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Biological Clocks

The timekeeping mechanisms of life

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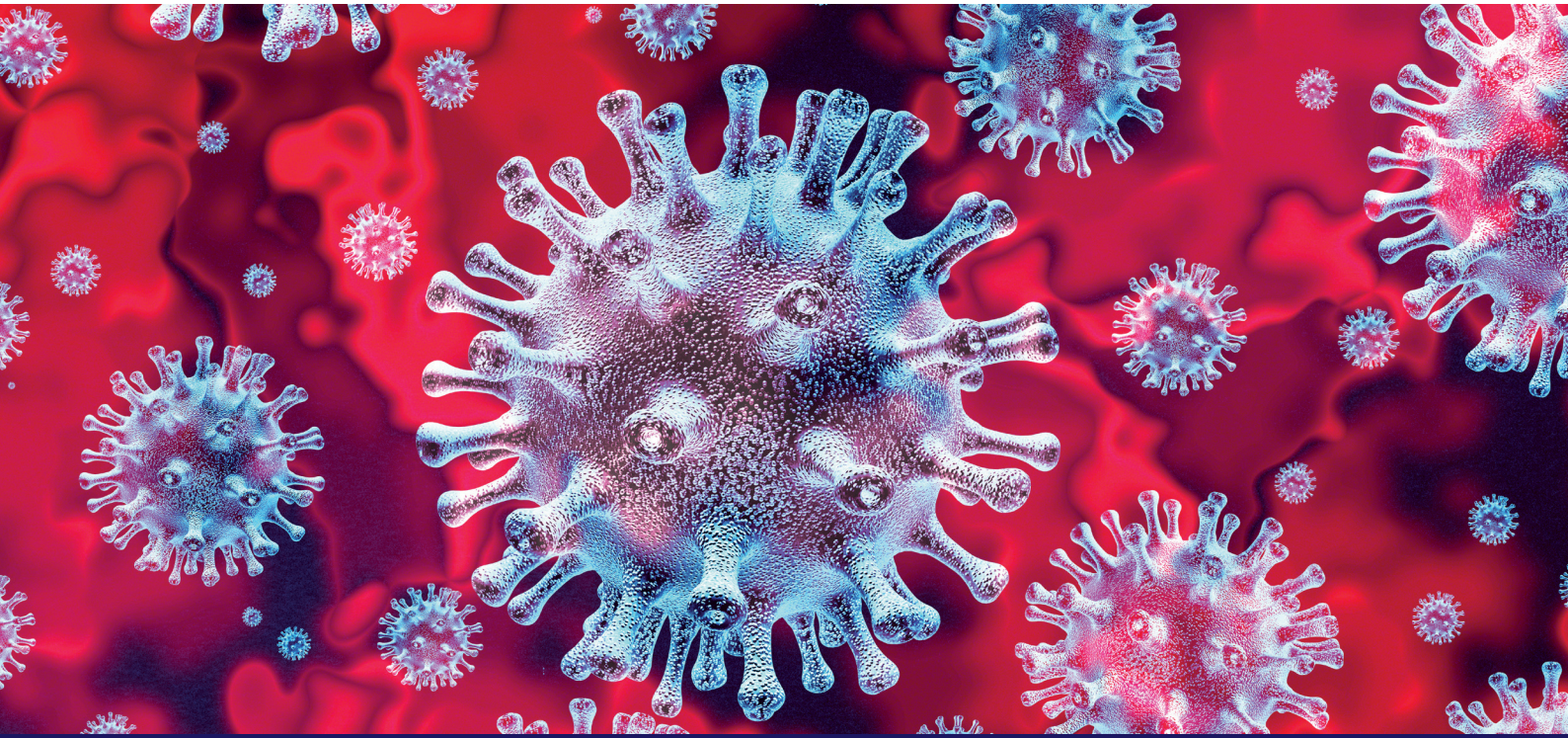

The Biochemical Society and its publisher, **Portland Press**, have signed a joint statement committing to rapid and open sharing of research data and findings relevant to the coronavirus (2019-nCoV) outbreak.

As of 31 January 2020, we've made relevant published articles free to read. We're also making new papers related to the outbreak available via open access on publication and are encouraging authors to post submitted versions of articles as preprints.

Our reach on this is global. Given the unprecedented speed and number of papers in response to the outbreak, our intention is to ensure research findings and relevant data are shared quickly and widely.

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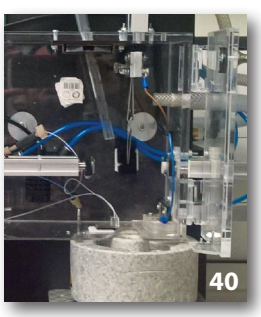

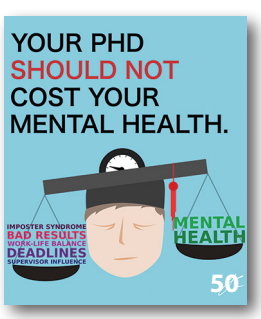

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Research Culture (June)
Plant Genomics (August)
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Pharma 4.0 - Drug Discovery (December)

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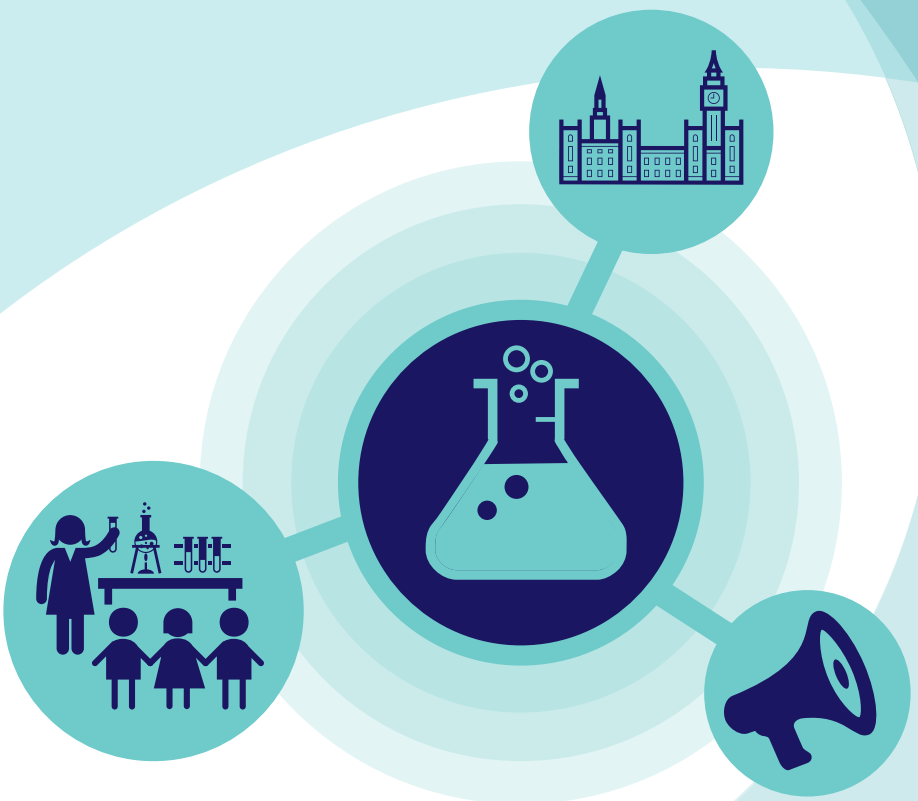

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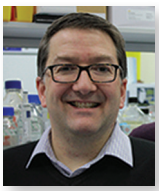
Let's be open about this

by Chris Willmott, Science Editor



Despite the emergence of new media, publication of peer-reviewed papers remains the principal way by which scientific research is disseminated. For those of us with British sensibilities about discussing money, it is an uncomfortable truth that publication is not a zero-cost enterprise; articles need to go through various editorial processes, as well as being made available via electronic and/or printed format. All these stages incur costs.

Historically, the burden of paying for this process fell on institutional and individual subscribers, which, crudely speaking, represents a 'reader pays' model. For a number of reasons, not least the fact that much of the research has been funded from the public purse and therefore 'belongs' to everyone, there is a widespread commitment to move to Open Scholarship, in which papers are freely available to any interested parties.

I do not imagine that many readers of *The Biochemist* will be unaware that this revolution is underway. However, fewer may have thought about the complexities of making the transition from traditional subscriber-based publication to open access (OA) models. In the August 2019 issue of this magazine, Malavika Legge, Director of Publishing for the Biochemical Society and our wholly owned publisher Portland Press, introduced us to some of the Society's thinking about this process. Portland Press publishes seven journals plus *The Biochemist*. Two of the journals are already fully OA, but the other five are currently 'hybrid' titles in which most of the content is behind a paywall, with approximately 20% available to everyone as a consequence of authors (or someone on their behalf) opting to pay an article publishing charge (APC) for OA.

The Biochemical Society shares the ambition to transition to OA. One of our journals *Bioscience Reports* was flipped to full OA in 2012. This was an overnight 'cliff-edge' switch to 'Gold' OA, with articles appearing under a CC BY licence. This was a risky strategy, which might have caused the journal to perish. It did not, but it was a bumpy ride that took 5 years to stabilize.

In 2020, the Society is undertaking an exciting and innovative pilot project. As an alternative to renewing their existing contracts, current institutional subscribers to our journals are being offered 'Transformative renewal' in which, for an additional amount (often totalling less than one APC fee), their employees have unlimited capacity to submit APC-free publications, on top of their access to read all of the paywalled content. This '*Read & Publish*' model, also being trialled by the Microbiology Society for their journals, is transitional, an interim solution which, it is hoped, offers an equitable and sustainable route towards full OA. Many of the Society's approximately 700 subscribing institutions have elected to take part in this pilot. If your's has not, why not raise the possibility with your librarians and/or Open Publication coordinators?

For more information see: http://bit.ly/Read-Publish ■

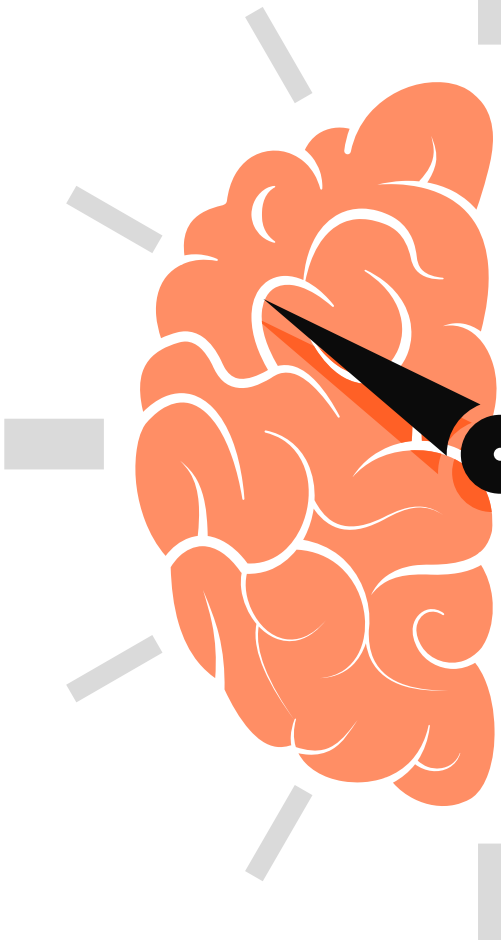
Biological clocks: insights from Portland Press and beyond

Biological rhythmicity has relevance to almost every aspect of our lives, from sleep–wake cycles and everyday metabolism to physical and mental health implications. We experience jet lag because our biological clocks can only shift by a certain amount, treatment for which has been explored but is perhaps not as effective as we thought (https://www.theguardian.com/society/2020/jan/14/melatonin-should-not-be-made-available-on-nhs-to-treat-jet-lag).

This issue explores the molecular and wider regulation of these rhythms in mammals, plants and insects, and the behavioural effects we see as a result. We hope you enjoy this issue.

A recent article in *Biochemical Society Transactions* explores the unique transcription mechanisms in cyanobacteria. As explained throughout this issue of *The Biochemist*, transcription factors play a huge role in regulating circadian rhythms. The authors explain how key differences in transcription mechanisms in cyanobacteria enable them to synchronize cell activities to a circadian rhythm, a function that has not yet been observed in other eubacteria.

Biochem Soc Trans (2019) 47 (2): 679–689.
https://doi.org/10.1042/BST20180508



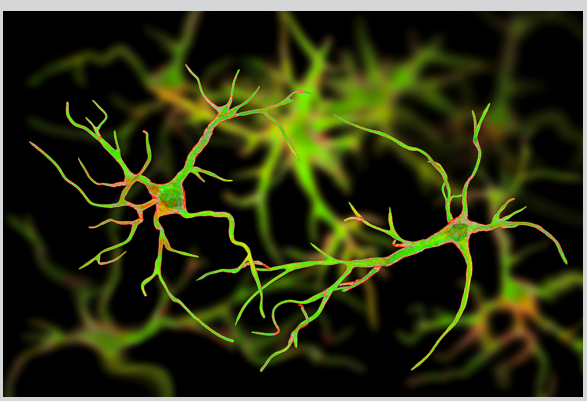
Glossary – A handy guide for the rest of the issue

- **SCN:** Suprachiasmatic nucleus, a region of the hypothalamus that regulates circadian rhythms. It has input from the optic chiasm and extensive endocrine and autonomic outputs to the rest of the body.
- **Clock genes:** A group of genes expressed in the SCN. CLOCK, BMAL, PER and CRY are expressed in the mammalian clock, while in *Drosophila*, analogues CYCLE and TIM are expressed instead of BMAL and CRY.
- **Circadian:** A rhythm occurring naturally on a 24-hour cycle, for example, the sleep–wake cycle.
- **Diurnal:** A rhythm occurring within a day/within daylight hours, for example, cortisol secretion.
- **Oscillator:** A mechanism, process or component that produces biological rhythms, for example, the SCN or a clock gene.
- **Zeitgeber:** An external cue that entrains biological rhythms, for example, light. Non-photic zeitgebers also exist, such as social cues. See entrainment.
- **Entrainment:** The synchronization of the internal biological clock to external cues.

You may be aware that altering your circadian rhythms, for example, through shift work or jet lag, has adverse effects on metabolic health. A key explanation of this is gut dysbiosis, an imbalance of microbiota caused by consuming food at suboptimal times, which alters the natural diurnal rhythms of microbes. An article published in *Clinical Science* in 2018 explores the links between gut microbiota and the circadian cycle, gut dysbiosis and the development of various metabolic disorders.
Clin Sci (2018) 132 (7): 791–811.
https://doi.org/10.1042/CS20171328

Within our master clock, the SCN, neurons play a vital role in relaying information to the rest of the brain and body. Astrocytes also express clock genes and constitute a large part of the SCN. They play a fundamental role in our biological rhythmicity, mostly achieved by supressing the circadian activity of neurons.

Authors of a recent article published in *Science* showed how astrocytic transcription factors can drive molecular oscillations in the SCN and circadian behaviour of mice in the absence of neuronal input. This contradicts what we previously thought about the control and initiation of complex behaviour being strictly neuronal. doi: 10.1126/science.aat4104

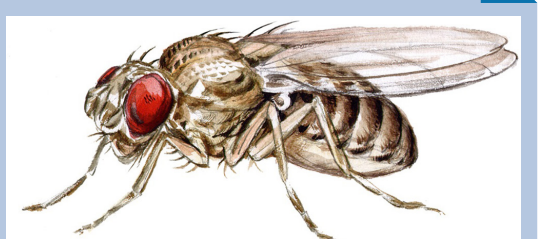


Interested in the history of the molecular clock? A mini-review from *Biochemical Society Transactions* offers an insight into how the molecular clock has impacted our understanding of evolution and the timescales of life on earth.
Biochem Soc Trans (2018) 46 (5): 1183–1190.
https://doi.org/10.1042/BST20180186

Drosophila – why are these tiny creatures so important for circadian research?

Professor Bambos Kyriacou from the University of Leicester gives us an introduction to his research into circadian rhythms and explains the importance of *Drosophila* in this, and other, behavioural research.

Take a look here: https://youtu.be/LOGeTdcnqFM



Clock-in, clock-out: circadian timekeeping between tissues

Jacob G. Smith and **Paolo Sassone-Corsi** (University of California, Irvine, USA)

Life evolved in the presence of alternating periods of light and dark that accompany the daily rotation of the Earth on its axis. This offered an advantage for organisms able to regulate their physiology to anticipate these daily cycles. In each light-sensitive organism studied, spanning single-celled bacteria to complex mammals, there exist timekeeping mechanisms able to control physiology over the course of 24 hours. Endowed with internal timekeeping, organisms can put their previously stored energy to the most efficient use, selectively ramping up biological processes at specific times of day or night according to when they will be needed. Humans have evolved to be more active during the day (diurnal), likely due to the increased opportunities for foraging or hunting in our evolutionary past, and this daily activity is accompanied by an up-regulation of genes involved in metabolism to increase the energy available for such behaviours. Remarkably, this happens without conscious thought—due to a complex organism-wide signalling apparatus known as the circadian clock network, which conveys time information between cells and tissues.

A clock in every cell

Circadian clocks are self-sustaining timekeepers, able to continue on repeat, cycling through a biological program of events lasting approximately 24 hours (hence circadian – from Latin circa, 'about', and diem, 'day'). Clocks tick due to a biological feedback loop present in almost every cell of the body, in which interlocked cycles of gene expression and protein production drive daily rhythms in nearly all aspects of cellular function. At the centre of this feedback loop in mammals are two nuclear proteins, the aptly named CLOCK (Circadian Locomotor Output Cycles Kaput) and its counterpart BMAL1 (Brain and Muscle ARNT-Like 1), which bind specific regions of DNA called E-boxes and promote nearby gene expression (Figure 1). In humans, during night-time (i.e. rest phase), CLOCK/BMAL1 bind E-boxes within regulatory regions of the genes *Per* (Period, 1–3) and *Cry* (Cryptochrome, 1–2). *Per* and *Cry* messenger RNAs are produced and then translated into PER and CRY proteins in the cytoplasm. As the day begins, PER and CRY interact and pass back into the nucleus, where they repress CLOCK/BMAL1, switching off circadian gene expression including their own. Towards the end of the day, enzymes phosphorylate the PER/CRY complex, which targets PER/CRY for degradation and frees up CLOCK/BMAL1 to start the cycle again. This core feedback loop cooperates with a host of additional regulatory mechanisms to form a cellular clock that drives daily rhythmic expression of a large fraction of mammalian genes. Not only does the clock control gene expression, essentially every other subcellular process has been shown to exhibit some level of daily regulation through a variety of cooperative signalling mechanisms (see Further reading).

One of the most important features of circadian clocks is their ability to adapt to changes in the environment. Within the body, specific signals derived from the external environment can act as zeitgebers ('time givers' in German) and reset the timing of cellular clocks. This phenomenon is critical, allowing clock timing to be realigned in response to changing conditions. In addition, cellular clocks throughout the body must also remain synchronized to one another, in order to allow coordination of physiology at the systemic level. Achieving alignment of clocks therefore represents a colossal signalling challenge for organisms, the mechanisms of which are still being revealed.

The circadian network

The first level of functional organization of cellular clocks occurs at the tissue level; clocks within a particular tissue must behave synchronously in order to cohesively regulate organ function. In the pancreas for example, insulin-producing β-cells influence clock timing in glucagon-emitting α-cells so that insulin and glucagon release is timed appropriately in response to food. Despite the importance of cell-to-cell clock coordination within tissues, how this is achieved is not yet well understood. To date, research suggests that such coordination may depend on either the exchange of soluble signalling factors between cells or the physical contacts between cells themselves.

