ELSEVIER

Mechanisms of Development 109 (2001) 3–12

www.elsevier.com/locate/modo

Axon guidance of *Drosophila* SNb motoneurons depends on the cooperative action of muscular *Krüppel* and neuronal *capricious* activities

Sarah Abrell*, Herbert Jäckle

Max-Planck-Institut für biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, D-37070, Göttingen, Germany

Received 2 April 2001; received in revised form 25 July 2001; accepted 6 August 2001

Abstract

The body wall musculature of the *Drosophila* larva consists of a stereotyped pattern of 30 muscles per abdominal hemisegment which are innervated by about 40 distinct motoneurons. Proper innervation by motoneurons is established during late embryogenesis. Guidance of motor axons to specific muscles requires appropriate pathfinding decisions as they follow their pathways within the central nervous system and on the surface of muscles. Once the appropriate targets are reached, stable synaptic contacts between motoneurons and muscles are formed. Recent studies revealed a number of molecular components required for proper motor axon pathfinding and demonstrated specific roles in fasciculation/defasciculation events, a key process in the formation of discrete motoneuron pathways. The gene *capricious* (*caps*), which encodes a cell-surface protein, functions as a recognition molecule in motor axon guidance, regulating the formation of the selective connections between the SNb-derived motoneuron RP5 and muscle 12. Here we show that *Krüppel* (*Kr*), best known as a segmentation gene of the gap class, functionally interacts with *caps* in establishing the proper axonal pathway of SNb including the RP5 axons. The results suggest that the transcription factor Krüppel participates in proper control of cell-surface molecules which are necessary for the SNb neurons to navigate in a *caps*-dependent manner within the array of the ventral longitudinal target muscles. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Axon guidance; *capricious*; *Drosophila*; Muscular *Krüppel*

1. Introduction

The body wall musculature of the *Drosophila* larva consists of a repeated array of 30 muscles which can be distinguished by position, morphology, size and innervation pattern in each hemisegment of the abdomen (reviewed in Bate, 1990, 1993). Each muscle derives from a single mesodermal muscle founder cell (Bate, 1990; Rushton et al., 1995) and is targeted by specific motoneurons to precisely coordinate the movements of the larvae in response to signals from the central nervous system (CNS). The formation of the stereotyped pattern of the neuromuscular system (Landgraf et al., 1997; Schmidt et al., 1997; Schmid et al., 1999) involves growth cones of approximately 40 different motoneurons, searching through a variety of different tissues for their specific muscle targets (reviewed in Mitchison and Cramer, 1996; Chiba, 1999; Rose and Chiba, 1999). The growth cone-directed search involves a number of different and specific cues along specific 'choice points' which allow the axons to navigate along a stereotyped pathway which is characteristic for each motoneuron (van Vactor et al., 1993; Landgraf et al., 1997; Schmidt et al., 1997). Upon reaching the target region, the growth cones of the motoneurons transiently search through potential targets within a distinct muscle field and form synaptic connections with one or more muscles (van Vactor et al., 1993; Tessier-Lavigne and Goodman, 1996).

Within each segment of the embryo, about 30 neuroblasts are generated from the neuroectodermal region, which delaminate internally and give rise to both glia cells and neurons of the CNS (Bossing et al., 1996; Schmidt et al., 1997; Schmid et al., 1999). Axons of the motoneurons leave the CNS via two nerve roots, the intersegmental (ISN) and the segmental nerve (SN) root, respectively. In the exit junction region, the SN and ISN nerve roots defasciculate into five nerves (ISN, SNa, SNb, SNc, SNd), each of which then navigates towards a different set of muscles in the dorsal, lateral and ventral muscle fields (Landgraf et al., 1997; Schmidt et al., 1997; Schmid et al., 1999). Axon guidance as well as the spatially defined defasciculation process is precisely coordinated by local signals and cell-adhesion molecules (CAMs), which either promote or decrease intercellular adhesion of

* Corresponding author. Georg-Speyer-Haus, Paul-Ehrlich-Strasse 42-44, D-60596 Frankfurt, Germany.
E-mail address: sabrell@em.uni-frankfurt.de (S. Abrell).

0925-4773/01/$ - see front matter © 2001 Published by Elsevier Science Ireland Ltd.
PII: S0925-4773(01)00511-1

both nerve–nerve contacts and nerve–muscle surface interactions (reviewed in Chiba and Keshishian, 1996; Goodman, 1996; Chisholm and Tessier-Lavigne, 1999). Ligand-dependent activation of receptors present on motoneurons has been shown to trigger intracellular signal transduction pathways necessary for proper axon guidance (reviewed in Korey and van Vactor, 2000). Thus, it appears that the identity of nerves is in part established via the combination of expressed CAMs, receptors and secreted proteins (Patel et al., 1987; Nose et al., 1992, 1994; Chiba et al., 1995; Fambrough and Goodman, 1996; Desai et al., 1996; Raghavan and White, 1997; Rose et al., 1997).

Muscles not only contribute to the pathfinding and final target selection of motoneurons in the muscle fields, but also participate in early axon guidance by providing defasciculation signals in the exit junction region (Landgraf et al., 1999). Genes involved in pathfinding and synaptic targeting include a putative signal molecule encoded by *sidestep* (*side*) (Tessier-Lavigne and Goodman, 1996; Sink et al., 2001) as well as the CAMs encoding genes *capricious* (*caps*) and *Fasciclin II* (*FasII*) (Grenningloh et al., 1991; van Vactor et al., 1993; Shishido et al., 1998). *caps* encodes a member of the 'leucine-rich repeat' family of CAMs (Shishido et al., 1998) and a detailed analysis of *caps* mutant third instar larvae showed that *caps* activity is required for proper axon pathfinding and muscle-specific synapsing of part of the SNb, the RP5 motoneuron. The gene is expressed in both the RP5 neuron and the single target muscle implying that it provides a recognition substrate both on RPs axons and their targets (Shishido et al., 1998; Taniguchi et al., 2000). We have previously found that the P-insertion mutant *l(3)02937* genetically interacts with *Krüppel* (*Kr*), when *Kr* was ectopically expressed during eye development (Carrera et al., 1998). *Kr* codes for a zinc finger-type transcription factor (Preiss et al., 1985; Rosenberg et al., 1986; reviewed in Sauer et al., 1996), which plays a key role in segmentation (reviewed in Sauer et al., 1996), neurogenesis, myogenesis and organ development (Gaul and Weigel, 1991; Schmucker et al., 1992; Hoch et al., 1994; González-Gaitán et al., 1995; Romani et al., 1996; Ruiz-Gomez et al., 1997).

Here we report that *l(3)02937* is a novel *caps* allele and that *Kr*, which is expressed in muscles along the SNb pathway, genetically interacts with both *caps* and the more general CAM FasII. The results suggest that *Kr* activity regulates a muscular programme of cell-surface components which participate in *caps*-dependent SNb axon guidance and defasciculation in the ventral muscle field.

2. Results and discussion

We have recently made use of a *Kr* gain-of-function mutation, termed *Irregular facets* (*If*), to identify modifiers of *Kr* activity during eye morphogenesis which turned out to be required for specific aspects of *Kr*-dependent organogenesis (Carrera et al., 1998; Abrell et al., 2000). One suppressor of ectopic *Kr* activity in the eye, the P-element insertion *l(3)02937* (Spradling et al., 1995), was found to reside within the gene *caps* (Fig. 1A). *caps* was previously shown to encode a CAM of the 'leucine-rich repeat' family and shown to be required for proper pathfinding and synapsing of the RP5 motoneuron with muscle 12 (Shishido et al., 1998; Taniguchi et al., 2000; for details see below).

