SMN and Gemins: 'We are family' … or are we?

Insights into the partnership between Gemins and the spinal muscular atrophy disease protein SMN

Ruben J. Cauchi

Gemins 2–8 and Unr-interacting protein (UNRIP) are intimate partners of the survival motor neuron (SMN) protein, which is the determining factor for the neuromuscular disorder spinal muscular atrophy (SMA). The most documented role of SMN, Gemins and UNRIP occurs within the large macromolecular SMN complex and involves the cytoplasmic assembly of spliceosomal uridine-rich small nuclear ribonucleoproteins (UsnRNPs), a housekeeping process critical in all cells. Several reports detailing alternative functions for SMN in either motor neurons or skeletal muscles may, however, hold the answer to the extreme neuromuscular tissue specificity observed in SMA. Recent discoveries indicate that collaboration between SMN and Gemins also extends to these non-canonical functions, hence raising the possibility that mutations in Gemin genes may be the cause of unlinked neuromuscular hereditary syndromes. This review evaluates the functions of Gemins and UNRIP inside the SMN complex and discusses whether these less notorious SMN complex members are capable of acting independently of SMN.

Keywords:
Gemin proteins; neuromuscular disease; snRNP assembly; spinal muscular atrophy; survival motor neuron

Introduction

First described by Guido Werdnig at the close of the nineteenth century, spinal muscular atrophy (SMA) is a recessively inherited disease characterised by degeneration of the anterior horn α-motor neurons of the spinal cord, as well as progressive muscle weakness and wasting, and can be fatal. The genetic mapping of the disease to 5q11.2–13.3 by Conrad Gilliam's team and the subsequent discovery of the determining gene by Judith Melki's group, a novel gene named *survival motor neuron* (*SMN*), happened at the end of the following century and was triggered by the revolutions in molecular biology in the preceding decades [1–3]. The hunt for the function of SMN began in earnest and in just a year following its discovery, Qing Liu and Gideon Dreyfuss [4] reported that they had stumbled unexpectedly on the SMN protein whilst searching for heterogeneous nuclear ribonucleoprotein (hnRNP)-interacting proteins. In the same paper, they eventually described that, in the nucleus, SMN is concentrated in several intense foci that are frequently found neighbouring or overlapping Cajal bodies (CBs), hence being named Gemini of CBs or simply, gems. From this point onwards, SMN biochemistry became the leitmotif of the Dreyfuss laboratory and in the following decade, in tandem with other groups, they elegantly demonstrated that SMN exists as an oligomer [5–7] but, more importantly, it is complexed with several components that were identified in subsequent studies and termed collectively as Gemins for protein components of gems [8–18]. The SMN

DOI 10.1002/bies.201000088

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Abbreviations:
AGO2, Argonaute2; **CB**, Cajal body; **hnRNP**, heterogeneous nuclear ribonucleoprotein; **IRES**, internal ribosome entry site; **miRNAs**, microRNAs; **mRNP**, messenger ribonucleoprotein; **PHAX**, phosphorylated adaptor for RNA export; **PRMT5**, protein arginine methyltransferase 5; **scaRNAs**, small Cajal body-specific RNAs; **SMA**, spinal muscular atrophy; **SMN**, survival motor neuron; **TGS1**, trimethylguanosine synthase 1; **UNRIP,** unr-interacting protein; **UsnRNP**, uridine-rich small nuclear ribonucleoprotein.

complex was later implicated in the biogenesis of the uridine-rich small nuclear ribonucleoproteins (UsnRNPs), a crucial housekeeping function [19]. Each UsnRNP particle consists of a short non-coding RNA molecule bound to a set of seven Smith (Sm) or Sm-like (LSm) proteins, and a unique set of UsnRNP-specific proteins [20]. UsnRNPs together with numerous non-UsnRNP splicing factors form the spliceosome, which comes in two forms. The major spliceosome, composed of U1, U2, U4/U6 and U5 snRNPs, is responsible for splicing the vast majority of pre-mRNA introns or those introns that have common consensus sequences near their 5′ and 3′ ends (U2-type introns). The less abundant minor spliceosome, composed of U11, U12, U4atac/U6atac and U5 snRNPs, splices a rare class of metazoan introns that have non-canonical consensus sequences (U12-type introns) [21]. Despite the plethora of evidence favouring a role in UsnRNP biogenesis, recent years have seen a surge in reports detailing alternative or non-canonical functions of SMN in either motor neurons or skeletal muscle, two tissues that are specifically affected in SMA. This review aims at probing the relationship between SMN and Gemins, and specifically evaluates whether collaboration between these proteins extends beyond UsnRNP biogenesis.

Architecture of the SMN complex

The human SMN complex counts within its fold at least nine members including the eponymous member SMN, Gemins 2–8 and Unr-interacting protein (UNRIP) [8–18]. In view of protein-protein interactions observed in at least two independent systems, the Fischer laboratory established that at the backbone of the SMN complex lie SMN, Gemin7 and Gemin8, which form a binding platform for other components. Hence, SMN binds to Gemins 2, 3 and 8. Gemins 2 and 3, in turn interact with Gemins 5 and 4, respectively. Finally, Gemin8 interacts with Gemins 4 and 7, whilst the latter recruits UNRIP and Gemin6 *via* direct interactions [22] (Fig. 1). Non-consensus protein-protein contacts confirmed in later studies include the Gemin2-Gemin7 interaction [23]. Self-associations were independently observed for SMN, Gemin2 and Gemin8 [6, 7, 22, 24] with the Gemin2-Gemin2 interaction stabilising SMN self-association, and hence SMN oligomerisation [24].

Based on their efficient co-immunoprecipitation with epitope-tagged SMN, SMN, Gemins 2–4 and 6–8 are core SMN complex members. In contrast, Gemin5 and UNRIP are peripheral components as a result of their weak co-precipitation with SMN, and in the case of Gemin5, its dissociation on treatment of immobilised purified SMN complexes with high salt concentrations [22]. In accordance with this, although all components of the SMN complex are present throughout the cytoplasm, only the core SMN complex members are highly enriched in gems [8–18], whereas Gemin5 is rarely detectable [25, 26] and UNRIP is undetectable in this nuclear structure [16, 18]. Indeed, both UNRIP and Gemin5 have been found to associate with the SMN complex only in the cytoplasm, hence being the only compartment-specific members of the SMN complex [18, 26]. So far, only SMN, Gemins 2, 3 and 5 knockouts have been generated, resulting in lethality in a variety of organisms including yeast, worm, fly and mouse, thereby indicating that these SMN complex components are essential for viability [27–36].