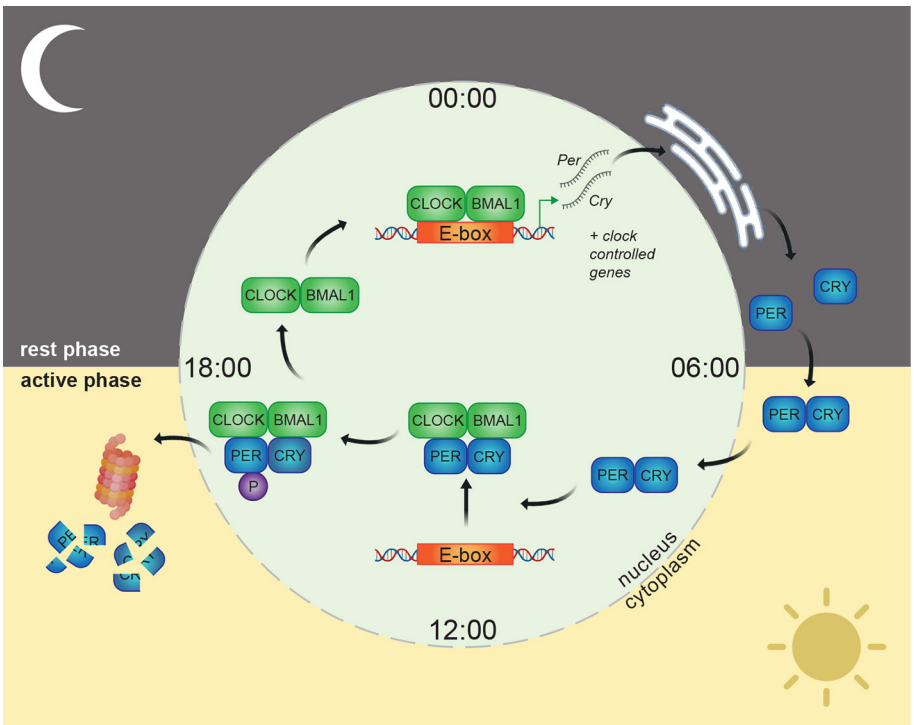


Figure 1. The molecular clockwork. The transcription factors CLOCK and BMAL1 bind DNA E-boxes as a pair, inducing expression of Per and Cry mRNAs. After export to the endoplasmic reticulum, Per/Cry mRNAs are translated into PER/CRY proteins, which build up in the cytoplasm before passing back into the nucleus and repressing CLOCK/BMAL1 transcriptional activity by several mechanisms, including direct binding and dissociation of CLOCK/BMAL from DNA. Phosphorylation (P) of PER2 by specific kinases triggers degradation of the PER/CRY complex by the ubiquitin–proteasome pathway. CLOCK/BMAL1 are subsequently derepressed, and the cycle starts anew. Additional mechanisms, including auxiliary feedback loops, chromatin topology, protein modifications and mRNA processing, work in concert with the core loop to drive 24-hour rhythms at the genome, proteome and signalling levels. Figure created with BioRender.

At the next level of organization, tissue clocks in different organs form a network. In mammals, the clock network is arranged as a hierarchy, with the central clock in the hypothalamic suprachiasmatic nucleus (SCN) at the top (Figure 2). The SCN clock communicates with other brain clocks and clocks in the rest of the body (i.e. periphery) and can synchronize their rhythms.

Top-down synchronization

The SCN is made up of a mere 45,000 neurons (less than 0.0001% of the 86 bn neurons in the human brain), which work together with their supporting glial cell counterparts to form the body's central clock. The dominant zeitgeber for the central clock is light; neuronal input from the retina allows daily readjustment of SCN clock timing to align with the light–dark cycle. At a cellular level, this timing shift is thought to be caused by light changing the electrical firing activity of SCN neurons, which increases the expression of PER proteins, the negative regulators of BMAL1 shown in Figure 1. The SCN clock then passes on time-of-day information to other brain regions via neuronal connections and release of neuropeptides. To communicate with the periphery, the SCN uses diverse routes including neuronal connections, hormonal release from nearby endocrine glands and control of daily behaviours such as the sleep–wake cycle (Box 1).

A key synchronizing signal for peripheral tissues, such as liver, muscle and adipose (fat), is the daily release of hormones called glucocorticoids from adrenal glands, secretory organs that sit on top of the kidneys. The SCN signals to the adrenals directly through neuronal connections and indirectly via control of hormonal release from the nearby pituitary gland. Using these routes, SCN timing cues are translated into daily oscillations in the amount of glucocorticoids released into the bloodstream. Binding of glucocorticoids to their receptors in peripheral tissues triggers a cascade of intracellular signalling events, culminating in changes in the timing of circadian gene expression.

The SCN also influences peripheral clocks indirectly though control of the sleep–wake cycle and its associated behaviours. During waking hours, mammals are more active and consume the majority of their calories,

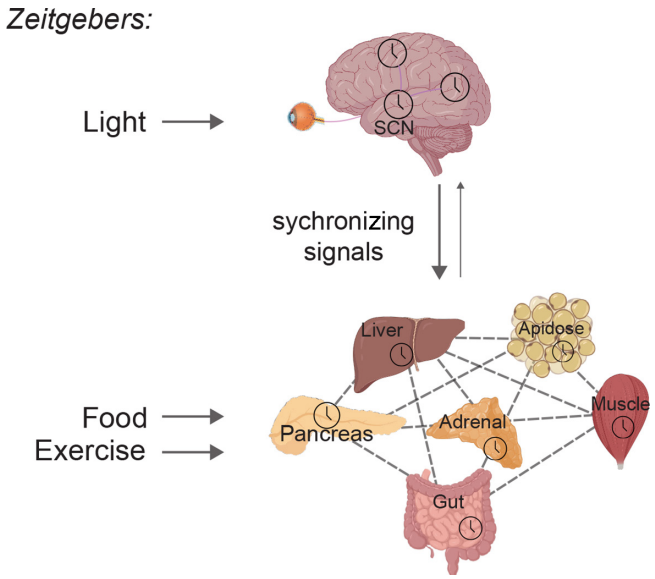


Figure 2. Mammalian circadian network organization. Light is the major zeitgeber for the central clock in the SCN, readjusting timing daily to be aligned with the light–dark cycle. The SCN communicates with clocks in the rest of the body to synchronize their timing. Clocks in peripheral tissues also influence synchronization through the release of soluble factors into the bloodstream. For peripheral tissues, food intake and exercise are key zeitgebers. Figure created with BioRender.

whereas during sleep, there is low movement and food intake is mostly absent. This cycle of feeding and fasting is a major zeitgeber for clocks in peripheral tissues (Box 2). One way feeding signals to clocks in peripheral tissues, is by stimulating the release of hormones such as insulin into the bloodstream. Similar to the synchronizing effect of light in the SCN, insulin alters the timing of peripheral clocks through modulating the levels of the negative regulators of BMAL1. Decades of research has now clearly established that the SCN can impact timing in the rest of the body in a variety of ways. Yet, recent evidence shows that the SCN clock does not act alone in this endeavour; peripheral clocks themselves play decisive roles in influencing each other's timing.

Box 1. Synchronizing signals from brain to peripheral clocks

Control of behavioural cycles
- Sleep–wake
- Locomotor activity
- Feeding–fasting

Control of body temperature

Control of hormonal release
- Pituitary hormones
- Glucocorticoids (via adrenal gland)

Neuronal innervation

Box 2. The feeding–fasting cycle and peripheral clocks

Under normal conditions, SCN-driven feeding rhythms result in mice consuming ~80% of their calories during their active phase (when lights are off). Under these conditions, clock timing in the SCN and peripheral tissues is aligned. However, if researchers only give mice food during their rest phase (when lights are on), inverting their natural feeding–fasting rhythm, peripheral clocks set their time to this new feeding rhythm, whereas timing of the central clock in the SCN is unaffected. This leads to a state of desynchrony between central and peripheral clocks, which can promote dysregulation of metabolism including excessive fat storage. Research has now revealed that clock function in human tissues follows similar rules, highlighting the importance of carefully timed food intake.

The power of peripheral clocks

Clocks in the periphery regulate important aspects of metabolic function in each tissue (Box 3). Research is now revealing that signalling between these peripheral clocks is critical in regulating clock timing as well as metabolic output. For example, the local pancreatic clock controls the responsiveness of the pancreas to food intake, so that the amount of insulin released differs according to the time of day that food is ingested. As insulin can act as a synchronizer in the periphery, the pancreatic clock thereby influences peripheral clock timing and metabolic output. A similar phenomenon occurs with the adrenal glands, in which the local clock determines whether glucocorticoids are released according to the time of day that SCN input is received.

Uncovering additional signals that relay timing information between peripheral clocks is an area of ongoing research. Two potential key players in peripheral clock-to-clock signalling are the liver and muscle (Figure 3). The liver clock controls blood glucose levels, thereby influencing systemic energy use through changing the amount of glucose available for other tissues. In addition, the liver clock controls the secretion

Box 3. Major metabolic roles of peripheral clocks

- Liver: carbohydrate metabolism
- Muscle: carbohydrate, amino acid and fat metabolism
- Adipose: fat metabolism
- Gut: lipid metabolism

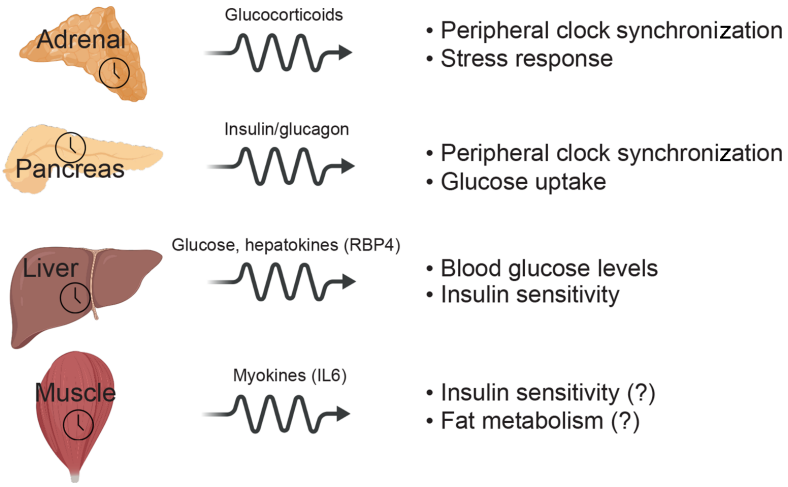


Figure 3. Peripheral clock signalling output. Local clocks in peripheral tissues control the rhythmic release of soluble signalling factors into the bloodstream. For many peripheral tissues, the effect of clock-controlled output on timing in other tissues is not well defined. Figure created with BioRender.

of small proteins called hepatokines, which have been shown to influence the ability of other tissues to take up glucose after a meal. How such liver-clock-driven signals affect clocks in other peripheral tissues is not yet well defined. Like the liver, the muscle has its own arsenal of signalling proteins, in this case called myokines. Boosted by exercise, myokines have been shown to regulate energy use in other tissues. Like hepatokines, our understanding of the cellular mechanisms employed by myokines is hazy at best. Intriguingly though, from experiments on muscle cells grown in culture, the muscle clock has been shown to affect the daily rhythmic release of myokines. Excitingly, the influence of the muscle clock on myokine release *in vivo* remains unexplored.

Peripheral clock feedback to the brain

Apart from their direct roles in synchronizing each other, peripheral clocks can also signal back to the brain and regulate daily control of behavioural cycles (and thereby indirectly affect their own timing). Using mice in which peripheral clocks have been turned off in specific tissues, researchers have revealed that the liver, adipose and muscle clocks can all affect behavioural cycles in this way (Figure 4). In adipose tissue, for example, the local clock influences secretion of the hormone leptin, which travels in the bloodstream to the hypothalamus and supresses food intake. In addition, peripheral clocks control the secretion of metabolites, chemical intermediates in energy use, many of which can cross the blood–brain barrier and therefore potentially affect brain function. In cases such as the muscle, the identity of molecules that signal back to the brain to regulate circadian timekeeping remains a mystery.

Future directions

Very few examples of peripheral clock communication have been described to date. However, the recent development of genetic mouse models in which clocks

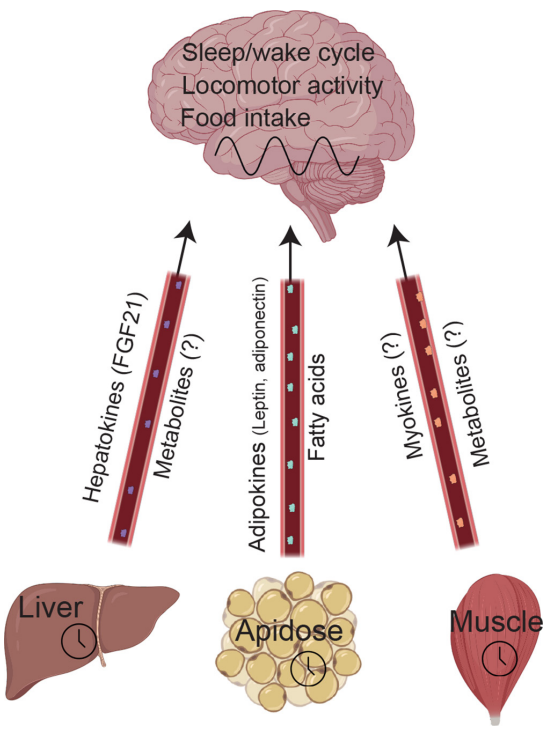


Figure 4. Peripheral clock signalling to brain. Peripheral clocks can feed back to the brain and alter timing of circadian behavioural cycles, through as-of-yet poorly defined mechanisms. Figure created with BioRender.

are active only in specific tissues, in otherwise 'clockless' mice, offers a new approach to studying links between peripheral clocks. Using this methodology, work from our laboratory has revealed that peripheral clocks certainly cannot go it alone; mice with only a liver clock have just 20% of their normal rhythmic liver function. The next step is to restore clocks in multiple tissues to identify the key routes of communication that support the majority of circadian function in our organs.

Human relevance

Modern lifestyles present our circadian networks with a variety of zeitgebers on a daily basis, acting on central clocks, peripheral clocks or both. If we mistime our exposure to zeitgebers, e.g. by eating late at night or disrupting our sleep–wake cycle, this can lead to desynchronization between brain and peripheral clocks. This happens every time we experience jet lag, as peripheral clocks align to the new environment almost immediately whereas the SCN clock takes much longer to adjust (approximately 1 day per hour of time difference). While jet lag may cause some discomfort short term, long-term or repeated desynchronization may be highly detrimental to our health; night-shift workers, for example, are at a higher risk for obesity, metabolic diseases and cancer. However, there are reasons to be optimistic. The importance of circadian rhythms in overall health is increasingly being recognized, as is the notion that our circadian rhythms can be harnessed to treat disease more effectively. The emerging field of circadian medicine, for example, has revealed that the effectiveness of drugs can be dramatically increased by administration at certain times of the day. It is our hope that by understanding how timekeeping between tissues is achieved, new ways to promote synchronous circadian function and optimum health will be revealed. ■

Further reading

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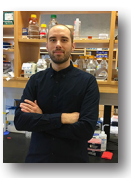

Jacob G. Smith is a postdoctoral researcher in the laboratory of Professor Paolo Sassone-Corsi at University of California, Irvine. Jacob's research is aimed at uncovering new communication routes for clock-dependent signalling between organs. Email: jacob.smith@uci.edu

Paolo Sassone-Corsi is a professor of circadian biology at University of California, Irvine. Research from his laboratory has revealed the role of peripheral clocks and nutrition in plasticity of circadian function. Email: psc@uci.edu

To code or not to code?

That is the question for RNA in timekeeping

Rebecca A. Mosig and **Shihoko Kojima** (Virginia Tech, USA)

Elementary cellular biology teaches that proteins are the main actors within cells. In addition, the DNA carries genetic information and the mRNA serves as the go-between molecule to create protein using the genetic code from the DNA. Recent advances in sequencing technologies have revealed that a large portion of our DNA is transcribed even though it does not appear to encode proteins. Further studies have shown that many of these non-coding RNAs (ncRNAs) serve regulatory functions within cells. Here, we examine the role of such molecules in the regulation of the circadian clock system as illustration of the wider ways in which ncRNAs can influence cellular processes.

What are non-coding RNAs?

In genetics class, we have all learned that a gene is 'the basic physical and functional unit of heredity' and that 'genes provide the instructions for the synthesis of proteins, the action molecules of the cell and organisms as a whole'. Thus, it was a surprise when the Human Genome Project revealed that only approximately 2% of the human genome is dedicated to protein-coding genes.

The remaining 'non-genic' regions were originally assumed to be mostly junk and artefacts that had been carried along and inexplicably expanded throughout evolution. Even after studies revealed that over 80% of these regions are actively transcribed, there has been persistent debate whether these transcripts are genetic 'noise' or whether they have any biological function. In time, however, some of these non-coding transcripts (hereafter, ncRNAs) have been shown to exert regulatory functions in a variety of biological processes, such as stress response, cell differentiation, fertility, locomotor activity, memory formation and immune response.

The importance of ncRNA can be further inferred from the observation that the number of ncRNAs is significantly increased in higher organisms and their location and/or sequence are evolutionarily conserved. The variety of functional ncRNAs has grown dramatically in the past 15 years, and more recent studies have started to shed light on the role of ncRNAs even in disease mechanisms from cancer to neurodegeneration, further lending credit to their biological relevance.

How do regulatory ncRNAs function without producing a protein?

Even before the completion of the Human Genome Project, we already knew that some RNAs were functional. For example, ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) facilitate protein synthesis. Other ncRNAs are categorized by their length and structure.

- Short ncRNAs include regulatory ncRNAs such as microRNA (miRNA) and PIWI-interacting RNA (piRNA) (~21–30 nucleotide [nt]), as well as housekeeping RNAs such as small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA) (~30–170 nt) (Figure 1).
- Long ncRNAs (lncRNAs) are, by definition, longer than 200 nt, and include enhancer RNA (eRNA), intra- and inter-genic RNA, sense RNA and antisense RNA (asRNA), based on their genomic location in reference to protein-coding genes (Figure 1).
- New types of ncRNAs, such as circular RNA (circRNA), keep emerging, making this an exciting and rapidly advancing area of research.

Regulatory ncRNAs can alter the expression, processing, translation or stability of target mRNAs. There are two possible mechanisms by which ncRNAs can act on target mRNAs (Figure 2). The transcript model, as the name implies, assumes that the *transcript* (i.e. RNA molecule itself) is the functional unit, in which transcripts bind to target mRNA, DNA or proteins to alter gene expression of target mRNAs. The most well-defined example is microRNA (miRNA), which can post-transcriptionally stall translation or trigger mRNA degradation by directly binding to target sequences. LncRNAs can serve as an adapter for chromatin looping and also as a scaffold, decoy or guide for histone/DNA modification enzymes, transcription factors and RNA-binding proteins among others to ultimately alter target mRNA expression (Figure 2). In contrast, the transcription model assumes *transcription* of the ncRNA's own gene as the functional unit. The transcription machinery moves along the DNA and inhibits target gene expression, which is a mechanism called transcriptional interference. RNA polymerase on the ncRNA strand can prevent, dislodge, or stall RNA polymerases on the target mRNA strand, depending on the directions of transcription of ncRNAs and target mRNAs (Figure 2).

