Gemin3, also known as DDX20 or DP103, is a DEAD-box RNA helicase which is involved in more than one cellular process. Though RNA unwinding has been determined in vitro, it is surprisingly not required for all of its activities in cellular metabolism. Gemin3 is an essential gene, present in Amoeba and Metazoa. The highly conserved N-terminus hosts the helicase core, formed of the helicase- and DEAD-domains, which, based on crystal structure determination, have key roles in RNA binding. The C-terminus of Gemin3 is highly divergent between species and serves as the interaction site for several accessory factors that could recruit Gemin3 to its target substrates and/or modulate its function. This review article focuses on the known roles of Gemin3, first as a core member of the survival motor neuron (SMN) complex, in small nuclear ribonucleoprotein biogenesis. Although mechanistic details are lacking, a critical function for Gemin3 in this pathway is supported by numerous in vitro and in vivo studies. Gene expression activities of Gemin3 are next underscored, mainly messenger ribonucleoprotein trafficking, gene silencing via microRNA processing, and transcriptional regulation. The involvement of Gemin3 in abnormal cell signal transduction pathways involving p53 and NF-κB is also highlighted. Finally, the clinical implications of Gemin3 deregulation are discussed including links to spinal muscular atrophy, poliomyelitis, amyotrophic lateral sclerosis, and cancer. Impressive progress made over the past two decades since the discovery of Gemin3 bodes well for further work that refines the mechanism(s) underpinning its multiple activities.

Introduction
DEAD-box RNA helicases or unwindases are distinguished by the presence of several conserved motifs within the helicase core including the Asp-Glu-Ala-Asp or DEAD (in one-letter code) motif, which gives the protein family its name. DEAD-box family members use energy from adenosine triphosphate (ATP) hydrolysis for RNA chaperoning and RNPase activity. The former entails rearrangement of inter- or intra-molecular RNA structures, whereas the latter involves the association or dissociation of RNA–protein complexes, also known as ribonucleoproteins (RNPs) [1,2]. Genetic and biochemical studies in several organisms revealed that DEAD-box proteins have roles in every aspect of the cellular RNA metabolism. Thus, they are crucial and, in many cases, essential for several physiological RNA-centric functions including transcription, spliceosome assembly, pre-mRNA splicing, ribosome biogenesis, mRNA export, protein translation, mitochondrial gene expression, and RNA turnover and quality control (reviewed in refs [1,3]). Although DEAD-box proteins have generally been ascribed to specific processes, increasing evidence suggests that several are involved in more than one cellular process and, at times, they perform roles that do not directly involve RNA (reviewed in ref. [4]). Gemin3, also referred to as DDX20 or DP103, is one such multifunctional DEAD-box RNA helicase. Its complete loss is incompatible with life in several model systems including Caenorhabditis elegans [5], Drosophila [6,7], and mouse [8], highlighting its essential role in cellular
metabolism. Here, we review the involvement of this highly conserved helicase in multiple pathways and the clinical implications of its disruption including links to disease states, namely motor neuron degenerative disease and cancer.

