The cell adhesion molecule, connectin, and the development of the 
Drosophila neuromuscular system

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SUMMARY

The connectin gene of Drosophila has been identified as a candidate direct target of homeotic gene control and has also been implicated in the formation of specific neuromuscular connections. The gene product, connectin, is a member of the leucine-rich repeat protein family and we show that it is attached to the cell surface via a glycosyl-phosphatidylinositol linkage and that it can mediate homotypic cell-cell adhesion in vitro. The expression of connectin protein during Drosophila embryogenesis provides support for a role in adhesion in vivo. In the central nervous system, it is initially expressed on longitudinal glia and on a few identified neurons. These cells extend processes and connect up to form a continuous scaffold of connectin-expressing cells, presaging the development of axonal pathways. Later, connectin is expressed on specific axons as they track along the connectin scaffold. Glial expression then declines and connectin appears on axons that fasciculate with pre-existing connectin-positive bundles. Thus scaffold formation, axon pathfinding and fasciculation involve specific contacts between connectin-positive cells. The timing and pattern of connectin expression suggest that it may play an important role in mediating specific interactions through homotypic cell adhesion.

Key words: cell adhesion molecule, leucine-rich repeat family, neural development, muscle development, Drosophila development, homeotic gene

INTRODUCTION

Homeotic genes in Drosophila control the development of segment-specific pathways by acting on sets of downstream genes (Lewis, 1963; Garcia-Bellido, 1975; Lewis, 1978). Understanding how homeotic genes regulate morphogenesis will require the analysis of the functions mediated by these downstream target genes. At present, few target genes have been identified, and we have little idea of the range of molecular functions that are regulated by homeotic genes. Targets identified so far include genes for putative transcription factors, a tubulin gene and also growth factor genes: decapentaplegic encodes a TGF-β homologue under homeotic control in the visceral mesoderm (Immergluck et al., 1990; Reuter et al., 1990); Distal-less is a homeobox gene showing homeoetrically regulated expression in the primordia of certain imaginal discs (Vachon et al., 1992); empty spiracles is a homeobox gene regulated by the homeotic gene Abdominal-B and is required for generation of filzkörper in the posterior spiracles in the eighth abdominal segment (Jones and McGinnis, 1993); split is under the control of the homeotic gene Antennapedia (Wagner-Bernholz et al., 1991) and encodes a putative transcriptional regulator of the zinc-finger class and the β3 tubulin gene is regulated by the homeotic gene Ultrabithorax (Ubx) in the visceral mesoderm (Hinz et al., 1992).

Using a chromatin immunopurification strategy designed to identify direct targets of the homeotic gene Ultrabithorax, we have isolated several candidate downstream genes by virtue of their proximity to in vivo binding sites for Ubx protein (Gould et al., 1990). For one of these genes, connectin, we have shown that the immunopurified site is located within a regulatory element that is under the control of several homeotic genes including Ubx (Gould and White, 1992). The connectin gene encodes a member of the leucine-rich repeat family (Lopez et al., 1987) that is expressed in the embryo in a segmentally modulated pattern in both the nervous system and the somatic mesoderm. Analysis of the expression of connectin protein at the time of muscle innervation has revealed that it is expressed on a subset of motor neurons and on their specific target muscles (Nose et al., 1992).

Here we investigate further the developmental function mediated by this homeotic target gene, connectin. We show that connectin functions as a cell-cell adhesion molecule in vitro. Analysis of the connectin expression during embryogenesis provides support for an in vivo role for connectin as an adhesion molecule and reveals that connectin is expressed in...
Production of fusion proteins and monoclonal antibodies

A 1.7 kb SalI fragment from the cDNA gt35.8 (Gould and White, 1992) containing most of the connectin open reading frame was blunt ended with Klenow polymerase and inserted, in the sense orientation, into the SalI site of pGEX-3x (Smith and Johnson, 1988; Pharmacia) to give pGEX3x-35, and into the Klenow-filled BamHI site of pET3c (Rosenberg et al., 1987) to give pET3c-35. Balb/c mice were immunised i.p. with purified and washed inclusion bodies containing 50 µg pGEX3x-35 fusion protein in complete Freund's adjuvant. Five days prior to fusion a further i.p. boost was given with 50 µg. Fusion supernatants were screened on protein blots of lysates of pET3c-35 cultures.

