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Understanding the biochemical activities of galectin-1 and galectin-3 in the nucleus

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Nuclear extracts (NE), capable of carrying out splicing of pre-mRNA, contain galectin-1 and galectin-3. NE depleted of galectins-1 and -3 concomitantly lose their splicing activity. The activity of the galectin-depleted extract can be reconstituted by the addition of either galectin-1 or galectin-3. These results suggest that galectins-1 and -3 serve as redundant splicing factors. Consistent with this notion, immunofluorescence staining showed that both galectins yielded a diffuse nucleoplasmic distribution, matching that of nascent transcripts and consistent with the hypothesis that bulk transcription and pre-mRNA processing occur throughout the nucleoplasm. Under some conditions, the galectins could be found in speckled structures and nuclear bodies but the prevailing thought is that these represent sites of storage and recycling rather than sites of action. Galectin-1 and galectin-3 bind directly to Gemin4, a component of the SMN core complex, which plays multiple roles in ribonucleoprotein assembly, including the biogenesis, delivery, and recycling of snRNPs to the spliceosome. Thus, galectin-1 and galectin-3 constitute a part of an interacting dynamic network of many factors involved in the splicing and transport of mRNA.
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Keywords: **lectins; pre-mRNA splicing; spliceosomes; ribonucleoprotein complexes; gems and nuclear bodies**

Abbreviations: **RNP, ribonucleoprotein complex; hnRNP, heterogeneous nuclear RNP; snRNP, small nuclear RNP; SR splicing factors, Ser-, Arg-rich family of splicing factors; NE, nuclear extract; SMN, survival of motor neuron protein.**

Introduction

Galectins were initially isolated as galactose-specific carbohydrate-binding proteins, which provided the basis for coining the nomenclature for this family of proteins [1]. In addition to binding glycoconjugates, many members of the galectin family share another key property: they exhibit dual localization, being found in both the intracellular (cytoplasm and nucleus) as well as the extracellular (cell surface and medium) compartments [2]. From a historical perspective, extracellular lectin-carbohydrate mediated functions predominated the literature on the galectins [3].

On the other hand, many members of the galectin family are predominantly intracellular proteins. For example, galectin-1 was shown to be synthesized on free ribosomes [4] and both galectin-1 and galectin-3, purified from tissue extracts, were acetylated at the NH_2-terminus [5,6]. These findings are more typical for cytosolic proteins than secreted proteins. In addition, observations of nuclear localization have been reported for eight of the 14 known galectins at this writing: (a) galectin-1 [7–9]; (b) galectin-3 [10–14]; (c) galectin-7 [15,16]; (d) galectin-10 [17]; (e) galectin-11 [18]; (f) galectin-12 [19,20]; (g) galectin-13 [21]; and (h) galectin-14 [22]. In some cases, the proteins are found in discrete nuclear domains (see below).

What do these observations reveal about the physiological activities of the galectins? Do the localization data offer insight on how to approach the challenge of learning more about their function? In the context of the present special issue of the Glycoconjugate Journal dedicated to the galectins, it is the purpose of this article to review the information on the intracellular localization of galectin-1 and galectin-3, the most extensively studied of the galectins. It seems timely to take a fresh look at what hints are offered by these data in terms of analyzing the activities of the two proteins.

Galectin-3 in the nucleus

Depending on the culture conditions, staining protocol, and antibody reactivity, immunofluorescence studies have yielded

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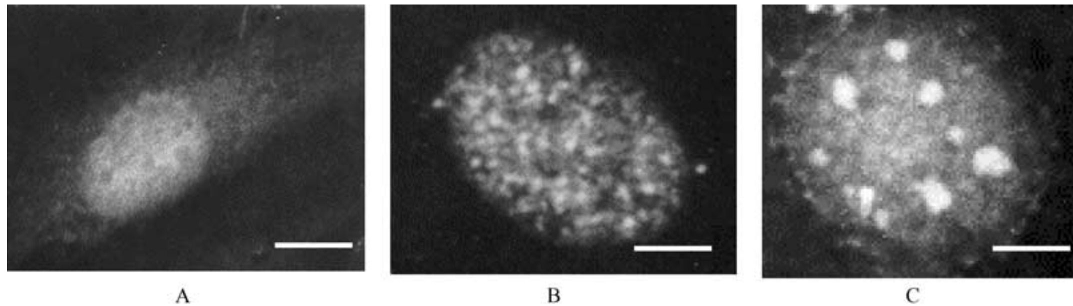

