toxicity. This conclusion is in part supported by recent studies in mammals. While mice with genetic defects in *Sod1* and *Sod3* have relatively mild phenotypes[16,17], mice lacking mitochondrial Sod (Sod2) die at about eight days of age, of cardiac failure and hepatic lipid accumulation, secondary to $O_2^{-}\bullet$ inhibition of the respiratory chain[18]. Treatment of these animals with a chemical SOD mimic (MnTBAP) rescues the visceral defects, but because MnTBAP does not cross the blood barrier, the animals subsequently die of neuronal degeneration resulting from loss of motor control[9]. Treatment with drugs that do cross the blood-brain barrier can partially protect the *Sod2*-deficent mice against the neurodegenerative effects of the mutation and further extend their lifespan[12].

Taken together, these observations suggest that cumulative ROS toxicity may be an important factor in determining longevity and that the nervous system, and specifically motor neurons, may be important targets. The differences between the *Drosophila* and mouse results, however, caution against the allure of over-generalization. For example, Sod1-deficient *Drosophila* are severely affected, whereas *Sod1*-deficient mice are relatively unaffected. Such inconsistencies may reflect differences between classes of animals—most likely, the major cellular sites of production and attack of the ROS. Even so, these results imply that the ROS detoxification enzymes are good candidates for longevity-assurance genes, with the relative importance of the different enzymes varying between species. It is therefore reasonable that different alleles of these genes can have an important influence on human degenerative diseases and possibly longevity.

In this rapidly advancing field, it seems likely that further investigation of both endogenous and exogenous regulators of ROS will improve our understanding of the molecular basis of ageing and senescence and help to identify drugs that can counteract ROS effects. While these investigations are likely to have an impact on the science of ageing, one can only speculate as to their effect on the art of ageing. □

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Mind the GAP, Rho, Rab and GDI

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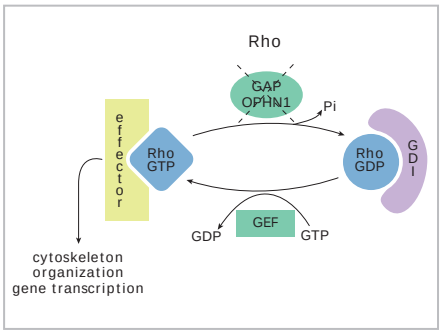
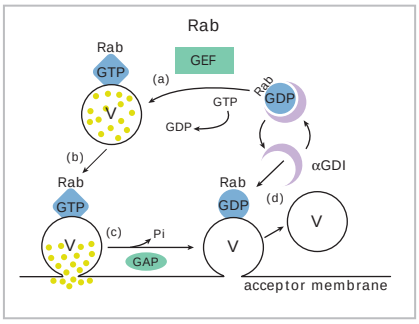
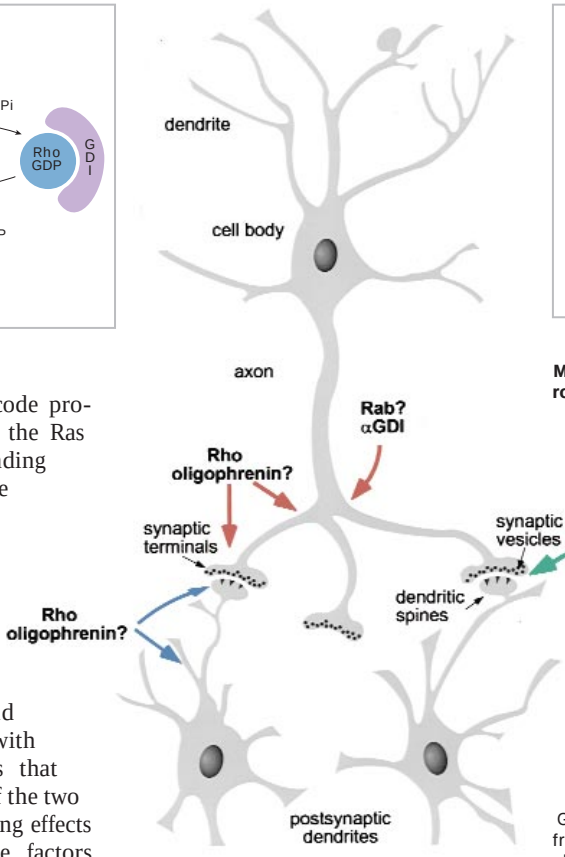
Mental retardation is a common phenotype that is caused by defects in a large number of genes—the updated McKusick catalogue of genes and phenotypes (OMIM; http://www3.ncbi.nlm.nih.gov/omim) responds with 828 entries when queried with "mental retardation". Mapping the position of the corresponding loci, the first step towards cloning of the genes involved, is much easier on the X chromosome because of the 'males only'-affected pedigrees. This translates to an inflated number of OMIM entries that cite the X chromosome, which contains approximately 3% of the human gene complement[1]. Considerable effort has been invested in the systematic identification of X-linked loci that segregate with non-specific mental retardation (MRX; ref. 2). The MRX phenotype is frequent; it affects approximately 1 in 500 males. Linkage analyses of more than 60 well-characterized MRX families, however, implicate at least a dozen loci, and until now, only one of these, the *FMR2* gene, has been cloned[3].

