Cis-activation of L1-mediated Ankyrin Recruitment by TAG-1 Homophilic Cell Adhesion*

(Received for publication, August 27, 1998, and in revised form, September 30, 1998)

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Neural cell adhesion molecules (CAMs) of the immunoglobulin (Ig) superfamily mediate not only cell aggregation but also growth cone guidance and neurite outgrowth. In this study we demonstrate that two neural CAMs, L1-CAM and TAG-1, induce the homophilic aggregation of Drosophila S2 cells but are unable to interact with each other when expressed on different cells (trans-interaction). However, immunoprecipitations from cells co-expressing L1-CAM and TAG-1 showed a strong cis-interaction between the two molecules in the plane of the plasma membrane. TAG-1 is linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor and therefore is unable to directly interact with cytoplasmic proteins. In contrast, L1-CAM-mediated homophilic cell adhesion induces the selective recruitment of the membrane skeleton protein ankyrin to areas of cell contact. Immunolabeling experiments in which S2 cells expressing TAG-1 were mixed with cells co-expressing L1-CAM and TAG-1 demonstrated that the homophilic interaction between TAG-1 molecules results in the cis-activation of L1-CAM to bind ankyrin. This TAG-1-dependent recruitment of the membrane skeleton provides an example of how GPI-anchored CAMs are able to transduce signals to the cytoplasm. Furthermore, such interactions might ultimately result in the recruitment and the activation of other signaling molecules at sites of cell contacts.

The navigation of growth cones to their targets in the developing embryonic nervous system is a critical step in the patterning of neuronal projections. Substantial evidence suggests that axonal guidance depends in part on cell surface and on extracellular matrix molecules, which are expressed along the path of the advancing growth cones (1, 2). Axonal extension and pathway choices are influenced by membrane receptors to these molecules, which are expressed on the growth cone.

One group of such molecules are cell adhesion molecules (CAMs) belonging to the immunoglobulin (Ig) superfAMILY that are expressed by advancing growth cones and are able to recognize and transduce environmental signals (3). Ig domain CAMs have been implicated to act both as receptors as well as substrates for growing axons (4, 5). For example, members of the L1 family of neural CAMs, such as mammalian L1-CAMs and chicken Ng-CAM, not only exhibit a strong Ca²⁺-independent homophilic adhesive activity (6,7), they also promote neurite outgrowth in culture, probably by the activation of neuronal FGF receptors (8). Through the interaction with the cytoplasmic linker protein ankyrin, L1 family members are also connected to the membrane skeleton (9). Human L1-CAM and the Drosophila L1 homologue neuroglian both recruit ankyrin and other components of the membrane skeleton to cell contact sites in Drosophila S2 cell aggregates (10, 11). This interaction strictly depends on the extracellular L1 adhesive activity, and ankyrin binding in turn stabilizes the L1 adhesive interaction (12).

Members of another subgroup of Ig domain neural CAMs are anchored in the plasma membrane by a glycosylphosphatidylinositol (GPI) moiety. These include TAG-1 in mammalian species and its chicken homologue axonin-1 (13, 14). TAG-1/axonin-1 has been shown to mediate homophilic cell adhesion and is able to promote neurite outgrowth in culture (13, 15, 16). However, TAG-1-induced neurite growth is not only mediated by but also requires other neuronal membrane proteins, such as L1- or β₁ integrin-type molecules (17, 18). TAG-1/axonin-1 interacts with a number of different heterophilic binding partners, including several members of the L1 family, NCAM, nervous tissue-specific chondroitin sulfate proteoglycans, and several extracellular matrix molecules (18, 19). It has been suggested that some of these heterophilic interactions might enable TAG-1 to induce or influence intracellular signaling processes (5). Axonin-1 and Ng-CAM expressed in the same plasma membrane engage in a strong cis-interaction, forming larger multimeric complexes that are also associated with several intracellular protein kinase systems (18, 20). This cis-interaction between TAG-1/axonin-1 and L1-type molecules is essential for the stimulation of neurite outgrowth on TAG-1/axonin-1 substrates in culture, and homophilic TAG-1 cell adhesion appears to activate TAG-1-associated L1-CAM molecules (16, 17). Although several lines of evidence suggest that the interaction of TAG-1 with L1-CAM is an important link in TAG-1-initiated signal transduction, a direct demonstration that TAG-1 is capable of altering the functional state of L1-CAM has been missing. In our present study we demonstrate that TAG-1-mediated homophilic cell adhesion induces an intracellular restructuring of the membrane skeleton by the cis-activation of human L1-CAM.