Figure 1. Panel A: Unsynchronized cultures of 3T3 cells were fixed with paraformaladehyde (4%) and permeabilized with Triton X-100 (0.2%) and then stained with rabbit anti-galectin-3 followed by fluorescein-conjugated goat anti-rabbit immunoglobulin. Bar, 20 μm. Panel B: 3T3 cells were permeabilized (0.5% Triton X-100), extracted with 0.25 M ammonium sulfate, and then fixed. The residue was incubated with rabbit anti-galectin-3 followed by fluorescein-conjugated goat anti-rabbit immunoglobulin. Bar, 15 μm. Panel C: 3T3 cells were synchronized by serum starvation (0.2% serum, 48 h) and stimulation (re-addition of serum to 10%). Sixteen hours post serum reactivation, the cells were fixed with paraformaldehyde (4%) and permeabilized with Triton X-100 (0.2%). The cells were stained with rabbit anti-galectin-3 followed by fluorescein-conjugated goat anti-rabbit immunoglobulin. Bar, 12.5 μm.

several, apparently disparate, staining patterns for galectin-3. Of these, three staining patterns are particularly prominent and instructive in terms of providing hints regarding the protein's activity: (a) diffuse nucleoplasmic; (b) nuclear speckles; and (c) discrete nuclear bodies over a nucleoplasmic background. We will illustrate and discuss these three staining patterns using the mouse 3T3 fibroblast (Figure 1).

Mouse 3T3 cells, fixed with paraformaldehyde and permeabilized with Triton X-100, yielded both nuclear and cytoplasmic staining with rabbit anti-galectin-3. In general, the nuclear staining appeared to be diffuse, covering the entire nucleus with the exception of about five circles, devoid of fluorescence, which correspond to nucleoli (Figure 1A) [11]. A similar diffuse nucleoplasmic and cytoplasmic staining is also obtained with human HeLa cells [7]. This staining pattern is obtained most generally with unsynchronized cultures of 3T3 cells. The nuclear *versus* cytoplasmic distribution of the protein was dependent on the proliferation state of the cells under analysis. In quiescent cultures of 3T3 fibroblasts (*e.g.* serum-deprived or density-inhibited), galectin-3 was predominantly cytoplasmic; proliferating cultures of the same cells, on the other hand, showed intense nuclear staining [23]. These conclusions were substantiated by quantitative immunoblotting of subcellular fractions derived from quiescent and proliferating cultures.

Figure 1B shows the labeling pattern obtained when 3T3 cells were permeabilized with Triton X-100 without prior fixation, subjected to extraction with 0.25 M ammonium sulfate, followed by fixation and staining. This procedure extracts the majority of the non-histone nuclear proteins, leaving chromatin, nuclear matrix, and associated RNAs [24,25]. A distinct speckled fluorescence pattern was observed in 3T3 cells [11,26], as well as in HeLa cells [7,27]. Such speckles have been observed for a number of nuclear antigens, including the Sm polypeptides of the small nuclear ribonucleoprotein particles (snRNPs) that play crucial roles in splicing of pre-mRNA and the Ser-, Arg-rich (SR) family of splicing factors [28]. Indeed, double immunofluorescence experiments have shown that galectin-3 can be colocalized in speckled structures revealed by antibodies against *bona fide* splicing factors, including snRNP proteins (anti-Sm) and SC35, representing the SR family of splicing factors [7,27]. The association of the speckled structures with ribonucleoprotein components of the nuclear matrix is also consistent with the observation that treatment of unfixed and permeabilized cells with ribonuclease removed the nuclear staining of galectin-3 while parallel treatment with deoxyribonuclease failed to yield the same effect [11,26].

Cultures of 3T3 fibroblasts were made quiescent by serum starvation (0.2% serum, 48 h). The quiescent cells were stimulated to re-enter the cell cycle in a synchronous fashion by the re-addition of serum (10%). DNA synthesis, as monitored by the incorporation of [^{3}H]thymidine, began 16–20 h after serum stimulation. At this time, immunofluorescence staining for galectin-3 showed the protein to be prominent in 5–6 dot structures, over a general nucleoplasmic background (Figure 1C). The diameters of these nuclear dots ranged from 0.5–5 μm. In terms of number and size, these structures resembled two subnuclear domains reported by other investigators: (a) coiled (Cajal) bodies, whose signature protein is p80 coilin [29]; and (b) gems, whose signature protein is the Survival of Motor Neuron (SMN) protein [30]. At present, we have no direct evidence, such as double immunofluorescence labeling with antibodies against galectin-3 and p80 coilin or SMN, to indicate that galectin-3 is found in coiled bodies or gems. We will refer to the observed nuclear dots as galectin-containing nuclear bodies.

