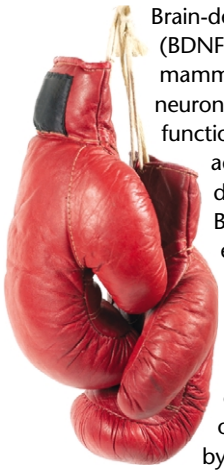
Biology

Neuroscience

It's a knockout!



Brain-derived neurotrophic factor (BDNF) is widely expressed in the mammalian brain and regulates neuronal development and function. However, its impact on adult behaviour has been difficult to discern because BDNF-null mice display early postnatal lethality and there is a lack of effective pharmacological alternatives.

Monteggia and colleagues have successfully overcome these difficulties by generating a sophisticated tetracycline-sensitive Cre/loxP model system, in which BDNF can be deleted in an inducible manner (upon withdrawal of the tetracycline derivative, doxycycline) from the forebrain of mice at desired stages of development [1]. Using *lacZ*/β-Gal and immunohistochemical staining, the authors showed that doxycycline withdrawal induced brain-specific recombination in certain neuronal populations; BDNF mRNA levels were reduced by ~70% in the hippocampus of knockout mice.

Adult knockout mice (in which BDNF expression was attenuated from the age of 12 weeks) exhibited impaired context-dependent memory, a function that is critically dependent upon hippocampal function. At the neurophysiological level, these mice showed specific impairments in long-term potentiation (LTP) in the hippocampus, suggesting a role for BDNF on LTP induction, and providing a possible explanation for the behavioural effects. Like adult knockout mice, early knockout mice demonstrated a context-dependent memory deficit, albeit more severe. However, in contrast to adult knockout mice, early knockouts were hyperactive and displayed a cue-dependent fear conditioning impairment.

Loss of BDNF expression in the hippocampus has been suggested to result in 'depressive' behaviours, therefore, the authors tested knockout mice using the forced swim test, in which the proportion of time a mouse spends immobile in a beaker of water relates to its level of 'depression'. The knockout mice performed equivalently to control mice under baseline conditions but showed a reduced sensitivity to the antidepressant desipramine.

This study revealed important dissociations between BDNF loss during early development and adulthood on behaviour. Perhaps most interestingly, it suggested a modulatory role for persistent BDNF expression on LTP in the hippocampus and context-dependent memory independent of developmental effects. Moreover, although the data showed that loss of forebrain BDNF expression was not sufficient to cause depressive behaviours, it suggested that this process could be an important factor in antidepressant efficacy. Thus, this model will undoubtedly clarify the role of BDNF on brain and behavioural phenotypes throughout development and could be used to identify and characterize factors underlying antidepressant activity. More generally, the study highlights the dangers inherent in using conventional knockout mice for behavioural experiments.

1 Monteggia, L.M. *et al.* (2004) Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10827–10832

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Molecular Biology

Beyond the double helix

DNA exists as a double-stranded helix, as described by Watson and Crick's seminal paper of 1953. However, just over a decade ago, Sen and Gilbert reported that, *in vitro*, long stretches of guanines (Gs) fold to produce a four-stranded structure, known as G4-DNA, which is stabilized by non-canonical bonds formed between the Gs. There are many G-rich sequences in the human genome, leading to the intriguing possibility that G4-DNA might form *in vivo*. However, despite the recent isolation of an enzyme, GQN1, from mammalian cells, which specifically cleaves G4-DNA, direct evidence for its existence *in vivo* has been elusive.

Genomic DNA is double-stranded, thus Gs are complemented by cytosines (Cs) on the opposite strand. It has been shown recently that transcription of a C-rich template leads to a stable RNA–DNA hybrid, thus, in dsDNA, this might leave the G-rich strand free to form G4-DNA. Duquette *et al.* now report that that is exactly what happens – both *in vitro* and *in vivo* [6].

Using plasmids containing G-rich sequences from human, the group observed that, following transcription, loops were formed consisting of one RNA–DNA hybrid strand (as shown by RNaseH treatment, which specifically cleaves RNA–DNA hybrids) and one G4-DNA strand (shown by GQN1 cleavage). These loops formed both *in vitro*, and when plasmids were introduced into *Escherichia coli* cells. However, loops were only seen in *E. coli* deficient for RNaseH and RecQ (a helicase that actively unwinds G4-DNA), suggesting that G4-DNA is likely to form only transiently in normal cells.

Many G-rich sequences in the human genome are prone to high recombination rates, thus, the authors suggest that targeting of nucleases and helicases to G4-DNA might lead to recombination at these sites. The system used by Duquette *et al.* should facilitate future studies into the dynamics of G4-DNA *in vivo*, and into its potential function.