Two positional-cloning efforts—one recently published in *Nature* by Pierre Billuart and colleagues, and the other, reported on page 134 of this issue by Patrizia D'Adamo and colleagues—identify the genes responsible for two different forms of MRX that map to different regions of the X chromosome. The gene hunts were conducted using the full armamentarium available to the positional cloner of the late '90s. Billuart *et al.* took full advantage of an MRX patient with translocation t(X;12); they characterized the breakpoint junction and used sequence-sampling methods to identify potential transcripts mapping to the precise translocation breakpoint. D'Adamo *et al.* used a positional-candidate strategy. Using appropriate families with MRX, they searched for mutations in genes that map to Xq28 and are predominantly expressed in the brain. The gene sequences had been made previously available by genomic sequencing, cDNA selection and exon-trapping experiments. Two genes, *OPHN1*, which encodes oligophrenin-1 (ref. 4) and *GDI1* (ref. 5), were found to be mutated in 4 of the more than 60 MRX families identified so far, underscoring the extensive locus heterogeneity of the MRX phenotype. Furthermore, not all affected families whose loci map to the same chromosomal regions as *OPHN1* and *GDI1* have mutations in these genes—suggesting that the total number of MRX loci may well be about two dozen or more. This is bad news with respect to diagnosis and counselling, but excellent news for those interested in the biology of mental retardation, as it promises the identification of different genes, each of which will play a role in the obscure and complicated molecular and developmental pathways that result in mental retardation.







Model for role of Rho and Rab GTPases in neuronal process formation or synaptic transmission. In a typical neuron, the dentrites serve as the main apparatus for receiving input from other nerve cells, whereas the axon transmits output. Signalling between neurons occurs at the synapse where vesicle fusion at the presynaptic axon terminal results in transmitter release into the synaptic cleft. The transmitter molecules then bind to the postsynaptic receptors located mostly on opposing dendtritic spines. Mutations in *OPHN1* (left-hand side) may result in increased activation of the Rho GTPases. The Rab cycle is illustrated at the right. A GEF catalyses the exchange of GDP to GTP on Rab (a). Rab in its GTP-bound form moves to the acceptor membrane where it docks, fuses and releases the vesicle contents (b), during or after which a GAP triggers the hydrolysis of Rab-bound GTP to GDP (c). GDI then extracts GDP-bound Rab from the membrane and deposits it into the cytoplasm (d). Mutations in *GDI1* may result in an increased GTP/GDP ratio and indiscriminate membrane-binding. Perturbing the activity of the Rho and Rab GTPases results in defects in either axonal outgrowth, dendtritic development or synaptic transmission. GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; OPHN1, oligopherin-1; V, vesicle.

