Cell Tissue Res (1999) 297:169–186

REVIEW

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Integrating bits and pieces: synapse structure and formation in *Drosophila* embryos

Received: 12 August 1998 / Accepted: 17 November 1998

Abstract During the development of the nervous system, numerous neurons connect to form complex networks. In order to build a functional network each neuron has to establish contacts with appropriate target cells, and at these contacts synapses of the right quality and strength have to be formed. Gaining insight into the mechanisms underlying this complex development is an important step towards a better understanding of how the nervous system is formed and behaviour generated. One model system in which synapse formation can be studied at the morphological, physiological and molecular level is that of the fruitfly *Drosophila*, and insights gained from *Drosophila* embryos are reviewed here. The first part of this review deals with the neuromuscular junction as the best-known synaptic contact in *Drosophila*. It describes: (1) its structure, (2) mechanisms underlying the formation of the neuromuscular cell junction and the arborisation of the presynaptic terminal, and (3) our present understanding of signal-dependent and -independent processes during synapse formation at the neuromuscular junction. The last part of this review deals with the question of how particular neurons can adopt specific synaptic properties, stating as an example the development of the neural lineage of NB7-3, which gives rise to two serotonergic neurons.

Key words Adhesion · Cell lineage · Development · Neuromuscular junction · Serotonin

Introduction

The nervous system lies at the heart of behaviour, receiving, integrating and processing sensory information, storing information and generating distinct patterns of motor or gland activity. The cellular basis for this ability lies in the complex but highly ordered networks consisting of numerous neurons with distinct properties, connected to one another via synaptic cell junctions. In order to build these networks, neurons have to carry out at least three main developmental tasks. Firstly, they have to establish connections with the right target cells, thus determining the paths of information flow. Secondly, the connecting neurons have to form the correct types of synapses at the contacts they establish. This can be electrical synapses, chemical synapses with excitatory, inhibitory or modulatory characteristics, or they can be of mixed nature. Each type of synapse will have a particular impact on the neuronal circuit, as it transmits and modifies information in distinct ways. Finally, the strength of transmission between cells has to be regulated, for instance by the number of synapses formed between two cells.

The nature of synapses seems to depend on properties which the synaptic partner cells have acquired independently, as a function of their developmental history. In addition, during the process of cell contact formation, cell signalling is required to align synaptic structures at precisely apposed sides of the cell junction. Furthermore, cell signalling might influence the specificity of the synaptic components. For example, neurons in the sympathetic peripheral ganglia of vertebrates initially acquire adrenergic properties as a function of their developmental history (see for example Hirsch et al. 1998 and references therein), but the adrenergic quality can be changed into a cholinergic fate, depending on the type of target cells that are innervated (Kuno 1995).

In order to understand synapse formation we have to: (1) identify the structural and molecular components required at developing and functional synapses, (2) unravel the signalling and assembly processes involved in their formation and (3) ask by which gene regulatory events the required molecules are provided. One model system in which different aspects of synaptogenesis can be studied and hopefully one day be integrated comprehensively is the embryonic nervous system of the fruitfly *Drosophila melanogaster*. The first part of this review focuses on

The author is funded by the Deutsche Forschungsgemeinschaft

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the neuromuscular synapse as the most intensely studied synaptic contact in *Drosophila*, and describes the current knowledge about its structure on one hand and its development on the other. The second part deals with neurogenesis in the central nervous system of the *Drosophila* embryo, describing how regulatory cascades leading to the expression of synaptic components of identified cells can be studied.

A close look at an identified synapse: the structure of neuromuscular junctions and synapses in *Drosophila*

The structure of neuromuscular junctions

So far the best-characterised synapses in *Drosophila* are those at the neuromusclar junction (NMJ). NMJs are amenable to light-microscopic, electrophysiological, ultrastructural and experimental analysis, and they can be analysed in the embryo, so that phenotypes of embryonic lethal mutations can be studied. Thus, the NMJ has turned into a valuable model system, and numerous genetic tools and techniques established for *Drosophila* can be used to

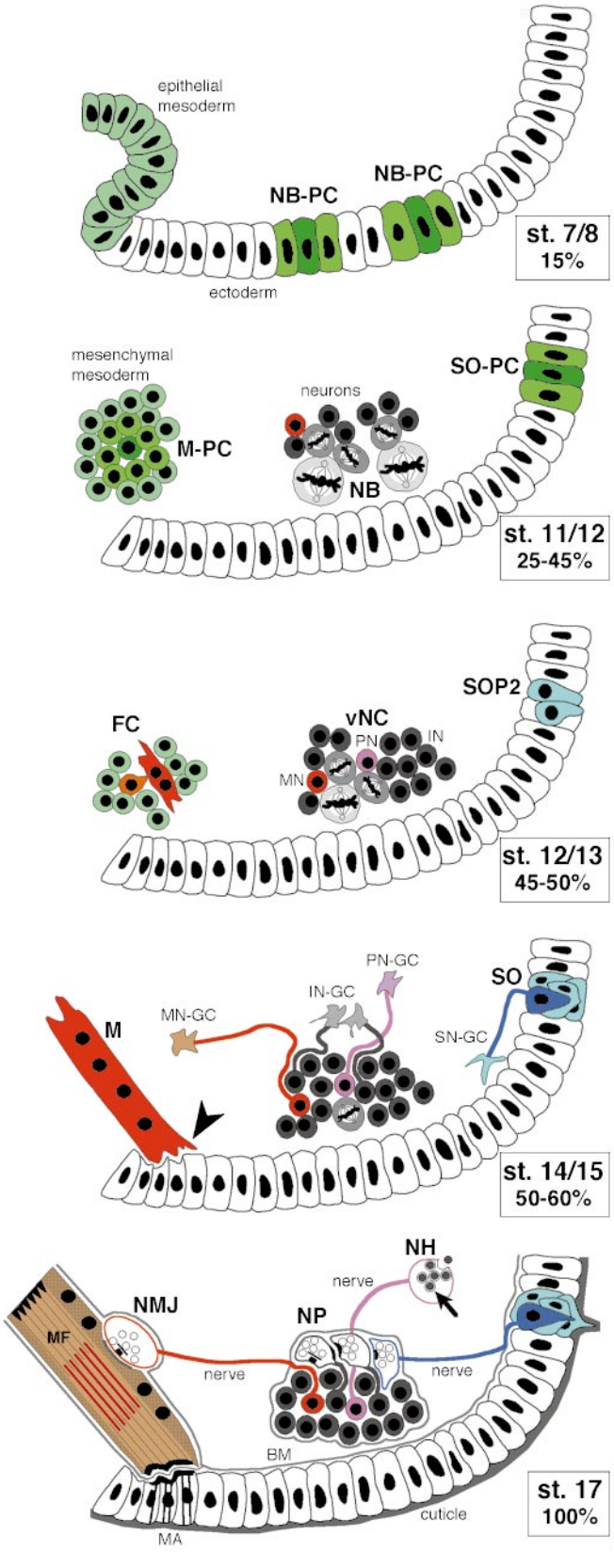


Fig. 1 Schematic representation of neurogenesis and myogenesis in *Drosophila* embryos. *Drosophila* embryogenesis lasts 21 h at 25°C and can be subdivided into 17 stages (stages indicated *at bottom right corner of every drawing*; percentage embryonic development is given). Precursor cells (*dark green*) for about 30 neuroblasts (*NB-PC*), 42 sensory neurons/organs (*SO-PC*) and 30 muscles (*M-PC*) are singled out from clusters of cells expressing proneural genes (*green*). NB-PC and SO-PC derive from a monolayer of ectodermal cells; M-PCs are derived from the mesoderm, which initially invaginates as an epithelial layer that disintegrates into a mesenchymal cell mass. Neuroblasts (*NB, light grey*) segregate from the ectoderm and divide repeatedly (schematic metaphase) to give rise to ganglion mother cells (*medium grey*), which in turn divide once to give rise to postmitotic neurons (*dark grey IN* interneurons, *red MN* motoneuron, *pink PN* peptidergic neuron, peripheral peptidergic neurons are omitted for clarity), which associate into the densely packed ventral nerve cord (*vNC*), surrounded by a glial sheath (not shown) and basement membrane (*grey line*). Sensory organ precursors (*SO-PC*) remain in the epidermis, divide twice and give rise to sheath/support cells (*light blue*) and one sensory neuron (*SN, dark blue*). Starting at stage 12, neurons form axons by sending out growth cones (*GC*) that follow cell-specific paths. At stage 15/16 these growth cones establish contacts with their appropriate target cells (not shown) and differentiate into synapses (active zones with clustered vesicles). Motoneurons establish neuromuscular junctions (*NMJ*), interneurons and sensory neurons form central synapses in the neuropile (*NP*) of the ventral nerve cord, and peptidergic neurons contain dense-cored vesicles (*arrow*) and often have peripheral processes with neurohemal release sites (*NH*). Muscle precursors (*M-PC*) give rise to founder cells (*FC*), which express specific markers (*red*) and fuse with surrounding mesenchymal myoblasts into a multinucleated muscle (*M*), which inserts into the epidermis (*arrowhead*). During stage 16/17 somatic muscles form myofilaments (*MF*), establish elaborate muscle attachment sites (*MA*) and contribute to NMJ differentiation. At stage 16/17, basement membrane (*BM, grey line*) forms around the muscles, ventral nerve cord and on the basal side of the epidermis, whereas the apical epidermal surface is covered by cuticle. Nuclei of all tissues are indicated as *black dots*. (For further details, see Campos-Ortega and Hartenstein 1997)

dissect the mechanisms underlying its formation and function (Keshishian et al. 1996).