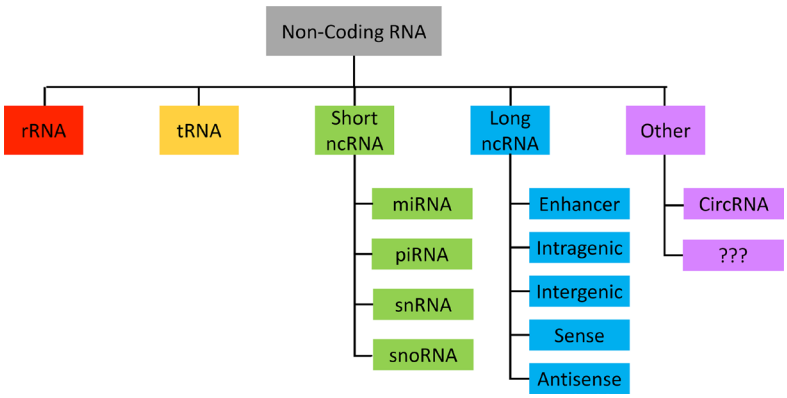

Figure 1. Classes and types of ncRNA.

Circadian rhythms and ncRNAs

A circadian rhythm is an approximately 24-hour self-sustaining oscillating system that allows organisms to anticipate and adapt to environmental changes that occur on a daily cycle. Circadian cycling is observed in nearly all organisms on Earth, and its timekeeping mechanism is evolutionarily conserved. The information from the environment (input), such as light, nutrient availability and temperature, is transmitted to the 'core' oscillator that generates cell-autonomous circadian rhythmicity via transcription–translation feedback loops (see the article by Smith and Sassone-Corsi, this issue). This core oscillator, in turn, drives the circadian rhythms of downstream cellular activities (output), such as locomotor activities, reproduction and metabolism as well as rhythmic gene expression (Figure 3).

Just like any other biological process, the circadian system requires many protein-coding genes. However, ncRNAs also have important roles in regulating circadian rhythms, affecting all three processes: input, the core and output. Some ncRNAs are rhythmically expressed or are acutely induced by the input, while others are not, although the rhythmic expression pattern is not a requirement for their functions. Below, we will discuss examples of ncRNAs that are involved in regulating circadian rhythms.

Input control

The ability to reset the core oscillator by external stimuli (input) is one of the important characteristics of the circadian system and is called entrainment. Light is one of the most powerful inputs in many species including plants, fungi, flies, mice and humans. In mammals, light information is perceived by the retina and transmitted to the suprachiasmatic nucleus (SCN), the master pacemaker cells in the hypothalamus of the brain. Interestingly, miRNA-132 (miR-132), a brain-specific miRNA, is rhythmically expressed and acutely induced by light in SCN. Because its knockdown impairs the clock's ability to adequately respond to the light stimulus, miR-132 appears to play a pivotal role in circadian entrainment, although its target mRNA(s) remain unclear.

Core regulation

MicroRNAs also regulate the core oscillator in various organisms that determines the period length of the circadian

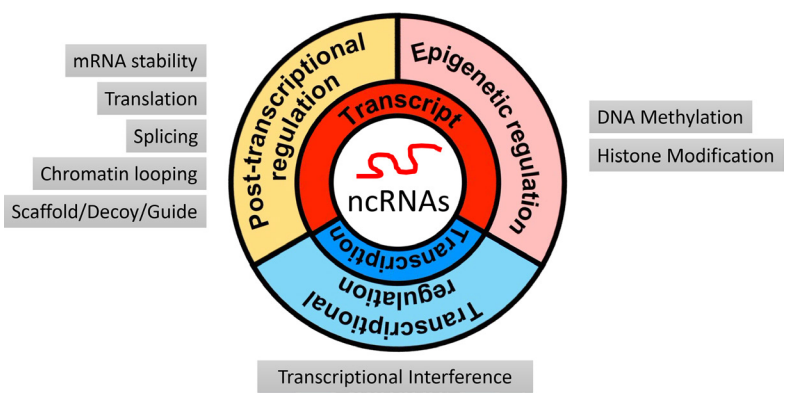

Figure 2. Gene regulatory mechanism of ncRNA. Both transcript and transcriptional models can have effects at the epigenetic, transcriptional and post-transcriptional levels.

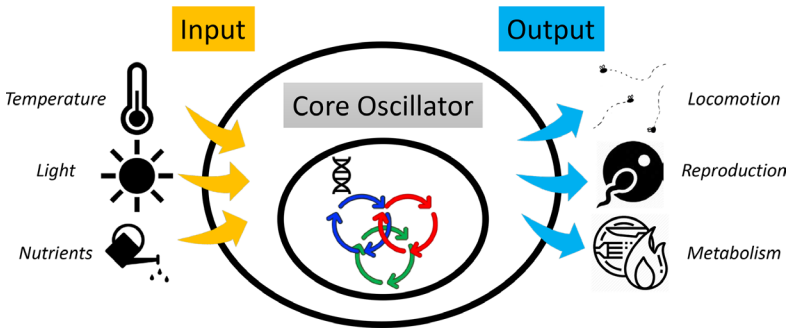

Figure 3. The circadian clock system composed of input, the core oscillator and output.

clock. In *Arabidopsis*, miR-397b overexpression leads to a longer period. This is because miR-397b represses its target Casein Kinase II Subunit Beta-3 (*CKB3*), resulting in hypophosphorylation of the negative element and the core clock gene, *CCA1* (Figure 4a). In *Drosophila*, disruption of miR-276a leads to a loss of circadian rhythms. This is because miR-276a directly targets the negative element and the core clock gene *timeless* (*tim*) to promote its mRNA degradation (Figure 4c). In mouse SCN, the expression of another brain-specific miRNA, miR-219, is rhythmic and its knockdown led to a longer free-running period. Even though its target mRNA(s) have not been identified, this indicates that miR-219 targets one of the core clock genes essential for setting the period length. MiR-24, a more ubiquitously expressed miRNA, also plays a pivotal role in regulating period as a knockdown of miR-24 shortens the clock period *in vitro* (Figure 4d). Interestingly, miR-24 directly targets *Period 2* (*Per2*), the negative element and the core clock gene, whose rhythmicity, proper phase and expression levels are critical to maintaining cellular rhythmicity.

In addition to miRNAs, lncRNAs also are important to sustain and maintain the functions of the core oscillator. In *Neurospora*, the core clock gene and the negative element *frequency* (*frq*) has an asRNA named *qrf*. *Qrf* is rhythmically expressed and anti-phasic to *frq*, and *qrf* is involved in regulating both circadian rhythmicity and light entrainment (Figure 4b), utilizing both transcript and transcriptional mechanisms to regulate *frq*. Similar to *frq* and *qrf*, the mammalian core clock gene *Per2* also has an asRNA, *Per2AS*, that is expressed rhythmically and antiphasic to *Per2* (Figure 4d). The function of *Per2AS* is yet to be determined. The sense–antisense pair of the core clock genes is also observed between the putative core clock component *period* (*per*) and its antisense transcript in the silkmoth, *Antheraea pernyi*. Although the precise regulatory mechanism between sense and antisense transcript pairs is still to be elucidated, conservation of antisense transcripts to core clock genes across kingdoms further supports their biological relevance and suggests that they may constitute a common mechanism for circadian clock regulation.

Output control

Various ncRNAs play an important role in the output process as well. Many ncRNAs are rhythmically expressed in tissues, including mouse liver, pineal gland and fly neurons. The rhythmic ncRNA expression certainly implicates their potential roles in regulating circadian rhythmicity. However, it is not entirely clear whether the rhythmic expression of ncRNAs is regulated by the same mechanism as protein-coding genes. Typically, the rhythmic expression pattern of mRNAs is regulated by a set of circadian transcription factors in a time-dependent manner. Interestingly, rhythmic transcription of eRNA in mammals is also controlled by the circadian transcription factors that drive rhythmic transcription of protein-coding genes. This indicates that rhythmic transcription of ncRNAs can be directly regulated by the core clock genes.

Housekeeping RNAs are also rhythmically expressed. In mouse liver, rRNAs are more highly expressed at night as compared to the day, thereby affecting the global

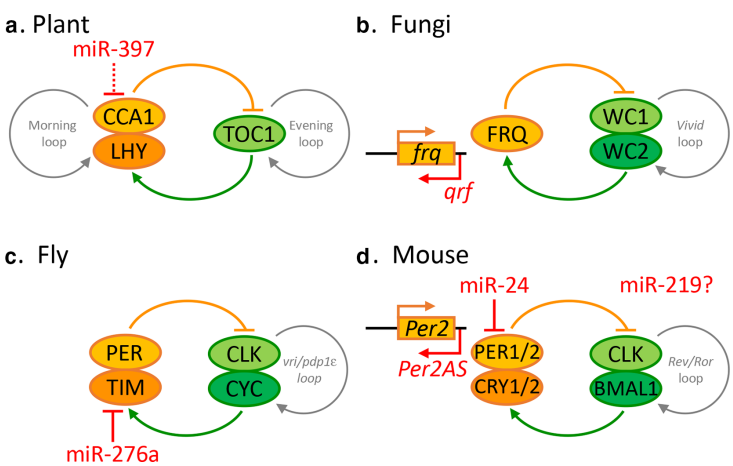

Figure 4. Simplified core circadian feedback loops of **a)** Plant, **b)** Fungi, **c)** Fly and **d)** Mouse showing positive elements (green), negative elements (orange) and selected points of ncRNA regulation (red).

translation level of the cell. In addition, the expression of snRNP complexes that contain snRNAs is rhythmic, and this globally affects the processing and alternative splicing of core clock and clock-controlled genes in *Arabidopsis*, *Neurospora*, and *Drosophila*.

Other ncRNAs modulate the rhythmic expression pattern of protein-coding genes that ultimately drive downstream rhythmic physiological processes. *CYCLIC DOF FACTOR 5* (*CDF5*) and its asRNA *FLORE* in *Arabidopsis* mutually inhibit each other's transcription to create an antiphasic circadian expression pattern and together control the time-of-day dependent process of flowering. In mice and flies, miRNAs also modulate the rhythmic expression pattern of mRNAs that are involved in diverse rhythmic processes such as metabolism, cellular differentiation, adipogenesis and sleep–wake patterns. Furthermore, in mouse liver, an lncRNA acts as a scaffold to bring together target gene promoters, circadian transcription factors and histone-modifying enzymes to co-regulate multiple genes that are rhythmically expressed within the same chromosome but located distantly. This long-distance regulation by lncRNA provides a novel mechanism for rhythmic mRNA expression without being directly regulated by circadian transcription factors.

Conclusions and future directions

Advances in sequencing technology were instrumental in discovering new types of ncRNAs even though they were originally thought to be mere transcriptional noise. Recent examples have shown that some, if not all, are biologically functional. However, it can still be technically challenging to perturb ncRNAs to test their functionality, depending on the mode of action, genomic location and regulatory mechanisms, as this can also directly affect target mRNA expression. Nevertheless, the development of CRISPR gene targeting techniques that can specifically target ncRNA expression with relative ease will dramatically assist in understanding their roles and regulatory mechanisms. There is no doubt that future studies will uncover the functions of these new types of ncRNAs even further and create a more complete picture of the role of ncRNAs in circadian and other systems. ■

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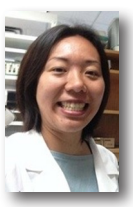

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Seasonal rhythms of energy metabolism

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Most environments on our planet are highly seasonal, reflecting the tilt in the Earth's axis relative to the sun. As a consequence, the majority of life forms have evolved profound seasonal variations in their behaviour and physiology that allow them to anticipate these patterns in food supply and to optimize their reproductive strategy. Reproduction is an energetically costly process in mammals, for example, in supporting pregnancy and then lactation in females. There has been strong selective pressure to ensure births occur in the optimal seasonal for survival. Terrestrial mammals indigenous to temperate and polar regions tend to give birth in spring, when the climatic conditions and food availability are conducive to survival. Seasonal cycles of reproduction also occur in equatorial regions, where they may be linked to wet and dry seasons. Only the species that have been domesticated by man or intensively bred, such as the laboratory strains of mice and rats, fail to display this seasonality. Given the intimate link between energy availability and reproductive success, it is no surprise that body systems regulating energy intake, storage and expenditure are themselves highly seasonal.

Photoperiod and circannual rhythmicity

Huge advances in our understanding of the mechanisms underlying the seasonal rhythms have occurred in the last few decades and thrown up quite a few surprises. The importance of the annual change in daylength as a proximate cue to synchronize seasonal rhythms in vertebrates was understood from the first half of the 20th century. Accordingly, sheep respond to the decreasing days in autumn to activate the hypothalamo-pituitary-gonadal axis, promoting spermatogenesis in males and follicular development in the ovary and ovulation in females, and consequently, sexual behaviour and copulation. Gestation is 147 days in sheep; therefore, lambs are born 5 months later in the following spring.

In a commonly studied laboratory rodent model for photoperiodic control of reproduction, the Siberian hamster, it is the long days of spring that promote activation of the reproductive axis, with the gestation being just 21 days. The seasonal cycle of energy balance is finely honed in these hamsters. Under spring–summer conditions that can be simply mimicked in the laboratory by keeping hamsters in long photoperiods of 16 hours of light and 8 hours of darkness, the hamsters display hyperphagic anabolic physiology, and so, they maintain an increased body weight (Figure 1a). However, exposing the hamsters to short photoperiods comprising 8 hours of light and 16 hours of darkness results in a winter survival strategy. Reproductive function ceases, and the animals display a markedly reduced voluntary food intake, whilst increasing lipolysis, resulting in catabolism of visceral fat reserves. This occurs to sustain life through winter. Daily torpor also occurs to preserve the caloric reserves. During 12 weeks in short days (Figure 1a), the body weight is reduced by up to 30%. Interestingly, this hypophagic catabolic state spontaneously reverts to the anabolic state after prolonged exposure to short days, resulting in a return to the spring–summer body weight. Thus, the seasonal changes in energy metabolism are not simply direct responses to the annual changes in daylength, but also reflect innate long-term timing mechanisms.

In vertebrates that have longer life spans, these mechanisms are truly circannual rhythms in that they persist in constant environmental conditions. An elegant study in chipmunks kept in the absence of changes in photoperiod and at constant ambient temperature demonstrated the persistence of a circannual rhythm in hibernation (Figure 1b), with innate periodicities slightly shorter than a year (227–367 days). There are some clear analogies between circannual and circadian timing systems; but whereas circadian timing relies upon intracellular transcriptional feedback loops, the mechanisms underlying circannual timing are not known. Current hypotheses revolve around cyclical histogenesis and tissue remodelling in the pituitary–hypothalamic network.

Melatonin

We now understand that for most mammals, it is the pineal gland that is key in transducing daylength information into a neurochemical signal – the nocturnal secretion of melatonin (Figure 2). Descartes considered

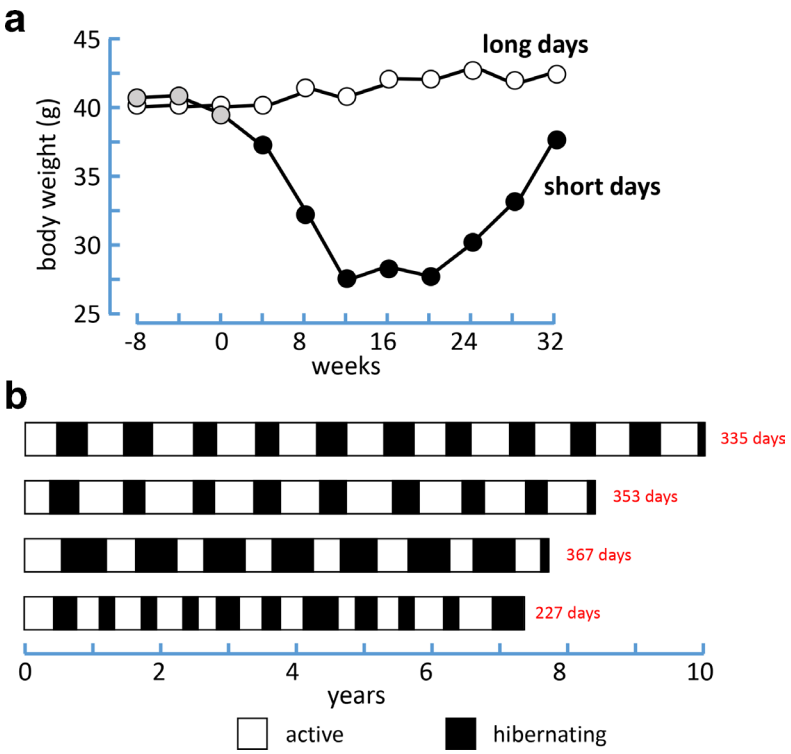

Figure 1. a) Photoperiodic effects on the body weight of adult male Siberian hamsters maintained throughout on long days of 16 hours of light:8 hours of darkness (O), or transferred at week 0 to short days of 8 hours of light: 16 hours of darkness (black filled circles). Note how the body weight declines substantially in short days, but after 20 weeks begins to revert back to the long-day phenotype. Data from Ebling, unpublished. **b)** Persistence of circannual rhythms of hibernation in male chipmunks maintained at constant temperature (5°C) and constant photoperiod of 12 hours of light:12 hours of darkness. Note that cyclicity persists in all individuals, but the periodicity defined as the mean time between successive onsets of hibernation (values in red) varies between individuals. Data redrawn from Kondo et al. (2006) Cell **125**, 161–172.

the pineal to be the seat of the soul, and for many centuries, this gland was thought to be vestigial. However, the loss of response to changes in photoperiod in mammals such as Siberian hamsters, ferrets and sheep after surgical removal of their pineal gland confirms its importance in mediating the effects of photoperiod on reproductive and metabolic cycles. Although it was well known that the retina perceives the light–dark cycle in mammals, it was one of several recent surprises when Russell Foster and David Berson discovered in the early 2000s that a new class of photoreceptors in retinal ganglion cells use melanopsin rather than rhodopsin as the

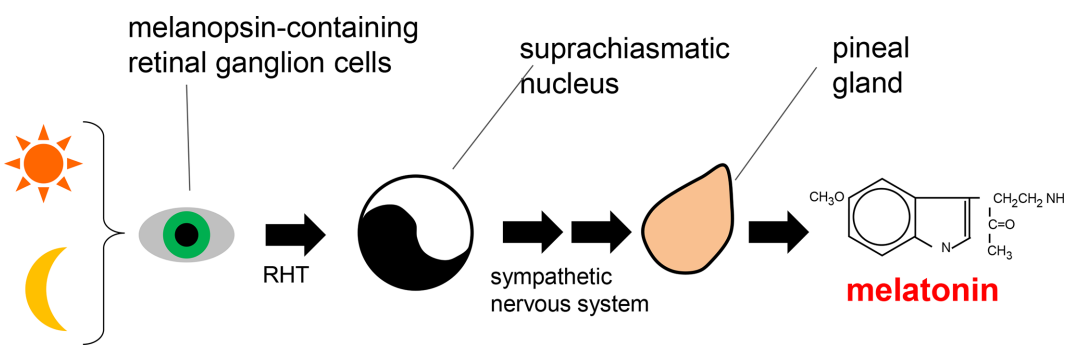

Figure 2. Pathway by which the change in daylength is perceived by mammals and transduced into a change in the duration of nocturnal melatonin secretion. Retinal ganglion cells expressing the photopigment melanopsin project directly via the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN), a master circadian pacemaker. The SCN regulates sympathetic innervation via the brainstem and intermediolateral column of the spinal cord of the pineal gland, restricting melatonin synthesis and secretion to the dark phase.

