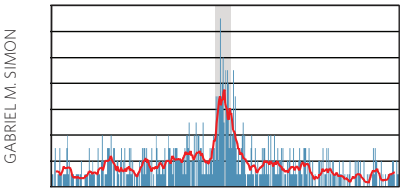
PROTEOMICS

Cross-talking modifications

Cell **150**, 429–440 (2012)



Caspases are proteases that orchestrate programmed cell death. Substrates for caspases include kinases, and although it is known that cleavage of a kinase can activate or deactivate its enzymatic activity, a systems-wide view of the relationship between caspase-mediated proteolysis in apoptosis and phosphorylation is lacking. Dix *et al.* now report a proteomic method—combining PROTOMAP, which is used to characterize proteolytic events in cells, with SILAC, which is a quantitative, isotopic in-cell labeling method—called quantitative phospho-PROTOMAP (qP-PROTOMAP) to address this knowledge gap. After validation of the qP-PROTOMAP method, the authors assessed crosstalk between caspase activity and phosphorylation in staurosporine (STS)-induced cell death. The authors compiled all known caspase cleavage sites, aligned by their scissile P1 aspartate, and found that many phosphorylation sites associated with apoptosis clustered within six residues of this cleavage site. An ATP-binding activity–based proteomic analysis revealed that DNA-dependent protein kinase (DNA-PK) had strong activity in STS-treated cells. DNA-PK–selective inhibitors or short hairpin RNA–mediated DNA-PK knockdown blunted phosphorylation at these sites. The authors found that caspase-dependent relocalization of DNA-PK from the nucleus to the cytoplasm was necessary for phosphorylation adjacent to caspase cleavage sites during STS-induced apoptosis. In addition, the authors found that phosphorylation and caspase cleavage are interrelated during apoptosis, with caspase cleavage exposing phosphoryation sites and phosphorylation promoting caspase cleavage. Taken together, these data indicate that large-scale functional crosstalk occurs between phosphorylation and caspase cleavage during apoptosis. *AD*

VIROLOGY

Cholesterol manipulation

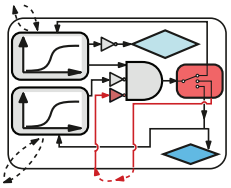
Cell Host Microbe **12**, 86–96 (2012)

As with all viral infections, infection by the enveloped human cytomegalovirus (HCMV) causes changes in expression in numerous host cell genes, presumably to the benefit of the virus. To characterize the changes that occur in host cell-surface proteins, Gudleski-O'Regan *et al.* evaluated the proteomes before and after HCMV infection by MS after treatment of intact cells with a cell-impermeable amine-reactive probe. The authors detected 114 proteins differentially expressed on the cell surface after HCMV infection. They focused on LRP1, an LDL receptor–related protein involved in cholesterol uptake, which was transiently increased at 24 h after infection. Both knockdown of LRP1 and HCMV infection are known to lead to increased intracellular cholesterol in fibroblasts; the authors verify these relationships and also find that LRP1 knockdown increases HCMV infectivity and increases cholesterol in released virions. Blocking cholesterol biosynthesis or depleting cholesterol from cells decreased infectivity, verifying the connection between infectivity and increased cholesterol content due to LRP1-mediated uptake. Finally, the authors showed that envelope cholesterol is critical for HCMV fusion with the host cell. These results suggest that increased cellular and virion cholesterol content leads to more efficient fusion of the virion envelope with the plasma membrane and therefore increased virion infectivity. *MB*

SYNTHETIC BIOLOGY

Beta testing

PLoS Comput. Biol., published online 19 July 2012; doi:10.1371/journal.pcbi.1002579



Synthetic biologists often seek to create gene networks that execute defined tasks, but the application of these networks in undefined systems offers important orthogonal tests of our ability to engineer biology. Miller *et al.* now explore the interplay of design and disorder in a conceptual framework suitable for creating a stable population of stem cells and differentiated β-cells. The authors first designed and modeled a system including four known modules that determine whether stem cells should undergo renewal or differentiation into β-cells but observed that delays in the differentiation process could cause unwanted oscillations in population homeostasis. To counter this, a second system included a 'commitment' module, but this model was also prone to oscillations because of the potential for multiple simultaneous commitments to drain the pool of stem cells. To avoid this synchronization, two final systems explored mechanisms to generate variability in decision making, using either a newly designed and tested toggle switch or an oscillator. The authors further developed several computational methods such as an 'intermodular coupling analysis' to define the best integration of the varying time scales of the different modules and a 'phenotypic sensitivity analysis' to identify whether input modules would achieve a desired phenotype even though specific parameters of the system could not be defined. These results offer new guidelines for systems where heterogeneity is a feature, not a bug. *CG*

REGULATION

Positively alarming

EMBO Rep., published online 20 July 2012; doi:10.1038/embor.2012.106

During the stringent response, *Escherichia coli* use the alarmone ppGpp to rapidly respond to environmental changes. RelA, which makes ppGpp, is known to depend on the ribosomal protein L11 and to be activated by ribosomally bound deacylated $tRNA^{Phe}$ and mRNA, but it was not clear how these factors could explain a previous observation that production of ppGpp is nonlinear. Instead, Shyp *et al.* hypothesized that this amplification might be driven by the direct action of ppGpp on RelA. To test this idea, the authors added ppGpp to mixtures of RelA and 70S ribosomes at different time points, observing increased activity upon ppGpp addition. Activation was seen with concentrations of ppGpp that were consistent with availability of the molecule in the cell. Measurements of turnover rate with varying concentrations of ribosomes indicated that ppGpp increases product formation by affecting k_{cat}, not K_m. The effect of ppGpp is synergistic with that of other activators, with 10- to 20-fold activation of RelA observed with combinations of ribosomes, mRNA and deacylated $tRNA^{Phe}$. The authors further show that L11 and ppGpp can serve as a minimal regulation system, though elevated concentrations of L11 were required for activation compared to regulation with intact ribsomes. This unusual feedback mechanism of activation rather than inhibition by a reaction product provides a sensitive means by which cells can quickly sense and react to changes. *CG*