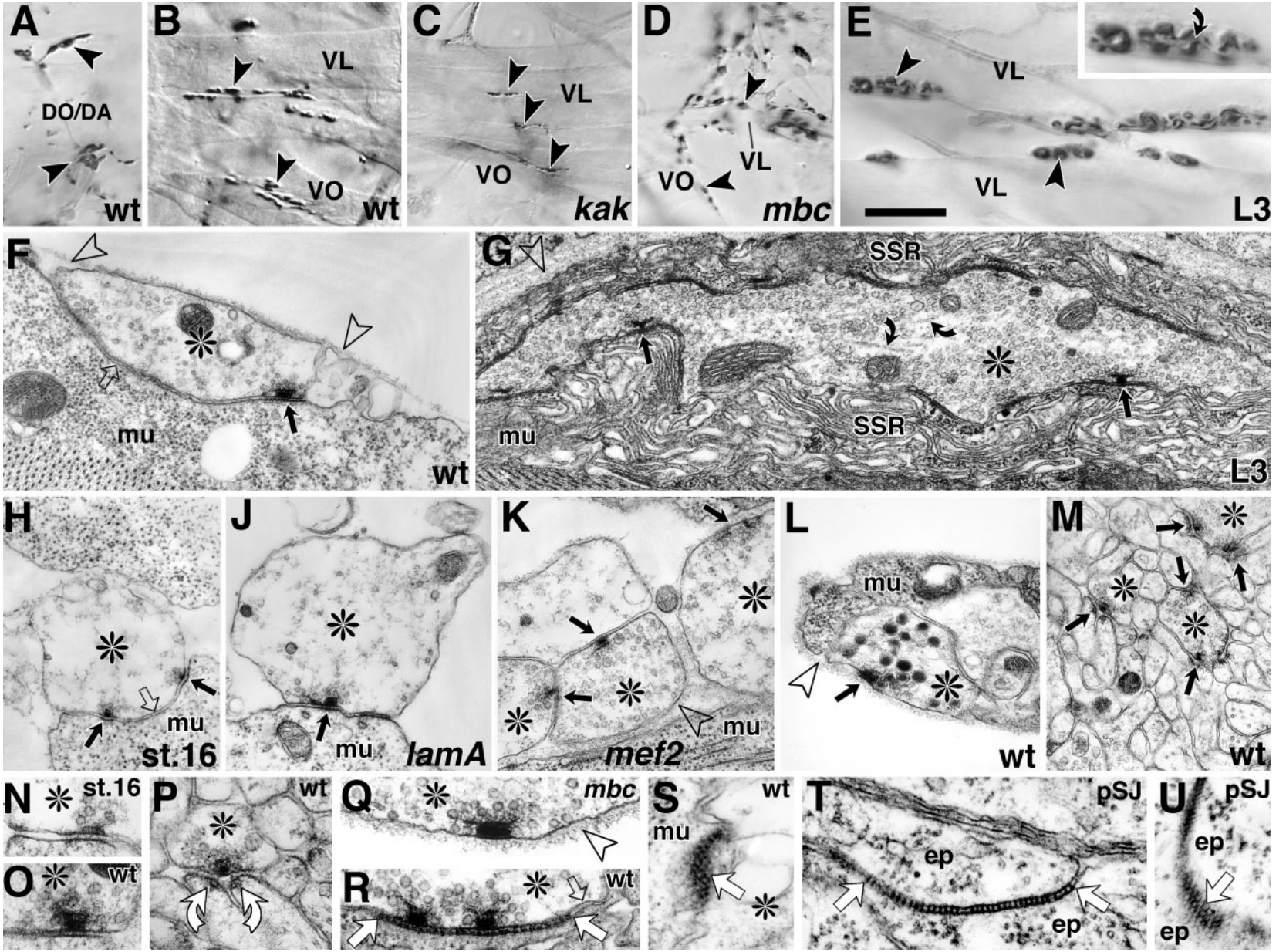
In the *Drosophila* embryo a stereotypical pattern of 30 somatic muscles forms per abdominal hemisegment, and each muscle is individually identifiable by its characteristic shape and position. *Drosophila* muscles are single polynucleated fibres and much of their development is well studied (Fig. 1; Bate 1993; Baylies et al. 1998; Ruiz-Gómez 1998). Each muscle is innervated by at least one identified motoneuron, of which most can be assigned to a particular neural lineage (Landgraf et al. 1997; Figs. 1, 5; see later). Motoneurons have a characteristic soma position in the ventral nerve cord and distinct dendritic and axonal projections. Most (if not all) embryonic neuromuscular terminals are glutamatergic, and they form branches on the muscle surface, with reproducible shape differences between different muscles (Johansen et al. 1989a; Fig. 2A vs. B). Also the point of nerve entrance and position of the NMJ on the muscle surface are reproducible, although position does not appear to be essential to neuromuscular transmission, as shown by displaced NMJs in *prospero* mutant embryos (Broadie and Bate 1993d). The branches of motoneuronal terminals show more or less reproducible numbers of varicosities, called boutons (Fig. 2A,B,E; Johansen et al. 1989a; Broadie and Bate 1993c). The embryonic boutons, which have a diameter of up to 1 µm, are attached to the muscle surface on one side and on the other side face the haemolymph, covered only by basement membrane (Fig. 2F). The attachment between muscle and neuronal surface is a non-specialized junction with a cleft of about 15 nm. This junction is interspersed with synapses, which are stretches of membrane with characteristic structural specialisations and that are believed to be the sites of neural transmission (Figs. 2F,R, 3A; Broadie et al. 1995; Prokop et al. 1996). Interestingly, and in contrast to vertebrates, the fully functional embryonic *Drosophila* NMJ is not covered by glial cells, leaving 10–20 µm of neuronal surface without insulation from the hemolymph[1] (Fig. 2F; Hall and Sanes 1993; Auld et al. 1995). However, after larval hatching, NMJ morphology changes, and the neuronal terminal submerges below the muscle surface, so that the type I boutons appear to be relatively shielded from the hemolymph by the muscle cell (Fig. 2G; Atwood et al. 1993; Jia et al. 1993). Further changes occurring after larval hatching are: (a) immense growth of the NMJ, (b) increase in numbers of synapses, (c) more than twofold increase in the number of vesicles[2] (own observations), and (d) extensive infolding of the muscle membrane opposite to the neuronal terminal (called subsynaptic reticulum, SSR; see Fig. 2F vs. G; for review, see Budnik 1996). Mechanisms regulating these postembryonic changes are under intense investigation, turning the larval NMJ into an important model system to study mechanisms underlying neuronal plasticity (reviewed in Budnik 1996; Davis and Goodman 1998).

The structure of neuromuscular synapses

Although the architecture of NMJs changes dramatically during postembryonic development, the characteristics of neuromuscular synapses are similar between embryo and larva (Fig. 3Ai vs. ii, D vs. inset in D). This also holds true for NMJs which develop in vitro (Seecof et al. 1972). At neuromuscular synapses the membranes appear smoother and more electron dense compared to the extrasynaptic areas of the neuromuscular junction (Fig. 2F,R), suggesting that the molecular composition of synaptic membranes differs from extrasynaptic membranes. The intracellular face of the presynaptic membrane harbours active zones, which contain T-shaped structures of electron-dense material (T-bars) surrounded by clusters of synaptic vesicles (Fig. 3, white arrowheads). T-bars can still be seen in neuronal terminals that are depleted of synaptic vesicles and therefore appear to be independent molecular structures (Poodry and Edgar 1979). The molecular composition and function of T-bars remains unclear, although studies of larval NMJs indicate a correlation between the number of T-bars and the strength of synaptic transmission (Jia et al. 1993; Stewart et al. 1996). In NMJs of the moth *Manduca* or the lobster *Homarus* which are similar to neuromuscular synapses of *Drosophila* (by criteria of conventional transmission electron microscopy), freeze-fracture analysis reveals T-bars to be associated with clusters of particles within the presynaptic membrane (arrow in Fig. 3B,C; Rheuben and Reese 1978; Rheuben 1985; Walrond et al. 1993). Similar electron-dense presynaptic particles have been found in vertebrates and other invertebrates. They are thought to be calcium channels which, upon stimulation, mediate the presynaptic high calcium peaks that induce exocytosis of synaptic vesicles (Heuser et al. 1979; Pumplin et al. 1981; Robitaille et al. 1990; Llinás et al. 1992). Therefore, T-bars may contain intracellular portions of calcium channels and/or molecules involved in their clustering, and might thus be a key element of synaptic release (Rheuben 1985; Walrond et al. 1993). Alternatively, T-bars might be involved in the trapping and clustering of vesicles at the active zone, as vesicles often appear to be physically attached to T-bars (Fig. 3A; Osborne 1966; Koenig and Ikeda 1996). Similarly, vesicles cluster at presynaptic dense material of vertebrate NMJs, and loss of this dense material (in *s-laminin* mutant mice) correlates with a failure of vesicle clustering (Noakes et al. 1995). A third potential function of T-bars might be an involvement in one specific pathway of reformation of synaptic vesicles: In photoreceptor neurons synaptic vesicles bud off the presynaptic membrane ei-

[1] This may be different for some imaginal NMJs; for example in the moth some NMJs show wrapping by glial processes (Stocker and Nüesch 1975; Rheuben and Reese 1978).

[2] At the larval NMJ there are two pools of vesicles, a readily releasable pool at the active zone and an actin- and *shibire*-dependent reserve pool located in the centre of the bouton (Wu and Bellen 1997; Kuromi and Kidokoro 1998). In late embryonic boutons vesicles tend to concentrate mainly at the active zone (Broadie et al. 1995; see Fig. 2).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ther below the T-bar or extrasynaptically, and both populations can be distinguished (Koenig and Ikeda 1996). In contrast to extrasynaptic reformation, the vesicles at T-bars form more rapidly, without cisternal intermediates and can be blocked by high-Mg^{2+}/low-Ca^{2+} solution.

A further feature of neuromuscular synapses in *Drosophila* embryos and larvae is the presence of regularly structured electron-dense material in the synaptic cleft over a distance of several hundred nanometres (between bent arrows in Fig. 3). In transverse sections a continuous fine line can be seen in the middle of the cleft, and a thick dashed line closely associated with the postsynaptic membrane (Fig. 3A). Oblique sections through the synaptic cleft show a patch of honeycomb-like pattern (Fig. 3D). Freeze-fracture analyses of comparable neuromuscular synapses in the moth *Manduca* and the lobster *Homarus* reveal areas of stippled material in the external leaflet of the muscle membrane opposite the T-bar, suggesting that the structured material in the cleft is of postsynaptic nature and anchors in the muscle membrane (black arrowheads in Fig. 3B,C,E; Rheuben and Reese 1978; Walrond et al. 1993). Synaptic proteins likely to form part of these postsynaptic structures are transmembrane molecules like the ionotropic glutamate receptors, voltage-gated Shaker potassium channels and the cell adhesion molecule (CAM) Fasciclin 2 (Fas2; Fig. 4C; Broadie and Bate 1993c; Saitoe et al. 1997; Tejedor et al. 1997; Thomas et al. 1997a; note that Fas2 is expressed also on the presynaptic side). Interestingly, the honeycomb-like pattern in oblique NMJ sections is very similar to patterns seen in oblique sections through another type of cell junction, the so-called pleated septate junction (Fig. 2S vs. U; Baumgartner et al. 1996). In transverse sections of pleated septate junctions transverse lines can be seen spanning the cleft every 20–25 nm, and this frequency of repetition is similar to that of the thick dashed line at NMJs (Fig. 2R vs. T). In spite of a similar organisation, the extracellular material at pleated septate junctions looks different from that at NMJs and is likely to include Neurexin IV, an unusual member of the Neurexin family of proteins which has not been found at NMJs (Baumgartner et al. 1996). However, common cytoskeletal elements might arrange the different structural elements at both junction types into similar honeycomb-like patterns. A likely candidate for such an organisational cytoskeletal element is the Discs large (Dlg) protein, which is localised both at NMJs and pleated septate junctions and has been shown to organize transmembrane proteins at junctional sites (Budnik 1996).