In addition to *l(3)02937* (Spradling et al., 1995), we identified a second P-element insertion, *l(3)05121* (Spradling et al., 1999), that resides in the first exon of the *caps* gene (Fig. 1A). Genetic analyses showed that the two P-element insertions failed to complement each other and the previously identified $caps^{65.2}$ null-mutation, indicating their allelism to *caps* (Shishido et al., 1998). Furthermore, precise excision of the P-element insertion *l(3)05121* resulted in a reversion of the *caps* mutant phenotype (see below) to wildtype (data not shown), indicating that the P-element insertion was the cause of the mutation. Moreover, the phenotype caused by the newly identified *caps* alleles was indistinguishable from

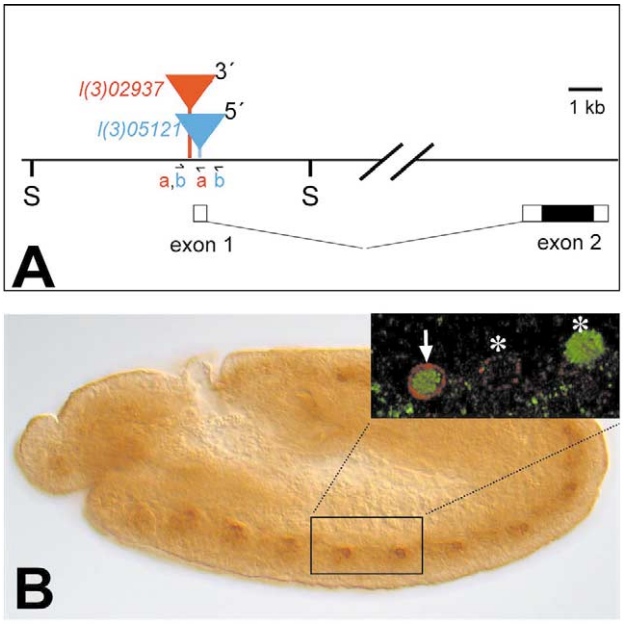

Fig. 1. Physical map of the *caps* gene and its expression in wildtype and Kr^{res} mutant embryos. (A) Schematic representation of the *caps* locus and the sites of P-element insertions as determined by plasmid rescue and PCR experiments. P-element *l(3)05121* is inserted in the first exon in position 175 of the 2.9-kb *caps* cDNA. The P-element of *l(3)02937* is inserted 119 bp 5′ of the first exon of the gene and is associated with a small deletion that removes 244 bp 3′ to its insertion site which include 125 bp of the first exon. → and ← indicate binding positions of the primer pairs used for PCR (a, primers for *l(3)05121* DNA; b, primers for *l(3)02937* DNA; S, SpeI). Positions refer to Shishido et al. (1998). (B) Wildtype embryo (stage 10; anterior is left, dorsal up) stained with anti-Caps antibody shows expression of Caps in neuroblasts. *Inset:* Confocal image of the neuroectoderm of a stage 10 embryo (equivalent to boxed region) stained with anti-Krüppel (nuclear signal; green) and anti-Caps (red) antibody. Note coexpression (arrow) and differential expression (asterisk) of the two genes in a distinct set of neuroblasts of the two proteins.

the *caps*$^{65.2}$ loss-of-function phenotype (see below), suggesting that they represent either strong hypomorphic or lack-of-function *caps* mutations.

2.1. caps is required during embryogenesis

While the innervation of muscles occurs during embryogenesis, previous studies on *caps* function have focussed on mutant defects that were observed in the motoneuronal pattern of third instar larvae, showing that Caps is necessary for proper pathfinding of the RP5 axons (Shishido et al., 1998; Taniguchi et al., 2000). RP5 axons are part of the SNb fascicle (see Fig. 2A,B). During embryogenesis, the SNb enters the ventral muscle field between muscle 15 and 28 (choice point 'target entry'). Its axons pass the ventral oblique muscle field towards the ventral longitudinal muscle targets in the most internal muscle layer (Fig. 3C). Close to the second choice point of the SNb nerve (see Fig. 2B), the RP3 axon separates from SNb to target the cleft between muscles 7 and 6. The SNb continues along muscle 14 and enters the ventral longitudinal muscle field. At a position close to muscle 30, the RP1 and RP4 axons separate to target muscle 13. The remaining RP5 bypasses muscle 13 to synapse with muscle 12. This muscle as well as the RP5 axons are characterized by *caps* expression both in the embryo and in larvae (Shishido et al., 1998). A schematic representation of the innervation pattern of the motoneurons and nomenclature is shown in Fig. 2A,B.

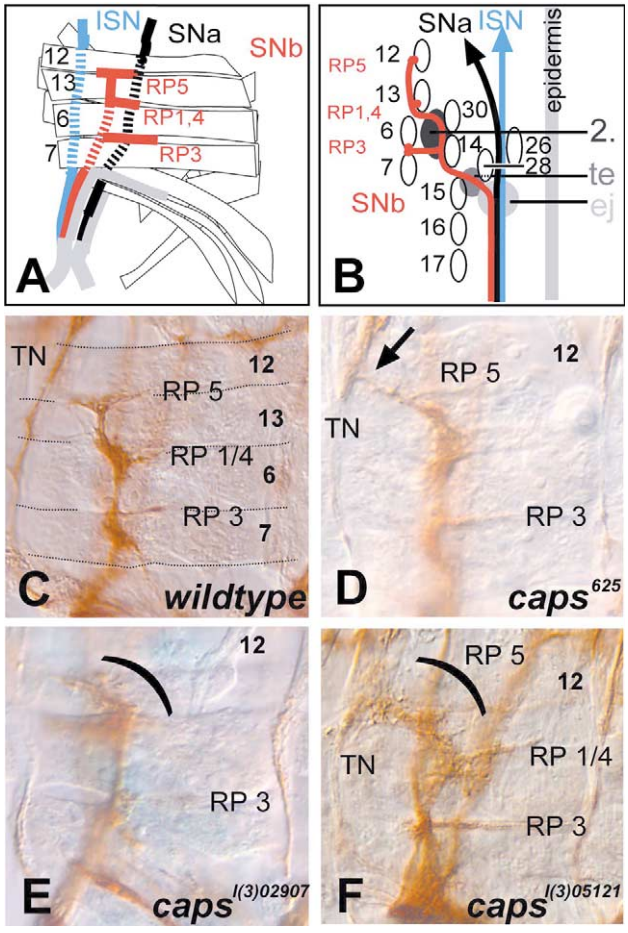

Fig. 2. Innervation of wildtype and *caps* mutant ventral muscles of stage 17 embryos. (A, B) Schematic representation of the innervation pattern (for details and nomenclature, see Landgraf et al., 1997). (A) The ISN (blue) and the SNa (black) pass the ventral muscle field, the SNb (red) defasciculates and single RP axons are formed (for details see text). (B) Corresponding cross-section showing the SNb pathfinding and its choice points. The SNb defasciculates in the exit junction (ej) region and enters the ventral muscle field between muscles 15 and 28 (target entry choice point, te). Single RP axons defasciculate at the second choice point (2). For details see text. (C–F) Stainings with anti-FasII antibodies showing the SNb and RP nerves of stage 17 wildtype (C) and *caps* mutant embryos (D–F). Dotted lines delineate muscle borders (6,7,12,13). (D) RP5 axon with an improper contact with the TN in *caps*$^{65.2}$ mutants. (E) *caps*$^{l(3)02937}$ and (F) *caps*$^{l(3)05121}$ mutants showing the growth cone-like morphology of the RP5 axons (brackets) and contact with TN (F, arrowhead). Details are described in the text.

