Implications of the elevated activity of protein kinase DYRK1A in Down syndrome

by Dr Ulf Soppa, Dr Chrisovalantis Papadopoulos and Dr Walter Becker

DYRK1A is a gene located on chromosome 21 and is thus overexpressed in individuals with Down syndrome, who carry a third copy of chromosome 21. Several lines of evidence suggest that, in Down syndrome, higher than normal levels of DYRK1A contribute to the aberrant brain development underlying mental retardation and to premature neurodegeneration. The functional characterisation of the protein kinase DYRK1A has provided insights into the mechanisms by which the *DYRK1A* gene exerts its deleterious effects. Because the elevated activity of DYRK1A is key to its pathological effects, inhibitors of DYRK1A may offer a therapeutic option for the treatment of Down syndrome.

Implication of DYRK1A in Down syndrome

With an incidence of approximately 1 in 800 live births, Down syndrome is the most common serious genetic disorder in humans. Although the range and severity of abnormalities is highly variable among individuals, all have some level of intellectual disability and show the neuropathological features of Alzheimer's disease by age 30–40 years. The *DYRK1A* gene on chromosomes 21 is strongly suspected to be at least partly accountable for the neurological alterations in Down syndrome. Mammalian DYRK1A and the orthologous genes in chicken and *Drosophila*, called *minibrain*, regulate neuroblast proliferation and neuronal differentiation [1].

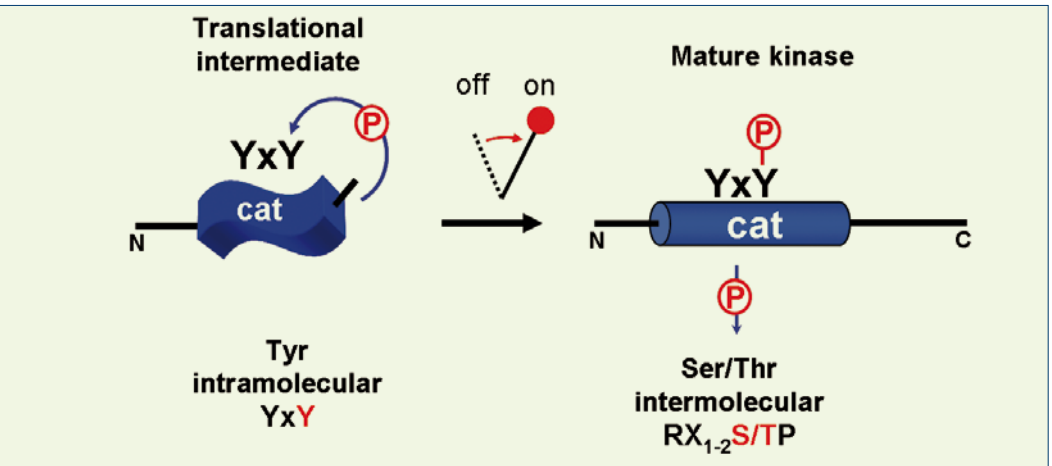

Figure 1. One-off activation of DYRK1A. Autophosphorylation of the conserved activation loop tyrosine (YxY, Tyr-x-Tyr motif) is only catalysed by a translational intermediate form of DYRK1A. After a conformational change of the catalytic domain (cat), mature DYRK1A phosphorylates substrates only on serine or threonine residues. The different conformations of the translational intermediate and the mature form of the kinase exhibit differential sensitivity towards kinase inhibitors [3].

Overexpression of DYRK1A in transgenic mice results in disturbed neuronal development and brain function. Strikingly, reduced expression of DYRK1A due to heterozygous loss-of-function of *DYRK1A* also results in severe developmental abnormalities in mice and humans. These observations support the conclusion that the function of DYRK1A in neurodevelopment is particularly sensitive to gene dosage effects and that the activity of DYRK1A must be exactly tuned. It is also interesting to note that the analysis of the Neanderthal genome identified *DYRK1A* as one of the genes positively selected during the early history of modern humans, leading to the hypothesis that the "modern" alleles were selected due to their importance for cognitive development.

Constitutive activation

Why is it that the level of expression of DYRK1A is so critical for its function? Protein kinases serve as versatile regulators of cellular functions and play key roles in the intracellular transduction and processing of extracellular signals such as those involving hormones, growth factors or neurotransmitters. To fulfil this role, the activity of the kinases themselves must be regulated. Most kinases reside in a basal inactive state until switched on by the appropriate stimulus.

