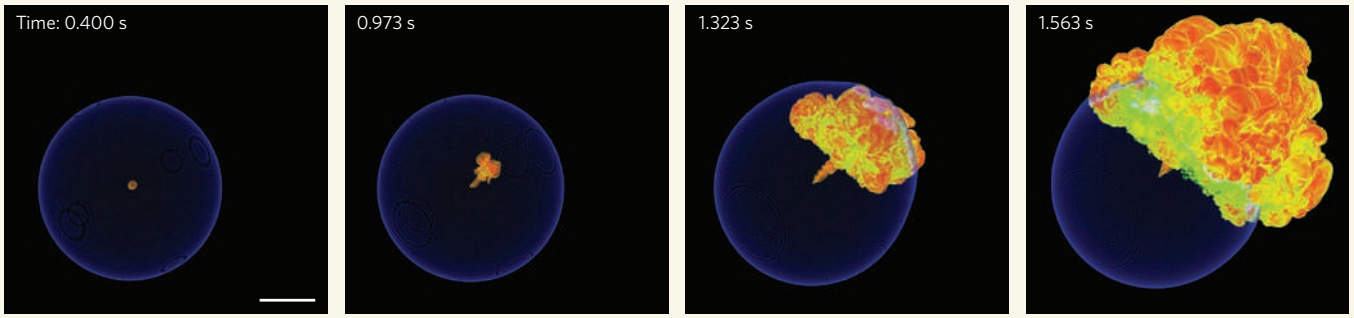

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Figure 1 | Lopsided supernova explosion. Maeda and colleagues' analysis[1] of type Ia supernova observations implicates a lopsided explosion mechanism for these objects. In the simulation shown, a white dwarf is initially ignited slightly away from the centre, and a thermonuclear flame begins to consume the star. The burned material is hot and buoyant, and so the flame and ash quickly float upward, resulting in one side of the star being more completely incinerated and more rapidly expelled than the other. Scale bar, 1,000 km.

Such lopsided explosions have been popping off in computer simulations for years, but it has been hard to tell whether this is the way they occur in the real world. The supernovae we observe are, with few exceptions, too distant to be resolved, looking from Earth like structureless points of light. Often that light is polarized[15], implying a breaking of spherical symmetry, but the exact shape of the debris cloud has been difficult to deduce.

The clever analysis of Maeda and his team[1] has now helped to flesh out the debris geometry. Apparently, the trick was just to be patient. Astronomers, like paparazzi, tend to quickly lose interest in burnt-out stars that have passed their peak, turning their cameras to chase some younger spectacle on the rise. These new results, though, came from following up supernovae years after their prime, when they had faded more than a hundredfold and by which time the debris had expanded to such low densities as to become translucent. Then, peering through the cloud of stellar ash, the observer can quantify the complete distribution of material throughout. The ageing supernova can no longer hide its second face.

When Maeda and colleagues[1] compiled new and archival data of this kind for a large number of supernovae — each presumably seen from a random viewing angle — a unifying picture emerged. For the high-speed supernovae, the bulk of the debris was moving towards Earth. For the low-speed ones, it was just the opposite — most of the debris was moving away. Apparently the difference was not of kind, but of perspective. The inferred asymmetry is generally consistent with that expected from off-centre explosions, and may provide a means of discriminating between specific theoretical models.

A lopsided geometry does not explain all the diversity seen in type Ia explosions. The peculiarities of a few supernovae do not easily fit into the picture, and may require a genuinely different explosion process or progenitor channel[16]. But at least now, observations and theory both suggest that much of the common variation is due to asymmetry[17]. Because supernovae are randomly oriented with respect to Earth, this effect should introduce statistical (not systematic) deviations in their perceived brightness. For cosmologists, that is comforting news — simply observe a large number of supernovae and they are effectively averaged over all viewing angles. Our measure of the expanding Universe then won't be fooled by their shifting faces. ■

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AUTOPHAGY

Snapshot of the network

Beth Levine and Rama Ranganathan

Autophagy is an essential cellular process for protein and organelle quality control. Analyses of proteins that interact with the human autophagic machinery provide an outline of the molecular organization of this pathway.

To survive, cells must get rid of damaged, obsolete or dangerous components. They do so by the process of autophagy, which involves a series of dynamic membrane-rearrangement reactions mediated by a core set of proteins — the Atg proteins[1]. Up to now, these proteins have largely been analysed in the context of discrete macromolecular complexes that function at specific steps in the autophagy pathway. In this issue, Behrends *et al.*[2] (page 68) report a tour de force of proteomic analysis that has identified human proteins that interact with the core autophagic machinery and related molecules.

During autophagy, unwanted cellular components — proteins, lipids, entire organelles and invading pathogens — are delivered by double-membraned vesicles called autophagosomes to the lysosome, where they are destroyed. This degradative process is essential for cellular homeostasis, and defects in autophagy lead to a wide range of disorders in model organisms and probably in humans[3,4].

The dawn of the modern era of autophagy research dates to the 1990s, when genetic screens in yeast[1,5,6] identified a set of evolutionarily conserved gene products essential for autophagy-related processes. The screens revolutionized our understanding of autophagy, and stimulated genetic and biochemical analyses of the pathway. Studies in model organisms that involved deletion and depletion of *Atg* genes uncovered a crucial function for autophagy in nutrient and energy homeostasis,

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

greatest impact of this screen — laying the groundwork for the discovery of the unknowns about autophagy. The prospect of such discoveries heralds a rewarding journey into the post-proteomic era of autophagy research. ■

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CANCER STEM CELLS

Invitation to a second round

Peter Dirks

Tumour cells are non-uniform. The question is whether a distinct subpopulation of the cells drives tumour growth and generates cellular variation. To answer this, the data must be interpreted carefully.

