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Systems biology: Integrating in silico and experimental approaches to study signaling networks

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The systematic analysis of dynamic signaling networks provides a new grammar[1,2] for drug discovery by considering the complex biological context of drug targets. Such analyses are ideally suited for a systems biology approach that integrates experimental with computational approaches with the aims of discovering and validating new drug targets and biomarkers, as well as predicting potential "off target" effects.

A systems biology investigation is an iterative process that we generally start with the identification of known network components and their relationships by extracting such information from the scientific literature[2,3] as well as specialized databases. We use this information to derive process maps and the mathematical models of the functional relationships. We then validate and calibrate these models by comparing simulated predictions with existing data, and test them against new experiments. Iterations of modeling, simulation and experimentation are applied as required by specific studies.

A number of techniques can be employed to generate the quantitative data, crucial to this approach. Among others, we have implemented a proteomics platform based on "reverse protein arrays" (RPA)[2] onto which we spot protein mixtures such as cell lysates. We then probe these arrays with selected fluorescent antibodies in a multiplexed manner, using the planar waveguide (PWG) technology for improved sensitivity[4]. PWG RPA makes it feasible to obtain reproducible and quantitative protein expression information about the dynamic aspects of cell signaling. This application is illustrated by measuring how insulin stimulates the Akt/PKB pathway[5] in a time resolved manner (Figure 1) and how wortmannin[6] affects this signaling cascade (Figure 2). The attenuated response to the compound, as evidenced by the increase in apparent IC50, suggests that between Akt/PKB and S6 ribosomal protein, part of the signal is diverted to other branches of the pathway.

PWG RPA can rapidly deliver signaling data obtained from large scale perturbation experiments of great value for a systems biology approach. Eventually, the integration of text mining, computational modeling and the systematic measurement of signaling events will results in a new mathematico-experimental paradigm. One of the outcomes will be new types of databases that integrate biological observations gathered from traditional experimental approaches, detailed biological process maps, their associated mathematical models and validating data sets. These compendia[7] will include cell-specific and "perturbagen"-specific signalomes that represent not only a new grammar of drug discovery, but also many examples of how this grammar is used to construct complete sentences, paragraphs and even entire stories about the complex biology of health and disease.

References

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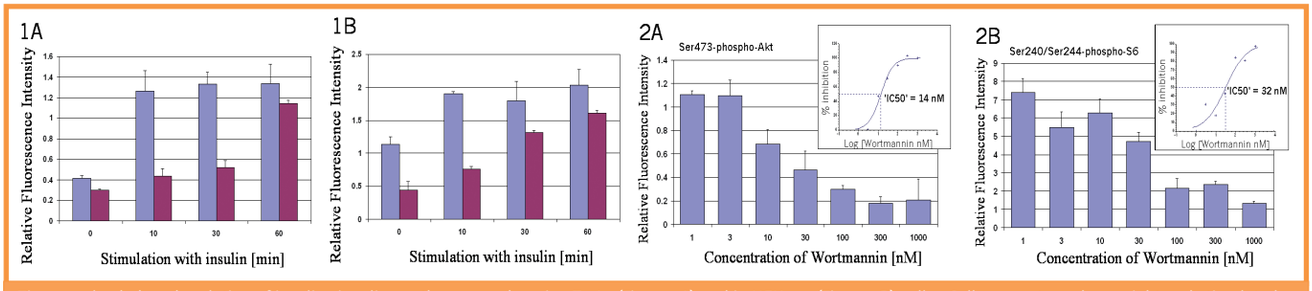

Time-resolved phosphorylation of insulin signaling pathway members in 3T3-L1 (Figure 1A) and in HEK293 (Figure 1B) cells. Cells were starved overnight and stimulated with 100 nM insulin for the indicated time. Cells were then lysed and the phosphorylation levels of Akt/PKB and S6 ribosomal protein were assessed with antibodies specific for Ser473-phospho-Akt (blue) and Ser240/Ser244-phospho-S6 ribosomal protein (red) in our PWG RPA platform.
Inhibition of PI3 kinase by wortmannin results in reduced phosphorylation of Akt/PKB and S6 ribosomal protein, downstream of PI3 Kinase in the insulin signaling pathway, with apparent IC50 values of 14 nM and 32 nM, respectively. 3T3-L1 cells were starved overnight, preincubated for 30 min with the indicated concentrations of wortmannin and stimulated with insulin (1 mM). Cells were then lysed and the phosphorylation levels of Akt/PKB (Figure 2A) and S6 ribosomal protein (Figure 2B) were assessed with antibodies specific for Ser473-phospho-Akt and Ser240/Ser244-phospho-S6 ribosomal protein respectively in our PWG RPA platform. By plotting the percent inhibition versus log wortmannin concentrations, one can derive IC-50 like data from such experiments (insets).