Electron microscopy analyses yielded immunogold labeling of galectin-3 in both the nucleus and cytoplasm of cells [11,12]. In the nucleus, galectin-3 could be found in the dense fibrillar component of the nucleolus and at the periphery of fibrillar centers [11]. This is in direct contrast to the immunofluorescence results, which had suggested that the nucleoli were devoid of galectin-3 (*e.g.*, see Figure 1A). In addition to the nucleolus, the ultrastructural data showed galectin-3 localization in the nucleoplasm: in interchromatic spaces and at the borders

of condensed chromatin [11]. The latter region contains the perichromatin fibrils, which are labeled with short pulses of [^{3}H]uridine [31] and anti-RNA polymerase II antibodies [32]. Thus, this region is thought to be the site of both mRNA synthesis [33] and early events in pre-mRNA splicing [34], a notion consistent with the finding that hnRNPs and snRNPs containing the Sm antigens have been localized in perichromatin fibrils [35,36]. Finally, this localization of galectin-3 to the interchromatic spaces and perichromatin fibrils of the nucleoplasm is also similar to the reported distribution of the SR splicing factor SC35 [28].

Lessons learned from the galectin-3 localization

The correspondence in localization between galectin-3 and snRNPs and SR family of splicing factors raised the possibility that the lectin might be associated with some of these components in functional activity, as well as in subnuclear structures. The critical question is how to determine the active *versus* inactive pool of splicing factors. The "bed linen" analogy of Neugebauer seems instructive (see reference [37]). Bed linens are found in hotel rooms where they "function," in laundry rooms where they undergo "recycling," and in storage closets, where they are most probably the highest in concentration. By simply looking at the steady-state distribution, it is difficult to determine which is the functional pool. Thus, the most striking staining pattern, galectin-3 associated nuclear bodies, may reflect the sites of greatest concentration but these may not be the sites of action.

Recent studies have shown that bulk transcription and splicing occur throughout the nucleoplasm during periods of active transcription [38,39]. On the other hand, the speckled structures observed under immunofluorescence have been found to correspond, at the ultrastructural level, to interchromatin granule clusters which are not labeled with short pulses of [^{3}H]uridine [31,32,35]. On this basis, it has been hypothesized that factors involved in transcription and pre-mRNA processing coalesce to the speckles for recycling or reactivation. On the other hand, the dispersed nucleoplasmic staining of galectin-3, snRNPs, and SR splicing factors matches the distribution of nascent transcripts [40,41]. Thus, the least striking galectin-3 staining pattern, diffuse nucleoplasmic as seen Figure 1A, may represent the most important distribution in terms of function.

Although most of the detailed analyses discussed above were carried out, at least initially, with galectin-3, similar considerations apply to galectin-1.

Biochemical assays on the activities of galectins-1 and -3 in the nucleus

The association of galectin-3 with ribonucleoprotein components of the nuclear matrix, as revealed by immunofluorescence staining of nuclear residues after extraction with ammonium sulfate and by the ribonuclease sensitivity of such staining, is further supported by biochemical fractionation studies. When nucleoplasm was subjected to sedimentation on a cesium sulfate density gradient, galectin-3 was found not as a free polypeptide, but at a density (ranging from 1.3 to 1.35 g/ml) consistent with that of hnRNPs and snRNPs [26], major factors required for the processing of pre-mRNA. On this basis, two assays were used to test for a functional role of galectins in splicing.

