

used. Such HF animals show elevated expression of MMP-2 and MMP-9 at 23 weeks without LV dilatation. However, LV dilatation, LV systolic dysfunction, and pulmonary edema occur at 26 weeks with further enhancement of the expression and activity of MMPs. Administration of the ACE inhibitor (enalapril, 5 mg/kg/d) from 9 weeks prevents such geometrical and functional deterioration. Thus, MMPs appear to promote LV remodeling, and ACE blockade inhibits MMP activation by reducing Ang II formation, thereby preventing LV remodeling and dysfunction. These recent *in vitro* and animal studies have provided promising findings on the reduction of hypertension and LVH through blockade of MMPs and the EGF-R. The extent to which inhibition of these molecules is clinically applicable to the prevention and treatment of hypertension and LVH in human patients remains to be determined.

References

- 1 Armiento, J.D. (2002) Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. *Trends Cardiovasc. Med.* 12, 97–101
- 2 Spinale, F.G. (2002) Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ. Res.* 90, 520–530
- 3 Tayebee, M.H. *et al.* (2003) Extracellular matrix biology: a new frontier in linking the pathology and therapy of hypertension? *J. Hypertens.* 21, 2211–2218
- 4 Prenzel, N. *et al.* (1999) EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402, 884–888
- 5 Shah, B.H. and Catt, K.J. (2003) A central role of EGF receptor transactivation in angiotensin II-induced cardiac hypertrophy. *Trends Pharmacol. Sci.* 24, 239–244
- 6 Rockman, H.A. *et al.* (2002) Seven-transmembrane-spanning receptors and heart function. *Nature* 415, 206–212
- 7 Virdis, A. and Schiffrin, E.L. (2003) Vascular inflammation: a role in vascular disease in hypertension? *Curr. Opin. Nephrol. Hypertens.* 12, 181–188
- 8 Hao, L. *et al.* (2004) Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ. Res.* 94, 68–76
- 9 Peterson, J.T. (2004) Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail. Rev.* 9, 63–79
- 10 Kagiya, S. *et al.* (2003) Antisense to epidermal growth factor receptor prevents the development of left ventricular hypertrophy. *Hypertension* 41, 824–829
- 11 Florian, J.A. and Watts, S.W. (1999) Epidermal growth factor: a potent vasoconstrictor in experimental hypertension. *Am. J. Physiol.* 276, H976–H983
- 12 Schlessinger, J. (2002) Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* 110, 669–672
- 13 Seals, D.F. and Courtneidge, S.A. (2003) The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* 17, 7–30
- 14 Thomas, W.G. *et al.* (2002) Adenoviral-directed expression of the type 1A angiotensin receptor promotes cardiomyocyte hypertrophy via transactivation of the epidermal growth factor receptor. *Circ. Res.* 90, 135–142
- 15 Asakura, M. *et al.* (2002) Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat. Med.* 8, 35–40
- 16 Eguchi, S. *et al.* (2001) Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metalloproteinase-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK. *J. Biol. Chem.* 276, 7957–7962
- 17 Saito, S. *et al.* (2002) Metalloproteinase inhibitor blocks angiotensin II-induced migration through inhibition of epidermal growth factor receptor transactivation. *Biochem. Biophys. Res. Commun.* 294, 1023–1029
- 18 Flamant, M. *et al.* (2003) Epidermal growth factor receptor transactivation mediates the tonic and fibrogenic effects of endothelin in the aortic wall of transgenic mice. *FASEB J.* 17, 327–329
- 19 de Gasparo, M. *et al.* (2000) International Union of Pharmacology: XXIII, The angiotensin II receptors. *Pharmacol. Rev.* 52, 415–472
- 20 Sakata, Y. *et al.* (2004) Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats. Its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. *Circulation* 109, 2143–2149

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Flying through the genome: a comprehensive study of functional genomics using RNAi in *Drosophila*

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Sequencing the DNA of an entire mammalian genome now seems routine. The human sequence along with the mouse – the model for mammalian genetics – and the rat – the model for mammalian physiology – are now part of the data archive. However, the real challenges for the 21st century are what to do with this information and how to test the function of so many different genes in so many different cellular contexts. The potential payoffs are enormous. Examples include a better understanding of disease pathologies with effective strategies for therapeutic interventions that cause few, if any, side effects.

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To understand why the fruit fly, *Drosophila melanogaster*, remains an important genomic model system, it is important to remember that the formulated science of genomics did not begin at the end of the 20th century with the advent of high-throughput sequencing technologies. As outlined in Table 1, for more than 90 years the *Drosophila* community has made substantial contributions to our knowledge base of how genes are organized, expressed and regulated at the genomic level.