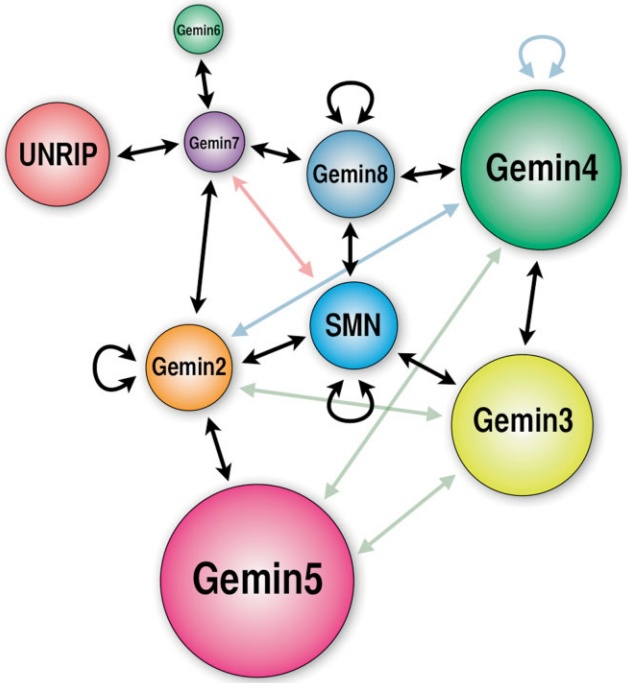

Figure 1. Interaction map of the human SMN complex. Consensus interactions or protein-protein associations observed in at least two independent experimental systems are depicted with black arrows. Non-consensus interactions or protein-protein associations observed in a single experimental system are depicted in red (GST pulldown assay), green (co-immunoprecipitation) or blue (yeast two-hybrid assay) (adapted from Otter *et al*. [22] with additional findings from Ogawa *et al*. [23, 24]).

The most primitive and ancestral version of the SMN complex, observed in the yeast *Schizosaccharomyces pombe*, is composed of only SMN and Gemin2 [34, 37]. Such a simple SMN complex is thought to have opened up its membership to additional proteins throughout the tortuous journey of evolution. In view of such an overall evolutionary trend towards a multisubunit SMN complex in metazoans, it is, however, enigmatic why orthologues of Gemins are missing in the genomes of certain species but present in organisms at lower evolutionary branches (Fig. 2). This could indicate gene loss, probably because of loss of a role outside of the SMN complex [37], and begs the question of whether Gemins have an interchangeable function.

Chronicles of spliceosomal uridine-rich small nuclear ribonucleoproteins biogenesis

Birth and nuclear departure

LSm-class U6 and U6atac snRNPs are assembled within the confines of the nucleus, most probably promoted by SMN complexes charged with a core formed of LSm 2-8 [38]. In

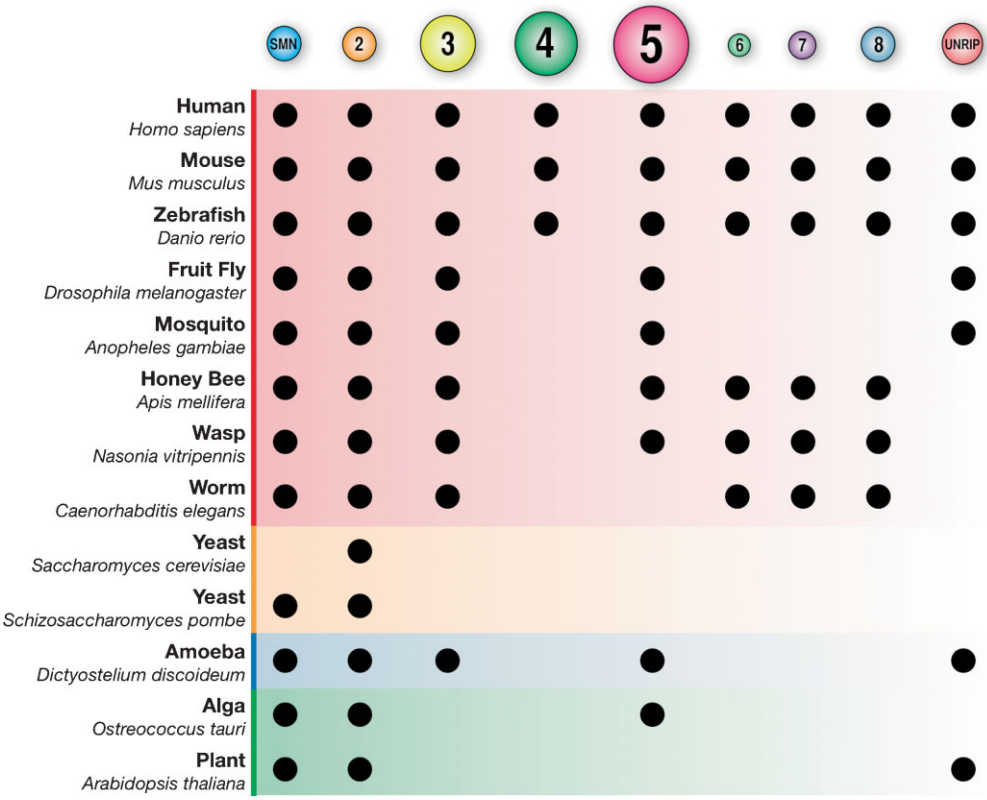

Figure 2. Orthologues of human SMN complex components in different organisms. The simplest SMN complex, composed of just SMN and Gemin2, is found in single-celled organisms such as the fission yeast *S. pombe* [34] and most probably in the plant *A. thaliana*. The stepwise addition of novel components occurred only later in evolution, thereby increasing the complexity of the SMN complex, though baffling exceptions are obvious. For instance, Gemins 6–8 are apparently missing in dipterans, such as the fruit fly *D. melanogaster,* but are present in the genome of closely-related eukaryotes at lower evolutionary branches, such as the honey bee *A. mellifera* and the wasp *N. vitripennis*. These Gemins are also present in the worm *C. elegans*; but the absence of Gemin5 in the worm genome and its retention in distant organisms, such as the amoeba *D. discoideum* and the green alga *O. tauri,* is yet again perplexing. Although remote, one should not exclude the possibility that such Gemin genes reside in an unsequenced heterochromatic region, or that their sequence is so highly divergent that it is not detectable through similarity comparisons. The presence of Gemin4 only in higher metazoans indicates that it joined the SMN complex only recently in evolution [37]. Organisms are listed from bottom to top according to their evolutionary complexity, with colour groupings: green, *Plantae*; blue, *Amoebozoa*; orange, *Fungi*; and red, *Metazoa*. Figure is based on data presented in Kroiss *et al*. [37] with additional data on UNRIP derived from BLAST genome searches.

contrast, Sm-class UsnRNPs are forged within the cytoplasm in an energy-demanding process facilitated by the SMN complex, though assembly is spontaneous *in vitro* [39, 40]. Sm-class UsnRNAs are 'born' on transcription by RNA polymerase II and their co-transcriptional acquisition of a 7-methylguanosine (m^7G) cap allows interaction with the cap-binding complex (CBC). This in turn facilitates binding to the phosphorylated adaptor for RNA export (PHAX), which is an adaptor for the co-operative addition of RanGTP and export receptor exportin1 (XPO1). Interaction of XPO1 with the components of the nuclear pore complexes spanning the nuclear envelope ensures nuclear export (Fig. 3) [20].