The first functional assay reports on the splicing activity using denaturing gel electrophoresis to analyze the RNA species. Thus, nuclear extract (NE) and a ^{32}P-labeled pre-mRNA substrate are incubated at 30°C for 30–45 min. The RNA is then extracted and resolved on a 10% acrylamide—8.3 M urea denaturing gel system. The starting pre-mRNA substrate, the mature mRNA product, as well as the intermediates of the splicing reaction (free exon 1, lariat-exon 2, and the excised lariat) can be discriminated by this gel system [42]. The second assay, which reports on the assembly of the spliceosome, essentially corresponds to a gel mobility shift assay. It detects complexes formed by the splicing factors, which retard the mobility of the radiolabeled pre-mRNA on non-denaturing or native polyacrylamide gels [43]. Initially, the pre-mRNA is complexed with the hnRNP proteins to form the H-complex. Addition of U1 snRNP and the non-snRNP splicing factor U2AF results in the formation of the E complex, a commitment step in spliceosome assembly [44]. This is followed by the orderly addition of other uracil-rich snRNPs in an ATP-dependent fashion, leading to the higher order complexes, A-, B-, and C-complexes (the active spliceosome). In our assays, the native gel system cannot distinguish the H-complex from the E-complex so they are lumped together. The H- and E-complexes are well resolved, however, from the higher order A- and B- complexes.

The criteria of depletion and reconstitution were used to test for a role of galectin-1 and galectin-3 in splicing activity and in spliceosome assembly. In addition, perturbation experiments were also carried out to test for an effect of addition of saccharides and polypeptide fragments on the activities of the complete, endogenous NE. The key findings of these studies include [27,45,46]:

- NE derived from HeLa cells, capable of carrying out the *in vitro* splicing reaction, contain galectins-1 and -3;
- Addition of saccharides that bind galectins with high affinity inhibits the pre-mRNA splicing activity in NE;
- Removal of galectins from NE, either by lactose affinity chromatography or by antibody-mediated depletion, abolishes the splicing activity;
- Addition of either galectin-1 or galectin-3 to a galectin-depleted NE restores splicing activity;
- NE devoid of galectins do not assemble active spliceosomes, but only form H-/E-complexes upon addition of the pre-mRNA substrate;
- Addition of the NH_2-terminal domain of galectin-3 to a complete NE inhibits splicing and arrests spliceosome assembly at the H-/E-complex whereas full-length galectin-3 or the COOH-terminal domain does not have the same effect.

On the basis of these results, we conclude that galectins-1 and -3 are redundant splicing factors [47]. In assigning any specific function to galectin-1 or to galectin-3, it is important to acknowledge that mutant mice with the galectin-1, galectin-3, or both galectin genes disrupted have been generated [48]. All three strains of mutant mice were viable and fertile, thus implying that there is no absolute requirement for these proteins in the fundamental activities assigned to them.

Several possibilities might be considered to rationalize the apparently disparate results of the *in vitro* splicing assays (galectins required) *versus* the phenotype of the knock-out mice (no embryonic lethality or gross developmental defects). Clearly, the cell-free studies have limitations: (a) the nuclear extract (as everybody in the field prepares it) could be deficient in another factor, which makes galectins-1/-3 stringently required; or (b) the protocol we use to deplete galectins may take out other factors, making galectins-1/-3 necessary. Alternatively, the galectin-1/-3 null mice could involve compensatory measures arising from redundant mechanisms. These considerations provide a strong impetus to continue characterization of the *in vitro* system, with the possibility of finding alternative, compensatory mechanisms. In fact, the galectin-1 and galectin-3 null mice [48] could serve as an useful resource for the comparative analysis of the composition and the assembly of spliceosomes. For example, is a splicing extract generated from cells derived from the galectin-null mice sensitive to "galectin depletion"? The potential role of the other members of the galectin family in pre-mRNA splicing may also be an important area of investigation.

In contrast to mammalian systems, a galectin gene does appear to be required for viability in the nematode *Caenorhabditis elegans*. Two galectins have been isolated and well characterized in *C. elegans* [49]: (a) a prototype galectin (∼16 kDa); and (b) a tandem-repeat type galectin (∼32 kDa). Although the genome project of this organism has revealed a number of other possible members of the galectin family, the use of RNA-mediated interference (RNAi) technology to inhibit a galectin gene resulted in embryonic cell death [50]. Thus, *C. elegans* may have a more limited number of galectin family members, making the requirment for any given member easier to demonstrate than in the mouse system.

Nuclear partners for galectin-1 and galectin-3

To identify interacting partners for the galectins, a yeast two-hybrid genetic screen was carried out, using galectin-1 as the "bait." Of the ten positive colonies isolated in this screen, one was of particular interest: it was identitical to the reported sequence encoding the COOH-terminal 50 amino acids of Gemin4 (this clone was thus designated as Gemin4(C50) [46]). Gemin4 is a member of nuclear (and cytoplasmic) complexes containing the SMN protein, the gene product responsible for the pathogenesis of spinal muscular atrophy [51].