The abbreviations used are: CAM, cell adhesion molecule; DiI, 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate; FGF, fibroblast growth factor; GPI, glycosylphosphatidylinositol; Ig, immunoglobulin; NCAM, neural cell adhesion molecule; Ng-CAM, neuron-glia cell adhesion molecule; PAGE, polyacrylamide gel electrophoresis.
unlabeled cells at a ratio of 1:1 to a final cell concentration of 3, as described by Hortsch. The vital fluorescent membrane dye DiI (Molecular Probes, Eugene, OR) was added to the cell culture medium to label the cell surface. Labeled cells were mixed with unlabeled cells to form mixed aggregates. To assess cell adhesion, mixed aggregates were incubated on a shaking platform for 4 hours. After the incubation, the aggregates were washed and permeabilized with 0.5% Triton X-100 in TBS buffer and fixed with 3% paraformaldehyde. FACS analysis was performed to determine the percentage of DiI-labeled cells.

**Materials and Antibodies**—A mouse polyclonal serum was raised against a glutathione S-transferase-Drosophila ankyrin fusion protein, which has been previously described (21). A rabbit polyclonal serum against human L1-CAM was raised by Dr. Vance Lemmon (Case Western Reserve University, Cleveland, OH), and the rabbit anti-TAG-1 serum was characterized previously (22, 23). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG, Texas Red-conjugated goat anti-rabbit IgG, and horseshadish peroxidase-conjugated goat anti-rabbit IgG were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). The rabbit anti-TAG-1 serum was characterized previously (22, 23). Subcloned cDNAs under the control of the Drosophila metallothionein promoter (24) were cloned into the pSP65 vector (Promega, Madison, WI) and used as templates for in vitro transcription and translation. The recombinant human L1-CAM and human TAG-1 proteins were expressed in transfected Drosophila S2 cells. The pRmHa3 constructs expressing human L1-CAM and human TAG-1 were induced overnight with 0.7 mM CuSO4 to the cell culture medium. Without inducing cell aggregation by an incubation step on a rotary shaker, the cells were pelleted and solubilized in cold dilution buffer (60 mM Tris/HCl, pH 7.5, 80 mM NaCl, and 1.25% Triton X-100, 6 mM EDTA, and a mixture of protease inhibitors). The soluble fraction was incubated overnight with rabbit anti-TAG-1 antibodies. Secondary antibodies were further incubated with Protein A-Sepharose beads (Amersham Pharmacia Biotech) for 2 hours, and immunoprecipitates were eluted with SDS gel electrophoresis buffer after three washing steps. After separation on 10% SDS-PAGE gels, proteins were transferred to a nitrocellulose filter and then probed with anti-L1-CAM antibody.

**RESULTS**

**Drosophila S2 Cells Which Express Human TAG-1 Do Not Form Mixed Aggregates**—Human L1-CAM and TAG-1 have both been shown to induce the aggregation of Drosophila S2 cells by a homophilic mechanism (23, 27). Although initial experiments using the chicken homologues of TAG-1 and L1-CAM, axonin-1 and Ng-CAM, coated to Covaspheres, suggested that these molecules might also be able to interact with each other when expressed on different surfaces (30), follow-up experiments using axonin-1 and Ng-CAM expressed by tissue culture cells indicated that these molecules are unable to engage in an adhesive trans-interaction (18). To investigate whether human L1-CAM and TAG-1 are able to interact with each other, we performed experiments using the chicken homologues of TAG-1 and L1-CAM, axonin-1 and Ng-CAM, coated to Covaspheres. We found that these molecules do not form mixed aggregates, as evidenced by FACS analysis. This result suggests that the ability of human L1-CAM and TAG-1 to interact with each other is specific to the chicken homologues and does not extend to the human homologues.

