_i, was identified in mouse brain. This isoform includes a premature stop codon located upstream of the last exon-exon junction (33-35), making this mRNA a putative target for the nonsense-mediated decay (NMD) pathway (reviewed in (47)). A similar regulatory mechanism for the double-stranded RNA-dependent protein kinase (PKR) was described (48). Alternative splicing in Staufen 2 is limited to the generation of differentially extended N-termini and C-termini, with longer variants of the former resembling *Drosophila's* dsRBD1

sequence. The different N-terminal variants of this paralog are able to bind distinct mRNA subsets (49) (see below) but more profound analyses regarding the physiological relevance of these differences is still pending. Similarly, the relevance of variations at the C-terminus still awaits further analysis.

Deletional studies have allowed the mapping of some Staufen functions regarding the regulation of maternal and zygotic mRNAs in *Drosophila*. The hydrophobic loop that interrupts the dsRBD2 consensus motif is important for the localization of the maternal messenger *oskar* at the oocyte posterior pole, likely by mediating the recruitment of the transport machinery. Notably, this region is moderately conserved in most of the protein's orthologs studied so far, suggesting a similar function in RNA transport for this domain in all species. The *Drosophila* dsRBD5 is responsible for the release of the translational blockage imposed on the maternal *oskar* mRNA. The RNA-binding protein Bruno keeps *oskar* mRNA silent during its transport, and Staufen contributes to its derepression once the messenger arrives its destination at the posterior pole (41). Additionally, *Drosophila's* dsRBD5 is required for the *Miranda*-dependent delivery of *prospero* mRNA to the developing ganglion mother cells in *Drosophila's* embryos, as described previously (50). This domain is present in Staufen 1 but virtually absent from most Staufen 2 molecules, suggesting the two vertebrate paralogs are not redundant in their dsRBD5-associated functions. Finally, and consistently with a role in mRNA transport, a C-terminal-truncated mammalian Staufen 2 construct impairs the accumulation of RNA in neuronal dendrites in rodent cells (15).

4. STAUFEN REGULATES TRANSLATION AND mRNA STABILITY

Double-stranded RNA-binding domains recognize RNA duplexes or hairpins, without sequence specificity. Structural analysis of dsRBD-RNA complexes indicates the lack of base-specific interactions. Accordingly, SELEX enrichment of preferred RNAs yields several highly structured molecules which are not discriminated by distinct dsRBDs (51). It has been suggested that the cooperation among several dsRBDs would be responsible for the recognition of specific ligands (52). In addition, other Staufen-associated proteins might also contribute to the specificity in RNA binding. The mammalian Staufen 1 dsRBD3 directly binds double-stranded motifs as short as 12 bp (12, 53), and similar binding properties were shown previously for the *Drosophila* molecule (45, 54).

The isolation of Staufen RNPs by immunoprecipitation followed by the recovery of Staufen-associated RNAs and their subsequent hybridization on microarrays allowed to identify hundreds of mRNAs, which represent 10% of cellular mRNA species, and are involved in diverse functions (49, 55-57). The pull-down assays selective for Staufen 1 and Staufen 2 in HEK293 cells, indicated the presence of two subsets of transcripts, with less than 21% of common species, supporting the

notion that Staufen 1 and Staufen 2 have marked functional differences (49). It is not known whether the presence of these transcripts is the consequence of direct binding by Staufen proteins or rather due to the presence of additional RBPs in Staufen RNPs. In this regard, FMRP, Pur alpha and TDP43 –among others– are usually found in Staufen-containing particles.

The consequences of Staufen binding on the fate of the target mRNA are variable, and range from translational regulation to decay, in addition to transport. Mammalian Staufen 1 is able to enhance translation when bound to the 5'UTR. It has been shown that the presence of structured double-stranded motifs or the HIV trans-activating response element (TAR), both of which mediate Staufen recruitment, enhance translation up to 5-fold when located at the 5'UTR (35). Moreover, the translation of weak transcripts in rabbit reticulocyte lysates is upregulated two-fold under the presence of high levels of Staufen. Details of the mechanism are yet to be solved but it is likely to involve Staufen's interaction with ribosomes. Since Staufen 1 is capable of binding preferentially to 60S ribosomal subunits, the possibility is open that Staufen 1 stimulates protein synthesis by enhancing the recruitment of ribosomal large subunits to 43S initiation complexes (35). Relevantly, *Drosophila* Staufen functions as a translational enhancer by helping to relieve the Bruno-dependent *oskar* mRNA-translational blockage at the oocyte posterior (41). Here again, the molecular mechanisms are not known in detail.

Paradoxically, in parallel to this enhanced-expression effect, specific mechanisms have evolved, leading to the elimination of Staufen-bound transcripts from the actively translating pool, by means of their degradation. Briefly, Maquat and co-workers demonstrated that certain mRNAs containing Staufen 1 binding sites in their 3'UTR are promptly degraded in the presence of the protein. This decay pathway is termed *staufen-mediated decay* or SMD, and it involves recruitment of Upf1, the last effector of NMD, thus ultimately dictating mRNA decapping and degradation (58, 59). Not surprisingly, microarray analyses indicate that a large number of transcripts are up or downregulated upon Staufen 1 knockdown in cell lines, with variations of at least two-fold in 2% of the analyzed mRNA species (59, 60). All this reveals both enhancement and inhibition of the overall expression of specific transcripts by Staufen proteins. SMD appears as an important pathway during development, and affects the differentiation of C2C12 mouse myoblasts into myotubes (61). It was speculated that SMD is also instrumental to mRNA regulation at the synapse (62). SMD requires active translation for an mRNA to be degraded and it has been speculated that mRNAs being transported along dendrites in association with Staufen will be degraded if expressed prematurely, and allowed to be translated only once SMD is relieved at the synapse (63).

5. MAMMALIAN STAUFEN GRANULES: FUNCTIONAL UNITS FOR mRNA REGULATION?

Staufen activity is believed to be linked to its ability to form granules. As many other Staufen features, this was shown first for the *Drosophila* molecule. Fly

Staufen forms granules when bound to *bicoid* or *oskar* mRNA (7, 8). More recently, it was shown that mammalian Staufen 1 oligomerizes (32). This multimerization is in part mediated by RNA, and RNA recognition by the dsRBD2 and dsRBD3 is crucial. In addition, the dsRBD2 and the dsRBD5 mediate protein-protein interactions that help multimerization (32). The presence of alternative splicing variants affecting these domains is predicted to introduce subtle variations in granule formation, a property expected to be conserved in Staufen 2.

