Differential roles of multiple adhesion molecules in cell migration: Granule cell migration in cerebellum

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Summary. The migration of cerebellar granule cells from the external granular layer to the internal granular layer is mediated by the radial Bergmann glial fiber. Recent works have shown that cell adhesion molecules, extra-cellular matrix proteins and proteolytic enzymes or their activators are involved in this process. Immuno-localization studies showed differential temporal and spatial expression patterns of different adhesion molecules, their isoforms, and post-translational modification during different stages of granule cell migration. Functional perturbation experiments using cerebellar explant cultures demonstrated that several adhesion molecules as well as plasminogen activator are involved in granule cell migration and are required in different stages. Other systems used to study granule cell migration including dissociated microwell cultures and granule cell deficient mouse mutants are discussed in the context of adhesion molecules. The results accumulated so far suggest that the migration of granule cells is a complex process in which the cooperation of a group of molecules with different functions, some for adhesion some for de-adhesion, are required to fulfill the different needs during the migratory course.
Key words. Adhesion molecules; N-CAM; cytotactin; tenascin; cell migration; neuronal migration; granule cells; cerebellum development; plasminogen activator.

One of the most prominent features of the vertebrate brain is the well organized horizontal layers of cell bodies and neurites in the cortex. How these layers form during development is one of the major questions in developmental neurobiology. This process includes sequential events of cell proliferation, cell migration, neurite outgrowth, cell contact, cell recognition, and synaptic activity[18]. For the study of cell migration in the developing brain, cerebellar granule cell migration has been considered to be one of the best models. This is because neuroanatomical data in cerebella are well documented and the organized cytoarchitecture makes any disrupted patterns recognizable whether they are produced by in vivo mutation or in vitro perturbation[27, 55, 62]. Indeed several neurological mutants with abnormal cerebellar morphology involving disrupted granule cell migration do exist in mice *(weaver, staggerer,* and *reeler)*[67] and humans (ataxia telangiectasia, tuberous sclerosis)[17]. They provide model systems for analyzing molecular defects in granule cell migration.

In addition, cerebellar granule cell migration takes place late in development, occurring postnatally in mice, and thus is more accessible to in vitro experimentation. Recent works using cell aggregation assays[6] have led to the identification of several adhesion molecules involved in neuron-neuron and neuron-glia adhesion (for review, see Edelman[23] and Jessel[45]. They can be tested by two assays for cerebellar granule cell migration. Microwell cultures developed by Trenkner[69] and Hatten[35] provide a system to study the behavior of neurons and glia, and allow recombination of cells from different sources such as co-culture of normal neurons and *weaver* glia[39] for further analyses. Cerebellar explant cultures developed by Moonen[53] provides an assay for cell migration in a more physiological setup than dissociated cells.

A recent comprehensive review by Burgoyne and Cambray-Deakin[9] has covered the cell biology of granule cells, particularly those of the cytoskeleton and surface molecules. It is the purpose of this review to focus on the expression and differential roles of three of the adhesion molecules present in developing cerebella: neural cell adhesion molecules (N-CAM), neuron-glia cell adhesion molecule (Ng-CAM, which is the same or very similar to the L1 molecule, see below) and cytotactin (which is the same or very similar to tenascin, J1 and others; see below). We will first summarize the process of granule cell migration, the known facts of these adhesion molecules, then provide a current review of these three adhesion molecules including their expression during cerebellar granule cell migration. The effects of incubation with antibodies to these adhesion molecules, extracellular matrix molecules and their enzymes, as well as plasminogen activator inhibitors on granule cell migration in cerebellar explant cultures will be discussed.