**Immunoprecipitation of L1-CAM and TAG-1**—Immunoprecipitations were performed using a modification of the protocol by Anderson andBlobel (29). For each immunoprecipitation, 1 × 10^6 cells expressing either L1-CAM or both L1-CAM and TAG-1 were induced overnight with 0.7 mM CuSO4. Without inducing cell aggregation by an incubation step on a rotary shaker the cells were pelleted and solubilized in cold dilution buffer (60 mM Tris/HCl, pH 7.5, 80 mM NaCl, and 1.25% Triton X-100, 6 mM EDTA, and a mixture of protease inhibitors). The soluble fraction was incubated overnight with rabbit anti-TAG-1 antibodies. Secondary antibodies were further incubated with Protein A-Sepharose beads (Amersham Pharmacia Biotech) for 2 hours, and immunoprecipitates were eluted with SDS gel electrophoresis buffer after three washing steps. After separation on 10% SDS-PAGE gels, proteins were transferred to a nitrocellulose filter and then probed with anti-L1-CAM antibody.

**Experimental Procedures**

**Materials and Antibodies**—A mouse polyclonal serum was raised against a glutathione S-transferase-Drosophila ankyrin fusion protein, which has been previously described (21), and used at a dilution of 1:200 for indirect immunofluorescence microscopy. Rabbit anti-L1-CAM was a gift from Dr. Vance Lemmon (Case Western Reserve University, Cleveland, OH), and the rabbit anti-TAG-1 serum was characterized previously (22, 23). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG, Texas Red-conjugated goat anti-rabbit IgG, and horseshadish peroxidase-conjugated goat anti-rabbit IgG were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). The rabbit anti-TAG-1 serum was characterized previously (22, 23). Subcloned cDNAs under the control of the Drosophila metallothionein promoter (24) were cloned into the pSP65 vector (Promega, Madison, WI) and used as templates for in vitro transcription and translation. The recombinant human L1-CAM and human TAG-1 proteins were expressed in transfected Drosophila S2 cells. The pRmHa3 constructs expressing human L1-CAM and human TAG-1 were induced overnight with 0.7 mM CuSO4 to the cell culture medium. Without inducing cell aggregation by an incubation step on a rotary shaker, the cells were pelleted and solubilized in cold dilution buffer (60 mM Tris/HCl, pH 7.5, 80 mM NaCl, and 1.25% Triton X-100, 6 mM EDTA, and a mixture of protease inhibitors). The soluble fraction was incubated overnight with rabbit anti-TAG-1 antibodies. Secondary antibodies were further incubated with Protein A-Sepharose beads (Amersham Pharmacia Biotech) for 2 hours, and immunoprecipitates were eluted with SDS gel electrophoresis buffer after three washing steps. After separation on 10% SDS-PAGE gels, proteins were transferred to a nitrocellulose filter and then probed with anti-L1-CAM antibody.

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**Results**

**Drosophila S2 Cells Which Express Human TAG-1 Do Not Form Mixed Aggregates**—Human L1-CAM and TAG-1 have both been shown to induce the aggregation of Drosophila S2 cells by a homophilic mechanism (23, 27). Although initial experiments using the chicken homologues of TAG-1 and L1-CAM, axonin-1 and Ng-CAM, coated to Covaspheres, suggested that these molecules might also be able to interact with each other when expressed on different surfaces (30), follow-up experiments using axonin-1 and Ng-CAM expressed by tissue culture cells indicated that these molecules are unable to engage in an adhesive trans-interaction (18).
to induce the aggregation of cells by a heterophilic mechanism, populations of S2 cells expressing either human L1-CAM or both human L1-CAM and human TAG-1 were induced overnight with 0.7 mM CuSO₄ and solubilized with a Triton X-100-containing buffer. As indicated at the bottom of the figure immunoprecipitations from these extracts were performed using either a rabbit non-immune serum or a rabbit antiserum against TAG-1. Immunoprecipitated proteins were separated on 10% SDS-PAGE gels and transferred to nitrocellulose filters, which were probed with a rabbit anti-L1-CAM antiserum.