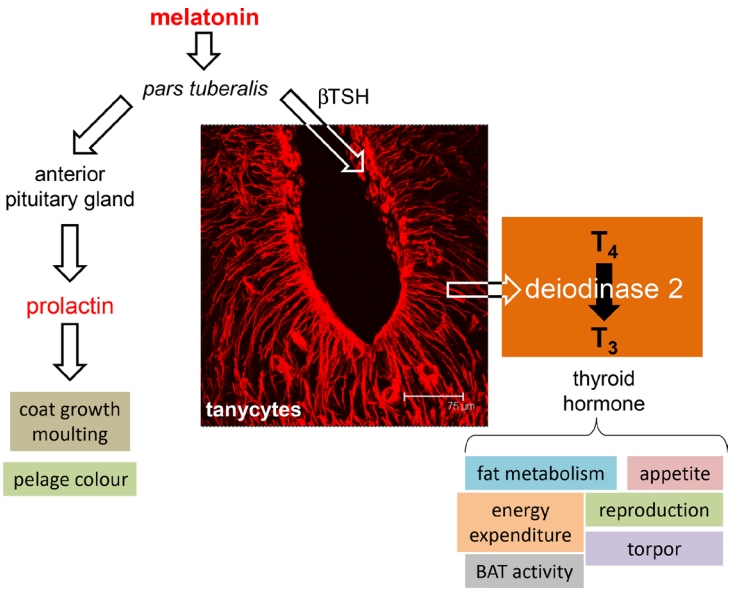

Figure 3. Pathways by which the nocturnal melatonin signal regulates seasonal cycles. The primary target is the pars tuberalis (pituitary stalk). Paracrine factors from this tissue either regulate prolactin-secreting cells in the anterior pituitary or act on tanycyte processes. The β subunit of thyrotropin stimulating hormone (βTSH) is the main signal acting on tanycytes, and ultimately regulates the expression of deiodinases 2 and 3. These regulate the availability of tri-iodothyronine (T3), the active form of thyroid hormone. Intrahypothalamic T3 is a major determinant of seasonal cycles of energy intake and expenditure, and of reproduction.

photopigment (Figure 2). These then directly innervate the suprachiasmatic nucleus in the hypothalamus that in turn regulates pineal melatonin synthesis and secretion via the sympathetic nervous system (Figure 2).

Another surprise was that the identification of melatonin-sensitive structures in the brain and pituitary of mammals indicated that the pars tuberalis of the pituitary stalk is the common site of action across most mammalian species. The pars tuberalis likely communicates via paracrine signals to lactotrophs in the anterior pituitary, thereby regulating the seasonal patterns of prolactin secretion, the major determinant of annual cycles of coat growth and moulting (Figure 3). A final surprise is the pathway by which the energy balance is affected, because the influence of photoperiodic information encoded by melatonin is then relayed from the pars tuberalis back to the brain! The secretion of β–thyrotropin stimulating hormone (βTSH) is the primary signal from the pars tuberalis, and even more surprising is that its target is not neuronal, but specialized glial cells known as tanycytes that line the third ventricle but extend projections into the surrounding hypothalamus (Figure 3).

Tanycytes and thyroid hormone

Although we are familiar with the metabolic importance of the influence of thyroid hormone in the periphery on the basis of the symptomology of hypo- and hyperthyroidism, the notion that the thyroid hormone exerts actions on energy balance via processes within the central nervous system is relatively novel. Tanycytes express the TSH receptor and respond to seasonal alterations in the βTSH signal from the pars tuberalis by regulating local availability of thyroid hormone (tri-iodothyronine [T3]) in the surrounding hypothalamus. Their role in this is complex; they express thyroid hormone transporters such as MCT8 and OATP1C1, and therefore are a principal route by which the thyroid hormone in the bloodstream gets actively transported into the cerebrospinal fluid. In effect, tanycytes are a functional component of the blood–brain barrier. More importantly, they express deiodinase enzymes, as found in all target tissues of thyroid hormone. Deiodinase 2 converts the relatively inactive precursor thyroxine (T4) into the bioactive form T3, whereas deiodinase 3 inactivates T3 and T4 (Figure 4a) – an important protective mechanism in the developing brain. βTSH is secreted from the pars tuberalis in response to long-day patterns of melatonin secretion, and up-regulates *DIO2* expression but down-regulates *DIO3* expression, thus increasing local T3 concentrations. Experimental studies in sheep, hamsters and quail have demonstrated the functional importance of this seasonal change in hypothalamic thyroid hormone availability for both reproductive activity and control of energy balance. In the case of the Siberian hamster, for example, taking

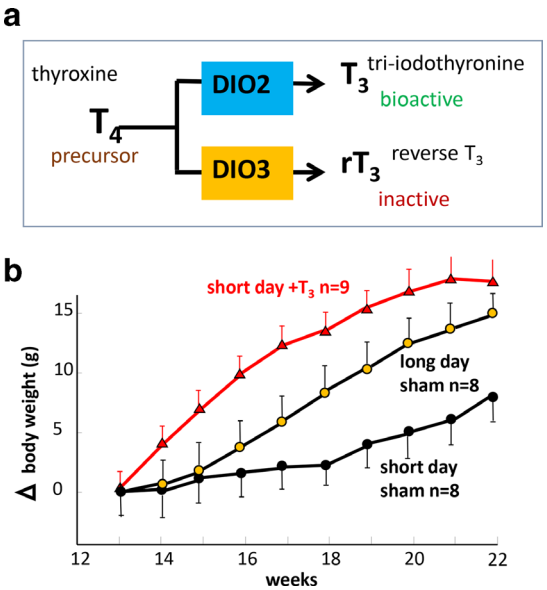

Figure 4. a) The regulatory roles of deiodinase 2 (DIO2) and deiodinase 3 (DIO3). DIO2 catalyses the conversion of thyroxine (T4) into the bioactive form of thyroid hormone, tri-iodothyronine (T3). DIO3 inactivates T4 to produce reverse T3 (rT3) and also inactivates T3. **b)** Experimental evidence that locally manipulating T3 concentrations in the hypothalamus by means of surgically placed microimplants affects energy balance. Adult male hamsters that had been maintained in short days for 13 weeks and therefore had a low body weight were either implanted with T3 and maintained on short days (red triangle) or received sham implants and were kept in short days (black circle) or long days (yellow circle). Note that the T3 implants induce a very rapid gain in body weight, even more swift than that in hamsters with sham implants but transferred to long days. Data redrawn from Murphy et al. (2012) Endocrinology **153**, 101–112.

animals in the short-day catabolic state but increasing the thyroid hormone locally by the surgical placement of microimplants directly within the hypothalamus induces the long-day hyperphagic and anabolic state (Figure 4b). Conversely, preventing the short-day–induced decline in hypothalamic thyroid hormone concentrations in hamsters using the same microimplant technology blocks the hypophagia and loss of body weight normally induced by short photoperiods.

Perspectives: the relevance to man

There are a number of reasons why an understanding of the mechanistic basis of seasonal cycles in energy balance has importance for the human condition. First, despite our evolutionary heritage as equatorial primates and our exposure to man-made environments where we can override the natural cycle in daylength, we still display elements of seasonality in many aspects of our lives. These tend to manifest as population-level changes in fertility rates or incidence of infectious diseases, but at an individual level, seasonal changes in mood and affect disorders are well documented. The prevalence of these symptoms increases at more northerly latitudes. The most recent diagnosis manual of the American Psychiatric Association defines seasonal affective disorder (SAD) as a recurring major depression that occurs usually in the autumn and winter and spontaneously remits in spring. SAD is an atypical depression in that it is usually associated with increased appetite, craving for carbohydrates and increased sleep, and hence is a further example of seasonal cyclicity in energy metabolism. In the UK, the Royal College of Psychiatrists considers around 3% of the population to have SAD, making it a substantive healthcare issue. Understanding the biological basis of SAD, which may be viewed as a type of hibernation strategy, has benefits for its treatment; for example, the use of bright light illumination to promote a return to the long-day/summer state.

The second reason is of perhaps even greater importance: by understanding the mechanisms by which long-term changes in appetite and energy expenditure are controlled in a seasonal context, we may gain new insights into the strategies to help people eat less, lose weight and, therefore, gain the associated health benefits. Unquestionably, obesity is a worldwide issue; yet, behavioural strategies rarely work in the long term and pharmaceutical approaches have had almost no success. Genetic approaches in mice have identified a myriad of homeostatic pathways involved in the short-term development of satiety after meal ingestion. These encompass gut–brain signals, and downstream pathways in the hypothalamus and brain stem, but translation to clinically successful strategies has been disappointing. The observation that seasonal mammals can suppress appetite over a period of many months and correspondingly adjust their peripheral metabolism suggests that homeostatic mechanisms can be overruled by longer-term 'rheostatic' mechanisms. This article has highlighted the key role of hypothalamic thyroid hormone in the seasonal rheostatic process in hamsters, a finding that seems to apply to all vertebrate taxa. Some of the actions of thyroid hormone in regulating the seasonal cycles of appetite may be manifest through regulation of the pro-opiomelanocortin (POMC) mechanisms in the brain; but given the crucial role of thyroid hormone in initial brain development, it seems likely that some of its actions in adult seasonality are via plasticity and remodelling of neural circuits. The relatively recent appreciation of the plastic capabilities of the adult hypothalamus in seasonality and in pregnancy that encompasses altered neurogenesis, synaptogenesis and glial ensheathment should give us hope that the development of obesity is not a one-directional process. Perhaps better appreciation of the underlying mechanisms in seasonal cycles will lead to strategies to return the hypothalamic circuitry to a pre-obese state.

Finally, although the focus of this article has been the contribution of hypothalamic and pituitary mechanisms in seasonal cycles, study of the peripheral adaptations during the cycles of fat gain and loss might inform therapies to treat type 2 diabetes and obesity. One recent example relates to the actions of the hepatokine, fibroblast growth factor 21 (FGF21), an endocrine factor that has attracted the attention of multiple pharmaceutical companies. In preclinical studies in rodents, treatment with FGF21 was found to improve glucose homeostasis and induce weight loss. Our studies in the Siberian hamster suggest that increased production of FGF21 in the liver and brown fat may be part of the mechanism by which this species survives winter. Treatment of hamsters in the long-day anabolic state with FGF21 induces a number of features of the short-day state: reduced appetite, weight loss and catabolism of visceral fat depots. Studies using positron emission tomography (PET) scans reveal that white adipose tissue is a major target of FGF21, and so FGF21 promotes fat oxidation rather than carbohydrate oxidation, a key component of the winter survival strategy. Perhaps this seasonal biology of FGF21 could be exploited further in man? ■

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Katharina Beer (University of Würzburg, Germany)
Guy Bloch (Hebrew University of Jerusalem, Israel)

Circadian rhythms of about a day are ubiquitous in animals and considered functionally significant. Honey bees show remarkable circadian plasticity that is related to the complex social organization of their societies. Forager bees show robust circadian rhythms that support time-compensated sun-compass navigation, dance communication and timing visits to flowers. Nest-dwelling nurse bees care for the young brood around the clock. Here, we review our current understanding of the molecular and neuroanatomical mechanisms underlying this remarkable natural plasticity in circadian rhythms.

Introduction

Our planet completes a rotation around its axis every 24 hours creating day–night oscillations in many environmental variables. Many organisms have evolved circadian clocks, enabling them to anticipate these predicted changes and align their physiology and behaviour to their constantly changing environment. Perturbations to normal clock functions reduce performance and increase the risk of illness. Circadian clocks are endogenous, meaning that they tick even without environmental signals, but their phase is repeatedly reset by ecologically relevant time cues such as sunlight, food consumption and temperature cycles. The circadian clock influences many downstream molecular, biochemical and physiological processes in numerous cells and tissues throughout the body. Given the importance of normal circadian rhythms to animal health and performance, it was quite perplexing to discover that honey bees and other social insects can show relatively extended periods of activity around the clock with no circadian rhythms and no apparent ill-effects.

Honey bees live in complex societies (known as 'advanced eusociality') typically consisting of a single reproductive 'queen', several tens of thousands of non-reproducing 'worker' bees that are typically the queen's daughters and up to several hundred males. They show elaborate communication systems, allowing them to coordinate almost any aspect of their life. Worker bees generally specialize in certain tasks, forming a division of labour that is related to their age. Young workers usually care for (nurse) the brood and the queen, at later ages they perform nest maintenance activities and at the age of about three weeks, they switch to outside activities such as nest guarding and foraging for resources such as nectar, pollen, water and propolis.

Circadian rhythms in bees

The honey bee was one of the first models for research on circadian rhythms. By the early 1900s, it had been discovered that honey bees have a 'time sense', allowing them to visit flowers at times of highest reward availability. Later studies have implicated the circadian clock in additional complex behaviours such as sun-compass orientation and dance communication, which use directional information relative to the sun location above the horizon. The bees consult their circadian clock to compensate for the sun movement during the day. Given the importance of this clock for successfully foraging and communicating the location of floral resources, it is not surprising that foragers have strong circadian rhythms with activity during the day and sleep during the night. This remains true even when isolated under constant laboratory conditions, indicating that the rhythms are endogenous. Only in the late 1990s it was discovered that strong circadian activity rhythms are not typical to all the bees in a colony.

Socially mediated plasticity in circadian rhythms

When individually tagged bees were placed in observation hives with transparent glass walls, it was discovered that nurse bees tend larvae around the clock without daily activity cycles. It is assumed that around-the-clock care improves brood development, enabling faster colony growth. The premise that task-related division of labour is adaptive is supported by comparative studies showing similar patterns in bumble bees, in which division of labour relates to body size rather than to age, and in ants in which sociality has evolved independently of bees. Remarkably, there is substantial plasticity in division of labour. For example, when there is a shortage of foragers (e.g. due to high predation outside the nest), some young worker bees mature faster and start foraging at an early age compared to typical colonies. If there is a shortage of nurses, some of the older foragers revert to care for the brood (Figure 1). This remarkable plasticity in circadian rhythms is regulated by contact with the brood. Workers in the hive, in small groups or individually isolated, can be induced to switch to activity around the clock if placed with pupae or larvae, and switch to strong circadian rhythms if separated from the brood. Workers that are enclosed in a mesh cage on a broodless piece of wax comb show strong circadian rhythms even if they can smell the odour of larvae.

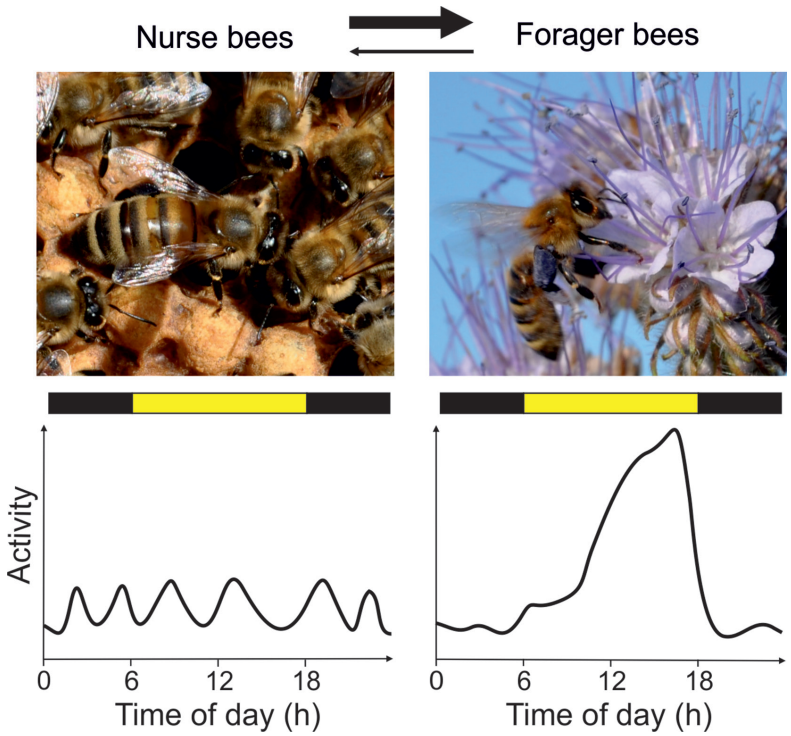

Figure 1. Socially regulated plasticity in the circadian activity of honey bees. Nurse bees care for the brood without daily rhythms, whereas foragers are typically more active during the day and sleep at night. Division of labour is plastic and socially regulated. Young bees develop circadian rhythms with age (thick arrow). However, under certain conditions such as a shortage of young nurses, foragers can revert to nursing (thin arrow). The yellow and black bars on top of the activity plots indicate day- and night-time, respectively.

We now know that extended periods of activity around the clock, and plasticity in circadian rhythms, are not limited to bees and other social insects. For example, it is common in Arctic birds and mammals during the long, 24-hour light, summer days. Marine mammals need to breathe air at the sea surface, and many open-sea fishes such as tuna and some sharks need to constantly move in order to get sufficient flow of oxygen over their gills. Given that the molecular circadian 'clockwork' (i.e. the biochemical 'cogs' driving the clock) is similar in animals as diverse as mammals and insects, it is not clear why some animals can be active around the clock with no apparent ill-effects, while others like humans, suffer from perturbations to the normal rhythms of their body. Studies with bees have started to explore the molecular and neuronal mechanisms underlying natural plasticity in circadian rhythmicity.

The molecular clockwork of bees

Many clock-related genes are known. The honey bee genome encodes the mammalian-type *Cryptochrome* (*Cry-m* or Insect *Cry2*) but not *Timeless1* and the *Drosophila*-type *Cryptochrome* (known as Insect *Cryptochrome 1* [*Cry1*], or *Cryptochrome-d* [*Cry-d*]), which are essential components of the fly clockwork. Consistent with these findings, additional bioinformatics and expression analyses suggest that the honey bee clockwork is more similar to that of mammals than to *Drosophila* flies. The molecular clockwork is based on transcriptional–translational feedback loops. In the honey bee model, the proteins encoded by the *Cycle* (*Cyc*) and *Clock* (*Clk*) genes interact with E-box sequences in the promoters of *Cry-m* and *Period* (*Per*) and activate their transcription. The translated PER and CRY-M proteins are translocated into the nucleus, in which they inhibit the transcriptional activity of the CLK:CYC protein complex. This negative feedback loop downregulates the transcription of *Per* and *Cry-m* (Figure 2). In the bee, as in mammals, it appears that *Cyc* functions in a 'positive' loop that is thought to stabilize the clockwork. This is different from *Drosophila* in which *Clk* forms the positive loop. The circadian expression patterns of additional known clock genes of the positive loop, such as *Par domain protein* 1 and *Vrille*, have not yet been studied with sufficient detail in honey bees. Given that in *Drosophila, Tim1* and *Cry-d* are necessary for light resetting of the clock, their absence from the honey bee genome suggests that bees use different light

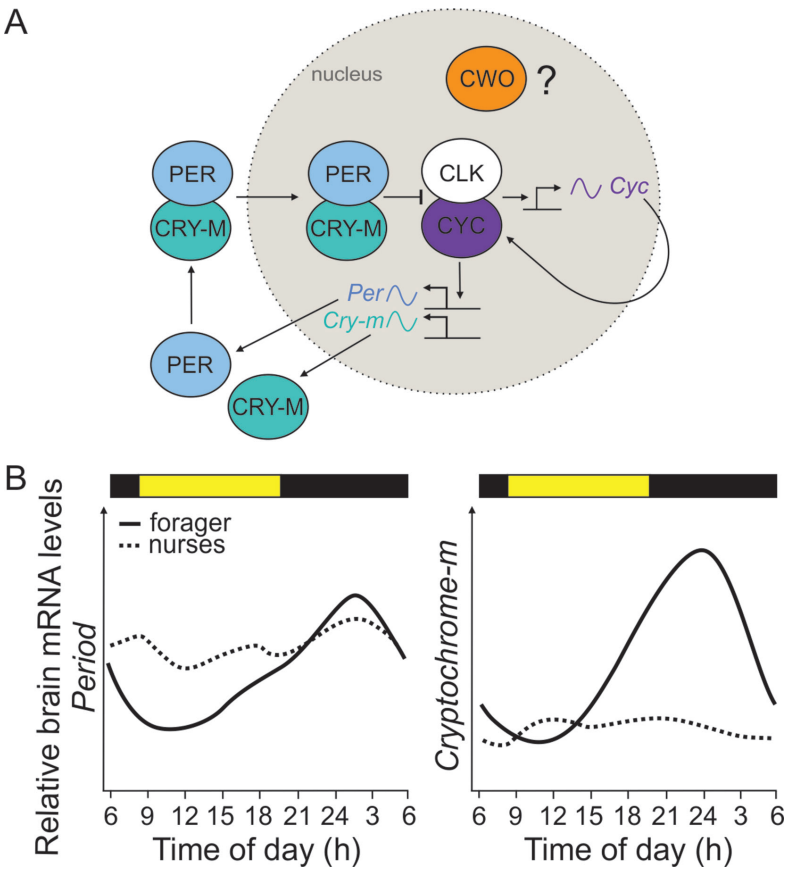

Figure 2. The molecular clockwork of the honey bee. **A)** A model for the circadian clockwork in the honey bee brain. The Cycle (CYC) and Clock (CLK) proteins form a complex that activates the transcription of the *Period* (*Per*) and *Cryptochrome-m* (*Cry-m*) genes. Their translated proteins are translocated into the nucleus in which they inhibit the transcriptional activity of the CYC:CLK protein complex. This down-regulates their own expression, forming a negative transcriptional/translational feedback loop. *Cyc* is part of a second feedback loop that is thought to stabilize the molecular clockwork and is not yet characterized in detail in bees. *Clockwork Orange* (*Cwo*) shows strong oscillations in the bee brain and may have a similar transcriptional repression function as in *Drosophila*. **B)** Whole brain transcript abundance of the clock genes *Per* and *Cry-m* oscillate in foragers but not in nurses. The yellow and black bars are as in Figure 1. A similar pattern of mRNA levels over the day is seen for foragers and nurses, which are collected after the bees experienced constant conditions, indicating that the pattern is endogenous and not driven by the light–dark illumination regime.

input pathways. *Clockwork orange* (*Cwo*), which represses transcription in *Drosophila*, cycles with a similar phase in the bee brain, consistent with the premise of a similar function. The characterization of the molecular clockwork of the bee sets the stage for comparing its function in foragers that show circadian rhythms and nurses that do not.