Cerebellar granule cell migration

Classical studies, including histological and autoradiographic analyses, have detailed the morphogenesis of the cerebellar cortex at the cellular level[1, 30, 62]. At the stage of granule cell migration from the pial surface to the internal granular layer, the developing cerebellar cortex is composed of the external granular, molecular, Purkinje cell, and internal granular layers (fig. 1). The external granular layer is composed primarily of external granule cells which later segregate into the proliferative and premigratory zones. The proliferative zone is composed of the external granule cells immediately beneath the pia mater. These cells are round in shape and actively dividing with a cell cycle of 19 h determined by ^{3}H-thymidine

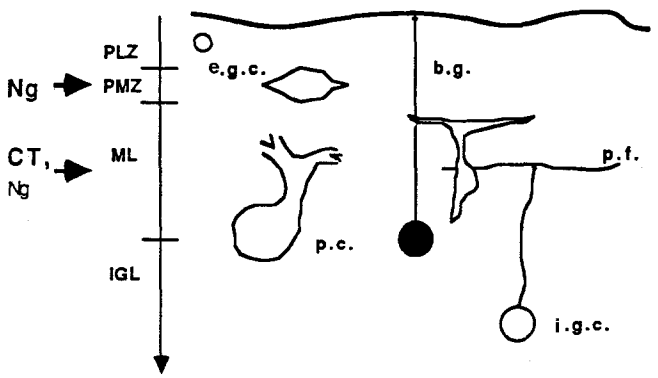

Figure 1. Schematic diagram of the events during migration of cerebellar granule cells. Long arrow points to the direction of cell migration. Cells toward the right side of the figure are more mature cells that have migrated a longer distance. Thick arrows point to the sites where the specific adhesion molecules act. In the pre-migratory zone, Ng-CAM plays a major role. In the molecular layer, cytotactin plays a major role while Ng-CAM plays a minor role. Cerebellar layers: PLZ, proliferative zone; PMZ, pre-migratory zone; ML, molecular layer; IGL, internal granular layer. The external granular layer is composed of PLZ and PMZ. Names of structures: b.g., Bergmann glia; e.g.c., external granule cell; i.g.c., internal granule cell; p.c., Purkinje cell; p.f., parallel fiber. Not to scale.

incorporation[30]. After a final mitosis, the cells descend to the pre-migratory zone and stay there for 28 h to prepare a set of molecules necessary for migration (see below for the many cell surface molecules specifically turned on during this stage). The cell shape undergoes dramatic changes during this period. Initially, it elongates into a spindle shape, then the cell soma moves internally very rapidly (it takes only 4 h for it to reach the internal granular layer), perpendicular to the previous cellular axis, to form a 'T' shape, with the cell nucleus at the bottom and its axon forming the cross ot the 'T' (fig. 1)[30]. Rakic[62] observed that the granule cell soma and its leading process are closely apposed to the Bergmann glial fiber which spans radially from its cell body in the Purkinje cell layer to the pia mater. These glial cells may serve as a guide for the granule cell to migrate from the external to the internal granular layer[63].

Adhesion molecules

The molecular mechanism of granule cell migration is unknown, although cell-cell adhesion during the process has been thought to play an important role. Recently, several molecules involved in cell adhesion have been identified, purified and characterized. Molecular cloning of these adhesion molecules followed by nucleotide sequence analysis has revealed that they can be grouped into several gene families. The most notable one related to granule cell migration is the immunoglobulin superfamily[71]. Identification of these adhesion molecules has been achieved mainly through the application of immunological assays using antibodies reacted with cell surface components to inhibit cell adhesion in vitro[24,45,65]. Immuno-localization of these adhesion molecules in development usually gives good indications of their in vivo function. Their physiological functions were further explored with perturbation experiments using antibodies to these adhesion molecules in a variety of in vitro or in vivo assays.

The neural cell adhesion molecule (N-CAM), was the first to be identified using cell aggregation assays. It is a cell surface glycoprotein, mainly composed of mol.wt 180, 140 and 120 kDa species, and is expressed in the nervous system and developing muscle as well as transiently in sites of induction during embryogenesis[19]. The primary sequence contains immunoglobulin-like domains, each consisting of approximately one hundred amino acids folded in a beta-sheet structure usually linked by a disulfide bond. There is only one N-CAM gene, but several tissue specific isoforms of N-CAM are generated through differential splicing[56]. The major variations are located near the C-terminus with some forms having different cytoplasmic domains (e.g. the 180 kDa polypeptide) or missing the transmembrane domain (e.g. the 120 kDa polypeptide chain). Another variation to N-CAM is generated by post-translational modification. N-CAM purified from embryonic brains (the E form of N-CAM[25]) appears as a diffuse band on polyacrylamide gel electrophoresis rather than the three distinct bands of mol.wt 180, 140 and 120 kDa of adult brains (the A form of N-CAM). Later studies showed that the E form of N-CAM is distinct from the A form in that it contains more of a characteristic poly-sialic acid residue (N-CAM PSA) and other carbohydrate epitopes[25]. Using a monoclonal antibody specific to N-CAM PSA, it was found that N-CAM PSA is also present restrictively on some neurons in the adult brain[13] and during regeneration.