Structure–function relationship

Gemin3 first appears in the genome of the amoeboid Dictyostelium discoideum, a facultative multicellular organism [9], and was retained in metazoa [9] but probably not in fungi. The protein shows significant variation in amino acid (aa) length, ranging from 728 aa in the honey bee to 1028 aa in the fruit fly. At 824 aa, the length of human Gemin3 is at mid-range (Figure 1). Gemin3, like its relatives within the DEAD-box RNA helicase family, is characterized by nine conserved motifs including Q, I, Ia, Ib, II, III, IV, V, and VI. These are present within the ‘helicase’ core located in the N-terminus (Figure 2). Based on mutational, biochemical, and structural studies, different functions have been assigned to these motifs. Motifs I and II are required for ATP binding and hydrolysis. Motifs Ia, Ib, IV, and V are involved in RNA substrate binding. Motifs III and VI couple ATP binding and hydrolysis with helicase activity. They also serve as binding sites for γ phosphate or the phosphoryl group furthest from the ribose sugar of ATP. A role as a sensor of the ATP-bound state and, thereby, a regulator of ATPase activity has been proposed for motif Q [1,10]. Motifs are distributed across two Escherichia coli recombinase A (RecA)-like domains joined by a short flexible linker. They are commonly referred to as domain 1 or DEAD-domain, and domain 2 or helicase-domain (Figure 2). Although Gemin3 shows considerable sequence and structural homology within the ‘helicase’ core, the flanking C-terminus is highly divergent. As expected, it shows no unwinding activity; nonetheless, it is required for Gemin3’s enzymatic activity [11]. Sequences flanking the helicase core of DEAD-box helicases are thought to provide specificity of function through interaction with specific RNA substrates or other interacting factors. Such accessory factors (Table 1) might answer the question of how distinct functions ascribed to RNA helicases are temporally and spatially regulated within the cell. Therefore, cofactors could recruit Gemin3 to its target substrates and/or they might stimulate or inhibit its enzymatic activity in specific cellular compartments or tissues.

RNA unwinding by Gemin3 was determined in vitro making use of model RNA/RNA duplex substrates, which are considered an excellent proxy for the remodelling of more complex RNA and RNP structures that the protein is thought to perform in vivo (see below). RNA helicase activity for Gemin3 is ATP-dependent and occurs in a 5′- to 3′-direction when the substrate has a 5′-single-strand overhang [11]. Bidirectional unwinding activity, typical for several helicases, remains to be determined. Crystal structures of single domains from several human DEAD-box helicases including Gemin3 implicated direct involvement of the helicase-domain in the activation of the RNA-binding site on the DEAD-domain. When substrate-free, the RNA-binding sites of the DEAD-domain and the helicase-domain are partially blocked by motifs II and V, respectively. ATP binding is thought to prime Gemin3 for RNA substrate binding by bringing both domains together, a state that facilitates the interaction of motifs II and V, thereby unblocking the RNA-binding site. The binding of the RNA substrate further stabilizes the conformation, hence allowing ATP hydrolysis to proceed [12]. Following hydrolysis, the DEAD-domain of Gemin3 was found to selectively bind adenine diphosphate since no binding to guanosine, cytidine, or the uridine equivalent can be detected. This is the result of extensive speciﬁcity coupled ATP binding and hydrolysis with helicase activity. They also serve as binding sites for γ phosphate or the phosphoryl group furthest from the ribose sugar of ATP. A role as a sensor of the ATP-bound state and, thereby, a regulator of ATPase activity has been proposed for motif Q [1,10]. Motifs are distributed across two Escherichia coli recombinase A (RecA)-like domains joined by a short flexible linker. They are commonly referred to as domain 1 or DEAD-domain, and domain 2 or helicase-domain (Figure 2). Although Gemin3 shows considerable sequence and structural homology within the ‘helicase’ core, the flanking C-terminus is highly divergent. As expected, it shows no unwinding activity; nonetheless, it is required for Gemin3’s enzymatic activity [11]. Sequences flanking the helicase core of DEAD-box helicases are thought to provide specificity of function through interaction with specific RNA substrates or other interacting factors. Such accessory factors (Table 1) might answer the question of how distinct functions ascribed to RNA helicases are temporally and spatially regulated within the cell. Therefore, cofactors could recruit Gemin3 to its target substrates and/or they might stimulate or inhibit its enzymatic activity in specific cellular compartments or tissues.

