

# News & Views

## Cancer Research with *C. elegans*

Research with the nematode worm, *Caenorhabditis elegans*, has uncovered several genes that could be used as targets for anti-cancer therapies. The work involved a strain of *C. elegans* with a mutation in a particular gene, LIN-35, which is similar to the *pRb* gene that is inactivated in many human cancers. By using a genome-wide RNA-interference-feeding screen for suppressors of the gene, individual genes were inactivated, in order to identify those which reverse the effects caused by the loss of LIN-35.<sup>1</sup> According to the report published in *Genetics*, “Suppressors of multiple *lin-35* phenotypes may be candidate targets for anticancer therapies. Moreover, screening for suppressors of phenotypically distinct synthetic interactions, which share a common altered gene, may prove to be a novel and effective approach for identifying genes whose activities are most directly relevant to the core functions of the shared gene”.<sup>2</sup>

<sup>1</sup>Anon. (2012). *Worms help solve cancer mystery*. [GEN News, 10.08.12]. Available at: <http://www.genengnews.com/gen-news-highlights/worms-help-solve-cancer-mystery/81247172/> (Accessed 17.08.12).

<sup>2</sup>Polley, S.R. & Fay, D.S. (2012). A network of genes antagonistic to the LIN-35 retinoblastoma protein of *Caenorhabditis elegans*. *Genetics* **191**, 1367–1380.

## Ad Libitum Feeding

Confirming the work of Dr Francis Roe more than 30 years ago, an investigation published in *Chemical Research in Toxicology*<sup>1</sup> indicates that laboratory rodents with unlimited access to food, which is the standard protocol for toxicology studies, are physiologically and metabolically different from rodents fed controlled amounts.

Laboratory rodents are usually fed *ad libitum* and tend to overeat. As a result, they can develop a range of disorders, such as hypertriglyceridaemia, hypercholesterolaemia and nephropathy, that can affect the outcome of experiments. In addition, meal-feeding synchronises biological rhythms, and peripheral clocks in all organs of the body, especially the GI tract and liver, are particularly sensitive to food intake — the feeding schedule has been shown to affect gene expression, and it has also

been shown to alter the toxicity and metabolism of certain drugs. According to the *Society for Toxicologic Pathology*, the feeding schedule is an important variable that should be controlled in toxicology experiments, and research continues to underscore this position.

<sup>1</sup>Carey, G.B. & Merrill, L.C. (2012). Meal-feeding rodents and toxicology research. *Chemical Research in Toxicology* **25**, 1545–1550.

## Dieting Fruit Flies

Adult fruit flies fed an unbalanced diet, developed metabolic alterations and insulin resistance, a hallmark of type 2 diabetes.<sup>1</sup> In order to find out whether diet could cause metabolic changes in *Drosophila melanogaster*, the animals were fed two types of nutrient-high diets (in an attempt to mimic overeating in humans) — a carbohydrate-loaded and a protein-loaded diet. Flies in both groups gained weight, but upon extreme overfeeding, those on the protein-rich diet lost weight. Surprisingly, the protein-loaded diet induced insulin resistance at a quicker and more severe rate than the carbohydrate-loaded diet. According to the principal investigator of the study, Dr Bauer, “the two diets have exactly opposite effects on metabolism... But too much of either one of them causes insulin resistance”.<sup>2</sup> Both diets had a negative impact on the flies’ health and physiology — decreased egg-laying and shortened longevity were recorded. Similar changes were observed in ageing flies, with an insulin-resistant phenotype detectable at early middle-age. This *in vivo* model of insulin resistance might be an alternative to rodent studies for investigating the molecular mechanisms behind insulin resistance and for the development of therapies.

Further research involving fruit flies has shown that, when confronted with a decreased diet, the flies become hyperactive. This happens because an AMP-activated protein kinase stimulates the secretion of the adipokinetic hormone (the equivalent of mammalian glucagon), which acts as the opposite of insulin by signalling the body to release sugar. When the function of the AMP-activated protein kinase was reduced, sugar release decreased and the hyperactive response was halted.<sup>3</sup> According to lead author of the study, Dr Johnson, “this discovery could inform metabolic research in general and dia-

betes research specifically".<sup>4</sup>

<sup>1</sup>Morris, S.N., Coogan, C., Chamseddin, K., Fernandez-Kim, S.O., Kolli, S., Keller, J.N. & Bauer, J.H. (2012). Development of diet-induced insulin resistance in adult *Drosophila melanogaster*. *Biochimica et Biophysica Acta* **1822**, 1230–1237.