The binding region of N-CAM is near the N-terminus and is highly conserved. The major binding mechanism is homophilic (N-CAM binds N-CAM)[42]. This is best demonstrated by reconstitution experiments using transfection of mouse sarcoma cells with plasmid containing N-CAM cDNA sequence[52]. The functional characteristics of the E forms of N-CAM were further explored by in vitro aggregation assays using lipid vesicles reconstituted with purified adhesion molecules. The A form of N-CAM aggregates much faster than the E form, while the neuraminidase-treated E form of N-CAM, which loses its N-CAM PSA, aggregates faster than the non-treated E form[42]. Therefore the highly negative charged polysialic acid residues may repel each other and weaken the binding between developing or regenerating neurites, so that neurites can contact each other but remain mobile. After specific connections are established, the A form of N-CAM, which has a higher binding affinity, is expressed[23]. Other mechanisms modulating N-CAM binding may also exist and may involve other E form specific carbohydrate epitopes (e.g. 9E11[13,25]) or heparin-like molecules[16,47].

Another member of the immunoglobulin superfamily is Ng-CAM. It was first defined by the inhibition of neu-

ron-glia binding by a corresponding antibody[34] and it is probably the same as L1[54], NILE, 8D9 and G4 antigen[45]. Later, Ng-CAM was also found to be involved in neurite fasciculation[45] with a distribution much more restricted than N-CAM[22].

Contactin[64] or F11[8] is another nervous system adhesion protein belonging to the immunoglobulin family. It also contains domains homologous to fibronectin type III repeats. It is present mainly on neurites but not on cerebellar granule cells. The other members of the immunoglobulin superfamily include molecules of general immunological importance such as T cell receptor, HLA antigens, Fc receptors, molecules involved in myelin formation such as myelin associated glycoprotein and P_0, growth factor receptor such as the PDGF receptor, and molecules that work as receptors for neutrophils and rhinovirus such as I-CAM[68].

In invertebrates, members of the immunoglobulin superfamily are found to be involved in neurite fasciculation[37]. The wide range of molecular heterogeneity suggests that an evolutionary ancient molecule with a single immunoglobulin domain (e.g. Thy 1) may have carried out some primitive cell interaction functions. During evolution the progress of gene duplication, diversification, somatic recombination and double chain formation have resulted in a variety of molecules involved in diverse functions in cell-cell interaction[71].

Cytotactin[35] also termed tenascin[11] or J1 is an extracellular matrix protein with molecular weights between 190 and 240 kDa, and that tends to form hexamers (termed hexabrachion[27]). It was first detected in the myotendinous junction[10] and later was discovered in active mesenchyme such as teeth, hair and mammary gland and in regions of active cell migration[11,20]. In the nervous system, cytotactin/tenascin is produced by astrocytes[35], oligodendroglia[32] and some glioma cell lines[5], and was shown to be involved in neuron-glia adhesion[35]. Antibodies against tenascin have been shown to perturb cephalic neural crest cell migration in the chicken embryo[7]. The most interesting feature of cytotactin/tenascin is that it is composed of three domains homologous to epidermal growth factor, fibronectin type III repeats, and fibrinogen[32,46,59]. This unique composition suggests that it may serve in a dual fashion as an adhesion molecule as well as a growth factor. Recently a cell surface receptor for tenascin was identified and their binding appears to be mediated by an RGD dependent mechanism[4].

