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Modelling cellular signal communication mediated by phosphorylation dependent interaction with 14-3-3 proteins

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ABSTRACT

The 14-3-3 proteins are important effectors of Ser/Thr phosphorylation in eukaryotic cells. Using mathematical modelling we investigated the roles of these proteins as effectors in signalling pathways that involve multi-phosphorylation events. We defined optimal conditions for positive and negative cross-talk. Particularly, synergistic signal interaction was evident at very different sets of binding affinities and phosphorylation kinetics. We identified three classes of 14-3-3 targets that all have two binding sites, but displayed synergistic interaction between converging signalling pathways for different ranges of parameter values. Consequently, these protein targets will respond differently to interventions that affect 14-3-3 binding affinities or phosphorylation kinetics.

1. Introduction

Cellular adaption and decision-making rely on the communication between multiple signalling pathways. Post-translational protein modifications on multiple sites is one important mechanism for signal cross communication. To execute a response specific to such multisite modifications, it often has to be "read" by specialized domains in the receiving signal transduction protein. Among the many specialized cellular signal transduction molecules that have been discovered during recent decades, the family of 14-3-3 proteins occupies a remarkably ubiquitous role as downstream effectors of phosphorylation events. Essentially being soluble dimers of single phospho-Ser/Thr binding domains, the 14-3-3 proteins are reported to bind hundreds of different cellular proteins, although there are also examples of binding to non-phosphorylated target proteins (for review see [1–3]). Upon binding to their phospho-recognition site, they are reported to affect the target protein (TP) by modulating its activity [4], interaction with other molecules [5], intracellular localization [6] or stability [7].

Dimeric 14-3-3s are required for binding to many targets, and several TPs like the PKCε, Cdc25B, c-Raf and Foxo4 require two sites to be phosphorylated for high affinity binding of 14-3-3 [6,8–10] (see [11] for more examples). The term "gatekeeper site" refers to the primary role of one phospho-site in determining 14-3-3 binding. A secondary phosphorylation site, which in some cases can be more divergent from the consensus sequence, can further contribute to increased affinity binding or induction of structural changes in the TP. The latter is referred to as the "molecular anvil" hypothesis [12]. Multi-phosphorylation events have also been reported to negatively regulate binding of 14-3-3 proteins, by phosphorylation of TPs at a site close to the 14-3-3 interaction site, thereby preventing complex-formation [10,13,14].

Context dependent signalling mechanisms can act at the level of signalling pathways or at their downstream targets. Examples exist for the action of 14-3-3 proteins at both levels [4,13]. We wanted to investigate in more detail how 14-3-3 proteins may influence signal transduction, particularly in multi-pathway communication executed through phosphorylation at multiple sites. A mathematical modelling approach was chosen for generality, but the modelling was based on previously reported mechanisms of interaction

Abbreviations: AANAT, aralkylamine N-acetyltransferase; Bad, Bcl-2 antagonist of cell death; Cdc25B, cell division cycle 25B; Foxo4, forkhead box protein O4; MAPKAP-K1, mitogen activated protein kinase activated protein kinase 1; p27Kip1, cyclin-dependent kinase inhibitor 1B/p27; PAK1, p21 protein (Cdc42/Rac)-activated kinase 1; c-Raf/Raf-1, Raf proto-oncogene ser/thr protein kinase; Rap1GAP2, Rap1 GTPase activating protein 2; RGS18, regulator of G-protein signalling 18; r_n, phosphorylation rate constant ratio site n; r_S, synergy ratio; S1, signal 1; S2, signal 2; TP, target protein; pSn-TP, TP phosphorylated on site n

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between 14-3-3 and TPs and experimentally derived affinity constants. We first investigated 14-3-3 binding to TP downstream of one signalling pathway and its regulation by phosphorylation kinetics and binding affinities. Next, we modelled inhibitory signal communication for 14-3-3–TP interactions where the TPs in addition had a phosphorylation site that inhibited 14-3-3 binding, a mechanism reported for several TPs. The capability of dual phosphorylation site recognition of 14-3-3 proteins has been suggested to make them functional logic AND-gates or coincidence detectors [11]. Based on the nature of their signal output, we defined three functional classes of dual site 14-3-3 TPs. The influence of phosphorylation rate kinetics of TPs and the binding affinities of 14-3-3 to different phosphorylated states on the signalling response of the different classes was then investigated. In particular, we report on conditions that gave optimal synergistic cross-talk between the two signalling pathways, mediated by 14-3-3 interactions. These conditions varied substantially between the three classes of 14-3-3 TPs. Our findings provide new insights into the regulation of 14-3-3 target proteins and open up for new strategies for therapeutic modification of 14-3-3 regulated processes.

2. Materials and methods

2.1. Modelling of protein phosphorylation and 14-3-3 binding

We refer to the Supplemental text for details on the modelling approach. The reactions were implemented in the simulation software Copasi (v4.8) [15]. We used the LSODA algorithm for numerical integration using an absolute tolerance of 10^{-12}.

3. Results and discussion

3.1. Signalling interaction through disrupted 14-3-3 binding

Several hundred binding partners have been reported for the 14-3-3 proteins. Most of the available 14-3-3 proteins in a cell can therefore be expected to be bound to different target proteins. To tackle this in our models we classified the bulk cellular 14-3-3 TPs as high and low affinity binders, and used a buffered protein interaction modelling approach for all the calculations (Supplemental text, Fig. S1).