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snRNP biogenesis

Gemin3 is known to be a core member of a large multiprotein assemblage known as the survival motor neuron (SMN) complex [14–16], which also includes the eponymous SMN protein and seven diverse proteins, namely Gemin5, Gemin6, Gemin7, Gemin8, and Unrip [17]. Comprehensive biochemical studies revealed that the SMN complex has a modular composition with the SMN/Gemin8/Gemin7 module placed at its centre, thereby allowing the
recruitment of the Gemin2/Gemin5 subunit via SMN, the Gemin6/Unrip subunit via Gemin7, and the Gemin3–Gemin4 subunit via both SMN and Gemin8 ([18] and reviewed in refs [17,19]). Additional interactions and self-associations are possible and are thought to further stabilize the complex [18,20–23]. Besides physical associations, Gemin3 was found to interact genetically with several SMN complex members in the Drosophila model system (Table 1). Hence, disruption of SMN, Gemin2, Gemin4/Gaulos, Gemin5, and Gemin8/Valette was found to enhance the phenotype of flies expressing a Gemin3 allele with marginal function [24,25]. In addition to confirming that Gemin3 functions within the SMN complex, at least in Drosophila, this finding shows that components of the SMN complex were conserved during evolution not as independent proteins but rather as a genetic network.

The most documented function of the SMN complex is the assembly of small nuclear RNPs (snRNPs), which form the building blocks of the spliceosome (Figure 3). Excluding snRNP type-specific proteins, core
elements of snRNPs are a short noncoding RNA (snRNA) and a heptameric Sm/Lsm protein ring (reviewed in ref. [26]). These are brought together in a stepwise order of events involving cooperation between the SMN complex and the protein arginine methyltransferase 5 (PRMT5) complex, which is itself composed of WD45, PRMT5, and pICln (reviewed in ref. [27]). The involvement of numerous factors is thought to ensure the faithful assembly of snRNPs in view that Sm/Lsm proteins have a low intrinsic selectively for snRNAs. Furthermore, by limiting the major part of the snRNP production cycle to the cytoplasm, cells prevent the contact of partially assembled snRNPs with their nuclear substrates. Hence, following transcription, 7-methylguanosine

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### Table 1 Accessory proteins that interact with the C-terminal domain of Gemin3

<table>
<thead>
<tr>
<th>Accessory protein</th>
<th>Key function</th>
<th>System</th>
<th>Interaction technique</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMN</td>
<td>RNP assembly function</td>
<td>Human, Drosophila</td>
<td>GST-pulldown, Y2H, co-IP, genetic interaction</td>
<td>[6,7,14,18,24,44]</td>
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<td>Gemin2</td>
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<td>Human, Drosophila</td>
<td>co-IP, genetic interaction</td>
<td>[18,24]</td>
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<tr>
<td>Gemin4/ Gaulios</td>
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<td>Human, Drosophila</td>
<td>Y2H, co-IP, genetic interaction</td>
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<td>Gemin8/Valette</td>
<td>SMN complex member</td>
<td>Drosophila</td>
<td>Genetic interaction</td>
<td>[25]</td>
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<tr>
<td>Gemin7/ Sabbat</td>
<td>SMN complex member</td>
<td>Drosophila</td>
<td>Y2H</td>
<td>[25]</td>
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<tr>
<td>Sm B</td>
<td>snRNP component</td>
<td>Human</td>
<td>GST-pulldown</td>
<td>[14]</td>
</tr>
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<td>snRNP component</td>
<td>Human</td>
<td>GST-pulldown</td>
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</tr>
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<td>pICln</td>
<td>RNP assembly function</td>
<td>Drosophila</td>
<td>Y2H, genetic interaction</td>
<td>[51]</td>
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<tr>
<td>Tgs1</td>
<td>snRNP cap hypermethylatation enzyme</td>
<td>Drosophila</td>
<td>Y2H, genetic interaction</td>
<td>[51]</td>
</tr>
<tr>
<td>EBNA2</td>
<td>Epstein–Barr virus-encoded nuclear antigens</td>
<td>Human</td>
<td>Y2H, co-IP</td>
<td>[73,98]</td>
</tr>
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<td>EBNA3C</td>
<td>Epstein–Barr virus-encoded nuclear antigens</td>
<td>Human</td>
<td>Y2H, co-IP, GST-pulldown</td>
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<td>p53</td>
<td>Tumour suppressor</td>
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<td>GST-pulldown</td>
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<tr>
<td>TAK1</td>
<td>Regulator of NF-kB signalling</td>
<td>Human</td>
<td>co-IP</td>
<td>[100]</td>
</tr>
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<td>AGO1</td>
<td>RNA silencing</td>
<td>Human</td>
<td>co-IP</td>
<td>[66]</td>
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<tr>
<td>AGO2/elf2C2</td>
<td>RNA silencing</td>
<td>Human</td>
<td>co-IP, GST-pulldown</td>
<td>[63,66,67,70]</td>
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<td>M2H, His-pulldown</td>
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<td>Y2H, GST-pulldown</td>
<td>[77]</td>
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<td>Y2H, GST-pulldown</td>
<td>[77]</td>
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<tr>
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<td>Histone deacetylase</td>
<td>Human</td>
<td>Y2H, GST-pulldown, co-IP</td>
<td>[77]</td>
</tr>
<tr>
<td>HDAC-5</td>
<td>Histone deacetylase</td>
<td>Human</td>
<td>co-IP</td>
<td>[77]</td>
</tr>
<tr>
<td>FOXL2</td>
<td>Transcription factor</td>
<td>Human</td>
<td>Y2H</td>
<td>[76]</td>
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</tbody>
</table>