In pioneering reports describing the presence of Staufen molecules in vertebrates, it was first observed that Staufen 1 forms granules that associate to the ER, identified by several molecular markers (11-13). As Staufen interacts with mRNAs and polysomes, the interaction with the ER is believed to be –at least partially– indirectly mediated by ER-associated polysomes. The association with ER membranes appeared to be specific, as no colocalization with endosomes or Golgi compartments was apparent. Afterwards, electron-microscopy analysis helped to understand that both Staufen 1 and Staufen 2 paralogs form RNA-containing granules which associate to the endoplasmic reticulum and microtubules. This is in accordance with the well established role for the cytoskeleton and the endolysosomal pathway on the cytoplasmic transport of mRNA ((64) and references therein). Also –as discussed below– Staufen seems to stabilize polysomes during stress, and ER-associated polysomes are stress-resistant, thus suggesting a direct role for Staufen in protecting ER-associated polysomes against stress-induced disruption (reviewed in (65)).

Both neurons and oligodendrocytes are profusely loaded with Staufen 1 and Staufen 2 granules, which form two different sets of *foci* (16, 33). Accordingly, biochemical analyses of rat brain showed that both proteins co-purify only partially (17), further suggesting the presence of distinct macromolecular structures. Staufen granules localize in the somatodendritic compartment of hippocampal cells (13, 15, 16). In oligodendrocytes, Staufen 1 and Staufen 2-containing granules are localized in the cell body, as well as primary, secondary and distal myelinating processes (33). Staufen associates to the ER membranes located at the distal processes, as well to the intricate arrays of microtubules and microfilaments that support the myelinating extensions. All this evidence suggests that myelin mRNAs are associated to Staufen along the transport pathway and in their final location, namely the cytoplasmic channels where myelin proteins are translated. Notably, Staufen associates to myelin polysomes, which are highly abundant in this cellular compartment.

The formation of Staufen-containing granules is significant to RNA transport, and several other RBPs involved in cytoplasmic RNP transport are also found in dendritic granules. RNA transport is also important in axons (66-71) and more than 300 distinct transcripts are present in mammalian axons (72). Given its general function as RNA transport factor, a role for Staufen in axonal RNA transport is expected and, indeed, Staufen was

found to form RNA granules in axonal growth cones (73), and in peripheral and central axons, apparently mediating RNA localization (27).

Like RNA transport, mRNA silencing and decay are as well linked to RNA granules, specifically, to the cytoplasmic *foci* termed Stress Granules (SGs) and P bodies (PBs), which are collectively known as mRNA silencing *foci* (reviewed in (65)). PBs were described recently as cytoplasmic compartments specialized in mRNA storage and destruction. How translation regulation by Staufen or SMD depends on the assembly of RNA granules or silencing *foci* remains unknown.

In recent years, we reported that Staufen granules located at the myelinating processes of cultured oligodendrocytes remodel upon stress induction. Strikingly, both Staufen 1 and Staufen 2 granules virtually vanished from the cell periphery, to accumulate in large *foci* preferentially located at the cell soma (Figure 2A and reference (33)). These novel Staufen accretions were identified as SGs, which specifically form in cells undergoing acute stress, as we shall review in the following section. Most Staufen molecules are recruited to SGs upon stress induction, and this apparently does not affect the movement of newly transcribed mRNAs (33). All these observations are compatible with the notion that Staufen plays an important role in the regulation of mRNAs engaged in polysomes, and that the participation of Staufen in controlling free mRNA molecules is less important (33). Supporting these speculations, Staufen is absent from PBs, which lack translation elements and polyadenylated RNA (36, 74).

6. STAUFEN IN THE STRESS RESPONSE

The cellular response to stress is an evolutionarily conserved defense strategy against a plethora of noxious stimuli, including exposure to oxidants, hypoxic conditions, or the intracellular accumulation of unfolded proteins, among others. All these factors are relevant in several diseases ranging from cancer to neurodegeneration. Tumor cells are exposed to hypoxia and their survival and aggressiveness largely depends on their capacity to mount a powerful stress response, thus evading apoptosis. Oxidative stress compromises neuron health in post-ischemic oxidative stress. Finally, abnormal protein aggregates are associated to a growing number of neurodegenerative conditions that affect distinct brain areas, causing a variety of neurological deficits.

The stress reaction comprises an adjustment of cell metabolism, translation and transcription to confront these harmful conditions, and to allow the mounting of a protective response. A novel arm of the stress response is the formation of SGs. A number of comprehensive reviews describing SG assembly, and their contribution to the stress response have been presented recently (65, 75, 76). Briefly, SGs are large macromolecular aggregates of ribonucleoproteins that form in the cytoplasm as a

consequence of the global translational silencing triggered by acute stress. The translation initiation blockage typically initiated by eIF2- α inactivation by specific kinases, ultimately leads to polysome disassembly and to the aggregation of mRNA and translation initiation factors in SGs. In addition, SGs contain a large number of RNA binding proteins involved in mRNA silencing and stability (65, 75). Besides being important in reprogramming translation upon stress, work from several laboratories indicates that SGs contain almost a hundred proteins with very distinct cellular functions, including signaling and splicing. Relevantly, SGs sequester pro-apoptotic factors, thus contributing to escape cell death.

More recently, SGs were shown to be involved in neurodegeneration. Several forms of Amyotrophic Lateral Sclerosis, Alzheimer's disease and Frontotemporal Dementia are characterized by the presence of intracellular aggregates containing, among other components the RNA binding proteins TDP-43 and FUS/TLS/hnRNP P2. Moreover, it was shown that pathologic TDP-43 or FUS/TLS/hnRNP P2 aggregates contain SG markers (77) (reviewed in (65)) and strikingly, pathogenic forms of these proteins induce SG formation in cell lines (77-82), and are recruited to these *foci*. A working hypothesis currently under intense investigation is that the irreversible aggregation of these altered proteins is initiated by SGs that eventually form as part of what would be a normal stress response, and that should otherwise assemble only transiently (82) (reviewed in (65)).