Embryo injections

Embryos were injected at 6-7 hours after egg laying (staged from the onset of gastrulation) with 5-10 nl of C1.427 ascites fluid containing approximately 4 mg/ml antibody. Embryos were allowed to develop for 12-14 hours at 18°C following injection. The embryos were labelled to visualise connectin, either as above or omitting the primary C1.427 antibody incubation.

RESULTS

Connectin function in vitro

The sequence of the connectin gene predicts a protein product of the leucine-rich repeat family with a signal sequence but no transmembrane domain (Gould and White, 1992; Nose et al., 1992). The carboxy-terminal hydrophobic region is consistent with a lipid anchor linkage to the cell surface. Although leucine-rich repeat molecules serve a variety of functions (see Rothberg et al., 1990a), two cell surface leucine-rich family members in Drosophila have been shown to act as cell-cell adhesion molecules; the transmembrane Toll protein (Keith and Gay, 1990) and the glycosyl-phosphatidylinositol (GPI)-linked cell suface molecule, chaoptin (Krantz and Zipursky, 1990). We therefore tested the ability of connectin to mediate cell-cell adhesion by expressing connectin in the non-adhesive Drosophila tissue culture cell line S2 (Fig. 1A). Figs 2 and 3 show that induction of connectin expression results in considerable aggregation of the cells as seen by the formation of cell clumps (Fig. 2A) and the decrease in particle number (Fig. 3A). We further showed that the aggregation mediated by connectin is homophilic as adhesion assays with mixtures of connectin-expressing and non-expressing cells resulted in the clumping together of only the connectin-expressing cells (Fig. 2). In order to directly implicate the connectin molecule in adhesion, we investigated whether an antibody against connectin would block the cell aggregation. We used a monoclonal antibody, C1.427, generated against connectin expressed as a fusion protein in bacteria. On protein blots of S2 cells expressing connectin, or Drosophila embryos, this antibody recognizes bands corresponding to connectin and also a cross-reacting protein in bacteria. On protein blots of S2 cells expressing connectin, or Drosophila embryos, this antibody recognizes bands corresponding to connectin and also a cross-reacting protein in bacteria. For antibody inhibition, cells were allowed to recover from heat shock and then a 1 in 5 dilution of monoclonal antibody supernatant C1.427 (or FP3.38 as control; White and Wilcox, 1984) was added to a suspension of cells in BSS to a final cell density of 3×10^6 to 4×10^6 cells/ml. For analysis of GPI-linkage, 7.5×10^5 cells, after recovery from heat shock, were resuspended at a density of 5×10^7 cells/ml in M3 without FCS either with or without PI-PLC (Boehringer-Mannheim; 0.6-10 units/ml) and incubated at 25°C for 2 hours. After centrifugation, the pellet and supernatant fractions were analysed by SDS-PAGE followed by western blotting using monoclonal antibody C1.427.
neurons and on their target muscles, correlates well with a role aspect of connectin expression, its expression on specific motor al., 1992). Nose et al. (1992) have also demonstrated that one previously (Gould et al., 1990; Gould and White, 1992; Nose et RNA and protein expression has been described pre- connectin expression during embryogenesis to investigate whether its distribution supported an in vivo role in homotypic cell-cell adhesion. We analysed the expression of connectin using the monoclonal antibody, C1.427. The overall pattern of cell-cell adhesion. We analysed the expression of connectin using the monoclonal antibody, C1.427. The overall pattern of expression by heat shock, the cells were heat shocked to induce connectin expression, then treated with phosphatidylinositol-specific phospholipase C (PI-PLC; 10 units/ml) (+) or with enzyme buffer only (−). After incubation, the cells were spun down and pellet (P) and supernatant (S) extracts were analysed for connectin by protein blotting and probing with C1.427. Connectin is specifically released into the supernatant by enzyme digestion. The asterisk marks the position of the major connectin band at 61 kDa.

for the induction of adhesiveness. We also showed that adhesion is independent of calcium (data not shown).

The connectin expression system in S2 cells allowed us to test biochemically for evidence of GPI-linkage. After induction of connectin expression by heat shock, the cells were digested with PI-specific phospholipase C, centrifuged and the cell pellet and supernatant were assayed for connectin activity on protein blots (Fig. 1B). The results show clear enzyme-dependent release of connectin into the supernatant fraction, indicating that at least a substantial fraction of connectin is linked to the cell surface via a GPI-anchor.