Regulating Rho and Rab

Amazingly, both new genes encode proteins that regulate members of the Ras superfamily of small GTP-binding proteins, implicated in a wide variety of essential biological functions[6]. GTPases function as molecular switches that cycle between two states: one in which they are inactive and bind GDP, and another in which they are active and bind GTP. Only in their GTP-bound state are they able to interact with downstream effector molecules that mediate their effects. The ratio of the two forms is regulated by the opposing effects of guanine-nucleotide exchange factors (GEFs) which enhance the exchange of bound GDP for GTP, and the GTPase-activating proteins (GAPs) which increase the intrinsic rate of hydrolysis of bound GTP. In addition, both the Rho and Rab subfamilies (of the Ras superfamily) are regulated by guanine-nucleotide dissociation inhibitors (GDIs) which stabilize the GDP-bound conformation (ref. 6; see figure). Oligophrenin-1 is a novel GAP of the Rho subfamily, whereas αGDI is a GDI that acts specifically on the Rab subfamily of small GTPases. Both are highly expressed in brain. How does their loss of function result in the cognitive impairment manifested in MRX? A plausible hypothesis can be wrought from current knowledge of Rho and Rab GTPase function.

Rho GTPases

Members of the Rho-subfamily, including RhoA, Rac and Cdc42, have emerged as key regulators of the actin cytoskeleton and, more recently, as regulators of gene transcription in response to extracellular stimuli. The involvement of Rho GTPases in cellular events such as motility, growth control, membrane-trafficking and development has been well documented[6,7]. In the nervous system, perturbation of the Rho GTPase activity interferes with the formation of neuronal processes (see figure). For example, the cerebellar expression of activated Rac in transgenic mice affects dendritic spine formation and axonal growth; in particular it reduces axon terminals[8]. Expression of constitutively activated mutants of RhoA, Rac and Cdc42 in cultured cortical neurons leads to an increase in the number of primary and basal dendrites[9]. In *Caenorhabditis elegans* and *Drosophila melanogaster*, a role for the Rho GTPases in axonal guidance has been demonstrated[10,11].

Billuart *et al.* demonstrated that oligophrenin-1 stimulates GTP hydrolysis of the Rho GTPases *in vitro*. One possible scenario is that loss of oligophrenin-1 results in constitutively active Rho GTPases and consequent stimulation of downstream effector pathways in the subcellular compartments of the neurons. This may result in aberrant formation of neuronal processes which may, in turn, account for MRX. It cannot be excluded, however, that cognitive impairment associated with loss of oligophrenin-1 activity results from loss of interaction with other yet unidentified proteins.

Rab GTPases

The Rab GTPases are primarily implicated in vesicle transport[12]. Rab3, a neuronal member of the Rab GTPases, is essential to synaptic-vesicle exocytosis and neurotransmitter release[13] and recent studies have demonstrated that Rab3 regulates a late Ca^{2+}-dependent step in synaptic-vesicle fusion at the synapses[14]. This regulation is physiologically important for the synaptic plasticity believed to underlie certain forms of learning and memory[15]. The function of αGDI is crucial in maintaining the balance between the GTP- and GDP-bound forms to Rab3 (see figure); mutations identified in the *GDI1* gene from MRX patients either abolished the synthesis of αGDI or reduced its ability to associate with Rab3. This perturbation of αGDI function is therefore likely to interfere with synaptic transmission. Additionally or alternatively, the cognitive impairment associated with mutations in *GDI1* may result from perturbations in neuronal development. D'Adamo *et al.* observed an increase in expression of both Rab3 and αGDI at the onset of brain differentiation before synaptic activity takes place, and that suppression of αGDI expression in cultured hippocampal neurons resulted in an inhibition of axonal outgrowth.

The finding that a RhoGAP and a RabGDI are involved in determining human intellectual abilities points towards new avenues that lead to the molecular mechanisms underlying MRX. Effector molecules downstream of the Rho and Rab GTPases that mediate their effects on neuronal development and synaptic function are now candidate genes for MRX, as are those involved in their regulation. It may also inspire the development of rational therapeutic modalities aimed at the correction of the dysregulation of the lives and actions of these proteins—and those who carry them. □

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A chilled-out knockout

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Stress is the most common disorder of our century—most of us know too well what it means to be 'stressed out'. The word 'stress' is often used to mean anxiety regarding conditions that may be physical, environmental or emotional. In biological and molecular terms, however, stress is a critical physiological response of the body to threatening situations; it is a response that results in enhanced vigilance and allows our bodies to react to a striking number of diverse cues.