<sup>2</sup>Allen, M. (2012). *Human diabetes has new research tool: Overfed fruit flies that develop insulin resistance*. [SMU Research, 04.06.12]. Available at: <http://blog.smu.edu/research/2012/06/04/human-diabetes-has-new-research-tool-overfed-fruit-flies-that-develop-insulin-resistance/> (Accessed 13.08.12).

<sup>3</sup>Braco, J.T., Gillespie, E.L., Alberto, G.E., Brenman, J.E. & Johnson, E.C. (2012). Energy-dependent modulation of glucagon-like signaling in *Drosophila* via the AMP-activated protein kinase. *Genetics* **191**. [E-pub ahead of print.]

<sup>4</sup>Neal, K. (2012). *Hormone in fruit flies sheds light on diabetes cure, weight-loss drug for humans*. [EurekAlert, 09.08.12]. Available at: [http://www.eurekalert.org/pub\\_releases/2012-08/wfu-hif080712.php](http://www.eurekalert.org/pub_releases/2012-08/wfu-hif080712.php) (Accessed 13.08.12).

## NATO Countries Still Using Animals for Military Training

Most of the North Atlantic Treaty Organisation (NATO) nations do not use animal laboratories for military medical training, but Canada, Denmark, Norway, Poland, the UK and the USA confirmed that they did use animals.<sup>1</sup>

In an attempt to examine the prevalence of animal use in military medical training exercises, officials in the 28 NATO countries were surveyed during 2010 and 2011.<sup>2</sup> A variety of training methods are employed to prepare military medical personnel to treat injured civilians and soldiers, including simulators, moulage scenarios, didactics and live animal studies. Of the 28 countries, 22 confirmed that they use exclusively non-animal methods, such as life-like human simulators in realistic battlefield scenarios.

<sup>1</sup>Perle, D. (2012). *Study finds US among few NATO nations that use animals for military training*. [PETA, 08.08.12]. Available at: <http://www.peta.org/mediacenter/news-releases/Study-Finds-US-Among-Few-NATO-Nations-That-Use-Animals-for-Military-Training.aspx> (Accessed 13.08.12).

<sup>2</sup>Gala, S.G., Goodman, J.R., Murphy, M.P. & Balsam, M.J. (2012). Use of animals by NATO countries in military medical training exercises: An international survey. *Military Medicine* **177**, 907–910.

## Model of a Synapse

Understanding how neurotransmitter receptors work is critical for the development of drugs to treat disorders in which altered brain chem-

istry plays a part, such as epilepsy and anxiety. In order to study the behaviour of the GABA-A receptor, which interacts with the neurotransmitter GABA, Wu *et al.* developed a model synapse between a nerve cell and a HEK cell.<sup>1</sup> The work focused on elucidating the role of the  $\alpha$  subunits of the GABA-A receptor protein, and how they might affect the location of the receptor on the cell membrane. They found that the receptor's response to the neurotransmitter GABA varied with the type of the  $\alpha$  subunit present — GABA-A receptors with an  $\alpha 2$  subunit tended to cluster at the synaptic region on the cell membrane, while those with an  $\alpha 6$  subunit tended to migrate to the extrasynaptic region of the cell membrane.

According to Dr Chen, the lead author of the paper, understanding the differences in the behaviour of receptors can be particularly important in predicting what side-effects a drug might cause. For example, drugs such as Valium and Xanax, which target the GABA-receptor and are used to treat anxiety, appear to directly change the GABA neurotransmitter's synaptic transmission, significantly altering nerve cell activity and causing side-effects such as confusion, agitation, and memory loss. Therefore, targeting extrasynaptic receptors by modulating the  $\alpha 6$  subunit might be a step toward creating new drugs with fewer side-effects.<sup>2</sup>

<sup>1</sup>Wu, W., Wu, Z., Ning, G., Guo, Y., Ali, R., Macdonald, R.L., De Blas, A.L., Luscher, B. & Chen, G. (2012).  $\gamma$ -Aminobutyric acid type A (GABA<sub>A</sub>) receptor  $\alpha$  subunits play a direct role in synaptic versus extrasynaptic targeting. *Journal of Biological Chemistry* **287**, 27,417–27,430.