Intricacies and intrigues of the cytoplasmic passage

The cytoplasmic passage of UsnRNAs centres on the role of the SMN complex in the assembly of a stable 'ring'-shaped heptameric core made of Sm protein B, D1, D2, D3, E, F and G, on the short, single-stranded, uridine-rich Sm site of Sm-class UsnRNAs. Hinting at a possible UsnRNP assembly function, early experiments showed that antibodies directed against components of the SMN complex or over-expression of a dominant-negative SMN mutant strongly interfered with Sm core assembly onto UsnRNAs, while abolition of assembly in extracts immunodepleted of SMN complexes was restored on addition of affinity-purified macromolecular SMN complexes [19, 39, 41, 42]. All SMN complex members, bar UNRIP, are essential for the Sm core assembly reaction since knockdown of SMN and Gemins 2–8 in HeLa cells has been reported to disrupt the assembly reaction [14, 18, 24, 43, 44]. The SMN complex is capable of binding directly to the Sm proteins in view of studies demonstrating that all components of the SMN complex, except Gemins 2 and 8, interact directly with a distinct subset of Sm proteins [8–13, 15, 16]. A methyltransferase complex consisting of the proteins arginine methyltransferase 5 (PRMT5), WD45/MEP50 and pICln has been shown to recruit Sm proteins and enable symmetric dimethylation of an Sm protein subset (D1, D3 and B) on designated arginine residues by PRMT5, a modification

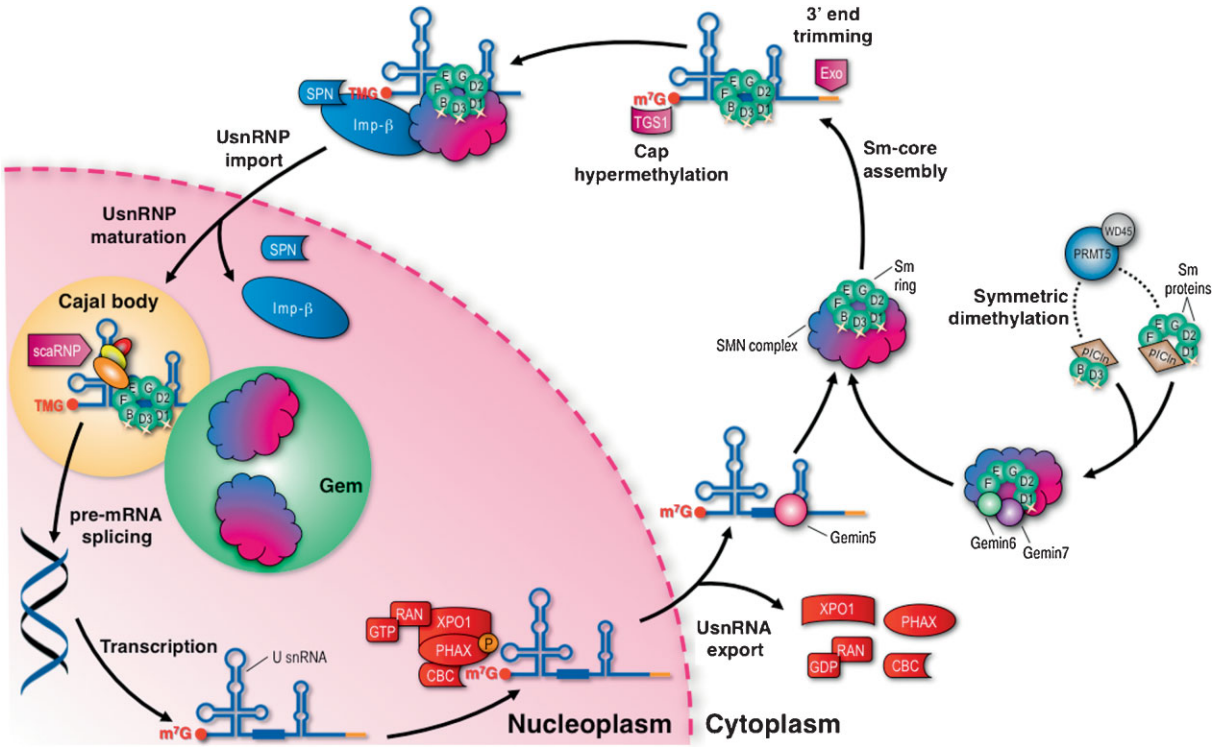

Figure 3. Spliceosomal UsnRNP biogenesis pathway. Export of all spliceosomal UsnRNAs (except U6 and U6atac) to the cytoplasm requires assembly of a transport complex composed of CBC (formed of subunits CBP80 and CBP20), PHAX, RanGTP and XPO1 onto the m^7G cap of the nascent UsnRNAs. Once in the cytoplasm, hydrolysis of RanGTP to RanGDP, promoted by cytoplasmically-localised factors and dephosphorylation of PHAX, not only results in the release of the UsnRNAs but also contributes to the directionality of export. PHAX and CBC are subsequently shuttled back to the nucleus to initiate a new round of transport. The released UsnRNAs associate with free Gemin5, which directs such snRNAs to the SMN complex, charged with a ring-shaped heptameric Sm protein core. The assembly of the Sm ring is initially chaperoned by pICln and is finalised within the SMN complex. Following processing and nuclear import, and prior to participating in pre-mRNA splicing, UsnRNPs stop temporally at the CB where further maturation processes take place, including association of UsnRNP-specific proteins and nucleotide modification.

thought to enhance the interaction between Sm proteins and SMN oligomers [45]. pICln was recently found to induce the formation of higher order Sm protein structures including D1-D2, E-F-G, and B-D3, and to prevent their premature association with RNA before transfer of Sm proteins from two pICln-Sm protein complexes (pICln·D1-D2·E-F-G and pICln·B-D3) onto the SMN complex [46] (Fig. 3). In addition to binding Sm proteins, the SMN complex binds UsnRNAs through the stringent recognition of a code formed of sequences and structural motifs in UsnRNAs. Hence, cells are protected from illicit and potentially deleterious, non-specific binding of Sm proteins to random RNAs whilst ensuring that Sm cores only assemble on the correct RNAs [47].

