An integrative tool for phosphoproteomics

A key in systems biology

Systems biology is a data-driven science in which a combination of experimental and computational approaches are used to understand complex dynamic biological systems from a systems-wide perspective. Systems biology integrates various omics data, including genome, transcriptome, proteome and metabonome information, and explores the complex interactions among many levels of biological information to understand how these levels work together. Knowledge regarding interacting protein and signaling networks provides researchers with a basic understanding of the molecular mechanisms of cell and tissue function and can also contribute to the development of new drug targets, drugs and combination therapeutic strategies.

Protein functions determine cellular functions; therefore, the proteome, which was first introduced in 1996 by Mark Wilkins as an analog to the genome and is defined as the entire complement of proteins expressed in a cell, tissue or organism in a specific state, provides the most important type of omics data. Post-translational modifications (PTMs) are protein modifications that occur in almost all proteins and play important roles in various biological processes. Over 200 protein PTMs have been described. Protein phosphorylation is one of the most important PTMs in cells. Protein phosphorylation modifies protein substrates at specific amino acid residues to regulate cell signaling transduction and modulate protein-protein interactions. Analysis of the dynamic phosphoproteome is crucial for understanding cell signaling but remains challenging. Professor Hsueh-Fen Juan's team developed an integrative user-friendly web tool, DynaPho (http://dynapho.jhlab.tw/), for the analysis of time-series phosphoproteomics data.

DynaPho contains six analytical modules: data summary, profile clustering, function enrichment, dynamic network, kinase activity and correlation analysis. In the "data summary" module, DynaPho provides the distribution of phosphorylated residues and phosphorylation ratios as well as the phosphorylation trend for a set of phosphosites. Users can obtain clustered profiles in the "profile clustering" module. The "function enrichment" module performs a Gene Ontology (GO) enrichment analysis of phosphoproteomic data for each time point. The "dynamic network" module provides time-dependent interaction networks based on users' phosphoproteomics data and protein-protein interactions. The "kinase activity" module of DynaPho helps users infer kinase activity at each time point. The "correlation analysis" module of DynaPho facilitates the identification of known and novel kinase/phosphatase-substrate relationships and the visualization of kinase/phosphatase-phosphosite association networks.

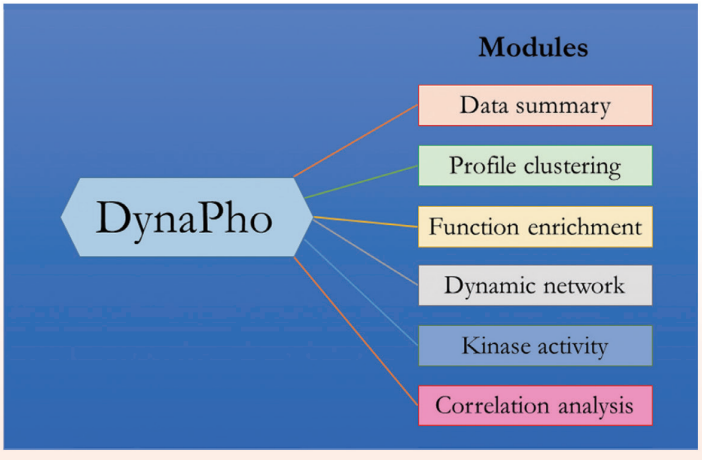

Figure 1. The six modules in the DynaPho web tool: data summary, profile clustering, function enrichment, dynamic network, kinase activity and correlation analysis.

DynaPho can be applied in studies of various organisms and has successfully facilitated the exploration of drug response networks in cancer cells, hormone-regulated phosphosignaling in plants and molecular functions in cells. The drug response network research revealed that the drug citreoviridin induced mitogen-activated protein kinase/extracellular signal-regulated kinase signaling in lung cancer cells to suppress cancer cell growth and provided perspectives regarding cancer therapeutic strategies. The aforementioned research on hormone-regulated phosphosignaling in plants dissected the dynamic regulation of brassinosteroids in *Arabidopsis* and expanded our knowledge of protein phosphorylation regulation. In studies of molecular functions in cells, DynaPho can be used to identify key phosphoproteins and their regulated networks. This tool contributes to the enhanced use of time-series phosphoproteomics data by providing easy-to-understand visuals and information regarding condition-specific cellular signaling.

References

1. Chia-Lang Hsu, Jian-Kai Wang, Pei-Chun Lu, Hsuan-Cheng Huang, Hsueh-Fen Juan (2017). DynaPho: a web platform for inferring the dynamics of time-series phosphoproteomics. *Bioinformatics, 33*(22), 3664-3666. DOI:10.1093/bioinformatics/btx443.
2. Hsueh-Fen Juan and Hsuan-Cheng Huang (2018). *A Practical Guide to Cancer Systems Biology.* Singapore: World Scientific Publishing.

Professor Hsueh-Fen Juan
Department of Life Science
yukijuan@ntu.edu.tw

Assembly of recombination-mediated DNA repair machinery

How cells maintain their genome integrity is our long-term interest. The genome is constantly exposed to various endogenous and exogenous insults, ranging from endogenous replication stress to exogenous ultraviolet light and carcinogens. These insults cause a variety of DNA damage. DNA double-strand breaks (DSBs) are the most lethal chromosomal lesion if not repaired properly. DSBs trigger genomic instability and halt DNA replication. Cells have developed evolutionarily conservative mechanisms to repair DSBs and maintain genome stability. Dysregulation of DSB repair pathways causes cell death or diseases such as cancers.

Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two major DSB repair pathways. NHEJ is an error-prone repair pathway that simply promotes religation of two broken ends. In marked contrast, the homologous recombination-mediated repair pathway is a precise repair mechanism. Notably, recombination machinery is also a prerequisite for stabilizing and reinitiating stalled/collapsed replication forks during replication stress. Thus, dysfunction in HR leads to chromosome fragility and cancer susceptibility.

Mechanistically, HR induced by DSBs is catalyzed by RAD51 recombinase. RAD51 polymerizes ssDNA generated from DSB sites to form a helical filament known as the presynaptic filament and then catalyzes the homology search and DNA strand exchange reaction (Figure 1). During HR, RAD51 activity is tightly regulated by several associated partners, including BRCA1/2, PALB2, RAD51 paralogs, RAD51AP1, and the SWI5-SFR1 complex. These interactions raise intriguing questions regarding how these accessory factors influence RAD51 activity mechanistically and how they coordinate with each other. My laboratory is dedicated to addressing this issue using biochemistry, biophysics and cell-based approaches.

Our research work has made a significant contribution to the understanding of the mechanism