

RESEARCH HIGHLIGHTS

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NETWORK BIOLOGY

A protein network of one's own proteins

Network biology has taken off with the advent of high-throughput, parallel data collection and analysis. The term interactome has been coined to describe an organism's total set of protein-protein interactions, and interactome maps have been created for many model organisms. Knowing all protein-protein interactions is seen as a crucial prerequisite to understanding how cells function and the general principles that govern this function. Importantly, such information should also help us to understand disease processes. Two recent reports provide the first experimentally derived description of a human protein-protein interaction network. The network, although preliminary, is a useful resource and provides interesting insights into the nature of protein-protein interactions on a global scale.

Two groups led by Erich Wanker and by Marc Vidal used similar high-throughput, stringent yeast two-hybrid strategies and identified 3,186 and ~2,800, mostly novel, protein-protein interactions, respectively. The data sets were each evaluated for technical and biological false positives. Whereas the former were dealt with rigorously, using co-affinity purification and pull-down assays, the authors admit that the latter are more difficult to deal with. To this end, Vidal's group compared their interactions with other known biological relationships such as expression correlation, shared gene ontology or phenotype annotation. Wanker's group

used orthologous interactions as well as topological and gene ontology criteria to develop a confidence-scoring system to evaluate the biological relevance of the interactions. They also compared the human interaction network with 22 human regulatory pathways from the Kyoto Encyclopedia of Genes and Genome, KEGG. The authors mapped 150 human proteins to KEGG pathways and using more stringent criteria mapped 66 of those to specific pathways.

The human interaction network seems to have scale-free properties. Most proteins are separated by only a few links, indicating that the network has 'the small world property'. The network is also hierarchical, showing local clusters that are coordinated by hubs. Similar organization has been observed in model organisms, in which hubs are likely to correspond to essential proteins.

Vidal's group also provided an insight into how the interactome might evolve. Because interactions between proteins of the same evolutionary class are more frequent, the network seems to evolve by preferentially adding interactions between lineage-specific proteins.

The human protein-protein interaction network is an invaluable resource to build on (Vidal's group estimate that their data set reveals 1% of the human interactome). Perhaps the most exciting aspect of an interactome map in humans is that it provides

direct information about molecular processes that are related to disease.

The interaction network is a template onto which other information will need to be superimposed. Determining the location and the timing of the interactions, and their regulation, are just some of the challenges that lie ahead.

Magdalena Skipper

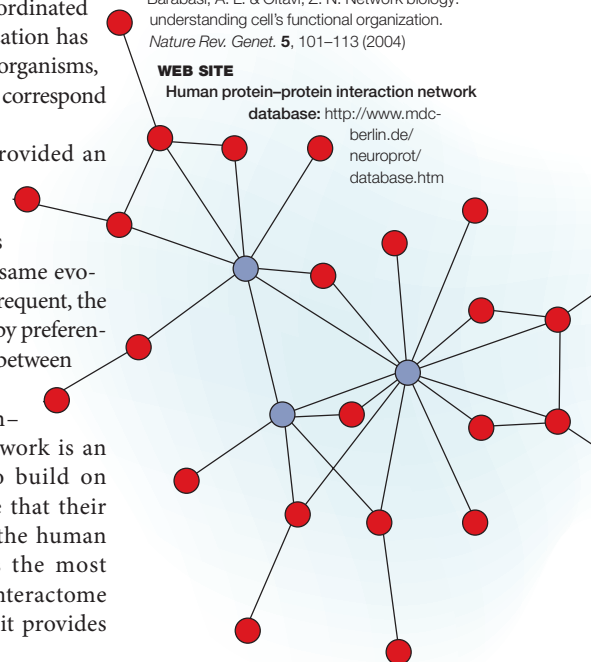
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WEB SITE

Human protein-protein interaction network database: <http://www.mdc-berlin.de/neuroprot/database.htm>



EVOLUTION

A helping hand

Heat shock protein 90 (HSP90) is a well-known molecular chaperone, but is also a therapeutic target in cancer. Recent studies of the evolution of drug resistance in pathogenic fungi show that HSP90 might also influence the evolution of new traits by potentiating the phenotypic effects of genetic variation.