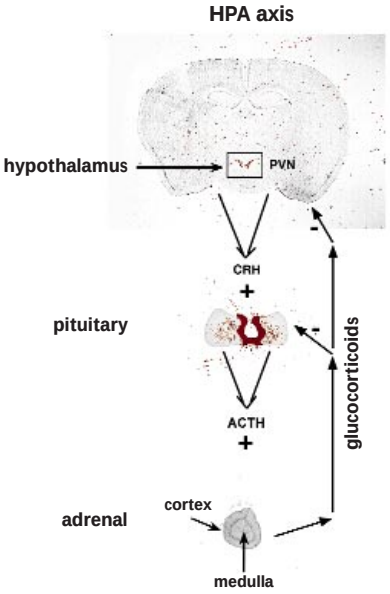
The best-characterized response to stress affects the hypothalamus-pituitary-adrenal (HPA) axis (see figure). Signals originating in the central or peripheral nervous system are conveyed to centres located in the hypothalamus. A molecular cascade of events is then triggered by one molecule: the corticotropin-releasing hormone (CRH; also known as CRF). CRH is a peptide, most of which is synthesized in small nuclei of the hypothalamus, whereupon it is released in the portal-vein system which delivers it to the hypophysis or pituitary gland. Here, CRH interacts with membrane receptors present on the membrane of two pituitary cell types: the corticotrophs and the melanotrophs. Interaction of CRH with the cognate receptor (CRHR) evokes the production of second-messenger cyclic AMP in the target cells. Stimulation of pituitary corticotrophs by CRH results in the production of the adrenocorticotropic hormone (ACTH) and the primary target tissue of ACTH is the cortex of the adrenal gland, from which it elicits the release of glucocorticoids (cortisol in humans and corticosterone in rodents) through interaction with ACTH receptors. Glucocorticoids in turn signal to the pituitary and the brain to turn off the response to stressful stimuli. In addition, a direct effect of CRH on other regions of the brain has also been described, indicating that this peptide might act not only as a neurohormone but also as a neuromodulator[1].

Two different receptors have been isolated: CRHR-1 and CRHR-2 (refs 2–7). Both bind CRH, albeit with different affinities, and each has a specific expression pattern—both are expressed in the brain[8], but CRHR-1 is expressed mainly in the brain (compared with its expression elsewhere), while CRHR-2 is most abundant in peripheral organs (compared with its expression in the brain). Notably, CRHR-1, and not CRHR-2, is expressed by the pituitary. In addition, CRHR-2 has a stronger affinity than CRHR-1 for urocortin, a newly isolated peptide[9].

CRHR-1, stress and anxiety

The existence of more than one CRH receptor has raised the question: which is responsible for mediating the stress response? Peter Timpl and colleagues now provide an answer to this question on page 162 (ref. 10). These authors have engineered mice carrying mutations in the *Crhr1* gene and have documented their behaviour in response to stress. Would they be less anxious? Do the classical responses to stress require the stimulation of the Crhr1 receptor or could the mice compensate for the lack of such an important component?

To test these different possibilities, Timpl *et al.* performed biochemical and behavioural tests on the *Crhr1* mutant animals. The most convincing test involved forced swimming. Imagine being hydrophobic or a poor swimmer, and having to swim to survive. As you might imagine, this is an extremely stressful test (and it would appear that mice agree). Using this test, it is possible to quantify the stress response by direct measurement of circulating ACTH and glucocorticoid levels. Not only are these good



The hypothalamus-pituitary-adrenal axis. Stress induces *CRH* mRNA synthesis in the paraventricular nuclei (PVN) of the hypothalamus, which is indicated in red in the boxed region. CRH interaction with CRHR-1 receptors in the pituitary stimulates synthesis of the proopiomelanocortin gene (*POMC*), leading to the production of ACTH from the corticotrophs. Corticotrophs and melanotrophs that stain positive for *POMC* RNA transcripts are indicated in red. ACTH stimulates the synthesis of glucocorticoids from the adrenal cortex. Glucocorticoids provide negative feedback to the pituitary and brain to arrest the response.