Temporal expression of adhesion molecules during granule cell migration

To see if N-CAM, Ng-CAM and cytotactin are involved in cerebellar granule cell migration, it is necessary to determine if they are present where migration occurs. The results from several papers are summarized in table 1. Immunocytochemical analysis using anti-N-CAM on the developing cerebella reveals that N-CAM is present in all layers (fig. 2 A and Chuong et al.[15]), staining Bergmann glial fibers and all neurons including granule cells[60]. It is unlikely to have a specific guiding function if all cells express N-CAM homogeneously. But when antibodies specific to different epitopes of N-CAM were tested, heterogeneity in the distribution of these epitopes was discovered. As described earlier, the major isoforms in the brain are of mol.wt 180, 140 and 120 kDa. N-CAM 180 (the 180 kDa polypeptide of N-CAM) contains an extra cytoplasmic domain which may have special interaction with the cytoskeleton[56,61]. This isoform is not expressed in non-neural tissues such as the skin[14] and in neuroblastoma cells it has been shown to be concentrated in the inter-cellular junctional region[61]. In various brain regions, there are different ratios of the three N-CAM isoforms[13]. When antibodies specific to N-CAM 180 are used to stain cerebella, the staining pattern is much more restrictive than when antibodies to all forms of N-CAM are used. N-CAM 180 is present only on the parallel fibers and the cell bodies of post-migratory granule cells (fig. 2 B and Chuong et al.[15]). N-CAM 180 is absent in the external granule cells, the migrating granule cell bodies, and the Bergmann glia[15,60]. Differences in post-translational modifications have also been examined. During the first to third week of post-natal development in mice, most of the E form of N-CAM is replaced by the A form. This is judged by the loss of N-CAM PSA and a non-sialic acid carbohydrate epitope 9E11[25]. This time period corresponds to the period of active cell migration and neurite outgrowth. Nagata and Schachner[57] initially reported no differences between the external and internal granular layer with immuno-blotting and micro-dissection, but later Hekmat et al.[41] were able to show the differential distribution of N-CAM PSA with remarkable resolution using ultrastructural methods. N-CAM PSA is absent in the proliferative zone, but begins to be expressed in the external granule cells when they enter the pre-migratory zone. N-CAM PSA remains on the granule cell body and leading process during migration. Once migration is complete, N-CAM PSA disappears from the granule cell body but is still present on the granule cell axons and dendrites until the synapses

Table 1. Expression of adhesion molecules in cerebellar granule cell migration

	N	N PSA	N 180	Ng	AMOG	CT
Proliferative zone	+	–	–	–	+	+
Premigratory zone	+	+	–	+	+	+
Molecular layer	+	+	+	+	+	+
Internal granular layer	+	+	+	–	+	+

Expression is indicated by '+'. N and N PSA are on both the neuronal and glial side. N 180 and Ng are on the neuronal side. AMOG and CT are on the glial side. N, neural cell adhesion molecules; N PSA, poly-sialic acid residue on N-CAM; N 180, the epitope of 180 kDa N-CAM polypeptide chain that is specific to the 180 kDa isoform; Ng, neuron-glia adhesion molecule; AMOG, adhesion molecule on glia; CT, cytotactin.

