DARPP-32 is a robust integrator of dopamine and glutamate signals

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Abstract

Integration of neurotransmitter and neuromodulator signals in the striatum plays a central role in the functions and dysfunctions of the basal ganglia. DARPP-32 is a key actor of this integration in the GABAergic medium-size spiny neurons, in particular in response to dopamine and glutamate. When phosphorylated by cAMP-dependent protein kinase (PKA) DARPP-32 inhibits protein phosphatase-1 (PP1), whereas when phosphorylated by cyclin-dependent kinase 5 (CDK5) it inhibits PKA. DARPP-32 is also regulated by casein kinases and by several protein phosphatases. These complex and intricate regulations make simple predictions of DARPP-32 dynamic behaviour virtually impossible. We used detailed quantitative modelling of the regulation of DARPP-32 phosphorylation to improve our understanding of its function. The models included all the combinations of the three best characterized phosphorylation sites of DARPP-32, their regulation by kinases and phosphatases, and the regulation of those enzymes by cAMP and Ca^{2+} signals. Dynamic simulations allowed to observe the temporal relationships between cAMP and Ca^{2+} signals. We confirmed that the proposed regulation of protein phosphatase-2A (PP2A) by calcium can account for the observed decrease of Threonine 75 phosphorylation upon glutamate receptor activation. Sensitivity analysis showed that CDK5 activity is a major regulator of the response, as previously suggested. Conversely, the strength of the regulation of PP2A by PKA or by calcium, had little effect on the PP1-inhibiting function of DARPP-32 in these conditions. The simulations showed that DARPP-32 is not only a robust signal integrator, but that its response also depends on the delay between cAMP and calcium signals affecting the response to the latter. This integration did not depend on the concentration of DARPP-32, while the absolute effect on PP1 varied linearly. *In silico* mutants showed that Ser137 phosphorylation affects the coincidence detector function, and that constitutive phosphorylation in Ser137 transforms DARPP-32 in a quasi-irreversible switch. This work is a first attempt to better understand the complex interactions between cAMP and Ca^{2+} regulation of DARPP-32. Progressive inclusion of additional components should lead to a realistic model of signalling networks underlying the function of striatal neurons.

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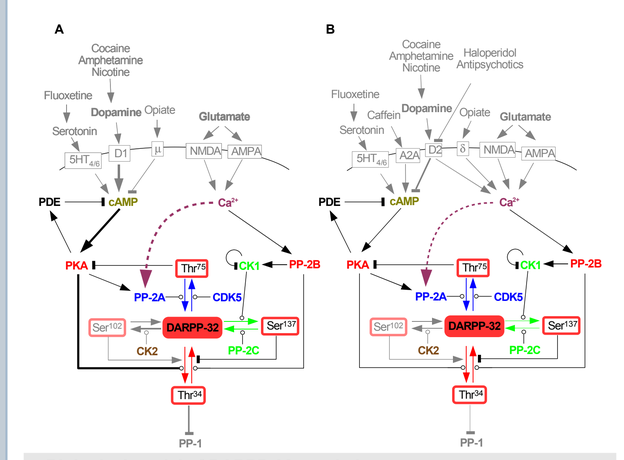
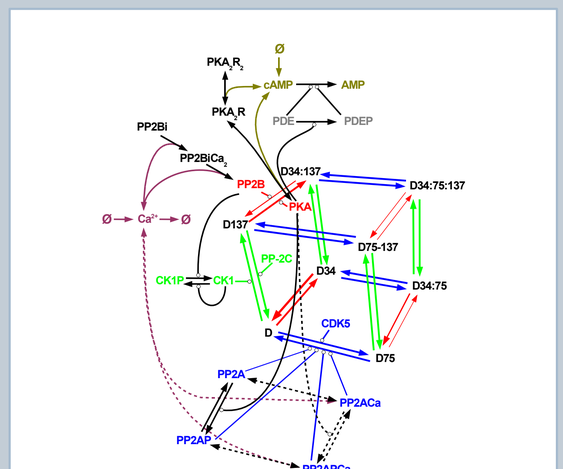


Biological model of DARPP-32 regulation

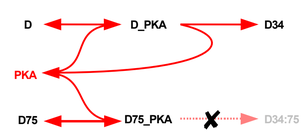
The various endogenous external signals affecting DARPP-32 through cAMP and calcium are represented, as well as external drugs. A, nigro-striatal medium-size spiny GABAergic neuron; B, nigro-pallidal medium-size spiny GABAergic neuron. Arrow-ending lines represent stimulation, bar-ending lines represent inhibition, circle-ending lines represent enzymatic reactions. Dashed lines represent reactions only present in model B. Grey reactions are not present in the model. Source: Svenningsson P, Nishi A, Fisone G, Girault J, Nairn AC et al. (2004) DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol 44: 269-296 [Swenningston et al (2004)].