Starting with the simple case where only one signalling pathway targets the TP 14-3-3 binding site, we show how different signalling strengths (kinase/phosphatase rate ratios, r_R) and K_d-values of 14-3-3 binding can modulate outputs such as complex formation and TP phosphorylation (Figs. S2 and S3). This simple model would apply to many 14-3-3 TPs. Multiple signalling inputs occur when several kinases or phosphatases target the same phosphorylation site. As such, additional input signals will change the r_R-value, leading to altered response (Fig. S3).

Alternatively, signalling pathways may modulate the affinity of 14-3-3 binding, e.g., by expression of different 14-3-3 isoforms, modification of 14-3-3s [16,17] or by additional modification of their TPs [18]. The latter mechanism has been shown for the proteins Cdc25B, RGS18, Rap1GAP2 and Bad, where phosphorylation-mediated inhibition of 14-3-3 binding by direct phosphorylation of the TP close to the 14-3-3 binding site has been reported [10,13,14,19]. This provides a mechanism for inhibitory signalling communication, and we investigated this mechanism more closely where one signalling pathway downstream of signal 1 (S1) controls phosphorylation of site 1 on TP, necessary for 14-3-3 binding (Fig. 1A, Fig. S4A). We modelled the situation where a strong inhibitory phosphorylation was included downstream of signal 2 (S2), that targeted a site 2 on TP and rendered it incapable of binding 14-3-3 (Fig. 1A, Fig. S4A). As the proteins reported to be regulated

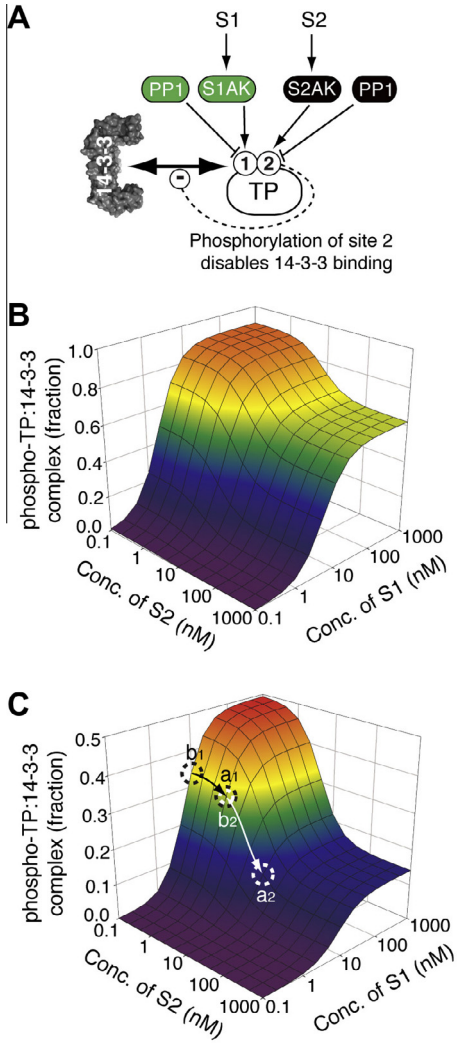

Fig. 1. Inhibitory signal communication. We modelled a TP with two phosphorylation sites (see Fig. S4A for details on the model) where site 1 was phosphorylated downstream of signal 1 (S1) and site 2 downstream of signal 2 (S2) (A). Phosphorylation of site 1 (pS1-TP, $r_1 = k_{K1}/k_{P1} = 0.1$) initiated binding of 14-3-3 with high affinity, which was necessary for the regulation of TP. Phosphorylation of TP on site 2 (pS2-TP and pS1pS2-TP, $r_2 = k_{K2}/k_{P2} = 5$) inhibited any binding to 14-3-3. Phosphorylation and dephophorylation of the two sites was independent and did not occur on pS1-TP bound to 14-3-3 (Fig. S4A). In (B), the amount of TP (phosphorylated only on site 1) in complex with 14-3-3 (pS1-TP:14-3-3 complex, fraction of total TP) is shown for varying signalling strengths of S1 and S2. A K_d of 1 nM was used for the binding of 14-3-3 to pS1-TP. The results in panel (C) are obtained using the same model as in (B), but the K_d of 14-3-3 binding was changed to 10 nM. The dotted circles illustrates changes in TP:14-3-3 complex expected going from basal states of 0.1 or 1.0 nM S2, b1 or b2, respectively, to activated states a1 and a2. The signal strength from S1 is kept constant (10 nM).

by this mechanism have the inhibitory phosphorylation site (site 2) placed adjacent to the 14-3-3 binding site (site 1), we assumed that site 2 was unavailable for phosphorylation in the complex between 14-3-3 and pS1-TP.

Expectedly, for high 14-3-3 binding affinities (K_d = 1 nM) only modest inhibition was obtained (Fig. 1B) even at phosphorylation conditions favouring high phosphorylation stoichiometry on the inhibitory site (rate constant ratio of kinase/phosphatase of site 2, $r_2 = 5$). This low inhibition occurred as most of the TP resided in a complex with 14-3-3 unavailable for inhibitory input. This is shown in Fig. S4B, where increasing S1 generated more pS1-TP:14-3-3 complex that was unresponsive to S2 signal input. A lower affinity (K_d = 10 nM) opened for more sensitive pathway communication (Fig. 1C). In comparison, the influence of 14-3-3