

Structure-function correlations in agrin.

The neuromuscular junction (NMJ), is a highly organized apparatus responsible for communication between nerve to muscle [26]. Agrin is a multi-domain, multi-functional heparan sulfate proteoglycan (HSPG) that plays key roles in the development, maintenance, and remodeling of NMJ synapses [27]. Mechanistic information on these processes could pave the way for therapies to regenerate synapses damaged by injury or disease. Agrin has additional, less well-understood roles in the central nervous system, in non-neural tissues, in immunological synapses, and in Alzheimer's disease.

To achieve a timely and efficient response to the neurotransmitter released into the synaptic cleft, acetylcholine receptors (AChRs) are organized into dense clusters on muscle plasma membranes at sites of contact with the nerve terminals [26]. The aggregation of AChRs together with other proteins such as rapsyn is a hallmark of NMJ development. Agrin orchestrates this process by activating a muscle specific kinase (MuSK), that through a series of steps leads to the phosphorylation and clustering of AChRs [27].

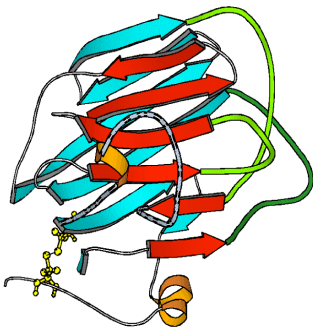


Fig. 2: NMR structure of Ca²⁺-bound agrin-G3 (B0)

The C-terminus of agrin contains three 'G' (globular) domains. The last of these domains is necessary and sufficient to induce agrin's receptor clustering activity, as long as it has 'B' sequence inserts of 8, 11, or 19 residues, derived from alternative mRNA splicing of the agrin gene. Our lab has solved the NMR structure of the B0 form of the agrin-G3 domain, in a joint effort with a crystallographic group that solved the structures of the B8 and B11 isoforms [28]. The ~22 kDa G3 domain has a 13-stranded β -jellyroll structure. We were the first to show that this domain binds calcium [29], which has subsequently been demonstrated to be needed for agrin's AChR clustering activity [30]. Our structural studies [31] showed that the calcium-binding site (light green loops in Fig. 2) is close to the B-insert site (dark green). NMR was used to extensively characterize the dynamics of the B0 and B8 isoforms. It was shown that the regions surrounding the calcium-binding site and insert loops are flexible on μ sec-ms timescales, and that flexibility is reduced when calcium is bound. The insert-bearing loop, however, remains flexible even in the calcium bound form and presumably only adopts a fixed structure when the domain binds to its receptor through an induced fit. The results from NMR agree with the crystallographic data, which show the inserts segments in B8 and B11 remain disordered in the calcium-bound state.

A long-standing issue in the agrin field is the mechanism by which neural isoforms activate MuSK. The agrin-G3 domain that induces AChR aggregation also induces phosphorylation of MuSK, while splice variants inactive in AChR aggregation fail to trigger MuSK phosphorylation. Cross-linking experiments suggest that agrin binds directly to MuSK in the myotube progenitors of mature muscle fibers. In other types of cells, however, agrin fails to activate MuSK. These observations led to the hypothesis of a co-receptor called 'MASC' (myotube-specific accessory component) [32]. The identity of MASC has remained elusive for nearly a decade, although it has recently been suggested that MASC may be a carbohydrate [33]. This hypothesis led us to examine the binding of a number of carbohydrates to agrin.

Long-term goals:

- We will continue to examine the mechanism by which agrin exerts its AChR clustering activity. Our immediate goals are to complete the characterization of interactions with carbohydrates and to understand the folding of agrin insert sequences that occur as a result of alternative splicing of agrin mRNA.
- We recently started NMR work on the N-terminal domain of agrin, NtA, and plan to study complexes with laminin peptides that will model the structural basis for agrin-laminin recognition. This interaction is important, in light of recent findings that a synthetic agrin mini-gene circumvents the symptoms of merosin deficient muscular dystrophy in a mouse model of the human disease. [37]. In addition to the functions described above, agrin has been found tightly associated through its glycosaminoglycan chains with amyloid deposits [38]. These deposits are typically localized to the basal lamina of brain microvasculature. Because the NtA domain serves to anchor agrin to the basal lamina, this interaction could be a target for structure-based inhibitors that interfere with amyloid deposition.

Recent publications

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- 2) Stetefeld, J., Alexandrescu A. T., Maciejewski, M. W., Jenny, M., Rathgeb-Szabo, K., Schulthess, T., Landwehr, R., Frank, S., Ruegg, M.A., & Kammerer, R.A. (2004) "Modulation of agrin function by alternative splicing and Ca²⁺ binding". *Structure* 12, 503-515.

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