Cell adhesion molecules (CAMs) are now known to mediate much more than adhesion between cells and between cells and the extracellular matrix. Work by many researchers has illuminated their roles in modulating activation of molecules such as receptor tyrosine kinases, with subsequent effects on cell survival, migration and process extension. CAMs are also known to serve as substrates for proteases that can create diffusible fragments capable of signaling independently from the CAM. The diversity of interactions is further modulated by membrane rafts, which can co-localize or separate potential signaling partners to affect the likelihood of a given signaling pathway being activated. Given the ever-growing number of known CAMs and the fact that their heterophilic binding in cis or in trans can affect their interactions with other molecules, including membrane-bound receptors, one would predict a wide range of effects attributable to a particular CAM in a particular cell at a particular stage of development. The function(s) of a given CAM must therefore be considered in the context of the history of the cell expressing it and the repertoire of molecules expressed both by that cell and its neighbors.

Cell migration, axon extension and dendrite arborization are all essential processes in creating the complex neural architectures of the developing brain. A number of cell adhesion molecules (CAMs), including those of the immunoglobulin superfamily (IgCAMs), integrins and cadherins, are known to mediate signaling between cells and between cells and the extracellular matrix (ECM). Because the greatest amount of IgCAM research has focused on L1-CAM and NCAM and their invertebrate homologs Neuroglian and Fasciclin II, these molecules will be the primary focus of this Commentary & View. IgCAMs are so named because their extracellular domains contain immunoglobulin repeats (usually 5–6). The Ig repeats are usually followed by fibronectin type-3 (Fn III) domains (2–5) and either transmembrane plus cytoplasmic domains or a glycosyl-phosphatidylinositol (GPI) linkage (reviewed in refs. 1–3). IgCAMs can bind homophilically and heterophilically via their Ig and/or Fn III domains to achieve cell-cell and cell-ECM adhesion, which can simply stabilize the architecture of neural tissue, but can also transmit information to the cell interior. For example, a number of IgCAMs are known to bind to the cytoskeleton via linker molecules including Ankyrin, Doublecortin and members of the Ezrin-Radixin-Moesin family (ERMs). Some of these linking interactions are thought to allow engagement or disengagement of a molecular "clutch module" (reviewed in ref. 13) similar to coupling of integrins to F-actin flow via focal adhesion proteins and are believed to be important in growth cone function and synaptogenesis. Work from these groups suggests these linker molecules are expressed in different developmental windows, so that ERMs and Doublecortin are important in L1-mediated neurite outgrowth and suppression of neurite branching, while subsequent Ankyrin expression and binding to L1 blocks outgrowth and fosters axon stabilization and synaptogenesis. Another
important example of outside-in signaling by IgCAMs is their ability, via Ankyrin’s multivalent binding sites, to cluster and position many receptors and channels at specific cellular locations such as axon initial segments and nodes of Ranvier (reviewed in ref. 12 and 16). IgCAMs have also been shown to be substrates for matrix metalloproteases (MMPs), which can cleave extracellular domains, allowing the fragments to act as diffusible signaling molecules and changing the signaling effects of the remaining membrane-bound moieties.17,18

**IgCAMs are Important Activators of Receptor Tyrosine Kinases**

Understanding of the diversity of roles played by CAMs was dramatically advanced by the work of Williams, Saffell, Walsh, Doherty and coworkers in the 1990s.19,20 These researchers showed that the IgCAMs L1 and NCAM, as well as N-cadherin, can affect neurite outgrowth by activating fibroblast growth factor receptors (FGFRs, members of the Receptor Tyrosine Kinase (RTK) family) directly, without requiring FGF. Indeed, subsequent work pointed to the ability of CAMs and FGF to have different effects on cell signaling pathways, both mediated through FGFRs.21 More recent work has shown that NCAM (or a small fragment derived from NCAM) can competitively inhibit FGF binding to FGFRs, biasing cells toward neurite outgrowth rather than proliferation.22 Significantly, the phosphorylation state of key tyrosine residues in the IgCAM cytoplasmic domains, shown to play a role in regulating binding to ankyrin and doublecortin, is thought to be mediated by MAP kinases, which in turn can be activated by RTKs, thus allowing IgCAM-RTK interactions to affect linkage to the cytoskeleton.1,23 On the other hand, L1 binding to ERM family proteins or to AP2 is thought to be mediated by Src-family kinases.7,24

