Mechanisms of postsynaptic development

During recent years, considerable insights have been gained into the development of the postsynaptic organisation of the NMJ, in particular the formation of ionotropic glutamate receptor (GluR) fields opposite presynaptic release sites. Ionotropic glutamate receptors at the NMJ are hetero-tetrameric and contain each of the three subunits GluRIIC, D and E together with either GluRIIA or GluRIIB. GluRIIA and B exclude each other, and define two GluR subtypes which localise in partially overlapping patterns and display distinct properties [20, 49, 62]. At newly forming embryonic NMJs, postsynaptic GluR clustering depends on signals from presynaptic terminals [10, 13]. The organisation of this machinery depends on presynaptic action potentials [8, 70] in ways not yet understood. Vesicular transmitter release may or may not be involved [7, 48, 69], but certainly levels of the neurotransmitter glutamate are important for GluR clustering [25, 26]: enzymatic up-regulation of glutamate contents in the presynaptic terminal negatively influences clustering of functional GluRs at the postsynaptic membrane. The corresponding glutamate release might occur via non-vesicular neurotransmitter transporters [25, 26]. Therefore, given that the amount of presynaptic glutamate released is instructive for GluR clustering, we would expect that GluRs themselves should trigger their own localisation. Calcium signalling events might mediate such processes, since it has been shown that calcium/calmodulin-dependent kinase (CaMKII) phosphorylates and removes Discs large (Dlg) from the postsynaptic site at larval NMJs [38] - although this might differ during embryogenesis [36]. Dlg is a PDZ-domain containing scaffolding protein, which becomes enriched at postsynaptic sites in response to innervation [13]. Its homologues are involved in GluR clustering at synapses in the CNS of mammals [75]. In the absence of Dlg, GluRIIB but not GluRIIA receptors, fail to localise synaptically, but this role of Dlg is likely to be indirect [13]. Conversely, GluRIIA but not GluRIIB localisation depends on the cytoskeletal linker 4.1 protein Coracle [14], whereas both Dlg and GluRIIA localisation can be regulated through muscular functions of Pix and Pak kinases and the adaptor protein Dreadlocks [2, 56]. In addition, postsynaptic GluR levels are adjusted through expression regulation of GluR-encoding genes, e.g. via translational control [77, 78]. Control of GluR expression is already observed at late embryonic stages, induced by presynaptic innervation [9]. Additional genes required for clustering at postsynaptic sites have been identified [44] and will provide us with the genetic tools to test to what degree the regular cleft material at neuromuscular synapses (Fig. 1H,I) represents the extracellular domains of GluRs.

Compared with these advances at the NMJ, the postsynaptic organisation at photoreceptor tetrad synapses awaits investigations based on the recently reported histamine receptor gene, DmHisCl1 [27, 28, 100].

Table 1. Factors underlying the regulation of NMJ structure

A) Genetic factors mutations or manipulations of which have been reported to cause defects at the EM level of synaptic structures reviewed here. **B)** Genetic factors mutations or manipulations of which have been reported to cause structural defects of larval NMJs at any microscopic level; factors have been assigned to only one group of proteins (underlined in B), although for some of the factors different assignments would have been possible. Abbreviations indicate the structural feature regulated by the respective genetic factor: bs, bouton size; bn, bouton number; sd, synapse density; tl, terminal length; gr, glutamate receptor mislocalisation; sa, structural aberrations (shape aberrations, altered numbers of satellite boutons, cytoskeletal defects, synaptic defects, abnormal inclusions, abnormal distribution of organelles, SSR aberrations etc.). A source of further mutations potentially affecting NMJ morphology is provided by an over-expression screen published previously [40].