Western blots stained with anti-L1-CAM

**Fig. 3. Co-immunoprecipitation of L1-CAM and TAG-1 protein from S2 cell protein extracts.** S2 cells expressing either human L1-CAM, TAG-1, or the unrelated CAM *Drosophila* fasciclin I were stained with the fluorescent dye DiI and mixed with unlabeled S2 cells expressing the same set of homophilic adhesion molecules. Most cell aggregates were comprised of several hundred cells, but no significant inclusion of cells expressing TAG-1 into cell clusters expressing L1-CAM and vice versa was observed (Fig. 2). As a result approximately 50% of all cell clusters consisted of DiI-labeled cells expressing one adhesive molecule, whereas the other clusters consisted of unlabeled cells expressing the other CAM. Similar results were obtained mixing induced S2:L1-CAM or S2:TAG-1 cells with S2 cells expressing the unrelated CAM, fasciclin I. *Drosophila* fasciclin I was used as a negative control in these experiments, because it is not known to interact with L1-type molecules and a TAG-1 homologue has not been identified in *Drosophila*. In contrast, more than 90% of all cell clusters contained labeled as well as unlabeled S2 cells when both populations expressed either human L1-CAM or TAG-1. This is in agreement with the homophilic adhesive properties that have been demonstrated for both molecules (23, 27). These results also indicate that cells expressing either human TAG-1 or L1-CAM do not form mixed aggregates and that these two adhesive molecules do not engage in a measurable heterophilic trans-interaction with each other.

**Human L1-CAM and TAG-1 Engage in a Cis-interaction within the Plane of the Plasma Membrane**—Stable S2 cell lines expressing both L1-CAM and TAG-1 were used to demonstrate that the two molecules interact when expressed in the same cell. Immunoprecipitations with anti-TAG-1 antibodies followed by immunoblotting with an anti-L1-CAM antiserum indicated that L1-CAM and TAG-1 form stable complexes when present in the same plasma membrane (Fig. 3, lane 2). As shown in lane 1, no L1-CAM was immunoprecipitated from these cells with non-immune rabbit serum. The observed co-immunoprecipitation of L1-CAM with TAG-1 was also not caused by a cross-reactivity of the anti-TAG-1 antiserum toward human L1-CAM, because this antiserum failed to precipitate L1-CAM from cells that did not express TAG-1 (Fig. 3, lane 3).

**Fig. 4. Ankyrin is recruited to cell contact sites in S2 cells expressing human L1-CAM but not in cell aggregates expressing human TAG-1.** After protein induction from the transfected cDNA constructs S2 cells expressing human L1-CAM (A and B) or human TAG-1 (C and D) were allowed to aggregate, fixed, and fluorescently stained using a mouse anti-*Drosophila* ankyrin antiserum. Scale bar is 25 μm.

**Fig. 5. Ankyrin recruitment to human L1-CAM is induced by homophilic TAG-1-mediated cell adhesion.** S2 cells expressing human TAG-1 protein were mixed at a ratio of 20:1 with S2 cells co-expressing human TAG-1 and human L1-CAM (A-D) or with S2 cells expressing only human L1-CAM (E and F). This ratio of cells was selected to maximize the number of cell contacts between cells expressing both adhesion molecules with cells expressing only TAG-1, rather than contacts between cells expressing L1-CAM and TAG-1. After protein induction cells were briefly allowed to aggregate and subsequently processed for double immunofluorescence using a rabbit anti-L1-CAM antiserum (left panels) and a mouse anti-*Drosophila* ankyrin antiserum (right panels). The arrow in A marks a cell contact between two cells expressing L1-CAM as well as TAG-1. Cell contacts between cells expressing TAG-1 with cells expressing both adhesion molecules are indicated by arrowheads. Scale bar is 25 μm.
Homophilic, TAG-1-mediated S2 Cell Aggregation Does Not Induce Ankyrin Recruitment at Cell Contact Sites—Members of the L1 family induce the specific recruitment of ankyrin and other components of the membrane skeleton to sites of cell contact in S2 cell aggregates (10, 11). As shown for human L1-CAM in Fig. 4, A and B, endogenous S2 cell ankyrin is specifically recruited to cell contact sites by L1-CAM-mediated cell adhesion. No ankyrin staining of the plasma membrane can be detected in non-contact areas or in cells that have not joined cell aggregates. S2 cells expressing human TAG-1 exhibit robust homophilic cell aggregation (Fig. 2). However, no ankyrin recruitment to cell contacts or to other areas of the plasma membrane was ever observed in TAG-1-expressing cells (Fig. 4, C and D). In some cells, especially cells exhibiting no ankyrin recruitment to cell contact sites, ankyrin staining appears in a punctate pattern. The reason for this punctate intracellular distribution of ankyrin in S2 cells is unknown but has been observed and described before (10, 12).

