neuron that expresses the protein odorant receptor 22a (Or22a) was more abundant in *D. sechellia* than in any other fly species they analysed. And in both *D. sechellia* and *D. simulans*, these neurons were attuned to a class of compound prevalent in noni odour. Subsequent work revealed similar changes in further sets of noni-sensitive neurons[3,4]. Could these olfactory alterations underlie the specialists' appetite for the smelly fruit? It seemed likely, but an inability to precisely manipulate *D. sechellia*'s olfactory system prevented scientists from moving beyond correlational evidence.

Now, Auer *et al.* have finally cracked the case using the genome-editing tool CRISPR–Cas9. This technology is commonly used in model organisms such as *D. melanogaster* and the mouse, *Mus musculus*, to manipulate genes at will. However, importing the technique into other species is not always straightforward. Other animals might take poorly to life in the laboratory, or it could be difficult to obtain enough viable embryos during the crucial time frame when genome editing takes place. The authors cleared these obstacles, thus gaining the precise genetic control necessary to begin rigorous causality testing.

Auer *et al.* focused on how changes in Or22a contribute to *D. sechellia*'s selective diet. Inactivating the *Or22a* gene left the fly almost completely unable to locate its favourite fruit from just under one metre away. This result confirmed that neurons expressing Or22a process cues that help *D. sechellia* to target noni. But removing a receptor completely is a drastic manipulation — more extreme than the receptor 'tuning' that occurred as Or22a evolved greater sensitivity to noni compounds. It is similar to asking whether you can still perform a concerto on a violin missing a string. The missing string is clearly important, but you cannot tell how its tuning would have affected your performance.

The authors therefore sought to explicitly test how tuning changes in Or22a affect noni-seeking behaviour. They substituted Or22a in *D. melanogaster* with the version of the receptor from *D. sechellia*, and vice versa. The two species' receptors are nearly identical, harbouring just a few changes in amino-acid residues that tweak sensitivity to different compounds. To continue the musical analogy, we might compare this experiment to swapping strings between a violin and a viola and asking how the mismatched, differently tuned strings on each instrument affect the recital. Remarkably, the receptor swap gave *D. melanogaster* a slight taste for noni and diminished *D. sechellia*'s attraction to the fruit. This definitive test, made possible by the group's cutting-edge toolkit, clearly showed that changes in Or22a tuning contribute to *D. sechellia*'s partiality for noni.

Of course, evolution of Or22a tuning is only part of the story. One of the most interesting aspects of Auer and colleagues' study is just how many evolutionary changes might contribute to this apparently simple behavioural shift. The authors confirmed[2] that, besides tinkering with Or22a's tuning, evolution has amplified its ability to trigger downstream signalling in *D. sechellia* by doubling or tripling the number of Or22a neurons. Further receptor-deletion experiments strongly suggested that previously documented changes[3,4] in two other key classes of sensory neuron are also involved. And remodelling of downstream circuits might play a part, too: Auer *et al.* discovered a structural branch on neurons deep in *D. sechellia*'s brain that could alter how the fly processes information about noni odour.

Unfortunately, it remains difficult to directly test causality for many of these evolutionary changes. We can cleanly manipulate the activity of sensory neurons by altering the receptors they express, and can even modify the activity of neurons deeper in the brain — as demonstrated by recent work on the evolution of central brain circuits in two other non-model *Drosophila* species[11,12]. However, it is difficult, if not impossible, to precisely manipulate structural features such as the number of neurons in a circuit or the connections between them. This wiring is established early in an animal's development, and has a genetic basis that is not yet well enough understood to allow custom manipulation. As our neurogenetic toolkits expand, it will be exciting to continue piecing together the puzzle of *D. sechellia*.

We are entering an era in which genetic tools are available to alter precise targets in the nervous systems of diverse organisms. At the same time, we have countless observations of variations in animal behaviour at our disposal, gathered over the past century and more. By combining these two resources, as Auer *et al.* have done in *D. sechellia*, we can finally begin to test long-standing hypotheses about behavioural evolution across a diverse range of organisms. Even humble flies that love stinky fruit can provide powerful insight into how brains evolve to shape complex behaviours.

