

## RESEARCH HIGHLIGHT

# Making brundlefly, one gene at a time

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*Drosophila melanogaster* has proved to be a remarkable organism for studying complex biological phenomena. One of the great virtues of *D. melanogaster* is its amenability to large scale genetic screens. Many types of genetic screen have been performed in *D. melanogaster* in the past with great success, to explore myriad biological processes such as tissue growth, differentiation, patterning, morphogenesis, and immunity. Different types of genetic screens employed include: (1) Homozygous mutant. A striking example of which is the embryonic patterning screen performed by Wieschaus and Nusslein-Volhard, where multiple genes important for the development and patterning of the *D. melanogaster* embryo were identified [1]. (2) Dominant modifier. These have most commonly been used to identify new components of existing signalling pathways. A highly successful example was the identification of components of the Ras-Map kinase signalling pathway as downstream effectors of the Sevenless receptor tyrosine kinase [2]. (3) Overexpression. These screens typically employ a collection of randomly inserted transposable elements that activate the expression of different genes when crossed to tissue-specific drivers [3]. (4) Flp/*FRT* clonal. Homozygous

mutations in many genes cause lethality at early stages of development. A major advantage of clonal screens is that they provide a mechanism to generate patches of homozygous mutant tissue in an otherwise heterozygous animal, thus allowing phenotypic characterisation of lethal genes at later stages of development [3]. (5) RNA interference. First performed in cell culture, and more recently in transgenic *D. melanogaster*; these screens utilise the phenomenon of RNA interference to systematically ablate the function of individual genes in a given experimental setting. Cell culture screens have largely focussed on identification of novel modulators of existing signalling pathways [4, 5].

In the November issue of *Cell Research*, Tian Xu and colleagues [6] describe a genetic overexpression screen whereby they aim to classify the function of uncharacterised human genes by overexpressing them in *D. melanogaster*. The screen was conceived based on the premise that most important biological processes are conserved between human and flies, and that although the identity of all human genes is now known, the function of many is still obscure [7-9].

Xu *et al.* [6] generated transgenic flies expressing 236 different human cDNAs under the control of the yeast *GAL4 UAS* control element, allowing expression of these upon crossing the flies to tissue-specific *GAL4* drivers. They used drivers that expressed ubiquitously, in the developing eye or wing epithelia, or in differentiating neural cells, and screened for cDNAs that when overexpressed, produced visible phenotypes. They found that 51 of the human cDNAs analysed in flies gave a reproducible phenotype. Through comparing the phenotypes generated to those obtained in other studies, by searching the vast *D. melanogaster* literature (see <http://flybase.bio.indiana.edu/>), it can be inferred as to whether the over-expression of the gene affects processes such as cell growth, proliferation, death, differentiation or morphology. Moreover, the phenotype generated by over-expression of the human cDNA can then be interrogated against a bank of available *D. melanogaster* mutations to provide insight into the pathway by which the gene operates. This approach can be scaled-up to a global context to analyse all ~30 000 human cDNAs in *D. melanogaster*, and since the function of at least 40% of all predicted human coding cDNAs is not yet known, this strategy has the potential to rapidly provide valuable information on the function of “pioneer” human genes.

While conceptually this approach has immense power in revealing the function of unknown human genes, there are many caveats to the success of this approach and whether the information revealed in the *D. melanogaster* system will be meaningful for mammalian biology. Firstly, it is likely that only highly conserved genes will give phenotypes

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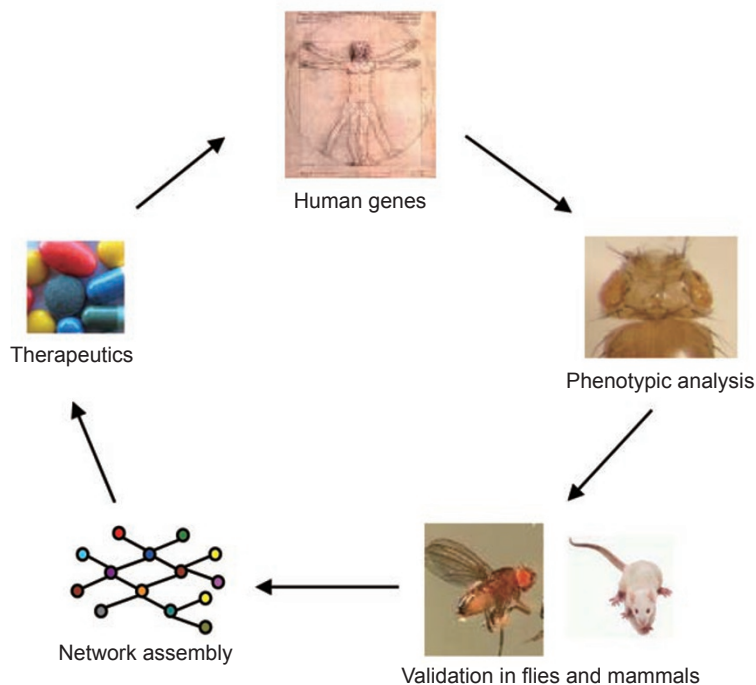
when mis-expressed in a heterologous system (e.g. Rpl8 which is 80% identical between human and *D. melanogaster*). Secondly, even amongst the most highly conserved genes, only those that are rate-limiting for a particular process in *D. melanogaster* may be expected to give phenotypes, rather knockdown of gene-function is more likely to create phenotypes. In this regard, it is pertinent to point out that one of the cDNAs (the ribosomal protein gene, *RPL8*) that Xu *et al.*, discovered to produce strong phenotypes when expressed from all drivers was shown to be a truncated dominant-negatively acting protein. Thirdly, due to divergence of critical domains in homologous proteins between *D. melanogaster* and humans, and differences in protein complexes or signalling pathways between organisms, many human protein functions may be

