that a five-minute incubation allowed peptides to exchange for native protein? Also, the authors saw no effect of SNAP-25 peptides. Is this because SNAP-25 peptides lack the ability to interact with binding partners, such as snapin[18] or snip[19], that regulate the interaction of SNAP-25 with VAMP? Answers to these questions are likely to reveal additional targets for plasticity-dependent modification of regulated secretion.

A US politician once remarked that 'all politics is local'. Given the difference in the order of SNARE-complex assembly observed *in vitro* compared with *in situ*, this remark can be adapted to the analysis of protein interactions. Although many models of molecular events germinate from the results of *in vitro* studies, Chen *et al.*'s findings illustrate that protein interactions need to be considered in their local environments before a model is mature. The ability to move between *in vitro*, *in situ* and *in vivo* approaches is allowing rapid progress in the field of neural secretion. Undoubtedly, many future surprises await that will have important implications for our understanding of both normal and abnormal neuronal processes.

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Caught in the matrix: how vitronectin controls neuronal differentiation

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Cerebellar granule cells are the most abundant neurons in the brain and are crucial to the circuitry that controls motor coordination. The proliferation of granule cell precursors (GCPs) is controlled by the secreted signaling molecule Sonic hedgehog (Shh), but the factors that regulate GCP differentiation remain a mystery. A recent study suggests that the extracellular matrix protein vitronectin might tell GCPs when to stop dividing and begin differentiation.

If you have ever been on the New York subway at rush hour, you have a sense of what it is like to be a granule cell precursor (GCP): crammed into a small space, jostled around by hordes of other passengers, bombarded by flashy signs and crackling, ambiguous announcements, your only goal is to figure out where your stop is and when to get off.

But somehow, amidst the confusion, granule cells manage to find their way. Starting out on the outside of the cerebellum in a region called the external germinal layer (EGL), they undergo waves of proliferation to generate millions of GCPs. As these cells proliferate, they begin to move inwards, migrating or shoved down by the mass of cells being generated above them. Then, at a specific point in their journey – about halfway through the EGL – they abruptly stop dividing and differentiate. They extend axons (the parallel fibers that will form synapses with Purkinje cells), express markers of differentiated neurons and migrate downwards to their final destination, the internal granule layer (IGL)[1,2].

Some of the signals that direct granule cells along their route have recently been identified. For example, the initial proliferation of GCPs appears to be controlled by Sonic hedgehog (Shh), a secreted glycoprotein that plays a crucial role in many aspects of nervous system development[3]. In the cerebellum, Shh is made by Purkinje cells, and acts as a powerful mitogen for GCPs *in vitro*[4–6].

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Antibody-blocking studies indicate that Shh is also necessary for expansion of these cells *in vivo*. Moreover, mutations in elements of the Shh signaling pathway are associated with medulloblastoma, a tumor that originates from GCPs[7–10].

But if Shh controls proliferation, what tells GCPs when to stop proliferating and differentiate? One possibility is differential localization of Shh itself. Shh might be present only in the outer EGL, and might make cells in that region proliferate; then, as cells move inwards and away from the mitogenic signal, they would have no choice but to exit the cell cycle. Although this model has not been ruled out, there is no evidence to support it. In fact, because Shh is made by Purkinje cells (which reside beneath the EGL) and can induce proliferation of cells in the outer EGL, it seems likely that the protein permeates the entire germinal layer. Why then, would GCPs in the middle of this layer suddenly stop dividing? A recent paper[11] suggests that the signal to exit the cell cycle might come from an unexpected source: the extracellular matrix (ECM).

Previous studies by these investigators had revealed an important role for the ECM glycoprotein vitronectin (VN) in differentiation of motor neurons in the developing spinal cord (then called the neural tube)[12,13]. They showed that VN is expressed in the ventral neural tube at the time when motor neurons are generated, and that addition of VN to neural tube explants could promote motor neuron differentiation. Moreover, treatment with anti-VN neutralizing antibodies caused a dramatic reduction in the number of motor neurons generated *in vitro* and *in vivo*. Because motor neuron differentiation was known to depend on Shh (Refs 14,15), they examined the relationship between Shh and VN. Surprisingly, they found that the two molecules could bind to one another. And in cultures of dissociated neural tube cells, the combination of Shh and VN induced motor-neuron differentiation much more effectively than did either molecule alone. The authors proposed that VN might help transport or present Shh to its target cells and thereby enhance motor-neuron generation.

To find out whether vitronectin had a similar function during granule cell development, Pons *et al*. examined the expression of VN and other ECM molecules in the developing cerebellum[11]. They found that VN and its major receptors (α_v integrins) were expressed in the inner EGL and in the IGL, where granule cells are undergoing differentiation. By contrast, laminin (LN) and its receptors (α_6 integrins) were found primarily in the outer EGL, where GCPs are rapidly proliferating. This striking difference in localization raised the possibility that vitronectin and laminin might regulate distinct aspects of granule cell differentiation.

To test this idea, the researchers isolated GCPs and plated them on dishes coated with laminin or vitronectin. When cells were cultured in the absence of Shh, there was little difference in proliferation between the cells on different substrates. But in the presence of Shh, ECM molecules had a significant effect on the GCP response: cells cultured on laminin showed a much stronger proliferative response to Shh than cells cultured on vitronectin. Conversely, expression of differentiation markers (β-tubulin and TAG-1) was enhanced in cells grown on VN compared with those grown on LN. These findings suggest that the abrupt differentiation of GCPs in the inner EGL might result from their direct encounter with vitronectin.