Stress-induced Staufen accretions were identified as SGs by the presence of several marker molecules, and by their sensitivity to polysome-stabilizing drugs known to halt SG formation (33). Later on, several reports indicated that Staufen molecules are always recruited to SGs induced in very different cell types by a variety of stimuli ((65) and references therein). After all these observations, Staufen is currently considered a reliable SG marker. Not surprisingly, Staufen 1 is also present in SGs induced in cultured neurons upon exposure to oxidative stress conditions (Figure 2B,C) or in oligodendrocytes under treatment with pro-inflammatory cytokines. Noteworthy, in both cell lines and neurons, mammalian Staufen molecules are excluded from PB (Figure 2C and ref (36, 74); reviewed in (65)). Finally, Volkering and co-workers identified Staufen 1 as a component of the TDP-43 aggregates present in ALS tissue (83), again suggesting that pathological aggregates are related to SGs.

The significance of Staufen to SG physiology is linked to its ability to interact with polysomes. Paradoxically, despite being a conserved SG component, and a RNA granule forming protein, Staufen interferes with SG formation, and moreover, promotes SG disassembly. Work from our lab indicates that Staufen 1 overexpression impairs SG formation, whereas Staufen 1 knockdown impairs their dissolution (36, 37). Relevantly, the protein regions that mediate SG dissolution comprise the ribosome-binding regions. Moreover, Staufen 1 remains associated to stress-resistant, apparently stalled polysomes,

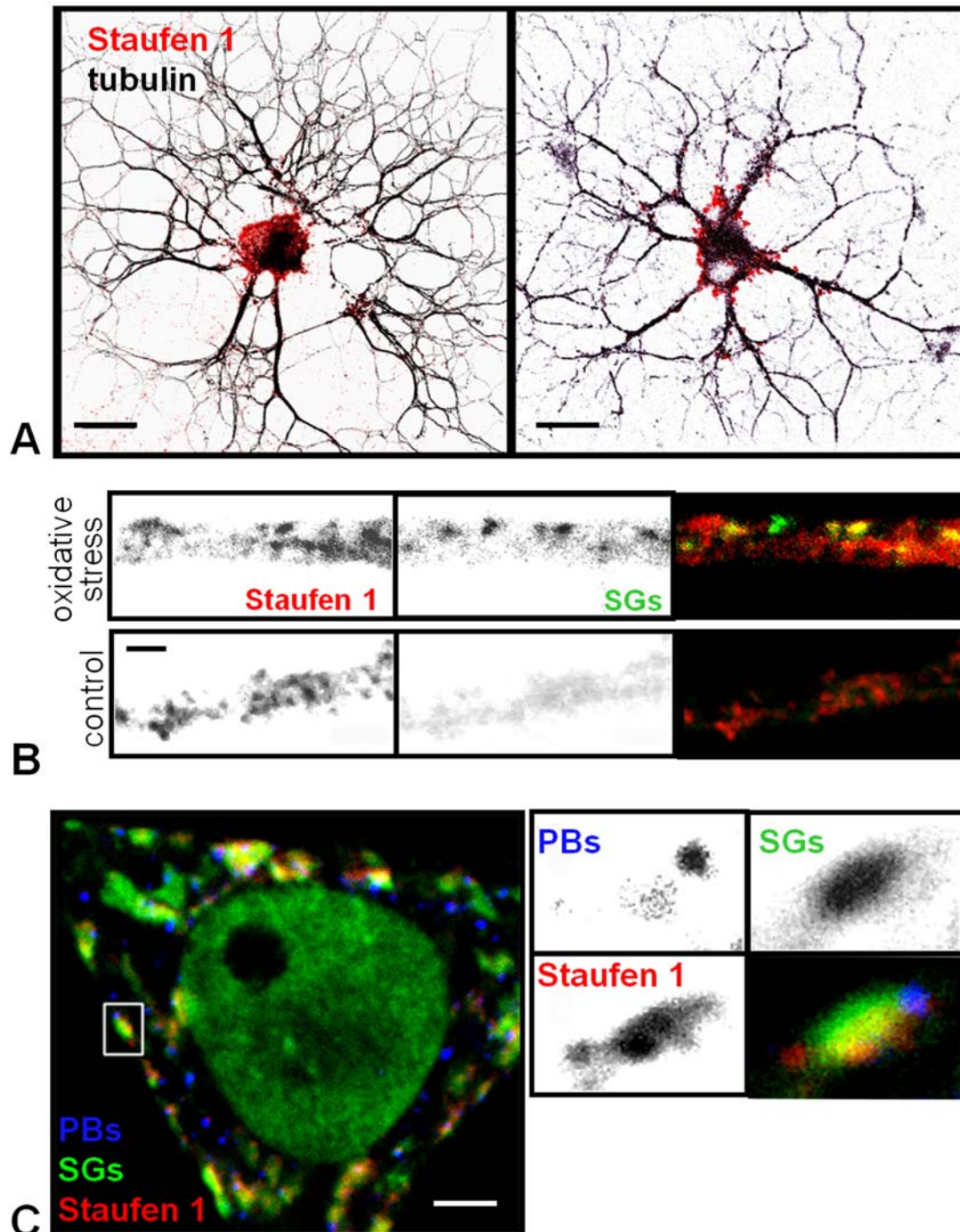


Figure 2. Staufen granules present in mammalian CNS cells remodel and form SGs upon oxidative stress. A, Cultured rat oligodendrocytes extend long myelinating processes loaded with Staufen 1 granules (in red), thought to mediate RNA transport to myelinating regions (left). Upon oxidative stress induction, Staufen 1 granules relocalize to the cell body and primary processes, forming SGs (right). The oligodendrocyte's cytoskeleton is depicted in black. Size bar: 10 µm. (see text for details and ref (33)). B, Magnifications of single dendrites of cultured rat hippocampal cells. Staufen 1 (red) forms granules involved in dendritic RNA localization (see text for details). Upon stress induction, most Staufen 1 collapses into SGs, identified by the SG marker TIA1 (green), which is normally absent from dendrites. Size bar: 0.5 µm. C, Left, SGs are abundant in the neuronal soma, and Staufen (red) colocalizes with SGs (green). In contrast, Staufen 1 is absent from PBs, identified by the marker molecule DCP1a (blue) (see text for details and ref (36)). Size bar: 5 µm. Right, magnification of a single SG growing in close vicinity to PBs, a usual observation for these kind of *foci*. Size bar: 0.5 µm.

of relative large size. Thus, it was suggested that Staufen 1 regulates SG formation by stabilizing polysomes in a non-productive state (33). Given that the N-terminal part is enough to achieve SG dissolution, and that this part of the molecule mediates oligomerization (32), the possibility is open that Staufen helps SG dissolution by directing mRNAs to a distinct subcellular compartment consisting of large RNPs, distinct from polysomes, but with similar biochemical behavior on sedimentation assays.