DYRK1A depends on the phosphorylation of a certain amino acid in the so-called activation loop to obtain full catalytic activity. In contrast to many other kinases, members of the DYRK family are activated by autophosphorylation independent of regulatory signals [2]. Moreover, analysis of *Drosophila* DYRKs revealed that the autophosphorylation is irreversible and can only occur during protein synthesis, thus acting as a one-off switch that leads to constitutive activation of the kinase [3] [Figure 1]. Strikingly, although the mature form of DYRK1A only phosphorylates serine or threonine residues in substrate proteins, the autoactivation occurs on a tyrosine residue. This unusual feature is reflected in the acronym DYRK that stands for "dual-specificity tyrosine(Y)-phosphorylation regulated kinase". Although this mechanism does not exclude modulation of activity by other methods, current evidence indicates that at least DYRK1A is catalytically active under all conditions studied so far. Due to its constitutive activity, all changes in the amount of DYRK1A in the cell will directly translate into changes in its activity and alter the downstream effects in cellular regulation.

DYRK1A and neurodevelopment

DYRK1A is a pleiotropic kinase that phosphorylates a plethora of substrates involved in cellular processes, such as cell cycle control, gene transcription, mRNA splicing and synapse function [4]. DYRK1A harbours a nuclear localisation signal and is predominately localised in the nucleus when overexpressed in cultured cells. However, in accordance with the phosphorylation of non-nuclear substrates, endogenous DYRK1A protein is present both in the nucleus and the cytoplasm, and this distribution is also seen with exogenous GFP-DYRK1A when expressed at low levels [Figure 2A].

Recent progress has revealed several pathways by which DYRK1A acts in neuronal development. Transcription factors of the nuclear factor of activated T cells (NFAT) family are among

the targets of DYRK1A. Enhanced phosphorylation by DYRK1A attenuates the gene regulatory effects of NFAT, which are, among many other functions, critically involved in neuronal differentiation. Another downstream effect of elevated DYRK1A activity is the dysregulation of expression of NRSF/REST (neural-restrictive silencer factor/RE1-silencing transcription factor), which controls the expression of genes for fundamental neuronal functions and is necessary for the transition from embryonic stem cells to neural progenitor cells, and thence to mature neurons. Proliferation and differentiation of neural progenitors can also be dysregulated by overexpressed DYRK1A through its effect on cell cycle exit, leading to premature neuronal differentiation and depletion of cycling progenitor cells. In contrast, elevated levels of DYRK1A result in an increased number of retina inner cells because DYRK1A regulates the number of cells that die by intrinsic apoptosis. Recently DYRK1A was shown to regulate asymmetric neural stem cell division in the adult brain by modulating EGF receptor signalling. Although the question of the relative importance of these pathways is not yet conclusively elucidated, the available evidence shows clearly the deleterious consequences of reduced or increased cellular levels of DYRK1A.

DYRK1A and neurodegeneration

Apart from its role in development, it is being increasingly recognised that overexpression of DYRK1A in the adult may contribute to cognitive deficits and Alzheimer-like neurodegeneration in Down syndrome. Enhanced phosphorylation of proteins involved in vesicle transport (dynamin, amphiphysin, synaptojanin) might contribute to synaptic dysregulation observed in DYRK1A-overexpressing mice. Moreover, overexpression of DYRK1A causes hyperphosphorylation of the microtubule-associated protein tau and subsequent formation of neurofibrillary tangles. Other substrates of DYRK1A have also been identified as components of protein aggregates that are hallmarks of neurodegenerative diseases, such as amyloid plaques in Alzheimer's disease and Lewy bodies in Parkinson's disease [Figure 2]. Although the exact role of DYRK1A in the development of these diseases has not been fully elucidated, a number of studies suggest that elevated DYRK1A activity may play a part in the formation of protein aggregates in neurodegenerative diseases.