There are many reasons why *Drosophila* has been so popular for genomic studies (reviewed in [17]). First, the genome is organized into only four chromosomes, which was an asset for establishing the first linkage maps. A

Table 1. Timeline of important genomic studies using *Drosophila* as a model organism

Genomic milestone	Authors	Selected reference
First linkage map generated (X chromosome)	Sturtevant	[1]
First complete drawings of polytene chromosomes	Painter	[2]
First defined deficiencies and duplications generated using X-ray induced chromosomal aberrations	Dubrovsky and Kelstein	[3]
First systematic study to induce puffs on polytene chromosomes after <i>in vitro</i> treatments	Ashburner	[4]
First use of whole genome scanning to screen for phenotypic perturbations caused by changes in gene dosage	Lindsley <i>et al.</i>	[5]
First description of chromosomal walking technique to clone genomic DNA	Bender <i>et al.</i>	[6]
First genome-wide mutational screen to identify all genes involved in embryonic development	Nusslein-Volhard and Wieschaus	[7]
First enhancer-trap screen based on pattern of reporter-gene expression	O'Kane and Gehring	[8]
First large-scale mutagenesis screen using engineered transposable elements	Cooley <i>et al.</i>	[9]
First development of a binary expression (GAL4/UAS) system for controlling ectopic gene expression	Brand and Perrimon	[10]
First report of a systematic gain-of-function study to identify important genes	Rorth <i>et al.</i>	[11]
First comprehensive microarray study of <i>Drosophila</i> development	White <i>et al.</i>	[12]
First published sequence of the <i>Drosophila</i> genome	Adams <i>et al.</i>	[13]
First report of gene-trapping strategy to randomly label endogenous proteins with GFP	Morin <i>et al.</i>	[14]
First protein-interaction map of <i>Drosophila</i>	Giot <i>et al.</i>	[15]
First RNAi study in cultured <i>Drosophila</i> cells using a comprehensive genome-wide approach	Boutros <i>et al.</i>	[16]

second reason is that *Drosophila* has polytenized tissues with giant interphase chromosomes that were used in microscopic studies to map mutant alleles to cytological locations. Polytene chromosomes also made it possible to generate defined duplications and deletions of chromosomal segments to systematically test regions of the genome for gene-dosage effects before recombinant-DNA technologies. A third reason is that flies are easy to culture in the laboratory and they have a short generation time. This has allowed for a sufficient number of individuals to be mutagenized and screened so that most of the genes necessary for embryonic development could be identified and subsequently cloned. Finally, the fact that the organism can be easily transformed with transposable-element vectors has made it feasible to 'trap' genomic enhancers and use them to drive transgenes in a spatial- and temporal-specific manner to test gene functions.

Version 1.0 of the *Drosophila* genome was completed in the year 2000. With current revisions and current algorithms (<http://flybase.net/annot/release3.html#reannotate>), 13 666 protein-encoding genes are predicted. Gene chips with genome-wide coverage are now widely available and microarray analyses are affordable for many laboratories. As a result, a plethora of publications has appeared in the last few years using this technology to identify genes with altered transcription profiles in different tissues, before and after developmental stimuli or environmental exposures, and in wild-type versus mutant backgrounds.

To test the function of these genes, the power of *Drosophila* genetics and its 90-year history come into play. Because of the large number of pre-existing mutants, it is often possible to test animals that are defective in a gene of interest for a specific phenotype. In those circumstances where mutants are not available, they can be generated with a variety of strategies including using homologous recombination to make specific gene-knockout lesions [18]. However, it can still take several

months to make the desired reagents before an experiment can be formulated. Recently, the University of California, Berkeley *Drosophila* Genome Project has committed the resources to gather and/or generate transposable-element-disruption mutations in every gene predicted from the genomic sequence. Remarkably, more than 40% of the genes have already been targeted (<http://flybase.bio.indiana.edu/bin/fbidq.html?FBrf0173867>).

However, even if the entire genome were saturated with loss-of-function mutations, most genes are pleiotropic, and thus analyzing their mutant phenotypes might be difficult or impossible under the conditions of interest. For example, a gene that is involved in adult learning and memory could not be analyzed if mutants display an embryonic-lethal phenotype. Also, there is the added difficulty of combining mutations into a single animal if one wants to test more than one gene interaction. Such disadvantages can often be circumvented with RNA interference (RNAi).