The interaction of galectin-1 with Gemin4(C50), revealed by the yeast two-hybrid assay, was confirmed in direct binding assays. The cDNA for Gemin4(C50) was expressed as a fusion protein with glutathione S-transferase (GST). GST-Gemin4(C50) was incubated with purified recombinant galectin-1 and their binding to each other was tested in "pull down" assays using glutathione beads. GST-Gemin4(C50), but not GST alone, pulled down galectin-1. In parallel experiments, it was found that galectin-3 also bound directly to GST-Gemin4(C50). In contrast, bovine serum albumin failed to do so and thus, GST-Gemin4(C50) was not pulling down any irrelevant protein subjected to the test [46].

The SMN protein forms a core complex with six other proteins, five of which (Gemin2, Gemin3, Gemin5, Gemin6, and Gemin7) bind directly to SMN while the remaining one (Gemin4) associates indirectly via binding to Gemin3 [51–53]. Immunoprecipitation experiments using anti-SMN antibodies or antibodies to the Gemins lead to their co-purification, even under high salt conditions. Similarly, antibodies directed against SMN and any of the Gemins exhibit colocalization under immunofluorescence experiments. The SMN complex is found in the cytoplasm and the nucleus, where it is present throughout the nucleoplasm and is highly concentrated in discrete nuclear bodies called gems (see Figure 2D). Using anti-SMN as a gem marker and p80 coilin as a marker protein for coiled bodies, double immunofluorescence experiments demonstrated that gems are similar in size and in number to coiled bodies. Although gems and coiled bodies are coincident in most cells, they appear as distinct entities in fetal tissues and certain cultured cells (thus, the nomenclature of gems—gemini or twins of coiled bodies) [30,52].

In addition to the tight interactions among the components of the core SMN complex, the SMN protein also interacts, perhaps less strongly or more transiently, with many other proteins, including the Sm and Lsm polypeptides of snRNPs, fibrillarin and GAR1 of the small nucleolar RNPs (snoRNPs), heterogeneous nuclear RNP U and Q, and RNA helicase A [52]. A common theme among these substrates of the core SMN complex is that each of the polypeptides contains an RG-rich domain. In addition, the SMN protein also interacts with a number of other proteins without RG motifs, most notably RNA polymerase II [54]. The SMN complex functions in RNP assembly. In the cytoplasm, it mediates the assembly of the Sm polypeptides with the newly synthesized snRNAs (see Figure 2A) and chaperones the assembled snRNPs back into the nucleus [55,56]. In the nucleus, the SMN complex is important in the function of the spliceosome (see Figure 2B) [57] as well as the components of the RNA polymerase II transcription and processing machinery [54], as revealed by the dominant negative mutant of SMN, SMNΔN27.

The demonstration of a direct interaction between galectin-1 and Gemin4 suggested the possibility that the lectin might also be a component of nuclear complexes containing SMN. Indeed, when NE of HeLa cells is subjected to immunoprecipitation