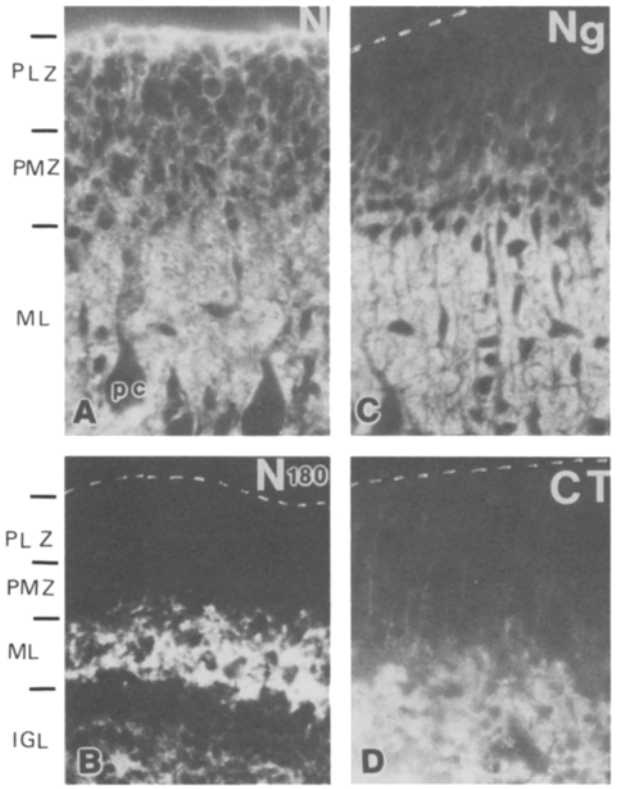

Figure 2. Immunolocalization of adhesion molecules in developing cerebella (chicken, E 15–16). Frozen sections of E 15 chicken cerebella were stained with antibodies to adhesion molecules and visualized with fluorescent secondary antibodies[15]. *A* N-CAM (all isoforms); *B* N-CAM 180; *C* Ng-CAM; *D* cytotactin. Note that N-CAM is present in all layers but appears to be expressed more in the proliferative zone. N-CAM 180 is expressed only in the molecular layer and the internal granular layer. Ng-CAM is expressed on the external granule cells when they enter the premigratory layer. Cytotactin is present in the Bergmann glial fibers and the molecular layer. The white dot line indicates where pia mater was. Abbreviations are the same as in fig. 1.

mature. The Bergmann glial fibers also express N-CAM PSA until after postnatal day (P) 15 in the mouse when it gradually disappears. Thus correlations at the subcellular level further support the hypothesis that N-CAM PSA is required for the migration of cell bodies and the movement of neurites.

Ng-CAM is absent in the proliferative zone of the external granular layer. It begins to be expressed in the pre-migratory granule cells. During migration it remains expressed on the granule cell body including the leading process (fig. 2C and Chuong et al.[15]). Persohn and Schachner[60] observed L1 to be present on the granule cell body but only on the side facing neurons, not the side facing the Bergmann glia. After migration, Ng-CAM disappears from granule cell bodies, but remains on their axons. L1/Ng-CAM is not present on the Bergmann glial fiber or on neurons such as basket neurons, stellate cells or Purkinje cell bodies.

Cytotactin first appears as a speckled pattern in the external granular layer in embryonic day (E) 14 chicken, with higher reactivity located at the border between the proliferative and pre-migratory zone. The pattern of distribution of cytotactin changes and by E 17 is organized radially along the Bergmann glial fiber (fig. 2D and Chuong et al.[15]). There is abundant cytotactin in the molecular layer, but it is not clear whether this cytotactin is associated with the glial membrane, the migrating granule cell body, the parallel fiber or the extracellular space.

Differential function of adhesion molecules during granule cell migration

The distribution of these adhesion molecules strongly suggests that they are involved in granule cell migration. Their functions in granule cell migration were enhanced using the cerebellar explant assay developed by Moonen[53]. The assay takes advantage of two facts: the granule cells are the only neurons still dividing at the time of explantation and they initiate migration after their last mitosis. Developing cerebellar explants (P 7 in mice or E 15 in chicken) were dissected, pulse labeled with ^{3}H-thymidine (1 h), and cultured for three days. The labeled cells were visualized by autoradiography. At time 0, most of the labeled cells (86%) were in the external granular layer, while by day 3, the vast majority (73%) resided in the internal granular layer, indicating a movement from the external granular layer to the internal granular layer[15]. Thus, the effects of various adhesion molecules or their antibodies, and enzymes or their inhibitors can now be tested (table 2). Exposure of cells to the Fab' fragments of N-CAM antibodies has only a mild effect on migration[15]. After three days in culture, 24% of labeled cells remain in the external granular layer, compared with 12% in control explants incubated with non-immune Fab'. Lindner also reported a 15% inhibition of 'migratory index' (see below) using similar assays with antibodies to mouse N-CAM[49]. The expression of N-CAM PSA in the pre-migratory zone suggests that it could play a role in enhancing migration by reducing inter-molecular binding strength[23] but neuraminidase treatment of the explant did not have any apparent effect[49]. It will be interesting to test the effect of antibodies to N-CAM PSA.