What is the precise contribution of each member of the SMN complex to the UsnRNP assembly reaction? The answer to this question is still a work in progress, though in recent years several interesting insights have been uncovered. Gemin5 was identified as the factor that allows the SMN complex to specifically recognise, as well as bind to, UsnRNAs [48], and it does so *via* its WD repeats [49]. Crystal structure studies have revealed that the Gemin6-Gemin7 heterodimer exhibits a structure that resembles Sm core protein dimers though both Gemins 6 and 7 lack significant sequence similarity with Sm proteins [50]. In this context, Gemins 6 and 7 may serve as a surrogate for the SmB-SmD3 dimer around which Sm hetero-oligomers are arranged following their transfer from the pICln-Sm protein complex. UNRIP, another SMN complex component with WD-repeats, might be important for the exchange of the Gemin6-Gemin7 heterodimer by the SmD3-SmB particle, which should ensure Sm ring closure within the SMN complex in preparation for uploading onto UsnRNAs [23]. The exact functions of the remaining SMN complex components in UsnRNP assembly are, as yet, unknown. Forming the backbone of the SMN complex, SMN, Gemin2 and Gemin8 oligomers may provide the platform for the final stages of Sm core formation and, subsequently, its uploading onto a UsnRNA once snRNA-charged Gemin5 docks into the SMN complex [51]. Gemin3 (formerly DP103/DDX20) is a DEAD-box RNA helicase that has been shown to exhibit an ATP-dependent and a 5′-3′ RNA unwinding activity *in vitro* [52], that might be crucial in chaperoning RNA, and eventually RNP complexes, during the assembly reaction with the intimately bound component, Gemin4, probably acting as a cofactor for such activities.

Nuclear return of UsnRNPs

Newly assembled UsnRNPs undergo cytoplasmic processing, including m^7G cap hypermethylation to a 2,2,7-trimethylguanosine (TMG) cap catalysed by trimethylguanosine synthase 1 (TGS1), and nucleotide trimming of the 3′ terminus, both of which processes are dependent on proper Sm core assembly. The TMG cap and the Sm core serve as the nuclear localisation signal necessary for nuclear import *via* the import receptor importin-β (Imp-β). Snurportin-1 (SPN) is the adaptor that specifically recognises the TMG cap, whereas the SMN complex remains bound to the Sm core to serve as the adaptor for the Sm core [20]. In view of their cytoplasmic predominance, SMN complex members Gemin5 and UNRIP are thought to either shuttle back to the cytoplasm following their import into the nucleus with the SMN complex or else they dissociate from the SMN complex prior to nuclear import. Upon their return to the nucleoplasm, the import proteins dissociate and the newly imported UsnRNPs transiently accumulate in CBs, whereas SMN complexes relocate to gems. In CBs, UsnRNPs are thought to undergo the final steps of maturation before their participation in pre-mRNA splicing, including association of species-specific UsnRNP proteins, UsnRNP coupling, such as the formation of the U4/U6-U5 tri-snRNP, as well as site-specific dyskerin-catalysed pseudouridylation and fibrillarin-catalysed 2′-*O*-ribose-methylation directed by small CB-specific RNAs (scaRNAs) [53].

The special relationship

Gems and Cajal bodies

The discovery of gems in a HeLa (PV) subline, as nuclear structures that are frequently near, touching or overlapping CBs, underlined a special and possibly functional relationship between these two nuclear bodies [4]. The CB, named after its discoverer, the Noble laureate Santiago Ramón y Cajal, contains a bewildering array of proteins and RNAs. Common to a wide range of eukaryotes, the CB, which is identified by the signature protein coilin and scaRNAs, hosts several important steps in the maturation of the RNA-processing machinery [53]. Homozygous knockouts of coilin do not lead to lethality in *Drosophila* and are only semi-lethal in mice; although, in the absence of coilin, CBs are not formed [54–56].

Quenching the doubt cast upon the authenticity of gems as distinct nuclear bodies, later studies substituted immunofluorescence for immunoelectron microscopy to prove this point. Gems were found to be coilin-negative, fibrillarin-negative, SMN complex-positive, circular nuclear bodies of intermediate electron density and bearing a granular texture [57, 58]. Furthermore, in contrast to early immunofluorescence studies that reported that gems were devoid of UsnRNPs [4, 58, 59], immunoelectron microscopy demonstrated that gems have small amounts of UsnRNPs, whilst CBs are highly enriched in these spliceosomal components [57]. CBs and gems were also shown to be kinetically autonomous compartments, since dissociation kinetics of their respective components, coilin and SMN, remain unchanged upon separation of the two structures [60].

Early studies detected gems in a variable, but small proportion, of rapidly proliferating cells in culture [59]. Laborious experimentation on mammalian tissues by Young and colleagues [61, 62] demonstrated that the majority of gems and CBs occur as separate nuclear structures in foetal tissues, but their co-localisation increases with foetal age and is almost complete in the adult. Furthermore, gems and CBs were found in all foetal tissues, even those like heart, skin and spleen, which lack these nuclear bodies in the adult. Co-localisation of gems with CBs depends on the symmetrical dimethylation of the coilin arginine- and glycine-rich (RG) domain [63], which was previously found to mediate direct interaction with SMN [64]. In this respect, gem formation was enhanced on inhibition of methylation or mutation of the coilin RG domain, and HeLa cells with prominent gems were found to contain hypomethylated coilin [63–65]. SMN self-association [66], essential zinc-finger ZPR1 [67], as well as the nuclear phosphatase implicated in the dephosphorylation of SMN and Gemin3 (PPM1G) [68] were shown to be important in shifting the subcellular distribution of SMN complexes to nuclear bodies, whereas the opposite was found to be true for UNRIP [18, 68]. Interestingly, canonical CBs and, as expected, gems were lost on SMN knockdown and coilin was dispersed in the nucleoplasm into numerous small foci that lack UsnRNP components [43, 69, 70]. Knockdown of TGS1 and PHAX gave a similar phenotype except that gems were unaffected, suggesting that CB integrity is dependent on ongoing UsnRNP biogenesis, whereas formation and stability of gems is independent of CBs [69] (Fig. 4).

U and P bodies

Recent studies in the fruit fly *Drosophila melanogaster* have captured another special relationship between two organelles that also involves the SMN complex, but occurs beyond the nuclear border in the cytoplasm. In the *Drosophila* nucleus, SMN was found to localise with coilin, U85 scaRNA, spliceosomal UsnRNPs and fibrillarin in CBs [71] but, as yet, SMN has never been described in nuclear bodies separate from CBs, hence indicating that gems may be absent in *Drosophila*. Notably, the *Drosophila* SMN complex concentrates with UsnRNPs in discrete structures named U bodies in the cytoplasm, which are invariably associated with processing or P bodies as well as the endoplasmic reticulum, and are most abundant in mitochondria-rich cytoplasmic zones [72, 73]. P bodies are discrete cytoplasmic domains enriched with proteins and small non-coding RNAs, which function in mRNA silencing, quality control and degradation [reviewed in ref. 74]. Probing the relationship between P and U bodies, Lee *et al*. [75] showed that loss of either P or U body components in the *Drosophila* female germline leads to surprisingly similar defects in nuclear organisation. Furthermore, formation and/or organisation of P and U bodies are disrupted in the ovaries of flies mutant for components of either organelle [72, 75].