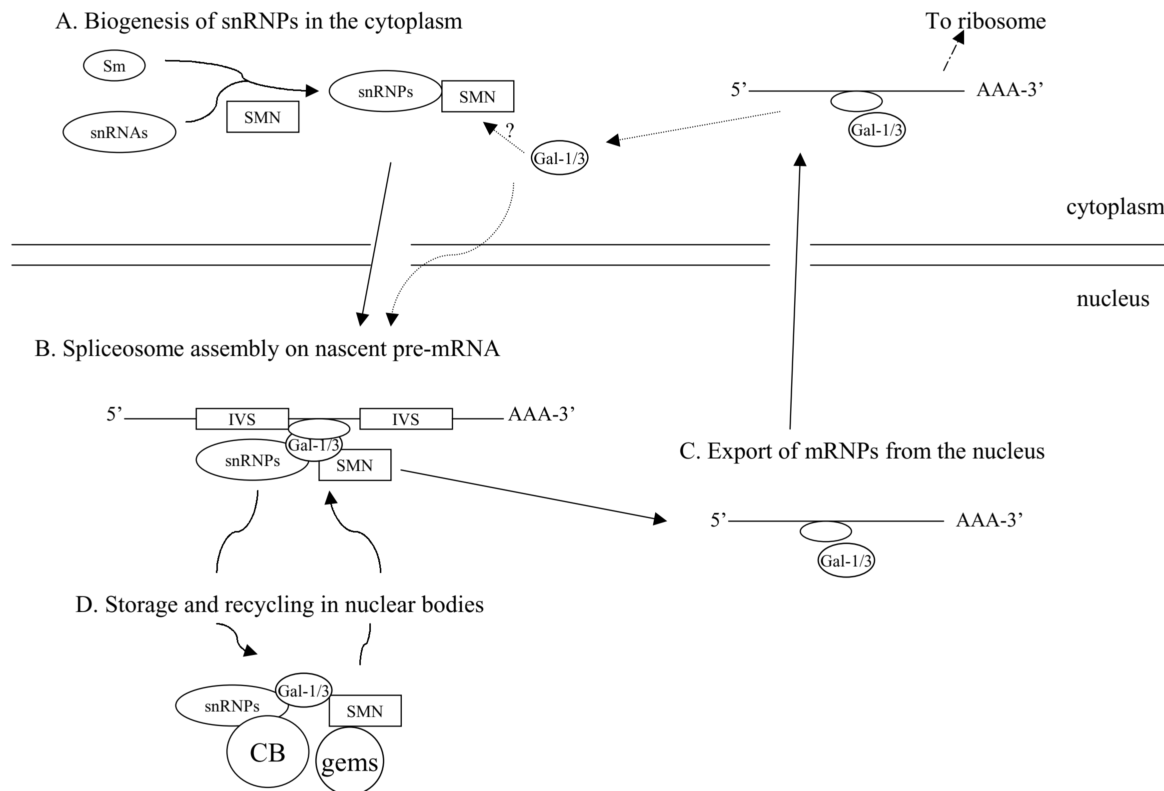

Figure 2. Schematic diagram illustrating the possible roles of galectin-1 and galectin-3 in the pathway of pre-mRNA processing. (A) The SMN-mediated assembly of Sm and snRNAs into snRNPs and their import into the nucleus. (B) Spliceosome assembly on a pre-mRNA, containing introns (shown as intervening sequences, IVS) and a poly A tail at the 3′-end. (C) Assembly of messenger RNPs and their export from the nucleus into the cytoplasm, where they are targeted to ribosomes. The mature mRNA contains the 3′-end poly A tail but is now devoid of introns. (D) Storage and recycling of splicing factors in nuclear bodies such as gems and coiled bodies (CB).

with anti-galectin-1, a number of polypeptides were found to be co-precipitated; these include the SMN protein, Gemin2, the Sm B/B' and D polypeptides of the snRNPs, as well as galectin-3. The anti-galectin-1 immunoprecipitate did not contain, however, irrelevant nuclear proteins such as the high mobility group (HMG) 14/17 proteins [46]. The finding of galectin-1 (and galectin-3) in complexes containing SMN and the observation of galectin-3 in nuclear bodies similar in size and number to gems and/or coiled bodies (Figure 1C) provide additional lines of evidence that these galectins are involved in the pathway of RNP assembly and pre-mRNA processing.

Challenging questions

It is perhaps important to emphasize that not all nuclear (and cytoplasmic) galectins are associated with SMN complexes. The immunofluorescence micrographs of Figures 1A and 1B emphasize this point; there are many cells stained with anti-galectin-3 that do not show nuclear bodies corresponding to gems/coiled bodies. As discussed above, the least striking staining pattern, diffuse throughout the nucleoplasm, may represent the most important distribution in terms of function. In addition, the inventory of the core components of the SMN complex appears essentially complete and neither galectin-1 nor galectin-3 has been identified in this complex [52,53]. Finally, the presently available evidence indicates that the association of galectins-1 and -3 with complexes containing SMN is through Gemin4, the only member of the SMN core complex that does not bind SMN directly. Thus, its seems possible that the association of galectins-1 and -3 with the SMN complex is only transient. This, in turn, raises a number of challenging questions related to how many facets of the SMN complex function really involve galectin-1 and/or galectin-3.