When anti-Ng-CAM polyclonal antibodies were tested, there was a remarkable accumulation of labeled cells in

Table 2. Effects of molecules on cerebellar granule cell migration

Inhibition	No effect
Surface molecules	
Anti-N-CAM	Heparan sulfate
Anti-Ng-CAM (L1)	Anti-laminin
Anti-cytotactin	Anti-J1
AMOG	Glycosaminoglycans
L2 monoclonal	Hyaluronic acid
Con A	Wheat germ agglutinin
Enzymes	
Plasminogen activator inhibitor	Chondroitinase ABC
Plasmin inhibitor	Neuraminidase

Cerebellar explant assay[53] was used. (Compiled from refs 15, 44, 48, 49, 53).

the external granule layer in the chicken cerebellum (64%). A similar effect was achieved when a monoclonal antibody to Ng-CAM was used [15]. These results are consistent with Lindner's earlier finding that L1 inhibits granule cell migration in the mouse [48].

When antibodies to cytotactin (tenascin) were added to the explants, we found a marked inhibition of cell migration, with only 35% of cells reaching the internal granular layer versus 73% in the control. Sections through the explant showed that 55% of the labeled cells accumulate in the molecular layer [15]. Antonicek et al. [3] reported no inhibition of migration using an antibody to J1 (the mouse equivalent to cytotactin) known to inhibit neuron-astrocyte adhesion in vitro and suggest that either J1 is not involved in migration or that the antibodies may not recognize the functionally critical epitopes for granule cell migration. It is also possible that they do not detect an effect because of their use of a 'migratory index' to interpret their results. This parameter compares the average migratory distance between pia mater and labeled granule cells. Due to the accumulation of granule cells by anti-cytotactin, the molecular layer becomes abnormally thickened in these explants compared to controls. If only the travel distance is monitored, cells arrested in the thickened molecular layer could be counted as migrating normally, judged by such a 'migratory index'. In addition, this analysis does not take into consideration that the granule cell migratory course is a heterogeneous path; cells migrate relatively slower in the pre-migratory zone, but faster in the molecular layer [30]. Therefore, inhibition at the later stage could be overlooked if the assay is stopped too soon.

Adhesion molecule on glia (AMOG), an integral cell surface glycoprotein of Mr 45–50 kDa, is another newly defined cell surface molecule that mediates neuron-glia binding in vitro [3]. When tested in this assay, antibodies to AMOG also inhibit granule cell migration. The gene has recently been cloned and sequenced, and surprisingly it is homologous (40%) to the beta subunit of the Na,K-ATPase [31]. Antibodies to AMOG, which cross-react with Na,K-ATPase, can increase the enzyme activity in astrocytes. Gloor et al. [31] speculate that the adhesion between AMOG and the hypothetical AMOG receptors could be a new form of cell interaction in which cell recognition is followed directly or indirectly by signal transduction, possibly mediated by ion pumps or channels, to lead to physiological responses.

Several other molecules have also been tested in the cerebellar explant assay. L2 is a carbohydrate epitope present on several neural adhesion molecules including N-CAM, Ng-CAM and cytotactin. It would be interesting if such an epitope itself is involved in cell migration. Monoclonal antibodies to L2 do have significant inhibitory effect on cerebellar migration [49], but steric hinderance on binding sites by antibodies cannot be ruled out. Heparan sulfate has been suggested to modulate N-CAM binding [16] but does not have an effect when added to this culture system. Hyaluronic acid and other glycosaminoglycans were also non-inhibitory when added to the assays [49]. This however does not rule out the possibility that they may be functionally important, because these extracellular molecules may have to be incorporated in the right confirmation to have observable effects. Laminin has been shown to be produced by cultured astrocytes and to promote granule cell migration in vitro [66]. Antibodies to laminin however do not inhibit granule cell migration in these explants [49].