Fig. 2A–U Phenotypes of NMJs, central synapses and other release sites. All images except **E,G,H** and **N** are late-stage 17; **A–E** light-microscopic images of NMJs stained with synaptic markers (**A** anti-Kakapo, **B–D** anti-Synaptotagmin, **E** anti-cysteine string protein; see Table 1); **F–M** ultrastructural images of boutons; **N–U** details of synaptic or junctional structures. **A,B** NMJs (*black arrowheads*) on different muscles show characteristic shape differences (**A** dorsal, **B** ventral muscle field). **C,D** NMJs in mutant embryos (same area as in **B**): *kakapo* mutant NMJs are reduced **(C)**, *myoblast city* mutant muscles and NMJs are reduced but ectopic presynaptic terminals form in the area **(D)**. **E** NMJ boutons and branches have grown at late larval stages (same NMJ as in B); unstained line (*bent black arrow in close-up*) represents most likely vesicle-free axolemma (cf. **G**). **F** At late embryonic NMJs, neuronal boutons (*) are half covered by basement membrane (*open arrowhead*) and half attached to muscle (*mu*) via an unspecialised junction (*small open arrow*), which is interspersed with synapses (*black arrow*; cf. **R**). **G** At late larval NMJs boutons are completely surrounded by folds of the muscle membrane (*SSR*) and enriched with vesicles, except in the axolemma (*bent black arrows* indicate microtubules in axolemma; cf. **E**). **H** At late stage 16, the neuromuscular cell junction (*small open arrow*) consists of only short stretches of apposed neuronal and muscle surfaces and harbours immature synapses (cf. **N**). **J** *laminin*A mutant NMJs lack basement membrane attachment and also neuromuscular contact is reduced. **K** At *mef2* mutant NMJs boutons are separated from the muscle by basement membrane (*open arrowhead*) and presynaptic structures are mislocalised (*black arrows*). **L** Classical neurohemal release site (*black arrow*) of a peptidergic varicosity with dense-cored vesicles attached to muscle, most likely corresponding to larval-type III boutons (Jia et al. 1993; Martínez-Padrón and Ferrús 1997). **M** Synapses in the central nervous system. **N** Neuromuscular synapse at late stage 16 exhibiting a presynaptic dense body with clustered vesicles and a stretch of electron-dense membranes. **O** Neuroneural synapse outside the CNS with presynaptic T-bar (see also Yoshihara et al. 1997). **P** Central synapse with postsynaptic densities (*bent white arrows*). Note that synapses in **N–P** lack structured material in the synaptic cleft (in contrast to R). **Q** Neurohemal active zone in *myoblast city* mutant embryo covered by basement membrane (*open arrowhead*; rare cases exist also in wild type; Prokop et al. 1996). **R,S** Neuromuscular synapse with structured material in the cleft (*between white arrows in* **R**), which appears as a honeycomb-like pattern in oblique sections (*white arrow in* **S**). **T,U** Ribbons of septa (*between white arrows*) in synaptic cleft of epidermal pleated septate junctions (*pSJ*) are similarly spaced as cleft material at NMJs (cf. **T** with **R**), and look similar in oblique sections (cf. **U** with **S**) [*bent black arrows* core of the bouton (axolemma), *bent white arrows* postsynaptic density, *DO/DA* dorsal oblique and acute muscles, *black arrows* synapses or release sites, *black arrowheads* boutons, *asterisks* presynaptic or releasing boutons, *ep* epidermis, *kak kakapo* mutant embryo, *L3* third larval instar, *mbc*, *myoblast city* mutant embryo, *mu* muscle, *open arrowheads* basement membrane, *pSJ* pleated septate junction, *small open arrows* extrasynaptic cell junction, *SSR* subsynaptic reticulum, *st.16* embryonic stage 16, *VL* ventral longitudinal muscles, *VO* ventral oblique muscles, *white arrows* demarcate or indicate material in junctional cleft, *wt* wild type]. Muscle nomenclature as in Bate 1993. *Scale bars* 15 µm (**A–E**), 480 nm (**F–M**), 255 nm (**N–U**)

Formation of the neuromuscular junction

Initiation of neuromuscular contact

In *Drosophila*, NMJ formation has been studied most thoroughly on the ventral longitudinal muscles VL3 and VL4 (nomenclature according to Bate 1993). However, all or most embryonic NMJs might develop in a similar way, as they are glutamatergic and share a common structural organisation. At stage 15/16 muscles send out filopodia preferentially from the future site of innervation (Suzuki et al. 1999), and these are contacted by filopodia sent out from the motoneuronal growth cones in a random fashion (Fig. 4A). Those motoneuronal processes which are in contact with inappropriate muscles are soon withdrawn (Broadie et al. 1993; Yoshihara et al. 1997). Selection for the right muscle is thought to be mediated by repulsion from inappropriate and contact-medi-

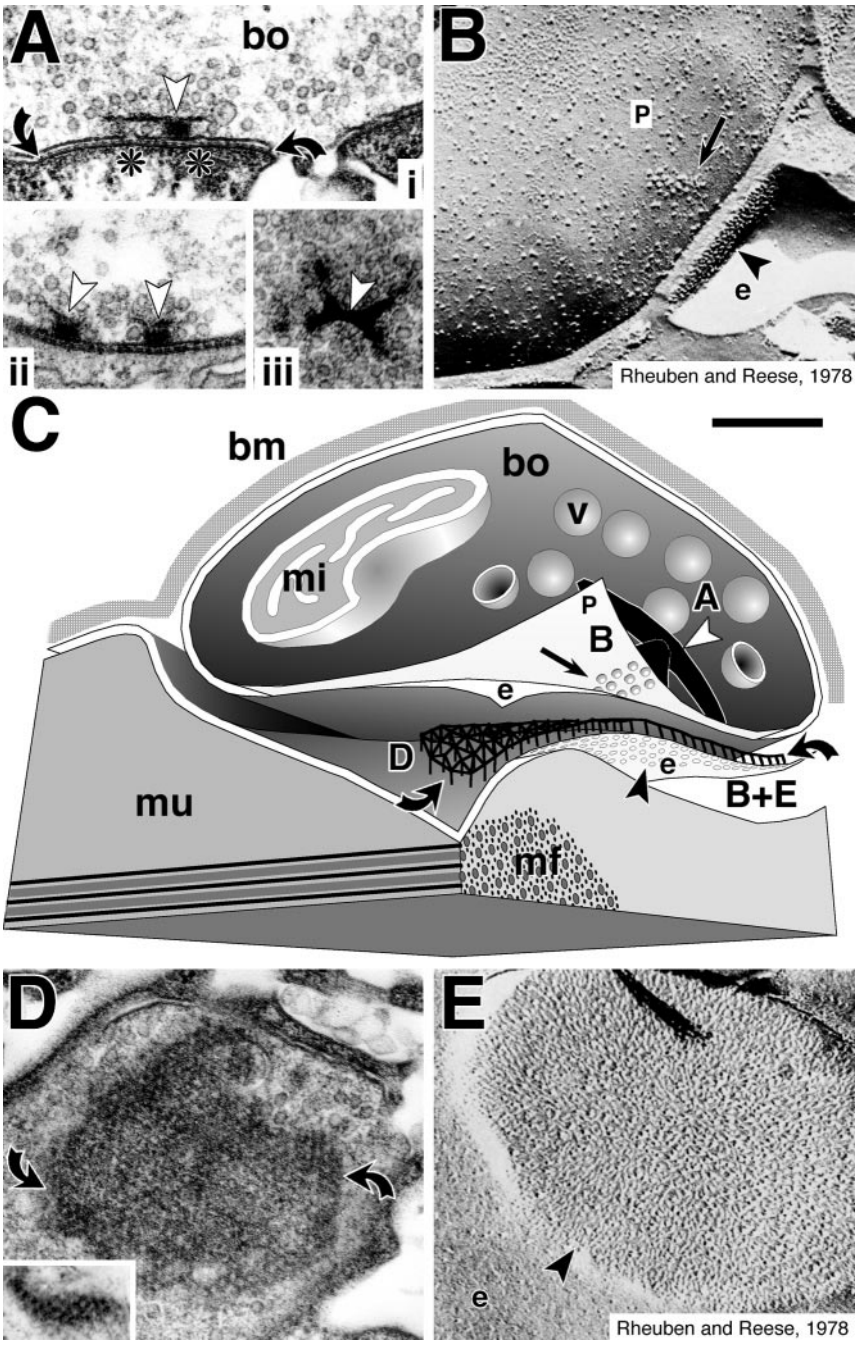


Fig. 3A–E Ultrastructural details of neuromuscular synapses. Images show details of neuromuscular synapses analysed with conventional transmission electron microscopy in *Drosophila* (**A,D**) or freeze-fracture technique in *Manduca* **(B,E). C** Schematic representation of an attachment between bouton (*bo*) and muscle (*mu*) opened up on two sides towards the front. The muscle exhibits myofilaments (*mf*), the bouton contains mitochondria (*mi*), vesicles (*v*) and T-bars (*white arrowhead*), and both are covered by basement membrane (*bm*). Pre- and postsynaptic membranes are partially unfolded, and external (*e*) and protoplasmic (*p*) membrane leaflets shown. Positions of the plates **A,B,D** and **E** are indicated in the scheme; chosen symbols indicate the same distinct structures throughout the figure. **A** Transverse section through a larval (*i*) or late embryonic (*ii*) contact between neuronal bouton (*bo*) and muscle (*bottom*), forming synapses with similar dimensions and features (width of the synapse defined by *a dashed line* of material in the synaptic cleft; *between bent arrows*). *White arrowheads* indicate presynaptic T-bars (with a roof and a stem) surrounded by synaptic vesicles (* indicates fusion or reformation events of vesicles at the presynaptic membrane). Obliquely sectioned T-bars (*iii* larval example) reveal the pronged nature of their horizontal roof. **B** Freeze-fracture images of comparable synapses in *Manduca* show concentrations of membrane particles (*black arrow*) on the protoplasmic membrane leaflet (*p*) believed to be accumulations of calcium channels at the base of T-bars. **D** Oblique sections through the dashed material in the synaptic cleft of the late larva or late embryo (*inset*) reveal honeycomb-like patterning of the material (*between bent arrows*; see also Fig. 2S). **E** Freeze-fracture images of the external leaflet of the postsynaptic membrane reveal oval particle fields (*black arrowhead*, cf. B), suggesting that the extracellular material may be anchored in the postsynaptic membrane. (**B** and **E** reprinted with permission from Rheuben and Reese 1978). *Scale bar* 300 nm