RNAi is an effective gene-silencing technique that employs sequence-specific double-stranded RNA (dsRNA) complementary to a target gene. As a phenomenon, it was first described in plants, but was later shown to be effective at compromising gene functions in animals including *Drosophila* (reviewed in [19]). Presumably, RNAi works by eliciting an antiviral response within the cell that leads to a catalytic degradation of the dsRNA. During the degradation, short interfering RNAs (siRNAs) of 21–25 nucleotides are generated that can anneal with mRNAs from the targeted gene. The transcripts (now partially double stranded) are then selected for efficient destruction and are prevented from being translated. Double-stranded RNAs can be introduced into *Drosophila* tissues by injection or osmotic shock, or they can be added directly to the medium where they will be endocytosed into tissue-culture cells [20], a method that has not been effective for mammalian cell lines. The RNAs can also be internally generated from transgenes by cloning an inverted repeat

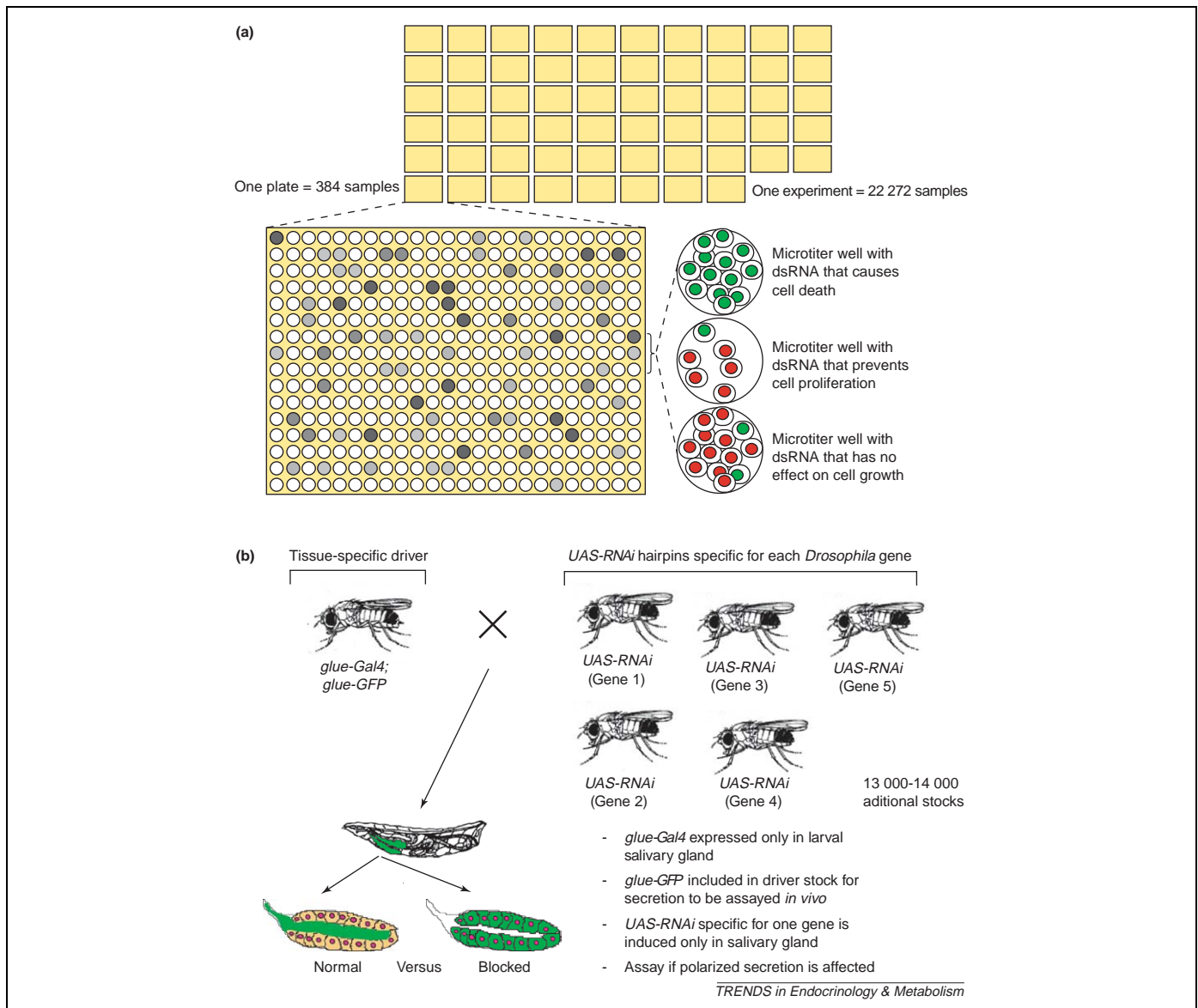


Figure 1. Genome-wide strategies for testing *Drosophila* genes using RNAi. **(a)** A high-throughput screen used to test the function of putative *Drosophila* genes in controlling cell proliferation and viability is shown. Tissue-culture cells derived from embryonic hemocytes are plated at uniform density into several microtiter dishes containing thousands of wells. Double-stranded RNA synthesized for each putative *Drosophila* gene is generated by *in vitro* transcription from a PCR product and added to each well. Cells are incubated with the dsRNA for several days and then stained with fluorescent dyes to detect viable (Hoechst-red) or dying (SYTOX-green) nuclei. The ratio of the two markers is used to generate a z-score that indicates the severity of the RNAi phenotype. **(b)** A theoretical screen that could be used to assay gene functions in individual tissue types in a developing animal. The salivary gland is used as an example because the *glue-Gal4* driver (derived from *sgs3*) is known to be exquisitely temporally and spatially specific for that tissue. To be thorough, the progeny of more than 13 000 crosses should be analyzed. The experiment is less daunting if a microarray analysis is used to prescreen for genes with interesting expression patterns.

that can form double-stranded hairpins or snap-back molecules when expressed [19].

A recent collaborative effort involving the Harvard Medical School in the USA and the Heidelberg Fly Assay Consortium and the Max-Planck Institute for Molecular Genetics in Germany has demonstrated the power of this technique when combined with a whole-genome approach in a single cell type [16]. In their publication, the researchers used PCR primers to generate clones that could be transcribed and annealed into double-stranded RNAs against nearly all (91%) of the known *Drosophila* genes predicted from the genomic sequence. They then incubated each dsRNA in a microtiter well containing cultured cells. The experiment was performed in duplicate using two different *Drosophila* lines (S2R+ and Kc167)

