Extracellular Crosstalk: When GDNF Meets N-CAM

N-CAM has now been identified as a receptor for glial cell line-derived neurotrophic factor (GDNF). This finding solves a long-standing question regarding RET-independent GDNF signaling, and reveals a novel pathway distinct from both GDNF–RET and N-CAM–N-CAM signaling. Functional assays of Schwann cell migration and axon growth of CNS neurons suggest physiological significance for this GDNF–N-CAM pathway.

The GFRαs are soluble or GPI-linked cell surface molecules that bind GDNF family ligands (GFLs) with high affinity and interact with the receptor tyrosine kinase RET to elicit intracellular signaling (Baloh et al., 2000). However, in several regions of both CNS and PNS, GFRαs are highly expressed while RET is undetectable. This discrepant expression has suggested the existence of additional signal-transducing GFL receptors. However, whether GDNF receptors exist that signal cooperatively with GFRαs, but independently of RET, remains an unresolved issue.

Paratcha, Ledda, and Ibanez (2003) in this issue of *Cell* now identify a transmembrane isoform of the neural cell adhesion molecule N-CAM ($p140^{N\text{-}CAM}$) as a RET-independent receptor for the GFLs. They demonstrate that GDNF binds directly to N-CAM with high affinity. In cultures of Schwann cells and cortical neurons, both of which express GFRαs but not RET, this binding activates Fyn and FAK, signaling mediators downstream of N-CAM (Panicker et al., 2003). This activation is distinct from the RET signaling pathway that involves Ras and PLCγ (Airaksinen and Saarma, 2002). Even though GDNF can bind N-CAM independently of GFRα1, both receptors are required for GDNF to activate N-CAM downstream signaling. This might be due to the recruitment of N-CAM by GFRα1 to lipid raft domains, where Fyn is also concentrated.

These new results raise an intriguing possibility that in cells expressing both N-CAM and GFRα1, some biological functions previously ascribed to homophilic N-CAM interactions could actually be mediated by GDNF. In support of this idea, coimmunoprecipitation assays reveal constitutive binding between N-CAM and GFRα1, which is in significant contrast to the GFRα1-RET interaction that requires GDNF binding (Tansey et al., 2000). Moreover, crosslinking studies show that GFRα1 can act as a negative regulator of homophilic N-CAM interactions. Indeed, N-CAM-mediated Jurkat cell aggregation is significantly suppressed when GFRα1 is coexpressed. This role of GFRα1 in silencing N-CAM homophilic interactions but promoting GDNF–N-CAM binding, together with developmentally regulated changes in N-CAM and GFR α1 expression, may allow cells to fine-tune their responses to short- and long-range signals.

Is this new type of GFL signaling physiologically relevant? The work reported here demonstrates that GDNF stimulates Schwann cell migration in wild-type and RET(−/−) sciatic explants in an N-CAM and Fyn-dependent manner. Furthermore, GDNF promotes axon extension from cultured wild-type and RET-deficient hippocampal neurons, but not from neurons lacking N-CAM. Although the in vitro data are suggestive, a role of GDNF-GFRα1–N-CAM-Fyn signaling during nervous system development is difficult to establish at present because of early lethality of GFRα1 null mice. N-CAM null mice display a reduction in the size of the olfactory bulbs. The neurons in the olfactory bulb are replenished throughout life by newborn cells migrating in from the subventricular zone, a process that requires N-CAM expression on the migrating neurons. The current work demonstrates the presence of N-CAM and GFRα1 expression along the rostral migratory stream (RMS), while Ret expression is undetectable. Consistent with a role for GDNF signaling via N-CAM, morphometric analysis shows that the anatomy of the RMS is disturbed in newborn GFRα1 null mice, similar to N-CAM nulls, but not to pups lacking RET. However, these findings can only be considered suggestive at present because of the rudimentary stage of olfactory bulb development at P0 when GFRα1(−/−) mice die. Determining whether GDNF signaling is a critical mediator of N-CAM effects on olfactory bulb development will require conditional inactivation of the GFRα1 gene.

Crosstalk between signaling pathways triggered by cell contact and those triggered by growth factors has been well documented. Such crosstalk has previously been characterized intracellularly, at, or downstream of, receptors. At the receptor level, for instance, integrin engagement can trans-activate many growth factor receptors in the absence of their ligands via a Src-dependent pathway. On the other hand, growth factor signaling can modify integrin conformation and thus its activation through an inside-out mechanism (Schwartz and Ginsberg, 2002). Downstream, both growth factors and integrins share common elements, most prominently, ERK and PI3K pathways, that provide multiple points of intersection between integrin and growth factor signaling. The importance of this paper is that Paratcha, Ledda, and Ibanez have uncovered a novel intersection between growth factor and contact-mediated signaling that occurs extracellularly. One consequence of growth factor ligand binding to a cell adhesion molecule could be to allow direct access of soluble factors to the adhesion molecule signaling pathways which mainly involve locally regulated cytoskeletal reorganization. Thus, long-range soluble factors could act locally to modify behaviors such as migration and growth cone advance.