In addition to its role as a polysome-stalling factor, Staufen may contribute to the recruitment of anterograde motors, which are strictly required for the dispersion of SG components during disassembly (36). As expected, disruption of SG dynamics impairs cell survival, and Staufen depletion induces a poor resistance to stress insults.

Apart from this immediate mechanism for controlling the stress response, Staufen may have a delayed effect, regulating the stability of translation of specific transcripts. Notably, several stress-related transcripts showed increased levels upon Staufen 1 depletion, namely TIA-1, which is a key SG component, the pro-apoptotic factor TRAF2, Dcp-2, which is required for the degradation of transcripts in PBs, and c-Jun, a well known transcription factor which plays a pivotal role in the stress and apoptotic signaling cascades (59), and Arf1, an ADP-ribosylation factor likely mediating the inhibition of the eukaryotic elongation factor 2 (84, 85). Thus, besides the direct regulation of SG disassembly, Staufen may modulate the stress response by additional pathways involving the modulation of the expression of a number of key molecules.

7. STAUFEN AFFECTS SYNAPSE MORPHOLOGY AND FUNCTION

The functional relevance of Staufen granules in mammalian neurons appears to be linked to several aspects of mRNA regulation, including subcellular localization, silencing and decay. It is now well established that both mammalian Staufen paralogs are implicated in mRNA transport in neurons. More than ten years ago, Kiebler and collaborators performed live-imaging experiments to show that human Staufen 1-GFP forms motile granules in the somatodendritic compartment of hippocampal neurons. These granules contain RNA, as demonstrated by SYTO14-staining. The speed and overall manner of Staufen granule movement strongly suggest the participation of microtubule-dependent motors. Indeed, Staufen-granule transport is affected by nocodazole, a drug that disrupts microtubules, but not by latrunculin B, a drug that affects microfilaments (22). It has been suggested that Staufen mediates the interaction with kinesin motors, thus allowing anterograde movement (17, 60, 86). As it is the case for other neuronal RNA granules, the recruitment of Staufen granules to the dendrites depends on synaptic activity ((26, 28, 29, 62, 87-112) among others). Hippocampal neuron depolarization increases dendritic Staufen 2 levels, and this is modulated by the MAPK pathway, a signalling cascade directly linked to synapse activity (86).

The identity of the mRNA cargo of these motile granules begins to be unveiled. High-throughput analysis of localized mRNAs has led to the identification of hundreds of dendritic mRNAs pertaining to very distinct molecular functions. Signalling molecules, neurotransmitter receptor subunits, ion-channels, and components and regulators of the actin cytoskeleton are among the most relevant categories (28, 96-122). Functional studies have confirmed that the localization of β -actin and CaMKII α are impaired upon interference of Staufen expression and/or function (29, 60). Given the wide variety of Staufen-associated mRNAs identified in cell lines (49) or embryonic rat brains (56, 57), we can expect a large number of dendritic transcripts to be actually transported in Staufen granules.

Staufen-mediated transport appears to be of great relevance to neuron physiology (26, 28-30). In a recent study, RNP transport was analyzed in mutant mice that express a Staufen 1 protein lacking dsRBD3 and thus unable to bind RNA efficiently (29). The results show a serious impairment in the accumulation of polyadenylated RNA and β -actin mRNA in distal dendrites. As a consequence, the affected neurons show impaired dendritic spine formation, and the typical mushroom spines are replaced by long filopodium-like structures, with fewer functional synapses. The size and growth of the spine head correlate with synaptic strength, and a morphology with elongated spine and filopodium-like protrusions is typical of silent synapses (123). Thus, Staufen 1 loss-of-function affects synaptic connectivity, and mutant mice exhibit decreased locomotor activity (29). Further suggesting a key role for Staufen 1 in long-term plasticity and maintenance of mature synaptic connections, Staufen 1 depletion in hippocampal slices leads to defective L-LTP (30). In this experimental model, the amplitude and frequency of synaptic potentials through non-NMDA glutamate receptors is diminished, suggesting either a reduction of postsynaptic efficacy at individual synapses or a decrease in the number of functional synapses. Without excluding these defects at the post-synapse, where Staufen operates, changes in presynaptic transmitter release may contribute to the mutant phenotype. Unraveling the mechanisms underlying altered L-LTP will require further work, but defective transport and/or translation of yet unknown mRNAs is speculated to be the initial defect (30).

The role of Staufen 2, which is preferentially expressed in the CNS, was also analyzed in cultured hippocampal cells (15). Here, the expression of a dominant negative form of Staufen 2 lacking the C-terminal domain, provokes a decrease of messenger and ribosomal RNA content in dendrites. In contrast, the overexpression of full-length Staufen 2 induces an increase in dendritic mRNA content. These observations suggest that Staufen 2 mediates the transport of a broad spectrum of neuronal mRNAs to dendrites (15, 86). Similarly to the above-mentioned observations for Staufen 1, Staufen 2 depletion leads to a lower number of protrusions with extended filopodial appearance, suggesting that Staufen 2 is crucial to dendritic spine formation and maintenance. This is apparently the consequence of reduced β -actin expression in dendrites and defective F-actin organization. Whether β -

actin mRNA transport or stability is directly affected by Staufen 2 is unknown, but F-actin disruption at the spines provokes an impaired excitatory synaptic transmission, primarily due to a low responsiveness of postsynaptic glutamate receptors (26).

