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SPOTLIGHT

NuMA1 facilitates the assembly of the axon initial segment by promoting the retention of neurofascin-186

Amr Abouelezz and Pirta Hotulainen

Axon initial segment (AIS) functionality relies on a specific organization of the AIS with high enrichment of structural and functional proteins. In this issue, Torii et al. (2019. *J. Cell. Biol.* https://doi.org/10.1083/jcb.201907048) describe a mechanism for achieving a high density of proteins in the nascent AIS.

The axon initial segment (AIS) is a specialized region at the proximal ends of the axons of vertebrate neurons. The AIS regulates the entry of macromolecules into the axon, forming a barrier between the somatodendritic and axonal compartments. In addition, voltage-gated ion channels cluster at the AIS, making it the site of initiation of action potentials. Shortly after the establishment of neuronal polarity, several AIS-specific proteins accumulate at the proximal end of the nascent axon and assemble the AIS complex—a highly-organized complex of scaffolding proteins and cell adhesion molecules that underlies the different functions of the AIS. At the heart of this complex is the AIS-specific adaptor protein ankyrin G, which recruits and binds several AIS proteins and links the AIS complex to microtubules (1). While recent work has provided insight into the structure and function of the AIS in unprecedented detail, a rapidly growing list of questions remains unanswered. In particular, the processes governing the initial assembly of the AIS are poorly understood. In a new study by Torii et al. (2), the authors take an ingenious proteomic approach to identify proteins involved in the early stages of AIS development.

In contrast to synapses, proteomic approaches to study the AIS in isolation have not been technically feasible. To circumvent this technical difficulty, Torii et al. (2) compared the detergent-insoluble fractions of the proteomes of ankyrin G–deficient neurons to wild-type neurons. For this purpose, the authors used both conditional ankyrin G knockout mice as well as shRNA-mediated ankyrin G silencing in cultured neurons. Given the importance of ankyrin G to AIS structure, these neurons effectively lack an AIS. The levels of several proteins were lower in the detergent-insoluble fractions of ankyrin G–deficient neurons, potentially indicating their involvement in the AIS. Importantly, the authors conducted their experiments in conditional ankyrin G knockout mice at P0, as these mice die shortly after birth, and shRNA-induced ankyrin G silencing experiments at 12 days in vitro (DIV). Therefore, the potential AIS proteins identified are likely to play a role in the early stages of AIS development. Using this method, the authors identified nuclear mitotic apparatus protein 1 (NuMA1) as a promising candidate that was significantly reduced in ankyrin G–deficient neurons. NuMA1 plays a role in cell division (3, 4) and has not been previously implicated in AIS assembly.

Torii et al. (2) first demonstrate that NuMA1 exhibits basic characteristics of an AIS assembly protein: (a) NuMA1 localizes to the AIS in early development, (b) NuMA1 localization in the AIS is resistant to detergent extraction, and (c) silencing NuMA1 by introducing shRNA in cultured neurons at 2 DIV resulted in a reduced accumulation of ankyrin G and neurofascin-186 (NF186) at 6 DIV. Similarly, in vivo silencing of NuMA1 at embryonic day 15.5 resulted in more transfected neurons lacking an AIS compared to controls. NuMA1 expression strongly decreases after 9 DIV and silencing NuMA1 at 9 DIV had no effect on transfected neurons. These results indicate that NuMA1 is important for the assembly of the AIS, but not for its maintenance.

To determine the mechanism behind the localization of NuMA1 to the AIS, Torii et al. (2) tested the binding of NuMA1 to several AIS proteins using immunoprecipitation. NuMA1 coimmunoprecipitated with protein 4.1B, a structural AIS protein that binds β-spectrins. The authors further show that the C terminus of NuMA1 is responsible for the interaction with protein 4.1B, and this interaction is sensitive to phosphorylation. Thus, these data suggest that NuMA1 localizes to the AIS through binding protein 4.1B. Consistent with this, the silencing of protein 4.1B at 2 DIV led to a reduction in the accumulation of ankyrin G and NF186, while silencing at 9 DIV had no effect on the AIS. Furthermore, as NuMA1 is known to interact with the dynein/dynactin complex, the

Minerva Foundation Institute for Medical Research, Helsinki, Finland.

Correspondence to Pirta Hotulainen: pirta.hotulainen@helsinki.fi.

