Developmental neurobiology

Unscrambling a disabled brain

André M. Goffinet

The orderly development of neurons in the mammalian brain is a conundrum that defies imagination: the billions of neurons that are generated around the cerebral ventricles must migrate through the tissue to find their destination, before forming exquisite cell patterns. The formation of these patterns is abnormal in mice with the *reeler* phenotype, showing that it is controlled by the *reeler*, *scrambler* and, probably, several other genes.

Although the mechanisms by which pattern formation is regulated remain largely unknown, the gene that is mutated in *reeler* mice — *reelin* — was cloned in 1995[1]. Reelin is an extracellular-matrix glycoprotein that is secreted by a few embryonic brain neurons, such as Cajal–Retzius cells in the marginal zone of the cerebral cortex[2]. Yet most of the neurons that show the *reeler* phenotype do not express the *reelin* messenger RNA and protein[3,4], suggesting that Reelin acts at a distance in a juxtacrine fashion on target cells, radial glial cells and/or neurons.

The latest breakthrough is reported by Sheldon *et al.*[5] and Howell *et al.*[6] on pages 730 and 733 of this issue, and by Ware *et al.*[7] in *Neuron*. The authors clearly identify the mouse *disabled homologue 1* (*mdab1*) gene as an essential component of the Reelin response in target neuronal cells. The story is a textbook example of convergent science, and it vividly illustrates the lightning speed of current molecular genetics.

The *scrambler* mutation was isolated a few years ago at the Jackson laboratory[8]. It produces a *reeler* phenotype but, whereas *reelin* maps on chromosome 5, *scrambler* maps on chromosome 4. *Yotari* (which is Japanese for tottering) is a similar mutation which maps to the same locus as *scrambler*[9]. The *scrambler/yotari* locus was considered to be a prime candidate for a Reelin receptor, and it was eagerly pursued using positional cloning. But earlier this year, Howell and colleagues[10] reported that *mdab1* is expressed in the embryonic brain and, like *scrambler*, it maps to mouse chromosome 4. *mdab1* is one of two mouse homologues of the *Drosophila* gene *disabled* which, in turn, was isolated in a screen for mutations that modify the neurological Abelson null phenotype in flies[11].

Although the map location of *mdab1* did not suggest an immediate link with *scrambler*, Howell *et al.*[6] have now elegantly shown that when they inactivate the *mdab1* gene using homologous recombination, the result is a *reeler*-like phenotype. This finding, communicated before publication, proved directly useful to the positional-cloning initiatives of Sheldon *et al.*[5] and Ware *et al.*[7], who demonstrated that *scrambler* and *yotari* are mutations of *mdab1*.

mdab1 mRNA and protein are expressed in neurons in the cortical plate, and in cerebellar Purkinje cells[5,6] — two main targets of the *reeler* mutation. Although it is expressed in neurons that respond to Reelin, mDab1 is not a Reelin receptor, but a cytoplasmic protein that is phosphorylated during neural development. mDab1 binds to the Src-homology (SH2) domains of non-receptor tyrosine kinases such as Src, Fyn and Abl, and it has a protein interaction/phosphotyrosine-binding domain, providing potential for another class of protein–protein interactions.

The work of Sheldon *et al.*, Howell *et al.* and Ware *et al.* indicates that extracellular Reelin signals to nearby neurons by activating a kinase cascade. The involvement of kinases was already suspected through observations that mice deficient in cyclin-dependent

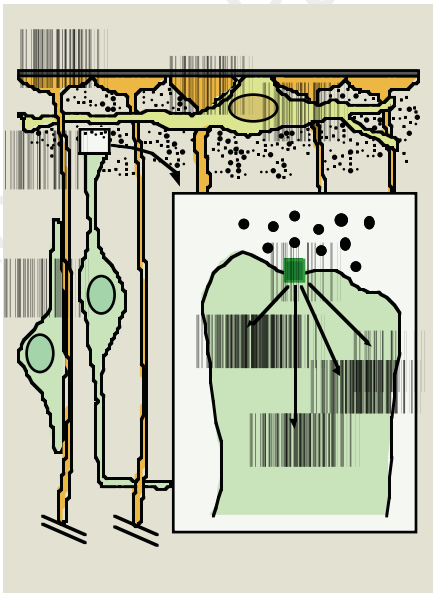
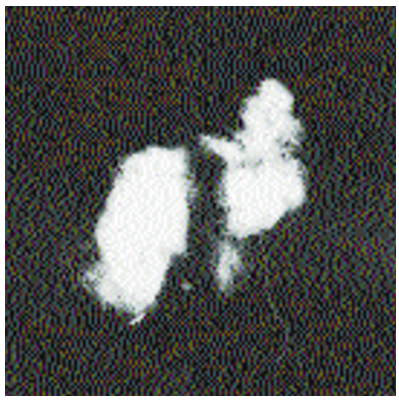