Subtle but relevant differences are found upon depletion of either Staufen 1 or Staufen 2. Staufen 2 knockdown provokes a reduction in spine density, which is not observed upon Staufen 1 depletion. This suggests that Staufen 2 is linked to spine morphogenesis, and that Staufen 1 is linked to synaptic plasticity and associated spine morphology changes. As described above, a non-redundant, complementary role for the two vertebrate Staufen versions is supported by the fact that these molecules form two separate kinds of granules, likely representing physiologically distinct units to control mostly distinct subsets of mRNAs. The investigation of the specific role of the mRNA species present in the distinct Staufen granules will require intensive work. At the moment, imaging approaches and co-IP strategies indicate that Staufen 1 granules contain transcripts coding for CaMKII α , Arc and MAP2, among others (23, 59, 88). With regard to Staufen 2, a plethora of associated mRNAs has been recently identified, opening multiple possibilities for the probing of new physiological roles in the regulation of these transcripts (49, 56, 57).

8. A ROLE FOR STAUFEN IN NEURODEGENERATION?

8.1. RNA-binding proteins linked to neurodegeneration are present in Staufen granules

Several observations indirectly suggest that Staufen functions in connection with three distinct proteins involved in neuronal diseases, namely FMRP, TDP-43 and huntingtin. Staufen and each one of these molecules were shown to colocalize in dendritic RNA granules. Mutations in the gene encoding the RNA binding protein FMRP provokes serious alterations in spine morphogenesis, thus leading to dramatic neurological deficits in the disease termed Fragile X Mental Retardation Syndrome. FMRP granule motility is regulated by synaptic activity and by cellular stress. Remarkably, FMRP and Staufen granules partially overlap and a number of cellular transcripts co-purify with both proteins (19, 27, 49, 124-126). Upon stress insults, FMRP and Staufen granules remodel and both proteins are recruited to SGs, thus regulating SG dynamics (reviewed in (65), and references therein).

TDP-43 is an RNA-binding protein that forms cytoplasmic inclusions in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (127, 128). This protein has been linked also to Alzheimer (129), Parkinson (130) and Huntington diseases (131). In normal conditions, TDP-43 form dendritic RNA granules, and a large proportion of them contain Staufen (73). A strong depolarizing stimulation of cultured neurons enlarges TDP43 granules and enhances colocalization with Staufen, suggesting a similarity with SGs. More recently, Staufen was linked to the function of huntingtin, responsible of Huntington's disease when mutated. Savas and colleagues (121, 132) showed that under non-

pathogenic conditions, huntingtin forms dendritic granules. Remarkably, more than half of these huntingtin *foci* contain Staufen. These granules are motile, contain RNA and moreover, huntingtin knockdown impairs the global accumulation of RNA in dendrites. Collectively, these observations highlight a role for Staufen as a general component of neuronal RNA granules, which include a wide collection of proteins causative of neurodegenerative disorders by either gain- or loss-of-function mechanisms. The exact role of Staufen in the neuronal granules containing TDP-43, FMRP or huntingtin remains to be investigated, but is likely connected to its capacity of self-multimerization.

Finally, Staufen may indirectly contribute to the pathogenesis of spinocerebellar ataxia 2 (SCA2), a kind of ataxia linked to polyglutamine expansions in ataxin-2 (ATXN2), a PB component. ATXN2 and its yeast homologue, Pbp1, function in global RNA silencing and decay. Adding evidence of a role in translation regulation, ATXN2 associates to polysomes and to PABP. ATXN2 is required for PB and SG assembly (133)(reviewed in (65)), and partial knockdown of the protein impairs SG assembly (134, 135). As mentioned above, these granules are negatively regulated by Staufen, thus, deregulation of mRNA-silencing *foci* by Staufen may aggravate the condition triggered by pathogenic ATXN2.

8.2. Staufen in nucleotide-repeat disorders

Staufen interaction with double-stranded hairpin RNA appears to be relevant in CUG repeat disorders. Among nucleotide repeat diseases, a few of them arise as a consequence of a gain of function of the mutated RNA. In these cases, pathogenic mechanisms involve nucleotide repeats in non-coding RNAs or in non-coding regions of mRNAs, and protein alterations are not causative of the disease. As for polyglutamine-based disorders, pathology onset and severity both correlate with repeat length. The molecular mechanisms underlying RNA-based CUG repeats disorders is intensely studied. Apparently, oligonucleotide expansions titrate specific RBPs involved in RNA processing, thus indirectly affecting the expression of relevant molecules, and provoking a plethora of cellular disorders (136-140). The activity of the main splicing factor muscleblind-like (MBNL/MBL) is affected by RNA with CUG repeats. MBNL/MBL binds GC-rich RNA hairpins present in their natural targets and also in pathogenic RNAs (136, 141, 142). Several reports show that, depending on the conditions, CUG expansions form metastable or highly stable hairpins *in vitro*, with single or multiple stems of varied length (139, 143, 144). Titration of the splicing factor MBNL/MBL by CUG repeats greatly contributes to disease onset (136, 139, 145).

Both neurons and muscle cells are susceptible to this kind of pathologies, which show progressive cell degeneration. We discuss below the role of Staufen in fly model diseases and speculate its putative relevance in human diseases involving nucleotide repeats.