Abbreviations: AGO: argonaute; FOXL2: forkhead box L2; HDAC: histone deacetylase; Hsp: heat-shock protein; METS: mitogenic Ets transcriptional suppressor; N-CoR: nuclear receptor corepressor; PE1: PU-Ets related-1; SF-1: steroidogenic factor-1; SMN: survival motor neuron; TAK1: TGF-β-activated kinase-1; Tgs1: trimethylguanosine synthase 1.
(m$^{7}$G)-capped nascent snRNAs are exported to the cytoplasm where they are captured and channelled to the SMN complex by Gemin5 [28,29], though the U1-70K protein can act as a substitute factor in a U1-exclusive snRNP assembly pathway [30]. Sm proteins are pre-arranged into the spatial position they occupy in the assembled snRNP by pICln [31,32], which is also required for the release of newly translated Sm proteins from the ribosome [33]. Gemin2 then captures and holds the Sm D1/D2/F/E/G pentamer in a configuration that blocks RNA-binding capacity until delivery of snRNAs [32,34]. Sm ring closure might involve Unrip and the Gemin6/Gemin7 dimer with the latter acting as a temporary substitute for the Sm B/D3 dimer [20,35]. Finally, the SMN complex recruits trimethylguanosine synthase 1 (Tgs1), an enzyme that hypermethylates the cap of assembled snRNPs [36,37]. The resulting 2,2,7-trimethylguanosine (TMG) cap and the Sm ring act as a localization signal for nuclear import. In the nucleus, prior to their involvement in pre-mRNA splicing, snRNPs are transferred to Cajal bodies (CBs) where they undergo maturation including the association of snRNP type-specific proteins, nucleotide modification, and formation of di- or tri-snRNP complexes (reviewed in ref. [38]).