SMN complex-rich aggregates: Raison d'être

The formation of SMN complex-rich spherical aggregates in either the nucleus or cytoplasm and their association with

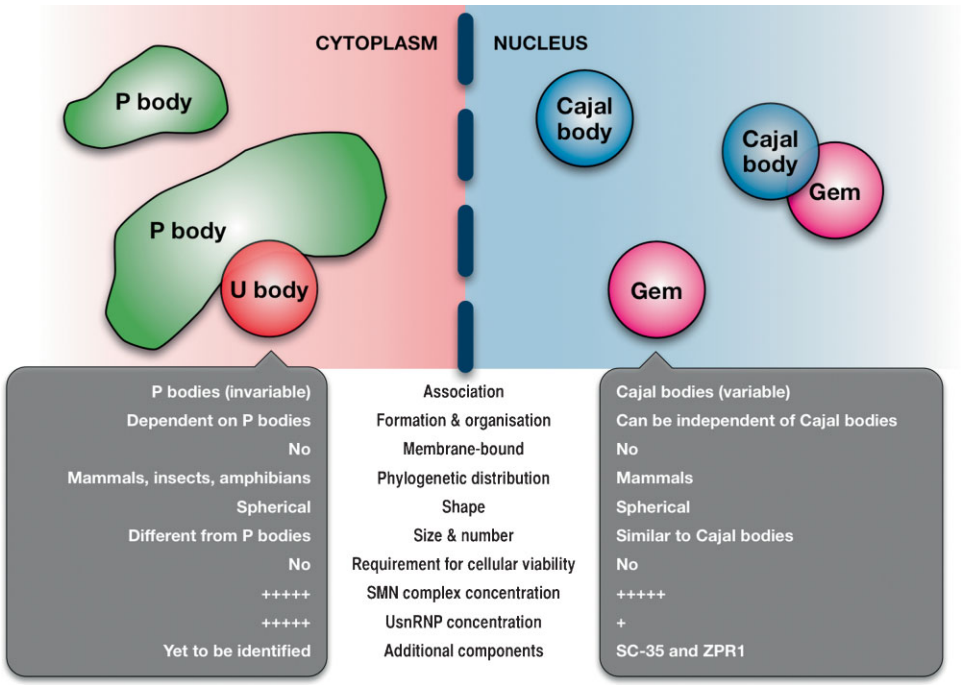

Figure 4. Characteristics of subcellular aggregates rich in SMN complexes. SMN complexes are enriched in several spherical organelles in the cell, with compartment-specific features and possibly, functions. Nuclear gems can be either separate, touching and/or overlapping CBs, whereas their cytoplasmic brethren, U bodies, are invariably associated with P bodies. The relationship of both gems and U bodies with cellular domains involved in RNA biology is likely to define, or at least influence, their function. SC-35, a non-snRNP splicing factor, and ZPR1 were reported as components of gems in [57] and [67], respectively.

organelles involved in RNA metabolism is quite intriguing (Fig. 4). Several authors favour the idea that nuclear gems are storage sites for excess nuclear SMN complexes that might be recruited to CBs for some late UsnRNP assembly reaction and/or recycling of these spliceosomal building blocks. On the cytoplasmic side, U bodies could be sites for the cytoplasmic UsnRNP assembly reaction and/or UsnRNP storage following their assembly, but before nuclear import. In view of the invariable association of U bodies with P bodies, SMN complexes resident in U bodies might assemble and/or transport mRNPs present in the P body [72, 73]. Cross talk between both these two cytoplasmic structures might also allow the regulated release of UsnRNPs from U bodies, depending on the rate of mRNA degradation. Alternatively, UsnRNP assembly/storage in U bodies could be balanced by UsnRNP degradation in the associated P bodies [72]. Although the exact role of gems and U bodies is as yet unknown, it is interesting to point out that neither structure is essential for viability. For instance, gems (and CBs) are absent from several adult mammalian tissues [61], whereas U bodies were not detected in egg chambers that are homozygous for a viable mutation in *dart5*, the *Drosophila* orthologue of *PRMT5* [72].

Recent work on the intensively studied CBs offers some interesting lessons on the formation of subcellular aggregates. In this respect, Deryusheva and Gall [76] observed normal levels of scaRNAs and no disrupted snRNA modification in viable coilin-null flies that lack CBs. Similar to CBs (and possibly P bodies [74]), the concentration of SMN complexes in cytologically detectable organelles may not be required for functionality. However, the formation of such structures is hypothesised to increase the concentration of macromolecules in a discrete cellular locale, with the aim of accelerating reaction rates based on the principle of mass action, as well as increasing the specificity of individual interactions [77]. Recent work suggests that the role of cellular bodies in promoting the rate and specificity of individual interaction reactions may only be revealed when the step affected is rate-limiting for the process being examined. This happens, for example, in zebrafish embryogenesis where rapid assembly and/or maturation of large amounts of new snRNPs are heavily required. Indeed, coilin knockdown during zebrafish embryogenesis leads to CB dispersal, deficits in snRNP biogenesis, disrupted pre-mRNA splicing and, consequently, reduced cell proliferation followed by developmental arrest [78].

Dispatches from the periphery

SMA is clinically heterogeneous and this reflects its genetic aetiology. In this respect, humans and closely related species such as chimpanzees are unique amongst metazoans because the nine exon-long SMN gene (*SMN1*) is duplicated. However, due to a nucleotide difference, the majority (though not all) of the mRNAs transcribed from the SMN gene duplicate (*SMN2*) skip exon-7, hence generating a truncated protein (SMNΔ7) that is not functional and rapidly undergoes degradation. In this context, SMA is the result of reduced levels but not

complete absence of the SMN protein since patients typically harbour deletions or loss-of-function mutations in *SMN1* but retain at least one *SMN2* copy capable of producing extremely reduced amounts of full-length SMN. In line with the observed correlation between SMA severity and SMN protein levels, an increased *SMN2* copy number dampens disease severity though other modifying factors might be involved [2, 3].