As a chaperone for many signal transducers, HSP90 buffers the effects of genetic variation by enabling the cell to tolerate mutations. Although HSP90 is highly inducible following environmental stress, the demand that occurs due to stress-induced protein misfolding can outpace its induction, enabling previously silent mutations to act combinatorially and generate new phenotypes. It now seems that HSP90 has another role in the emergence of new traits — by allowing a mutation to have immediate effects, rather than buffering against it, HSP90 might potentiate the appearance of new phenotypes.

Cowen and Lindquist examined the role of HSP90 in the evolution of resistance to antifungal drugs. Using rapid selection of three strains of *Saccharomyces cerevisiae* with varying levels of HSP90, they showed that the development of resistance depended on high-level expression of HSP90. Moreover, HSP90 was required to maintain resistance.

The HSP90-dependent effect was specific to rapid selection, which favours mutations that prevent the accumulation of toxic metabolites, rather than gradual selection, which involves upregulation of a multidrug transporter. However, enhanced resistance in all 11 previously identified *S. cerevisiae* drug-resistant deletion strains was HSP90-dependent, showing that HSP90 can influence resistance that is caused by a range of genetic lesions.

How does HSP90 achieve this? One possibility is that a common regulator mediates HSP90-dependent effects on different mutations. An



obvious candidate was calcineurin, one of the targets of HSP90, which regulates the cell's response to certain antifungal agents. Satisfyingly, inhibition of calcineurin strongly reduces fluconazole resistance in all HSP90-dependent resistant strains.

This suggests an attractive therapeutic strategy against fungal infection as similar results were seen with several fungal pathogens, including *Candida albicans* isolates that were collected from an HIV-infected individual. With continued exposure to fluconazole, the fungi evolved towards HSP90-independent resistance, prompting speculation that HSP90 initially allows the phenotype, but that environmental stress drives the cell towards stabilizing the resistant phenotype. Inhibiting HSP90 early in infection could therefore render resistant fungal pathogens sensitive to conventional treatment, or could prevent the initial development of resistance.

Joanna Owens, Associate Editor, Nature Reviews Drug Discovery

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IN BRIEF

GENOME EVOLUTION

Metabolic functions of duplicate genes in *Saccharomyces cerevisiae*.

Kuepfer, L., Sauer, U. & Blank, L. M. *Genome Res.* **15**, 1421–1430 (2005)

Why are duplicated genes maintained in the genome? Although many duplicate genes can provide a back-up role when their paralogue is knocked out, this is unlikely to be the reason that selection maintains duplicates. These authors applied a systems biology approach to duplicated genes that are involved in yeast metabolism. Using *in silico* predictions, single-mutant phenotypes and network analysis, they show that back-up function and increased gene dosage are less important in the maintenance of duplicates than functional divergence, which gives the paralogues distinct, although overlapping, roles.

REGULATORY NETWORKS

Regulated cell-to-cell variation in a cell-fate decision system.

Colman-Lerner, A. *et al. Nature* **437**, 699–706 (2005)

There is considerable variation in the response to signals between seemingly identical cells — how much of this is due to noise in the system? The authors used pheromone-induced expression of a fluorescent reporter gene to answer this question in budding yeast. Little of the cell-to-cell variation could be attributed to noise; instead, it was due to differences in the cells' capacities to transmit signals through the pathway and to express proteins. They also found that the variation was regulated in several ways.

FUNCTIONAL GENOMICS

Second-generation shRNA libraries covering the mouse and human genomes.