Despite recent findings ([39] and see below), several lines of in vivo evidence support a role for Gemin3 in snRNP assembly. First, snRNP assembly is disrupted on RNAi (RNA interference)-mediated knockdown of Gemin3, a result confirmed by two independent studies [40,41]. Second, poliovirus-infected cells have reduced Sm ring assembly activity as a result of intracellular Gemin3 proteolysis by poliovirus-encoded proteinase 2Apro [41]. Third, within the cytoplasmic compartment, SMN complex components, including Gemin3, localize to discrete universal organelles termed U bodies because they are enriched in snRNPs, which are themselves Uridine-rich [42]. U bodies are probably the sites where snRNP assembly takes place in vivo and their invariable association with P bodies, which are involved in RNA surveillance and decay, suggests that the two structures co-operate in regulating aspects of RNA metabolism [43]. Fourth, loss of Gemin3 function in either muscle or neurons phenocopies SMN disruption in Drosophila, hence, resulting in organisms with impaired motor abilities [6,7,44] (Table 2). In agreement, like SMN loss [45,46], perturbation of Gemin3 in C. elegans has a negative impact on several parameters attesting to neuromuscular function [47]. Also, similar phenotypes...
were uncovered in egg chambers mutated for either SMN [48] or Gemin3 [49]. SMN is the causative factor of spinal muscular atrophy (SMA), a disorder characterized by muscle weakness and selective degeneration of lower motor neurons [50]. Intriguingly, poliovirus-induced poliomyelitis has overlapping clinical features. A
plethora of in vivo studies point towards perturbation in snRNP assembly downstream to SMN deficiency and the consequential transcriptome abnormalities as the primary drivers of the progressive neuromuscular degeneration underpinning SMA (reviewed in ref. [19]). Fifth, Gemin3 associates both genetically and physically with snRNP biogenesis factors pICln and Tgs1. Moreover, disruption of either pICln or Tgs1 was found to induce phenotypes that mirror those resulting from reduced Gemin3 activity, further strengthening the evidence favouring a role for Gemin3 in the snRNP assembly pathway [51].

Surprisingly, in vitro studies using purified reconstituted systems have recently shown that Gemin3 is dispensable for the assembly and proofreading of snRNAs [39]. This finding may, however, not be contradictory to the in vivo data, rather it points to a different function of Gemin3 in the snRNP biogenesis pathway. Such function remains elusive since structure-based studies making use of snRNA or snRNP as a substrate are lacking. It is possible that Gemin3 is more required in the nuclear compartment than in the cytoplasm. In the nucleus, the SMN complex, including Gemin3, is highly concentrated in gems which are frequently associated with CBs [14,52,53]. Hence, it is plausible that Gemin3 might be key for displacing the SMN complex from the assembled newly imported snRNP. Alternatively or additionally, the SMN complex, particularly Gemin3, is recruited for remodelling activities during the ongoing recycling of snRNPs once these are ready from their activities in pre-mRNA splicing. Supporting this hypothesis, we note that in adult tissues, a Gemin3 mutant lacking the helicase core or the full-length functional version of Gemin3 mostly localizes to the nucleus in Drosophila [44,53]. Interestingly, numerous studies have revealed that depletion of gems is a signature feature not only of SMA but also of amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease (reviewed in ref. [54]). In this context, future studies on the nuclear snRNP-centric activities of Gemin3 within the SMN complex are warranted.

**Gene expression**

Whether disruption of snRNP biogenesis and the consequential splicing defects give rise to motor dysfunction in the form of either SMA, poliomyelitis or even ALS remains debatable. Should this be the case, it is not fully clear why the neuromuscular system is particularly vulnerable. Increasing evidence is suggesting that the SMN complex has a more universal role in RNP complex assembly that goes beyond its well-documented function in snRNP assembly (reviewed in refs [55,56]). Predominantly supported by the localization of its constituent members including Gemin3 in transport granules within neuronal processes [57–60], the SMN complex has been implicated in the assembly and transport of messenger ribonucleoprotein (mRNP) complexes (Figure 3). In this context, an alternative hypothesis proposing that disruption of this function is responsible for the selective neurodegenerative phenotype observed in SMA patients has gained traction in recent years [61]. SMN promotes the interaction between mRNA-binding proteins and their transcripts, a key step in the assembly of mRNP complexes. Thus, SMN deficiency was found to impair mRNP assembly, leading to decreased association of mRNPs with the cytoskeleton and, therefore, reduced trafficking [62]. The role of other SMN complex components including Gemin3 in this process still needs to be determined. Nonetheless, Gemin3 is well known to have more direct roles in gene expression in view of its implication in transcription and post-transcriptional gene silencing.