The nucleocentric dogma

In view of the extensively characterised role of the SMN complex as an assembly machine, it is not surprising that various authors hypothesise that SMA is the result of disrupted UsnRNP biogenesis. Supporting this view, various reports have shown that the SMN mutants found in SMA patients have reduced UsnRNP biosynthesis activity [5, 43, 79]. However, it is presently unclear how deficiencies in a function required by all cells leads to preferential neuromuscular degeneration. It is possible that, relative to other cell types, neuromuscular tissues have a greater requirement for SMN function in UsnRNP assembly. In this regard, the activity of the SMN complex in UsnRNP assembly was reported to be temporally regulated in spinal cord tissue relative to other tissues during mouse development [80]. An alternative hypothesis explaining the extreme tissue-specificity observed in SMA patients proposes that inefficient UsnRNP synthesis could lead to inappropriate and/or inefficient splicing of one or more specific mRNA transcripts crucial for the survival of neuromuscular tissues. The latter hypothesis was recently substantiated by Zhang *et al*. who report that in a moderately severe SMA mouse model ($Smn^{-/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$), reduced UsnRNP assembly is associated with tissue-specific alterations in the repertoire of UsnRNAs ('snRNPertoire'), with brain, spinal cord and heart (though not kidney and skeletal muscle) exhibiting a reduction in several minor spliceosomal UsnRNAs, including U11, U12 and U4atac. Intriguingly, the authors link such defects to the various tissue-specific perturbations in pre-mRNA splicing uncovered in SMN-deficient mouse tissues [81]. Bäumer and colleagues have argued that since such analyses were carried out at the late symptomatic stage, the widespread splicing abnormalities were not the primary cause of neuromuscular degeneration and, consequently, they reanalysed SMN deficient spinal cord tissues at pre-, early- and late-symptomatic stages. Although the majority of splicing changes were found to occur late in SMA, some missplicing was still detectable at the early stages, hence indicating that the link between splicing defects and survival of neuromuscular tissue is still plausible [82].

Happenings at the periphery: Axons

Despite evidence linking SMN loss to splicing abnormalities, in recent years enough support has been garnered to suggest that the SMN complex may have a function specific to motor neurons and/or skeletal muscles. Such a role, which might be the one that is impaired in SMA, is thought to be independent of UsnRNP assembly. The possibility of a neuronal-specific role for the SMN complex first stemmed from immunohistochemical analyses of mammalian central nervous tissues that demonstrated the association of SMN with cytoskeletal elements in spinal dendrites and axons [83, 84]. These early findings were supported by the observed presence of SMN at branch-points and growth-cones of neurites in cultured mouse primary motor neurons [85] and neuronal-like cells [86], together with the observed rapid, bidirectional and microtubule-dependent movement of enhanced green fluorescent protein (EGFP)-SMN granules in processes and growth cones of neuronal cell cultures [87]. Later work revealed that SMN significantly co-localises in large, stationary, and small, actively-transported neurite granules with various SMN complex members, including Gemins 2–7 but not Sm proteins. This suggests that any neuronal-specific role is first, independent of UsnRNP assembly and, second, it must be interpreted in terms of the SMN complex [88–90]. In line with this view, larval-lethal *Drosophila smn* or *gemin3* mutants exhibit strikingly similar phenotypes before they perish, including defects in motility and neuromuscular junctions [28, 29, 36, 91].

A plethora of work focussed on unravelling the exact role of the SMN complex in the neuron periphery. SMN was shown to bind to and co-localise with hnRNP-R, and the neuronal tissue-specific protein, profilin IIa, in neuronal processes [92–94]. Consistent with its role as a regulator of actin dynamics, profilin IIa is involved in correct neurite outgrowth [93], whereas hnRNP-R, an mRNA binding protein of which the targets include β-actin mRNA, was recently shown to be crucial for axon growth of spinal motor neurons in zebrafish embryos and isolated embryonic mouse motor neurons [94, 95]. Corroborating this evidence, *in vitro*-cultured Smn-deficient motor neurons derived from a severe SMA mouse model ($Smn^{-/-}$; $SMN2^{+/+}$) had reduced axon growth that correlated with a reduction in β-actin mRNA and protein in distal axons and growth cones [94]. Such neurons were also found to have defects in spontaneous excitability because of reduced integration of voltage-gated calcium channels into axonal growth cones and this, in turn, could be secondary to disturbed presynaptic synthesis of β-actin [96]. All these studies strengthen the hypothesis that, in addition to its role in UsnRNP assembly, the SMN complex may also interact with UsnRNP-independent proteins such as hnRNP-R and profilin IIa to assemble, sort and/or transport localised mRNP complexes needed for axonal growth and/or formation, as well as maintenance of neuromuscular junctions (Fig. 5). The 'dual-role' SMN complex hypothesis was surprisingly refuted by Winkler *et al*. [97] who reported that the motor axon defects observed after silencing Smn and Gemin2 in zebrafish embryos were rescued upon injection of purified UsnRNPs, suggesting that the motor neuron degeneration observed in SMA-afflicted patients is a direct consequence of impaired UsnRNP production. These findings were, however, rebutted by the Beattie laboratory who identified SMN mutations that failed to rescue the erroneous outgrowths of motor axons caused by Smn reduction in zebrafish but retained UsnRNP function, and *vice versa*. Thus, they successfully demonstrated dissociation of the UsnRNP biosynthesis function of the SMN complex from its function in motor axons [98]. Both studies have their pitfalls: rescue of the motor axon defects by UsnRNP reintroduction in the former study was most probably secondary to rescue of morphological defects; whereas the SMN mutations assessed in the latter study were linked to UsnRNP assembly, not through the assessment of assembly in fish extracts, but