Silva, J. M. *et al. Nature Genet.* 2 October 2005 (doi:10.1038/ng1650)

This paper reports the construction of new and improved small-hairpin RNA (shRNA) libraries that cover a large proportion of mouse and human genes. The design of the shRNAs was modified from previous libraries in the light of recent advances in understanding microRNA biogenesis and other factors that affect the efficiency of RNAi. Validation using biochemical and phenotypic assays showed that the new constructs are significantly more efficient than first-generation versions. The collection can be accessed at <http://codex.cshl.edu/>.

EVOLUTION

Urochordate $\beta\gamma$ -crystallin and the evolutionary origin of the vertebrate eye lens.

Shimeld, S. M. *et al. Curr. Biol.* **15**, 1684–1689 (2005)

This study furthers our understanding of the evolutionary origins of the vertebrate eye. The lens — a key eye component — exists only in vertebrates. However, the authors showed that the urochordate *Ciona intestinalis*, which diverged from the vertebrate lineage before lens evolution, encodes a member of the $\beta\gamma$ -crystallin family of proteins that give the lens its unique properties. The promoter region of the gene was able to drive reporter-gene expression in the *Xenopus laevis* visual system, indicating that pre-existing regulatory mechanisms were co-opted during vertebrate eye evolution.

WEB WATCH

Your cup of ZF-espresso?

- <http://zf-espresso.tuebingen.mpg.de>

Zebrafish has quickly established itself as an important model not only for developmental biologists, but also for those studying human disease. To satisfy the growing need for analysis of data from large-scale, high-throughput experiments, Robert Geisler's group at the Max Planck Institute in Tuebingen has developed an online database for zebrafish expression-profiling data — *ZF-Espresso*.

The database is being developed as part of the ZF-MODELS project, an Integrated Project that is funded by the European Commission. The aim of *ZF-Espresso* is to give biologists access to publicly available expression-profiling data. Experiments from different investigators can be compared and combined in a single expression profile. It is also possible to search for genes with similar expression profiles across experiments.

The information is organized under three headings: Select Conditions, Select Probes and Draw Expression Profile. The user can select from a list of experimental conditions and a list of microarray (or qPCR) probes. Because the probes have been mapped to UniGene IDs comparisons can be made across microarray platforms. Line and bar graphs and dot and distribution plots help to visualize expression profiles. The data (from the spreadsheet view) can also be downloaded and imported for further analysis.

ZF-Espresso launched only a couple of months ago. Eventually, it will include all the zebrafish expression-profiling data from public repositories. The plan is to add links to external image databases, provide raw and processed data and to add statistical tools for cross-experiment validation. It is based on freely available PHP and MySQL software and will be easily adaptable for other organisms.

Magdalena Skipper

DEVELOPMENTAL BIOLOGY

Shapely organs require clear orientation



Cell migration and orientated cell division contribute to the spatial distribution of cells. Careful analysis of fly wild-type and mutant embryonic organ precursors for orientation of cell division implicates such orientation in determining organ morphology and points to planar cell-polarity genes as important players in this process.

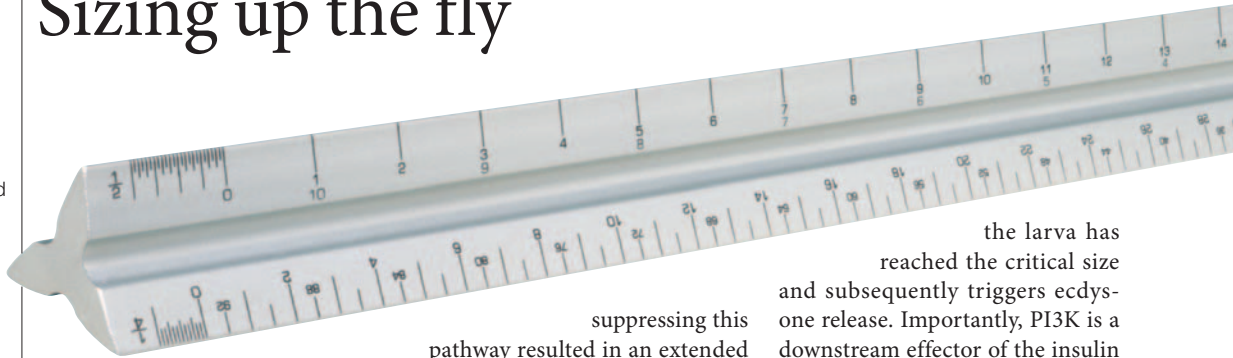
The authors analysed the orientation of cell divisions in marked clones within imaginal discs — the epithelial, embryonic