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This article was published online on 4 March 2020.

Flow chemistry

Automated synthesis on a hub-and-spoke system

Klavs F. Jensen

Organic compounds can be synthesized in a continuous flow of solutions, but the need to balance mass flow across multiple reactors complicates the development of such systems. A new platform for flow chemistry addresses this issue. **See p.379**

The desire to perform chemical synthesis quickly and without tedious manual manipulations has long driven the development of automated chemical synthesizers. On page 379, Chatterjee and colleagues[1] report an automated approach that they describe as radial synthesis. In their system, individually accessible compartments for performing reactions are arranged around a central hub that coordinates reagent delivery, product sampling and chemical analysis, and the temporary storage of compounds produced as intermediates. The authors' approach not only promises to reduce manual manipulation, but also eliminates the need to customize a synthesizer for each target molecule.

The synthesis of structurally complex organic molecules is the first task in the discovery of functional compounds needed for new technologies, including those in medicine and flexible electronics. Starting with relatively simple, purchasable reactants, the process

involves sequences of chemical reactions in which the complexity of the molecules produced gradually increases with each step towards the final target. The sequences can be linear, or convergent (different parts of the target molecule are made in separate sequences and then joined together). To ensure that large amounts of the final product are prepared, each synthetic step must be high-yielding and reproducible, and must generate few side products.

Conventional chemical synthesis is performed 'in batch' (in a flask). However, this requires multiple manual interventions from chemists to prepare, run and stop each reaction, and to isolate the desired product from any by-products that cannot be tolerated in subsequent steps. An approach known as flow chemistry[2] has therefore been developed to address this problem. Flow chemistry can also increase the productivity of synthetic routes compared with batch procedures, and is safer for syntheses that involve potentially hazardous components.

In flow chemistry, a continuous stream of reactants is pumped through a heated or cooled reactor (typically, a tube) to form the product. To carry out multistep synthesis, multiple reactor units are usually connected in series, so that the output of one reactor becomes – along with any further reagents – the input to the next (Fig. 1a). In-line separation systems can be added to purify intermediate compounds or to switch solvents.

The mass flow must be balanced so that the flow exiting each reaction or separation step equals the sum of the input flows from the previous step and from any added reagents and solvents, minus any diverted streams (which occur when compounds are extracted from one solvent into another). This mass-flow constraint means that different reactor types and volumes are needed to achieve the best-possible product yield in each step: slow reactions require large reactors, whereas fast reactions need small reactors.

Because reaction rates increase with temperature, flow-reactor temperatures can sometimes provide flexibility in the choice of reactor sizes. For example, high temperatures can make relatively slow reactions fast enough to enable the use of a small reactor. However, high temperatures can also promote side reactions, including those that cause decomposition of the desired product. Therefore, the need to balance mass flow often dictates a specific sequence of reactor volumes and temperatures for a particular multistep synthesis. This, in turn, means that the platforms used for flow chemistry must be reconfigured – reactors must be replaced with others that are more appropriately sized – for each new target molecule, decreasing flexibility and increasing the development time (the time needed to optimize the platform and chemistry for each new synthesis).