ated attraction to the right target muscle (Fig. 4A; reviewed in Bate and Broadie 1995; Keshishian et al. 1996). For example, the RP3 neuron grows past Toll-expressing muscles VO4 and VO5 to innervate the non-Toll-expressing muscles VL3 and VL4. Taking away expression of the transmembrane molecule Toll from VO4 and VO5 causes some extra branching in that area, whereas misexpression of Toll on VL3 and VL4 will repel the RP3 motoneuron and retard the neuromuscular contact (Rose et al. 1997). In contrast, the homophilic CAM Fasciclin 3 (Fas3) is specifically expressed on the RP3 motoneuron and at the future innervation site on muscles VL3 and 4. Ectopic expression of Fas3 on neighbouring muscles is sufficient to redirect RP3 innervation to these non-target cells, but not if Fas3-expression is eliminated on the RP3 growth cone (Kose et al. 1997). Thus, Fas3 appears to function as a homophilic synaptic recognition molecule mediating attractive cell-cell interactions between RP3 and muscles VL3/4 (stippled lines in Fig. 4A). However, loss of function of Fas3 has no apparent effect on targeting and innervation, and this might be explained by the fact that the target recognition code is redundant (see, for example, Speicher et al. 1998). Observations similar to those of Fas3 have been made for Connectin, another homophilic CAM specifically expressed on a different subset of muscles and neurons (Nose et al. 1992; Raghavan and White 1997). Thus, specificity of innervation is believed to involve combinatorial functions of several cell-specific CAMs or repellents. In addition, this first specific contact requires general factors, such as the transmembrane protein commissureless on all muscles (not shown in Fig. 4). In the absence of commissureless the motoneurons stall in close vicinity of their target muscles or project beyond (Wolf et al. 1998). Once a tolerable neuromuscular contact is established, its further differentiation appears to depend on properties which appear to be common to most or even all muscles and motoneurons. This is suggested by the observation that persistent functional NMJs differentiate, even when motoneurons are misrouted to contact wrong muscles (Keshishian et al. 1996; Kose et al. 1997).

Adhesive properties at the differentiating neuromuscular contact

If particular combinations of specifically expressed CAMs participate in the neuromuscular recognition code, they might also mediate adhesion at newly formed neuromuscular cell junctions (connected rectangles in Fig. 4B). Usually these junctions consist of short stretches of apposed membranes, often interrupted by stretches of non-connected cell surfaces (Schuster et al. 1996b; Yoshihara et al. 1997; Prokop et al. 1998a). Such contacts develop into an extended cell junction at the mature NMJ of late stage 17 embryos (Fig. 4B vs. C), suggesting that adhesive properties change during NMJ differentiation. Micrographs of vertebrate NMJs in vivo and in vitro also show an initially narrow and often discontinuous junctional cleft of about 10–20 nm width, which later on widens to about 50 nm and accumulates a basement membrane (Kullberg et al. 1977; Takahashi et al. 1987; Hall and Sanes 1993). This clearly indicates a molecular reorganisation of cell adhesion properties. At the *Drosophila* NMJ, the width of the junctional cleft does not change, but nevertheless molecular changes of adhesive properties occur during NMJ maturation, as suggested by several observations:

During NMJ maturation, expression of Fas3, which potentially contributes to the early phase of adhesion, vanishes from neurons, muscles and NMJs (Broadie and Bate 1993c). Connectin vanishes from the extrajunctional muscle surfaces but remains in the neuron, and staining at the NMJ persists into late larval stages (M. Landgraf, personal communication; own observations). This could either mean a complete downregulation of Connectin postsynaptically, or that Connectin becomes restricted to the postsynaptic site (Fig. 4C). Furthermore, the homophilic CAM Fas2 is initially expressed strongly on the surfaces of all motor axons and at low levels on all muscles but, during NMJ formation, Fas2 is progressively restricted to the NMJ in pre- and postsynaptic cells (Schuster et al. 1996b). The late neuromuscular restriction of Fas2 is mediated by the cytoskeletal element Discs large (see later; Thomas et al. 1997a; Zito et al. 1997; Fig.4C), which itself is not detectable at the NMJ until late stage 17 (Guan et al. 1996). Deleting Fas2 function does not affect NMJ formation in the embryo but strongly affects postembryonic stabilisation and maintenance of NMJs, suggesting a late requirement for Fas2[3] (Schuster et al. 1996b). Even more, it seems to be of developmental importance that Fas2 function is restricted to the late phase of NMJ differentiation: Overexpression of Fas2 in muscles during the early phase of NMJ formation seems to render muscle surfaces too „sticky“ and interferes with neuromuscular target recognition, in that ectopic motoneuronal branches (which normally occur only transiently) become trapped and form NMJs on inappropriate muscles (Davis et al. 1997). Taken together, NMJ differentiation appears to involve dynamic but regulated changes in expression and localisation of CAMs, and different CAMs appear to serve distinct functions at different times during this process.

A second argument in favour of a switch in adhesive properties at the NMJ comes from analysis of *mef2* mutant embryos, where the initial NMJ contact can be genetically separated from the late phase of neuromuscular adhesion. In *mef2* mutant embryos, muscle founder cells (FC in Fig. 1) form and express muscle-specific markers including the CAMs Connectin and Fas3, and motoneurons establish contact with their appropriate target cells (Prokop et al. 1996). However, the muscle founder cells remain immature and fail to acquire properties of general

[3] During larval life the adhesion of Fas2 not only stabilises NMJs but even inhibits their further growth, and postembryonic growth at the NMJ is promoted by a modest downregulation of Fas2 (Schuster et al. 1996a).

muscle differentiation such as contractile filaments, their proper attachment to the epidermis, and their ability to fuse into multinucleate fibres. Micrographs of late-stage 17 *mef2* mutant embryos never revealed proper cell junctions between motoneurons and improperly differentiated muscle fibres, but instead their cell surfaces are covered by basement membrane (Fig. 2 K; Prokop et al. 1996). This phenotype could be explained by loss of *mef2*-dependent late synaptic CAMs, directly maintaining the neuromuscular cell junction. Alternatively, *mef2*-function might be required to exclude basement membrane receptors from the NMJ (e.g. via synapse-specific cytoskeletal properties), thus preventing separation of the pre- and postsynaptic membranes by competitive invasion of basement membrane material (white arrowheads in Fig. 4B).

So far, basement membrane receptors have not yet been identified. Loss of cell surface adhesion to basement membrane in *laminin A* mutant embryos suggests that basement membrane receptors bind to Laminin or Laminin-dependent extracellular matrix components (Prokop et al. 1998a). In addition, lack of Laminin A leads to a partial detachment of neuromuscular boutons[4] (Fig. 2J), even though basement membrane is normally absent from the narrow neuromuscular cleft in *Drosophila* (Fig. 3A; Prokop et al. 1998a). This suggests that a thin layer of Laminin in the synaptic cleft might exist and mediate cell adhesion via appropriate receptors at the NMJ. Alternatively, basement membrane, which spans over the neuronal terminal and adheres closely to surrounding muscle surfaces, might press the terminal against the muscle (Fig. 4B,C). If this were the case, weak adhesion at the neuromuscular cleft during the process of NMJ formation would suffice (provided *mef2*-dependent factors kept basement membrane receptors away from the NMJ; white arrowheads in Fig. 4B). Low neuromuscular adhesion would facilitate the extensive reorganisation of the terminal's shape during NMJ formation. This would be consistent with the fact that plastic reshaping of neuronal terminals in *Drosophila* larvae or in the sea slug *Aplysia* is at least partly dependent on a decrease in synaptic CAMs, i.e. a reduction in cell adhesion (Schuster et al. 1996a; Thompson et al. 1996; Bailey et al. 1997).