As shown before, the early route of SNb pathfinding and the defasciculation patterns of RP1, RP3 and RP4 are not affected in *caps* homozygous larvae, whereas the pathway of RP5 is altered (Shishido et al., 1998; Taniguchi et al., 2000). RP5 loses its target specificity and synapses with both muscle 12 and 13 instead of muscle 12 only (Shishido et al., 1998). Furthermore, overexpression of *caps* in all muscles causes the same phenotype (Shishido et al., 1998). These results were taken to indicate that *caps* plays an important role in selective target recognition and synapse formation by the motoneuron RP5 (Shishido et al., 1998; Taniguchi et al., 2000).

In order to see whether and to what extent *caps* affects neuromuscular connectivity in the embryo, we examined the phenotypes of the different *caps* mutants. Of a total of 111 homozygous *caps*$^{65.2}$ mutant individuals examined, all embryos developed into normal looking larvae. However, the majority of the *caps*$^{65.2}$ mutant larvae (62%) failed to hatch. Out of 81 of hatched larvae examined, only two individuals survived to pupal stages and developed into adults. Similar results were obtained with homozygous *caps*$^{l(3)02937}$ (64% unhatched larvae; 3% adults) and *caps*$^{l(3)05121}$ (73% unhatched larvae; 3% adults) mutants. The two newly identified *caps* alleles are therefore similar in strength to *caps*$^{65.2}$, previously shown to be a lack-of-function mutation. Moreover, these data indicate that *caps* functions primarily during embryogenesis and that previous studies on *caps* function in larvae (Shishido et al., 1998; Taniguchi et al., 2000) have been performed with the fraction of mutants which develop into larvae.

We therefore examined SNb development in homozygous mutant embryos using monoclonal antibodies directed against the CAM FasII (van Vactor et al., 1993). In all three *caps* mutants, the SNb entered the ventral muscle field and the RP axons defasciculated normally. Furthermore, RP1, RP3 and RP4 properly synapsed with their respective target muscles (Fig. 2D–F), whereas the RP5 axons stalled, showed enlarged growth cone-like structures and failed to contact the target muscle 12 (Fig. 2D–F). Instead, the RP5

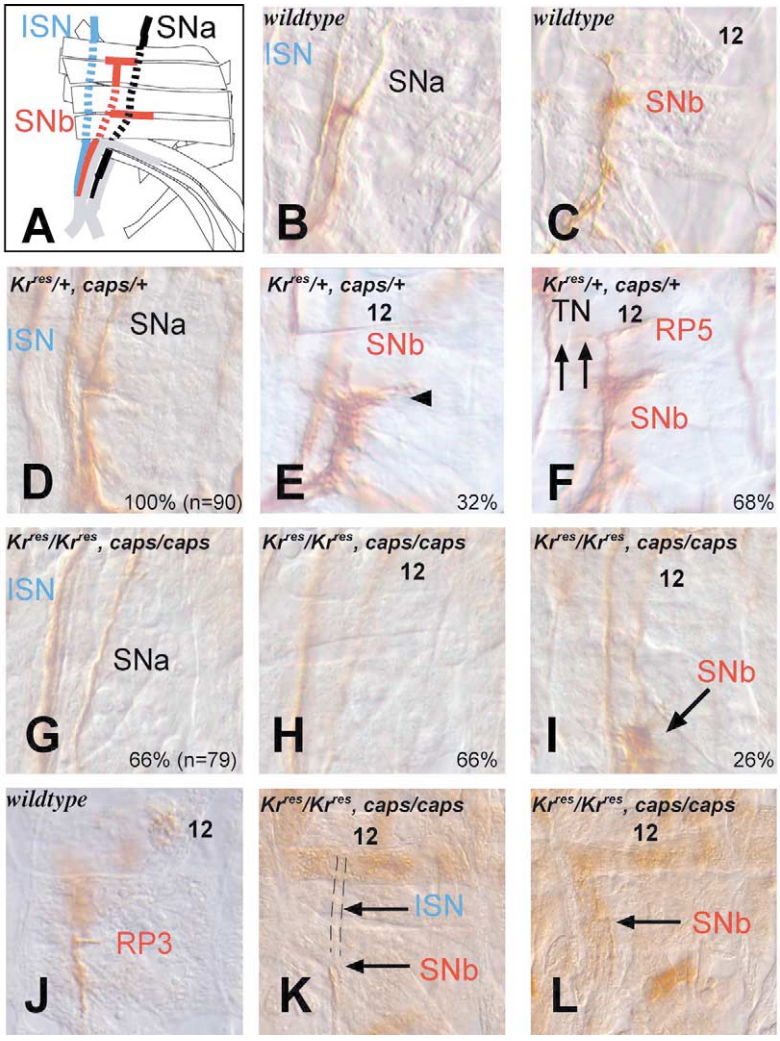

Fig. 3. Innervation of ventral muscles of wildtype and *Kr*; *caps* double-mutant stage 17 embryos. The genotype of embryos and the frequency of phenotypes are indicated; *caps* refers to the $caps^{l(3)05121}$ allele, 12 to muscle 12. (A) Schematic representation of ISN (blue), SNa (black) and SNb (red) nerve pattern in ventral muscle fields (see also Fig. 2A). (B, C) Focal plane showing the ISN, SNa and SNb in the exterior (B) and internal (C) muscle layer of a wildtype embryo. (D–F) Kr^{res}/+; $caps^{l(3)05121}$/+double heterozygous mutants showing that the ISN and SNa are normal (D), the SNb stops at the second choice point (E, arrowhead) or RP axons form improper contacts with the TN (F; arrow). (G–I) Double homozygous Kr^{res}; $caps^{l(3)05121}$ mutants showing that the ISN and SNa are formed normally, but the ISN is thicker than in wildtype (G, compare to B), the SNb is not formed (H), or stalls before entering the ventral muscle field (I, arrow). (J) *islH*-τ-myc marker showing the RP3 nerve innervating wildtype muscles 6 and 13. (K, L) Double homozygous Kr^{res}; $caps^{l(3)05121}$ mutant embryos showing in different focal planes that the *islH*-τ-myc-labelled RP nerves form, but fail to separate from the ISN (K; indicated by the dotted line) and stall in the entrance region of the ventral muscle field (L; arrow). Motoneurons are stained with either anti-FasII or anti-myc antibodies showing *islH*-τ-myc marker expression.

axons were split and found also in direct contact with the transversal nerve (TN), a link never observed in wildtype (Fig. 2D,F). These phenotypes suggest that pathfinding of RP5 axons during embryogenesis is retarded and in cases the RP5 axons defasciculate and elongate, they show erratic targeting and synapsing as has been described for third instar larvae.