In Drosophila, recent studies have discovered two FGF ligands for the FGFR known as Heartless (Htl).25,26 In the developing eye, Pyramus, produced by glial cells, acts on glial cell Htl FGFRs to induce migration and proliferation. Thisbe, produced by retinal axons, acts on these same glial FGFRs to halt migration and induce ensheathment of axon bundles.27 How two different ligands for a single receptor could have such different effects is not yet clear, but certainly one possibility is that the retinal axons also present one or more CAMs, which then interact with the glial FGFRs and/or glial CAMs in trans to alter the resulting cellular response. FGFRs and EGFRs are known to activate numerous identical signaling cascades, including those converging on Akt, MAPK/ERK and the generation of diacyl glycerol (DAG), Inositol trisphosphate, 2-arachidonyl glycerol and arachidonic acid by Phospholipase Cγ and DAG lipases. An intriguing scenario reviewed in reference 28 is that the end result of RTK activation can depend on a cell’s history due to the numerous feedback loops that determine the presence or levels of the many intermediates in these pathways.

These insights into CAM-FGFR interactions led to the discovery, by other groups, of similar interactions between CAMs and the epidermal growth factor receptor (EGFR) in vertebrates, while studies in invertebrates showed that similar interactions occur between invertebrate RTKs and the L1 and NCAM homologs, Neuroglian and Fasciclin II.29,30 In particular, Hortsch and coworkers have provided evidence for homophilic binding, in trans, of L1 molecules (expressed in cultured Drosophila S2 cells) activating EGFRs in cis.30 Furthermore, they have shown that in vivo expression of an artificial construct of Neuroglian, in which the transmembrane and cytoplasmic domains were replaced with a GPI linkage, still leads to activation of EGFRs, proving that the CAM extracellular domains are sufficient to cause EGFR activation.30 In a similar vein, Doherty and coworkers have shown that soluble or substrate bound Fc constructs of Neuroglian and Fasciclin II extracellular domains can promote neurite outgrowth, via activation of Htl FGFRs, in Drosophila embryonic cells growing in culture.31

In the developing adult olfactory pathway of the moth, *Manduca sexta*, centrally-derived glial cells migrate outward to define a sorting zone region just distal to the antennal (olfactory) lobe. In this region, we have found that interactions between axons of olfactory receptor neurons (ORNs, whose cell bodies are located peripherally in the antennae) and the sorting zone glial cells result in activation of EGFRs on the portion of the ORN axons which come in contact with the glial cells.32 Neuroglian molecules, present along the entire axon length and on the glial cells, become stabilized against detergent extraction in this same region, but not elsewhere on the axons (see ref. 6 for an earlier discussion of this phenomenon). These results suggest that homophilic binding of neuronal and glial Neuroglian molecules activates axonal EGFRs in the sorting zone and that this is accompanied by anchoring of Neuroglian molecules to the cytoskeleton in the same region. Pharmacologic blockade of EGFR activation resulted in axon stalling, suggesting that EGFR activation is necessary for axon extension through the glial cell zone, recalling results reported in Drosophila.29,31,32 Further experiments revealed that blocking EGFR activation also leads to loss of Neuroglian resistance to detergent extraction in the sorting zone, suggesting that anchoring of Neuroglian to the cytoskeleton requires activation of a signaling pathway, possibly the MAPK pathway,1,23 downstream of the EGFR.33