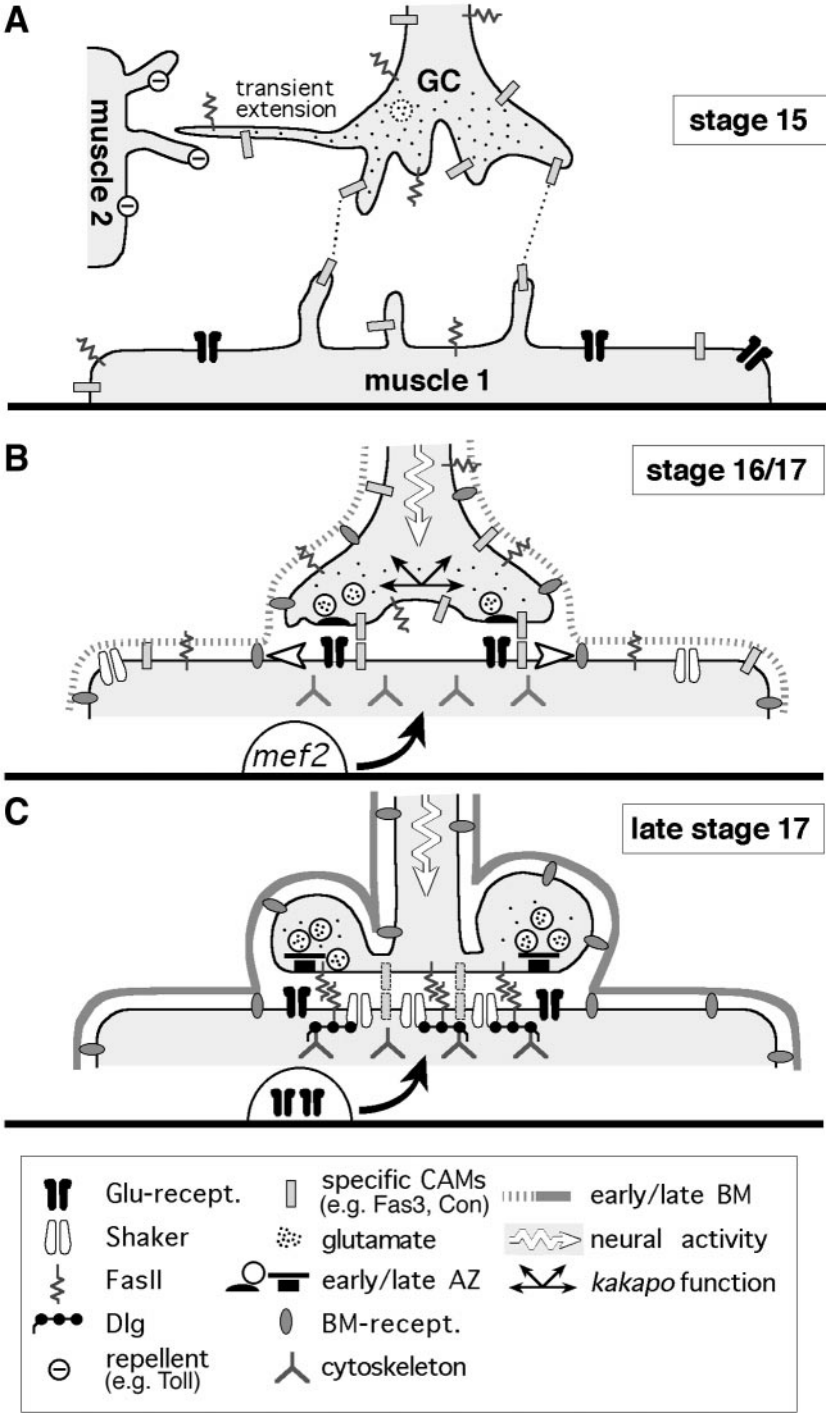
Differentiation of nerve terminal shape and size

Differentiation and shape changes of growth cones into bouton-forming terminals take place during 4–5 h after initial neuromuscular contact at early stage 13 (Broadie and Bate 1993c; Yoshihara et al. 1997). These changes appear to be accelerated or facilitated by *late bloomer* gene function, which encodes a member of the tetraspanin family of receptor-complex-associated proteins. The *Drosophila late bloomer* protein is localised in motoneuronal axons (Kopczynski et al. 1996), suggesting that motoneurons receive signals. Such signals might come from the target muscles, inducing the differentiation process of the presynaptic terminal (not shown in Fig. 4). In agreement with this interpretation, retrograde signalling at the onset of NMJ differentiation has been described in other systems. For example, at the vertebrate NMJ muscle-released Agrin seems to serve such a function (Rüegg and Bixby 1998). Also certain *Heliosoma* motoneurons in culture show presynaptic calcium influx upon contact with appropriate muscle fibres (Funte and Haydon 1993; Zoran et al. 1993). However, in the case of *Drosophila*, loss of *late bloomer* function only delays NMJ differentiation but does not block it (Kopczynski et al. 1996), suggesting that a potential muscle-derived signal might only be of minor importance. This is in accordance with other findings that differentiation of bouton-like motoneuronal structures can take place even in the complete absence of muscles (Prokop et al. 1996).

Also the size of the motoneuronal terminal appears relatively independent of the target muscle. This is suggested by observations in *myoblast city* mutant embryos, where myoblast fusion is blocked due to lack of *myoblast city* function within the muscle (Erickson et al. 1997). As a result *myoblast city* mutant muscles are mononucleated and much smaller, and also NMJ size is severely reduced. However, the motoneuronal terminals do not adjust to this fact, but instead grow ectopic branches which contain displaced presynaptic structures

Fig. 4A–C Summary model of embryonic NMJ formation in *Drosophila.* The scheme shows the situation before contact **(A)**, in the immature **(B)** and the mature junction **(C)**. Stages are indicated *top right*, symbols *in the box below*. **A** At about stage 15 the growth cone (*GC*) approaches the muscle, both forming filopodial extensions and expressing cell-specific cell adhesion molecules (specific CAMs) and non-specific Fas2 (*Fas2*). Repellents (on muscle2) prevent establishment of false contact and transient extensions are retracted. Early CAMs (on growth cone and muscle1) might attract each other (*stippled lines*) during the process of target recognition. Glutamate (*little dots*) is detectable in the terminal around the time of contact formation and vesicles (*stippled circle*) must form soon, as first transmission occurs within 30 min after neuromuscular contact. **B** The early cell junction might be formed by the specific CAMs involved in target recognition (*connected rectangles*). Basement membrane (*BM*) forms and adheres to receptors on muscle and neuron (*BM-recept.*), perhaps holding the presynaptic terminal against the muscle surface. Basement membrane appears to be excluded from the synaptic cleft (*white arrowheads*) by a function downstream of the transcription factor *mef2* (*arrow from nucleus*). Glutamate receptors (*Glu-recept.*) are initially evenly distributed but, upon neuromuscular contact, cluster in response to presynaptic electrical activity (*zigzag arrow*). *kakapo* function mediates branching of the presynaptic terminal, and active zones (*AZ*) start assembling. **C** Specific CAMs either fade from the NMJ or they become restricted to the NMJ (*stippled rectangles*). Fas2 is restricted to the NMJ pre- and postsynaptically and appears to manifest the status of the synapse at late embryogenesis. On the postsynaptic side, Fas2 is clustered, together with Shaker channels, by Discs large (*Dlg*). Dlg might localise to the NMJ via binding to synapse-specific cytoskeletal elements (*three-pronged stars*; so far purely hypothetical) or to synaptic transmembrane proteins (not shown). The amount of Glu-Rs is upregulated as a function of presynaptic electrical activity (*arrow from nucleus*)

[4] Reduction of neuromuscular contact was also found in s-laminin mutant mice; s-laminin is part of the basement membrane within the ca. 50-nm-wide neuromuscular cleft and might well mediate adhesion to synaptic receptors (Noakes et al. 1995).



(Fig. 2D,Q; Prokop et al. 1996). A comparable intrinsic determination of neurons to elaborate a typically sized terminal has been demonstrated in vivo for sensory neurons of crickets, cockroaches and *Drosophila* (Murphey and Lemere 1984; Bacon and Blagburn 1992; Canal et al. 1998) and in vitro for motoneurons of crayfish (Arcaro and Lnenicka 1995; Zoran et al. 1996).

Like in *myoblast city*, *kakapo* mutant embryos show severely reduced NMJs (and reduced dendritic trees in the central nervous system; see Fig. 2C for NMJ phenotype). In contrast to *myoblast city*, embryos carrying strong alleles of *kakapo* have normal-sized muscles and motoneuronal terminals form no ectopic branches. These observations suggest a presynaptic requirement for *kakapo* function and, accordingly, anti-Kakapo antisera detect the protein at motoneuronal terminals (Prokop et al. 1998b). Cloning data suggest that *kakapo* encodes a cytoskeletal element with actin-binding properties (Gregory and Brown 1998; Strumpf and Volk 1998). Further molecular components involved in the growth of the em-

bryonic *Drosophila* NMJ are unknown. As insects appear to lack intermediate filaments (Bartnik and Weber 1989), growth and maintenance of the motoneuronal terminal are likely to involve mainly actin and tubulin and those elements interacting with these structural proteins (Caroni 1997; Suter and Forscher 1998).

Synapse formation at the NMJ

In addition to nerve terminal attachment to target muscles, the assembly of synaptic structures has to be induced at the neuromuscular contact zones and pre- and postsynaptic sites have to be precisely aligned. A hypothetical model of this process would envisage cleft-spanning adhesion molecules to function as tags which localise additional synaptic proteins. For example, at central synapses in vertebrates, Neuroligins and Neurexins (both transmembrane proteins) seem to bind to each other across the synaptic cleft and associate intracellularly with protein complexes of synaptic proteins such as voltage- or transmitter-gated channel subunits (Irie et al. 1997; Missler and Südhof 1998). An essential linker protein within these complexes is the postsynaptic density protein PSD-95, which contains three protein-binding PDZ (common domain of PSD-95, Dlg and ZO-1) domains. While channel proteins bind to the first two PDZ domains (PDZ1, PDZ2), Neuroligins bind to the third PDZ domain (PDZ3) of PSD-95. The third PDZ domain of PSD-95 can additionally bind to CRIPT (cysteine-rich interactor of PDZ3), a factor which seems to interact with the microtubule cytoskeleton (Niethammer et al. 1998). Thus, PDZ domain proteins potentially function as linkers between: (1) cytoskeleton (see also Allison et al. 1998), (2) components involved in transmission, and (3) membrane-spanning proteins which bind to similar complexes on the other side of the synaptic cleft. Such complexes could explain the aggregation and alignment of pre- and postsynaptic components at the precisely apposed sites.

