SMN and SnRNP assembly

The first stage of SnRNP assembly involves the binding of the Sm proteins to pICln. *In vitro* reconstitution studies have suggested that pICIn binds the Sm proteins as two separate complexes: one composed of plCln, SmB and SmD3 and one composed of plCln, SmD1 and SmD2.[1] The latter complex subsequently binds SmE, SmF and SmG[1] The release of Sm proteins from the pICln complex and their assembly onto snRNA can be facilitated *in vitro* by the SMN complex on its own[1]; however, this reaction is only moderately efficient and does not require ATP, unlike the reactions that have been reported *in vivo*.[2,3] In contrast, SMN complexes that have been isolated from human cell lines performed snRNP assembly in an ATP dependent manner, similar to the reactions that have been reported *in vivo*[2,3]. Experiments have indicated that the PRMT5 complex can methylate the RG domain of certain Sm proteins (SmB, D1,and D3) and thus increase their affinity for SMN.[3,4] The role of methylation in snRNP assembly is controversial. *In vitro* reconstitution experiments of the SMN complex where concentration of components is high demonstrate that SMN, Gemin2 and Gemin8 can assemble Sm proteins complexed with pICln onto snRNA without methylation of the RG domain of the Sm proteins.[1] However, unlike the reactions involving SMN complex isolated from cells, this reaction was not dependent on ATP. Furthermore, the reaction did not require Gemins 5 and 3, which have been shown to play a key role in determining assembly activity.[5-7] Preventing Sm methylation in vertebrate cells by knocking down PRMT5 or PRMT7 disrupts snRNP assembly activity.[8] In Drosophila loss of PRMT5 does not affect snRNP activity and loss of methylation on a single Sm protein, SmD1, does not alter snRNP assembly.[9] Thus, the importance of Sm protein methylation for *in vivo* snRNP assembly reactions, particularly in vertebrates, is not fully resolved. The importance of different Gemin's in snRNP assembly has also been questioned.[1,6] Knockdown of various Gemins, including Gemin5 and Gemin3 indicates that these proteins play a key role in determining assembly activity.[5-7] In vertebrates, Gemin5 clearly binds snRNAs and the SMN complex allowing the snRNP to be delivered to the SMN complex.[6] However, as mentioned above, *in vitro* reconstitution experiments using only Gemin2 and Gemin8 indicated that only these proteins are required for assembly.[1] Furthermore, the *Gemin5* homologue (*rigor mortis*) has not been detected in the Drosophila SMN complex.[10] However, whether snRNA delivery is performed in a different manner in Drosophila and vertebrates is unknown. To date most assays have been performed using U1 and U4 snRNAs. It is therefore possible that the binding of other snRNAs of lower abundance to Sm proteins could have greater or lesser dependence on the components of the SMN complex.[1,5-7] The additional members of the SMN complex are likely to play specific roles in the assembly reaction. For example, Gemin8 is

critical for the association of the SMN complex with Sm proteins, yet its loss does not affect snRNA binding to the SMN complex.[5]

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