Expression of connectin protein in embryogenesis

Having demonstrated that connectin can act as a cell-cell adhesion molecule in vitro, we were interested in examining connectin expression during embryogenesis to investigate whether its distribution supported an in vivo role in homotypic cell-cell adhesion. We analysed the expression of connectin using the monoclonal antibody, C1.427. The overall pattern of connectin RNA and protein expression has been described previously (Gould et al., 1990; Gould and White, 1992; Nose et al., 1992). Nose et al. (1992) have also demonstrated that one aspect of connectin expression, its expression on specific motor neurons and on their target muscles, correlates well with a role in homotypic cell-cell adhesion. Here we have examined an early phase of connectin expression in the CNS. We will describe the connectin expression in terms of stages that may correspond to separable roles for connectin in the morphogenesis of the CNS. Stage one involves the construction of a glial/neuronal scaffold; in stage two this scaffold appears to provide a substrate for axonal pathfinding; in stage three connectin expression along axons may be involved in fasciculation; and finally, in stage four, connectin may be involved in motor neuron outgrowth and synaptogenesis.

Stage one begins at about 6 hours (developmental timing is given as hours after egg laying at 25°C), prior to axonogenesis, when connectin is expressed in a small number of cells in the CNS. These connectin-positive cells subsequently organise to form a continuous scaffold of connectin expression, involving both glia and neurons, in a pattern that prefigures the axonal ladder (Fig. 4). In each segment 4-6 longitudinal glial (LG) cells and two sets of identified neurons, a pair of VUM cells and a pair of neurons in the position of RP cells, are labelled (Fig. 4A; for naming of cells see Jacobs and Goodman, 1989).

At 6 hours the connectin-positive cells are isolated from each other but soon thereafter processes extending from these cells make specific contacts with other cells expressing connectin. By 8 hours the connectin-positive cells have connected together to form a continuous ladder-like array (Fig. 4C). This process involves the elongating LG cells making contacts with RP neurons in the same segment and also in the next more posterior segment. The rungs of the ladder are formed by the VUM cells and their contacts with both RP neurons and the LG cells.

In the second stage of expression, this connectin scaffold is utilised as a substrate over which connectin-expressing axons extend. Expression of connectin on axonal outgrowths begins in the anterior commissure. At about 8 hours, the VUM cells migrate ventrally leaving behind a wide process from the dorsal surface of the cells that maintains contact with the LG cells (Fig. 4C). Between 8.5 and 9 hours, the RP neurons extend processes that track across the midline along the dorsal surface of the VUM cells. Axons from connectin-positive interneurons, corresponding to the identified SP neurons, also appear to be amongst the earliest components of the anterior commissure and cross the midline on the dorsal surface of the VUM cells (Fig. 4D). From 8-10 hours, there is a considerable increase in the number of neuronal cell bodies expressing connectin, many of which are interneurons producing axonal outgrowths that track along the connectin scaffold (Fig 4C,D).

After 9 hours, two connectin-expressing axon tracts develop in the longitudinal connectives and subsequently thicken as other connectin-expressing axons fasciculate (Fig. 4D and F). From about 10 hours, there is a large increase in the number of cells expressing connectin outside the central ladder and this is concomitant with a general decrease in the expression levels in the LG cells. This would be consistent with the idea that glial cells act as guideposts for axon growth cones until a foundation of neurons is present to guide the subsequent axons by a selective fasciculation process. After 11 hours, connectin is more restricted to axons and there is decreased expression on cell bodies (Fig. 4G).

Connectin continues to be expressed on a subset of motor neuron outgrowths after exiting the CNS and may play a role
in peripheral pathfinding and neuromuscular connectivity (Nose et al., 1992).

Thus, in several phases of the development of the neuromuscular system, the expression of connectin on cell surfaces correlates with important interactions between the expressing cells.