Formation of postsynaptic structures of *Drosophila* muscles

At the postsynaptic face of the *Drosophila* NMJ, voltage-gated Shaker channels and the CAM Fas2 have similarly been shown to bind to the PSD-95 homologue Discs large (Dlg) in vivo and in vitro (Fig. 4C). As Fas2 molecules are likely to bind to each other across the synaptic cleft, they seem good candidates to localise Dlg at the NMJ. However, the PDZ1 and PDZ2 domains of Dlg to which Fas2 (and Shaker) bind are not required for the localisation of Dlg (Tejedor et al. 1997; Thomas et al. 1997a; Zito et al. 1997). Instead, the PDZ3 domain and/or the hook domain of Dlg are potentially involved in the localisation of Dlg to the NMJ (Tejedor et al. 1997; for parallel studies of Dlg localisation in the epidermis see also Hough et al. 1997). The hook domain may bind to actin-associated proteins of the protein4.1/ERM (common domain of Ezrin, Radixin and Moesin) family (Hough et al. 1997). PDZ3-binding proteins have not yet been identified in *Drosophila*, but might be homologous to transmembrane proteins such as Neuroligins or cytoskeletal components like CRIPT. Thus, Fas2 is not the initial targeting factor for Dlg, but Dlg function is required for the targeting of Fas2. Therefore, earlier events are likely to occur at the neuromuscular site, most likely installing tag molecules to which Dlg can bind (three-pronged stars in Fig. 4B,C). This is in agreement with the finding that NMJ-specific localisation of Fas2 and Dlg occurs relatively late (as discussed above), several hours after transmission is first detectable at the NMJ (Broadie and Bate 1993c). Thus, the *dlg* functions uncovered so far appear to be of little importance to embryonic but they are crucial for postembryonic NMJ development.[5]

What are the events essential to embryonic NMJ formation, preceding *dlg*-mediated clustering at the NMJ? Prior to innervation the muscles express glutamate receptors (GluRs), which are evenly distributed over the muscle membrane (Fig. 4A; Broadie and Bate 1993c; Currie et al. 1995; Saitoe et al. 1997). Clustering of GluRs at the postsynaptic site is one of the first events of synapse formation, and GluR clustering does not require Dlg (Fig. 4B; Broadie and Bate 1993c; Peterson et al. 1997; Saitoe et al. 1997). The incoming neuron instructs GluR clustering (Broadie and Bate 1993d) and this function is impaired if sodium-based action potentials of the neuron (zigzag arrow in Fig. 4; *Drosophila* muscles exhibit calcium currents) are blocked genetically or with tetrodotoxin (Broadie and Bate 1993a; Saitoe et al. 1997). Glutamate immunoreactivity can be detected in neuronal terminals around the time of first neuromuscular contact (small dots in Fig. 4A), and transmission sets in approximately 30 min thereafter (Johansen et al. 1989b; Broadie and Bate 1993c). Thus, early transmission may trigger GluR clustering. However, in manipulated or mutant embryos which lack transmission but not action potentials, the clustering of GluRs occurs and synapses form normally (Broadie et al. 1995). Therefore, GluR clustering at the postsynaptic site is neither merely contact mediated nor simply dependent on early glutamate release. The molecular mechanisms that underlie this process are subject to speculation. For example, presynaptic calcium signalling through voltage-gated calcium channels might be involved. A glance at cholinergic NMJs of vertebrates does not provide further insights: clustering of acetylcholine receptors at vertebrate NMJs does not require action potentials (Dahm and Landmesser 1991), but is instead dependent on release of presynaptic Agrin (Rüegg and Bixby 1998), which has so far not been identified in *Drosophila*.

Apart from clustered GluRs and Shaker channels, further channel proteins are inserted into the muscle mem-

[5] Note that taking away *dlg* function transiently during embryogenesis and reinstalling it afterwards causes postembryonic structural defects which become apparent more than a day later (Guan et al. 1996).

Table 1 Gene products and transmitters localised or expected to be at developing or mature synapses of *Drosophila*. This table lists more than 100 components found in *Drosophila*, which have either been shown to be localised at synapses or which are potentially synaptic. Some of these components are found at synapses as well as in extrasynaptic regions, others are specific to synapses but do not discriminate between different synapse types, but some are localised just at specific synapse classes (not distinguished). Synaptic components comprise: (a) molecules involved in synaptic architecture (e.g. clustering of synaptic elements, adhesion or shape), (b) molecules conferring the electrical properties to cell membranes, (c) releasable chemical substances, i.e. transmitters and neuropeptides, (d) proteins involved in synthesis, destruction or reuptake of transmitters, (e) components required for the trafficking, fusion and recycling of synaptic vesicles, (f) metabotropic and ionotropic receptors for transmitters or neuropeptides, (g) components involved in signalling and second messenger pathways, (h) a protein required for gap junctions at electrical synapses, and (i) several other components of yet undefined function. Most of these components are expected to be gene products [except biogenic amines and amino acids in (f)], and in many cases genes have been assigned (*in italics*). Abbreviations (*in brackets*) and/or full names are given *on the left side* and correspond to the nomenclature used in Flybase (Flybase 1998). Only one reference per component is given due to space limitations, but further references can be looked up in Flybase (http://flybase.bio.indiana.edu)

a) Adhesion/clustering/structure	*Fasciclin 1* (*Fas1*)	[1]
	Fasciclin 2 (*Fas2*)	Immunoglobulin superfamily (N-CAM) [2]
	Fasciclin 3 (*Fas3*)	Immunoglobulin superfamily [3]
	Toll (*Tl*)	Leucine-rich family [4]
	Connectin (*Con*)	Leucine-rich family [5]
	commissureless (*comm*)	Transmembrane protein [6]
	Neurotactin (*Nrt*)	Inactive esterase dom. (like neuroligin) [7, 8]
	Cadherin-N (*CadN*)	Neuronal form [9]
	discs large 1 (*dlg 1*)	MAGUK protein, PSD-95 homologue [10]
	Ca/calm. dep. prot. kin. (*Caki*)	MAGUK, CASK homologue [8, 11]
	kakapo (*kak*)	Actin-binding protein [12]
b) Electrical membrane properties	*ether a go-go* (*eag*)	Voltage-gated K channel [13]
	Shaker (*Sh*)	Voltage-gated K channel [14]
	hyperkinetic (*Hk*)	β-Subunit to Shaker [15]
	Shaker cognate b (*Shab*)	Voltage-gated K channel [16]
	Shaker cognate l (*Shal*)	Voltage-gated K channel [16]
	Shaker cognate w (*Shaw*)	Voltage-gated K channel [16]
	seizure (*sei*)	(=*erg*), voltage-gated K channel [17]
	(*Ca-α1D*)	Voltage-gated Ca channel α_1-subunit D [18]
	nightblind A (*nbA*)	(=*cac*) Ca-channel, α_1-subunit [19]
	paralytic (*para*)	Voltage-gated Na channel [20]
	slowpoke (*slo*)	Ca-gated K channel [21]
	temp.-induced paral. (*tipE*)	Membr. prot. interact. with *para* [22]
	Nervana 1+2 (*Nrv1, 2*)	ATP-dependent Na/K pump [23]
c) Transmitter/neuropeptides	Glutamate	Amino acid [24]
	Histamine	Biogenic amine [25]
	Serotonin	Biogenic amine [26]
	Octopamine	Biogenic amine [27]
	GABA	Biogenic amine [28]
	Nitric oxide	[29]
	amnesiac (*amn*)	PACAP-like peptide [30]
	FMRF	Cardioexcitatory neuropeptide [31]
	Small cardioactive peptide	Neuropeptide [32]
	Substance P	Neuropeptide [33]
	Corazonin (*Crz*)	Neuropeptide [34]
	(*CAP-2b*)	*Cardioaccel. pept.-2b* [35]
	Bombyxin (*Bom*)	Neuropeptide [36]
	Prothoracicotr. horm. (*Ptth*)	Neuropeptide[36]
	Diuretic hormone (*DH*)	Neuropeptide [36]
	Allatotropin (*Atn*)	Neuropeptide [36]
	Allatostatin (*Ast*)	Neuropeptide [36]
	Proctolin	Neuropeptide [37]
	Insulin-like peptide	Neuropeptide [38]
	Leucokinin I	Neuropeptide [39]

Table 1 continued

d) Transmitter metabolism	*Choline acetyl transf.* (*Cha*)	ACh synthesis [40]
	Acetylchol. esterase (*Ace*)	ACh removal [41]
	glut. acid decarb. 1 (*Gad1*)	GABA synthesis [42]
	glut. acid decarb. 2 (*Gad2*)	GABA synthesis [43]
	inebriated (*ine*)	Non-vesicular GABA (?) transporter [44]
	pale (*ple*)	(=TH); catecholamine synthesis [26]
	Dopa decarboxylase (*Ddc*)	Serotonin+catecholamine synthesis [45]
	Henna (*Hn*)	(=Pah) serotonin (?) synthesis [46, 47]
	α-methyl dopa-resist. (*amd*)	Similar to *Ddc* [48]
	Serotonin transp. (*SerT*)	Non-vesicular transporter [49]
	Histidine decarbox. (*Hdc*)	Histamine synthesis [50]
	Tyrosine decarboxylase	Octopamine synthesis [51]
	Tyramine β-hydrox. (*Tbh*)	Octopamine synthesis [52]
	Nitric oxide synth. (*Nos*)	[53]
e) Vesicle storage, release, recycling	*syntaxin 1A* (*syx1A*)	Core complex, cell membrane [54]
	n-synaptobrevin (*n-syb*)	Core complex, vesicular [55]
	synaptobrevin (*syb*)	Ubiquitous form of *syb* [56]
	Synapse prot. 25 (*Snap25*)	Core complex, cell membrane [57]
	synaptotagmin (*syt*)	Endocytosis+exocytosis, vesicular+cell membrane [58]
	Synapsin-1 (*Syn*)	Vesicular [59]
	Ras opposite (*Rop*)	sec1, munc-18; cell membrane [60]
	Sol. NSF att. prot. (*Snap*)	α-SNAP, soluble [61]
	comatose (*comt*)	NSF, soluble [62]
	Rab-protein 3 (*Rab3*)	Vesicular [56]
	Cysteine string prot. (*Csp*)	[63]
	leonardo (*leo*)	14-3-3 α-protein [64]
	α-Adaptin (*α-Adaptin*)	Recycling [65]
	β-Adaptin (*Bap*)	Recycling [66]
	stoned A+B (*stnA, stnB*)	(=*sesC*) recycling [67]
	shibire (*shi*)	Dynamin; recycling [68]
f) Transmitter/neuropeptide receptor	(*nAcRα–7E*)	(=*Dα3*) ionotropic ACh-R subunit [69]
	(*nAcRα–96Aa*)	(=*ALS*) ionotropic ACh-R subunit [69]
	(*nAcRα–96Ab*)	(=*SAD*) ionotropic ACh-R subunit [69]
	(*nAcRβ-64B*)	(=*ARD*) ionotropic ACh-R subunit [69]
	(*nAcRβ-96A*)	(=*SBD*) ionotropic ACh-R subunit [69]
	(*Glu-RIIA*)	Non-NMDA glutamate-R [70]
	(*Glu-RIIB*)	Glutamate-R [71]
	(*Glu-RI*)	Kainate-type glutamate-R [72]
	(*Nmdar1*)	NMDA-type glutamate-R [73]
	(*Glu-RA*)	Metabotropic glutamate-R [74]
	Octopamin-rec. (*Ocr*)	Metabotropic Tyramin/Octopamin-R [75]
	(*Oamb*)	(Metabotropic) Octopamine-R [76]
	(*5-HT1A+5-HT1B*)	2 Metabotropic Serotonin-Rs [77]
	Dopamine-rec. 2 (*DopR2*)	(=*Damb*) [78]
	Insulin-like-Rec. (*InR*)	[38]
	Resistance to dieldrin (*Rdl*)	Ionotropic GABA-R [79]
	(*Lcch3*)	Ionotropic GABA-R, β-subunit [79]
	Glycine rec. (*Grd*)	GABA-+glycine-R-like subunit [80]
	ora transientless (*ort*)	Glutamate-gated chloride channel [81]
g) Signalling/second messenger pathways	*dunce* (*dnc*)	Phosphodiesterase II [82]
	rutabaga (*rut*)	Ca/calmodulin-dependent adenylate cyclase [82]
	Ca/calm. dep. kin. (*CaMKII*)	Ca-binding protein [83]
	Calmodulin (*Cam*)	Ca-binding protein [84]
	Ras-Raf	[85]
	Calbindin 53E (*cbp53E*)	Ca-binding protein [86]
	Frequenin (*Frq*)	Ca-binding protein [87]
	Calcineurin A1 (*CanA1*)	Ca-sensitive Ser/Thr phosph. [88]
	myospheroid (*mys*)	βPS integrin [89, 90]
	multiple edem. wings (*mew*)	αPS1 integrin [90]
	inflated (*if*)	αPS2 integrin [90]
	scab (*scb*)	(=*Volado*); α-integrin [90]
	still life (*sif*)	GDP-GTP exchanger [91]