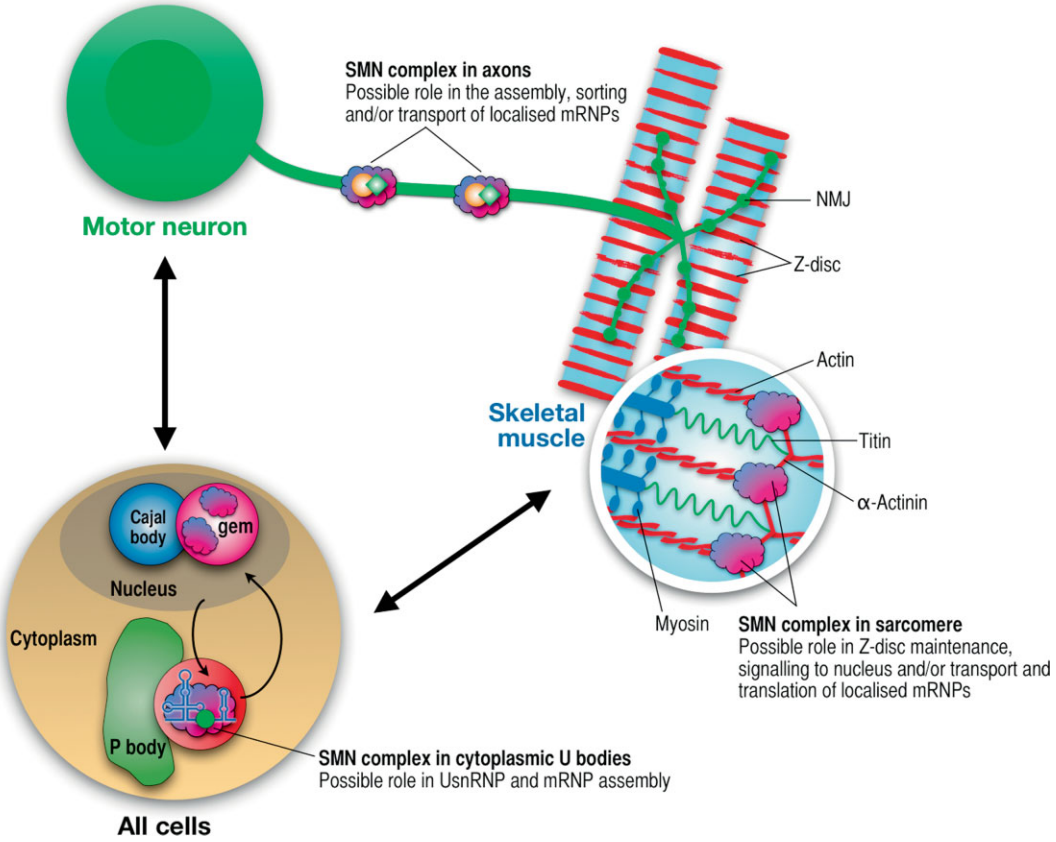

Figure 5. Hypothesised functions of the SMN complex. The SMN complex, localised in cytoplasmic U bodies, has a very well described role in UsnRNP assembly in all cells. So far, the proximity of U bodies to P bodies has only been described in the *Drosophila* egg chamber and might depend on specific metabolic conditions. In motor neurons, SMN complex movement in axons and binding with mRNP proteins, including hnRNP-R and profilin IIa, suggests an additional function in assembly, sorting and/or transport of localised mRNPs. In skeletal muscle, localisation of the SMN complex in sarcomeres hints at a role in the maintenance of Z-disc integrity, signalling to the nucleus and/or transport and translation of localised mRNPs.

based on the ability of the mutant proteins to oligomerise and bind Sm proteins [3].

Happenings at the periphery: Sarcomere

Interestingly, recent evidence has resurrected the idea that muscle might be the primary cause, or at least a major player, in the pathogenesis of SMA. Rajendra and colleagues [99] reported that the *Drosophila* Smn protein is localised at the I-band (actin enriched) as well as the Z-disc (α-actinin enriched) of the sarcomere, and the latter localisation pattern was conserved in mouse skeletal muscle. Notably, a later study showed that the entire SMN complex localises to the sarcomeric Z-disc of mouse skeletal and cardiac myofibrils [100]. Corroborating the role of the SMN complex in muscle, a hypomorphic *smn* mutant and a dominant-negative *gemin3* mutant were both shown to cause flightlessness and flight muscle degeneration [28, 99]. Although Gavrilina *et al.* [101] showed that expression of full-length Smn solely in skeletal muscles had no impact on the phenotype of severe SMA mice ($SMN2^{+/+}$; $Smn^{-/-}$), expression of Smn in neurons, in addition to muscle, had a major impact on the survival of the mice in question. Mirroring such findings, Chan *et al.* [29] noted that mesodermal expression of the wild-type Smn protein only led to occasional adult fly escapers; although, significant rescue of larval-lethal *smn* mutant flies to adulthood was observed on expression of Smn in mesodermal and nervous tissues. Furthermore, Chang *et al.* observed greater lethality when Smn expression was reduced in muscle compared to neurons [91], whereas a drastic impact on adult viability was observed on Gemin3 disruption in mesoderm and larval muscles but not nervous tissue [28]. In combination, these gripping findings suggest that a UsnRNP assembly-independent role for the SMN complex might also extend to the muscle. Walker and colleagues speculate a possible function in maintaining Z-disc integrity, a signalling role to the nucleus and/or transport as well as localised translation of mRNPs at the sarcomeric Z-disc [100] (Fig. 5).

The double life

Several studies have hinted that some components of the SMN complex are part of additional multiprotein complexes and likely perform important cellular functions outside the SMN complex. This is certainly the case for Gemins 3 and 4, which have been shown to form a less abundant complex that is

separate from the SMN complex, co-sediments with polyribosomes and contains Argonaute2 (AGO2), as well as numerous microRNAs (miRNAs) [102, 103]. miRNAs function within the RNA-induced silencing complex (RISC) to repress the translation of intracellular mRNAs with complementary nucleotide sequences. Remarkably, Murashov *et al.* [104] reported the presence of AGO2, fragile X mental retardation protein (FMRP), p100 and Gemin3 in the murine peripheral axons of the sciatic

Table 1. Proteins associated with Gemins and UNRIP outside of the SMN complex.

Binding partner	Function of binding partner	Significance of interaction	Ref.
Gemin2			
RAD51	Homologous recombination (HR)	Gemin2 enhances RAD51-DNA complex formation, hence stimulating RAD51-mediated homologous pairing; Gemin2 depletion in cell culture reduces HR efficiency and results in a decrease in the number of RAD51 subnuclear foci	[109]
HIV-1 integrase	Enzymatic catalysis of the integration of viral complementary DNA (cDNA) into host chromosome	HIV-1 integrase and Gemin2 synergistically augment viral cDNA synthesis by enhancing the assembly of reverse transcriptase onto viral RNA	[110]
Gemin3			
AGO2	RNA silencing	Gemin3 is amongst the several proteins present in mRNP and miRNP complexes containing AGO2	[102–104,111]
HspB8/Hsp22	Upregulated on heat shock	Motor neuron disease-associated mutant HspB8 forms have abnormally increased binding to Gemin3	[112]
EBNA2 EBNA3C	Epstein-Barr virus-encoded nuclear antigens	Unknown	[113]
SF-1	Nuclear receptor essential for the development of gonads, adrenal gland and ventromedial hypothalamic nucleus	Repression of transcriptional activity of SF-1	[114]
Egr1-4	Transcription factor family	Gemin3 represses Egr2-mediated transcriptional activation with significant promoter specificity	[115]
METS/PE1	Repression of Ets target genes involved in Ras-dependent proliferation	Anti-proliferative effects of METS require its interaction with Gemin3	[116]
N-CoR Sin3A	Transcriptional repression	N-CoR and Sin3A are probably recruited into a co-repressor complex required for the function of METS	[116]
HDAC-2 HDAC-5	Histone deacetylation	HDAC-2 and HDAC-5 are probably recruited into a co-repressor complex required for the function of METS	[116]
FOXL2	Forkhead transcription factor	Coexpression of Gemin3 with FOXL2 increases the cell death mediated by FOXL2	[117]
Gemin4			
Galectin-1 Galectin-3	mRNA splicing	Fragments of either Gemin4 or Galectin-3 exhibit a dominant negative effect on splicing	[118]
AGO1 AGO2	Protein involved in RNA silencing	Gemin4 is amongst the several proteins present in mRNP and miRNP complexes containing AGO1 and AGO2	[102, 103, 111]
Gemin5			
m^7G cap	In addition to UsnRNAs, m^7G is added co-transcriptionally to the 5′ end of mRNA transcripts synthesised by RNA polymerase II	Gemin5 associates with m^7G cap in the absence of eIF4E	[119]
eIF4E	Translation initiation factor	Gemin5 and eIF4E co-localise to cytoplasmic P bodies in human osteosarcoma U2OS cells	[120]
EcR USP DHR3 SVP βFTZ-F1	Nuclear receptors	*Drosophila* Gemin5 has an important role in larval development through gene-specific effects on ecdysone-regulated transcription, most likely as a cofactor for one or more nuclear receptors	[33]
UNRIP			
Unr	RNA-binding protein with 5 cold-shock domains	Unr and UNRIP stimulate translation, dependent on the rhinovirus IRES	[106]