The majority of Gemin3 and its associated protein, Gemin4, are found in the SMN complex. A less abundant Gemin3/Gemin4 complex, separate from the SMN complex, that co-sediments with polyribosomes and contains the eukaryotic initiation factor 2C2 or Argonaute (AGO) 2 and numerous microRNAs (miRNAs) was isolated from HeLa and neuronal cells [63–65]. The association of Gemin3/Gemin4 with AGO complexes was confirmed in several subsequent reports [66–68]. miRNAs are processed from long precursors by special enzymes called Drosha and Dicer, with subsequent initiation of the RNA-induced silencing complex (RISC), which is considered to be the effector mechanism of the RNAi-mediated gene silencing pathway (Figure 3). The strand of miRNA retained within the RISC serves as a template to identify the complementary nucleotide sequence of intracellular miRNAs, which are then translationally repressed. Although the core component of every RISC is a member of the AGO protein family, RISC-associated proteins include fragile X mental retardation protein (FMRP) and p100, the mammalian homologue of the Drosophila Tudor staphylococcal nuclease (reviewed in ref. [69]). Murashov et al. [70] implicated Gemin3 as an RISC component in vivo and speculated that Gemin3 may be the elusive RNA helicase of the RISC responsible for RNA unwinding or RNP restructuring events during miRNA maturation and/or downstream events such as target RNA recognition. In support of this view, the authors demonstrated the presence of AGO2, FMRP, p100, and Gemin3 proteins in the murine peripheral axons of the sciatic nerve, and their ability to form a multiprotein RISC in response to treatment
with siRNAs directed against neuronal β-tubulin, which is an integral component of the axonal cytoskeleton. The resulting RISC was functional since a concomitant decrease in the levels of β-tubulin mRNA and its corresponding protein product, as well as a significant impairment of axonal transport, were observed [70]. It is noteworthy that perturbation of the miRNA pathway in spinal motor neurons results in mice that exhibit hallmarks of SMA [71]. Interestingly, a recent report showed that increased levels of the Gemin3 ortholog mel-46 in C. elegans restored perturbed miRNA miR-2 function in SMN-deficient animals with a consequential amelioration of neuromuscular defects [47]. In C. elegans, miR-2 suppresses expression of the M2 muscarinic receptor m2R; hence, reduced SMN activity is associated with increased production of m2R. Interestingly, m2R inhibition in C. elegans or mouse SMA models was found to suppress neuromuscular defects [47]. This study implicates a central role for Gemin3 in SMA pathogenesis by suggesting that decreased SMN leads to defective microRNA function via Gemin3 misregulation. Importantly, Gemin3 or its substrate can potentially serve as a therapeutic target for SMA, thus complementing current therapies available to patients [72].

Gemin3 was originally isolated as a cellular factor that associates with the Epstein–Barr virus (EBV) nuclear proteins EBNA2 and EBNA3C, which play a role in the transcriptional regulation of both latent viral and cellular genes [73]. The role of Gemin3 as a transcriptional regulator was substantiated by reports that describe the ability of its non-conserved C-terminal domain to interact with and modulate a variety of cellular transcription factors (Figure 3). Hence, Gemin3 was found to repress steroidogenic factor 1 (SF-1), an orphan nuclear receptor that regulates expression of genes essential for reproductive and endocrine development [11,74], and early growth response protein 2 (Egr2/Krox-20), which is essential for myelination of the peripheral nervous system and for establishing segmentation in the developing vertebrate hindbrain [75]. In both cases, repression was not observed for all SF-1 and Egr2-responsive promoters, suggesting that this repression activity depended on the promoter context. Gemin3 has also been shown to interact with the forkhead transcription factor FOXL2 and to regulate its apoptosis-inducing ability [76]. Furthermore, Gemin3 was identified as a corepressor of mitogenic Ets repressor METS (mitogenic Ets transcriptional suppressor), which is induced during macrophage differentiation and represses transcription of Ets target genes involved in Ras-dependent cell proliferation. Since Gemin3 co-immunoprecipitates with histone deacetylases (HDACs) 2 and 5, transcriptional repression is thought to be partly mediated through the recruitment of HDACs [77]. Repression of SF-1 transcriptional activity by Gemin3 is, however, achieved through promotion of SF-1 sumoylation via the PIAS E3 SUMO ligases, which, in turn, result in subnuclear relocalization of SF-1 to discrete nuclear foci [78]. Intriguingly, the ATPase/RNA helicase activity of Gemin3 does not appear to be required for its function in transcription, thereby strongly suggesting that its role in transcription is distinct from its role in other processes where it may act as a bona fide helicase.