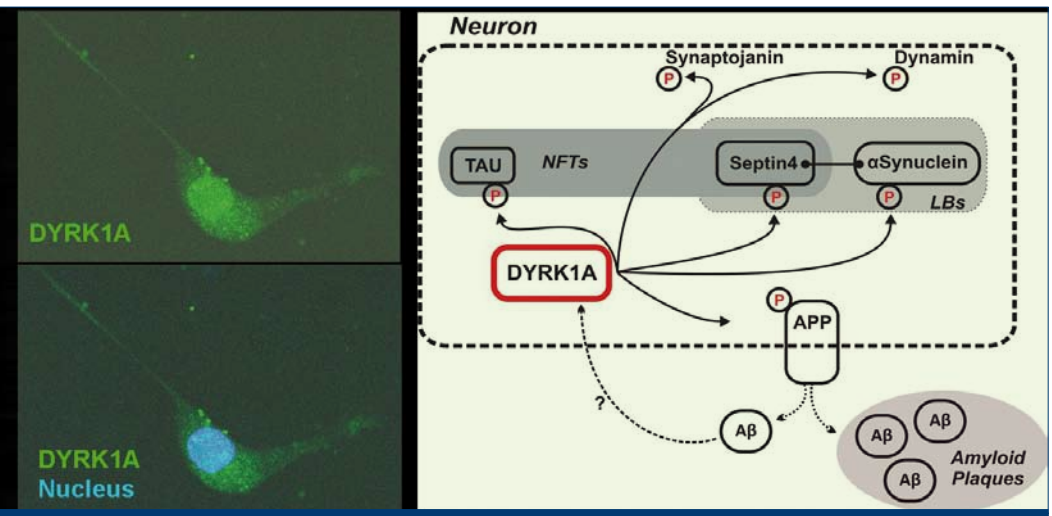

Figure 2. DYRK1A in neurons.
A) DYRK1A is localised both in the nucleus and the cytoplasm of the neuronal cell line PC12.
B) Substrates of DYRK1A in neurons include proteins involved in synaptic transmission (dynamin, synaptojanin) and components of protein aggregates that are hallmarks of neurodegenerative diseases (LB, Lewy body; NFT, neurofibrillary tangle). Phosphorylation of the amyloid precursor protein (APP) by DYRK1A enhances production of the Aβ peptide, which has been described to upregulate DYRK1A expression via an unknown pathway.

DYRK1A as a potential drug target/therapeutic target

The hypothesis that higher than normal activity of DYRK1A contributes to mental disabilities in Down syndrome and neurodegenerative diseases has suggested the idea of pharmacologically correcting the level of activity.

So far the most selective inhibitors of DYRK1A are two plant compounds epigallocatechin gallate (EGCG) and harmine. EGCG is the major polyphenolic compound of green tea and it inhibits DYRK1A quite specifically compared with other kinases. It must be noted however that EGCG also affects many other signalling pathways and is subject to rapid metabolism *in vivo*. EGCG has been successfully used to rescue brain defects of DYRK1A overexpressing mice, but that this result was achieved by effects on targets other than DYRK1A cannot be excluded.

Harmine is a β-carboline alkaloid that strongly and highly selectively inhibits DYRK1A compared with other protein kinases. Harmine is found in various plant species including the South American plant *Banisteriopsis caapi*. This vine is a component of *ayahuasca* ("spirit of the vine"), a concoction of plant extracts used in shamanic rituals and South American sects for its hallucinogenic effects. Harmine is

an essential ingredient of *ayahuasca*, because it inhibits monoamine oxidase in the liver and thus allows oral ingestion of the hallucinogenic drug dimethyltryptamine that is otherwise inactivated by the liver. It is interesting to note that plasma levels of harmine in *ayahuasca* users are sufficiently high to inhibit DYRK1A. Obviously, the inhibitory effect on monoamine oxidase precludes the therapeutic use of harmine as a DYRK1A inhibitor. However harmine appears to be a promising lead structure for the development of a more specific inhibitor, due to its activity with DYRK1A (30-80 nmol/L) and the small size of the molecule (M=212 Da) [5].

The large number (more than 500) of human protein kinases, as well as other ATP binding proteins, impedes the development of highly selective ATP-competitive inhibitors. Although several strategies have successfully addressed this problem, the particular activation mechanism of DYRK1A could provide new options for achieving specificity. Firstly, the three-dimensional structure of the translational intermediate of DYRK1A differs from the mature form in its sensitivity to kinase inhibitors [3,5]. Secondly, even compounds lacking optimal specificity for DYRK1A *in vitro* are expected to inhibit the cellular function of DYRK1A more strongly than that of most other kinases if the autoactivation by tyrosine phosphorylation is blocked [Figure 1]. It will be exciting to see whether this concept of irreversibly acting kinase inhibitors can be realised in practice.

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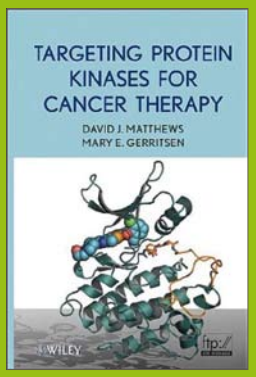
BOOK REVIEW

Targeting protein kinases for cancer therapy



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Research has shown that protein kinases can instigate the formation and spread of cancer when they transmit faulty signals within cells. Because of this, pharmaceutical scientists have targeted kinases for intensive study, and have been working to develop drugs to inhibit these signals.

Complete with full-colour presentations, this book defines the structural features of protein kinases and examines their cellular functions. Combining kinase biology with chemistry and pharmacology applications, the book also presents emerging data driving the discovery of new cancer-fighting drugs and provides valuable information including comprehensive overviews of the major kinase families involved in oncology, integration of protein structure and function. In addition the book describes important tools that can assist pharmaceutical researchers understand and work in this dynamic area of cancer drug research. There is a focus on small molecule inhibitors as well as other therapeutic modalities. A discussion of kinase inhibitors that have entered clinical trials for the treatment of cancer is presented, with an emphasis on molecules that have progressed to late stage clinical trials and, in a few cases, to market. Providing a platform for further study, this important work reviews both the successes and challenges of kinase inhibitor therapy, and provides insight into future directions in cancer therapy.

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