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Visions & Reflections (Minireview)

DARPP-32: molecular integration of phosphorylation potential

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Cells dynamically react to modifications of their environment by conveying extracellular signals to the cellular machinery able to produce new behaviours, such as movements, deformations or changes in gene expression. The propagation of these signals can be performed by diffusion or transport of material (e.g. small molecules such as calcium, cyclic AMP or IP3, but also small proteins), or transport of information (e.g. through cascades of covalent modifications or conformational spread). Cells must react concurrently to a variety of environmental signals of various nature, chemical (e. g. metabolites and pH) or physical (e. g. temperature, osmotic pressure and deformation), and intensities. Different signals can have opposite effects (e.g. attractors vs. repellents). Comparisons must therefore be made at each time point between complementary and conflicting information, and decisions taken based on the computed results. The analogy between intracellular signalling and computational or electronic processes has been suggested in the past [1–3]. Computational elements are most often associated with small regulatory units made up of several components [4], such as control loops. However, it was also suggested that proteins are themselves computational elements [5]. These proteins could act as logical gates, amplifiers, high-pass filters, switches etc. Most often, these functions are carried out by large, multimolecular complexes made of several identical or different subunits. In contrast, here we show that a fairly small, monomeric protein can perform very elaborate computations, taking into account many signals to decide how to influence neuronal behaviour.

The dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) is a protein phosphatase inhibitor highly expressed in medium-sized spiny neurons of the neostriatum [6]. These neurons are crucial relays in the cortico-striato-thalamic loop [7], and in particular, participate in action selection [8]. Depending on the striatal region this selection controls movements or complex behaviours (e.g. habits and motivation). Within this context, it has been shown that a small protein plays a role in the integration (in the layman sense) of various signals, including glutamate and dopamine signals [9, 10], and therefore in the response to drugs of abuse [11]. Because of its sequence similarity with inhibitor-1 [12] and its inhibiting function on protein phosphatase 1 (PP1) [13], DARPP-32 is often described in textbooks as an **on-off switch**, inhibiting PP1 or not, according to the phosphorylation of threonine 34 [14] (residue numbers in this article follow the human protein). The phosphorylation of T34 is carried out by protein kinase A [6] and is stimulated by an increase in cAMP levels, for instance following dopamine D1 receptor activation. The phosphate on T34 is removed mainly by calcineurin (PP2B) [15] and is thus sensitive to

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calcium increase, for instance upon glutamate stimulation [16].

However, a particularity of DARPP-32 is its ability to be phosphorylated on four different residues by at least four different kinases (Fig. 1). These phosphates can be removed by at least three different phosphatases. DARPP-32 can therefore be viewed as a *hub* for several kinases and phosphatases of the striatal neurons. When phosphorylated on threonine 75 by the cyclin-dependent protein kinase 5, DARPP-32 becomes an inhibitor of PKA [17]. That led to the idea that the protein may be a **changeover switch**, inhibiting either PP1 or PKA [9, 18]. A requirement to fulfill such a role would be a low level of dual phosphorylation on T34 and T75.

Because of the intricacy of kinase and phosphatase regulation, the situation is actually more complex (Fig. 1). Phosphorylation on serine 137 by casein kinase 1 inhibits the dephosphorylation of T34 by calcineurin [19], while phosphorylation on serine 45 and 102 by casein kinase 2 stimulates the phosphorylation of T34 by PKA *in vitro* [20], although the importance of this effect in cells is not known. Furthermore, cAMP signalling leads to increased phosphorylation on T34 and decreased phosphorylation of T75, through the activation of protein phosphatase 2A (PP2A) by PKA [21, 22], effectively disentangling both sites. Calcium not only activates dephosphorylation of T34 through calcineurin, but also dephosphorylation of T75 through PP2A [23, 24]. Therefore, calcium initially decreases both phosphorylations, through activation of PP2B and PP2A. At least in theory it could subsequently increase phosphorylation on T34 by relieving the inhibition of PKA (decrease of phosphorylation on 75) and increasing the inhibition of PP2B (increased phosphorylation of S137, through activation of casein kinase 1 by PP2B).

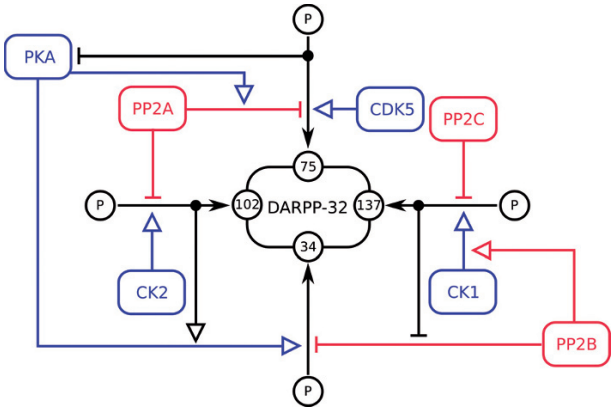

Figure 1. Regulation of DARPP-32 phosphorylation by four kinases and three phosphatases. The graphical conventions are those of the Entity Relationship idiom of the forthcoming Systems Biology Graphical Notation (http://www.sbgn.org/), derived from the Molecular Interaction Maps [28].