The specific SNb defect of the *caps* mutants on RP5 pathfinding and its failure to synapse exclusively with muscle 12 correlates with the observation that both RP5 neurons and their target muscle are characterized by *caps* expression both in embryos and larvae (Shishido et al., 1998). In addition, overexpression of *caps* in all muscles causes a phenotype similar to loss-of-function mutations, suggesting that relative levels of Caps are important for pathfinding. Thus, both lack-of-function and gain-of-function studies indicate a specific role for *caps* in selective target recognition and synapse formation (Shishido et al., 1998, Taniguchi et al., 2000). The results show that this function of *caps* is required during embryogenesis and that the previously reported phenotype observed in third instar larvae represents only a weak phenotype common to escapers. In all other cases, impairment of *caps* activity causes the RP5 axons to stall immediately after defasciculation from the SNb fascicle. This observation indicates that *caps* is a critical component specifically required for RP5 pathfinding after the defasciculation of the RP5 axons from SNb has occurred.

2.2. caps expression is not dependent on Kr and vice versa

caps is expressed in both motoneuron RP5 and its synaptic target muscle 12 in third instar larvae (Shishido et al., 1998). In view of the embryonic *caps* mutant phenotypes, we asked whether *caps* is also expressed during early embryogenesis and whether *Kr*, which is expressed in neuroblasts and in a distinct subset of embryonic muscles (Gaul et al., 1987; Romani et al., 1996; Ruiz-Gomez et al., 1997) could be responsible for the control of *caps* expression. We found that *caps* is indeed expressed in neuroblasts (Fig. 1B). However, this aspect of the *caps* expression pattern was not altered in *Kr* lack-of-function mutant embryos except in the central region which is distorted due to the earlier segmentation function of *Kr* (Romani et al., 1996). We therefore monitored *caps* expression in Kr^{res} mutant embryos in which the early segmentation function of *Kr* is specifically rescued (Romani et al., 1996). No difference was observed in the *caps* expression patterns of Kr^{res} mutant and wildtype embryos and the *Kr* neural expression pattern was unchanged in *caps* mutant embryos (data not shown), indicating that *Kr* and *caps* activities are independently controlled (see also below). Since *caps* acts as a dose-dependent modifier of ectopic *Kr* activity during eye formation (Carrera et al., 1998), we next asked whether later aspects of the expression patterns, *caps* expression in RP5 neurons and their target muscles (Shishido et al., 1998) and *Kr* expression in a subset of muscles along the SNb pathway (Ruiz-Gomez et al., 1997), might reflect the need of the two gene activities for the proper guidance of SNb axons.

2.3. Cooperation of Kr and caps activities are necessary for SNb guidance

Previous work has shown that in the absence of *Kr* activity, the SNb stalls at the second choice point and RP axons fail to defasciculate (Landgraf, 1996; Hartmann et al., 1997; see also Fig. 5C). This observation is consistent with the proposal that the two genes act in the same genetic pathway. In order to test this proposal, we performed genetic interaction studies using $caps^{l(3)05121}$ and Kr^{res} mutant combinations, asking whether a reduction of *Kr* and *caps* activities causes defects in neuromuscular connectivity.

Heterozygous $Kr^{res}/+$ or $caps^{l(3)05121}/+$ single mutant embryos developed a normal motoneuron pattern. Each of the RP axons was properly connected to its target muscle as revealed by anti-FasII antibody staining (data not shown). Thus, a reduction of either *Kr* or *caps* activity had no effect on motoneuron development and pathfinding. In contrast, double heterozygous $Kr^{res}/+$; $caps^{l(3)05121}/+$ embryos, where the dosage of both genes was reduced at the same time, developed a specific SNb nerve phenotype (Fig. 3D–F) without affecting the ISN and SNa (Fig. 3D). In about one-third of the cases (32%, $n = 90$), the SNb stopped along ventral longitudinal muscles, ending with a large growth cone-like structure (Fig. 3E). In addition, we noted that properly defasciculated RP axons failed to continue along their normal paths; a portion of them elongate and stall either in a position very close to the TN or is directly connected to it (68%; Fig. 3F). Double homozygous Kr^{res}; $caps^{l(3)05121}$ mutants developed an even stronger phenotype; the SNb was absent in most of the double mutants analysed (Fig. 3G,H; 66 %, $n = 79$) or did not extend beyond its second choice point close to muscle 28 (Fig. 3I; 26%). In only few cases (8%), the SNb stalled in the ventral muscle field as had been described for homozygous Kr^{res} embryos (Landgraf, 1996; Hartmann et al., 1997; see also Fig. 5C). These phenotypes were also obtained with similar frequencies in double homozygous Kr^{res}; $caps^{65.2}$ mutants (data not shown), indicating that the phenotype is not dependent on a particular *caps* allele. The results show in addition that the defects are stronger and more pronounced in double mutant embryos than those obtained with single mutant embryos.

The failure to detect the SNb nerve in the majority of homozygous Kr^{res}; $caps^{l(3)05121}$ mutant embryos correlated with a thickening of the ISN (Fig. 3G). In order to test whether SNb might have lost its identity due to a transformation into ISN identity, we labelled the SNb-derived RP neurons by virtue of the transgenic *islH*-τ-myc marker gene (Thor and Thomas, 1997; Thor et al., 1999; Fig. 3J). The results showed that the RP axons were present. However, the SNb failed to separate from the ISN (Fig. 3K) or in the cases separation occurred, it stalled shortly after the defasciculation (Fig. 3L). These observations indicate that the SNb was not transformed into ISN identity and suggest that the SNb has lost the capability to respond to guidance cues such as CAMs and repellents (Lin and Goodman, 1994; Fambrough and Goodman, 1996; Yu et al., 2000). Moreover, the results indicate that *Kr* and *caps* activities cooperate in a synergistic fashion necessary for proper defasciculation of the SNb axons at the exit junction and for RP axon guidance in the ventral muscle field.

2.4. Ectopic Kr activity affects defasciculation of the SNb and axon guidance

Previous studies have shown that overexpression of *caps* in all neurons causes a specific misrouting or stalling of RP5 at the second choice point near muscle 30 in about one-third of the embryos (Taniguchi et al., 2000). This effect of panneural *caps* expression was dependent on the extracellular domain of Caps, suggesting that Caps functions as a cell-adhesion component which participates in the guidance of the SNb at the specific choice point near muscle 30 (Taniguchi et al., 2000). In contrast, panmuscular expression of *caps* had no effect on SNb guidance and pathfinding, but severely interfered with synapsing of RP5 resulting in connections being formed not only with muscle 12, but also with the neighbouring muscle 13 (Shishido et al., 1998; Taniguchi et al., 2000).

In order to investigate whether misexpression of *Kr* can interfere with SNb formation, we ectopically expressed *Kr*

in all motoneurons or muscles using the Gal4/UAS system (Brand and Perrimon, 1993). To achieve this, we used the ftz_{NG}-Gal4 ('panmotoneuronal expression'; Thor et al., 1999) and the *24B*-Gal4 driver lines ('panmuscular expression'; Baylies and Bate, 1996), respectively, in combination with one and two copies of UAS-*Kr* transgenes (Hoch and Jäckle, 1998). Panmotoneural *Kr* expression from one transgene in wildtype embryos resulted in a minor phenotype of the SNb, in which the distal RP axons failed to reach their target muscles and maintained growth cone-like structures at their ends (data not shown). Panmotoneural *Kr* expression from two transgenes caused a stronger phenotype. In 36% of the cases ($n = 50$), the most distal RP axons stalled and the RP5 axon did not innervate the target muscle 12 (Fig. 4A), whereas in all other cases, the SNb stalled at the second choice point (Fig. 4B), a phenotype that is similar to the Kr^{res} homozygous mutant phenotype (Fig. 5C; Landgraf, 1996; Hartmann et al., 1997). This observation indicates that the *Kr* overexpression phenotype is dosage-dependent and that both the lack-of-function and gain-of-function effects of *Kr* interfere with SNb development. Since Krüppel is a cell-autonomous transcription factor, it is likely that it is required for and can interfere with the transcription of motoneuronal genes necessary for proper motoneuronal guidance.