Figure 1 **Model of Reelin action on developing cortex, based on the findings of Sheldon *et al.*[5], Howell *et al.*[6] and Ware *et al.*[7]. Immature neurons (light green) migrate along radial fibres (RF, orange). Reelin (dots) is secreted by Cajal–Retzius cells (CR, yellow) and incorporated in the local extracellular matrix. End-migration cortical-plate neurons (CP, dark green) respond to the presence of Reelin (which is sensed by their apical dendritic tip) by stopping migration, detaching from the RF and assuming a radial organization. The inset shows a neuronal tip. The mechanism by which this senses the Reelin signal is not known. Signal transduction and the neuronal response do, however, clearly require mDab1, Cdk5 in complex with its activator p35 (but also in association with another adaptor) and, presumably, hitherto unidentified molecules. PIA, meningeal surface and basement membrane; MZ, marginal zone.**

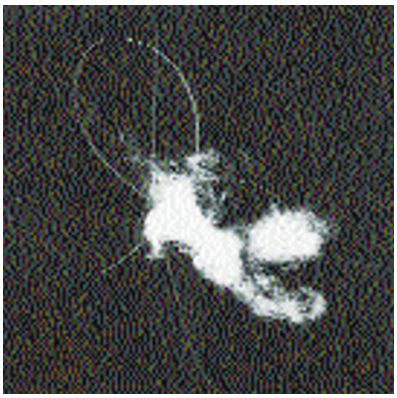
Another matter

Navel fluff **1: sailor (while at sea)**

2: farmer



3: architect



kinase 5 (Cdk5) have a *reeler*-like phenotype[12] — Cdk5 is a serine/threonine kinase that is specifically expressed in postmitotic neurons. Moreover, cortical development does not proceed normally in mice that are deficient in p35, a Cdk5 activator[13]. The neurological anomalies in *p35*-knockout animals are more subtle than in *cdk5*-knockout mice, suggesting that proteins other than p35 help in the regulation of Cdk5.

Based on these latest observations, it is tempting to propose an updated model of Reelin action on migrating neurons in the developing cerebral cortex (Fig. 1). This model raises several questions. First, what are the transcriptional mechanisms that regulate Reelin expression? Certainly, they must be very accurate if we compare the high expression in Cajal–Retzius cells with the absence of expression in adjacent cortical-plate neurons. Second, how does Reelin act on target neurons? Is there a surface Reelin receptor coupled to intracellular transduction, or does Reelin interact less directly with other receptor systems? Third, how is the signal that is generated by Reelin translated into a cell response? A proper arrangement of the cytoskeleton is needed for the formation of cell patterns, so how does the presence or absence of Reelin affect this cellular organization? Interestingly, whereas Reelin is purely extracellular, the characterization of mDab1 provides a tool to attack some of these questions from the inside of the cell.

Given the pace of recent progress, it cannot be long before these questions are answered. However, our past experience with this thorny problem has taught us that answers are likely to be quite different from what we imagine. □

André M. Goffinet is in the Département de Physiologie Humaine, Facultés Universitaires Notre-Dame de la Paix, 61 rue de Bruxelles, B-5000 Namur, Belgium.

1. D'Arcangelo, G. *et al. Nature* **374,** 719–723 (1995).
2. D'Arcangelo, G. *et al. J. Neurosci.* **17,** 23–31 (1997).
3. Lambert de Rouvroit, C., Bernier, B., Schiffman, S., de Bergeyck, V. & Goffinet, A. M. *Soc. Neurosci. Abstr.* **22,** 961 (1996).
4. Schiffmann, S. N., Bernier, B. & Goffinet, A. M. *Eur. J. Neurosci.* **9,** 1055–1071 (1997).
5. Sheldon, M. *et al. Nature* **389,** 730–733 (1997).
6. Howell, B. W., Hawkes, R., Soriano, P. & Cooper, J. A. *Nature* **389,** 733–737 (1997).
7. Ware, M. L. *et al. Neuron* **19,** 239–249 (1997).
8. Sweet, H. O., Bronson, R. T., Johnson, K. R., Cook, S. A. & Davisson, M. T. *Mamm. Genome* **7,** 796–802 (1996).
9. Yoneshima, H. *et al. Neurosci. Res.* (in the press).
10. Howell, B. W., Gertler, F. B. & Cooper, J. A. *EMBO J.* **16,** 121–132 (1997).
11. Gertler, F. B., Hill, K. K., Clark, M. J. & Hoffmann, F. M. *Genes Dev.* **7,** 441–453 (1993).
12. Ohshima, T. *et al. Proc. Natl Acad. Sci. USA* **93,** 11173–11178 (1996).
13. Chae, T. *et al. Neuron* **18,** 29–42 (1997).

