signaling cascades and connected network partners will also have major impacts on applications in metabolic engineering, drug design and synthetic biology.

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LIPIDS

The plasma membrane code

Epistatic maps are used to delineate the modes of interaction of genes in various cellular pathways. A new epistatic map of nearly 400 genes involved in plasma membrane biology has revealed unexpected modes of regulation of endocytosis and sphingolipid metabolism.

Anthony H Futerman & Maya Schuldiner

Just as cryptologists get a kick out of deciphering the meaning of ancient codes and symbols, biochemists and cell biologists get a kick out of dissecting the intricacies of the biochemical and metabolic pathways that regulate cell function. Fortunately for the biochemical cryptologist, a new tool has become available in the past few years to aid in these investigations: the so-called E-MAPs (or, more formally, epistasis mini array profiles), databases of selected subsets of genes that delineate their functional relationships. A new addition to the rapidly growing E-MAP family is an E-MAP of the plasma membrane in baker's yeast[1]. Using this E-MAP, Aguilar *et al.* found some unexpected components and regulatory mechanisms of the endocytic pathway, of eisosomes (organizing complexes found at the plasma membrane) and of sphingolipid metabolism (**Fig. 1**).

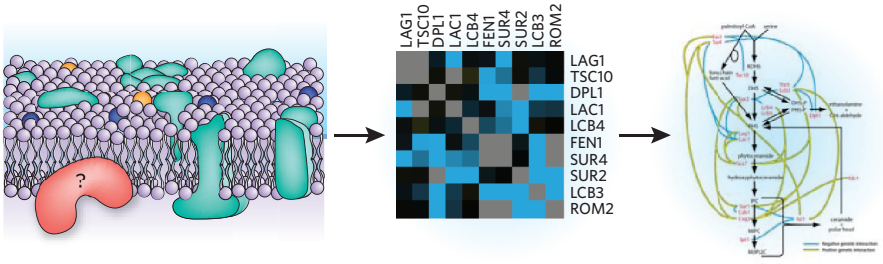

Figure 1 | The plasma membrane E-MAP uncovers sphingolipid regulatory pathways. Sphingolipids are major lipid components of the plasma membrane. Many of the regulatory proteins conserving sphingolipid levels on the plasma membrane have not yet been discovered (left: hypothetical proteins on the plasma membrane, red). The newly published plasma membrane E-MAP (center: small excerpt) has led to the discovery of regulatory steps in the sphingolipid biosynthetic machinery (right: the genetic interactions between all sphingolipid biosynthetic genes) that are likely to have profound effects on cell function. Middle and right images reprinted with permission from ref. 1.

Epistasis is the effect of a mutation in one gene on the phenotype of a mutation in a second gene. Aguilar *et al.*[1] measured genetic interactions for 374 yeast genes (generating nearly 60,000 genetic interactions) whose products either are localized to the plasma membrane or have functions that influence membrane function and architecture (such as those involved in the endocytotic pathway, eisosome biology, trafficking complexes and signaling pathways, as well as genes related to sterol and sphingolipid metabolism). To generate this map, they used ingenious genetic methods[2] to cross all deletion strains of the aforementioned proteins against each other, creating all possible double mutations. They then measured the colony sizes of each double mutant and designated the interactions[3] as either 'aggravating', in which the double mutant shows a more severe phenotype than expected by the phenotype of each single mutant, or 'alleviating', in which the double mutant has a less severe phenotype than expected. As genetic interactions are not dependent on physical interactions between the proteins under study, they provide a powerful tool for ascertaining functional relationships, such as those between one protein and other proteins in the same biochemical process. Previous E-MAPs—of the early secretory pathway[4], chromosome biology[5], RNA machinery[6] and kinases and phosphatases[7]—have shown that the technique can be used to uncover the function of unknown genes, to designate proteins as being part of complexes and pathways and to uncover the wiring or interdependencies between various cellular processes[8].

Given the exciting information arising from earlier E-MAPs, it was to be expected that the plasma membrane E-MAP would provide similarly novel insights into the workings of the cell. Indeed, some unexpected findings throw new light on endosome and eisosome function and sphingolipid metabolism. Sphingolipids are one of the three major lipid classes found in cell membranes, and in addition to being active in signaling processes, they are also essential components of membrane microdomains, or rafts, where they interact with sterols: ergosterol in yeast and cholesterol in mammalian cells. Sphingolipid and sterol biosynthesis take place mainly in the endoplasmic reticulum and in the Golgi apparatus, and their biosynthetic pathways have been described in painstaking detail. Moreover, feedback and cross-talk mechanisms appear to exist whereby changes in levels of either sphingolipids or sterols in the plasma membrane[9] may cause modulation of the level of their synthesis

in the endoplasmic reticulum. Additional unexpected regulatory pathways have emerged of late, as in the discovery of the regulation of sphingolipid synthesis by the Orm protein family[10].

Using their plasma membrane E-MAP, Aguilar *et al.*[1] demonstrated that tight genetic interactions exist between early and late steps in the sphingolipid biosynthetic pathway. For instance, the accumulation of putative toxic sphingolipid intermediates via gene deletion could be suppressed by modulating the activity of genes upstream in the pathway. Although this might seem like an obvious way to regulate a metabolic pathway, the E-MAP makes precise predictions about which steps in the pathway of sphingolipid synthesis are likely to be regulated and suggests experimental approaches for sphingolipid aficionados that would not have been apparent were it not for the availability of the E-MAP. Also, a gene with no previous known connection to sphingolipid metabolism, *ROM2*, which encodes a Rho1 GTPase exchange factor, had a strong genetic connection to genes involved in the early steps of the sphingolipid biosynthesis pathway. The clustering of Δ*rom2* with yeast genes that encode proteins involved in the synthesis of ceramide, a key metabolic intermediate and a signaling lipid, suggested a possible modulatory effect of Rom2 on the yeast ceramide synthases, Lag1 and Lac1. Ceramide synthesis in mammalian cells is much more complex than in yeast, as mammalian cells have six distinct ceramide synthases that each have a slightly different substrate specificity, leading to the formation of ceramides containing fatty acids of differing lengths[11]. Were Rom2 able to modulate ceramide synthesis in mammalian cells, this would add to the growing list of regulatory mechanisms of sphingolipid metabolism discovered over the past few years. The next challenge will be to integrate these regulatory mechanisms into a unified hypothesis of how sphingolipid metabolism is ultimately controlled. However, the fact that sphingolipid metabolism is regulated by so many seemingly diverse mechanisms implies that this pathway plays a more pivotal role in cell function than once thought.

Judging from the success of previous E-MAPS, the plasma membrane E-MAP is likely to guide many waves of exploration into the uncharted territory of plasma membrane biology. To facilitate this, the authors[1] have created a very useful website that will prove invaluable for plasma membrane cryptologists (http://acgt.cs.tau.ac.il/pmemap), hopefully leading to the deciphering of the plasma membrane code. ■

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