8.2.1. Spinocerebellar ataxias

Neurons are affected in spinocerebellar ataxias (SCA) where SCA8, SCA10 and SCA12 being due to the expansion of specific repeats (reviewed in (146)). SCA8 is provoked by CUG expansions longer than 74 in a

noncoding RNA, termed SCA8 RNA, which normally includes 17-34 repeats (147). SCA10 is due to the presence of large repeats –ranging from 500 to 4500– of a specific pentanucleotide, AUUCU in intron 9 of the ataxin 10 gene (148). Finally, SCA12 is the consequence of a moderate expansion –55 to 78 repeats– of the trinucleotide CAG in the gene encoding PPP2R2B, that encodes a regulatory subunit of the brain-specific protein phosphatase PP2A (149). SCA8 is particularly interesting as a model disease provoked by a mutant non-coding RNA carrying unstable triplet repeats. In SCA8 patients and model mice, transcripts containing CUG repeats accumulate in a subset of neurons forming large ribonucleoparticles, and these *foci* co-localize with MBNL. Moreover, MBNL silencing partially recapitulates the neurological symptoms, supporting that loss of activity of this splicing factor by the pathogenic RNA is causative of the disease (150). In contrast with other pathogenic CUG repeats, the expanded SCA8 repeat is remarkably variable in penetrance, and numerous individuals carrying an expanded repeat allele are unaffected (147). This strongly suggests that unknown regulatory factors are decisive for the disease outcome. In an elegant study performed in flies carrying a human pathogenic SCA8 gene, Rebay and co-workers identified Staufen as a negative modulator of neurodegeneration. They found that *Drosophila* lines that express the SCA8 transgene in photoreceptor neurons develop a progressive retinal degeneration (151). As expected, mutations in MBNL/MBL aggravate SCA8-induced retinal degeneration in these flies. Then, they performed a genetic screen to identify phenotype modifiers. Strikingly, Staufen and three additional genes, out of 22 genes encoding distinct RNA binding proteins, affect the neurodegeneration triggered by the human SCA8 gene. Three independent Staufen alleles, *stau*¹, *stau*⁹ and *stau*⁹⁹, dramatically enhance the disease. Relevantly, alleles known to be important to several neuron functions and involved in neurodegeneration, including FMRP, ELAV and musashi, had no effect on SCA8-induced degeneration. This leads to speculate that Staufen binds pathogenic CUG-containing transcripts. Using a simple experimental system, Mutsuddi and colleagues (151) found that fly Staufen form granules containing CUG-expanded transcripts *in vivo*. In light of current knowledge, two non mutually-exclusive hypothesis for the role of Staufen in CUG repeat-based neurodegeneration seem plausible. As mentioned, mammalian and *Drosophila* Staufen molecules are important to neuron plasticity and are involved in long-term memory formation (26, 28-30). The presence of long CUG expansions forming imperfect double-stranded stems may retain Staufen in the nuclei, where the pathogenic hSCA8 transcripts concentrate, thus impairing Staufen-mediated regulation of neuronal RNA, and aggravating the disease. Additionally, Staufen may bind double-stranded CUG expansions, thus masking these elements and avoiding titration of other relevant RBPs, such as MBL, which is known to mediate photoreceptor differentiation and muscle development.

8.2.2. A role in muscular dystrophies?

Paralleling the role of *Drosophila* Staufen in SCA8, preliminary observations suggest that Staufen may affect the outcome of a disease termed myotonic dystrophy type 1 (DM1), that compromise several organs in addition to muscle, and may include mental retardation (reviewed in

(146)). DM1 is the most common form of adult muscular dystrophy affecting 1 in 8000 individuals, and was the first example where the pathogenic potential of trinucleotide repeat expansions was identified (152-154). Individuals affected by this disease carry between fifty to ten thousand CUG repeats in the 3'UTR of a specific transcript encoding a serine threonine kinase termed myotonic dystrophy protein kinase (DMPK1), which normally bears less than 37 repeats. DMPK kinase activity itself does not affect the disease, and work from several labs demonstrate that the pathology is largely the consequence of the deregulation of the splicing of dozens of mRNAs from different tissues (146, 155).

Also in this case, the splicing proteins CUG-BP1 and MBNL/MBL are key factors in the disease mechanism. Relevantly, mammalian MBNL1 and CUGBP1 –another splicing factor that binds to CUG repeats and that is affected in these diseases– colocalize with Staufen in SGs in cell lines (156, 157)(reviewed in (75)), opening the possibility that the protein complement associated to CUG repeat-containing RNAs is remodelled in these *foci*. Suggesting a role for Staufen in regulating DM1, mammalian Staufen binds CUG-containing DMPK mRNA in muscle cells (Bélanger *et al* ,unpublished observations), and moreover, Staufen is overexpressed in a mouse model of DM1 carrying a 250 CUG expansion, suggesting a compensatory response to recover stoichiometry.

More recently, it was shown that Staufen nuclear functions may be relevant to the processing of dystrophin, another gene linked to muscular dystrophies (158). This study focused on Duchenne's Muscular Dystrophy (DMD), the most common muscular dystrophy with a relatively high incidence rate reaching 1/3500 males. Several defects in the gene encoding the muscle protein dystrophin, which is the largest human gene, lead to loss of expression of this protein, thus provoking DMD. In a screen aimed to identify splicing regulators that may compensate DMD mutations, Brinker and coworkers found that Staufen 2 enhances exon-skipping of a specific dystrophin exon (158). These observations highlight an important role of Staufen in regulating the expression of molecules key for the pathology; however, the potential relevance of a general splicing regulator in therapies against DMD is unclear.

8.2.3. Staufen binds pathogenic expanded RNA repeats

Though the precise relevance of Staufen in CUG-based diseases remains to be investigated, it is undoubtedly linked to its ability to bind stem-loop RNA structures. The CUG repeat-based hairpins formed by pathogenic transcripts are closely similar to fly and mammalian Staufen-binding motifs, which include, among others (see above) a 12-bp stem and a 5-nt loop (45, 53). The interaction of fly Staufen with SCA8 RNA was clearly demonstrated *in vivo* (151). In addition to Staufen, other dsRBD-containing proteins may be affected by the presence of CUG repeat hairpins. Indeed, the PKR dsRBD binds pathologically-expanded CUG repeats and this interaction leads to PKR activation. CUG expansions longer than 69 trinucleotides stimulate PKR autophosphorylation thus leading to eIF2 α inactivation and affecting global translation. Expansions of 35 trinucleotides

induced weak PKR activation, and this is not detectable below 20 trinucleotides (144). PKR activation by imperfectly matched duplexes may contribute to muscle or neuron degeneration (144, 159). All these observations suggest that additional dsRNA-binding proteins, as for example ADAR, RNA helicase A, PACT, NF90 or TRBP may mediate the toxicity of expanded CUG repeats, and that in all cases, Staufen may modulate the effect by playing an homeostatic role.