A) Ultrastructural mutant NMJ phenotypes:

- aberrations of vesicle pool: Lap/AP180 [37] ♦ StonedB [81] ♦ Dap160 [98] ♦ Discs large [33] ♦ Endophilin [90] ♦ Synaptojanin [91] ♦ Spectrin [58] ♦ Glued1 [21] ♦ Rab5 [96] ♦ Benchwarmer/Spinster [17] ♦ Shibire [23] ♦ αAdaptin [29] ♦ Comatose/dNSF1 [35] ♦ Syntaxin [7] ♦ Synaptotagmin [64] ♦ Slug-a-bed [66] ♦ Unc-13 [4]
- atypical membranous compartments: presynaptic FasciclinII expression [5] ♦ Benchwarmer/Spinster [17]
- aberrant SSR: Discs large [31, 41] ♦ Mod/mdg4 [30] ♦ CaMKII [38] ♦ Spectrin [58] ♦ dPix, dPak [56]
- aberrant NMJ adhesion: lamininA [60] ♦ mef2 [59] ♦ Wishful thinking [1, 47] ♦ Glued1 [21]
- aberrations of T-bar ribbons: Short stop [61] ♦ Wingless [55], Wishful thinking [1] ♦ liprin
- alteration of synapse size: Liprin-alpha, DLar [34]

B) Genetic factors involved in structural larval NMJ development:

- structural proteins: dX11/Mint (bn) [5] ♦ Nwk (bn, bs, sd) [15] ♦ Discs large (sa) [31, 41, 84, 85] ♦ Liprin-alpha (bn, sa) [34]
- cytoskeletal regulators: Wasp (bn) [15] ♦ Futsch (bn, bs, sa) [67] ♦ Spectrin (sa) [24, 58] ♦ Spastin (tl, sa) [76, 87]
- transmembrane molecules: Fasciclin2 (bn, bs, sa) [5, 73] ♦ APPL (bn) [86] ♦ Fasciclin1 (bn) [102] ♦ Volado (bn) [65] ♦ Myospheroid (bn, bs) [6] ♦ DLar (bn, sa) [34] ♦ Kekkon2 (bn) [32]
- intracellular trafficking: DVAP-33A (bn, bs, sa) [57] ♦ Dap160/Intersectin (sa) [37, 46] ♦ Endophilin (bs, sa) [90] ♦ Synaptojanin (sa) [91] ♦ Shibire (sa) [23] ♦ Dynamin-related protein (sa) [92] ♦ Benchwarmer/Spinster (bn, sa) [17, 83] ♦ Glued1 and Arp-1 (bn, sd, sa) [21] ♦ StonedB (sa, bn) [22, 81, 82] ♦ Sec5 (bn) [53] ♦ Rab5 (sa) [96] ♦ αAdaptin (sa) [29] ♦ Comatose/dNSF1 (sa) [35] ♦ Synaptotagmin (sa) [64] ♦ Hook, Deep orange (bn) [54]
- sinalling: CYFIP/Sra-1 (sa, tl) [72] ♦ Wingless pathway (bn, sa, gr) [55] ♦ aPKC (bn, sa, gr) [68, 97] ♦ Lethal (2) giant larvae (bn) [97] ♦ Dunce + Rutabaga (bn, sd) [74, 101] ♦ Ras1-MAPK pathway (bn) [39] ♦ BMP pathway (bn, sa) [1, 47, 50, 51, 63, 83] ♦ Gs alpha (bn) [94] ♦ GluRIIA+B (bn) [79] ♦ CaMKII (sa) [38] ♦ Still life (bn) [80] ♦ JNK pathway (bn) [71] ♦ Frequenin (bn, tl) [3] ♦ Hyperkinetic, Eather-a-gogo, Paralytic, Shaker, No action potential, Zydeco, Slowpoke, Seizure (bn) [11, 32] ♦ ecdysonless (bn, tl) [42, 43] ♦ dPix, dPak (sa) [56]
- regulation of gene expression: dFMR1 (bn, bs) [99] ♦ Fat facets (bn) [19] ♦ Mod/mdg4 (bn, sa) [30] ♦ eIF4E8 and poly(A)-binding protein (bn) [78] ♦ CREB (bn, sd) [16] ♦ cactus/dorsal (sa) [12] ♦ AP-1 (bn) [71] ♦ APC2/mr (bn) [89] ♦ Highwire (bn) [50, 93, 95] ♦ Nalyot (bn) [18] ♦ dCBP (bn) [45] ♦ Pumilio (bn, bs) [52]
- metabolism: Stress-sensitive B & Atpα [32, 88]

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