6 Duquette, M. L. *et al.* (2004) Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4-DNA. *Genes Dev.* 18, 1618–1629

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1359-6446/04/$ – see front matter PII: S1359-6446(04)03159-9

Steroids prescription for Niemann-Pick type C patients?

Niemann-Pick type C (NP-C) disease is a fatal neurodegenerative disorder affecting 1 in 150,000 individuals. About 95% of human NP-C is caused by mutations of the *NPC1* gene encoding for the NP-C protein, which transports cholesterol from lysosomes to the endoplasmic reticulum, where it undergoes esterification. Mutated NP-C proteins result in abnormal cholesterol trafficking, causing an accumulation of unesterified cholesterol and glycosphingolipids in the CNS.

The symptoms observed in naturally occurring BALB/c NP-C mice are similar to those of NP-C children, including Purkinje-cell degeneration, progressive demyelination and loss of motor function. In addition, male NP-C mice have low levels of androgen due to dysfunctional biosynthesis of steroid from cholesterol. In recent research, Griffin *et al.* show that the metabolism of steroids in the brain, or neurosteroids, is also disrupted in the NP-C mice [2]. The defect of normal cholesterol metabolism correlates with reduced activity of enzymes 5α-reductase and 3 α-hydroxysteroid deshydrogenase, which are responsible for the conversion of progesterone to the neurosteroid allopregnanolone, a modulator of $GABA_A$ and NMDA receptors that have an important role in neuronal proliferation and protection.

The authors conducted various steroid hormone replacement therapy regimens in NP-C mice. The most successful treatment, leading to a doubled survival period, was observed with a single injection of allopregnanolone administered shortly after birth. This suggests that allopregnanolone affects developmental processes just before or immediately after birth.

It is not known whether NP-C children have aberrant neurosteroids levels. However, if the mouse replicates the human disease, the use of allopregnanolone, which has been prescribed for years as an anxiolytic and antidepressant, is a promising candidate for the treatment of NP-C disease and potentially other neurodegenerative conditions.

2 Griffin, L.D. *et al.* (2004) Nieman-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat. Med.* 10, 704–711

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Structural Biology

A window of fluorescence

The dream of structural biologists is to see a macromolecular complex in action and directly monitor all the conformational changes that are responsible for its function. Because of its high sensitivity, fluorescence is an important and promising approach. Recently, Blunck *et al.* developed two techniques that enable time-resolved measurements of structural changes occurring upon activation and inactivation of voltage dependent ion channels [7].

These two techniques, called total internal reflection (TIR) and semiconfocal epifluorescence (EPI), combine a fluorescent/confocal microscope and a specific prism to direct a laser beam with a voltage-clamp apparatus for whole-cell electrophysiological measurements. Using these techniques, the authors could specifically excite a given region of the cell and measure fluorescence, while being able to maintain a negligible background under various conditions affecting the function of ion channels. By specifically labeling the bacterial sodium channel NaChBac with a fluorescent dye, the authors could, for the first time, record the voltage dependent gating process for this channel.

These advances enabled them to address an important question in the ion channel field, namely what is the range of movements of the voltage sensor domain in voltage-gated channels. The measurements could establish that not only does this domain not undergo a large movement upon channel activation, but that it also remains in its activated position when the channel becomes inactivated. EPI and TIR appear to be promising techniques that could be adapted to study many other systems aside from ion channels.

7 Blunck, R. *et al.* (2004) Detecting rearrangements of shaker and NaChBac in real-time with fluorescence spectroscopy in patch-clamped mammalian cells. *Biophys. J.* 86, 3966–3980

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Microbiology

Auf weidersehn, microbes…

Fimbriae are surface appendages that extend from a bacterial cell and are composed of polymerized, repeating protein subunits. Certain fimbriae are being investigated as possible targets for antimicrobial drugs or as antigens for use in vaccines.

Recently, Buckles *et al.* identified a new fimbrial gene cluster in the genome of the uropathogenic *Escherichia coli* strain CFT073 [3]. This genomic region, termed the *auf* gene cluster, is prevalent in other uropathogenic *E. coli* strains and contains genes encoding a typical chaperone-usher fimbrial system.

The researchers cloned the *auf* region, induced its expression using an arabinose promoter system, and visualized the accumulation of the prepilin and mature forms of the fimbrial subunit AufA. Using biochemical and microscopic assays, the group demonstrated the presence of a surface structure containing the AufA protein. Reverse-transcriptase PCR analysis of expression of the chromosomal *aufA* gene showed that it is expressed during exponential phase growth and during host infection, but not in stationary phase, suggesting the presence of a regulatory mechanism for these genes.