precursors of adult fly organs. They found a correlation between the orientation of cell divisions and the shape of the clones (and ultimately the adult organs); the correlation persisted throughout development.

Planar cell-polarity genes are known to define the polarity of cells within an epithelium, and flies that carry mutations in members of this family, such as *dachsous* and *fat*, have abnormally shaped organs. The authors show that this

DEVELOPMENTAL BIOLOGY

Sizing up the fly



The final size of an organism depends on both its rate and duration of growth — but how are these variables controlled and coordinated? A recent flurry of studies in the fruitfly has pieced together some important parts of this puzzle.

In *Drosophila melanogaster*, growth takes place over three larval stages, during which enough food needs to be consumed to survive the rest of development. Larvae at the third stage must reach a critical size to undergo pupariation, a process that marks the transition to metamorphosis and is triggered by the release of the steroid hormone ecdysone from the prothoracic gland (PG).

Caldwell and colleagues showed that expressing constitutively active components of the RAS signalling pathway in the PG led to a reduction in final body size. By contrast,

suppressing this pathway resulted in an extended duration of the larval stages and an increase in body size. Monitoring the expression of ecdysone-responsive transgenes revealed that activated RAS in the PG leads to premature ecdysone release. So, the RAS pathway regulates body size by regulating ecdysone production, and therefore the duration of growth.

However, the same study indicated that RAS signalling is not the only pathway that regulates body size. Expression of activated phosphatidylinositol 3-kinase (PI3K) was found to decrease body size independently of RAS activity. The authors postulated that this effect of PI3K is mediated through its ability to increase the size of PG cells, which would increase ecdysone production and terminate larval growth prematurely. This is consistent with a study by Mirth and colleagues, which showed that the PG assesses when

the larva has reached the critical size and subsequently triggers ecdysone release. Importantly, PI3K is a downstream effector of the insulin signalling pathway, which is known to couple fly body size to the availability of nutrients, and these findings provide new insights into the underlying mechanisms.

A paper by Colombani and colleagues reports similar effects on body size after genetic manipulation of PI3K in the PG. Interestingly, this study also establishes a new role for ecdysone in regulating animal growth rate, through a general repression of insulin signalling in peripheral tissues. So, insulin and ecdysone seem to antagonize each other's effects on growth rate, and thereby regulate body size.

Finally, Shingleton and colleagues investigated whether insulin signalling exerts the same effect on body size at all larval stages. Using a temperature-sensitive mutation in the insulin receptor (*Inr*) gene they showed that disrupting this

Of mice and men

For the first time, researchers have generated a mouse strain that also carries a single copy of human chromosome 21. O'Doherty *et al.* have overcome technical obstacles to create this new trans-species model of human Down syndrome, which is the result of chromosome 21 trisomy.

Previous attempts to model Down syndrome in mice have involved either introducing individual human transgenes or creating trisomies of mouse chromosomes. The one-gene-at-a-time approach does not correctly model the 3:2 gene dosage that is found in trisomy, and the mouse trisomies are only approximations to the human condition because genes that lie on human chromosome 21 lie on several mouse chromosomes.

Using injection into female mouse embryonic stem cells, the authors created an aneuploid strain that contains 92% of the gene content of human chromosome 21. The strain had several characteristics of Down syndrome such as heart defects and decreases in long-term synaptic potentiation and memory, neuronal density and T-lymphocyte activation, but only minor facial defects.