DARPP-32 cannot really be considered as a simple changeover switch that would 'decide' to inhibit PP1 or PKA according to cAMP and calcium signals. What DARPP-32 senses is the sum of kinase and phosphatase activities. It is primarily an adder. The increase in T34 phosphorylation reflects the activities of PKA, CK1 and CK2, while the decrease in T34 phosphorylation reflects mainly the activities of PP2B and PP2C, with a contribution of CDK5. PP2A acts in both directions through dephosphorylation of T75 and S102. DARPP-32 does not, however, translate these activities directly, but through integration, in the mathematical sense of the term. This function as an integrator is due to the fact that if we assume relatively fast equilibrium for the binding of DARPP-32 to the enzymes, the evolution of T34P concentration is merely a summation of the products of enzyme concentrations by their catalytic constants. The lower panel of Figure 2 represents the evolution of T34 phosphorylation after a cAMP signal followed, after 50 s, by 10 spikes of calcium [25]. The upper panel represents the derivative of T34 phosphorylation. The six phases represent mainly 1) the lack of phosphorylation in the absence of cAMP, 2) the activation of PKA, 3) the basal activity of PP2B, 4) the calcium-induced activation of PP2B, 5) the activity of CK1, and 6) the basal activities of PP2B and PP2C. In a first approximation, DARPP-32 can therefore be considered as taking the integral of the phosphorylation potential, with the phosphatases being seen as 'negative kinases'. Note that as a consequence, the intensity and the time course of T34 phosphorylation is fairly sensitive to the modifications of either the catalytic activity or the concentration of kinases and phosphatases. For instance, it was shown that increasing the activation of CDK5 decreased the phosphorylation of T34 triggered by cocaine [26]. This result was reproduced in quantitative models by varying the concentration of CDK5 [25].

In summary, in this model DARPP-32 is an adding hub for kinases and phosphatases, and an integrator for their activity. The resulting phosphorylation of Thr-34 is a fairly sophisticated computation of many converging signals, which should then translate into inhibition of PP1. The complexity of DARPP-32 function in neurons could be further increased by its differential location in the nucleus, the soma and the dendritic spine, modulated by drugs such as cocaine, D-amphetamine, morphine, and food-motivated learning [27]. That makes DARPP-32 an interesting building block for synthetic biology efforts in eukaryotic cells, able to link any pathway modulating any of the kinases and phosphatases acting on it. DARPP-32 is also potentially a versatile drug target, providing one can differentially target the different phosphorylation sites.

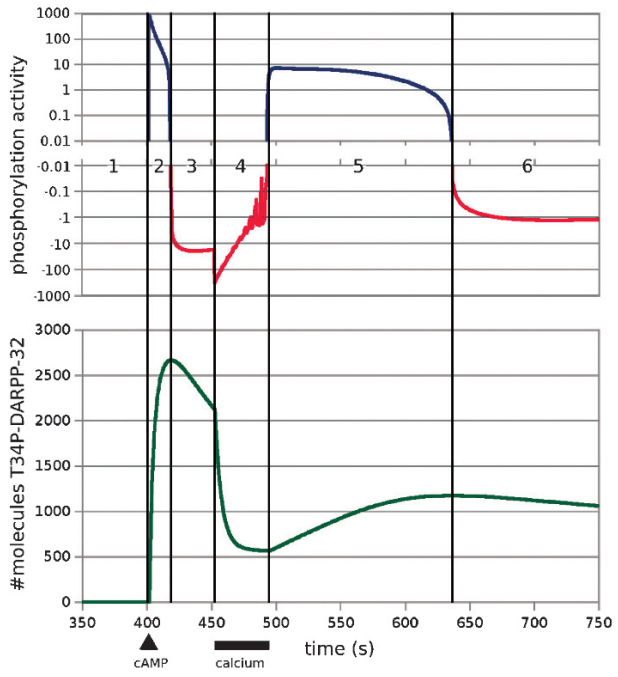

Figure 2. Lower panel, time course of T34 phosphorylation, with a cAMP stimulation at t = 400 s, and 10 calcium inputs from t = 450 to t = 490, as described in [25]. Upper panel, derivative of the T34P time course. The values are plotted on logarithmic ordinates for the sake of clarity. The positive values in blue mainly translate the activity of PKA and CK1, while the negative values in red represent the activity of PP2B and PP2C.

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