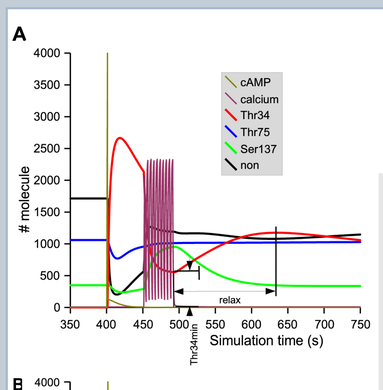
Biochemical model of DARPP-32 regulation

Graphical representation of the models implemented in this study. Arrow-ending lines represent transition, either phosphorylations or binding. Note that the bindings are reversible. Circle-ending lines represent enzymatic reactions The effects of kinases and phosphatases on DARPP-32 have been represented only once for clarity, but each couple of enzymes effectively acts on every pairs of arrows of the same colour. The different thickness of red arrows represent the catalytic rates for the various DARPP-32 species. Dashed lines represent reactions only present in model B.

Modelling and simulation were performed using the E-cell system version 3 [Takahashi et al (2004). Bioinformatics 20: 538-546.] release 3.1.103 http://www.e-cell.org/). A generic ODEStepper developed by Kazunari Kaizu was used for the elementary reactions, combining different types of single-step embedded Runge-Kuttas. XPP version 5.6 was also used to test specific features of the models [Ermentrout B (2002). Soc for Industrial & Applied Math] (http://www.math.pitt.edu/~bard/xpp/xpp.html)

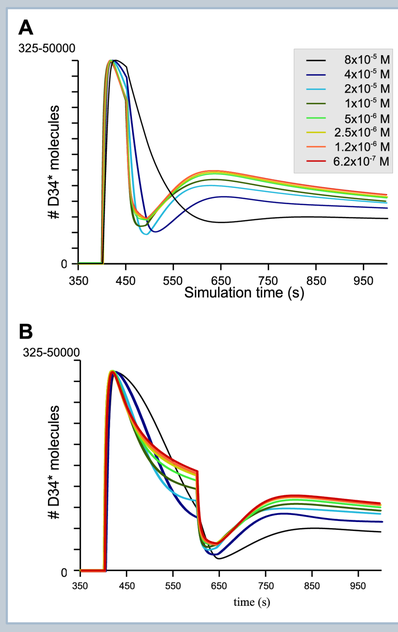


Mechanism of PKA inhibition by the DARPP-32 when phosphorylatie on threonine 75: competitive inhibition. Note that because there is no rebinding of the product, D34:75 and D34:75:137 do not inhibit PKA.



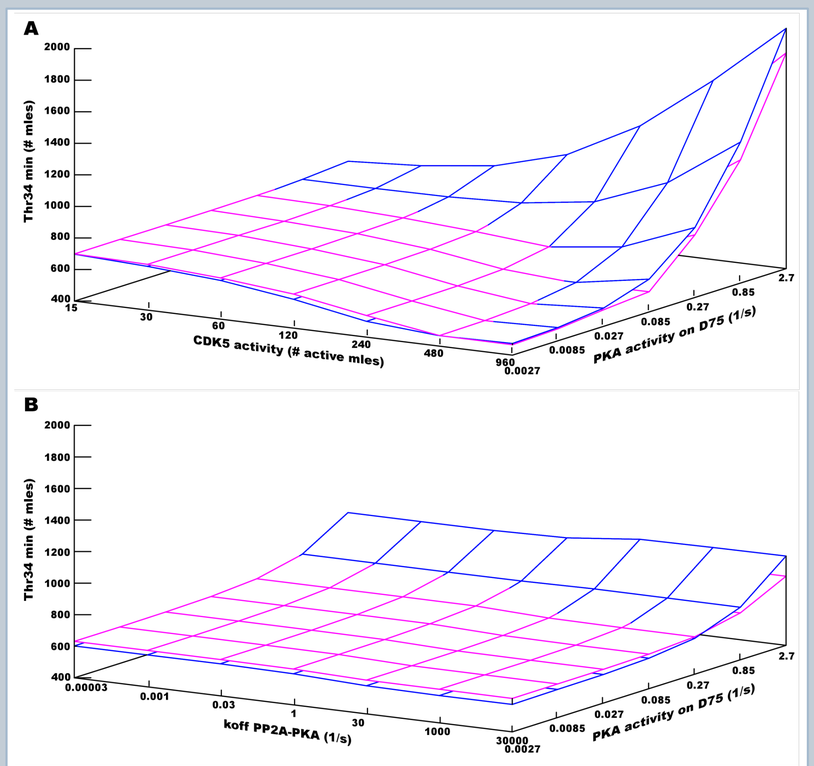
Effect of one pulse of cAMP followed by a train of Ca^{2+} spikes on DARPP-32 phosphorylation