Among the variety of cancer-cell subpopulations that make up a tumour, it is thought that only a select few — cancer stem cells — can drive tumour formation. Whether there is a hierarchy for tumorigenic potential among cancer cells, or whether every cell in the tumour has the same capabilities, is not yet certain. And can any cell within a tumour become a cancer stem cell? If so, a cancer-stem-cell model would be less attractive, particularly for cancer treatment: to cure a cancer, essentially all tumour cells, rather than a rare population of potent tumour-initiating cancer stem cells, would have to be killed. Two studies[1,2], including one by Boiko *et al.*[1] on page 133 of this issue, shed light on these points.

Boiko *et al.* study a type of human skin cancer called melanoma and, in particular, cancer cells enriched in a stem-cell marker called CD271. They find that, unlike other cells from the same tumour, CD271-expressing ($CD271^+$) cells could initiate and maintain tumour growth *in vivo* — an observation consistent with the existence of a melanoma-cell functional hierarchy.

This finding reflects a view different from that of an earlier study by Quintana *et al.*[3], which demonstrated that, in some cases, as many as 50% of human melanoma cells have tumorigenic potential. In addition, no marker tested identified a tumorigenic subpopulation. The authors[3] concluded that the frequency of cancer cells that can initiate tumorigenesis depends, in part, on the assessment techniques and assays.

Can these conflicting observations[1,3] be reconciled? One possibility is that, as a cancer progresses, more tumour cells acquire the attributes of cancer stem cells; a block on cell differentiation, for instance, could drive such a shift. Quintana and colleagues might have observed a high frequency of tumour-initiating cells because a high fraction of the melanoma tumours they studied were at an advanced stage. Moreover, the tumour cells that the authors[3] transplanted into animal models were first expanded by a process known as xenografting, to generate sufficient cells from the original patient samples; this procedure could also have selected for aggressive tumour cells. In addition, it could be that the initial tumour-cell dissociation process kills many cells, and if this is biased towards cells other than cancer stem cells, the technique may already select for a more rigorous cancer-stem-cell subpopulation.

Boiko *et al.*[1] examined a greater number of primary melanoma tumours at an early stage of disease than Quintana *et al.*, although their overall data — like the results of many studies of tumour-initiating cells — reflect analysis of relatively few patient samples. They report that, between each sample, the fraction of $CD271^+$ cells varies, ranging from 2.5% to 41%. Consequently, their investigation[1], like that of Quintana *et al.*, suggests that cells initiating melanoma tumours are not necessarily rare.

The authors[1] find that tumour-initiating ability is mainly, but not exclusively, a feature of the $CD271^+$ cells: 70% of animals injected with these cells developed tumours, compared with only 7% of those injected with cancer cells that did not express this marker. In addition, $CD271^+$ cells were more metastatic, and did not express melanoma-associated antigens such as MART-1 or tyrosinase, which are candidate targets for treatment.

Interestingly, xenograft tumours had higher fractions of $CD271^+$ cells. This could mean either that the xenografting process selects for a higher fraction of tumour-initiating cells, or that cells that do not express CD271 gain tumour-initiating potential with a change in their environment — from primary tumour to the xenograft. Exploring these two possibilities, and whether markers such as CD271 lose some of their 'meaning' in a xenograft setting (or in a cell culture), deserves investigation. In particular, to resolve some of the discrepancies, more human melanoma samples must be examined.

In a paper in *Cell*, Roesch *et al.*[2] also highlight issues that have implications for understanding tumour-cell variation and hierarchy. A key assumption of the cancer-stem-cell hypothesis is that, as well as self-renewing, these cells give rise to non-tumorigenic progeny, which cannot reacquire tumorigenicity. But researchers' ability to reprogram differentiated cells to a stem-cell-like state using defined transcription factors[4] suggests that — like any cell — the non-tumorigenic progeny could reacquire stem-cell properties. In fact, such reprogramming or de-differentiation might occur more readily in an abnormal tumour cell than in a healthy cell. Could the blocked-differentiation feature of cancer invite partially differentiated non-tumorigenic cells to become tumorigenic stem cells a second time around in response to intrinsic or extrinsic factors?

Roesch and colleagues[2] identify human melanoma-cell subpopulations in culture that express the enzyme JARID1B. Compared with melanoma cells that do not express this enzyme, $JARID1B^+$ cells cycled more slowly but also generated more progeny and were more tumorigenic. The possibility that, in a solid tumour such as melanoma, a slowly cycling cell population may be more tumorigenic implies that anticancer treatments should not focus only on rapidly cycling cell populations.

More intriguingly, the authors show that JARID1B expression is dynamic: even in experiments involving a single starting cell, $JARID1B^+$ cells could arise from cells that did not express it. So it seems that the stem-cell state can be acquired by any cell at any time — thus representing a moving target. The gloomy implication is that targeting each and every $JARID1B^+$ cell at any point in time would not be sufficient to target the melanoma, because more $JARID1B^+$ cells would emerge.

It is noteworthy that Roesch *et al.*[2] studied melanoma cell lines in culture. A culture system can differ from an *in vivo* setting in many ways, with considerable implications for interpretation of tumour hierarchy in patients. For instance, many primary-tumour cells do not survive to propagate in culture[5], and so a large bulk of the tumour (and perhaps the non-stem-cell fraction) may not be accessible in such an *in vitro* system. Moreover, for some tumour types, serum-based culture media propagate cell populations that do not resemble the patient's primary tumours in genetic make-up or in other characteristics. And non-serum-based culture media may selectively support the growth of cells with stem-cell features[6].

It is also known[7] for other essentially pure populations of stem cells in culture that cells