In the cytoplasm, does the SMN-mediated assembly of snRNPs require either of the two galectins (see Figure 2A)? If cytosol is subjected to fractionation on density gradients, do galectins-1/-3 co-fractionate with Sm proteins, snRNA components, and the SMN complex? In those fractions containing the requisite components for snRNP assembly, can anti-galectin antibodies co-immunoprecipitate the complex? It has been demonstrated that the SMN complex chaperones the nuclear import of assembled snRNPs [55]. Is this association a mechanism for galectins-1/-3 to gain entry into the nucleus? In this connection, it should be noted that two different portions of

the galectin-3 polypeptide have been identified as important for its nuclear localization [58,59]. Is either one of these sequences important for association with the SMN-snRNP nuclear import complex or with the snurportin importin-β receptor [60]?

Recently, preliminary evidence has been obtained to suggest that galectins-1/-3 are found on spliceosomes (J. Park, unpublished observations): (a) anti-galectin-1 antibodies co-immunoprecipitate the hnRNP proteins C1/C2, which are integral parts of the H-/E-complexes of the spliceosome assembly pathway; (b) when a splicing reaction mixture containing ^{32}P-labeled pre-mRNA is subjected to immunoprecipitation with anti-galectin-1, radioactive pre-mRNA substrate, free exon 1 and larait-exon2 (the intermediates of the splicing reaction), as well as the mature mRNA product are found to be co-precipitated with galectin-1. Thus, could galectins-1/-3 serve as docking sites to attract SMN machines via their affinity for Gemin4, thereby mediating the delivery of snRNPs to the spliceosome assembling on nascent transcripts (see Figure 2B)? As discussed earlier, the evidence suggests that bulk transcription and splicing occur throughout the nucleoplasm and the dispersed nucleoplasmic staining of galectin-3 (Figure 1A) and of galectin-1 [7] matches the distribution of nascent transcripts [38–41]. The fact that SMN interacts with RNA polymerase II [54] also provokes the question whether the nuclear galectins can interact with the transcriptional machinery as well.

The SMN protein and the Gemins constitute the major components of gems [52]. Are galectins partners in these nuclear bodies, as suggested by the immunofluorescence images of Figure 1C? What are the functions of these nuclear bodies (see Figure 2D)? It has been suggested that the SMN complex serves to "rejuvenate" snRNPs [57] but the chemical nature of the snRNPs that require "rejuvenation" has not been defined.

Finally, the association of galectins-1/-3 with the mature mRNA product raises the question whether these proteins may also serve as chaperones for exporting the spliced RNA into the cytoplasm (see Figure 2C). In this connection, it is important to note that galectin-3 shuttles between the nucleus and cytoplasm [61]. The protein is rapidly exported from the nucleus in a leptomycin inhibitable, and thus probably CRM-1 mediated, process. Moreover, gel filtration analysis of the exported fraction, derived from assays carried out in digitonin-permeabilized cells, showed that galectin-3 is exported in a high molecular weight ($\sim$650 kD) complex [62]. Is this complex sensitive to ribonuclease and does it contain poly A$^+$ RNA? Once exported from the nucleus, is the galectin a part of the mRNP that is ultimately targeted to the ribosome (see Figure 2C)?

Concluding remarks: Nuclear and other intracellular activities

Beside the splicing and spliceosome assembly activities that represent the focus of this review, galectins have been documented to play a role in other intracellular activities (*e.g.*, regulation of cell growth and apoptosis; see reference [63] for a review). What is the relationship, if any, between the various intracellular activities of galectin-3: regulation of cell growth, apoptosis, and RNA processing? Do they reflect separate functions of the same protein? Or, are they part of the same story: *i.e.*, the galectin participates in one fundamental process (*e.g.*, RNA processing), to which the other activities (cell growth regulation and anti-apoptosis) can be attributed? Quite strikingly, the SMN protein, aside from its role in snRNP biogenesis and pre-mRNA processing, has also been implicated as an inhibitor of apoptosis in a pathway involving Bcl-2 [64]. In fact, mutation and deletion of the SMN gene has been identified as the basis for the pathogenesis of the disease spinal muscular atrophy. In these patients, the level of the SMN protein is drastically reduced in the spinal cord motor neurons and, as a result, the cells undergo apoptosis [65]. Like the case of galectin-3, therefore, the relationship between the splicing function of SMN and its role in the survival of motor neurons from apoptosis (as seen in spinal muscular atrophy), is not clear. The dual roles of both galectin-3 and of SMN in RNA processing and in apoptosis remain as major challenges for future investigations.

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