In the wildtype embryos, *Kr* is not only expressed in the nervous system but also in specific subsets of muscle founder cells and muscles (Ruiz-Gomez et al., 1997). Relevant sites of *Kr* expression during the formation of neuromuscular connectivity are the ventral oblique muscles 14 and 16, the ventral acute muscle 27 and the ventral longitudinal muscles 6, 7 and 13 (Ruiz-Gomez et al., 1997). Heterozygous Kr^{res} mutant embryos develop a normal muscle pattern, whereas in homozygous Kr^{res} mutant embryos muscle 27 is transformed into a duplicated muscle 26. The other muscles that normally express *Kr* either appear to be normal or develop a variably altered morphology (Ruiz-Gomez et al., 1997). Overexpression of four copies of *Kr* in all muscles leads to the reverse result, i.e. muscle 26 is transformed into a second muscle 27 (Ruiz-Gomez et al., 1997). Thus, *Kr* is necessary to determine muscle identity and can alter muscle fate upon ectopic expression in muscles that normally do not express the gene.

Panmuscular expression of *Kr* in response to one or two copies of the *24B*-driven Gal4/UAS-*Kr* cDNA transgenes did not disturb the muscle pattern (Ruiz-Gomez et al., 1997; own observation). However, it had severe consequences for the formation and defasciculation of SNb. With one copy of *Kr*, the RP3 axon separated properly from SNb and found the cleft between target muscles 7 and 6, whereas the remaining RP axons failed to defasciculate (20%, $n = 80$; Fig. 4C). In the majority of cases (80%), however, the SNb passed the ventral oblique muscles and entered the ventral longitudinal muscles normally, but it stalled at the second choice point and RP axons failed to defasciculate (Fig. 4D). Two copies of transgene-derived *Kr* expression in all cases caused stronger defects that the SNb stalled at the position where RP3 would normally defasciculate (Fig. 4E, $n = 43$). The same *Kr*-dependent phenotypes were found in response to a different panmuscular Gal4 driver, namely the *twi* Gal4 line (Greig and Akam, 1993; data not shown). Thus, in contrast to panmuscular expression of *caps* (see above), panmuscular expression of *Kr* appears to interfere with a muscle-specific programme that regulates defasciculation of the RP axons and/or the elongation of SNb after the second

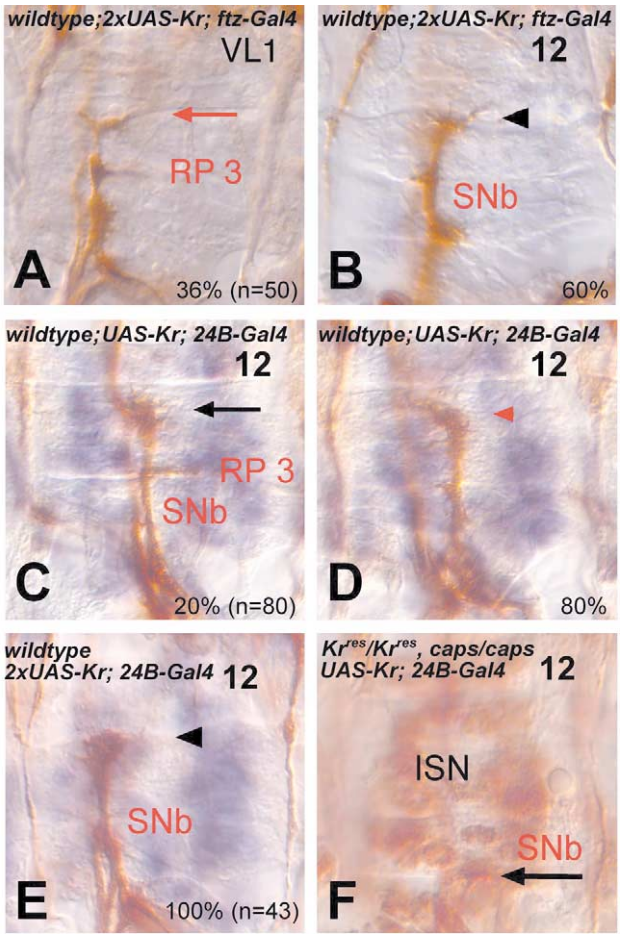

Fig. 4. Innervation of ventral muscles of wildtype and *caps* mutant stage 17 embryos which received panmotoneural (A, B) or panmuscular *Kr* activity (C–E); the genotypes of the embryos and the frequency of defects are indicated. Panmuscular *Krüppel* (blue) and neuronal *FasII* expression (brown) (C–E) and of panmuscular *Krüppel* and neuronal *FasII* both in brown (F) are shown. (A) In embryos which received panneural *Kr* expression, formation of the RP 3 axons is normal but the most distal RP axons stop their growth (arrow) and fail to reach their target muscle. (B) The SNb ends at the second choice point (arrowhead); no RP axons are formed. (C) Panmuscular *Kr* expression causes the most distal RP axons to fail reaching their target (arrow), whereas RP3 axons are properly connected. (D) In the majority of cases, the SNb stalls at the second choice point and RP axons fail to elongate. (E) In embryos that received panmuscular *Kr* activity derived from two copies of the transgene, the SNb stalls at the second choice point and forms a thickened growth cone-like structure (arrowhead). (F) In double homozygous Kr^{res}; $caps^{l(3)05121}$ embryos, which received panmuscular expression of *Kr* (nuclear staining), the SNb and the ISN defasciculate properly but the SNb stalls prior to entering the ventral muscle field (arrow).

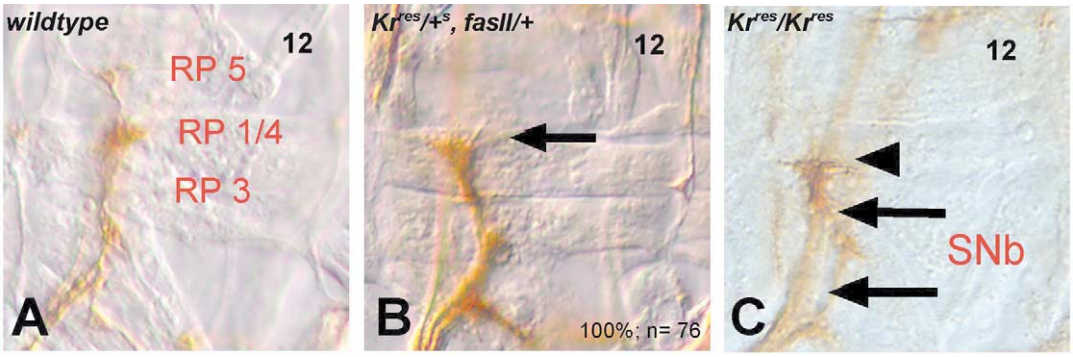

Fig. 5. Innervation of ventral muscles of wildtype. Double heterozygous *FasII*^{eb112}/+; *Kr*^{res}/+ and homozygous *Kr*^{res} embryos as visualized by anti-FasII antibody staining; the genotypes of the embryos and the frequency of defects are indicated. (A) Wildtype embryo. (B) In *FasII*^{eb112}/+; *Kr*^{res}/+ embryos, the SNb stalls at the second choice point (arrow) and RP axons fail to fasciculate in all examined cases. (C) Homozygous *Kr*^{res} mutant embryo showing a similar innervation defect; i.e. the SNb stalls at the second choice point (arrowhead; see also Landgraf, 1996).

choice point has been reached. The finding is consistent with the argument that *Kr* determines the spectrum of molecules expressed in muscles which are used to transmit signals to other cells, namely cell-surface and secreted molecules.