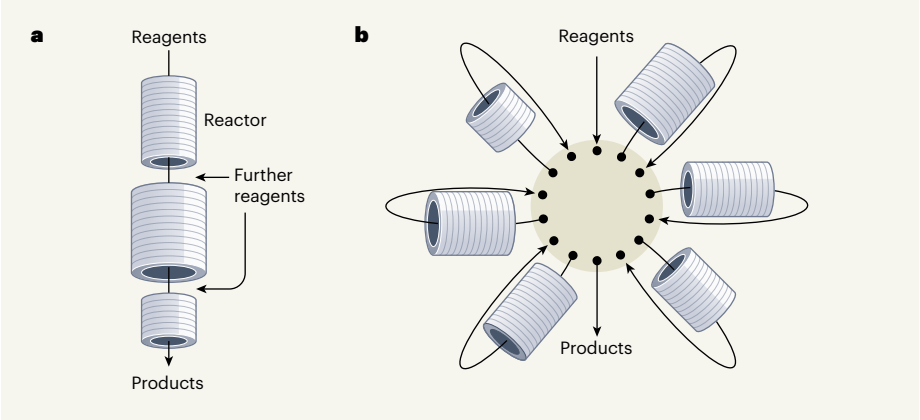

Figure 1 | Linear and radial flow systems for chemical synthesis. a, Multistep routes for synthesizing organic molecules can be carried out in a continuous flow of solvent. A solution of reagents is typically passed through a series of reactors; further reagents are introduced as needed. A different reaction occurs in each reactor, the product of which becomes the input for the next reactor. A solution of target molecules is produced as the output of the flow system. However, reactors and other modules in these systems often need to be manually replaced to optimize the platform for each synthetic route. **b**, Chatterjee *et al.*[1] report a flow platform in which a central hub is surrounded by individually accessible reactors. The hub coordinates reagent delivery to the reactors in any sequence, thereby allowing the most suitable reactor to be used for any particular reaction (black dots indicate ports through which solutions are passed from or to the hub). This removes the need to customize the system manually for each synthetic route.

Chatterjee and colleagues' radial synthesizer is designed to overcome this issue. A master controller in the central hub of their system uses multi-positionable valves to direct reagents to different reactor modules around it (Fig. 1b), so that each reaction in a synthesis can be performed at optimal conditions – for example, at the best temperature and concentration of reagents, and in the most appropriate reactor type. The effluent from each reactor returns to the central hub for in-line analysis and subsequent distribution to the next synthesis step, which could be in the same reactor unit or in a different one. This process repeats for each reaction step until the target molecule has been synthesized (in three steps, for most of the examples reported by Chatterjee and co-workers).

"The key advance of the authors' radial synthesizer is that it decouples the reaction steps."

The system allows both linear and convergent synthesis – in the latter case, intermediate products are stored and then joined together in the final step. But the key advance of Chatterjee and colleagues' radial synthesizer is that it decouples the steps by directing reagents to an appropriate module for each reaction, independently of the other steps. This eliminates the mass-flow constraints and the need to reconfigure the platform for different synthetic sequences, reducing the development time.

The need to store intermediate compounds produced from different steps until they are required for the next reaction increases the overall process time and the total volume of the flow platform, compared with linear sequences in conventional flow synthesis. The longer process times are likely to be offset by the shorter development times when synthesizing many different compounds in laboratory quantities (milligrams to grams). However, longer process times could be a disadvantage in the commercial synthesis of individual organic compounds, for which productivity per unit volume of the flow system matters.

The need to store intermediates also limits applications in which the intermediates are potentially hazardous – a disadvantage compared with conventional flow-chemistry platforms, which contain only small amounts of intermediates at any given time. For such cases, the radial synthesizer would need to be configured without interim storage and to have a short transfer time between successive reactor units, effectively creating a conventional linear system.

Previously reported automated synthesis platforms have been designed so that standardized reactors and other modules (such as those used for extractions) can be easily plugged into and taken out of reconfigurable linear sequences by hand, enabling simple customization for different syntheses[3,4]. More recently, a system has been reported[5] in which machine learning is combined with a chemist's expert knowledge to plan the synthesis of a target compound. The synthesis is automatically converted into a robotically assembled flow system that prepares the compound. Automated multistep reaction sequences can also

be carried out in robotically controlled batch reactors integrated with liquid handlers, purification and analytical systems[6]. And a robotic system that uses conventional laboratory apparatus, such as round-bottomed flasks, and which uses a standardized approach (the 'chemputer') to translate chemical-synthesis methods into physical operations, was reported last year[7].

Such automated systems ensure reproducibility, because a given instruction set for a synthesis will be carried out in exactly the same way on an identical system at another location, assuming that the input materials are of the same quality. Furthermore, these systems make it feasible for potentially dangerous compounds, such as potent pharmaceuticals or radioactively labelled compounds used for medical diagnostics, to be made without exposure to humans. Automated systems could also be used to generate reaction data – such as the reagents used, yields and conditions – for machine learning in organic chemistry. However, this will require further miniaturization of existing systems, which are currently too large, and therefore too slow to produce sufficient data (equivalent to tens of thousands of experiments) in a useful time frame (weeks) to enable machine learning. Modules for analysing reactions will also need to be incorporated into flow systems to produce such data.