TAG-1 Homophilic Cell Adhesion Induces L1-mediated Ankyrin Recruitment—Because TAG-1 is unable to directly interact with the intracellular membrane skeleton, we tested the possibility that it might activate the ability of L1-CAM to bind ankyrin. No recruitment of ankyrin to the plasma membrane was observed in single, non-aggregated S2 cells expressing L1-CAM as well as TAG-1 (not shown). However, when these co-expressing cells were mixed and co-aggregated with S2 cells expressing only TAG-1, a strong recruitment of ankyrin was observed at cell contact sites between these two cell lines (Fig. 5, A–D). The example shown in A depicts two cells that stain positive for L1-CAM (left side) and therefore express both L1-CAM and TAG-1. The corresponding micrograph on the right side of A shows the distribution of ankyrin in these cells. As indicated by the arrow, ankyrin was recruited to the cell contact between the two L1-CAM-positive cells. Ankyrin was also recruited to contact sites these two cells had developed to cells expressing only TAG-1 (marked by arrowheads). B, C, and D show a range of other examples of ankyrin recruitment to cell contact sites between the two different S2 cell types. A quantitative evaluation of these experiments indicates that approximately 75% of such cell contacts exhibited a recruitment of ankyrin (Table I).

Although we observed no trans-interaction between TAG-1 and L1-CAM in our S2 cell aggregation experiment shown in Fig. 2, we considered that a weak trans-interaction between TAG-1 and L1-CAM expressed by two different cells might be responsible for the observed induction of ankyrin binding to L1-CAM. We therefore determined the ankyrin distribution in mixtures of cells expressing either TAG-1 or L1-CAM. By analyzing a large number of immunostained slides, we identified

![Model of homo- and heterophilic interactions of TAG-1](image)

**Table I**

| Type of cell contact | Total number of cell contacts evaluated | Number of cell contacts with ankyrin recruitment | Cell contacts with ankyrin recruitment % |
|---------------------|----------------------------------------|-----------------------------------------------|---------------------------------------|
| L1/TAG-1            | 29                                     | 0                                             | 0                                     |
| L1 + TAG-1/TAG-1    | 115                                    | 86                                            | 75                                    |

![Ankyrin Recruitment](image)

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rare instances in which a TAG-1-expressing cell ended up in contact with a cell expressing just L1-CAM (Fig. 5, E and F). In none of the 29 examples we analyzed did we observe a recruitment of ankyrin to these cell contact points (Fig. 5 and Table I). This indicates that L1-CAM and TAG-1 must be co-expressed in the same cell for TAG-1-mediated cell adhesion to induce ankyrin binding to L1-CAM.

**DISCUSSION**

Complex homo- and heterophilic interactions between different adhesion molecules within the plane of the plasma membrane and between adjacent cells have been proposed to play an important role in regulating the growth and guidance of axons during embryonic neurogenesis (5). Our present *in vitro* study addresses how two different CAMs, human TAG-1 and human L1-CAM, interact and functionally regulate each other. Some groups of CAMs are transmembrane proteins and therefore potentially able to directly influence intracellular processes, e.g. by activating second messenger signaling cascades and/or by reorganizing components of the cytoskeleton. However, other CAM families, such as the TAG-1/axonin-1 and the F3/F11 groups, are anchored in the plasma membrane by a GPI moiety and therefore lack the means to interact with cytoplasmic proteins without additional linker proteins. Nevertheless, GPI-anchored CAMs are also able to operate as signal-transducing molecules during neuronal development. Although TAG-1/axonin-1 is fully functional as a homophilic CAM without engaging in any heterophilic cis-interactions, its ability to associate with other membrane proteins, especially L1-CAM, appears to be essential for its neurite outgrowth-promoting function (17, 31). Several models similar to the one displayed in Fig. 6 have been proposed in which TAG-1/axonin-1 associates with other membrane proteins expressed in the same plasma membrane in a cis-interaction to form a signal-transducing, multimeric protein complex (17, 18). A similar model has also been suggested to explain the adhesion-dependent activation of neuronal FGF receptors by L1-CAM, NCAM, and N-cadherin (8).