Genomic imprinting

Making sense or antisense?

Wolf Reik and Miguel Constancia

Genomic imprinting is a genetic mechanism by which genes are expressed from the maternal or paternal chromosomes[1], and loss of imprinting is involved in a variety of diseases and cancers. All the imprinted genes that have been studied so far have regions in which the maternal and paternal DNA copies are methylated differently at CpG residues — cytosine–guanine base pairs. So DNA methylation is suspected to be the signal from the parental germ cells that results in the allele-specific expression of imprinted genes. Indeed, in mice that do not have methylation (because of mutation in the *methyltransferase* gene), imprinting is altered[2].

The exciting work reported by Wutz *et al.*[3] on page 745 of this issue now shows that differentially methylated regions in a specific imprinted gene (*Igf2r*; the maternally expressed gene that encodes the type-2 receptor for insulin-like growth factor) can carry a crucial imprinting signal. When this region is deleted from *Igf2r*, the gene loses its imprinting and is expressed regardless of parental origin. Moreover, the authors show that the deleted region (which is in an intron of *Igf2r*) is normally the promoter of an antisense transcript that is expressed from the paternal chromosome. Intriguingly, when the antisense gene is expressed, the sense is not, and vice versa, indicating that some form of 'expression competition' regulates imprinting of *Igf2r*, as it apparently also does with other imprinted loci[4,5].

Methylation differences are found in two regions of the *Igf2r* gene. Region 1 (Fig. 1a, overleaf) is in the promoter, and it is methylated when the gene is not expressed. Region 2 is downstream in the second intron, and it is methylated in the expressed maternal copy. The maternal methylation patch (region 2) seems to originate in the germ cells, where eggs are methylated and sperm is not, leading to the suggestion that this region contains an 'imprinting box'. Moreover, because only the expressed allele is methylated, region 2 is thought to contain silencer sequences that can be suppressed by DNA methylation. Other imprinted genes have similar methylated regions in the expressed allele, indicative of similar mechanisms[1].

To show that the *Igf2r* gene contains local signals that are sufficient for imprinting, Wutz *et al.*[3] introduced a (marked) copy of the gene on a yeast artificial chromosome (YAC) into transgenic mice. In three of the four transgenic lines studied, they observed proper imprinting, with expression on maternal transmission and repression on paternal transmission. Hence, the new study,

100 YEARS AGO

The latest number of the *American Naturalist* is the first which has appeared under the new editors. Dr Robert P. Bigelow, of Boston, is now the responsible editor .... "Every scientific man, as such" [he writes], "may well read two general scientific journals – the weekly scientific newspaper and the monthly review of scientific progress." The *American Naturalist* will aim at providing investigators with the latter form of scientific information. Authors of papers intended for beginners, such as "Some Birds of the Garden," "Some Common Weeds," are politely informed that their contributions are not wanted, and very technical works of interest to only a limited number of specialists will be declined. What the editors desire is scientific papers written by scientific people and of interest to scientific workers in more than one field. The desire is a praiseworthy one, and we hope the fulfilment of it will exceed the editors' expectations.

From *Nature* 14 October 1897.

50 YEARS AGO

The lecture on "The Organisation of Industrial Research" which Dr. R. P. Russell, president of the Standard Oil Development Co., delivered on June 9 to the Industrial Research Committee of the Federation of British Industries was... an outstanding contribution to what may be termed the philosophy of research. Research activity in his own Company, he said, began in 1919 with a group of twenty-six people: to-day the staff includes 2,456 technologists, engineers, assistants and clerical personnel all engaged exclusively on research and development projects, as well as several hundred working on laboratory phases of direct operating problems and an engineering staff of more than five hundred. Dr Russell computed that this expansion had brought a return of £15,400 of additional profit for each £1,000 expended on research and development.

From *Nature* 18 October 1947.