GDNF-stimulated N-CAM signaling events are not only distinct from the RET-mediated pathway but also different from the reported N-CAM pathway involving FGF receptor (FGFR) signaling. It is of interest to note that GDNF activated N-CAM signaling alone is sufficient to promote CNS axon growth, whereas homophilic N-CAM interactions may require both N-CAM and FGFR downstream signaling for process outgrowth (Niethammer et al., 2002). Axon growth requires local regulation of the cytoskeleton and coordinated gene expression. Homophilic N-CAM-signaling-mediated axon growth requires both activation of the Fyn-FAK pathway downstream of N-CAM and calcium influx induced by FGFR signaling (Niethammer et al., 2002). Given the recent

exciting finding that calcium influx may activate a genetic program required for growth-factor-stimulated axon growth (Graef et al., 2003), it is tempting to suggest that homophilic N-CAM interactions might support axon growth through coordinating local Fyn-FAK activation with gene expression induced by FGFR activation and subsequent calcium influx. It will be of great importance to understand how the GDNF–N-CAM interaction activates both the local and the genetic programs required for axon growth.

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Selected Reading

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Closing Another Gap in the Plant SAR Puzzle

NPR1 is a key regulator of the salicylic acid (SA) dependent pathogen resistance pathway in plants. In this issue of *Cell*, Mou and Dong demonstrate that *Arabidopsis* NPR1 undergoes activation from an inactive oligomer to the active monomer as a result of cellular redox changes induced by SA during systemic acquired resistance.

Systemic acquired resistance (SAR) is a vital mechanism, which confers immunity throughout the plant toward a broad range of microorganisms following local infection by certain phytopathogens (Dong, 2001). The endogenous signal molecule salicylic acid (SA) has long been known to play a central role in plant defense with SA levels increasing in tissue upon pathogen infection. Genetic studies, mainly in the model plant *Arabidopsis thaliana*, have shown that SA is required for the induction of local defense responses, for activation of numerous defense-related genes including a set of pathogenesis-related (*PR*) genes, and in the establishment of SAR (Kunkel and Brooks, 2002). In addition, SAR can be induced in the absence of any pathogen by exogenous application of SA or its active analog 2,6-dichloroisonicotinic acid (INA).

Attempts by several laboratories to genetically dissect the SAR pathway downstream of the SA signal all resulted in the identification of numerous alleles of a single gene designated *NPR1*, *NIM1*, or *SAI1*. *NPR1* encodes a protein containing an ankyrin repeat domain and a BTB/POZ (*broad-complex*, *tramtrack*, and *bric-á-brac*/poxvirus, zinc finger) domain, both of which are involved in protein-protein interactions (Glazebrook, 2001). The importance of these domains for NPR1 function was solidified by the isolation of loss-of-function point mutations in highly conserved amino acids within these regions. *npr1* mutant plants fail to express several *PR* genes and display enhanced susceptibility to infection. They cannot be rescued by exogenous application of SA or INA consistent with an NPR1 function downstream of SA.

How does NPR1 exert its function and how does it transduce the SA signal? From the outset it was speculated that NPR1 could act as a transcription regulator to influence *PR* expression despite lacking any obvious DNA binding motif. Extensive work, including a series of elegant studies from X. Dong's laboratory, demonstrated that, in response to SA, NPR1 localizes to the nucleus via a functional nuclear localization signal (NLS), and that nuclear localization is a prerequisite for the activation of *PR-1* expression (Kinkema et al., 2000). Several yeast two-hybrid screens identified members of the TGA family of bZIP transcription factors as candidate interactors of NPR1. Indeed, NPR1/TGA2 interaction was subsequently directly visualized in plant protoplasts and also verified in planta, consistent with the fact that SA-dependent *PR-1* expression is positively influenced by the presence of an *as-1* element (a TGA factor binding site) within its promoter (Subramaniam et al., 2001; Fan and Dong, 2002).

Thus, pieces of the SAR puzzle are slowly beginning to fall into place. A major gap, however, concerns the mechanism by which SA accumulation directs NPR1 function within the SAR pathway. One should note that NPR1 protein is clearly present in uninduced plants and its concentration does not significantly increase upon SA or INA treatment. Furthermore, overexpression of *NPR1* alone does not activate *PR-1* expression nor induce resistance, clearly demonstrating the need for NPR1 activation by an unknown inducer (Cao et al., 1998). This suggests that SA somehow influences NPR1 function at the protein level. The article of Mou and Dong (2003) in this issue of *Cell* provides strong evidence that, upon SA/INA treatment or pathogen attack, alterations in the redox state of the cell may be the driving force leading to a transition from an inactive oligomer of NPR1 to an active momomeric form. Since the NPR1 protein contains 17 cysteine residues and a non-functional mutation (*npr1-2*) resulted in a cysteine to tyrosine conversion, the authors hypothesized that intra- or intermolecular disulfide bond formation could be important for NPR1 activity. Therefore, protein extractions in the presence/absence of the reducing agent dithiotreitol (DTT) were made from transgenic *npr1-1* mutant plants expressing a fully functional NPR1-GFP chimeric protein,