Chatterjee and co-workers' radial synthesizer and other automated platforms go a long way towards eliminating tedious manual operations from chemical synthesis. Nevertheless, challenges remain, particularly in the handling of solids – whether solid reagents or solids formed during reactions. Solids can be suspended in slurries by stirring reaction vessels, but the transfer of slurries through tubes (or even more problematically, through valves) leads to clogging.

Another problem is how to seamlessly integrate purification, isolation and analytical procedures. Robotic systems and the radial synthesizer offer opportunities to develop hybrid platforms that integrate the best elements of flow and batch technologies with purification and analytical methods. This would enable automated synthesis that incorporates state-of-the-art reactions and can produce more-complex molecular structures than have been achieved so far. As the hardware matures, the emphasis will shift to developing the control and artificial-intelligence infrastructure necessary to generate and implement chemical syntheses[5] – freeing chemists from carrying out routine procedures, so that they can focus on discovering new chemical reactions.

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Cell biology

Mitochondrial distress call moves to the cytosol

Bradford P. Tremblay & Cole M. Haynes

Cellular stress can result in dysfunction and disease, and mechanisms exist to combat this. Previously unknown steps have been uncovered in a pathway that signals when mitochondrial organelles are dysfunctional. **See p.427 & p.433**

Organelles called mitochondria are responsible for storing energy derived from the food that we eat, in the form of molecules called ATP. Although a mitochondrion has its own genome, 99% of this organelle's proteins[1] are encoded by nuclear genes and imported from the cytosol (the liquid part of the cytoplasm) into the mitochondrion. To function effectively, this process requires coordination and communication, and it must be able to respond to any mitochondrial dysfunction that might occur. Environmental toxins[2] and disease-causing agents[3], as well as diverse age-associated conditions, including Alzheimer's disease[4] and Parkinson's disease[5], are linked to mitochondrial dysfunction.

"The authors identified metabolites that were consistently released from all dying cells."

Now, Guo *et al.*[6] (page 427) and Fessler *et al.*[7] (page 433) report a previously unknown mechanism that is used by mitochondria to send a signal of their dysfunction to the cytosol and nucleus, enabling the cell to adapt to mitochondrial stress.

Studies of the nematode worm *Caenorhabditis elegans* indicate that coordination between the nucleus and mitochondria during stress is regulated by a combination of remodelling of chromatin (the complex of DNA and protein in the nucleus) and activity of a transcription-factor protein that responds to mitochondrial dysfunction[8,9]. Mammalian studies paint a different picture and implicate a process called the integrated stress response (ISR), which causes an overall reduction in protein production but an increase in the production of several transcription factors. The ISR is activated in response to diverse cellular stresses, including those that don't involve mitochondria. In 2002, researchers discovered[10] that mitochondrial perturbations drive the synthesis of a component of the ISR – a transcription factor called CHOP – and induce the expression of two types of mitochondrial protein that aid the ISR. These are chaperones, which aid protein folding, and proteases, which are enzymes that cleave proteins. One enduring mystery has been whether mitochondrial dysfunction also directly regulates kinase enzymes in the cytosol that are needed for the ISR, and that act by adding a phosphate group to proteins.

The ISR is regulated by such phosphorylation of the protein eIF2α (Fig. 1), which is involved in initiating the translation of messenger RNA during protein synthesis. eIF2α phosphorylation is mediated by four kinases – GCN2, PERK, PKR and HRI – that each phosphorylate eIF2α in response to different stressors. GCN2 is stimulated by depletion of amino acids; PERK responds to the presence of unfolded proteins in an organelle called the endoplasmic reticulum; PKR acts when double-stranded RNA accumulates in the cytoplasm during viral infection; and HRI is enlisted when the molecule haem is depleted[11,12]. The phosphorylation of eIF2α results in a reduction of total protein synthesis, but promotes production of the transcription factors ATF4, ATF5 and CHOP. These transcription factors harbour regulatory elements in their mRNA that facilitate translation when eIF2α is phosphorylated[11,13].

To understand how mitochondrial stress triggers an ISR, Guo *et al.* and Fessler *et al.* took similar experimental approaches using