In order to test whether panmuscular *Kr* expression interferes with SNb guidance in a *Kr*- and *caps*-dependent manner, we also examined the phenotype of double homozygous *Kr*^{res}; *caps*$^{l(3)05121}$ mutant embryos that received panmuscular *Kr* expression (see above). Upon panmuscular *Kr* expression, the SNb was absent in 70% of the cases ($n = 39$). In the other cases, the SNb has formed and separated from the ISN, but stalled in the region of the ventral longitudinal muscles (Fig. 4F). The observed phenotype is reminiscent of the phenotype obtained with *Kr*^{res}; *caps*$^{l(3)05121}$ double homozygous mutants (see Fig. 3K), but a higher proportion of axons defasciculate in the exit junction region. This observation indicates that panmuscular *Kr* expression can partially rescue the SNb phenotype of the double homozygous mutants. However, defasciculation from the stalled SNb occurred in an erratic manner, implying that axon guidance is still strongly impaired. The lack of a better rescue is likely to be due to the fact that *Kr* has not been expressed in its wildtype muscular pattern and/or that the correct levels and neuronal *Kr* activity are not provided through panmuscular expression. Nevertheless, the results are consistent with the proposal that *Kr* regulates a muscular programme which in turn regulates SNb axon guidance along the muscles. This proposal is supported by the notion that the phenotype is reminiscent of mutants affecting CAMs in motoneurons such as FasII (Lin and Goodman, 1994; Fambrough and Goodman, 1996; Yu et al., 2000).

2.5. Genetic interaction between Krüppel and Fasciclin II

The similarity of motoneuronal phenotypes of *Kr* and CAM mutants, and the interaction between *Kr* and *caps* activity suggested that *Kr* might also interact genetically with additional CAMs which are necessary for proper pathfinding. We tested this proposal using mutant combinations of *Kr*^{res} and the loss-of-function mutant *FasII*^{eb112}(Grenningloh et al., 1991). FasII is a more general CAM than Caps. It is expressed on all motoneurons during late embryonic stages and is necessary to maintain adhesion between the axons (van Vactor et al., 1993; Lin and Goodman, 1994). Heterozygous *Kr*^{res}/+ or *FasII*^{eb112}/+ (Grenningloh et al., 1991) embryos developed a normal SNb pattern and all RPs were properly connected to their target muscles. In double heterozygous *FasII*^{eb112}/+;*Kr*^{res}/+ mutants, however, the SNb entered the ventral muscle field normally in all cases ($n = 76$), but the nerve stopped at the second choice point by forming a growth cone-like structure. No individual RP axons could be observed (Fig. 5B). This phenotype is very similar to the one observed with homozygous *Kr*^{res} mutant embryos (Fig. 5C), implying that the two gene activities cooperate to allow for proper SNb development.

3. Conclusions

In addition to position, size and morphology, the innervation of muscles by specific motoneurons represents a diagnostic feature for the determination of muscle identity. Previous results have shown that *Kr* is expressed in a specific subset of muscle progenitors and is necessary for the acquisition of a specific muscle fate as shown by muscle transformations that occur in response to gain-of-function and lack-of-function *Kr* mutations. Muscle 27 is transformed into a duplicated muscle 26 in homozygous *Kr* mutant embryos whereas high level overexpression (four copies of *Kr*) in all muscles leads to the reverse transformation (Ruiz-Gomez et al., 1997). Our data suggest that *Kr* contributes not only to identifying characteristics of muscle 27 but also provides adhesion properties to other *Kr*-expressing muscles along the SNb pathway, i.e. muscles 14 and 16, the ventral acute muscle 27, and the ventral longitudinal muscles 6, 7 and 13 (Ruiz-Gomez et al., 1997; Fig. 2A,B). The genetic interactions between *Kr*

and the CAMs FasII and Caps support our hypothesis and imply that the adhesive properties of motoneurons and/or muscles are established in such a way that a concomitant reduction in adhesion of $Kr^{res}/+$; *caps*/+ or $Kr^{res}/+$; $FasII^{eb112}/+$ mutants results in a situation which no longer provides sufficient information to allow accurate axonal pathfinding and innervation. This proposal is also consistent with the finding that the presence of one muscle in an otherwise muscle-depleted embryo can be sufficient for the defasciculation of nerve bundles (Landgraf et al., 1999).

In the muscles 6, 7 and 13, *Kr* is known to maintain the expression of a direct target gene, *knockout* (*ko*) (Hartmann et al., 1997). *ko* mutant embryos display a *Kr*-like motoneuron phenotype, suggesting that the gene, which encodes a novel protein with unknown biochemical characteristics, plays a key role in SNb defasciculation and RP pathfinding by acting downstream of *Kr*. However, in contrast to *Kr*, ectopic misexpression of *ko* did not affect SNb branching and synaptic targeting of RP neurons (Hartmann et al., 1997), and no genetic interaction as observed between *ko* and *caps* could be found (S. Abrell, unpublished result). It is therefore likely that *Kr* transmits its signal not only via *ko*, but also through other factors that are still to be identified. We also found that in contrast to *caps*, ectopic panmotoneural expression of *Kr* causes defects similar to the *Kr* lack-of-function mutation, and a reduction of combined *caps* or *FasII* and *Kr* activities. We therefore speculate that *Kr* activity can also directly interfere with the spectrum of CAMs in motoneurons, resulting in non-compatible cell-surface characteristics between axons and muscles which in turn interfere with neuromuscular connectivity.

4. Experimental procedures

4.1. Mutant stocks and genetic analyses

Fly stocks were kept under standard conditions (Ashburner, 1989). The following mutants were used: Kr^{res} (Romani et al., 1996), $caps^{65.2}$ (Shishido et al., 1998), *l(3)05121* and *l(3)02937* (Spradling et al., 1995), $FasII^{eb112}$ (Grenningloh et al., 1991), UAS-*Kr* (Hoch and Jäckle, 1998), ftz_{NG}-Gal4 (Thor et al., 1999), *twi*-Gal4 (Greig and Akam, 1993), 24B-Gal4 (Baylies and Bate, 1996) and *islH*-τ-myc (Thor and Thomas, 1997). Recombination, combinations of mutations on different chromosomes and P-element mobilization experiments were performed according to Greenspan (1997). The mutation-bearing chromosomes were kept over balancer chromosomes carrying *ftz-lacZ* or *hb-lacZ* transgenes. This way, homozygous mutant embryos could be unambiguously distinguished from siblings by *lacZ* expression. To determine the lethal period of the *caps* mutants, the mutant alleles were kept over balancer chromosomes carrying a green fluorescent protein (GFP) transgene under the control of the constitutively active *actin 5* promoter (Reichhart and Ferrandon, 1998) which allows mutant individuals to be distinguished by the lack of GFP activity. For the expression of the *Kr* transgene via the UAS-Gal4 system (Brand and Perrimon, 1993), flies were kept at 25°C and *Kr* expression was monitored by staining with anti-Krüppel antibodies (González-Gaitán and Jäckle, 1997). Staging of embryos was according to Campos-Ortega and Hartenstein (1997).