Time-course of DARPP-32 isoforms triggered by a pulse of cAMP followed by a train of Ca^{2+} spikes. Relax and Thr34min show the two readouts used in sensitivity analysis. Panel A, model A; panel B, model B.



Dependency of the signal integration on the concentration of DARPP-32

Same simulation paradigm than the one depicted in figure 5, but with different concentrations of DARPP-32, all the other parameters being conserved. Only D34* of model B is plotted. While the X axis remains the same for all time-course, the Y axis is scaled to superpose all the traces. The vertical scaling is roughly linear, that is a twofold increase between successive values of DARPP-32. Panel A, calcium spikes started 50 s after the pulse of cAMP; Panel B, calcium spikes started 200 s after the pulse of cAMP.



Cross-sensitivity to the inhibition of PKA by DARPP-32 and the activity of CDK5 or the stimulation of PP2A by PKA

Values corresponding to model A are blue while values corresponding to model B are magenta. Panel A, cross-sensitivity to the inhibition of PKA by DARPP-32 and the activity of CDK5. Note the inverse relationship between CDK5 activity and Thr34min for strong inhibition of PKA (low kcat) while the relationship is reversed at weak inhibition. Panel B, cross-sensitivity to the inhibition of PKA by DARPP-32 and the stimulation of PP2A by PKA.

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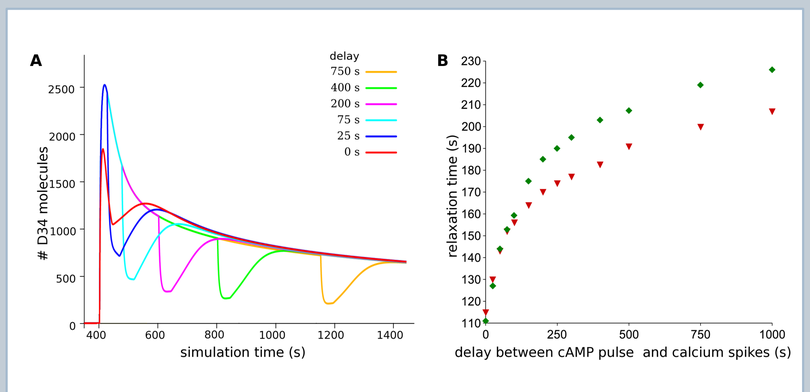
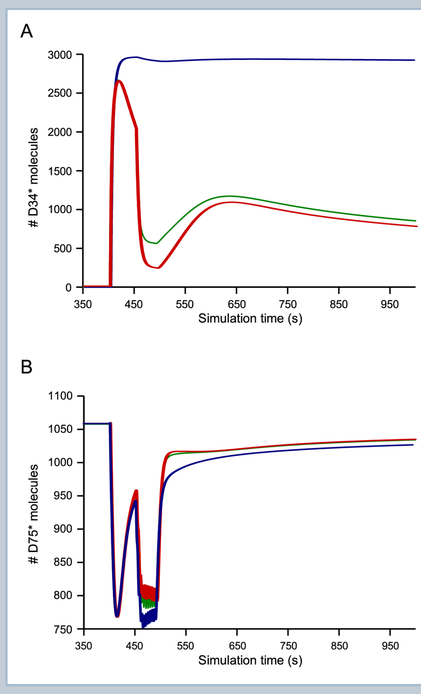


Effect of the delay between cAMP and calcium stimuli

Panel A, time-course of D34* in model B, triggered by a pulse of cAMP followed, after a variable delay, by a train of Ca^{2+} spikes. Panel B, relaxation time of DARPP-32 response to calcium in function of the delay between cAMP pulse and Ca^{2+} spikes. Green diamonds represent the response of "wild-type" DARPP-32 while red triangles represent the response of a mutant without Ser137 phosphorylation.



***In silico* site-directed mutagenesis of DARPP-32**

Same simulation paradigm than the one depicted in figure 5, but describing the predicted behaviour of mutants by model B. Wild-type DARPP-32 species are represented in green, Ser137Ala in red and constitutive Ser137P in blue. Panel A, D34*; Panel B D75*.

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