missed. Moreover, neomorphic effects may occur when the human protein is expressed in *D. melanogaster* that do not reflect the physiological function of the human protein. This issue is the most problematic in that it may lead to misconceptions in the understanding of gene function, and therefore it is highly important that all phenotypic effects are validated in the *D. melanogaster* system, as well as in mammalian cells (see Figure 1).

Importantly, Xu *et al.* provide convincing data to support the validity of their screening approach. They focus on three human proteins that give phenotypes upon expression from various drivers; the cell cycle inhibitor, p27, the SFRS3 splicing factor, and the ribosomal protein L8. p27-Kip1 is a homolog of the *D. melanogaster* protein Dacapo, which binds to and inactivates

G1-S phase Cyclin-dependent protein kinases, resulting in cell cycle arrest at the G1-S phase transition. Consistent with this, overexpression of p27 in the developing eye (via *eyeless-GAL4*) results in a small eye, while expression in the differentiating cells of the eye (via *GMR-GAL4*) results in a rough eye phenotype. Although they did not examine the effect of p27 expression on cell cycle progression in this study, these phenotypes are similar to that obtained when Dacapo or another human homolog of Dacapo, p21-Cip1 are overexpressed in the *D. melanogaster* eye, which have been shown to block S phase entry [10]. Moreover, they show the importance of a critical phosphorylation site on p27, Serine 10, which is important to allow nuclear entry and stability of p27, and for its activity in human cells and *D. melanogaster* tissues. This result demonstrates the functional conservation of Serine 10 phosphorylation for p27 function in *D. melanogaster* cells, and suggests that this system will be useful for further analyses to investigate p27 regulation.

Xu *et al.*, further demonstrate the validity of their approach to investigate human gene function in *D. melanogaster*, from their analysis of the splicing factor gene, *SFRS3*, and the gene encoding the ribosomal protein, *RPL8*. Expression of *SFRF3* in the differentiating region of the eye (via *GMR-GAL4*) results in a rough eye phenotype, while expression in the developing genital discs (via *Patched-GAL4*) leads to intersex genitalia. Importantly, overexpression of the *D. melanogaster* homolog of *SFRS3*, *Dx16*, in these tissues results in identical phenotypes. A dominant negative version of *RPL8* reduced tissue size and could be rescued by expression of *D. melanogaster RPL8*. In addition, Xu *et al.*, highlighted the functional conservation between human and *D. melanogaster RPL8* by showing that co-expression of either *D. melanogaster RPL8<sup>RNAi</sup>* or human *RPL8<sup>DN</sup>* could reverse overgrowth as-



**Figure 1** Theme for discovery of human gene function in *D. melanogaster*. Human genes are mis-expressed in different *D. melanogaster* tissues and the resultant phenotypes scored. Results are then studied in detail by assessing the function of phenotype-eliciting genes and their *D. melanogaster* orthologues (if available) in mammalian tissue culture, flies and mice. Signalling networks are assembled by grouping known and novel genes with related phenotypes. Suitable therapeutic targets are selected for the appropriate disease, and drugs developed.

sociated with hyperactivation of the insulin, or target of rapamycin pathways, which are known to stimulate ribosome biogenesis. These studies show that the expression of the human orthologues of SFRS3 and RPL8 are likely to be working by a similar manner to their *D. melanogaster* counterparts, and support the use of the *D. melanogaster* system to investigate the mechanism of action of SFRS3 and RPL8.

Currently the Xu laboratory has published a screen covering 1% of the human genome for overexpression phenotypes in *D. melanogaster*. An enormous expansion in effort and resources will be required to tackle the ambitious task of extending this approach to the entire human genome. Such a task is not insurmountable however, but will require a highly efficient approach. Recently the Vienna Drosophila Research Center, led by Barry Dickson, generated approximately 20 000 transgenic *D. melanogaster* lines expressing RNA interference constructs [5]. Approximately 30 000 transgenic overexpression lines would need to be produced to cover the human genome (assuming one transgenic fly line per gene). Based on the current study by Xu *et al.*, more than 1 500 transgenic fly lines were generated for 236 genes, (roughly 6 transgenic fly lines per gene), meaning that approximately 200 000 transgenic fly lines would need to be

produced to cover the whole human genome. To increase the efficiency of a genome-wide study it would be wise to employ recent technological advances in the production and expression of transgenes in *D. melanogaster*, such as site-specific integration, and transgenic vectors with gene insulator elements that boost expression from heterologous promoters [11]. Theoretically the utilisation of such technologies would reduce the time taken to generate transgenic *D. melanogaster*, guarantee robust and consistent transgene expression, and ensure that only one transgene would need to be generated for each human gene. If this approach of humanising the fly, one-gene-at-a-time, can be achieved, it has enormous potential for the discovery of new functions for human genes, and moreover, this bank of transgenic flies could be used in a “new-age” genetic interaction screen to discover novel human gene networks (see Figure 1).

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