4.2. Molecular biology

Plasmid rescue experiments were performed to obtain DNA adjacent to the P-element insertions *l(3)02937* and *l(3)05121* (Spradling et al., 1995). The integration sites of the P-elements were determined by polymerase chain reaction (PCR) (primers: 5′-TCGTGGTCTTGCTCTCCCGCTCTCGTG-3′ and 5′-GCCGATGACGATTGACGATTGACGATT-3′ for *l(3)02937*, 5′-CGTGGTCTTGCTCTCCCGCTCTGCT-3′ and 5′-GTAGCCCTATTTCAGTTCTAGTTGT-3′ for *l(3)05121* and sequencing of amplified DNA. For PCR reactions, 0.5 μg of genomic DNA were incubated with 10 pmol of each primer, 2.5 mM of each nucleotide and 2.5 U *TaqPlus* polymerase (*TaqPlus*® Precision PCR system, Stratagene, La Jolla, CA, USA) in a 50 μl reaction within 37 cycles (denaturating 30 s at 95°C, annealing 1 min at 60°C and extension 2 min at 68°C).

4.3. Immunohistochemistry

The following primary antibodies were used: rabbit anti-Krüppel (P. Carrera, unpublished), rat anti-Krüppel (Kosman et al., 1998), rabbit anti-Caps (Shishido et al., 1998), mouse anti-c-myc (Calbiochem), anti-FasII (van Vactor et al., 1993), anti-myosin heavy chain (MHC) (Kiehart and Feghali, 1986). The following secondary antibodies were used: biotinylated anti-rabbit and anti-mouse antibodies (Cappel, ICN Biomedicals, Inc., Costa Mesa, USA) peroxidase coupled anti-rabbit antibodies (Cappel, ICN Biomedicals, Inc., Costa Mesa, USA), anti-rabbit Alexa 563-coupled antibodies (Dianova, Hamburg, Germany), anti-rat fluoreseinisothiocyanat (FITC)-coupled antibodies (Dianova, Hamburg, Germany). Antibody reactions were performed as described (González-Gaitán and Jäckle, 1997). In the double-staining experiments, the peroxidase reaction was developed before the ABC system was applied (Vectastain, Vector, Burlingame, USA) to detect the biotinylated secondary antibody. Using fluorescent dyes for double-labelling, the embryos were examined with the Zeiss LSM 410 ‘inverted’ confocal microsocope applying the laser 488 with filter 510–520 for FITC and laser 543 with long-pass filter 570 for Alexa 543 dyes. The motoneuronal pattern was examined in flat preparations of embryos. Embryos were incubated in 70% glycerol (containing 50 mM NaCl, 100 mM Tris–HCl pH 7.4), dissected in 100% glycerol with a sharp tungsten needle and examined as described (www.its.caltech.edu/~zinnlab/motoraxons/protocols.html). We refer to SNb in cases when the RP nerves are not unambiguously identified. In cases we

refer to RP5, the other RPs (RP3, RP1 and RP4) are properly defasciculated and connected to their target muscles and thus, the absence or misrouting of RP5 axons could be unambiguously identified. When necessary, RP identity was established by transgene-dependent *islH*-τ-myc expression as outlined in the text.

Acknowledgements

We thank our colleagues in the laboratories for various important contributions, in particular M. González-Gaitán. We also thank S. Thor for the *islH*-τ-myc flies, A. Nose for anti-Caps antibodies and the original *caps*$^{65.2}$ mutant. We thank the Bloomington and Umeå Stock Centers, the Berkeley *Drosophila* Genome Project for fly stocks and clones. The work was supported by the Max-Planck-Society.

References

Abrell, S., Carrera, P., Jäckle, H., 2000. A modifier screen of ectopic Krüppel activity identifies autosomal *Drosophila* chromosomal sites and genes required for normal eye development. Chromosoma 109, 334–342.

Ashburner, M., 1989. *Drosophila*, a Laboratory Manual, CSHL Press, New York, NY.

Bate, M., 1990. The embryonic development of larval muscles in *Drosophila*. Development 110, 791–804.

Bate, M., 1993. The mesoderm and its derivatives. In: Bate, M., Martinez Arias, A. (Eds.). The Development of *Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1013–1090.

Baylies, M.K., Bate, M., 1996. twist: a myogenic switch in *Drosophila*. Science 272, 1481–1484.

Bossing, T., Udolph, G., Doe, C.Q., Technau, G.M., 1996. The embryonic central nervous system lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. Dev. Biol. 179, 41–64.

Brand, A.H., Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415.

Campos-Ortega, J.A., Hartenstein, V., 1997. The Embryonic Development of *Drosophila melangaster*, Springer, Berlin.

Carrera, P., Abrell, S., Kerber, B., Walldorf, U., Preiss, A., Hoch, M., Jäckle, H., 1998. A modifier screen in the eye reveals control genes for Krüppel activity in the *Drosophila* embryo. Proc. Natl. Acad. Sci. USA 95, 10779–10784.

Chiba, A., 1999. Early development of the *Drosophila* neuromuscular junction: a model for studying neuronal networks in development. Int. Rev. Neurobiol. 43, 1–24.

Chiba, A., Keshishian, H., 1996. Neuronal pathfinding and recognition: roles of cell adhesion molecules. Dev. Biol. 180, 424–432.

Chiba, A., Snow, P., Keshishian, H., Hotta, Y., 1995. Fasciclin III as a synaptic target recognition molecule in *Drosophila*. Nature 374, 166–168.

Chisholm, A., Tessier-Lavigne, M., 1999. Conservation and divergence of axon guidance mechanisms. Curr. Opin. Neurobiol. 9, 603–615.

Desai, C.J., Gindhart Jr, J.G., Goldstein, L.S., Zinn, K., 1996. Receptor tyrosine phosphatases are required for motor axon guidance in the *Drosophila* embryo. Cell 84, 599–609.

Fambrough, D., Goodman, C.S., 1996. The *Drosophila beaten path* gene encodes a novel secreted protein that regulates defasciculation at motor axon choice points. Cell 87, 1049–1058.

Gaul, U., Weigel, D., 1991. Regulation of *Krüppel* expression in the anlage of the Malpighian tubules in the *Drosophila* embryo. Mech. Dev. 33, 57–68.

Gaul, U., Seifert, E., Schuh, R., Jäckle, H., 1987. Analysis of Krüppel protein distribution during early *Drosophila* development reveals post-transcriptional regulation. Cell 50, 639–647.

González-Gaitán, M., Jäckle, H., 1995. Invagination centers within the *Drosophila* stomatogastric nervous system anlage are positioned by Notch-mediated signaling which is spatially controlled through wingless. Development 121, 2313–2325.

González-Gaitán, M., Jäckle, H., 1997. In situ localization of proteins in whole mount tissue. In: Crampton, J.M., Beard, C.B., Louis, C. (Eds.). Molecular Biology of Insect Disease Vectors: A Methods Manual, Chapman & Hall, New York, NY, pp. 283–294.

Goodman, C.S., 1996. Mechanisms and molecules that control growth cone guidance. Annu. Rev. Neurosci. 19, 341–377.

Greenspan, R.J., 1997. Fly Pushing, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Greig, S., Akam, M.E., 1993. Homeotic genes autonomously specify one aspect of pattern in the *Drosophila* mesoderm. Nature 362, 630–632.