Measurements of clock gene transcript abundance in the brain of foragers reveal robust circadian oscillations for *Per*, *Cry-m*, *Cyc*, *Cwo* and *Timeout* (a paralog of *Tim1* with no known role in the circadian clock), as can be expected for an animal showing strong activity rhythms. In similar analyses in sister nurse bees on the other hand, the same genes show no, or at best weak, oscillations in transcript abundance. These findings may suggest that the nurse clock stops ticking. However, nurses show strong circadian rhythms in locomotor activity and clock gene expression shortly after transfer from the hive (or brood-containing cages) into a constant laboratory environment. Moreover, the phase of their circadian rhythms is aligned with the day–night cycles outside the hive. How can clocks of nurses inside the dark and thermoregulated hive be in synch with ambient day–night cycles? Studies using several experimental approaches indicate that nurse bees are socially synchronized by foragers that are exposed to the environment outside the nest. Remarkably, when nurses experiencing conflicting social and light–dark cycles are removed from the hive and monitored in constant condition, they show circadian rhythms with a phase more similar to the social cycle. Thus, the honey bee is the first animal for which social synchronization was shown to override synchronization by light. How can this

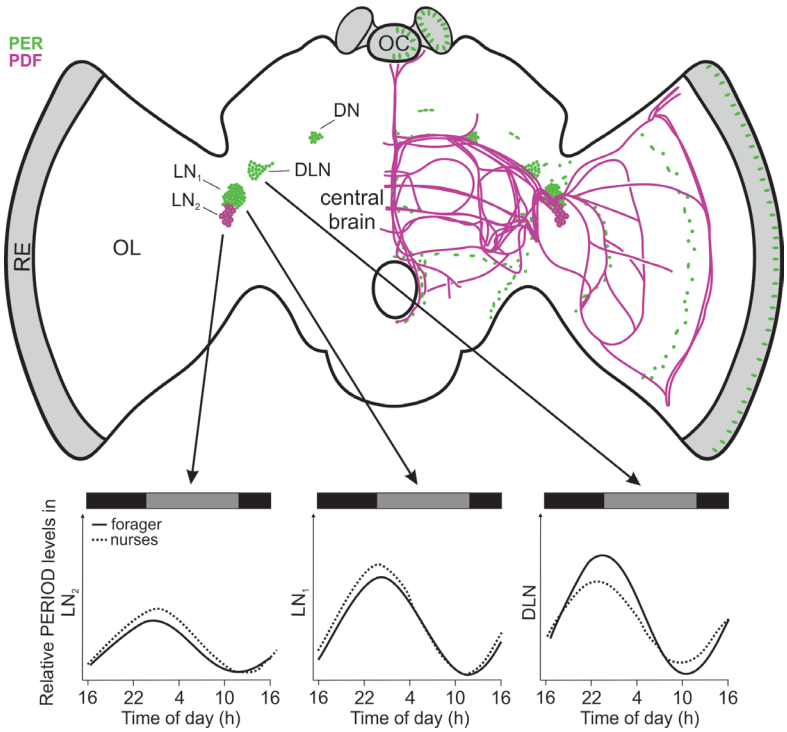

Figure 3. Neuronal clock protein oscillations in behaviorally rhythmic foragers and arrhythmic nurses. **Top.** Schematic organization of the circadian neuronal network in the honey bee brain. Green: cells expressing the clock gene PERIOD protein (PER); purple: cells expressing the circadian neuropeptide Pigment Dispersing Factor (PDF). The left hemisphere shows only the cell bodies of clock neurons. The LN_2 cluster expresses both PER and PDF. The right hemisphere shows the highly complex arborization network of PDF positive neurons as well as PER expressing glia cells. OC: ocelli; OL: optic lobe; RE: retina. **Bottom.** PER levels in clock cell clusters LN_2, LN_1 and DLN similarly cycle in nurses and foragers collected under constant conditions. The grey and black bars on top of the graphs, respectively, depict day- and night-time outside the hive.

evidence for phase resetting be reconciled with their lack of circadian rhythms in behaviour and whole brain clock gene expression? To answer this question, it is necessary to better understand the neuroanatomical organization of the circadian network in the honey bee brain.

Neuronal organization of the bee clock

To this end, the neuroanatomical description of the honey bee circadian clock network is based on immunostaining using antibodies specifically recognizing the core clock protein PER and the circadian neuropeptide Pigment Dispersing Factor (PDF). The honey bee clock network consists of several neuronal clusters centered in the dorsal and lateral brain (Figure 3). PER is also expressed in non-neuronal glia cells that are scattered throughout the brain. The neuronal organization of the circadian clock is similar to that of other insects consistent with a common ground plan of the insect circadian system. The LN_2 is the only cluster in which PER and PDF are co-expressed. These neurons build a highly complex arborization network (Figure 3), connected with many parts of the brain, including neuroanatomical structures regulating locomotion and endocrine systems. As neurons of the clock network also overlap with sun-compass pathway neurons, they may be involved in the integration of circadian and sun-compass information that is necessary for time-compensated sun-compass orientation and waggle dance communication.

Quantification of PERIOD protein levels in clock neuronal clusters revealed strong and similar circadian oscillations in behaviourally rhythmic foragers and arrhythmic nurses. The oscillations in the nurse bees are not consistent with their attenuated cycling in whole brain *Per* mRNA levels. One possible explanation for this apparent discrepancy is task-related variability in clock gene expression in glia cells that are included in whole brain analyses, but not in PER protein quantification that was limited to neurons. The oscillations in clock neurons lend credence to the behavioural studies suggesting that around-the-clock active nurse bees in the tightly regulated nest environment nevertheless have functional clocks that measure time and can be synchronized with environmental time cues.

Summary

The honey bee provides an excellent model for studying the interplay between the circadian clock and complex behaviours including those related to their elaborated social life style. The molecular clockwork of the bee is in many ways more similar to mammals than to fruit flies. Honey bees naturally switch between activity with and without circadian rhythms along with the task they perform. Nurse bees that are active around the clock nevertheless have a functional clock that is socially synchronized by the activity of rhythmic foragers. The temporal pattern of whole brain clock gene transcript abundance differs between rhythmic foragers and arrhythmic nurses suggesting that plasticity in circadian rhythms is mediated by functional modifications in the brain circadian clock system. ■

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Plants signal the time

Zeenat B. Noordally (University of Geneva, Switzerland)
Antony N. Dodd (John Innes Centre, UK)

Plants are generally sessile photosynthetic autotrophs; they depend on light for their existence and cannot move to escape challenging environmental conditions. This means that the lives of plants are intimately linked to daily fluctuations in environmental conditions caused by the rotation of the Earth on its axis. As a result, circadian regulation has an incredibly pervasive influence upon plant physiology, metabolism and development. For example, around 30% of the transcriptome of the model plant *Arabidopsis thaliana* is circadian regulated. In plants, the circadian clock influences processes of crucial importance such as photosynthesis, opening of the stomatal pores that allow gas exchange with the atmosphere, plant growth rates and organ position. It also contributes to the seasonal regulation of flowering. Taken together, this means that the circadian clock influences plant traits that are crucial to agricultural food production.

Circadian rhythms in plants (Figure 1) are generated by a series of interlocked transcription–translation feedback loops (TTFLs; Figure 2). The plant circadian clock has properties that are similar to mammalian and insect clocks, such as a free running period of about 24 hours, entrainment by environmental cues, and temperature compensation of the circadian period (Figure 3a). However, there are key differences between plant and animal circadian clocks. For example, in plants, the circadian system is relatively decentralized, with each cell thought to contain its own semi-autonomous circadian oscillator. This contrasts the structure in animals, where a central circadian oscillator – such as in the brain – communicates information to the peripheral or organ circadian oscillators. Furthermore, although plant circadian rhythms appear to be regulated by TTFLs, the majority of proteins within these feedback loops are different from the proteins having this function in mammalian clocks. At its core, the plant circadian oscillator comprises mutual repression of the morning-expressed myb-like transcription factors, *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), and the evening-expressed *PSEUDO-RESPONSE REGULATOR* (*PRR*), *TIMING OF CAB EXPRESSION1* (*TOC1*) (Figure 2). These are expressed in a cycle that has a period of approximately 24 hours. Fine tuning of this rhythm in response to external and internal cues is accomplished by additional transcription factors that feed into and out of this feedback loop, including but not limited to a suite of PRRs expressed during the day and components of the 'evening complex' (EC) at night (Figure 2).

The plant circadian system can be thought of as a series of signalling pathways. One set of signalling processes communicates information about the environment to the circadian oscillator to adjust its phase, so that it becomes synchronized with the phase of the environment. This process is known as entrainment (Figure 3a). Entrainment occurs in response to signals derived from red and blue light photoreceptors (phytochromes, cryptochromes and other blue light-sensing LOV-domain proteins), photosynthetic sugar-sensing mechanisms and components of the clock that provide sensitivity to ambient temperature. The circadian oscillator integrates these environmental inputs,

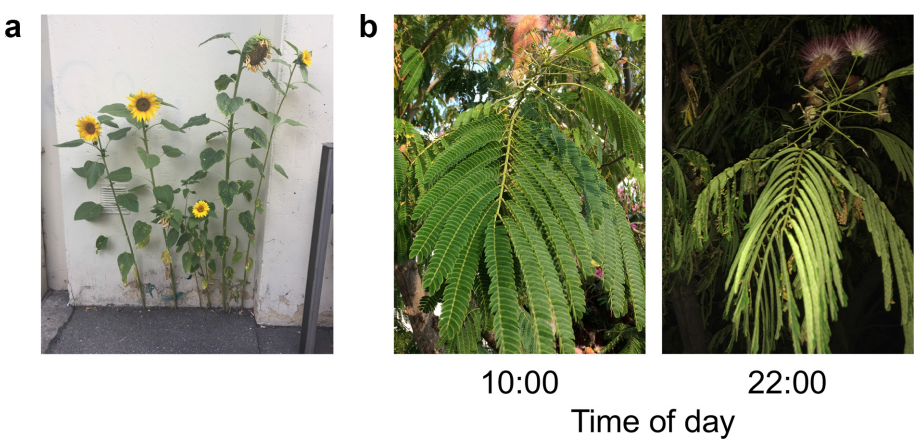

Figure 1. Importance of circadian rhythms in plants. **a)** These sunflowers growing between the paving slabs in a street in Geneva cannot move to escape environmental fluctuations. **b)** The circadian clock controls pronounced daily changes in the leaf position in many legumes, such as this silk tree growing in Sardinia.

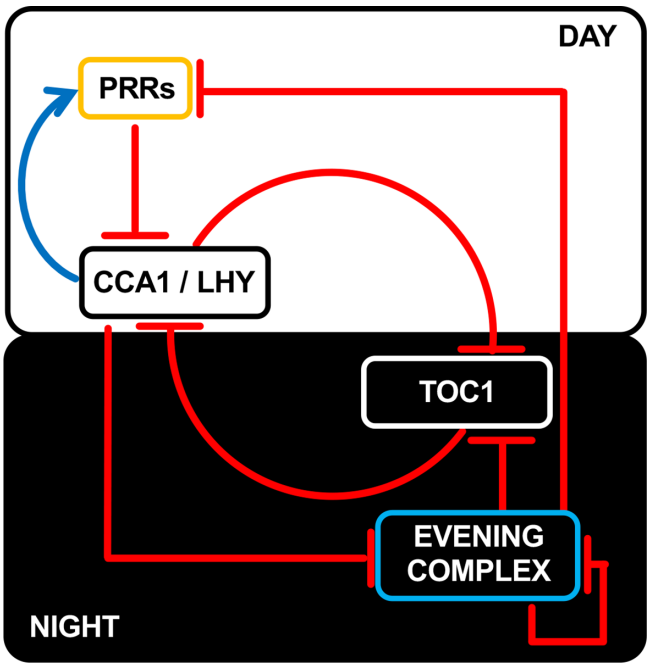

Figure 2. Simplified outline of the circadian clock in *Arabidopsis thaliana*. The core loop of the circadian clock comprises mutual transcriptional repression between morning-expressed *CCA1/LHY* and evening-expressed *TOC1*. The expression of the PRR proteins during the day is promoted by *CCA1/LHY*, which are in turn repressed by the PRRs. Proteins that form the 'evening complex' repress the PRR genes in the evening, so that *CCA1/LHY* abundance rises before dawn.

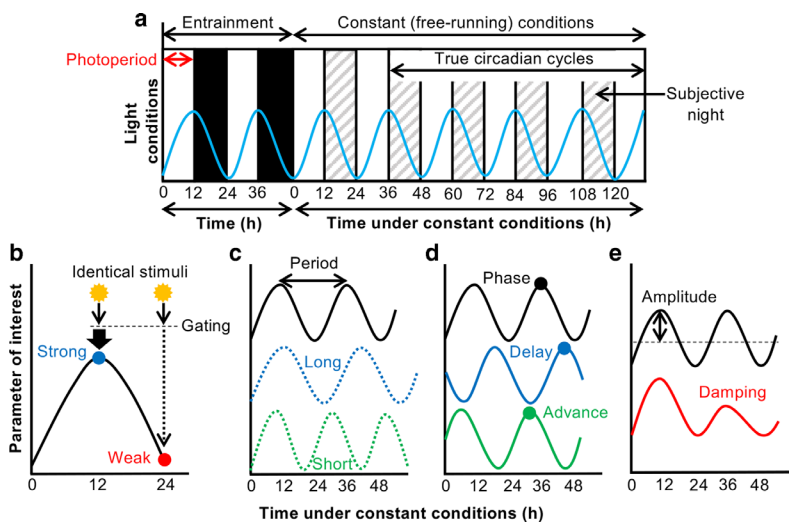

Figure 3. Experimental investigation of circadian rhythms. **a)** Cycles of light and dark can be used to entrain a circadian rhythm, which persists upon transfer into constant conditions, e.g. constant light. True circadian cycles begin 24 hours (h) after the last entrainment cycle, once transient effects from the final dawn become absent. Under constant conditions, the subjective day and subjective night correspond to the light and dark periods of entrainment, respectively. **b)** Circadian gating leads to identical cues applied at different times producing responses of different magnitudes. **c)** The period is the time taken to complete one cycle. Periods greater than 24 hours are said to be long and periods less than 24 hours are described as short. **d)** Phase is the time that a particular event occurs, such as the rhythm's peak. **e)** The amplitude is the difference between the peak (or trough) and the mean value of the oscillation. A progressively decreasing amplitude over time is described as a 'damping' rhythm.

so that it can produce an accurate measure of the time of day. The circadian oscillator produces another set of signals that communicate an estimate of the time of day to circadian-regulated processes within the plant cell. These output processes occur primarily by transcriptional regulation of sets of genes. The circadian oscillator also influences a variety of other cell signalling pathways through circadian 'gating'. This is a process whereby the circadian clock causes daily fluctuations in the output of a signalling pathway, such that a stimulus of identical magnitude will produce a response of differing magnitudes depending on the time of day of the stimulus (Figure 3b). For example, there is gating of the responses of plants to cold temperatures, light signals, pathogens and phytohormone signals that regulate plant growth. This appears to be a very common mode of circadian regulation in plants, although the underlying mechanisms are not always fully understood.

There is also spatial signalling of circadian timing information within the plants at organ, intercellular and intracellular scales. This includes communication between the circadian rhythms in distinct plant organs. For example, rhythms in roots have some properties that are distinct from the rhythms in aerial parts of the plant such as the leaves. It appears that root rhythms are regulated by circadian rhythms in the shoots of plants, with a mobile signal passing through the vasculature suggested to couple together circadian timing within these organs. There is also coupling between the circadian rhythms of different cell types within organs, with the circadian regulation in certain tissues, such as the vasculature, having a dominant influence upon the rhythms in adjacent cell types such as the mesophyll tissue. At an intracellular scale, there is communication of circadian timing information between distinct organelles of plant cells. For example, regulators of chloroplast transcription that are encoded by the nuclear genome and are imported into chloroplasts (sigma factors) are thought to signal circadian timing information from the nuclear-encoded clock to the chloroplast genome.

It is well known that signals from the circadian clock direct many aspects of plant primary metabolism, but there is also evidence that metabolic signals also have a critical role in timekeeping, giving rise to adjustments in circadian period (Figure 3c), phase (Figure 3d), amplitude and/or robustness (Figure 3e) of the rhythms of expression of the clock components. For example, photosynthetic sugars produced within the chloroplasts can lead to signals that entrain the nuclear-encoded circadian clock. Sucrose, sensed by a SnfI-related protein kinase (SnRK1), adjusts the circadian phase of the circadian oscillator through *PRR7*, and signalling through the central growth regulator target of rapamycin (TOR) kinase can modulate the circadian period length of *CCA1* expression in response to glucose.

In addition to sugars, a variety of other small signalling molecules have roles within plant circadian regulation. For example, it appears that signalling by reactive oxygen species (ROS), which are generated in large quantities during plant metabolism, is regulated by the circadian clock. ROS-detoxifying proteins, known as peroxiredoxins (PRXs), are evolutionarily well conserved across all domains of life. PRXs are hyperoxidized in cycles that have a 24-hour period which, interestingly, persist independently of the canonical circadian TTFLs. This could suggest the existence of an ancestral metabolic oscillator. Certain aspects of plant timekeeping are affected in the absence of PRXs, but the underlying mechanism of this elusive oscillator and its potential interactions with the transcriptional circadian clock are less well understood.