Although several molecular details of the TAG-1 interaction with L1-CAM have been elucidated, its regulatory and functional aspects are not well understood. L1-CAM/Ng-CAM not only can be co-immunoprecipitated with TAG-1/axonin-1, but cross-linking experiments suggest that both molecules directly bind to each other (18, 20). The part of the axonin-1 molecule responsible for this cis-interaction has been mapped to its amino-terminal Ig protein domains (32), whereas the homophilic adhesive activity of TAG-1/axonin-1 is mediated by its fibronectin type III domains (23). The protein domains of L1-CAM that mediate TAG-1 recognition and binding have not been identified yet. Also whether the homophilic adhesive activities of L1-CAM and TAG-1 regulate their cis-interaction is currently unknown. However, co-capping experiments reported by Buchstaller et al. (18) and the quantity of L1-CAM that co-immunoprecipitated with TAG-1 in our experiments suggest that a significant fraction of L1-CAM molecules forms heterodimeric complexes with TAG-1 before the two CAMs engage in cell adhesion and L1-CAM binds to the membrane skeleton. After L1-CAM interacts with ankyrin, it becomes resistant to Triton X-100 extraction as used in our immunoprecipitation experiments and is unavailable for precipitation with antibodies (10).

TAG-1/axonin-1 and L1-CAM/Ng-CAM are co-expressed in several locations during nervous system development, suggesting that their interaction is physiologically relevant (31, 33, 34). In cultures of chicken dorsal root ganglion neurons Ng-CAM and axonin-1 protein are found in overlapping areas on growth cone membranes, and axonin-1 expression by these cells is required for neurite outgrowth on both axonin-1 and Ng-CAM substrata (31). In contrast, in other developing neurons, e.g. in the rat embryonic spinal cord, TAG-1 and L1-CAM expression appear to be locally and temporally segregated (22, 35). This finding indicates that TAG-1/axonin-1 and L1-CAM/Ng-CAM are not obligatory co-receptors but are able to function independently. It might mean either that the signaling capabilities of TAG-1/axonin-1 are limited in certain areas of the developing nervous system or at certain developmental time points or that TAG-1/axonin-1 associates with other membrane proteins than L1-CAM/Ng-CAM to form functionally active protein complexes.

Although the cis-activation of neuronal FGF receptors by L1-CAM is thought to be the initial step in adhesion-induced neurite outgrowth, processes such as the recruitment of other signaling molecules to cell contact sites might also have an important, more indirect role. Our findings presented here would support the hypothesis that TAG-1 homophilic adhesion might also activate neuronal FGF receptor activity via L1-CAM. Other cellular changes that appear to be regulated by the TAG-1/axonin-1 interaction with L1-CAM/Ng-CAM are the recruitment and the release of several different protein kinase activities that are associated with the two CAMs in chicken dorsal root ganglion neurons (20). In the case of integrin- and cadherin-mediated cell adhesion, interactions with cytoskeletal elements induce the assembly of signal-generating and -processing multiprotein complexes at the cytoplasmic aspect of the adhesion contact site. This makes the observed assembly of membrane skeleton components in response to TAG-1-mediated homophilic cell adhesion especially significant. However, the most tantalizing aspect of the results reported here is the observation that TAG-1 homophilic cell adhesion directly regulates the functional status of L1-CAM. These findings suggest a mechanism that explains how GPI-anchored CAMs might actively participate in regulating the growth, organization, and differentiation of neuronal cells during development.

**Acknowledgments**—We thank Dr. Vance Lemmon for generously providing the rabbit anti-L1 antisera and the human L1 cDNA and Drs. Stephen Ernst and Robert Chandler for a critical reading of the manuscript. D. K. would also like to thank Dr. Joseph Papamatiakis for his support.