**Thoracic/abdominal differences**

During the extended germ-band the expression of *connectin* RNA in the somatic mesodem is considerably higher in thoracic versus abdominal segments (Gould and White, 1992). We have analysed the cellular basis for this difference in order to investigate the possible roles of a putative cell-cell adhesion molecule in constructing specific segmental morphologies. The expression pattern of *connectin* protein in the abdominal somatic mesoderm has been described by Nose et al. (1992). Expression of *connectin* in the somatic mesoderm differs between thorax and abdomen from its onset (Fig. 5A). The expression appears first at about 6 hours in segment-specific patterns in the thoracic segments. The labelled cells are muscle precursors; i.e. cells that will provide foci for fusion with surrounding unlabelled cells to give rise to identifiable muscles. In T2 and T3 there is a prominent group of four cells that are the precursors for the thoracic (Th) muscles (Th21-24; muscle numbering according to Bate (1990); Figs 5A-D). Subsequently a lower level of expression appears in two pairs of cells in each abdominal hemisegment; the precursors for abdominal (Ab) muscles Ab21-24 (Fig. 5A,B).

A major difference between abdominal and thoracic segments involves the ventral clusters of *connectin*-positive cells that appear after 7 hours (Fig. 5B-D). In the abdomen these ventral cells are the precursors of muscles Ab27 and Ab29. In the thorax the ventral clusters involve more cells and express higher levels of *connectin*. Also, these cells are not muscle precursors in the sense that they do not provide foci for fusion events generating multinucleate cells. Thus at about 9 hours, when the Th and Ab 21-24 and the Ab27 and 29 precursors grow larger through cell fusion (Fig. 5D), the cells in the thoracic ventral clusters remain unfused. The number of cells in the thoracic ventral clusters of *connectin*-expressing cells declines after 9 hours, probably due to loss of *connectin* expression in some cells, although fusion of some of these cells with muscle precursors might also be occurring. At least some of these cells are likely to be precursors for the adult muscles as, in double-label experiments, a pro-
Fig. 4. Expression of connectin protein in CNS development. Connectin expression is visualised by immunoperoxidase labelling of dissected embryo whole mounts. Anterior is to the left.

(A) At 6.5 hours: connectin is expressed in two centrally located VUM cells (large arrowhead), in clusters of 4-6 longitudinal glial (LG) cells per hemisegment (small arrowheads), and a pair of neurons per hemisegment (arrows), which subsequently take up a position similar to the identified RP motor neurons. (B) At 7.5 hours: a continuous cellular ladder of connectin expressing cells is formed due to contacts of processes between LG cells and VUM cells (large arrowhead) and also between the LG cells and the RP neurons (small arrowheads). Note that the major processes from the LG cells extend to other connectin-positive cells. Another pair of neurons per hemisegment begin expression; the SP interneurons (arrow).

(C) At 8.5 hours: between 8.5 and 9 hours the RP neurons (arrow) extend growth cones across the midline dorsal to the VUM cells. The VUM cells migrate ventrally (open arrow), leaving behind their axons, which, together with the RP axons, form a connectin-positive bundle within the anterior commissure (large arrowhead). The SP neurons also extend axons across the midline to form another axon bundle in the anterior commissure (small arrowheads).

(D) At 9.5 hours: two connectin-positive bundles in the anterior commissure are clearly defined (open arrows) and the first connectin-positive axons are visible in the posterior commissure (black arrow). Growth cones are also present in the longitudinal connectives (large arrowheads), forming the first interneuron connections between segments. Connectin-expressing cells provide some of the first axons to leave the CNS in the segmental nerve (small arrowhead). Interestingly, these axons exit the CNS and establish contact with the ventral somatic mesodermal cluster earlier in the abdomen than in the thorax. At this stage the connectin expression on glial cells is declining.

(E) At 9.5 hours: four new sets of cells begin to express connectin. Two pairs are shown here (small arrowheads) that lie dorsal to the main CNS ladder (out of focus).

(F) At 11.5 hours: the original glial and neuronal connectin ladder has been replaced by an axonal ladder of interneurons. There are two major connectin-positive tracts within the longitudinal connectives (small arrowheads). The large arrowheads indicate the SP neurons. SN, segmental nerve. ISN, intersegmental nerve.

(G) At 13 hours: at this stage most of the connectin expression is on axons, with cell bodies showing reduced expression, with the exception of neurons in the periphery of the CNS that send axons into the central ladder (small arrowhead).
portion of the connectin-positive cells also showed the persistent twist expression that characterises adult precursors (Bate et al., 1991; data not shown).

By 11 hours there is little difference in the intensity of connectin labelling between the thoracic and abdominal segments although the final muscle patterns are distinct (Fig. 5E).

