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Building and exploring an integrated human kinase network: Global organization and medical entry points☆

Jacques Colinge, Adrián César-Razquin, Kilian Huber, Florian P. Breitwieser, Peter Májek, Giulio Superti-Furga**

CeMM—Research Center for Molecular Medicine of the Austrian Academy of Sciences, Lazarettgasse 14, AKH-BT 25.3, 1090, Vienna, Austria

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ABSTRACT

Biological matter is organized in functional networks of different natures among which kinase–substrate and protein–protein interactions play an important role. Large public data collections allowed us to compile an important corpus of interaction data around human protein kinases. One of the most interesting observations analyzing this network is that coherence in kinase functional activity relies on kinase substrate interactions primarily and not on which protein complexes are formed around them. Further dissecting the two types of interactions at the level of kinase groups (CMGCs, Tyrosine kinases, etc.) we show a prevalence of intra-group interconnectivity, which we can naturally relate to current scenarios of evolution of biological networks. Tracking publication dates we observe high correlation of kinase interaction research focus with general kinase research. We find a similar bias in the targets of kinase inhibitors that feature high redundancy. Finally, intersecting kinase inhibitor specificity with sets of kinases located at specific positions in the kinase network, we propose alternative options for future therapeutic strategies using these compounds.

Biological significance

Despite its importance for cellular regulation and the fact that protein kinases feature prominent targets of modern therapeutic approaches, the structure and logic of the global, integrated protein phosphorylation network have not been investigated intensively. To focus on the regulatory skeleton of the phosphorylation network, we contemplated a network consisting of kinases, their substrates, and publicly available physical protein interactions. Analysis of this network at multiple levels allowed establishing a series of interesting properties such as prevalence of kinase substrate interactions as opposed to general protein–protein interactions for establishing a holistic control over kinases activities. Kinases controlling many or a few only other kinases, in addition to non-kinases, were distributed in cellular compartments differently. They were also targeted by kinase inhibitors with distinct success rates. Non-kinases tightly regulated by a large number of kinases were involved in biological processes both specific and shared with their regulators while being preferably localized in the nucleus. Collectively, these observations may provide for a new perspective in the elaboration of pharmacological intervention

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* *Corresponding authors.*
E-mail addresses: jcolinge@cemm.oeaw.ac.at (J. Colinge), gsuperti@cemm.oeaw.ac.at (G. Superti-Furga).

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strategies. We complemented our study of kinase interactions with a perspective of how this type of data is generated in comparison with general research about those enzymes. Namely, what was the temporal evolution of the research community attention for interaction versus non-interaction-based kinase experiments.

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1. Introduction

The organization of biological matter into functional networks with elements of modularity has been identified as key to warrant the accomplishment of a great variety of biochemical and cellular functions with a limited set of gene products [1–4]. In many instances, molecular networks have been very well studied, such as in intermediate metabolism, protein interactions and phosphorylation. In eukaryotes, and with the added layer of dedicated tyrosine phosphorylation, protein phosphorylation has evolved as a primary intracellular signaling strategy. It bears many intrinsic specificities over other posttranslational modifications, including reversibility, energetic convenience and the ability of changing polarity of protein surfaces, leading to allosteric changes as well as governing protein interaction [5]. As phosphorylation has many biochemical and cellular changes both as input and as outcome, it induces a network that intersects with a plethora of biological processes.

Continuous efforts and methodological developments have greatly augmented our knowledge of how human kinases interact with other proteins or small molecules. More than 11,000 protein–protein physical interactions (PPIs) involving at least one kinase can be retrieved from public databases that compile the individual efforts of a whole community. Different technologies were used to unravel these data with a strong contribution of affinity purification-mass spectrometry (AP-MS) [6–8] and the yeast 2-hybrid (Y2H) system [9,10]. Known kinase interactions were obtained thanks to a multitude of publications, which did not necessarily focus on kinases but nonetheless included some of those enzymes. Recently, dedicated campaigns mapped human protein kinase interactors specifically and on a large-scale. For instance, following a tandem AP–MS approach and using a tetracycline-inducible strep-hemagglutinin tag [7], the protein complexes formed around 32 commonly expressed kinases, i.e. kinases found in most cell types, were mapped in HEK293 cells [11]. Another study employing the same experimental protocol charted the complexes involving CMGC kinases in an unprecedented whole kinase group-wide effort [12].

Since kinases act primarily by regulating other proteins through their enzymatic activity, to map kinase PPIs is not sufficient for understanding their functional relationships. PPIs inform us on their collaboration with other proteins to form protein complexes or molecular machines, whereas kinase–substrate interactions (KSIs) picture what is regulated by their action. Human KSIs have been studied more sparsely than PPIs so far, although data exist from several databases and new large-scale efforts were undertaken. CEASAR, a protein microarray-based strategy unraveled 3,656 KSIs for 289 protein kinases [13], bringing the number of known KSIs to more than 8500. Furthermore, computer-inferred KSIs such as the 75,000+ KSIs predicted by NetworKIN [14], exploiting experimental phospho-proteomic data, known kinase substrate specificity, and kinase physical and genetic interactions, might provide a useful complement after stringent filtering before more experimental KSI are measured.

The regulatory function of kinases has been observed to be deregulated in many diseases, in particular cancer. Accordingly, a large number of kinase inhibitors have been developed by drug discovery laboratories and pharmaceutical companies, which are mostly small molecules or antibodies. To relate kinases with their PPI partners as well as their substrates calls for further annotating such a network with the perturbation entry points available for disease therapy or chemical biology. Several large *in vitro* kinase screens have provided comprehensive and quantitative drug–protein interaction data (DPIs) for a lot of cancer kinase inhibitors. In this category, 72 inhibitors were screened against 442 kinases by Ambit Biosciences [15] and another set of 178 inhibitors against 300 kinases by the Peterson's group [16]. Furthermore, specialized databases collect DPIs from a broad range of scientific reports, e.g. DrugBank [17] (78 DPIs). Finally, chemical proteomics has emerged as a very interesting, more physiologically correct alternative to *in vitro* screens, where immobilized compounds serve as bait in affinity purifications to identify kinase inhibitor protein targets in an unbiased and cell type-dependent manner [18,19]. This methodology has been applied successfully to small molecules inhibiting protein kinase activity [20–28].

Altogether, the availability of kinase PPIs, DPIs, as well as KSIs in unprecedented, large numbers created a unique opportunity for assembling a kinase-centered network combining these three kinds of interactions and to perform a global study of kinases in their environment. We hence collected and integrated data from the various sources mentioned above and computed such a network. We started our analysis by examining how protein interaction information was correlated with classical kinase research. Investigating the global topology of PPI and KSI networks we could obtain new insights in how they differ as well as refine previous hypotheses regarding the existence of global kinase communication ways. We finally investigated how existing kinase inhibitors actually cover different classes of kinases and how KSIs might help exploring new therapeutic approaches.

2. Materials and methods

2.1. Statistical analyses and data representation

All statistical analyses were performed with the R system (www.r-project.org). Cytoscape [32] was used to prepare network representations.