Carcinogenesis

Deregulation of numerous DEAD-box helicases are known to have deleterious effects on normal cellular homeostasis leading to uncontrolled proliferation, inappropriate survival of damaged cells, and invasion, which are events that lead to cancer development and progression (reviewed in ref. [79]). Focusing on the role of Gemin3 in carcinogenesis, a flurry of studies have probed for an association between Gemin3 polymorphisms and cancer susceptibility [80–92] or survival in cancer patients [93–96]. Results were contradictory, and a recent meta-analysis of eight studies found no statistically significant relationship between the Gemin3 rs197412 single-nucleotide polymorphism and increased cancer risk [97]. Nonetheless, Gemin3 has been implicated in abnormal cell signal transduction pathway activity, which is a key factor in cancer pathogenesis. One such report suggests that Gemin3 can be targeted by oncogenic viruses to drive cell proliferation and anti-apoptotic activities [98]. Hence, the interaction of EBNA3C with Gemin3 was reported to increase the stability of Gemin3 and its accumulation in both B lymphoma cells and EBV-transformed lymphoblastoid cell lines. Importantly, EBNA3C promotes the association of Gemin3 and tumour suppressor p53, specifically binding the DNA-binding domain of p53. This blocks p53-mediated transcriptional activity and apoptosis. In line with this finding, RNAi-based knockdown of Gemin3 was found to reverse the ability of EBNA3C to repress p53 transcriptional activity on its downstream genes in addition to increasing apoptosis of EBV-infected lymphoma cell lines [98]. In view that EBNA3C can also associate with SMN [99], it is plausible that the target of EBV is the SMN complex.

Gemin3 levels were found to be up-regulated in metastatic human breast cancers and they correlate with patient survival. Notably, depletion of Gemin3 in an invasive breast cancer cell line impeded cell migration and invasion. Conversely, forced overexpression of Gemin3 increased the invasive potential of a normal human
breast epithelial cell line. Additionally, mice inoculated with Gemin3-transfected breast cancer cells had a higher incidence of lung and liver metastases compared with controls [100]. Considering the Cancer Genome Atlas and cBioPortal data sets, multiple breast cancer subtypes were found to have genetic aberrations in the Gemin3 gene [79]. These findings establish that Gemin3 is not only a prognostic marker for breast cancer tumorigenesis and progression, but also a new predictive biomarker for cancer metastasis [101]. Interestingly, Gemin3 levels are also up-regulated in tissue samples derived from prostate cancer patients [102] and colorectal cancer patients who developed distant metastasis [100], a finding that strengthens the role of Gemin3 in metastasis. Elegant mechanistic studies showed that Gemin3 is a critical cofactor of TGF-β-activated kinase-1 (TAK1)-mediated activation of the IκB kinase 2 (IKK2), which, in turn, is key for activating the oncogenic transcription factor NF-κB (Figure 3). Heightened expression of active NF-κB (phospho-p65) and matrix metalloproteinase 9 (MMP9), an NF-κB target, mirrors the expression of Gemin3, thereby establishing a role for the Gemin3–NF-κB–MMP9 axis in breast cancer metastasis. Matrix metalloproteinases degrade the extracellular matrix, an essential step in metastasis. Gemin3 was recently found to enhance proliferation and metastatic potential of prostate cancer via a similar mechanism [102]. Importantly, these findings explain why, despite lack of overexpression of the TAK1 and IKK kinases, hyperactivation of NF-κB is apparent in the vast majority of cancers. Since levels of Gemin3 are transcriptionally controlled by NF-κB itself in breast cancer cells, this feedback loop maintains constitutive activation of NF-κB, which is linked to cancer progression and the acquisition of chemotherapy resistance. It is noteworthy that the helicase activity of Gemin3 is not required for its role in driving metastasis in breast cancer cells. Reduction of NF-κB signalling by down-regulating Gemin3 sensitized breast cancer cells to chemotherapy-induced cell death [100]. While this result seems to open the door for Gemin3 as a therapeutic drug target in breast cancer, caution is urged in view of Gemin3’s pleiotropic effects. In this regard, the role of Gemin3 in carcinogenesis differs between different cancer types. Hence, in contrast with its role as a tumour promoter in breast, prostate, and colorectal cancer, Gemin3 acts as a tumour suppressor in hepatocellular carcinoma. In this regard, RNAi targeting Gemin3 accelerated liver tumour growth [103]. Additionally, reduced levels of Gemin3 are frequently observed in human hepatocellular carcinoma. Gemin3 was found to reduce NF-κB activity by regulating the function of the NF-κB-suppressing miRNA-140 in order to inhibit liver cancer progression [104,105].

