in differentiation and development, and in the quality control of cellular proteins and organelles[3,4].

Biochemical studies in yeast and mammalian cells defined several protein complexes involved in autophagy. These include the ULK/Atg1-kinase complex and the autophagy-specific lipid-kinase complex, which both function in the initial nucleation of the membrane that eventually forms the autophagosome. Other complexes include two ubiquitin-like protein-conjugation systems that function in the elongation of the initial membrane to form a complete autophagosome, and a retrieval system that mediates the disassembly of Atg proteins from the mature autophagosome[5–8].

Despite these notable advances, however, several gaps remain in our understanding of autophagy. For instance, it has been unclear how the distinct protein complexes involved in autophagy talk to each other or to other cellular machinery involved in membrane formation and membrane trafficking. And how do key signals that trigger autophagy talk to the Atg proteins? Behrends and co-workers' proteomics screen[2] has the potential to foster the birth of another era of autophagy research that might answer these — and probably many other — questions.

The authors introduced tagged autophagy-related genes into human cells as part of retroviral vectors, and analysed the resulting protein interactions. Through the empirical statistical analysis of data generated by mass spectrometry, they identify what are called 'high-confidence interaction proteins', which in effect are proteins enriched for reproducible interactions with other abundant candidate proteins. Their results provide the framework not only for mechanistic studies that might challenge current thinking about the organization of core autophagy proteins, but also for investigations that could provide further insights into the molecular mechanisms and regulation of autophagy.

At a broad level, the screen[2] suggests a hitherto unappreciated level of interconnectivity between the different presumed modular components of the autophagy system. The analyses reveal 22 interactions between proteins in different autophagy subnetworks, with a convergence between subnetworks involved in vesicle nucleation (the ULK1/Atg1-kinase complex and lipid-kinase complex), membrane recycling (Atg2 complex), and mammalian proteins related to Atg8. (Atg8 is incorporated into autophagosomes, promotes autophagosome closure and functions in cargo recruitment.)

If validated with further experiments, the interconnectivity between autophagy subnetworks could prompt a revision of the current 'map' of the molecular components of the core autophagy machinery. More generally, a combination of theoretical analysis and experimental measurements of epistasis (regulation of a gene's activity by other genes) within and between components of the autophagy subnetworks might provide more rigorous tests of our intuitive notions of how to break down complex signalling systems into modular units. Behrends and colleagues' high-quality data set of molecular interactions forms the foundation for addressing this broader issue in systems biology in the context of the autophagy pathway.

At a more detailed level, a key finding of the screen[2] is the identification of 751 interactions between the 65 autophagy-related proteins (that the authors used as primary and secondary baits) and 409 other proteins. Further mechanistic analyses of these interactions may unearth a plethora of players involved in the regulation and execution of autophagy. Such analyses using the data set of autophagy-interacting proteins will also help to define how statistical measures of interaction strength between protein pairs determined by high-throughput proteomics relate to function in the context of a biological pathway.

Behrends *et al.* performed their proteomic analysis of the autophagy-interaction network (AIN) in human cells under conditions of basal autophagy. This type of autophagy mediates protein and organelle quality control and differs from stimulus-induced autophagy, which allows cells to respond acutely to stress. Their analysis therefore provides a single, albeit zoomed-out, snapshot of the AIN, which, because of the experimental design, is unlikely to reveal temporally and/or spatially regulated interactions that contribute to the dynamic regulation of stimulus-induced autophagy.

Of note, the authors[2] investigated how the stimulation of autophagy through inhibition of one of its potent negative regulators, the protein kinase mTOR, alters a subset of the interactions they identified in their proteomic analysis. Inhibition of mTOR did not cause large-scale changes in the core systems such as ubiquitin-like protein conjugation, the autophagy-specific lipid-kinase complex, and Atg-protein recycling. As the authors discuss, this observation suggests that post-translational modifications of Atg proteins may be key to the activation of autophagy. Another possibility, which is not mutually exclusive with that, is that activation of the autophagy pathway might involve increased or decreased interactions between positive or negative regulators that are not identified in the analysis of the AIN under basal conditions.

Numerous factors underscore the success of Behrends and colleagues' approach: identification of a high proportion of previously known interactions between autophagy proteins in yeast and in mammalian systems; reciprocal identification of about 50% of the interactions; the confirmation of a subset of interactions (those of Atg8 family members) in *in vitro* studies; and validation by RNA interference that a subset of the AIN genes functions in autophagosome formation. That the approach[2] revealed interactions that are already known bodes well for what will perhaps be the

50 YEARS AGO

Advances in Agronomy — What *is* agronomy? Certainly, like 'billion' and 'suspender', it suffers a potentially embarrassing change of meaning in crossing the Atlantic. In England, little would be left for agronomy when the claims of chemistry, entomology, plant pathology and so on had been stated — perhaps the study of green manuring, seed-rates and sowing dates. In the United States apparently the subject of agronomy comprises pretty well all agricultural science. Subjects covered by the present volumes range from liming to castor-beans and from wheat stem rust to water and its relation to soils and crops. "Advances in Agronomy" is written mainly by Americans about conditions in the United States ... The articles in these two volumes, with a few exceptions, read like a disjointed collection of condensed text-books, or chapters from text-books. The range of subjects covered is far too wide to justify the implied suggestion that they are all branches of one science. These volumes do not establish 'agronomy' as a science.

From *Nature* 25 June 1960.

100 YEARS AGO

Let me tell you of life-saving "eels" in vinegar. I was examining the creatures with a microscope when one of them became stranded, owing to its having strayed into the shallower portion of the vinegar-drop, and there it wriggled while the fluid grew shallower still. Just as it seemed on the point of giving its last expiring wriggle, what was my amazement to see three or four other "eels" make a dash from the deeper vinegar, and force themselves across the shallow to where lay their stranded comrade ... These tiny life-savers rushed with all the energy of desperation at their now quiescent comrade, and worked it slowly towards the deeper part of the fluid, and they reached it, too, in time to save their own and the other's life.

From *Nature* 23 June 1910.

50 & 100 YEARS AGO