Moderate CUG expansions in the Junctophilin gene – between 66 to 78 – provoke a disorder similar to Huntington, termed HDL2. The pathogenic mechanism is unclear, but the possibility that Staufen may affect disease severity also in this case is open. In the same line, is not clear whether Staufen significantly binds related polynucleotide expansions also linked to muscle or neuron degeneration. In particular the tetranucleotide CCUG, which is expanded 75–11,000 times in the gene encoding ZNF9 causing DM2, looks like a potential target. The putative role of Staufen molecules in these pathologies remains to be investigated.

9. VIRUSES EXPLOIT CELLULAR STAUFEN TO INFECT MAMMALIAN CELLS

Given the ability of Staufen molecules to regulate the localization, stability and translation of numerous cellular transcripts with low sequence specificity, an important role in regulating virus-encoded RNAs is expected. The direct relevance of Staufen molecules in viral infection has been studied in particular for two unrelated viruses: HIV and Influenza A (11, 160-165).

Apart from its role in the assembly of specific viruses (see below), Staufen may affect antiviral factors. Staufen and PKR bind similar dsRNA structures, and it has been speculated that the interaction of viral RNA with Staufen may contribute to avoid PKR activation during infection (35), thus escaping global silencing and facilitating virus-specific translation and viral proliferation. Another important cell defence mechanism against retroviruses depends on APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1-like 3G; A3G) and related deoxycytidine deaminases. Briefly, A3G incorporates to virus particles such as HIV-1 during their assembling, and extensively deaminates dC residues –converting them to dU– in the single-stranded viral DNA that transiently forms during reverse transcription. This leads to a G-to-A hypermutation, and to the lethal accumulation of uracil residues. Then, uracil DNA glycosylase and endonuclease activities destroy the aberrant DNA (166, 167), thus blocking virus proliferation. A3G is found in two different cytoplasmic complexes: a low-molecular-mass (LMM A3G) particle, where A3G remains active, and a high-molecular-mass (HMM A3G) where A3G enzymatic activity is blocked (167, 168). HMM A3G complexes contain Alu and small Y RNAs, two prominent human retrotransposons, and apparently their sequestration into HMM A3G complexes impairs the mobile elements' insertion process, thus promoting genome stability. Thus protection from endogenous retroelements by HMM A3G formation implies the loss A3G enzymatic activity and as a consequence, HIV replication ensues. Inactive HMM A3G

complexes can be converted into LMM A3G complexes by RNase A treatment (168, 169), indicating that RNA serves as a scaffold for the formation of the large complexes. Analysis of the protein composition of highly purified A3G complexes by mass spectrometry (168-170) allowed the detection of Staufen 1 in HMM particles, among other components. Given the capability of Staufen to mediate granule assembly (8, 21, 32, 171), the prediction is that Staufen will enhance HMM A3G stability by binding to structured retroelements or messenger RNAs present in HMM A3G complexes. This will negatively influence LMM A3G assembly and the concomitant protection against viruses. In addition, A3G ribonucleoparticles have a complex relationship with SGs and PBs (169, 170, 172), and the relevance of these interactions to the cell defense against retrovirus and endogenous retroelements is not completely understood. Staufen 1 is present in HMM A3G complexes and regulates SG and PB dynamics and thus, an effect on the interplay between A3G, SGs and PBs is anticipated.

9.1. Staufen and the human immunodeficiency virus

More than a decade ago, Gatignol and co-workers predicted the interaction of Staufen with a stem-bulge-loop structure that forms the TAR motif present in the R region of the HIV long terminal repeat (165). These authors started by analyzing the binding of another cellular factor, termed TAR-RNA Binding Protein (TRBP), to the viral TAR motif. Sequence alignments revealed that the TRBP domain that binds to the TAR-double helix motif is highly homologous to two regions within *Drosophila* Staufen, located at the C-terminal portion of dsRBD2 and at the dsRBD3 (165). More recently, DesGroseillers and co-workers demonstrated that mammalian Staufen 1 indeed binds to the TAR motif (35). Binding of TRBP stimulates translation of TAR-containing RNAs (173) and relevantly, this seems to be the case also for Staufen 1, as described above. Binding of Staufen to a TAR motif located at the 5'UTR of reporter transcripts greatly increases translation to levels comparable to those induced by TRBP binding (35). This supports the notion that cellular Staufen could be exploited by the virus to enhance the expression of viral proteins. Staufen-mediated translational stimulation would thus be mediated by concurrent mechanisms, such as the recruitment of ribosomes to the bound mRNA, as well as association to co-factors which promote translation. In addition, Staufen may destabilize the TAR-RNA structure, which is known to repress initiation, thus facilitating accessibility of eIF4E and additional initiation factors to the 5' cap.

In addition, Staufen associates to viral proteins involved in virus assembly. It was initially shown that human Staufen 1 is incorporated to mature HIV-1 particles (161). Moreover, this incorporation correlates with the content of genomic RNA, and Staufen overexpression can triple the amount of encapsidated viral RNA. The ordered assembly of viral particles is guided by a viral protein termed Pr55^{Gag}. Once assembled, a specific viral protease digests Pr55^{Gag} generating mature proteins and a mature HIV-1 virus is formed. Human Staufen 1 interacts with Pr55^{Gag}, but not with the mature Gag proteins. During virus assembly, Pr55^{Gag} multimerization is affected by human Staufen 1

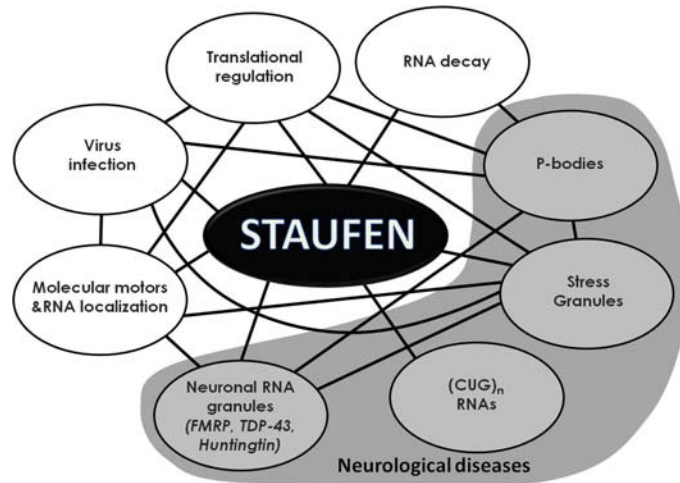


Figure 3. Staufen's network of physical and functional interactions. Staufen interacts with a variety of molecules and cellular structures (see text for details), and it is involved in post-transcriptional regulation at the levels of mRNA transport, translation or decay. In addition, Staufen associates to abnormal RNAs containing expanded CUG repeats, and to viral RNA components. Structures and molecules relevant to neurological diseases are shaded.