that are believed to be immortal derivatives of embryonic hemocytes. Cells were assayed days later for cell growth, proliferation and survival using a quantitative assay for cell number that relied on fluorescent probes specific for living or dying nuclei (Figure 1a). For example, SYTOX is a large dye that will not penetrate the membranes of living cells. Thus a well containing many dead cells should have a high number of SYTOX-green labeled nuclei compared with intact cells in which living nuclei are detected with a red conjugate of Hoechst 33342. The ratio of the fluorescent tags was used to generate a z-score that indicated the severity of the RNAi phenotype (a high z-score suggested a defect in cell survival or proliferation). As proof of principle, RNAi against genes involved in inhibiting apoptosis that should affect survival had high

z-scores, and were significantly different from those affecting proliferation. Also, duplicate samples of the same dsRNA displayed remarkably similar z-score values.

As expected, silencing genes encoding components of complexes that control protein translation, protein degradation and cell cycle regulation displayed severe phenotypes, as did genes encoding specific DNA-binding proteins and signaling molecules. Perhaps more interestingly, ~40% of the genes displaying severe phenotypes did not encode a predictive protein domain. This result indicated that the screen was useful in identifying a large number of essential genes that were previously uncharacterized. The function of these novel genes can now be tested phenotypically in live animals. The success of this screen for cell viability and proliferation raises the possibility that other cellular phenotypes could be assayed in a similar manner. In fact a pilot screen was performed using a subset of dsRNAs directed against specific candidate genes to test for cell-shape phenotypes resulting from defective organizations of the cytoskeleton [21].

In addition, the power of a classical modifier screen could be employed with this high-throughput RNAi approach to screen for suppressors or enhancers of a specific cell phenotype to identify molecules controlling a common pathway. One would simply start with a compromised cell and screen for those wells in which cell viability improved or deteriorated to possibly identify activator or inhibitor molecules from the pathway.

A similar genome-wide strategy is currently being attempted with human cell-culture reagents. Unfortunately, mammalian cells do not readily endocytose dsRNA from culture medium. As a result, these screens required tedious transfection protocols to introduce constructs that will express hairpin RNA molecules as short siRNAs.

However, a recent report documenting the success using a 'microarray of living cells' is very exciting [22]. With this approach, DNA-reporter vectors or short hairpin RNAs are first printed onto special glass substrates as a microarray using a modified lipid method. Next, living mammalian cells are added to the array so that when they attach to the glass substrate they are induced to take up the nucleic acids spotted beneath them. Widespread use of this or other modifications should open mammalian cell culture to loss-of-function and genetic-modifier screens, in addition to the obvious functional assays for predicted gene sequences.