**The Importance of Membrane Rafts in CAM-RTK Interactions**

An additional layer of complexity was introduced in the 1990s with the proposal from several investigators that cell membranes are not homogeneous, but rather include very small (10–200 nm in diameter, with the 200 nm figure referring to the surface area of caveolae) microdomains enriched in sterols, sphingomyelin and glycosphingolipids that “float” in a phospholipid “sea.”34,35 The microdomains became known as “lipid rafts,” later changed to “membrane rafts” to acknowledge the important contribution of proteins to the assemblies.36 Rafts were originally defined operationally as membrane subdomains which were resistant to solubilization by Triton X-100 at 4°C because of their composition. They are believed to be transitory, perhaps forming only in response to cell-signaling
of differing composition can also act in their ligands (reviewed in ref. 40). Rafts observation that RTKs often exhibit a dimerization. This may account for the domains may increase the likelihood of accumulation in small membrane sub - tion of their cytoplasmic domains, so by dimerization and cross phosphoryla tion of signaling partners while keeping others vide a mechanism for concentrating some transmembrane domains). Thus rafts pro\-\n\prenylated proteins and those with shorter with longer transmembrane domains) and exclude others (those lacking acyl chains, sial due to the difficulty in characterizing membrane rafts has remained controver-\nsical due to the difficulty in characterizing or imaging things that are, by nature, very small and transitory, so that any probe used for visualization, or technique used for purification, can alter the system from the natural state (the difficulties in studying rafts are reviewed in refs. 37 and 38). Nevertheless, numerous labs have sought to characterize the role of rafts in cell signaling and several principles have emerged. First, the composition of rafts causes their components to be tightly packed in a “liquid-ordered” phase, rather than the “liquid-disordered” phase typical of phospholipid bilayers. Their different composition also leads to rafts having a greater thickness in the membrane plane than phospholipid bilayers. These structural differences lead to predictions, based on free energy constraints (reviewed in refs. 37 and 39), that rafts will accumulate certain proteins (those anchored by GPI linkages, those modified by attachment of multiple acyl chains and those with longer transmembrane domains) and exclude others (those lacking acyl chains, prenylated proteins and those with shorter transmembrane domains). Thus rafts pro\-\nvide a mechanism for concentrating some signaling partners while keeping others apart. RTKs, for example, are activated by dimerization and cross phosphorylation of their cytoplasmic domains, so accumulation in small membrane sub-domains may increase the likelihood of dimerization. This may account for the observation that RTKs often exhibit a basal level of activation in the absence of their ligands (reviewed in ref. 40). Rafts of differing composition can also act in targeting particular molecules to different regions of a cell.41 It is interesting to note that while EGFRs have been described as being raft-associated or not by different groups working with various cell types, FGFRs have generally been described as not raft-associated, with one exception to date.40 However, unlike EGFRs, FGFRs require an adapter protein, FGFR Substrate 2 (FRS2), to activate some, but not all, signaling pathways and FRS2 may be restricted to rafts.42 Intriguingly, a number of researchers have found that molecules normally found outside of rafts, on binding their ligand and/or coreceptor, will be translocated into rafts.43,44 This can be important in coupling receptor activation to downstream signaling via, for example, non-receptor tyrosine kinases, such as Src and Fyn, which also show a differential distribution in inner leaflets of raft and non-raft domains, apparently due to differences in acylation. In an even more elaborate scenario, FGFR activation has recently been shown to cause palmitoylation of NCAM, caus-\ng its translocation into membrane rafts and subsequent neurite outgrowth.45 This apparently occurs without translocation of the FGFRs into rafts, and the palmitoylation may occur in endosomes which then return the palmitoylated NCAM to rafts on the cell surface.

In the developing brain of Manduca, we have found evidence that EGFRs are confined to a subset of rafts and that disrupting rafts in general (using the sterol sequestering molecule, methyl-β-cyclodextrin) again leads to loss of EGFR activation as well as absence of stabilization of Neuroglian against detergent extraction in the sorting zone.33 These results lead us to conclude that, in the developing anten-\nral lobe of Manduca, membrane rafts are an essential component of Neuroglian-\nEGFR signaling.

Summary

A review of the literature concerning the roles played by CAMs in various stages of development (as well as in cancer) reveals a wide variety of conclusions (e.g., out-\ngrowth vs. ensheathment, proliferation vs. apoptosis, etc.). In light of the above dis-\ncussion, in which we have seen that CAMs can link to the cytoskeleton with very different results depending on the develop-\mental stage and linker molecule, can modulate activation of RTKs (which in turn can have different effects on a given cell depending on its history) and can par-\nticipate in and out of membrane rafts based on intrinsic properties, on dynamic modi-\fications such as acylation, and on availability of ligands and co-receptors, it does not seem surprising to find a large number of divergent effects. Add to these variables the fact that a given cell will target rafts of differing composition (lipid and protein) to different areas (axon vs. dendrite, leading edge vs. uropod41) in a manner varying with developmental stage, and it becomes clear that a very large number of effects are possible. One would also predict that continued research will lead to a better understanding of the ways in which the growing number of known CAMs can interact with each other, both in trans and in cis, to cause yet a greater array of outcomes in cell-cell and cell-ECM sig-\nnaling (reviewed in ref. 46). As examples, heterophilic interactions in cis affecting homophilic interactions in trans has been described for NCAM and L1, while homophilic interactions in trans affecting heterophilic interactions in cis has been described for TAG-1 and L1.47,48

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