The model is a starting point for the study of the specific dosage effects of individual genes, although the precise consequences of heterologous interactions between human and mouse proteins need to be investigated.

Patrick Goymer

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HUMAN EVOLUTION

Brains under pressure

It has taken us many millions of years to evolve the big sophisticated brains that we are so proud of. But it's unlikely to be the best we will ever have. New work shows that two genes that are involved in brain development arose at culturally crucial times during human history and indeed might still be evolving.

It makes sense that genes involved in brain morphology, like so many other developmental genes, are subject to natural selection. Bruce Lahn's initial investigation into the subject was reported last year, when he and his colleagues found that two genes that regulate brain size — microcephalin (*MCPH1*) and abnormal spindle-like microcephaly associated (*ASPM*) — have been under strong selective pressure in the human evolutionary lineage since we split off from the chimpanzee lineage. New work has looked more closely at these two genes to see whether there are signs of more recent selection.

To do this, the distribution of haplotypes for the two genes was studied in a panel of ~90 cell lines that are representative of human diversity. In both cases, one haplotype stood out as being present in a large proportion of cell lines — a frequency that could not be explained by random or demographic factors and therefore might have been driven up in abundance by positive selection. The population distribution of polymorphisms at the two loci and the extent of linkage disequilibrium around each candidate positively selected region support this idea and also point to the occurrence of a recent selective sweep that still continues.

A statistical analysis that is based on estimating the past mutation rate of the genes placed the emergence of the high-frequency alleles at ~37,000 years ago for *MCPH1* and ~5,800 years ago for *ASPM*. These dates coincide with significant periods in recent human history: the first to the emergence of cultural traits such as music, art and symbolism, and the second to the building of the first cities in Mesopotamia.

The young age of the frequent *ASPM* variant makes it likely that brain evolution is still continuing. As the authors themselves point out, however, the results should not be overinterpreted. For example, as we cannot tell what force is driving the positive evolution of gene variants, we cannot ascribe it to variation in cognitive function (both genes are also expressed outside the brain). For the same reasons, we should be wary of reading any adaptive significance into the current geographical distribution of *MCPH1* and *ASPM* alleles.

Tanita Casci

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WEB SITE

Bruce Lahn's home page: <http://www.genes.uchicago.edu/fri/lahres.html>

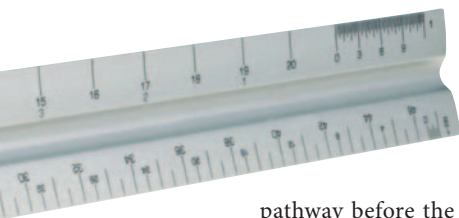
dismorphology is associated with changes in the orientation of cell division.

This work might have uncovered a general mechanism of organ morphogenesis: there is a correlation between the orientation of cell division and the shape of different organs and, importantly, planar cell-polarity genes are well-conserved throughout evolution. We now need specifically targeted experiments to confirm this possibility.

Magdalena Skipper

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Baena-López, L. A. *et al.* The orientation of cell divisions determines the shape of *Drosophila* organs. *Curr. Biol.* **15**, 1640–1644 (2005)



pathway before the critical size is reached extends total development time, but has no effect on the final size of the fly. The opposite was true when INR signalling was blocked after the critical size had been attained.

These studies represent important steps in understanding how body size is regulated in the fly. They might also have implications for understanding the coordination of nutrient availability and developmental transitions in mammals, where steroid hormones and insulin signalling also have key roles in regulating growth.