<sup>2</sup>Penn State (2012). *New model synapse could shed light on disorders such as epilepsy and anxiety* [ScienceDaily, 07.08.12]. Available at: <http://www.sciencedaily.com/releases/2012/08/120808093854.htm> (Accessed 16.08.12).

## Beagle Breeding Facility Temporarily Closed

An Italian court has ordered the temporary closure of one of the largest suppliers of dogs for research in Europe, after two animal rights groups, the Anti-vivisection League and Legambiente, alleged that dogs were maltreated. On 18 July 2012, the court judged that the allegations warranted further investigation, and gave the animal rights groups responsibility for the care of the 2500 beagles at the Green Hill facility (Montichiari, Italy).

Magda Chlebus, head of science policy at the European Federation of Pharmaceutical Industries and Associations, says that the closure

could be troublesome for drug developers, because the facility supplies many of the dogs used in Europe, and that studies could end up being conducted in countries with less stringent animal protection laws.<sup>1</sup>

<sup>1</sup>Abbott, A. (2012). Court orders temporary closure of Italian dog-breeding premises. [*Nature News*, 03.08.12]. Available at: <http://www.nature.com/news/court-orders-temporary-closure-of-italian-dog-breeding-premises-1.11121> (Accessed 17.08.12).

## Bacterial Mutant Screening

A common method for studying bacterial virulence involves infecting laboratory animals with specific bacterial strains and comparing the pathogenicity of the mutants with that of wild-type strains. A team from the University of Porto (Porto, Portugal), has developed a new strategy for screening targeted bacterial mutants that is fast and uses significantly fewer animals than the traditional method.

This new approach relies on PCR amplification to discriminate between a mixed bacterial sample in infected mouse organs. Provided that gene-specific primers are used, the intensity of the band of the PCR product is directly related to the quantity of the corresponding strain in a pool of mutants. This procedure was successfully employed to screen mutants in a murine model of listeriosis, and could be adapted to other bacterial systems. Overall, it provides a simple, rapid and sensitive method to screen targeted bacterial mutants, while reducing the number of animals required.<sup>1</sup>

<sup>1</sup>Henriques, A., Carvalho, F., Pombinho, R., Reis, O., Sousa, S. & Cabanes, D. (2012). PCR-based screening of targeted mutants for the fast and simultaneous identification of bacterial virulence factors. *BioTechniques Rapid Dispatches*. Available at: [http://www.biotechniques.com/multimedia/archive/00182/BTN\\_A\\_000113906\\_O\\_182002a.pdf](http://www.biotechniques.com/multimedia/archive/00182/BTN_A_000113906_O_182002a.pdf) (Accessed 17.08.12).

## Microfluidic Platform for Diabetes Research

Long-term exposure to excess glucose and fatty acids, a process referred to as glucolipotoxicity, can induce dysfunction of insulin-producing cells and lead to type 2 diabetes. Lam *et al.* have described an *in vitro* system that allows the study of the interplay between glucose and fatty acid oxidation in living pancreatic islets.<sup>1</sup>

The team developed a microfluidic system to move test solutions containing glucose or fatty acids around stationary pancreatic islets, the insulin-secreting tissue of the body. By holding the islets stationary in flow, confocal imaging of electron transfer flavoprotein (ETF) autofluorescence was successfully used to study the dynamics of fatty acid oxidation in  $\beta$ -cells within the islets. The results suggest that, in  $\beta$ -cells, glucose metabolism has a dominant role over fatty acid oxidation, since increasing the concentration of sugar in this system reduced the metabolism of fats within minutes. According to Dr Rocheleau, the lead author of the paper: "The  $\beta$ -cell is exquisitely sensitive to glucose, even with the availability of other nutrients; increasing the time to turn off fat metabolism after eating is critical to  $\beta$ -cell survival and a potential target for future therapies."<sup>2</sup>

<sup>1</sup>Lam, A.K., Silva, P.N., Altamentova, S.M. & Rocheleau, J.V. (2012). Quantitative imaging of electron transfer flavoprotein autofluorescence reveals the dynamics of liquid partitioning in living pancreatic islets. *Integrative Biology* **4**, 838–846.

<sup>2</sup>Anon. (2012). *Diabetes: Sugar reduces fat metabolism*. [UHN News, 03.08.12]. Available at: <http://uhnresearch.ca/news/php/readarticle.php?id=40788> (Accessed 22.08.12).