Table 1 continued

h) Electrical synapse	*shaking B* (*shakB*)	Gap junction component [92]
i) Unknown function	*hikaru genki* (*hig*)	ECM in synaptic cleft [93]
	Small synapt. boutons (*Ssb*)	Cross reaction with *dunce* antibody [94]
	late bloomer (*lbm*)	Tetraspanin [95]
	Syn.-ass. prot. 47kD (*SAP47*)	[96]

1 Zhong Y 1995 *J Neurosci* 15:6679; **2** Harrelson AL 1988 *Science* 242:700; **3** Halpern ME 1991 *J Neurosci* 11:3227; **4** Halfon MS 1995 *Dev Biol* 169:151; **5** Meadows LA 1994 *J Cell Sci* 107:321; **6** Wolf B 1998 *Development* 125:3853; **7** de la Escalera S 1990 *EMBO J* 9; **8** Missler M 1998 *Trends Genet* 14:20; **9** Iwai Y 1997 *Neuron* 19:77; **10** Budnik V 1996 *Curr Opin Neurobiol* 6:858; **11** Martin J-R 1996 *EMBO J* 15:1865; **12** Prokop A 1998 *J Cell Biol*, 143:1283; **13** Warmke J 1991 *Science* 252:1560; **14** Pongs O 1988 *EMBO J* 7:1087; **15** Chouinard SW 1995 *Proc Natl Acad Sci USA* 92:6763; **16** Tsunoda S 1995 *J Neurosci* 15:1741; **17** Wang XJ 1997 *J Neurosci* 17:882; **18** Zheng W 1995 *J Neurosci* 15:1132; **19** Smith LA 1996 *J Neurosci* 16:7868; **20** Salkoff L 1987 *Science* 237:744; **21** Elkins T 1986 *Proc Natl Acad Sci USA* 83:8415; **22** Feng G 1995 *Cell* 82:1001; **23** Sun B 1995 *Proc Natl Acad Sci* 92:5396; **24** Johansen J 1989 *J Neurosci* 9:710; **25** Buchner E 1993 *Cell Tissue Res* 273:119; **26** Beall CJ 1987 *Genes Dev* 1:510; **27** Monastirioti M 1995 *J Comp Neurol* 356:275; **28** Buchner E 1988 *Cell Tissue Res* 253:357; **29** Mueller U 1993 *Naturwissenschaften* 80:524; **30** Feany MB 1995 *Science* 268:869; **31** Schneider LE 1990 *J Biol Chem* 265:6890; **32** Truman JW 1993 In: *The development of Drosophila melanogaster.* Cold Spring Harbor Laboratory Press; **33** Nässel DA 1990 *Brain Res* 507:225; **34** Urban J 1997 *Semin Cell Dev Biol* 8:391; **35** Davies SA 1995 *Am J Physiol* 269:R1321; **36** Zitnan D 1993 *Dev Biol* 156:117; **37** Anderson MS 1988 *J Neurosci* 8:242; **38** Gorczyca MG 1993 *J Neurosci* 13:3692; **39** Cantera R 1992 *Cell Tissue Res* 1992; **40** Itoh N 1986 *Proc Natl Acad Sci USA* 83:4081; **41** Greenspan RJ 1980 *J Comp Neurol* 189:741; **42** Jackson FR 1990 *J Neurochem* 54:1068; **43** Phillips AM 1993 *J Neurochem* 61:1291; **44** Soehnge H 1996 *Proc Natl Acad Sci USA* 93:13262; **45** Schonick SB 1983 *Cell* 34:37; **46** Wright TRF 1987 *Adv Genet* 24:127; **47** Alcaniz S 1997 *Comp Biochem Physiol* 116:205; **48** Stathakis DG 1995 *Genetics* 141:629; **49** Corey JL 1994 *Proc Natl Acad Sci USA* 91:1188; **50** Burg MG 1993 *EMBO J* 12:911; **51** Livingstone M 1983 *Nature* 303:67; **52** Monastirioti M 1996 *J Neurosci* 16:3900; **53** Regulski M 1995 *Proc Natl Acad Sci USA* 92:9072; **54** Schulze KL 1995 *Cell* 80:311; **55** Deitcher DL 1998 *J Neurosci* 18:2028; **56** DiAntonio A 1993 *J Neurosci* 13:4924; **57** Weimbs T 1997 *Proc Natl Acad Sci USA* 94:3046; **58** Littleton JT 1994 *Proc Natl Acad Sci USA* 91:10888; **59** Klagges BRE 1996 *J Neurosci* 16:3154; **60** Harrison SD 1994 *Neuron* 13:555; **61** Ordway RW 1994 *Proc Natl Acad Sci USA* 91:5715; **62** Pallanck L 1995 *Nature* 376:25; **63** Zinsmaier KE 1994 *Science* 263:977; **64** Broadie K 1997 *Neuron* 19:391; **65** González-Gaitán M 1997 *Cell* 88:767; **66** Camidge DR 1994 *J Cell Sci* 107:709; **67** Smith M 1996 *Mol Biol Cell* 7:78; **68** van der Bliek AM 1991 *Nature* 351:411; **69** Gundelfinger ED 1992 *Trends Neurosci* 15:206; **70** Schuster CM 1991 *Science* 254:112; **71** Peterson SA 1997 *Neuron* 19:1237; **72** Ultsch A 1992 *Proc Natl Acad Sci USA* 89:10484; **73** Ultsch A 1993 *FEBS Lett* 324:171; **74** Parmentier ML 1996 *J Neurosci* 16:6687; **75** Arakawa S 1990 *Neuron* 4:343; **76** Han K-A 1998 *J Neurosci* 18:3650; **77** Obosi LA 1996 *FEBS Lett* 381:233; **78** Han KA 1996 *Neuron* 16:1127; **79** Aronstein K 1996 *Invertebr Neurosci* 2:115; **80** Harvey RJ 1994 *J Neurochem* 62:2480; **81** Cully DF 1996 *J Biol Chem* 271:20187; **82** Zhong Y 1991 *Science* 251:198; **83** Wang J 1994 *Neuron* 13:1373; **84** Nelson HB 1997 *Genetics* 147:1783; **85** Zhong Y 1995 *Nature* 375:88; **86** Reifegerste R 1993 *J Neurosci* 13:2186; **87** Angaut-Petit D 1998 *Eur J Neurosci* 10:423; **88** Cardenas ME 1995 *EMBO J* 14:2772; **89** Fernandes JJ 1996 *Dev Biol* 176:166; **90** Prokop A 1998 *J Neurogenet* 11:188; **91** Sone M 1997 *Science* 275:543; **92** Phelan P 1998 *Nature* 391:181; **93** Hoshino M 1996 *Development* 122:589; **94** Budnik V 1992 *J Neurobiol* 23:1054; **95** Kopczynski CC 1996 *Science* 271:1867; **96** Becker S 1995 *J Neurogenet* 10:18

brane during the period of NMJ differentiation (although most likely mainly at extrajunctional sites), so that the ionic currents of the late embryonic muscle membrane more or less match the properties of the larva[6] (Broadie and Bate 1993b and references therein). Apart from the addition of new channel types, a second stage of GluR expression begins several hours after the onset of NMJ formation (Broadie and Bate 1993c). This second phase of GluR expression depends on presynaptic electrical activity, similar to the initial clustering of GluRs (Broadie and Bate 1993a; Fig. 4C).

Formation of presynaptic structures at the motoneuronal terminal

Presynaptic structures can assemble independently of the postsynaptic muscle cell. In *twist* mutant embryos, which lack all muscles (Prokop et al. 1996), motoneuronal axons form bouton-like swellings, which are either unattached or attached to glial cells or to other neurons. Within these displaced swellings of twist mutant embryos at late stage 17, active zones can be found which stain for anti-Synaptotagmin (Littleton et al. 1993; see Table 1E) and contain T-bars and clusters of synaptic vesicles (in appearance similar to Fig. 2K,Q; Prokop et al. 1996). Interestingly, Synaptotagmin is incorrectly localised at NMJs of *dlg* mutant larvae and rescue experiments suggest a presynaptic requirement for *dlg* in these targeting and/or assembly processes (Thomas et al. 1997b). The fact that active zone assembly at *Drosophila* NMJs can take place independently of the postsynaptic target cell contrasts with the development of murine NMJs, where secretion of muscular s-laminin into the synaptic basement membrane is required for the formation of morphologically normal active zones (Noakes et al. 1995).