Although the above strategies are ideal for isolated cells in culture, they lack the ability to target a specific cell type within a developing or aging animal. In this regard, *Drosophila* could again be the model of choice because it is theoretically possible to target each gene in a specific cell-type during a specific developmental time. This would require combining RNAi with the GAL4/UAS binary expression-system (Table 1). Here the yeast transcription factor, GAL4, is under the control of a specific *Drosophila* enhancer that allows it to be expressed in a temporally and spatially defined manner. The target gene could then be expressed as an inverted repeat under control of the yeast upstream activating sequences (UAS), which are binding sites for GAL4. When the *Gal4* 'driver' fly is crossed with the *UAS-RNAi* 'responder' stock, the resulting progeny have both components of the system, and thus the

target gene is silenced in those tissues in which GAL4 is expressed. Figure 1b outlines the strategy using the larval salivary gland as the target cell type. Here the regulatory sequences for the glue gene, *sgs3*, can be used to drive GAL4 expression only in this tissue during the last half of the third instar. The salivary gland is a polarized epithelial cell that responds to the steroid hormone, 20-hydroxyecdysone, to secrete massive amounts of glue glycoproteins. Thus with a GFP reporter [23], it can be used as a model system to assay the requirement of genes necessary to respond to hormone, to mobilize calcium from intracellular stores, to sort secretory cargoes and to deliver granules to the apical side of the cell.

In order for the transgenic RNAi strategy to be comprehensive it might be necessary to generate 13 666 transgenic flies each carrying a RNAi-hairpin transgene. Ideally these could then be crossed to a driver line in which the GAL4 transcription factor is expressed only in the tissue and at the time of interest. Currently, few *Drosophila* genes have been identified that are so precisely restricted in their spatial expression, but as more promoter and enhancer regions are described it might be possible to generate such reagents for most tissue types. The problem of temporal specificity can theoretically be addressed using a temperature-sensitive ubiquitously expressed GAL80 transgene. When GAL80 is expressed in the same cell as GAL4, it prevents binding of the transcription factor to UAS sequences. Thus, the timing of GAL80/GAL4/UAS-RNAi activity can be controlled by the temperature at which the animals are exposed.

Currently, no laboratory or institute has committed the resources necessary to produce such a large collection of transgenic-RNAi stocks. However, given the enormous advancement and interest in this field, and the likelihood that such information will be applicable to human biology, it seems probable that at least a subset of these reagents will someday become available. Stay tuned.

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References

- 1 Sturtevant, A.H. (1913) The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *J. exp. Zool.* 14, 43–59
- 2 Painter, T.S. (1934) Salivary chromosomes and the attack on the gene. *J. Hered.* 25, 464–476
- 3 Dubovsky, N.V. and Kelstein, L.V. (1938) Reverse mutations of dominant genes. *Bull. Biol. Med. exp. URSS* 6, 733–735
- 4 Ashburner, M. (1971) Induction of puffs in polytene chromosomes in vitro cultured salivary gland of *Drosophila melanogaster* by ecdysone and ecdysone analogues. *Nature New Biol.* 230, 222–224
- 5 Lindsley, D.L. *et al.* (1972) Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. *Genetics* 71, 157–184
- 6 Bender, W. *et al.* (1979) Gene isolation by chromosomal waxing. *J. Supramol. Struct.* 8, 32
- 7 Nusslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287, 795–801
- 8 O'Kane, C. and Gehring, W.J. (1987) Detection in situ of genomic regulatory elements in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 84, 9123–9127