How can cells migrate through intact tissue? It has been proposed that extra-cellular proteolysis may be essential during cell migration in vivo. Using fibrin plates Moonen et al. [53] have shown that both plasminogen activator and plasmin are released by P7, but not adult cerebellum. They also showed that inhibitors to plasminogen activator and plasmin inhibit granule cell migration in cerebellar explants [70]. Recently, tissue plasminogen activator was shown to bind to granule cells, thus permitting these cells to 'arm' themselves with functionally active protease. The proteolytic enzymes could be involved in digesting the surrounding tissues and/or dynamically remodeling local adhesion attachments to allow the cells to migrate. The co-localization of cell surface urokinase with vinculin in the adhesion plaques of fibroblasts is consistent with this possible mechanism [40].

With the large number of molecules involved in granule cell migration, it is important to analyze their relationship with one another in development. As a first step, we attempted to test if the adhesion molecules may act at different points along the migratory course. Because granule cell migration proceeds in two phases (see above and Fujita [30]), we divided the cerebellar explant culture period into two intervals: from day 0 to 1.5 and from day 1.5 to 3, and tested their sensitivity to different adhesion molecules. When anti-Ng-CAM was present only in the early interval, the inhibitory effect on cell migration was similar to when antibodies were present for all three days. In contrast, when anti-Ng-CAM was present only in the late phase, it did not inhibit cells from leaving the external granular layer. Similar experiments were carried out with anti-cytotactin antibodies. When anti-cytotactin was present only in the early interval, the effect was minor compared to when anti-cytotactin was present throughout the culture period. However, when anti-cytotactin was present only in the late interval, it was enough to account for most of the inhibitory effect. Therefore, the major effect for anti-Ng-CAM was in the early interval, while that for anti-cytotactin was in the later interval. These results suggest that the two major stages of cerebellar granule cell migration can be differentially affected by Ng-CAM and cytotactin (fig. 1). Although, it is difficult to compare events exactly in explant cultures and in vivo the early interval corresponds closely to the stage involving the migration in the pre-migratory zone. This migration occurs at an average rate of 1.8 μm/h in vivo (estimated from Fujita [30]). It probably involves the initial

binding of granule cells to the Bergmann glia which may require Ng-CAM. Lindner et al. agreed that L1 is required for this process but considered the inhibition to be due to failure of parallel fiber fasciculation [48]. Cytotactin does not appear to be important at this stage. The late interval corresponds to the stage when cells migrate through the molecular layer along the glial fibers. This migration is fast with an average rate of 15 μm/h in vivo. The movement is sensitive to anti-cytotactin and to a small extent anti-Ng-CAM. It would be very interesting to use similar analyses to further locate the molecular action of plasminogen activator, plasmin and AMOG.

Other systems to study the roles of adhesion molecules in granule cell migration

Another powerful system for study of granule cell migration is the cerebellar microwell culture. By incubating dissociated cerebellar cells in different conditions, Trekner, Hatten and Mason [38, 69] were able to show that granule cell migration on glia in these cultures remarkably recapitulates in situ interactions [33, 51]. The in vitro system offers the unique opportunities to use time-lapse video-cinematography to study the behavior of granule cells and glial fibers. Hatten et al. [39] were also able to do recombination experiments between granule cells and glia from normal or *weaver* cerebellum in culture and showed that the *weaver* defect appears to be on the neuron. They also identified a new molecule called astrotactin using antibodies that inhibit the attachment of granule cells to glial cells in this system [26]. This activity can be neutralized by normal granule cells but not by granule cells from *weaver* mutants, suggesting defective expression of astrotactin in *weaver* neurons [26]. Inhibitory effects were not observed in these cultures using antibodies to N-CAM or Ng-CAM [26].