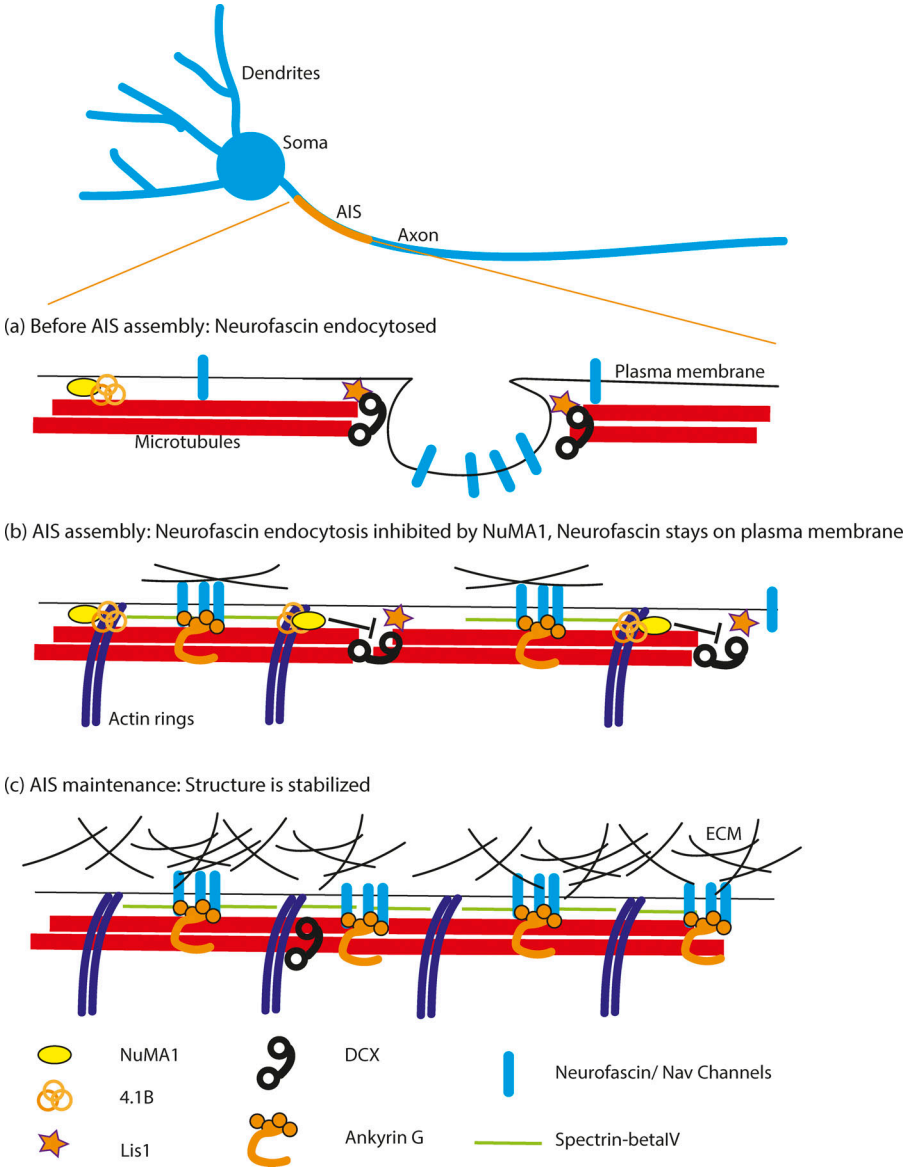

Figure 1. **Model for the role of NuMA1 in AIS assembly. (a)** In early development, DCX and Lis1 facilitate endocytosis of NF186, thus inhibiting the clustering of Neurofascin on plasma membrane. **(b)** During AIS assembly, NuMA1 inhibits the interaction of DCX and Lis1, leading to inhibition of endocytosis. Neurofascin will be retained on plasma membrane, thus promoting the enrichment of AIS proteins. **(c)** Mature AIS is well stabilized and can maintain its structure without proteins required for initial assembly.

authors tested if NuMA1 interacts with Lis1 in the AIS. Lis1 is present in the AIS where it interacts with Ndel1 and facilitates the activation of dynein, a process that is important for cargo trafficking (5). Torii et al. (2) report that NuMA1 coimmunoprecipitated with Lis1, through interactions with the N terminus of NuMA1. Interestingly, silencing Lis1 at 2 DIV had effects opposite to the silencing of NuMA1 or protein 4.1B: an increased accumulation of ankyrin G and NF186 at the AIS. Like NuMA1, however, silencing Lis1 at 9 DIV had no effect on the AIS. Finally, Torii et al. (2) elucidate the roles of NuMA1 and Lis1 in the endocytosis of NF186 through binding to doublecortin (DCX). DCX facilitates the endocytosis of NF186 (6). Torii et al. (2) demonstrate that Lis1 binds DCX and promotes the endocytosis and retrieval of NF186, while NuMA1 favors the retention of NF186 by inhibiting the interaction of Lis1 with DCX. Thus, these data reveal that NuMA1 plays an important role in AIS assembly by regulating NF186 endocytosis through inhibiting the action of Lis1.

The findings presented in this study represent a significant advance in our understanding of the AIS, particularly in its assembly. While ankyrin G can recruit and cluster various AIS proteins in the mature AIS, it is not clear how the accumulation of the different AIS proteins is initially achieved during the early stages of AIS development, when the levels of ankyrin G are not high enough. The question has traditionally been which individual protein localizes to the AIS first. However, it is more plausible that many proteins and interacting cells come together at a suitable time and space. It is likely that early interactions between the extracellular matrix and the axonal cytoskeleton—through adhesion molecules such as NF186—are the first scaffolding events that establish the AIS (Fig. 1). As shown in Torii et al. (2), the regulation of endocytosis rate seems to be one of the mechanisms that neurons use to organize scaffolding AIS proteins together. Increasing the time window to allow interactions seems to be enough to facilitate initial enrichment of different proteins in the same proximity. As the critical density of anchoring proteins and a sustainable structure are achieved, proteins required for initial assembly are no longer needed (Fig. 1).

The innovative proteomic approach used by Torii et al. (2) can inexpensively provide clues about other players in the AIS and significantly further assist our efforts to understand this important compartment. In the future, it will also be interesting to clarify the structural details of protein assemblies through studies empowered by super-resolution techniques aiming at understanding in detail how these proteins assemble and function together. Further studies on how the extracellular matrix and other cells in the brain (such as chandelier cells) regulate AIS assembly are also crucial for better understanding the mechanisms underlying AIS development.

Acknowledgments

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