- 9 Cooley, L. *et al.* (1988) Insertional mutagenesis of the *Drosophila* genome with single P-elements. *Science* 239, 1121–1128
- 10 Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415
- 11 Rorth, P. *et al.* (1998) Systematic gain-of-function genetics in *Drosophila*. *Development* 125, 1049–1057
- 12 White, K.P. *et al.* (1999) Microarray analysis of *Drosophila* development during metamorphosis. *Science* 286, 2179–2184
- 13 Adams, M.D. *et al.* (2000) The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185–2195
- 14 Morin, X. *et al.* (2001) A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 98, 15050–15055
- 15 Giot, L. *et al.* (2003) A protein interaction map of *Drosophila melanogaster*. *Science* 302, 1727–1736
- 16 Boutros, M. *et al.* (2004) Genome-wide RNAi analysis of growth and viability in *Drosophila* cells. *Science* 303, 832–835
- 17 Rubin, G.M. and Lewis, E.B. (2000) A brief history of *Drosophila*'s contributions to genome research. *Science* 287, 2216–2218
- 18 Rong, Y.S. and Golic, K.G. (2000) Gene targeting by homologous recombination in *Drosophila*. *Science* 288, 1973–1975
- 19 Montgomery, M.K. (2004) RNA interference: historical overview and significance. In *Methods in Molecular Biology* (Gott, J.M. and Totowa, N.J. ed.), pp. 3–21, Humana Press
- 20 Clemens, J.C. *et al.* (2000) Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6499–6503
- 21 Kiger, A.A. *et al.* (2003) A functional genomic analysis of cell morphology using RNA interference. *J. Biol.* 2, 1–15
- 22 Silva, J.M. *et al.* (2004) RNA interference microarrays: High-throughput loss-of-function genetics in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6548–6552
- 23 Biyasheva, A. *et al.* (2001) Glue secretion in the *Drosophila* salivary gland: A model for steroid-regulated exocytosis. *Dev. Biol.* 231, 234–251

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Resistin/ADSF/FIZZ3 in obesity and diabetes

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The role of adipocyte-secreted resistin/adipocyte-specific secretory factor (ADSF)/FIZZ3 in obesity and diabetes has been controversial at best. Recently generated *resn* knockout mice showed normal glucose and insulin sensitivity with lower fasting glucose levels. Upon feeding with a high-fat diet, the knockout mice exhibited increased glucose tolerance with decreased hepatic glucose output, possibly due to phosphorylation and activation of AMP-activated protein kinase and suppression of gluconeogenic genes. In comparison, transgenic mice overexpressing a dominant negative form of resistin/ADSF/FIZZ3 showed increased adiposity with elevated leptin and adiponectin levels, accompanying enhanced glucose tolerance and insulin sensitivity both on chow and high-fat diets. Although its underlying mechanisms need further elucidation, the *in vivo* studies demonstrate a role of resistin/ADSF/FIZZ3 in obesity and insulin resistance.

Obesity is a prevalent health hazard in industrialized countries and is closely associated with insulin resistance and type 2 diabetes. It results from increased size of adipocytes due to lipid accumulation, and from an increased number of adipocytes arising from differentiation of adipose precursor cells to mature adipocytes under the appropriate nutritional and hormonal conditions. Gene expression studies during adipocyte differentiation have firmly established that peroxisome proliferator-activated receptor γ (PPAR γ) and the CCAAT enhancer-binding protein (C/EBP) family of transcription factors play central roles in adipocyte

differentiation [1–3]. However, various factors in cell–cell and cell–matrix communications govern expression of the adipocyte transcription factors and therefore regulate conversion of preadipocytes to adipocytes. Although adipose tissue is the major energy reservoir in higher eukaryotes, the role of adipose tissue as a secretory organ has emerged through the discovery of leptin. In addition to leptin, other secretory factors including adiponectin, tumor necrosis factor- α (TNF- α) as well as the preadipocyte-specific preadipocyte factor-1 (Pref-1) are secreted from adipose tissue [1,4]. These factors are involved in regulating a variety of physiological functions including satiety and energy metabolism, as well as adipocyte differentiation and development.

Resistin/adipocyte-specific secretory factor (ADSF)/FIZZ3 is a protein secreted primarily from adipose tissue in rodents and belongs to a family of proteins named FIZZ (found in inflammatory zone) or RELM (resistin-like molecule) consisting of resistin/ADSF/FIZZ3, RELM α /FIZZ1, RELM β /FIZZ2 and RELM γ [5–9]. Resistin/ADSF/FIZZ3 forms homo-oligomers but can also interact with other RELMs/FIZZs [10,11]. A recent crystal structure study indicates that resistin/ADSF/FIZZ3 circulates in two distinct assembly states, which probably correspond to hexamers as well as more bioactive trimers [12]. By subtractive cloning, Steppan *et al.* originally identified resistin/ADSF/FIZZ3 as an adipocyte-secreted hormone whose expression is suppressed by the insulin-sensitizing PPAR γ agonists, the thiazolidinediones (TZDs), and found that the protein was detected in circulation at a higher level in obese mice [6]. Mice treated with recombinant resistin/ADSF/FIZZ3 had increased insulin resistance and administration of antibody against resistin/ADSF/FIZZ3

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