levels (174). Several Staufen domains are involved in this process: the N-terminal Staufen 1 moiety is required for Pr55^{Gag} multimerization and Staufen 1 dsRBD3 directly interacts with Pr55^{Gag} (164). Human Staufen 1 and Pr55^{Gag} form a complex that includes genomic viral RNA, and excludes spliced RNA species, and it has been suggested that human Staufen 1 plays a role in the selection of genomic RNA for encapsidation (163, 175). In addition, Staufen 1 helps Pr55^{Gag} expression and Staufen 1 silencing leads to reduced Pr55^{Gag} levels.

More recently, human Staufen 1, Pr55^{Gag} and viral RNA were shown to form a complex termed SHRNP, for Staufen HIV-1-dependent RNP (175). Very much like SGs, these novel *foci* are in dynamic equilibrium with the translational apparatus, as they dissolve upon pharmacological stabilization of polysomes, and conversely, are enhanced upon polysome-destabilization by specific drugs. As judged by the absence of several marker proteins, SHRNP are different from SGs and PBs (175). Thus, SHRNP are RNA silencing *foci* specific for HIV-1 RNA, and it can be speculated that their formation protects viral RNA from degradation. As is the case of SGs described above, Staufen 1 knockdown enhances SHRNP formation. These observations suggest that the two kind of *foci*, SGs and SHRNP, similarly respond to Staufen-mediated polysome stalling. Additionally, Staufen 1 may help delivering transcripts from the SHRNP to virus particles. Collectively, these results indicate that human Staufen 1 plays a critical role in HIV1 infection by helping several steps during genomic RNA encapsidation.

9.2. Staufen and the influenza A virus

Mammalian Staufen 1 also contributes to Influenza A virus proliferation. NS1, a non-essential component from influenza A virus involved in virus transcription and replication, was the first viral protein shown to be associated with human Staufen 1 (160). In a pioneer work by Ortin and colleagues, NS1 was found associated to human Staufen 1 in a

yeast two-hybrid screen (11). Direct interaction between these proteins was confirmed both *in vitro* and *in vivo*. NS1 and Staufen 1 cofractionate with polysomes in infected cells. More recently, Torreira and co-workers reported that human Staufen 1 interacts with additional Influenza virus components, and that this interaction is independent of the presence of NS1. Using a TAP-tag system, they demonstrated that human Staufen 1 associates to nucleoprotein (NP), to the polymerase components PB1 and PA, and to genomic viral RNA and transcripts (176). In a functional assay, they found that knockdown of cellular Staufen 1 dramatically reduces viral particle production, although the expression and localization of key viral proteins is not affected. Collectively, these results suggest that cellular Staufen 1 helps Influenza A virus replication. Future work will contribute to unveil the molecular mechanisms facilitated by Staufen 1, which likely include proper localization and expression of viral RNAs, as well as localization of RNPs at the plasma membrane and subsequent packaging.

10. SUMMARY AND PERSPECTIVE

Ever since its discovery in the *Drosophila* model, where it plays an essential role in the determination of embryo polarity by means of the localization of morphogenic mRNAs, Staufen has revealed a continuously increasing list of physiological tasks, in a wide variety of species. While showing a high degree of sequence conservation during evolution, the original Staufen gene has been duplicated in vertebrates, giving rise to two similar paralogs and several alternative splicing variants. The plethora of cellular mechanisms which implicate Staufen proteins as active components include cell fate determination, synaptic plasticity and neural morphogenesis, regulation of mRNA stability and translatability, modulation of the stress response and ensuing cell survival, incidence of nucleotide repeats-related diseases, the success of some viral infections, and presumably cancer-related cellular alterations in some cases (Figure 3). Although

it is clear that Staufen plays some more or less pivotal role in all the former, further research is still needed to pinpoint Staufen as a relevant prognosis marker, or as a potential therapeutic target. As new data regarding mRNA and protein composition of Staufen RNPs accumulates, it can be expected that novel Staufen functions –some of which will likely be relevant to human health– are yet to come.

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- Abbreviations:** A3G: apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1-like 3G (APOBEC3G); ALS: amyotrophic lateral sclerosis; ATXN: ataxin; CNS: central nervous system; DM1: myotonic dystrophy type 1; DMD: Duchenne's muscular dystrophy; DMPK: myotonic dystrophy protein kinase; dsRBD: double-stranded RNA-binding domain; FMRP: fragile X mental retardation protein; FTLD: frontotemporal lobar degeneration; HMM A3G: high molecular mass A3G; L-LTP: late long-term potentiation; LMM A3G: low molecular mass A3G; MBLN/MBL: muscleblind-like protein; NMD: nonsense-mediated decay; PABP: poly(A)-binding protein; PBs: P-bodies; PKR: double-stranded RNA-dependent protein kinase; PP2: protein phosphatase 2; RBP: RNA-binding protein; RNP: ribonucleoprotein; SCA: spinocerebellar ataxia; SGs: stress granules; SHRNPs: Staufen HIV-1-dependent RNP; SMD: Staufen-mediated decay; TBD: tubulin-binding domain; TRBP: TAR RNA-binding protein
- Key Words:** Staufen, SCA8, Stress Granules, FMRP, TDP-43, Huntingtin, FUS/TLS/hnRNP P2, HIV, Influenza Virus, mRNA transport, Oligodendrocyte, Neurodegeneration, Memory, Neuron, Review,

Staufen in cell stress and neurodegeneration

Send correspondence to: Graciela L. Boccaccio, Av
Patricias Argentinas 435, C1405 B WE Buenos Aires,
Argentina, Tel: 54 11 5238 7500 ext 3309, Fax: 54 11 5238
7501, E-mail: gboccaccio@leloir.org.ar

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