There are emerging roles for vitamins in the metabolic regulation of the plant circadian clock. Vitamin B_3 (niacin) is an important coenzyme regulator of redox homeostasis in the reduced forms of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NAD(P)H). Recently, nicotinamide has been shown to inhibit the period adjustments made to the circadian clock by glucose–TOR signalling. Vitamin B_3 has several redox-independent roles in the form of nicotinamide adenine dinucleotide (NAD^+), a precursor of the signalling nucleotide cyclic ADP-ribose (cADPR), and in NAD^+-dependent deacetylation by the signalling proteins, sirtuins. In mammals, sirtuins are involved in clock regulation, but a similar role in plants is yet to be elucidated. Furthermore, there are circadian rhythms in the concentration of cytosolic free calcium ($[Ca^{2+}]_{cyt}$) that encode the timing information and information concerning the light environment. These rhythms are generated by Ca^{2+} release from the internal stores by cADPR, and the $[Ca^{2+}]_{cyt}$ signals are sensed by calmodulin-like proteins to regulate specific components of the circadian oscillator. In addition to Ca^{2+}, there also appear to be circadian rhythms in plants in the cellular concentration of Mg^{2+}, which is a crucial regulator of metabolism by adenine 5'-triphosphate (ATP) hydrolysis.

The circadian clock regulates complex networks of signalling pathways, the products of which optimize plant physiology and can regulate the clock itself. Whilst our understanding of circadian rhythms in higher plants is mainly derived from experimentation with the model plant *Arabidopsis thaliana*, the community is also working to understand the circadian clock structure and its functions in both crops and naturally occurring plant populations. For example, selection for a longer circadian period during the domestication of tomato is thought to allow tomato to perform better at more northerly latitudes, raising the possibility that the latitudinal range of other crops might be extended by manipulation of the circadian clock. Fundamental research from *Arabidopsis* can be exploited to understand how the circadian regulation and metabolic homeostasis adapt plants to their fluctuating environments, and manipulate the circadian regulation of signalling and metabolism to sustainably increase agricultural production. ■

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Circadian neuronal networks

Özge Özkaya and **Ezio Rosato** (University of Leicester, UK)

Whatever you were doing just before reading this, walking to work, drinking a coffee after lunch or winding down in the evening in your favourite armchair, it is likely you will do it again tomorrow, more or less at the same time. This daily cycle is a reflection of the fact that we live in a 24-hour environment that, through rhythmic changes in light, temperature and social interactions, imposes temporal constraints on our lives. However, we do not simply react to those daily changes; rather, we anticipate them and behave accordingly.

It is all about good timing

We now know that the reason why we predictably allocate different activities to different times of the day is that we have evolved an endogenous biological timer that ticks with a period of about a day (*circa diem* in Latin) (Figure 1).

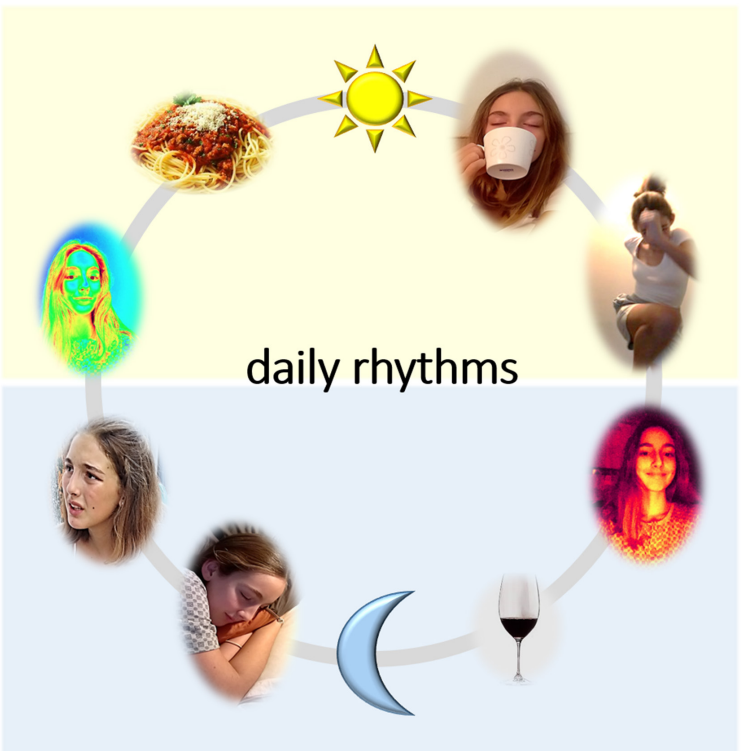

Figure 1. Daily rhythms. Our lives are marked by a constellation of activities that happen every day more or less at the same time. For instance, in the morning, our body temperature is at its lowest. Eating is under strong circadian control, and every day we feel hungry at about the same times, depending on when we normally have lunch and dinner. After lunch, we reach the trough of our attention, and many of us resort to caffeine to keep awake. A few hours later, our muscular tone is at its best, and we perform better in sports in the late afternoon. In the evening, our body temperature is at its warmest. Also in the evening, our ability to metabolize alcohol increases, and this is the time when we preferably consume alcoholic drinks. Night is our favourite time to sleep, but at night our sensitivity to pain is highest, so feeling pain may keep us awake.

There is abundant evidence suggesting that this circadian clock has adaptive value. Laboratory tests using different experimental systems (animals, plants, fungi and cyanobacteria) have shown that fitness is highest when organisms are placed in an environment that cycles with a rhythm that is the same as their inner rhythm. Additionally, field studies demonstrated that natural selection sculpts the clock; by sampling natural populations, we can identify clock variants that are best adapted to their local rhythmic conditions.

Since this synchrony between internal and external rhythms has been shown to confer benefits, we could argue, in contrast, that living in an environment that does not align with our inner clock might have consequences that are worse than not having a clock at all.

This prediction holds true; people exposed to continuous circadian insult, such as shift workers, have a higher incidence of psychiatric conditions, cardiovascular disease and cancer. Further, as industrial and post-industrial societies move inexorably towards a '24-hour life', where the difference between day and night is less defined, we observe an emerging epidemic of sleep and behavioural disturbances.

Clearly, 'circadian hygiene', or the harmony between external and internal rhythms, has real-life benefits. To ensure we implement it (both personally and at the level of society as a whole) and to suggest remedial measures for a broken clock, we must first learn, in detail, how the circadian clock works.

The fact that life on earth evolved from a common ancestor makes it possible to investigate the architecture of complex phenomena in simpler, more malleable, model organisms. On this premise, we discuss the circadian clock of the fruit fly *Drosophila melanogaster*, a small insect with a complex nervous system. Studying the circadian

clock of the fly has paved the way to understanding our own clock and, more generally, to unravelling how the nervous system works.

A cell-autonomous molecular clockwork

In 1971, through a mutagenesis screening in *Drosophila*, the first clock gene was identified. It was named *period* (*per*) and became the first-ever recognized component of the circadian clock. This started a concerted effort across many laboratories working on different species to use genetics to identify other genes involved in the clock and use biochemistry and molecular biology to describe the mode of action of their protein products.

Early efforts were specifically directed at identifying the cellular machinery of the clock, based on the assumption that the mechanism must be cell-autonomous since single cells, whether independent organisms or isolated mammalian cells in culture, show 24-hour rhythms in their physiology.

Multiple clock genes were identified in several model systems. This showed that the general design of the clock is conserved across kingdoms although the molecular components have evolved independently in animals, plants, fungi and cyanobacteria. (Other bacteria do not seem to have a recognizable clock.)

The general principle on which the clock is based is that of a negative feedback loop. Essential clock molecules change their status progressively across the 24 hours, thereby providing time information to the cell. As part of this process, they increasingly inhibit their own maturation or production until they either revert to their original state or are degraded so that a new cycle can begin. Since 24 hours is a particularly long time in terms of single chemical reactions, many biochemical processes come together to provide the necessary 'delay' in reaching the 24-hour period. This 'cooperation' provides robustness and increases the amplitude of the cycle, which is required for it to be able to influence the overall physiology of the cell.

In animals, the negative feedback loop is built around the control of key transcription regulators, such as PER (the protein product of the *per* gene), that can impart 24-hour rhythmicity to their own transcription and to that of downstream effector-encoding genes (Figure 2; for more information, see the article by Smith and Sassone-Corsi, this issue).

Three pioneers in this field, Jeffrey Hall (Brandeis University, USA), Michael Rosbash (Brandeis University, USA) and Michael Young (Rockefeller University, USA), were awarded the 2017 Nobel Prize in Physiology or Medicine for their discoveries of how *per* is regulated.

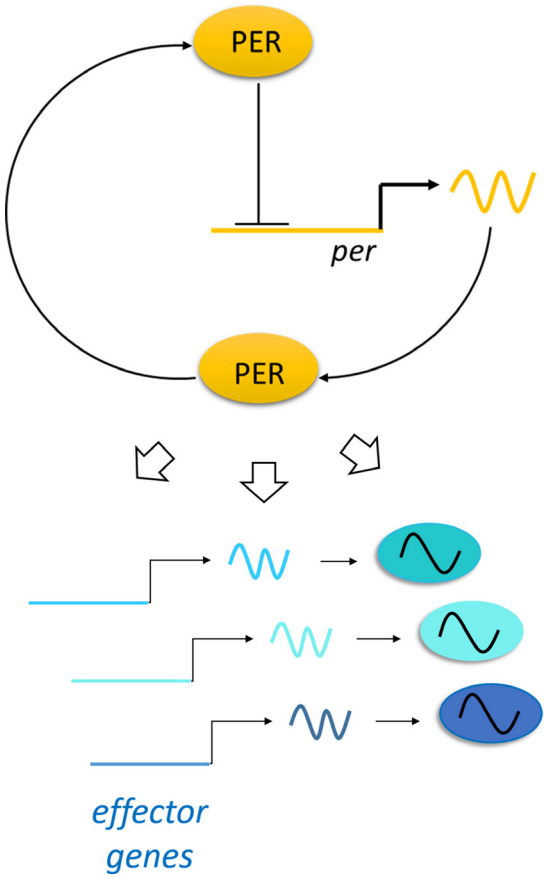

Figure 2. The basic functional unit of animal clocks. Cloning of the *per* gene and molecular characterization of its protein product–PER–revealed that this molecule is at the centre of a negative feedback loop. PER rhythmically inhibits expression of its own gene and of other genes encoding additional components of the clock. Mostly, these have been identified by mutagenesis screening and shown to have disparate molecular activities: transcription regulators, kinase/phosphatase, E3 ubiquitin ligases, translation regulators, miRNAs and so on. The clock then controls the expression of rhythmic genes and/or proteins responsible for imparting rhythmicity to physiology. Although there are some differences among species, the general design and the molecules that constitute the clock are evolutionarily conserved in the majority of animals analysed to date.

Many 'clock cells'

Although clock cells conform to a general design, animal clocks are made of cells that are not identical. For instance, there are molecular components that are expressed at a much lower level, if at all, in some clock cells compared to others in the same organism.

Experiments in mammals have shown that some isolated cells are more rhythmic than others *in vitro*.

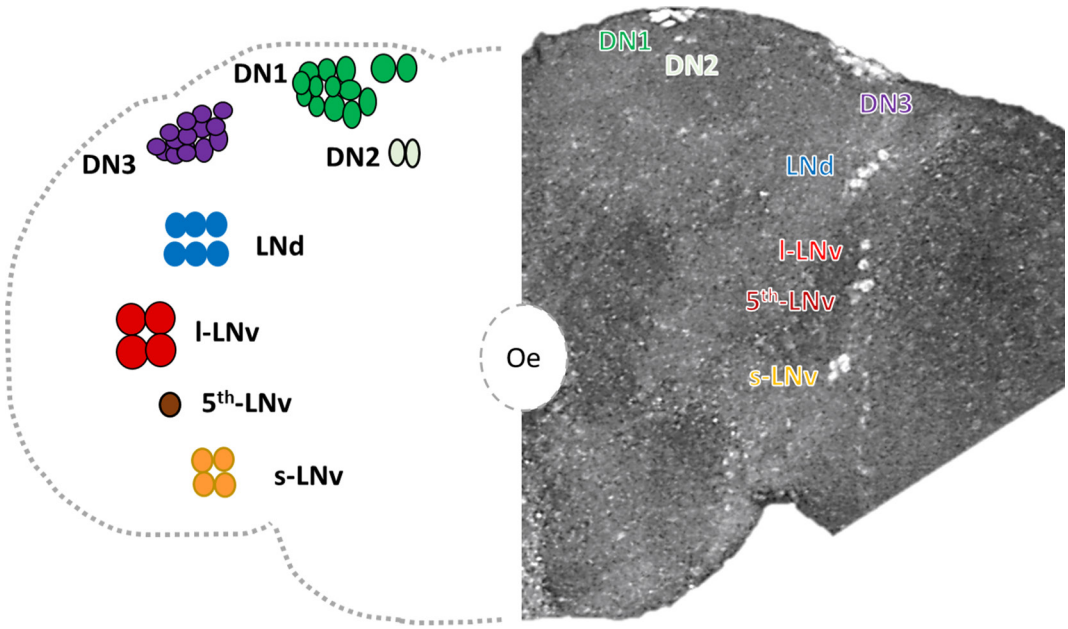

Figure 3. The anatomy of the circadian clock in *Drosophila*. The figure shows a representation of the clock neurons in the brain of the fly. The right hemisphere is a composite image obtained by combining several pictures of the same specimen acquired with a confocal microscope at different focal planes. The left hemisphere shows a diagrammatic representation. The clock neurons are named lateral neurons (LN) and dorsal neurons (DN), which are indicative of their relative position in the brain. The lateral neurons are divided into dorsal (LNd) and ventral (LNv). The latter are further distinguished into small (s-LNv), large (l-LNv) and 5th-LNv (because originally it was considered related to the four s-LNv). The dorsal neurons are numbered consecutively, e.g., DN1, DN2 and DN3. According to the dual-oscillator model (see text), the s-LNv form the morning oscillator while the 5th-LNv plus three LNd (the LNd are a heterogeneous group) constitute the evening oscillator. Additionally, the s-LNv function as a pacemaker. In flies, the oesophagus passes through the brain; Oe indicates the oesophageal foramen (opening).

Surprisingly, neurons derived from the suprachiasmatic nucleus (SCN), a brain centre that in mammals exerts control on the rhythmicity of the whole organism, are poorly rhythmic in dispersed culture. Conversely, SCN slices that maintain extensive communication among neurons are strongly rhythmic when grown *ex vivo*. This suggests that cellular communication is an additional, fundamental characteristic of animal clocks, at least in the brain.

In flies, about 150 clock neurons carry out functions similar to the approximately 20,000 neurons that constitute the SCN in mammals. This reduced complexity and the availability of tools to manipulate groups of clock neurons separately explain why our understanding of the circadian neuronal network is particularly advanced in *Drosophila* (Figure 3).

The 'clock network'

Since we can manipulate different groups of clock neurons independently, we can ask two fundamental questions:

1. Are different neurons in charge of the different features of the 24-hour rhythm?
2. Which neurons are necessary and/or sufficient for self-sustained rhythmicity?

Note that these questions assume a division of roles and a hierarchical relationship among the clock neurons; but how does it happen? One possibility is that roles and hierarchy are pre-defined to accommodate the features of different environments. Another is that the role and hierarchy adopted by different neurons depend on the configuration that the network assumes in the present condition and following its experience of previous environments.

The difference between the two hypotheses is fundamental. The first assumes that the functioning of the circadian system is predominantly stereotyped and that the characteristics of the clock as a whole reflect the characteristics of discrete neurons according to their leading role. The latter assumes that plasticity has a more important function and that the overall characteristics of the clock do not belong to any specific neuron but emerge from the configuration of the ensemble.

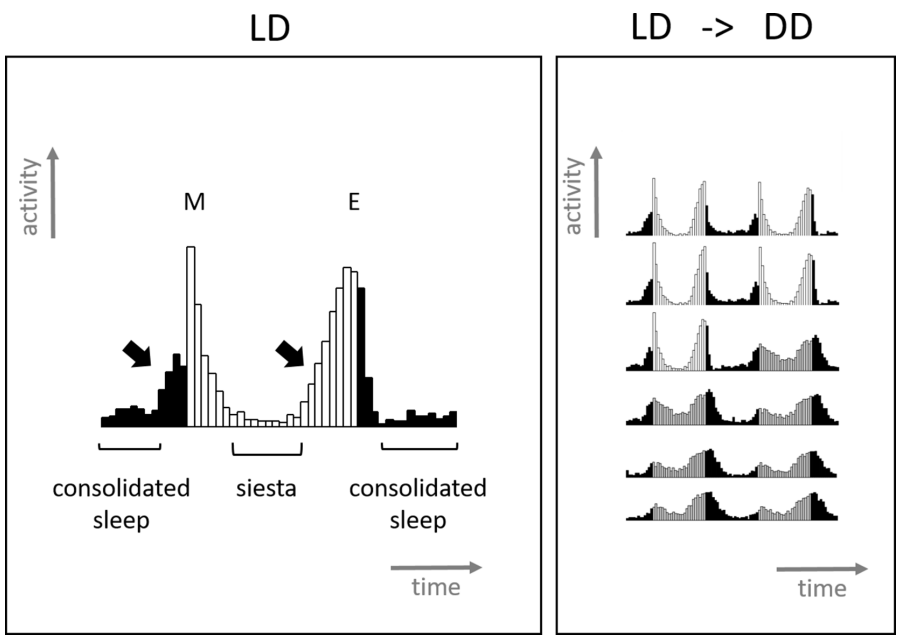

Figure 4. Circadian phenotypes in *Drosophila*. **Left panel**. One-day locomotor activity profile of several wild-type male flies averaged together (the activity profile of females is different) under 12-hour light and 12-hour dark conditions (LD). The temperature was constant at 25°C. The height of each bar corresponds to the amount of activity (measured by a commercial device that counts how many times a moving fly interrupts an infrared beam). The width of each bar is constant and represents 30 minutes. White bars denote the light phase and black bars the dark phase. M indicates the morning peak of activity and E the evening peak. Both peaks display 'anticipation' shown by an arrow. The phase of rest during the middle of the day is referred to as 'siesta'. During the night, there is a prolonged phase of consolidated sleep. **Right panel**. Average locomotor activity profiles of male flies. Three days under LD conditions followed by 4 days under constant darkness (DD) are shown. The temperature was constant at 25°C. The activity profiles are double-plotted (e.g. day 1–day 2, day 2–day 3, etc.) to help visualize the rhythm. White bars denote the light phase and black bars the dark phase. Grey bars correspond to subjective light. This means that the flies experienced dark but at a time when they would have been in light under LD conditions. Note that the flies are still rhythmic under DD, showing they have an endogenous clock.

Rhythms in locomotor activity and rest are the preferred circadian phenotype in *Drosophila*. Using small commercial devices, this phenotype can be monitored for hundreds of flies in parallel with little effort. Flies are crepuscular, meaning they are far more active in the morning and in the evening compared to the middle of the day or the middle of the night. Therefore, their activity profile shows a morning (M) and an evening (E) peak separated by a 'siesta' in the middle of the day and a phase of consolidated sleep at night. In the laboratory, light is usually presented as a symmetrical rectangular signal (either ON or OFF, 12 hours apart) under constant temperature (often at 25°C). Under such conditions, both M and E peaks reach their maximum at the time of the switch. The increase in activity before the switch is known as 'anticipation' and denotes the function of the circadian clock (Figure 4).

Flies carrying a mutation in the *per* gene, called per^0, have no functional PER protein. These flies cannot anticipate M or E peaks and are arrhythmic under constant darkness and temperature. We can test the function of different groups of neurons by reintroducing PER into those neurons only, while the rest of the animal still lacks functional PER.

Such manipulations showed that different groups of neurons rescued the 'anticipation' of the M and E peaks. Thus, we could suggest that the clock is composed of two connected oscillators: one morning oscillator controlling the M peak and one evening oscillator controlling the E peak.

Moreover, when PER expression was limited to the morning oscillator, flies were rhythmic under constant conditions. This was not the case for the evening oscillator, which led researchers to propose that the morning oscillator is the 'pacemaker'.