Although the system is useful in many ways, it may miss some processes important for granule cell migration. If an adhesion molecule acts on a mechanism not present in the culture system, its effect will not be detected. Some molecules may not be regenerated in these cultures and environmental constraints not present in the cultures may be important. For example, the well organized geometry of the Bergmann glial fibers only partially re-capitulates the situation in vivo. In addition, the axons of the granule cells do not form parallel fibers in culture. These could be important cues for the granule cells to organize their cytoskeleton and to distribute their surface molecules in the correct manner. Also the granule cells migrate bi-directionally in the microwell cultures. In vivo, they only move in one direction and the directionality may result from the passive displacement generated by cell growth in the proliferative zone of the external granular layer. Another point is that all granule cells from the cerebellum are used in these assays. Based on in situ immunohistochemical studies and time course experiments [69], however, the external and internal granule cells are very different in their expression of adhesion molecules (table 1) and their ability to migrate. Future attempts to isolate only the external granule cells, which is technically difficult, will help to improve this system.

Several mouse mutants in which cerebellar granule cells fail to migrate have been identified [67]. In addition to the *weaver* mutant, there are *staggerer* and *reeler*. All of these are autosomal recessive mutants. It would be interesting if the defect in any of these mutants directly or indirectly involves adhesion molecules. Faissner et al. [28] have examined the expression of N-CAM and L1 in these mutants with immunohistochemical methods but did not observe any apparent abnormality. With the importance of the different epitopes on adhesion molecules, detailed studies like that of Hekmat et al. [41] will have to be carried out to detect any potential abnormalities.

The replacement of the E form with A form of N-CAM occurs in *reeler* and *weaver* cerebella from E 7 to E 21, similar to the schedule in the wild type mice [13]. In contrast, large amounts of the E form of N-CAM remain in the E 21 *staggerer* cerebellum. We are not sure whether this accumulation of the E form of N-CAM is due to failure of cell migration or synapse maturation. Trekner has reported a decrease in neuraminidase activity in the *staggerer* cerebellum, which is consistent with the excess expression of N-CAM PSA in *staggerer*. Whether increased neuraminidase activity or decreased sialidase activity is responsible for the normal conversion from the E form to the A form of N-CAM has not yet been determined. It is interesting to note that the *staggerer* gene is located on mouse chromosome 9 and close to the N-CAM gene. The presumptive gene for ataxia telangiectasia, a disease involving abnormal cerebellar cell migration and abnormal immune function, is also in the corresponding region of human chromosome 11q [58]. The most direct approach to determine the pathology of these mutants is to isolate and clone the mutated genes; the gene products should provide invaluable insight into the molecular mechanism of cell migration and it would not be surprising if these gene products are related to new or already identified adhesion molecules.

Conclusion

The migration of neurons along radial glial fibers probably involves several adhesion molecules, extracellular matrix proteins, and enzymes. The mechanism may be similar to the molecular pathway in blood coagulation or complement activation in the sense that the cooperation of a series of molecules is required to achieve one physiological function. Various molecular interactions could lead to different kinds of protein conformational changes, enzyme activation, channel activity, cytoskeleton re-organization etc., which may be coordinated to achieve granule cell migration. For example, it has often been asked how an adhesion molecule can facilitate cell migration if it binds cells together and glues them to the

substratum. It may be that cell migration requires the coordination of a group of molecules, some for adhesion to provide friction and some for de-adhesion to allow translocation. De-adhesion, simply defined as the reduction of adhesive force, can be achieved in many ways: proteolytic cleavage activated by plasminogen activator, internalization through membrane recycling, or conformational change modulated by adjacent molecules. The emerging picture of modulation of the binding strength through cis or trans interaction among adhesion molecules or modifications of them is further strengthened by two recent findings. First, cytotactin/tenascin has the puzzling property of providing binding but not spreading for cells[29]. Now it has been shown that cytotactin can modulate fibronectin function by blocking fibronectin binding sites[50]. Second, L1/Ng-CAM binding has been shown to be much more potent in the presence of N-CAM[47]. Recent data suggest that N-CAM and L1/Ng-CAM may have cis interactions similar to the functional cooperation between CD4, CD8 and T cell receptors[47]. Thus, the complex pattern of different adhesion molecules, their iso-forms and their post-translational modifications expressed by different stages of granule cells may be necessary to fulfill the different needs during the migratory course. Through the dynamic modulation of multiple adhesion molecules, the external granule cell can bind the Bergmann glia, move along it and detach from the glia when it reaches its destination in the internal granular layer.

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