**REFERENCES**

1. Dodd, J., and Jessell, T. M. (1988) *Science* **242**, 692–699
2. Goodman, C. S. (1996) *Annu. Rev. Neurosci.* **19**, 341–377
3. Brumendorf, T., and Rutjens, P. E. (1993) *J. Neurochem.* **61**, 1207–1219
4. Lii-Mayer, C., and Lemmon, V. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 7753–7757
5. Sondergeld, P., and Rutjens, P. E. (1992) *J. Cell Biol.* **119**, 1387–1394
6. Lemmon, V., Farr, K. L., and Lagena, C. (1980) *Neuron* **2**, 1597–1603
7. Grumet, M., Hoffman, S., Chuong, C.-M., and Edelman, G. M. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 7889–7993
8. Dobert, P., and Walsh, F. S. (1996) *Mol. Cell. Neurosci.* **8**, 99–111
9. Davis, J. Q., and Bennett, V. (1994) *J. Biol. Chem.* **269**, 27163–27166
10. Dubreuil, R. R., Maevicar, G., Dissayake, S., Liu, C., Homer, D., and Hortsch, M. (1998) *J. Cell Biol.* **133**, 647–655
11. Hortsch, A., O’Shea, K. S., Zigel, U., Kim, P., Vallejo, Y., and Dubreuil, R. R. (1998) *Cell Adhes. Commun.* **5**, 61–73
12. Hortsch, M., Homer, D., Dhar Mahothra, J., Chang, S., Frankel, J., Jefford, G., and Dubreuil, R. R. (1998) *J. Cell Biol.* **142**, 251–261
13. Furley, A. J., Morton, S. B., Manalo, D., Karageorgos, D., Dodd, J., and Jessell, T. M. (1990) *Cell* **61**, 157–170
14. Zoellig, R. A., Rader, C., Schroeder, A., Kalschenk, M. B. V., von Bohlen und Halbach, F., Osterwalder, T., Ivan, C., Stoeckli, R. T., Afholter, H. U., Fritz, A., Hafen, E., and Sondergeld, P. (1992) *Eur. J. Biochem.* **204**, 453–463
15. Stoeckli, E. T., Kuhn, T. B., Dur, C. O., Ruegg, M. A., and Sondergeld, P. (1991) *J. Cell Biol.* **112**, 449–455
16. Rader, C., Stoeckli, E. T., Ziegel, U., Osterwalder, T., Kunz, B., and Sondergeld, P. (1993) *Eur. J. Biochem.* **215**, 133–141
17. Felsenfeld, D. P., Hynes, M. A., Skoler, K. M., Furley, A. J., and Jessell, T. M. (1994) *Neuron* **12**, 675–690
18. Buchstaller, A., Kunz, S., Berger, P., Kunz, B., Ziegel, U., Rader, C., and Sondergeld, P. (1996) *J. Cell Biol.* **135**, 1593–1607
19. Milew, M., Maurel, P., Haring, M., Margolis, R. K., and Margolis, R. U. (1996) *J. Biol. Chem.* **271**, 15716–15723
20. Kunz, B., Ziegel, U., Kunz, B., and Sondergeld, P. (1996) *J. Cell Biol.* **135**, 253–267
21. Dubreuil, R. R., and Yu, J.-Q. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10285–10289
22. Dodd, J., Morton, S. B., Karagogeos, D., Yamamoto, M., and Jessell, T. M. (1988) Neuron 1, 105–116
23. Tsotra, P. C., Theodorakis, K., Papamatheakis, J., and Karagogeos, D. (1996) J. Biol. Chem. 271, 29216–29222
24. Bunch, T. A., Grinblat, Y., and Goldstein, L. S. B. (1988) Nucleic Acids Res. 16, 1043–1061
25. Jokerst, R. S., Weeks, J. R., Zehring, W. A., and Greenleaf, A. L. (1989) Mol. Gen. Genet. 215, 266–275
26. Bieber, A. J. (1994) in Drosophila melanogaster: Practical Uses in Cell Biology (Goldstein, L., and Fyrberg, E., eds) Vol. 44, pp. 683–696, Academic Press, San Diego
27. Hortsch, M., Wang, Y. E., Marikar, Y., and Bieber, A. J. (1995) J. Biol. Chem. 270, 18809–18817
28. Hortsch, M., Avossa, D., and Meyer, D. I. (1985) J. Biol. Chem. 260, 111–120
29. Anderson, D. J., and Blobel, G. (1983) Methods Enzymol. 16, 1043–1061
30. Kuhn, T. B., Stoeckli, E. T., Condrau, M. A., Rathjen, F. G., and Sonderegger, P. (1991) J. Cell Biol. 115, 1113–1126
31. Stoeckli, E. T., Ziegler, U., Bleiker, A. J., Groscurbt, P., and Sonderegger, P. (1996) Dev. Biol. 177, 15–29
32. Rader, C., Kunz, B., Lierheimer, R., Giger, R. J., Berger, P., Tittmann, P., Gross, H., and Sonderegger, P. (1996) EMBO J. 15, 2056–2068
33. Honig, M. G., and Kueuter, J. (1995) Dev. Biol. 167, 563–583
34. Yamagata, M., Herman, J. P., and Sanes, J. R. (1995) J. Neurosci. 15, 456–457
35. Karagogeos, D., Morton, S. B., Casano, F., Dodd, J., and Jessell, T. M. (1991) Development 112, 51–67
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J. Biol. Chem. 1998, 273:33354-33359.
doi: 10.1074/jbc.273.50.33354

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