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**Fig. 5.** Connectin expression in thorax and abdomen. Immunoperoxidase labelling of connectin in dissected embryo whole mounts allowing an overview of expression within the CNS and mesoderm and focussing on segmental differences of expression within the somatic mesoderm. The thoracic segments, T2 and T3, and the abdominal segments, A1 and A2, are shown. Anterior is to the left. (A-C) Whole segments with the CNS centrally; (D-F) hemisegments with the CNS at the bottom. (A) At 7 hours: initially the intensity of expression is far higher in the muscle precursors of the thorax (arrow) than of the abdomen (small arrowhead indicates the pair of cells that are the precursors for Ab23 and 24). At higher magnification, the connectin expression can be seen to be in four cells: the precursors for muscles Th21-24. Out of the plane of focus are connectin-expressing cells in the visceral mesoderm (large arrowheads) that partially obscure the somatic mesoderm labelling. (B) At 7.5 hours: expression begins in ventral somatic mesoderm cells in the thorax (arrowheads) and more weakly in the abdomen (arrow). (C) At 8.5 hours: the abdominal expression is now approaching the intensity of the thoracic. Within the thorax the ventral cell clusters are approaching the intensity of the Th21-24 precursors. (D) At 9 hours: note the clear difference in the cellular arrangements within the ventral clusters in thorax versus abdomen. In the thoracic segments the ventral clusters consist of a mass of unfused cells (bracketted), whereas in the abdomen the clusters contain the precursors of muscles Ab27 (arrow) and 29 (large arrowhead) and a peripheral glial cell, PG4 (arrowhead). Note the connectin tends to be concentrated at regions of cellular contact. (E) At 11 hours: the muscle pattern is established. (F) At 13 hours: the staining intensity appears uniform across the segments. A branch of the SN (small arrowheads) innervates the muscles 21-24 in both thorax and abdomen. A branch of the ISN (arrows) innervates the dorsal glial structures (large arrowhead) and also muscle Ab18. Later staining becomes more localised to the synapses (Nose et al., 1992).
Connectin function in vivo

The demonstration that the anti-connectin monoclonal antibody, C1.427, could completely block connectin-mediated cell-cell adhesion in vitro (Fig. 3B) suggested that it might be a useful tool to probe connectin function in vivo. We injected over 50 embryos with C1.427 antibody. The embryos were injected at 6-7 hours of development; just prior to muscle fusion, establishment of the LG axonal scaffold in the CNS and axonogenesis by motor neurons. Development was allowed to proceed for 12-14 hours at 18°C, at which time the muscle pattern is complete, motor axons have found their target muscles and neuromuscular junctions are forming. The embryos were then dissected, fixed and labelled for connectin using C1.427 plus a 'second layer' anti-immunoglobulin reagent, or else by simply labelling the perduring injected anti-connectin antibody with the anti-immunoglobulin reagent. None of the embryos we examined showed any consistent defect in the development of the nervous or muscular systems or in neuromuscular connectivity. The embryos labelled only with the anti-immunoglobulin reagent also showed an apparently normal connectin expression pattern. Thus, the C1.427 anti-connectin antibody persisted at saturating levels for several hours during the development of the neuromuscular system.

DISCUSSION

We have shown that the connectin gene encodes a cell surface, GPI-linked member of the leucine-rich repeat family that can act as a homophilic cell-cell adhesion molecule in vitro. This proposed function for connectin as an adhesion molecule involved in interactions between specific connectin-expressing cells correlates well with the observed distribution of connectin in the embryo. Previous analysis has highlighted the correlation between the expression of connectin on particular motor neuron growth cones and on the surface of the specific muscles that they innervate (Nose et al., 1992). Connectin is transiently expressed on the muscles and motor neuron growth cones prior to and during the time of synapse formation. Thus, the timing of connectin expression fits well with a potential role as a homophilic cell adhesion molecule involved in the establishment of specific neuromuscular connections. Here we have shown that the expression of connectin also correlates with several key morphogenetic events in the development of the central nervous system and here too a role in cell-cell adhesion is indicated.

In the CNS prior to axonogenesis a skeleton of migration pathways prefigures the later axonal scaffold. In the development of the commissures, the surface of the VUM neurons located at the midline appears to provide critical migration cues (Klämbt et al., 1991) and the longitudinal connectives are prefigured by a set of six longitudinal glial cells per hemisegment (Jacobs and Goodman, 1989). Here we have shown that a small number of connectin-positive cells, including a pair of VUMs at the midline and the six longitudinal glial cells, form a continuous scaffold prior to axonogenesis. Thus connectin provides a molecular basis for the organisation of the scaffold based on homotypic adhesions between connectin-positive cells. Connectin is expressed prior to the establishment of connections between the units of the scaffold, and the cellular processes that form these connections extend specifically between connectin-positive cells. In the migration and linking together of the longitudinal glial cells, the connectin-positive neurons (RP neurons and VUM cells) may serve as guideposts.