Louisa Flintoft

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GENE NETWORKS

Grasping the essentials

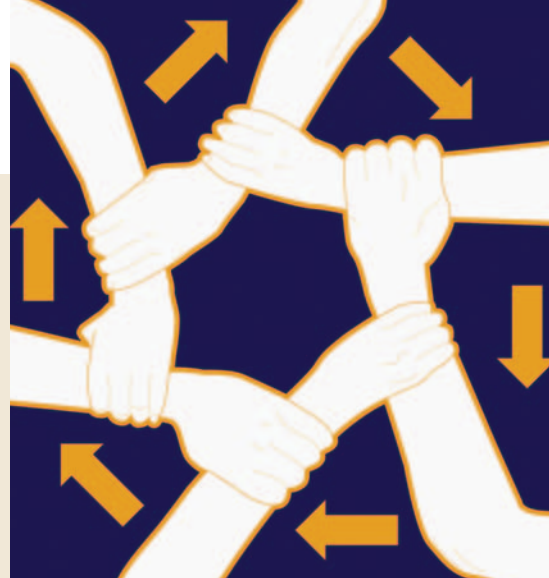
Understanding how essential genes function in networks presents a challenge — because knocking them out causes lethality, their function must instead be carefully manipulated to probe their interactions. Rising to this challenge, one group has now studied an extensive set of interactions for essential genes in *Saccharomyces cerevisiae*, indicating an unforeseen complexity of genetic networks.

Davierwala and colleagues made conditional expression alleles or conditional temperature-sensitive alleles for over half the essential genes in *S. cerevisiae*. Expression of both types of allele can be controlled so that levels of the gene products are decreased, but not abolished. These strains were crossed to a panel of 30 others, carrying similarly derived mutant alleles of either essential or non-essential genes. The

fitness of the double mutants was scored relative to the parent strains, as quantified by colony growth, which revealed a network of 567 interactions among 286 essential genes. Only two of these interactions were already documented, highlighting the power of this approach to identify new relationships.

The most remarkable aspect of the network was the number of interactions it contained. On average, essential genes take part in about five times as many interactions as their non-essential counterparts — significantly more than previously estimated. This indicates that the overall network in budding yeast might be twice as dense as previously thought.

Notably, many of the conditional alleles of essential genes had little or no phenotype as single mutants, but combined with interacting alleles they produced more severe



effects. If other gene networks are organized in a similar way, such interactions might underlie variation in many complex traits — including non-Mendelian disease in humans.

Louisa Flintoft

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STEM CELLS

Everything is possible if we work together

Three transcription factors — OCT4, SOX2 and NANOG — are essential for maintaining the pluripotency of human embryonic stem (ES) cells. Young and colleagues have now identified the target genes of these three

proteins, and have uncovered several regulatory circuits through which they fulfil their function.

Using a combination of chromatin immunoprecipitation and DNA microarrays, the authors assayed the regions near the promoters of 17,917 annotated human genes. They found that OCT4, SOX2 and NANOG bound near the promoters of 623, 1,271 and 1,687 genes, in that order. What was particularly surprising was that at least 353 of those genes were bound by all three transcription factors, which implies a significant co-ordination of function.

About half these coordinately regulated genes are activated by OCT4, SOX2 and NANOG, and half are repressed. The activated genes include components of the TGF β and WNT signalling pathways, both of which have a role in maintaining pluripotency; the repressed genes include many transcription factors that are important for differentiation.

The authors confirmed the presence of two types of regulatory circuit in human ES cells, using algorithms that had previously been devised in yeast. First, they found feed-forward loops, in which a first

regulator regulates a second regulator and both then regulate the target genes. If both regulatory steps are positive this gives stability against transient changes in input; if one step is negative a rapid switch in response to changed conditions is enabled.

The second type of circuitry that they describe is the autoregulatory loop, in which OCT4, SOX2 and NANOG regulate their own expression. This also offers stability of gene expression and rapid responses to environmental stimuli.

These approaches will allow researchers to elucidate the circuits that are controlled by other transcription factors, and also by chromatin regulators. Testing these circuits will be aided by advances in the culture and genetic manipulation of ES cells. A full understanding of the regulatory circuitry of ES cells will help researchers to promote stem cells to differentiate into a range of cell types, and possibly to reprogramme differentiated cells back into pluripotent ones.

Patrick Goymer

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WEB SITE

Richard A. Young's laboratory: <http://web.wi.mit.edu/young>