This interpretation that agrees with a deterministic model of the clock resonates well in the circadian field. Many years ago, researchers observed that small mammals placed under constant illumination split

their locomotor activity into two components: one with a long and the other with a short period. This led them to hypothesize that the clock is a dual-oscillator system.

Today, many investigators suggest that the results of the fly experiments provide further evidence for such a model as they map the morning and the evening oscillators to two distinct groups of neurons. This is why, in many circadian articles, the *Drosophila* clock is described using terms such as 'morning cells', 'evening cells' and 'pacemaker neurons'.

However, other researchers (including the authors) argue that such an interpretation is too simplistic. There is evidence of functional plasticity that should be taken into account in a model of the clock. For instance, it is possible to shift morning anticipation to an earlier time by 'speeding up' the clock of some other neurons (i.e. not the 'morning cells'). Additionally, increasing the excitability of these neurons under constant conditions makes the behavioural outcome worse (i.e. a higher proportion of flies are arrhythmic) than increasing the excitability of the 'pacemaker neurons'. These results do not fit the expectations of the dual-oscillator model, suggesting instead a functional switch in the role of neurons according to experimental conditions.

From 'clock network' to 'internal state networks'

In recent years, *Drosophila* researchers have described several neuronal circuits related to internal states. For instance, some circuits provide an internal representation of homeostatic needs such as sleep, hunger, thirst and sex drive. Others reflect social experiences, such as assuming a dominant/subordinate position after winning/losing several aggressive encounters or after multiple acceptance/rejection events following courtship. Some others depend on health/sickness status.

Internal states modulate how animals perceive and interpret the environment and motivate the selection of appropriate actions. Superficially, those circuits appear anatomically invariant, but they are functionally plastic. Some neurons respond to internal needs and changes in environmental variables by releasing neuromodulators. These molecules modify circuits by varying the excitability of neurons and/or the strength of synaptic connections, resulting in the physiological flexibility that is required for behavioural variability.

Internal state circuits interact with each other and sometimes even physically overlap. Yet, they regularly compete to take control of the overt behaviour. For instance, hungry flies search for food even at times when they should be asleep. Conversely, well-fed but sleep-deprived flies sleep at times when they should be alert.

Under this perspective, the concept of a distinct and almost 'self-contained' circadian network seems restrictive, and new questions are beginning to emerge. Can we physically separate the circadian network from other networks defining internal state? Is the presence of the molecular clock (i.e. the components of the negative feedback loop) necessary and/or sufficient to qualify a neuron as part of the clock network? Are there any conditions under which non-clock neurons (those that do not express clock genes) could take control of the circadian rhythmicity of the animal?

We suggest that unravelling how 'internal state networks' interact under 'good' or 'bad' timing (i.e. under conditions of harmony or disharmony between the inner clock and the external environment) is the new 'frontier' of circadian research. The main challenge will be to abandon a comfortable experimental and theoretical framework that considers the clock a highly stereotyped network built by 'clock neurons', and instead to embrace the unknown. ■

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A beginner's guide to flow kinetics

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For a liquid flowing through a tube at constant velocity, the distance from the point of origin can provide a measure of the reaction time. This concept of continuous flow, first applied over a century ago to follow biochemical reactions by absorption spectroscopy, is now being used in conjunction with high-resolution structural methods such as X-ray crystallography and cryo-electron microscopy. The resultant kinetic information is crucial to understanding the mechanism.

The 'Beginner's Guide' series covers key techniques and offers the scientifically literate, but not necessarily expert audience a background briefing on the underlying science of a technique that is (or will be) widely used in molecular bioscience. The series covers a mixture of techniques, including some that are well established amongst a subset of our readership but not necessarily familiar to those in different specialisms. This Beginner's Guide introduces flow kinetics.

Barely a week goes by without an article appearing in *Nature* with a title of the form 'A structural-based mechanism of ...', that presents a functional model of some biochemical mechanism, but contains no time-dependent measurements. In effect, structures are placed in a sequence that seems to make sense, but this does not constitute evidence that the assumed sequence is correct. This emphasis on structure is not a new phenomenon. Over 20 years ago, Cornish-Bowden complained: "And for all the current and rather silly emphasis on structural biology, understanding enzymes means understanding catalysis and catalysis is concerned with kinetics, not structure: as Jeremy Knowles aptly remarked, studying the photograph of a racehorse cannot tell you how fast it can run." No doubt, the over-the-top wording was chosen to attract attention, in an attempt to redress the balance of research. However, kinetics requires a signal, and if the signal comprises detailed structural information, so much the better. Kineticists should rejoice when novel detection methods are developed because it opens new avenues for time-resolved measurements and reinstates kinetic methodology at the cutting edge of research where it belongs.

The evolution of continuous flow

Recent examples of structural measurements that encompass the fourth dimension include time-resolved crystallography based on the continuous-flow concept, first developed in the 1920s to study the kinetics of oxygenation of haemoglobin (Figure 1a,b). Both assays faced the problem that the measurement process (recording the X-ray diffraction pattern or the visible absorption spectrum, respectively) took longer than the event under investigation. By flowing the reactants at a constant known velocity past the detector, the distance of recording point from the initiation point, d, provides a measure of reaction time on the milli- to microseconds timescale. The original continuous-flow method (Figure 1a) made extravagant use of materials, requiring litres of reactants. This led to development of stopped flow in the 1940s, where flow was used to achieve rapid mixing and the reaction was monitored in real time after bringing the newly mixed reactants to a halt within about 1 millisecond. This advance was only possible when optical detectors were fast enough to capture the signal in real time. Stopped-flow instruments with optical detection have been the mainstay for analysis of transient kinetics of enzymes and other macromolecules in the last half century. For reactions without an optical signal that require offline product analysis, the continuous-flow method was extended into the quenched-flow technique. Here, the reaction is brought to a halt by mixing with a third component (e.g. acid or another denaturant) that stops the reaction at a known time (determined by the length of the ageing tube and flow velocity) and the products are analysed subsequently. A variant of this method is to rapidly freeze the reaction mix after it flows through a calibrated ageing tube. This approach was particularly useful for electron paramagnetic (spin) resonance spectroscopy (EPR/ESR) because many seconds were needed to run the spectrum. More recently, the method has been applied to electron microscopy (Figure 1c), which has made a huge impact over the last decade

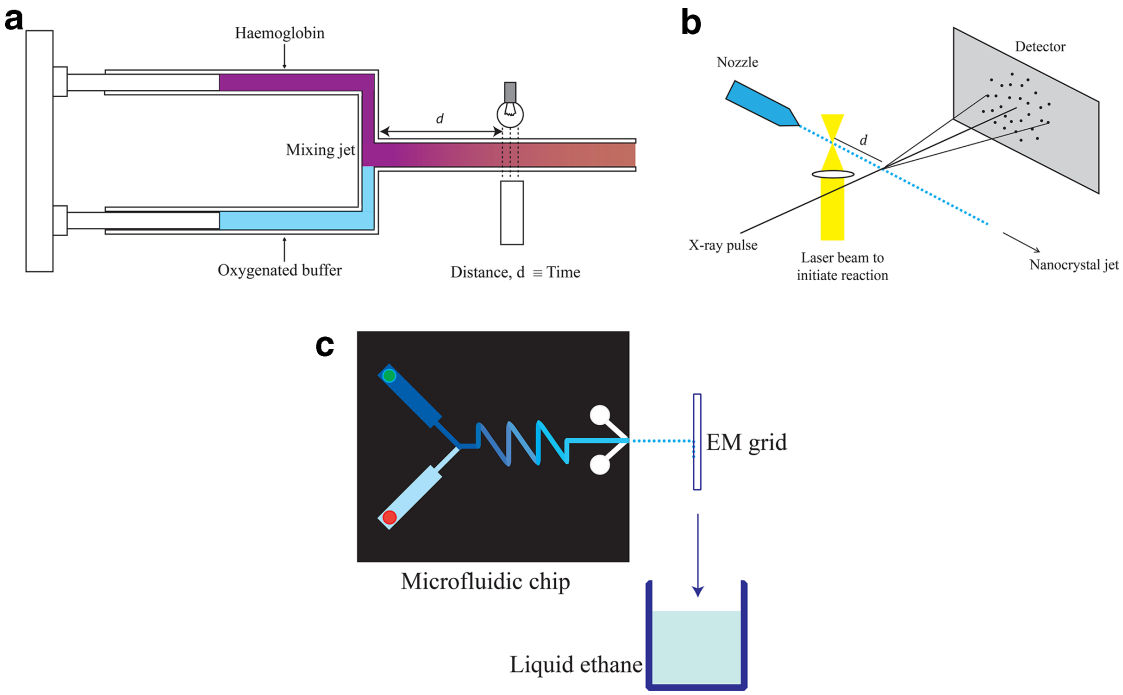

Figure 1. The evolution of continuous-flow methods. **a**) The original design used to study the oxygenation of haemoglobin. A hand-held spectroscope was used to monitor the colour change along the length of the ageing tube. **b)** A jet of nanocrystals is interrogated by X-rays, following the initiation of a photochemical reaction using a laser beam. **c)** A microfluidic device for time-resolved cryo-electron microscopy. Reactants are introduced via the red and green ports and pressurized gas is introduced via the white ports to spray the sample onto the grid. The latter is mounted on a plunger that quickly transfers the grid into liquid ethane. In the case of reactions involving a colour or fluorescence change, the microfluidic channel can be imaged to determine the time course along its length.

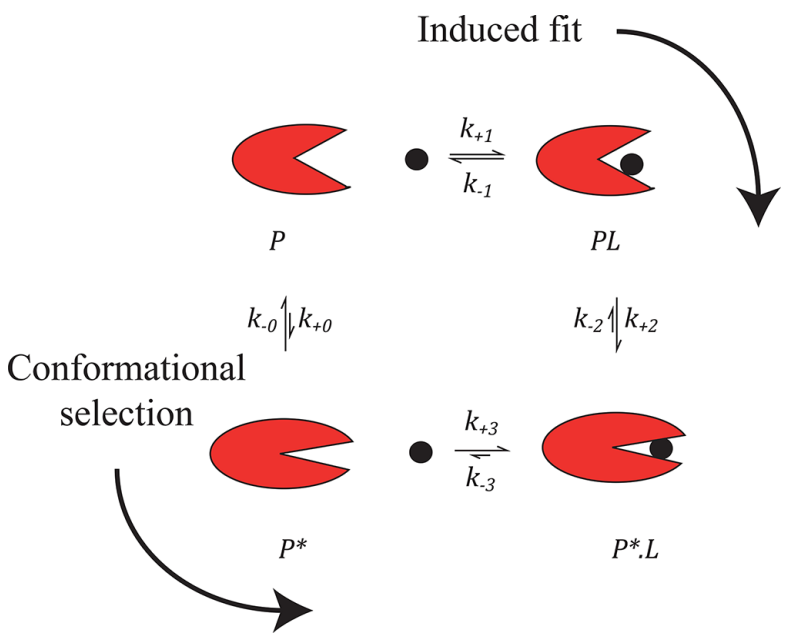

Figure 2. Conformational selection. A ligand (black circle) binding to a protein is often accompanied by closure of the binding site to make more favourable interactions. The closure may occur before or after binding. In real life, elements of both mechanisms are likely to apply, but one step (k_{+0} or k_{+2}) may limit the observed kinetics.

as structure determination has reached near-atomic resolution.

Cryo-electron microscopy has the potential advantage over X-ray crystallography in resolving heterogeneous class structures, which may suggest a dynamic system. However, the inadequacy of structural information alone to determine the mechanism is well illustrated by the long-standing discussions of induced-fit versus conformational-selection models for ligand binding (Figure 2). Conformational selection requires that the apo state of the binding partner is present in two or more conformations, one of which resembles the structure in the ligand-bound state and preferentially binds the ligand compared with the other conformation(s). However, this thermodynamic property does not translate directly to the kinetic pathway. Finding a bound-like conformation of the apo state amongst multiple conformations does not, in itself, prove conformational selection is the dominant pathway. Conversely, failure to find such a conformation does not rule out conformational selection because such a state may be below the detection limit. Only kinetic information can resolve this conundrum.

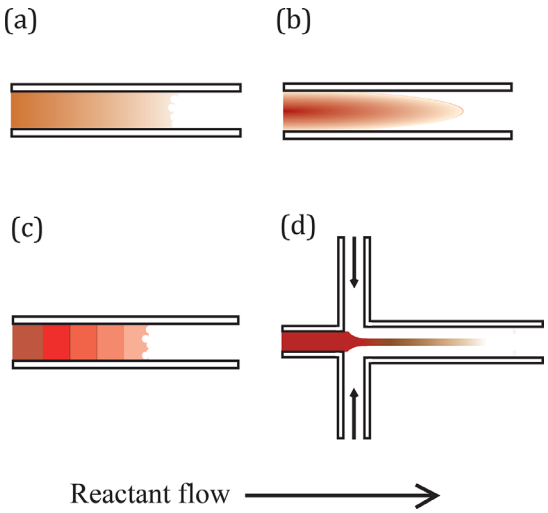

Figure 3. Flow characteristics in narrow-bore tubes.
a) Turbulent flow, **b)** laminar flow, **c)** pulsed turbulent flow and **d)** hydrodynamic focusing.

Some characteristics of flow

Given the renewed interest in applying flow methods in structural biology, it is pertinent to review some critical aspects of flow. In order for distance of fluid flow along a conventional ageing tube to be a proxy for reaction time, it is important that the flow is turbulent (Figure 3a). If the flow is too slow, it becomes laminar and the velocity of the liquid in contact with the side walls becomes zero (Figure 3b). Consequently, the age of the reaction mix at any point along the tube becomes ill-defined, even though the net flow is constant. Turbulence is also required during the mixing of reagents to bring the reactants into close proximity. The Reynold's number, R_e, defines the minimum flow velocity required through a tube to maintain turbulent flow.

$$R_e = \frac{\rho D v}{\eta}$$

where ρ is the solvent density, D is the diameter of the tube, v is the velocity of the fluid and η is the viscosity of the liquid. R_e is a dimensionless number, but care is required in using self-consistent units (e.g. kg, m and second) for the parameters in the above formula. An R_e value of <2000 will result in laminar flow, while >4000 is likely to be turbulent. It is evident that as instruments are scaled down in size (reduced D) to accommodate the limited availability of many biomaterials, so it becomes harder to achieve turbulent flow. Increasing v helps compensate, but this will shift the accessible time range to shorter values and may lead to excessive back pressure. One solution is to use pulsed flow where repetitive short but high-velocity pulses are applied to drive the solution through the ageing tube with a net slower velocity (Figure 3c).

In the case of microfluidic devices, where tube diameters are a few hundred micrometres or less, the turbulence criterion is more difficult to meet. The dispersion in timing due to laminar flow may be ignored, or at least tolerated, in cases where an approximate sampling time is sufficient. For example, in the device of Lu and colleagues used for rapid freezing of samples for cryo-electron microscopy, a mean reaction time of about 9 milliseconds was achieved using a 40 × 100 × 6000 µm channel with a flow rate of 6 µL /second^{-1}. The overall reaction time comprised 0.5 millisecond mixing time, about 4 millisecond of microfluidic flow, 5 millisecond spray time to the grid and <1 millisecond freezing time. For longer microfluidic flow channels, the dispersion of flow times, and hence reaction times, becomes more significant. This dispersion may be acceptable when characterizing the dominant species within a reaction mixture, but care is required in the interpretation of the kinetics of minor species. Laminar flow can be reduced by introducing one or more U- or Z-bends in the flow channel (Figure 1c).

The effect of laminar flow can also be minimized using hydrodynamic focusing (Figure 3d). For two-dimensional hydrodynamic focusing, buffer is introduced on either side of the new mixed reactants to give a thin ribbon of reactant that is largely clear of the side walls and proceeds along the centre of the tube linearly with time. In the case of three-dimensional hydrodynamic focusing, the reactants are introduced via the centre channel of concentric delivery tubes, which can focus the reactants into a core with a diameter of the order of 30 nm. Such an apparatus has been used to follow macromolecule refolding reactions, where the small molecule denaturant diffuses out of the core on the microsecond timescale, allowing a slowly diffusing macromolecule to refold as it progresses along the central core. The problem of side wall interactions with laminar flow can be avoided altogether by firing the reactant jet as microdroplets into air at a defined constant velocity and using the time of flight before sampling or detection as a measure of reaction time (Figure 4).

The initiation of the reaction also needs consideration. Usually, this is achieved by mixing two solutions together in a jet, so that the boundaries between the solutions are reduced to a few micrometres and diffusion completes the mixing process on the timescale a few hundred microseconds. Again, reducing the dimensions of the apparatus can assist in this process and some microfluidic devices can mix solutions within tens of microseconds. Photosensitive reactions can be initiated on an even shorter timescale with a brief light pulse.

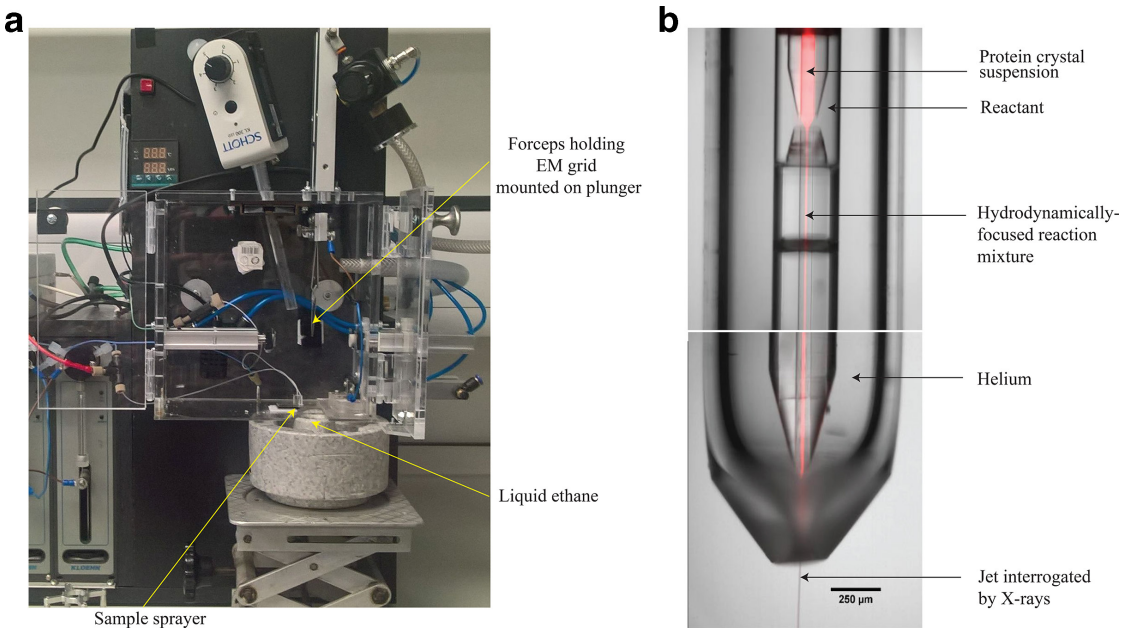

Figure 4. a) Time-resolved cryo-EM apparatus of Kontziampasis et al. (2019) (reproduced under Creative Commons License). In this apparatus, the spray is controlled by application of a high voltage rather than gas pressure as in Figure 1c. **b)** Mixing jet for time-resolved X-ray crystallography developed by Calvey et al. (2016) (reproduced under Creative Commons License), where a small molecule reactant is brought in close proximity to a suspension of crystals by hydrodynamic focusing and then fired into the X-ray beam using helium gas.