## Testing the Effects of Pesticides on Human Cells

A preliminary study conducted at Aston University (Birmingham, UK), with the aim of evaluating the health risks of mixtures *versus* single pesticides, suggests that the harmful effects of some commonly-used pesticides on human cellular health deserves further attention.<sup>1</sup>

In order to model the basic cell types of the human central nervous system, two cell lines were used: U251 cells as glial cells and SH-SY5Y cells as neuronal cells. The effects of three widely-used biocides (pyrimethanil, ciprodinil and fludioxonil) on cell viability, mitochondrial dysfunction and generation of oxidative stress were assessed. Professor Michael Coleman, who led the study, says that: "This work shows that some pesticides, alone or in combination, can induce stress and significantly affect human cells. They can also interfere with basic cellular processes, such as energy production. These effects were demonstrated at concentrations similar to those found in our food. This work suggests that we should make greater efforts to restrict pesticide use in crops intended for food."<sup>2</sup>

<sup>1</sup>Coleman, M.D., O'Neil, J.D., Woehrling, E.K., Ndunge, O.B., Hill, E.J., Menache, A. & Reiss, C.J. (2012). A preliminary investigation into the impact of a pesticide combination on human neuronal and glial cell lines *in vitro*. *PLoS One* **7**, e42768.

<sup>2</sup>Anon. (2012). *New scientific study reveals the adverse effects of mixtures of some commonly used pesticides*. [Antidote Europe, 07.08.12]. Available at: <http://antidote-europe.org/releases/pesticides> (Accessed 22.08.12).

## Comment on REACH

A recent report<sup>1</sup> suggests that the EU system for the registration, evaluation, authorisation and restriction of chemicals (REACH) is scientifically flawed. Even though it is a well-intentioned testing programme that aims to protect human health and the environment by placing the burden of proof on manufacturers to demonstrate the safety of their products, it could ultimately be self-defeating in that manufacturers must conform to invalid test methods to predict human health outcomes.

The report calls for immediate measures to be taken, including: a biomonitoring programme of EU citizens, so that priority can be given to the most dangerous substances; the use of biomarkers in biological fluids to help guide public policy; and an approach based on pollution prevention, rather than control, to limit environmental chemical pollution and risks to humans.

<sup>1</sup>Menache, A. & Nastrucci, C. (2012). REACH, animal testing, and the precautionary principle. *Medicolegal & Bioethics* **2**, 13–29.

## Awards presented at the EUSAAT 2012–Linz 2012 Congress

The EUSAAT 2012–Linz 2012 Congress was held in Linz, Austria, on 5–8 September 2012. At the Congress Social Evening a number of prizes were awarded.

Two EUSAAT Prizes, comprising an Award Certificate and €250, were presented to:

— A.R.M. von Bergh, M. Teunis, and C.A.M. Krul (Innovative Testing in Life Sciences and Chemistry, Research Centre Technology & Innovation, University of Applied Sciences Utrecht, Utrecht, The Netherlands), and S.C. van der Linden, B. Lussenburg, L. Jonker, and B. van der Burg (BioDetection Systems BV, Amsterdam, The Netherlands), for their poster entitled, *Pre-validation of a High-throughput Reporter Gene Assay to Detect Genotoxicity and Oxidative Stress*

— S.N. Kolle, B. van Ravenzwaay and R. Landsiedel (BASF SE, Ludwigshafen, Germany), K. Sullivan (Physicians Committee for Responsible Medicine, Oakland, CA, USA), and A. Mehling (BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany), for their poster entitled, *Applicability of In Vitro Test Strategies for Skin Irritation to Regulatory Classification Schemes: Substantiating Test Strategies with Data from Routine Studies*

The 4-Pfoten/4-Paws Prize, comprising an Award Certificate and €250, was presented to:

— Y. Kaluzhny, L. d'Argembeau-Thornton, P. Hayden and M. Klausner (MatTek Corporation, Ashland, MA, USA), and H. Kandarova (MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia), for their poster entitled, *Development of the EpiOcular™ Eye Irritation Test for Hazard Identification and Labelling of Eye Irritating Chemicals in Response to the Requirements of the EU Cosmetics Directive and REACH Legislation*

The ALTEX Award 2012 was presented to:

— Erwin van Vliet, of CAAT, Johns Hopkins University Baltimore, MD, USA, for the ALTEX 2011 publication, *Current Standing and Future Prospects for the Technologies Proposed to Transform Toxicity Testing in the 21st Century* (ALTEX **28**, 17–44).