The social architecture of an in-depth cellular protein interactome
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Nearly all cellular functions are mediated by protein-protein interactions and mapping the interactome provides fundamental insights into the regulation and structure of biological systems. In principle, affinity purification coupled to mass spectrometry (AP-MS) is an ideal and scalable tool, however, it has been difficult to identify low copy number complexes, membrane complexes and those disturbed by protein-tagging. As a result, our current knowledge of the interactome is far from complete, and assessing the reliability of reported interactions is challenging. Here we develop a sensitive, high-throughput, and highly reproducible AP-MS technology combined with a quantitative two-dimensional analysis strategy for comprehensive interactome mapping of Saccharomyces cerevisiae. We reduced required cell culture volumes thousand-fold and employed 96-well formats throughout, allowing replicate analysis of the endogenous green fluorescent protein (GFP) tagged library covering the entire expressed yeast proteome. The 4159 pull-downs generated a highly structured network of 3,927 proteins connected by 31,004 interactions. Compared to previous large-scale studies, we double the number of proteins (nodes in the network) and triple the number of reliable interactions (edges), including very low abundant epigenetic complexes, organellar membrane complexes and non-taggable complexes interfered by abundance correlation. This newly saturated interactome reveals that the vast majority of yeast proteins are highly connected, with an average of 16 interactors, the majority of them unreported so far. Similar to social networks between humans, the average shortest distance is 4.2 interactions. A web portal (www.yeast-interactome.org) enables exploration of our dataset by the network and biological communities and variations of our AP-MS technology can be employed in any organism or dynamic conditions.

Proteomic analysis to study the effect of 4-phenylbutyrate treatment on ABCA1 function and trafficking
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ATP-binding cassette A1 (ABCA1) is a transmembrane protein that exports cellular cholesterol to form high-density lipoprotein (HDL). Mutations in ABCA1 disrupt its trafficking to the plasma membrane and reduce its function. A chemical chaperone, 4-phenylbutyrate (4-PBA) was investigated for its potential to assist ABCA1 trafficking and cholesterol efflux function. 4-PBA is a short-chain fatty acid that acts as a histone deacetylase inhibitor affecting transcription. It has also been shown to promote trafficking of ABC proteins to the plasma membrane. This study aimed to identify proteins regulated by 4-PBA that could improve ABCA1 trafficking. Two mammalian cell lines, HEK293 and HepG2, were used as model systems. The wild-type and an ABCA1 mutant known to have defective trafficking, N1800H, were introduced to HEK293 cells via transient transfection. The HepG2 cells models were developed by a stable transfection approach to integrate wild-type and N1800H mutant ABCA1. The +/- 4-PBA cell lysates were analysed by SWATH-MS (sequential window acquisition of all theoretical fragment ion spectra-MS) to identify differentially regulated proteins in response to 4-PBA treatment. Proteins showing a >1.5 fold change were subject to bioinformatic analyses to categorise their compartmentalisation, function and potential interactions with ABCA1. The proteomic analysis of HEK293 cell models indicated that transcription, translation and signalling proteins were the most affected with some effects on both chaperones and trafficking proteins. The proteomic analysis of the HepG2 cell model also showed regulation of these classes of proteins, albeit different proteins within each class, with trafficking proteins more affected compared to that observed in HEK293. Three trafficking proteins, Rab10, Sec61β and sortilin were selected for further analysis based on their functional relevance to ABCA1. Immunofluorescence microscopy indicated a potential colocalisation of ABCA1 with all three selected proteins. An siRNA knockdown of these proteins showed that only Rab10 had a functional correlation with ABCA1. The siRNA knockdown of Rab10 significantly increased ABCA1-mediated cholesterol efflux in HepG2 cells which suggests that Rab10 has the potential to retard ABCA1 trafficking towards the plasma membrane. Altogether, this study has provided novel insight into the 4-PBA effects and generated a valuable resource for further research on this multitarget drug.