Although virulence assays indicated only a minor role for the *auf* genes in the colonization of the murine urinary tract, further analyses might reveal its possible role in virulence during human infection. The identification of new fimbrial gene clusters such as *auf* serve to increase the number of possible drug targets that can be exploited to fight microbial infections.

3 Buckles, E.L *et al.* (2004) Identification and characterization of a novel uropathogenic *Escherichia coli*-associate fimbrial gene cluster. *Infect. Immun.* 72, 3890–3901

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Biochemistry

Inhibitory role of GRK2 on insulin-induced glucose transport

The evidence of crosstalk between the actions of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) has been widely described. However, recent studies have shown that this interaction occurs not only at the receptor level, but it can also encompass components of both signaling pathways. Exciting work from Jerrold Olefsky´s group has described the participation of G protein-coupled receptor kinase 2 (GRK2) –an important protein that has a key role in desensitization of GPCRs – in the negative regulation of insulin-induced glucose transport [4].

The group previously reported that the heterotrimeric G protein α-subunit, G αq/11 can mediate insulin-stimulated glucose transport [5]. GRK2 contains a regulator of G protein signaling (RGS) domain with specificity for G αq/11, therefore, they decided to analyze the role of GRK2 in the inhibition of G αq/11 function using three mutants of GRK2: the GRK2 wild type, a kinase deficient mutant of GRK2 and a mutant without the RGS domain [4].

They demonstrated that the GRK2 wild type and the kinase-deficient GRK2 mutant inhibited the insulin-mediated GLUT4 translocation. Interestingly, the mutant GRK2 lacking the RGS domain had no effect, suggesting that GRK2 functions, through its RGS domain, as an endogenous inhibitor of insulin-induced glucose transport, by interfering with Gaq/11 signaling.

Moreover, inhibition of GRK2 by antibody microinjection, dominant-negative GRK2 expression, or siRNA-mediated GRK2 knockdown, all increased insulin sensitivity for stimulation of glucose transport. Thus, these results suggest that GRK2 has a new role as an endogenous protein inhibitor of the insulin-induced glucose transport, and can also be an important target for antidiabetic therapeutics acting as insulin sensitizer.

4 Usui, I. *et al*. (2004) GRK2 is an endogenous protein inhibitor of the insulin signaling pathway for glucose transport stimulation. *EMBO J*. 23, 2821–2829

5 Imamura, T. (1999) G alpha-q/11 protein plays a key role in insulin-induced glucose transport in 3T3-L1 adipocytes. *Mol. Cell. Biol*. 19, 6 765–6774

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Business

Collaborations

Phenomix signs major discovery collaboration with Genentech

Phenomix Corporation (http://www.phenomixcorp.com), have announced a collaboration with Genentech (http://www.gene.com) to identify and prioritize drug targets for the treatment of immune disorders.

Phenomix will also receive research funding and future payments on products emerging from the collaboration that are developed and commercialized by Genentech. In exchange, Genentech will obtain exclusive rights to research, develop, manufacture and commercialize potential therapeutics.

Laura Shawver, President and CEO of Phenomix, said: 'Our forward genetics approach differs fundamentally from genomics era attempts at novel target discovery… This partnership of our unique technology and Genentech's disease expertise presents a fantastic opportunity to identify novel intervention points and develop new treatments for debilitating diseases.'

'Forward genetics has been extremely productive in identifying critical genes in lower organisms such as drosophila and nematodes,' said Chris Goodnow, Founder and Chief Scientific Officer of Phenomix. 'We're excited to be collaborating with Genentech, given their leadership in immune-related research, development and commercialization.'

Abbott and Celera announce cancer therapeutics collaboration

Abbott Laboratories (http://www.abbott.com) and Celera Genomics Group (http://www.celera.com) have announced the formation of a strategic collaboration to discover, develop and commercialize therapies for the treatment of cancer. The collaboration will encompass the development of therapeutic antibodies and small-molecule drugs against over-expressed cell-surface proteins that have been associated with cancer and validated as therapeutic targets through proteomics research at Celera.

This collaboration pairs Celera's proteomics and target discovery efforts with Abbott's drug research, development, manufacturing and commercialization expertise. Abbott brings a full range of research capabilities and specific technology platforms, including expertise in RNAi for target validation, discovery of therapeutic small molecules and antibodies, and preclinical and clinical development.

'This collaboration is another important strategic step in Abbott's oncology research program,' said Jeffrey M. Leiden, President and COO,

1359-6446/04/$ – see front matter PII: S1359-6446(04)03152-6