Grenningloh, G., Rehm, E.J., Goodman, C.S., 1991. Genetic analysis of growth cone guidance in *Drosophila*: Fasciclin II functions as a neuronal recognition molecule. Cell 67, 45–57.

Hartmann, C., Landgraf, M., Bate, M., Jäckle, H., 1997. *Krüppel* target gene *knockout* participates in the proper innervation of a specific set of *Drosophila* larval muscles. EMBO J. 16, 5299–5309.

Hoch, M., Jäckle, H., 1998. *Krüppel* acts as a developmental switch gene that mediates *Notch* signalling-dependent tip cell differentiation in the excretory organs of *Drosophila*. EMBO J. 17, 5766–5775.

Hoch, M., Broadie, K., Jäckle, H., Skaer, H.I., 1994. Sequential fates in a single cell are established by the neurogenic cascade in the Malpighian tubules of *Drosophila*. Development 120, 3439–3450.

Korey, C.A., van Vactor, D., 2000. From the growth cone surface to the cytoskeleton: one journey, many paths. J. Neurobiol. 44, 184–193.

Kiehart, D.P., Feghali, R., 1986. Cytoplasmic myosin from *Drosophila melanogaster*. J. Cell Biol. 103, 1517–1525.

Kosman, D., Reinitz, J., Stephen, S., 1998. Rapid preparation of a panel of polyclonal antibodies to *Drosophila* segmentation proteins. Dev. Genes Evol. 208, 290–294.

Landgraf, M., 1996. Mechanisms underlying the development of neuromuscular connectivity in the *Drosophila* embryo. PhD thesis, University of Cambridge, UK.

Landgraf, M., Bossing, T., Technau, G.M., Bate, M., 1997. The origin, location, and projections of the embryonic abdominal motoneurons of *Drosophila*. J. Neurosci. 17, 9642–9655.

Landgraf, M., Baylies, M., Bate, M., 1999. Muscle founder cells regulate defasciculation and targeting of motor axons in the *Drosophila* embryo. Curr. Biol. 9, 589–592.

Lin, D.M., Goodman, C.S., 1994. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron 13, 507–523.

Mitchison, T.J., Cramer, L.P., 1996. Actin-based cell motility and cell locomotion. Cell 84, 371–379.

Nose, A., Mahajan, V.B., Goodman, C.S., 1992. Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. Cell 70, 553–567.

Nose, A., Takeichi, M., Goodman, C.S., 1994. Ectopic expression of *connectin* reveals a repulsive function during growth cone guidance and synapse formation. Neuron 13, 525–539.

Patel, N.H., Snow, P.M., Goodman, C.S., 1987. Characterization and cloning of Fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. Cell 48, 975–988.

Preiss, A., Rosenberg, U.B., Kienlin, A., Seifert, E., Jäckle, H., 1985. Molecular genetics of *Krüppel*, a gene required for segmentation of the *Drosophila* embryo. Nature 313, 27–32.

Raghavan, S., White, R.A., 1997. Connectin mediates adhesion in *Drosophila*. Neuron 18, 873–880.

Reichhart, J.M., Ferrandon, D., 1998. Green balancers. Drosophila Inform. Serv. 81, 201–202.

Romani, S., Jiminez, F., Hoch, M., Patel, N.H., Taubert, H., Jäckle, H., 1996. *Krüppel*, a *Drosophila* segmentation gene, participates in the specification of neurons and glial cells. Mech. Dev. 60, 95–107.

Rose, D., Chiba, A., 1999. A single growth cone is capable of integrating simultaneously presented and functionally distinct molecular cues during target recognition. J. Neurosci. 19, 4899–4906.

Rose, D., Zhu, X., Kose, H., Hoang, B., Cho, J., Chiba, A., 1997. Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in *Drosophila*. Development 124, 1561–1571.

Rosenberg, U., Schröder, C., Preiss, A., Kienlin, A., Cote, S., Riede, I., Jäckle, H., 1986. Structural homology of the product of the *Drosophila* Krüppel gene with *Xenopus* transcription factor IIIA. Nature 319, 336–339.

Ruiz-Gomez, M., Romani, S., Hartmann, C., Jäckle, H., Bate, M., 1997. Specific muscle identities are regulated by Krüppel during *Drosophila* embryogenesis. Development 124, 3407–3414.

Rushton, E., Drysdale, R., Abmayr, S.M., Michelson, A.M., Bate, M., 1995. Mutations in a novel gene, *myoblast city*, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. Development 121, 1979–1988.

Sauer, F., Rivera-Pomar, R., Hoch, M., Jäckle, H., 1996. Gene regulation in the *Drosophila* embryo. Philos. Trans. R. Soc. Lond. B 351, 579–587.

Schmid, A., Chiba, A., Doe, C.Q., 1999. Clonal analysis of *Drosophila* embryonic neuroblasts: neural cell types, axon projections and muscle targets. Development 126, 4653–4689.

Schmidt, H., Rickert, C., Bossing, T., Vef, O., Urban, J., Technau, G.M., 1997. The embryonic central nervous system lineages of *Drosophila melanogaster*. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Dev. Biol. 189, 186–204.

Schmucker, D., Taubert, H., Jäckle, H., 1992. Formation of the *Drosophila* larval photoreceptor organ and its neuronal differentiation require continuous *Krüppel* gene activity. Neuron 9, 1025–1039.

Shishido, E., Takeichi, M., Nose, A., 1998. *Drosophila* synapse formation. Regulation by transmembrane protein with leu-rich repeats CAPRICIOUS. Science 280, 2118–2121.

Sink, H., Rehm, E.J., Richstone, L., Bulls, Y.M., Goodman, C.S., 2001. *sidestep* encodes a target-derived attractant essential for motor axon guidance in *Drosophila*. Cell 105, 57–67.

Spradling, A.C., Stern, D.M., Kiss, I., Roote, J., Laverty, T., Rubin, G.M., 1995. Gene disruptions using P transposable elements: an integral component of the *Drosophila* genome project. Proc. Natl Acad. Sci. USA 92, 10824–10830.

Spradling, A.C., Stern, D.M., Beaton, A., Rhem, E.J., Laverty, T., Mozden, N., Misra, S., Rubin, G.M., 1999. The Berkeley *Drosophila* Genome Project gene disruption project: single P-element insertions mutating 25% of vital *Drosophila* genes. Genetics 153, 135–177.

Taniguchi, H., Shishido, E., Takeichi, M., Nose, A., 2000. Functional dissection of *Drosophila capricious*: its novel roles in neuronal pathfinding and selective synapse formation. J. Neurobiol. 42, 104–116.

Tessier-Lavigne, M., Goodman, C.S., 1996. The molecular biology of axon guidance. Science 274, 1123–1133.

Thor, S., Thomas, J.B., 1997. The *Drosophila islet* gene governs axon pathfinding and neurotransmitter identity. Neuron 18, 397–409.

Thor, S., Andersson, S.G., Tomlinson, A., Thomas, J.B., 1999. A LIM-homeodomain combinatorial code for motor-neuron pathway selection. Nature 397, 76–80.

van Vactor, D., Sink, H., Tomlinson, A., Famgrough, D.M., Tsoo, R., Goodman, C.S., 1993. Genes that control neuromuscular specificity in *Drosophila*. Cell 73, 1137–1153.

Yu, H.-H., Huang, A.S., Kolodkin, A.L., 2000. Semaphorin-1a acts in concert with the cell adhesion molecule Fasciclin II and Connectin to regulate axon fasciculation in *Drosophila*. Genetics 156, 723–731.