DHR, *Drosophila* Hormone Receptor; EcR, Ecdysone Receptor; Egr, early growth response; FTZ, Fuschi tarazu; HIV-1, human immunodeficiency virus type I; SF-1, steroidogenic factor-1; IRES, internal ribosome entry site; METS, mitogenic Ets transcriptional suppressor; N-CoR, nuclear receptor corepressor; PE1, PU-Ets related-1; SVP, Sevenup; USP, ultraspiracle.

nerve, and their ability to form a multiprotein RISC in response to treatment with short interfering RNAs (siRNAs) directed against neuronal β-tubulin. Gemin3, supported by Gemin4, may be responsible for RNA unwinding or RNP restructuring events during miRNA maturation and/or downstream events including, for instance, target RNA recognition. Through a combination of sedimentation and immunoprecipitation experiments on HeLa cell extracts, the Dreyfuss laboratory identified several additional stable subunits of the SMN complex including a Gemin3-Gemin4-Gemin5 complex, a Gemin5-Gemin7-UNRIP complex, and an SMN-Gemin2 complex, as well as free Gemin5 [25]. Gemin5 was recently reported to form part of two distinct complexes, a specific internal ribosome entry site (IRES)-ribonucleoprotein complex and an IRES-independent protein complex containing eIF4E [105], suggesting a role in the modulation of translation activity. Interestingly, UNRIP, which like Gemin5 is considered to be a peripheral SMN complex member, has been found to be required for IRES-dependent translation of human rhinovirus RNA [106].

The formation of SMN complex-independent complexes, in addition to the diverse protein associations reported for different SMN complex members, seems to suggest that on SMN reduction, the surplus of several SMN complex components has a negative or positive effect on a diverse number of functions that most probably contribute to the SMA phenotype. Recent SMN interactome studies [107, 108], together with previously reported protein interactions [reviewed in ref. 2], suggest that SMN may have a diverse number of cellular functions separate from those performed within the SMN complex including, for instance, transcriptional regulation, apoptosis, signalling, protein folding/trafficking and ubiquitin ligase regulation. The same holds true for other SMN complex components, with additional functions including homologous recombination, RNA silencing, transcriptional regulation and translation initiation (Table 1).

Conclusion

It is now obvious from several studies that SMN and Gemins, as well as UNRIP, can tightly associate to form a 'family' concerned with executing a common function. This is mostly apparent within the cytoplasmic compartment where the SMN complex performs UsnRNP biogenesis or additional non-canonical functions in motor neurons and muscle, the details of which remain to be defined (Fig. 5). The UsnRNP-independent functions uncovered in neuromuscular tissues favour a dual-role view of the SMN complex. However, recent work describing the close association of UsnRNP and SMN complex-rich granules with mRNP-rich aggregates [72, 73, 75] hints at a sole role that, in addition to UsnRNP generation, lends itself to processes exclusive to motor neurons and skeletal muscles. It is highly probable that such a function concerns RNP assembly and, hence, is essential for the generation of spliceosomal building blocks, as well as mRNP production for correct synapse function in motor neurons and sarcomere maintenance in skeletal muscles. Interestingly, several studies also report additional functions for the members of the SMN complex (Table 1), which are probably performed outside of the SMN complex. Furthermore, Gemin5 and UNRIP appear to be peripheral rather than core members of the complex. All this suggests that several SMN components may also act independently and thus, in some respect SMN, Gemins and UNRIP are not a 'family'.

More extensive studies are needed to define the 'politics' within the SMN complex with respect to functions performed. In the case of UsnRNP biogenesis, the exact role of SMN complex members remains unclear, with the exception of Gemin5. In addition, although we know the composition and architecture of the SMN complex, as well as a skeleton of the UsnRNP assembly mechanisms, it is still unknown how the large macromolecular SMN complex is itself assembled. The key to this question might lie in a member of the complex or an associated factor. Given the intimate association of Gemins with SMN in canonical and non-canonical functions, it is perhaps surprising that, so far, the Gemin genes have not been linked to SMA or other human motor disorders. The absence of an expressing gene duplicate or pseudogene, as is the case for the *SMN* gene, could be a possible explanation. Indeed, the SMA neuromuscular phenotypes are thought to be the result of insufficient levels of full-length SMN contributed by *SMN2* in the absence of functional *SMN1*. Lack of *SMN1* and *SMN2* genes presumably leads to embryonic lethality and such a possibility is probably also true in the presence of deleterious mutations in any of the Gemin genes. However, due to low levels of maternal contribution of the respective protein, *Drosophila smn* and *gemin3* mutants survive up to the late larval stages to develop similar motor phenotypes [28, 29, 36, 91, 99]. In addition, motor axon degeneration has been reported with either SMN- or Gemin2-knockdown in zebrafish [97]. Such findings indicate that hypomorphic or mild Gemin mutations in humans might give rise to motor deficiencies. Genetic studies of unlinked neuromuscular hereditary syndromes might hold the key to future studies concentrating on the molecular functions of Gemins.

Acknowledgments

Dedicated to the mothers and fathers of children afflicted with SMA. My heartfelt thanks go to Dr. Ji-Long Liu for his valuable comments on the manuscript, and his outstanding mentorship, generosity and infectious scientific aptitudes. I am immensely grateful to Dr. Michael Briese for the extensive discussions on SMN complex functions and SMA. I am also indebted to the anonymous reviewers whose criticisms significantly ameliorated the manuscript and I sincerely apologise to authors whose work was not cited due to space restrictions.

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