But how could vitronectin override the proliferative response to a potent mitogen like Shh? One explanation, in light of the physical association observed between these molecules in the neural tube, is that VN binds to Shh and inactivates it or alters its presentation to granule cells. Alternatively, VN might act independently, binding to its receptor on granule cells and inducing a signaling cascade that alters the response to Shh.

Pons *et al*. provide strong support for the latter view, and suggest that one important mediator of VN signaling might be the transcription factor CREB (cAMP-response element binding protein). CREB can be phosphorylated – and thereby activated – by several intracellular enzymes, including protein kinase A (PKA)[16]. Because earlier studies had indicated that PKA activation could block Shh-induced proliferation[4,6], the investigators speculated that the antiproliferative effects of PKA and vitronectin might be carried out by CREB. To test this, they first examined the effects of different ECM molecules on CREB phosphorylation. When GCPs were grown on laminin, exposure to Shh resulted in decreased phosphorylation of CREB, but when cells were grown on vitronectin, no such decrease occurred. In other words, VN appeared to maintain CREB in its phosphorylated state. Consistent with this, analysis of CREB phosphorylation *in vivo* revealed that it was most abundant in the cells exposed to VN, the differentiating granule cells in the inner EGL.

To determine the importance of CREB phosphorylation for granule cell function, the researchers then transfected CREB into GCPs and treated them with Shh. In spite of the presence of the mitogen, overexpression of wild-type CREB caused a significant increase in the expression of differentiation markers. By contrast, expression of a dominant-negative form of CREB (that cannot be phosphorylated, but can compete with endogenous CREB) resulted in decreased differentiation. Thus, CREB appeared to function as a switch that tells GCPs when to stop growing and differentiate.

CREB could regulate growth and differentiation in several ways. First, it could alter the response to Shh by interacting with elements of the Shh pathway. In fact, Gli-2, one of the transcription factors thought to mediate Shh effects, has been reported to bind directly to CREB (Ref. 17). In developing granule cells, such an interaction could alter the function or target specificity of Gli-2 and prevent it from inducing cell cycle progression. Alternatively, since Gli proteins and CREB both require the coactivator CBP (CREB binding protein) to activate transcription[16,18], it is possible that increased levels of phosphorylated CREB could compete for CBP binding and thereby terminate the Shh response. Finally, because CREB is a transcription factor, it could directly induce expression of target genes that are necessary for cell-cycle exit or differentiation, as has been shown in other types of neurons[19,20]. These mechanisms are certainly not mutually exclusive, and their relative contribution to granule cell differentiation remains to be determined.

The findings of Pons *et al*. clearly implicate ECM molecules and CREB as important regulators of granule cell differentiation, and suggest a simple model to explain the behavior of granule cells at various stages of development. In

the early part of their journey, in the outer EGL, GCPs are exposed to Shh and surrounded by laminin. This combination results in decreased phosphorylation of CREB and increased expression of Shh target genes (e.g. D-type cyclins[21]), which makes GCPs proliferate rapidly. As cells proliferate they move downward, and in the middle of the EGL, they encounter vitronectin. Although they continue to see Shh, exposure to VN promotes increased phosphorylation of CREB, and this in turn causes cells to stop dividing and to differentiate.

Although this model elegantly explains the timing of granule cell growth and differentiation, it might not represent the whole story. Vitronectin is not the only factor that can override Shh-induced proliferation of GCPs, or even the only one that might do so by promoting CREB phosphorylation. Previous studies have shown that Shh-induced proliferation of GCPs can be inhibited by basic fibroblast growth factor (bFGF)[6], which is known to induce CREB phosphorylation[19]. Similarly, pituitary adenylate cyclase-activating polypeptide (PACAP), a potent activator of both PKA and CREB[22,23], has been shown to regulate granule cell survival and differentiation[24]. Both of these factors are produced in the postnatal cerebellum[25,26], and could also contribute to the timing of cell cycle exit and differentiation.

Surrounded by this cacophony of directions, how do developing granule cells decide what to do? To understand this, it will be necessary to sort out the relationship between ECM molecules and soluble growth and differentiation factors. One possibility is that ECM molecules function indirectly by altering the presentation of soluble factors to GCPs. For instance, laminin might increase proliferation by binding to Shh and enhancing its ability to generate mitogenic signals, whereas vitronectin might induce CREB phosphorylation and differentiation by facilitating signaling by bFGF or PACAP. Alternatively, ECM molecules might act directly by binding to integrin receptors and inducing signals that affect growth and differentiation. In that case, granule cells would have to integrate signals (e.g. CREB phosphorylation) from a variety of growth factors and ECM molecules in order to decide how and when to respond.

Regardless of how granule cells interpret signals from the ECM, the findings of Pons *et al.* clearly show that such signals can play an important role in regulating granule cell growth and differentiation. It is therefore worth considering the contributions they might make to cerebellar tumorigenesis. Medulloblastoma is thought to arise from GCPs that proliferate indefinitely and fail to undergo normal differentiation. Mutations in components of the Shh signaling pathway presumably promote medulloblastoma by causing increased GCP proliferation. But why don't these cells ultimately stop dividing and differentiate? In light of the findings of Pons *et al.*, it will be important to examine the expression of ECM molecules in and around these tumors. If the environment is rich in laminin or deficient in vitronectin, this could help to explain why tumor cells fail to differentiate. Moreover, treatments that alter the ECM might promote differentiation of medulloblastoma cells. By learning to speak the language of the matrix, we might be able to convince misguided granule cells to alter their course.

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