**Conclusion and prospects**

The impact of Gemin3 deregulation can be gleaned from phenotypes observed in several animal models (Table 2). Information gained from human ex-vivo tissues was also useful. Nonetheless, we predict that the revolutions in human genomic sequencing technologies will be of value to identify direct contribution of Gemin3 to the pathogenesis of disorders with an as-yet-unidentified cause. Furthermore, the emergence of more sophisticated ways to generate animal models including the application of gene-editing tools like the CRISPR/Cas9 system will allow investigators to better manipulate the Gemin3 gene aiming at a better understanding of the role of its domains in vivo. To this end, it would be interesting to answer whether the divergent C-terminus is responsible for species-specific functions attributed to Gemin3. High-resolution structures of Gemin3 bound to its substrates are lacking and can potentially inform mechanistic aspects. Considering the findings uncovered to date, its involvement in several disease states including neuromuscular degeneration and cancer makes Gemin3, a potential biomarker for diagnosis, prognosis, and/or treatment response. Additionally, Gemin3 is an attractive target for the development of therapeutics that can potentially ameliorate phenotypes associated with these conditions. Our understanding of the precise mechanisms through which Gemin3 functions in multiple pathways including snRNP biogenesis, gene expression, and tumorigenesis remains incomplete. However, impressive progress has been made over the past two decades since the discovery of Gemin3. This is reason to be optimistic about what the future holds for studies that refine our understanding of the role of this key DEAD-box RNA helicase in cellular metabolism.

**Abbreviations**

aa, amino acid; AGO, Argonaute; ALS, amyotrophic lateral sclerosis; ATP, adenosine triphosphate; CB, Cajal bodies; EBV, Epstein–Barr virus; Egr2, early growth response protein 2; FMRP, fragile X mental retardation protein; HDAC, histone deacetylase; IKK2, IκB kinase 2; miRNA, microRNA; MMP, matrix metalloproteinase; mRNP, messenger ribonucleoprotein; PRMT5, protein arginine methyltransferase 5; RecA, recombinase A; RISC, RNA-induced silencing complex; RNAi, RNA interference; RNP, ribonucleoprotein; SF-1, steroidogenic factor;
SMA, spinal muscular atrophy; SMN, survival motor neuron; snRNA, small nuclear ribonucleic acid; snRNP, small nuclear ribonucleoprotein; TAK1, TGF-β-activated kinase-1; Tgs1, trimethylguanosine synthase 1.

Author Contribution
R.J.C. conceived the review focus. F.C. and R.J.C. conducted the literature review, wrote and edited the manuscript.

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Competing Interests
The Authors declare that there are no competing interests associated with the manuscript.

References

14. Cauchi, R.J. (2010) SMN and Gemins: ‘we are family’ … or are we? Insights into the partnership between Gemins and the spinal muscular atrophy disease protein SMN. J. Mol. Biol. 90, 1013–1021 https://doi.org/10.1074/jbc.M908528200