The independent assembly of active zones in *Drosophila* also means that the necessary genes have to be expressed on time, independent of retrograde signalling. This might be triggered by signalling from neurons pre-

[6] The existence of inhibitory glutamate/GABA-gated chloride channels has been reported for larval *Drosophila* muscles (Delgado et al. 1989). To my knowledge, such channels have not yet been looked for in the embryo, which would require alterations in the experimental procedure compared to analyses of glutamate receptor channels.

synaptic to the motoneurons,[7] by humeral signals or simply by cell intrinsic differentiation programmes, like those described for cultured *Xenopus* embryonic neurons (Gu and Spitzer 1995). Muscle-independent presynaptic differentiation might explain the *late bloomer* mutant phenotype (Kopczynski et al. 1996; see above), wherein impairment of potential signalling events leads to delay of presynaptic differentiation, but eventually the independent developmental capacity of the motoneurons will allow NMJ formation to occur. Although the assembly of presynaptic structures at the embryonic *Drosophila* NMJ is independent of the target muscle, their localisation at the neuromuscular site has to involve communication with the muscle, and this might involve *mef2*-dependent factors (Prokop et al. 1996).

From neural precursor to specific synaptic components: serotonergic differentiation in the lineage of NB7-3

The embryonic NMJ of *Drosophila* is a good model system to study general mechanisms underlying the formation of synaptic junctions and synapses. These studies benefit from the amenability of the NMJs and from their redundancy, in that most or all embryonic NMJs are similar or even the same with regard to their structure and the type of transmitter they use. In contrast, the *Drosophila* central nervous system harbours a greater variation in synaptic types as indicated by differences in their morphological appearance. For example, some central synapses show large postsynaptic densities and a less prominent presynaptic active zone (Fig. 2M,P); others show T-bars (Koenig and Ikeda 1996; Meinertzhagen 1996), or they contain gap junctions with closely associated membranes and a line of docked vesicles as shown for the giant fibre axon (Blagburn et al. 1999). Furthermore, many synaptic components are not found at the NMJ but in the central nervous system, indicating a greater complexity (not distinguished in Table 1). To address the variety of synapses and the way in which they can be formed at synaptic junctions between particular cells requires a very detailed model system. In this respect, one advantage of *Drosophila* is that its neurons are individually identifiable by their characteristic projections, as is typically the case for insect neurons (Burrows 1996). By now, almost all types of neural cells of an embryonic abdominal segment of *Drosophila* have been uncovered by clonal analysis and other staining and tracing techniques. This is true for the sensory system (Bodmer and Jan 1987; Jan and Jan 1993; Merritt and Whitington 1995), central neurons and glia cells (Bossing and Technau 1994; Broadus et al. 1995; Bossing et al. 1996; Schmidt et al. 1997), peptidergic neurons (references in Table 1) and motoneurons (Landgraf et al. 1997; see Fig. 1 for an overview). An increasing number of these cells can be singularly traced and identified, and their development can be studied. Research has now been initiated to capitalise on these detailed descriptions in order to investigate synapse formation in the central nervous system. Recently it was demonstrated that electrophysiology can be carried out on central neurons in situ in *Drosophila* embryos (Baines and Bate 1998), raising the hope that specific central synaptic contacts can be investigated at a functional level. Furthermore, there is an increasing list

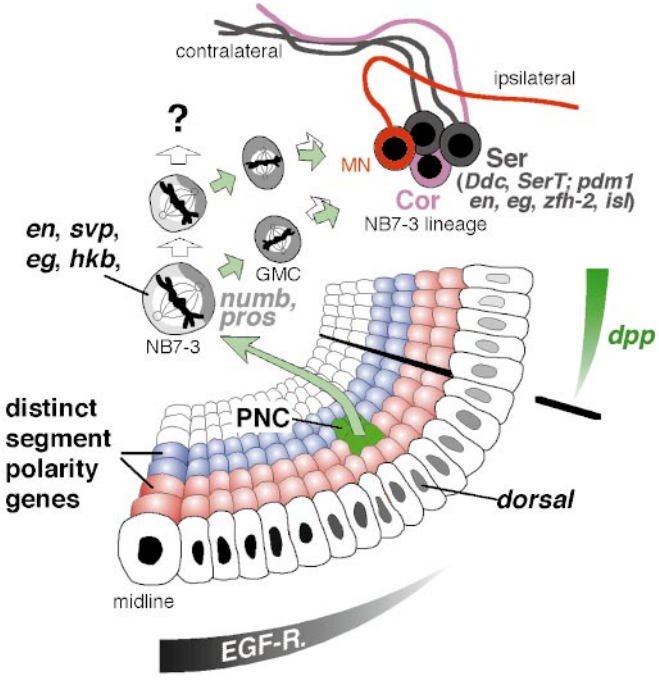


Fig. 5 Gene regulatory steps leading to the expression of cell-specific (synaptic) properties. A variety of patterning events lead to the formation of proneural clusters in defined positions of the ectoderm (*PNC, green*; cf. Fig. 1), all together giving rise (*long green arrow*) to 30 specific neuroblasts (NB). In the anterior-posterior axis, segment polarity genes (*red and blue stripes*) regulate NB identity (reviewed in Bhat 1998). In the dorsoventral axis, patterning is mediated by several interdependent gene regulatory events, for example a crude pattern is defined through a nuclear gradient of *dorsal* (Roth et al. 1989), the *dpp*-pathway defines the dorsal border of the neuroectoderm from which NB can arise (*below black bar*; Ferguson and Anderson 1992), and a gradient of EGF-receptor activity (*EGF-R*) helps to pattern the neuroectoderm (e.g. Udolph et al. 1998). Each NB is identifiable by position, time of segregation and expression of specific combinations of genes (Broadus et al. 1995; shown here *en*, *svp*, *eg*, *hkb* typical of NB7-3; for references concerning NB7-3 see main text). Segregated NB divide (*small arrows*) and give rise to ganglion mother cells (*GMC*), which themselves divide once. These divisions give rise to reproducible cell lineages. Cell differences can be generated by proteins encoded by *numb* or *pros*, which are asymmetrically distributed in NB or GMC (reviewed in Bhat 1998). The lineage of NB7-3 contains one ipsilaterally projecting potential motoneuron (*MN, red*), a cell expressing the neuropeptide corazonin (*Cor, pink*), and two serotonergic cells (*Ser, dark grey*) expressing genes involved in serotonin metabolism (*dopamine decarboxylase*, *Ddc*, non-vesicular *serotonin transporter*, *SerT*; see Table 1D) or transcription factors (*zfh-2*, *isl*)

[7] Embryos generally lacking neuronal transmission still form normal NMJs, arguing that motoneurons do not have to receive neuronal transmission in order to express „active zone genes“ (Broadie et al. 1995).

of gene products potentially localised at *Drosophila* synapses (Table 1). Mutations of the respective genes can be used to determine their potential requirement for synapse formation. On the other hand, synaptic markers can be taken to assay the mechanisms leading to their expression. One example is the serotonergic neurons in the central nervous system of the *Drosophila* embryo (Valles and White 1988). Serotonin is a transmitter or neuromodulator that, in the ventral nerve cord, is restricted to two cells per hemisegment which are derived from the neural precursor NB7-3 (see Fig. 5). Hence, serotonin is a highly specific marker for a particular synaptic property of two identified cells. These two serotonergic cells are a model with which to trace the development of late synaptic properties back to the neural precursor.

In vitro experiments with cultured neuroblasts lead to the differentiation of serotonergic cells, suggesting that, once NB7-3 has segregated, it carries out an intrinsic lineage programme culminating in serotonin expression (Huff et al. 1989). A variety of genes potentially involved in or activated by this lineage programme have been found to be expressed within the NB7-3 lineage, i.e. the genes *dopa decarboxylase*, *SerT*, *eagle*, *engrailed*, *huckebein*, *islet*, *pdm1*, *seven up* and *zfh-2* (Fig. 5; Corey et al. 1994; Demchyshyn et al. 1994; Lundell and Hirsh 1994; Broadus et al. 1995; Higashijima et al. 1996; Dittrich et al. 1997; Thor and Thomas 1997; Lundell and Hirsh 1998). Dopa decarboxylase is one of the enzymes catalysing Serotonin synthesis and SerT is a non-vesicular transporter required for reuptake of Serotonin into the nerve terminal. Hence these two genes are required very late in the serotonergic pathway, and all of them appear restricted to the two serotonergic cells. All of the other genes are (potential) transcription factors and therefore good candidates for components of a gene regulatory cascade. For example, *islet* is expressed late within the lineage and is not sufficient but necessary for Dopa decarboxylase and Serotonin expression, perhaps as a direct regulator of Dopa decarboxylase (Thor and Thomas 1997). The *eagle* gene is expressed throughout the NB7-3 lineage, and in its absence most serotonin expression is lost, although the NB7-3 lineage is still formed (Higashijima et al. 1996; Dittrich et al. 1997; Lundell and Hirsh 1998). The expression of *engrailed* and *huckebein* occurs prior to neuroblast segregation in the ectodermal layer and their absence might cause misspecification of NB7-3, as indicated by the loss of *eagle* expression (and lack of Serotonin; Dittrich et al. 1997). The principal mechanisms involved in the specification of neural precursors are summarised in Fig. 5.

It remains to be resolved to what extent other properties of the serotonergic cells such as their pathfinding and target selection are determined by these mechanisms, and how the postsynaptic partner cells develop and link to the presynaptic terminals. Furthermore, it remains to be seen to what degree rigid lineage mechanisms contribute to synaptic development, as opposed to cell communication and more flexible regulation or plasticity (see „Introduction“). Hopefully, future work on the embryonic nervous system of *Drosophila* will provide us with answers to these questions and help us to gain deeper insights into the fundamental mechanisms underlying the formation and function of neuronal circuits.

Acknowledgements I would like to thank Michael Bate, Nirupama Deshpande, Matthias Landgraf, Natalia Sánchez-Soriano and Joachim Urban for discussion and critical comments on the manuscript, Matthias Landgraf for personal communication of results, Rachel Drysdale for helpful comments on the table, Mary Rheuben for comments on Fig. 3, Yoshiaki Kidokoro for some special advice and the unknown reviewers for constructive criticism. I am grateful to Michael Bate and Kendal Broadie for their guidance, discussion and collaboration during my NMJ-related projects in Cambridge, and to Gerd Technau, who encouraged me to write this review. Given the amount of literature existing in the field, I apologise to those whose work is not cited or referred to by reviews due to space limitation or because it was missed by mistake.

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