Connectin continues to be expressed as a scaffold as axonogenesis commences and is found on several growth cones, including RP and SP neurons, that track along the connectin-labelled scaffold. Subsequently, as fasciculation interactions between axon processes become more prevalent, connectin expression is lost from the cell bodies and remains exclusively on the axonal processes.

Thus the episodes of connectin expression dramatically coincide both spatially and temporally with a set of specific morphogenetic events involving interactions between connectin-positive cells. These processes include the migration and interlinking of longitudinal glial cells, the pathway choice of pioneer growth cones, the establishment of specific axon tracts, the pathfinding of motor neurons growing into the periphery on particular peripheral glial cells and the generation of specific neuromuscular connections.

Does the likely cell-cell adhesion function for connectin allow us to make sense of the higher levels of connectin RNA and protein expression in thoracic than in abdominal segments? We have shown that the cellular contexts of the development of the connectin-positive muscles Th 21-24 and Ab 21-24 are quite distinct. In the thoracic segments the precursors for these muscles develop in proximity to a large cluster of connectin-positive cells that are not muscle precursors. In the development of neuromuscular connections, connectin might be one component of a system providing differential adhesive cues to differentiating muscles and outgrowing motor neurons. Perhaps more connectin is required on the thoracic muscles to provide a differential signal within the specific balance of adhesive cues in the thoracic landscape. Alternatively, the level of connectin expression may be irrelevant but the pattern may be important. If so, the variation in level may simply reflect that different homeotic genes are used to generate specific patterns of connectin expression in different segments.

We have shown that connectin can be linked to the cell-surface via a GPI-anchor; however, the functional significance of this is unknown. GPI-anchors are present in a broad range of cell surface proteins (reviewed by Ferguson and Williams, 1988; Ferguson, 1991). Their common feature is insertion in the outer membrane and their functions are diverse, but GPI-linked proteins have been shown to be involved in signal transduction (Stefanova et al., 1991; Bing et al., 1991), intracellular targeting (Dotti et al., 1991), and the endocytic process, phagocytosis (Rothberg et al., 1990b). As for connectin, other GPI-anchored molecules have been implicated in mediating cell-cell adhesion via a homophilic binding mechanism, e.g. NCAM (Edelman et al., 1987), fasciclin I (Elkins et al., 1990) and chaoptin (Krantz and Zipursky, 1990). Several GPI-linked molecules have been shown to interact with tyrosine kinases, suggesting a potential mechanism for signal transduction (Stefanova et al., 1991). In Drosophila, fasciclin I and the abl tyrosine kinase may interact together in a signal transduction pathway involved in growth cone guidance or extension (McAllister and Goodman, 1992). Hence, it is possible that connectin also plays a role in signal transduction. In comparison with a transmembrane protein, a GPI-linked protein may...
occupy a smaller volume in the membrane and have greater lateral mobility. Such mobility may be important for the plasticity of growth cones during migration and pathfinding, but clustering of connectin molecules at the final cell-cell contact sites may strengthen and stabilise the adhesion.

Using a monoclonal antibody that is capable of inhibiting connectin homophilic adhesion in vitro, we have been unable to demonstrate an effect in vivo. It is possible that the antibody concentration used was insufficient to maintain a constant blockade of connectin function. However, Nose et al. (1992) observed no obvious derangement of neuromuscular development in connectin mutants that produced only 1/20th of the normal amount of transcript. More recently an X-ray-induced deletion of the connectin locus has been generated; neuromuscular development is not clearly affected but the mutation is lethal and more analysis will be required to determine the basis of this lethality (A. Nose, personal communication). In several cases, null mutations in genes encoding cell adhesion molecules, e.g. fasciclin I and fasciclin III, have surprisingly little effect on development (see Hortsch and Goodman, 1991; Keshishian and Chiba, 1993). This may reflect the existence of back-up or parallel pathways. Thus, in the case of connectin, we may have to inactivate more than one adhesion system before we can unmask its developmental role.

REFERENCES


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