Data analysis

Data analysis is another important step in determining a mechanism. For example, consider a hypothetical reaction monitored by time-resolved cryo-electron microscopy, which is initiated by mixing an excess ligand (L) to a macromolecule (A). Excess ligand concentration ensures the binding reaction occurs under pseudo-first-order conditions with $k_1 = k_{bind}.[L]$, where k_{bind} is the second-order rate constant. Supposing the study identifies a novel transient structure formed with a half-time of about 0.07 second ($k_{obs} = \ln 2/t_{1/2} \approx 10$ second^{-1}) whose population decays to zero with a half-time of about 0.7 second ($k_{obs} \approx 1$ second^{-1}). The data are most simply analysed by a two-step mechanism:

$$A \xrightarrow[L]{k_1} B \xrightarrow{k_2} C$$

Equation 1

where A represents the apo state and B represents the novel intermediate structure. It might be tempting to assign the rate constant, $k_1 \approx 10$ second^{-1} to the binding step and $k_2 \approx 1$ second^{-1} to the second step. However, this assignment is ambiguous in the absence of amplitude information. Supposing it is observed that the transient conformation B only reaches a maximum of 8% of the total number of molecules. In this case, it is more likely that the assignment order is reversed with $k_1 \approx 1$ second^{-1} while $k_2 \approx 10$ second^{-1} (Figure 5). To distinguish these scenarios, we need to follow the kinetics of disappearance of the initial A state. If A disappears with an observed rate constant of 1 second^{-1}, then the latter assignment is favoured. Alternatively, it may be found that some A remains at the end of the reaction, indicating the presence of inactive molecules. If 90% remains as A, then the 10% active fraction of A would display a $k_1 = 10$ second^{-1} and a $k_2 = 1$ second^{-1}. The reaction profile of B could also arise if the first step is reversible. Any combination of rate constants that satisfy the conditions $k_1 + k_{-1} + k_2 = 11$ second^{-1} and $k_1.k_2 = 10$ second^{-2} with active A being between 10% and 100% can explain the profile of B (e.g. $k_1 = 3.3$ second^{-1}, $k_{-1} = 4.7$ second^{-1}, $k_2 = 3$ second^{-1} with the initial A being 30% active). Changing the ligand concentration to modulate k_1 would help to remove this ambiguity. Note $k_{-2} \approx 0$ because B approaches 0 at the end point of the reaction.

There are also technical problems that could affect determination of the populations of the different states. The analysis of electron micrographs attempts to assign each molecular image to a structural class, but some views may be ambiguous as to their assignment. Also, for longer times in a microfluidic continuous-flow device, laminar flow becomes more problematic, so that the peak in B may be broadened and the extended tail would bias the analysis towards slower kinetics. Three important conclusions can be drawn from this example:

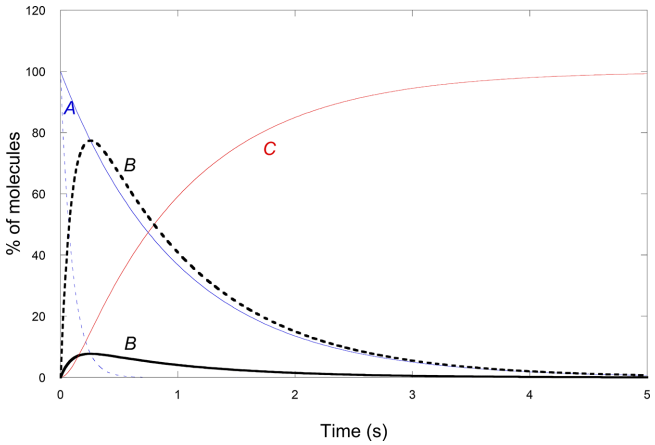

Figure 5. Reaction time course for a sequential reaction shown in Equation 1. Solid lines are for the condition k_1=1 second^{-1} and k_2=10 second^{-1}, while dashed lines correspond to k_1=10 second^{-1} and k_2=1 second^{-1}. Note the formation of *C* is independent of the assignment order, while the profiles for *B* only differ in amplitude and the half-times for the rising and falling phases are independent of the order. For *A* the amplitudes are the same, but the decay times depend on the assignment order.

(i) amplitude information is equally as important as the observed decay time in analysing kinetics; (ii) it is useful to explore the kinetics of reactions by two or more independent methods to check for instrumental or other technical contributions and (iii) assignment of an observed rate constant (i.e. one derived by fitting a time course to an exponential function) to a specific transition in a reaction can lead to erroneous conclusions. The latter often arises when the measured k_{on} and k_{off} values are assigned to the binding and dissociation steps of a mechanism, respectively. The observed k_{on} generally reflects the sum of several rate constants ($k_{+1}[L]+ k_{-1}$ at a minimum) and may be dominated by a first-order term (e.g. the reverse rate constant, k_{-1}). Also, when k_{off} is measured by displacement or washout, it is important to establish that k_{off} is limited by the dissociation step by varying the concentration of the displacing agent or the flow rate.

The recent advances in technology for time-resolved structural analysis described above have involved developments by specialized and multidisciplined teams and might seem an inappropriate topic for a 'Beginner's guide'. However, the concepts behind the analysis most likely will involve some fairly basic kinetic principles whose understanding is important for researchers, reviewers and literature readers alike, regardless of the level of instrumental sophistication. ■

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Clive R. Bagshaw is an Emeritus Professor of Physical Biochemistry at the University of Leicester and currently a Research Associate at the University of California at Santa Cruz. He spent most of his research career investigating the kinetics of myosin ATPases, but more recently has been involved in a number of collaborative projects involving telomerases, clock proteins and MHC–peptide interactions. He recently published a textbook on Biomolecular Kinetics, which covered both experimental and theoretical aspects. Email: cbagshaw@ucsc.edu

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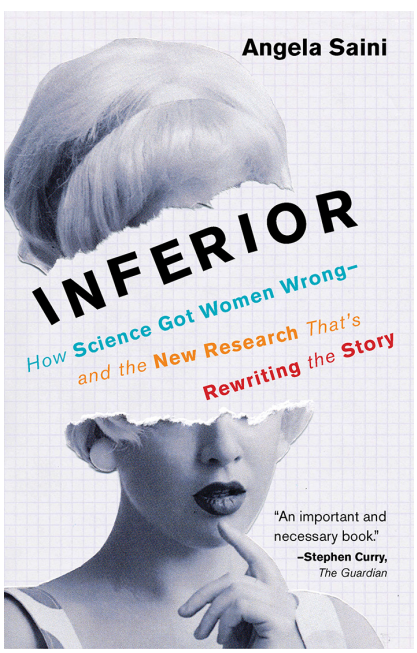
Angela Saini: dispelling the myths of science past

Angela Saini is a British science journalist and author. She presents radio and television programmes on the BBC, and her writing has appeared in *The Guardian*, *The Sunday Times*, *Prospect*, *New Scientist*, *New Humanist* and *Wired* among others. She has won a number of national and international journalism awards. She spoke to Emma Pettengale, Managing Editor of *The Biochemist*.

Thank you for taking the time to talk to us. For those readers who haven't read or heard of your two bestselling books, can you introduce yourself and tell us a little about what you do?

I am a science journalist and broadcaster; I cover a range of science topics but have focused the last few years on bias and what science tells us about race and gender.

There have been many mistakes made over the centuries, and it's important to understand how and why they were made. *Inferior* discussed how the male domination of science for many decades has impacted what science told us about women, and how misunderstandings are slowly being corrected. My new book *Superior* looks at the history and damaging legacy of race science.



What drew you to the world of science journalism? How did you get started?

I studied engineering at university, and as many people do, I got involved in student politics and started writing articles for student papers. I had every intention of becoming an engineer, but one career advisor suggested I consider journalism because of my passion for it, so I decided to give it a shot. Fortunately, engineering degrees are pretty bankable, so I felt I could always come back if I needed to. I was an everyday reporter at the start, covering news, politics, social affairs, but my science background was useful in interpreting statistics, things like that. When I entered science journalism later, I tried to bring the same kind of investigative skills to my reporting. There are so many issues that affect us all, which are based in science and technology and play out in our politics.

Science moves forward, evolving ever faster, and research culture is changing with it. What do you think is the biggest challenge in science over the next 5 years?

I don't know if I'm qualified to answer that! I am heartened to see that diversity and representation are being taken far more seriously – science is improved when we have better representation. Going forward, what we need is a more

nuanced understanding of inclusivity and what it means. It's not just about having different faces and equality – although that is important – we also need to understand the perspectives of people who have been historically marginalized or excluded. These perspectives are not always readily accepted because they can lie at odds with the current ways of doing things and the culture.

Institutions need to make concessions for people who have lives at home and still want high-flying careers, such as when they need to travel to conferences or arrange childcare or elder care. Gender roles are shifting, and this is becoming almost as much an issue for men as it is for women. I have a 6-year-old, and his dad, like many more men of my generation, is an active and involved parent alongside his partner. My husband does as much childcare as I do. 'Work at home' needs to be done by someone after all, and subsequent generations won't have the sexual division of labour we have historically seen – academia and industry need to come to terms with that. In most families, for financial reasons, both parents have to work. I grew up in a family where my parents split everything 50:50, so I didn't think that it was unusual for men to share the work, and this is now how I live.

I'm also concerned about power dynamics in academia. One of the reasons bullying and harassment occurs at such high rates in academic institutions is because of the hierarchical structures, which make it very easy to exercise power over more junior people. Those hierarchies need to be broken down.

Another big area is incentives – I am working now with journal editors, looking at how poor-quality research is published and can lead to overhyped claims and amplification of bad data. This reflects the problem with incentives in research – it's not just about getting 'sexy' results; science should also be accurate and responsible, with research done in an ethical way to minimize mistakes, misinterpretation or abuse.

Where do you feel there are improvements still to be made in sex difference research and understanding?

I look in *Inferior* at this, picking apart the historical work on sex differences. It is important to continue research into sex and gender. But we need to recognize that, historically, sex-based research has often been used to paint the sexes as fundamentally different and women as inferior, so we need to be cautious of people who make grand claims of differences between the sexes. At the same time, there are some biological differences that can't be ignored, for example in health, but I don't think they are as profound as some people claim they are, at least there's not the evidence to support that.

Sarah Richardson and Heather Shattuck-Heidorn (both at Harvard University) have shown that while it is important to investigate sex differences, sometimes they are conflated inappropriately with other factors. For example, the FDA recommended women take lower doses of the sleep drug zolpidem, thinking that women would clear the drug more slowly from their system. However, while women on average sometimes do clear the drug more slowly, this is likely to be due to their body weight – not their sex. Therefore, If we are using sex or race as a variable in research, we need to think about why and interrogate the reasons – are sex and race being used as proxies for other variables, such as weight, geography and if so, shouldn't we look at those instead? I prefer a more nuanced approach and a broadening of the variables we use. We need to be careful about essentialism when talking about the differences between 'men' and 'women' – there is no 'typical' man or women. Every person is different, and dividing all people into two buckets airbrushes over that complexity.

Recently, there have been efforts to revive the links between 'race' and IQ – via genetics. What are your views on this trend?

Intelligence research has a very fraught history, littered with racism, and there is still a strong thread of racism within the community. We have to be wary approaching the subject because of its legacy and the previous mistakes that have been made.

Race is a social construct, with no genetic or scientific basis showing consistent differences between groups of people. There are people outside science and academia who make claims of differences between natural groups in terms of IQ. We can't use the data we have now to make broad inferences about population-level differences. Work linking IQ to race is severely flawed on so many levels, it is hard to assume that the people making these claims are not politically motivated.

Dominic Cummings (Chief Adviser to Prime Minister Boris Johnson since July 2019) seems to be suggesting we stream children in education based on their IQ/aptitude for learning as predicted by genetic analysis. What are your thoughts on this?

What we know is that polygenic risk scores are not more reliable than school testing when it comes to measuring ability. So, number one, I don't see the point – I don't know what it would do in terms of efficiency in the education system. If there is a large heritable element of intelligence, even the most hereditarian of psychologists believe it accounts for only half of what we see, and even then only if the child is raised in a healthy family where their needs are taken care of. Sadly, this is not true for all children in the UK, with many living in poverty, and until you are able to meet their other needs, they cannot

meet their natural potential. It's just ridiculous and deeply, deeply problematic to assume otherwise.

Much of the 20th century was spent on the fallacious argument that all we are is our genetics – this deterministic approach to social policy was a huge mistake and historically disastrous. If we want all children to be the best they can be, we need to ensure a consistently high standard of education, make sure they are well fed and looked after and not neglected. Genetic testing is not the way to do it. There are many people who do very well in testing, and in life, because they have been trained very hard, tutored at the best schools and pushed well beyond other children, and therefore they overperform compared to if they were raised in an everyday family where children don't get this support. All inputs into a child's life have an impact.

In the current journalism culture, 'experts' are being shunned in favour of those who can shout the loudest. What is your take on this, and can you see a way forward?

This is what I am working on tackling at the moment. Last summer, I set up a group to challenge pseudoscience because this is an issue very close to my heart. Although a lot of my work critiques science, the reason I do this is because science is one of the best ways we have to understanding ourselves, and I want it to be the best it can be and produce the best quality of information.

Particularly on the internet, the culture has driven marginalized voices to the front and more moderate voices to the back. There's also the rise of clickbait articles, because people like to be shocked. The debate has become very simplified, so people on the extremes with simple arguments and a simple narrative control what we are exposed to – we need to deal with that.

I believe regulation is needed. Internet companies need to be made responsible for the content that appears on their sites, and subject to the same rules that society is held to offline, the standards for what is legally acceptable. Very many countries have such regulation or are considering bringing it in, and I think the days are numbered for the internet as we have known it – particularly social media. I hope scientists will step up and be part of the conversation as that regulation is debated.

What is next for you?

I'm working really hard to move forward with efforts looking at standards and ethics in journals, and I'm hoping to get more scientists involved in the discussion around internet regulation.

I will be spending the summer (or their winter) in Australia at the University of Sydney. As part of their new Resurgent Racism project, the university is bringing in scholars from all over the world to try and tackle the problem of modern racism in all its forms.

Sounds like a worthy but daunting challenge, we wish you all the best with it. ■

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Deadline: 28 May 2020

BIOCHEMICAL SOCIETY

Mental health in academia: what's it all about?

Jack Caudwell
(University of Leeds, UK)

Should your PhD sacrifice your mental health? Seems like a silly question to ask in 2020, maybe even rhetorical to some. However, a great deal of postgraduate researchers experience mental health issues as an unwanted by-product of their PhD. At what point does this become too much? Mental health has become one of the biggest issues put under the public spotlight in recent years, so why do we still have this archaic culture in academia? Maybe it is time for a change in how we think about what *is* and *is not* acceptable, and what resources are available to those of us who find ourselves struggling with our mental health during our research.

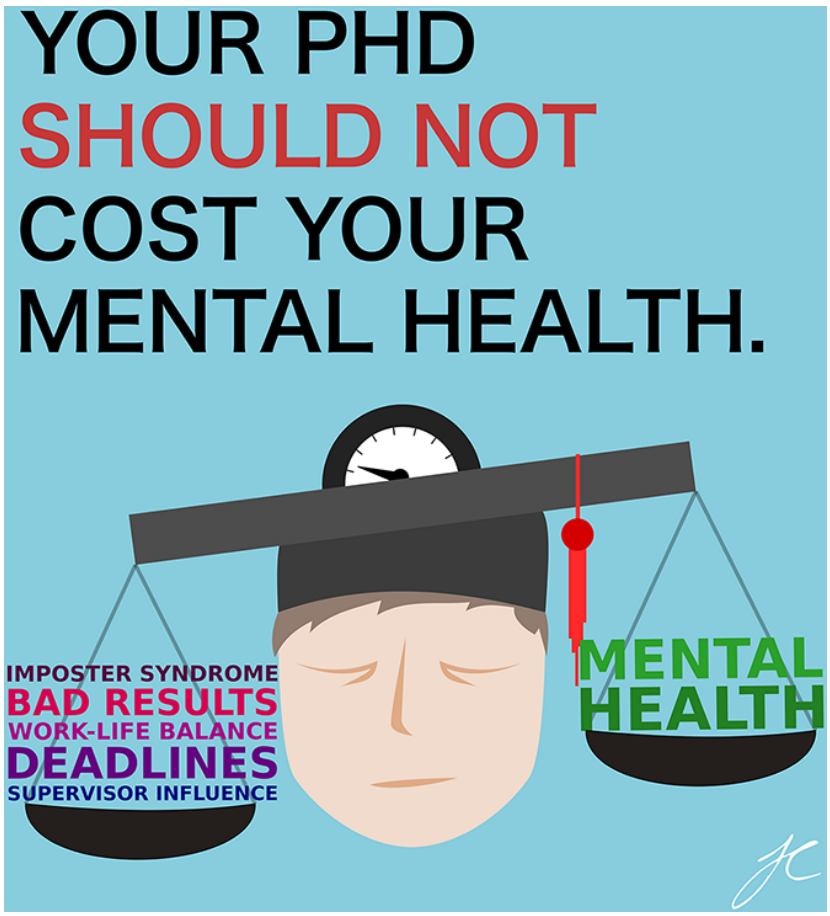
We all remember when we were fresh-faced, straight out of our undergraduate degrees, our certificate bristling with pride; the culmination of 3 to 4 years of hard work ends with the fated question, 'What do I do with my future?' For many, this is a route straight into industry to earn some serious money, maybe for the first time. Some of us weren't done with research; our dissertation projects just whetted the appetite, leaving us wanting more. Others had a clear idea of what they wanted, and a PhD was a step further on that journey. But some of us, let's be honest (myself included), saw it as an opportunity to delay having to decide what it is we wanted to do with our lives; and you know what? That's completely fine. Whatever your reason, we all had the daunting task of deciding what group to join. For many, we wanted to venture into pastures green, a change of scenery, maybe even a new university. For others, we'd had a blast working in our undergraduate labs and were lucky enough to secure a position with our old principal investigators (PIs). Did any of us know what we were signing up for? Yes, you had heard rumours about what PhD life was like, but did you truly understand what you were undertaking? How many hours is acceptable? Is my PI a good PhD supervisor? Is my project going to work well? For many, these questions were left unanswered, and students didn't know where to look for answers. Often, by the time you are able to find definitive answers, it is sometimes too late.

Nature Biotechnology published an article in 2018, 'Evidence for a mental health crisis in graduate education', in which they performed research on over 2000 postgraduate students worldwide and asked them about their mental health. Their results showed that 41% of the graduate students surveyed identified as having anxiety, with 39% identifying as being depressed. This statistic is beyond shocking. If we were to assume that this data represents the postgraduate community as a whole, then more than one in every three PGR students would identify as being depressed, compared with one in every fifty of the general population. This statistic alone should be enough to raise alarm bells, so let's discus some of the key issues surrounding mental health in academia.

Work–life balance

This is a tale as old as time; no matter what your job is, work–life balance is probably regarded as one of the major influencers of people's mental health. There seems to be so much ambiguity about how many hours constitute 'enough' at the PhD level. I had a friend who secured a PhD at a southern university that 'unofficially' expected you in from 08:00 until 19:00, Monday to Friday. Suffice to say, he eventually decided on a different career path after a few months, and who would have blamed him! Your working hours are largely up to the discretion of you and your supervisor, but most funding bodies expect you to work a 37- hour week; this is the normal Monday to Friday, 9–5 grind. PhD life can't always be 9–5; some days demand a little more of you than those 8 hours, often having to stay late or come in early. That's just part of the deal, but how you balance it is down to you. For example following a particularly long day, you may be able to take those hours out of another day. The problem is, sometimes it's hard to remind yourself that doing that is okay.

In the aforementioned *Nature* paper, when graduate students were asked to agree or disagree with the statement 'I have a good work–life balance', 52% disagreed with the statement. Everyone's work–life balance is different, but wherever your scales lie, making sure that you maintain them is important for your mental health. If it's socializing with your friends, going to see that new film or spending time with your family, do what you need to do to decompress and enjoy your free time.



My experience: relationship with your supervisor is key

In my opinion, the relationship between student and supervisor is a largely over-looked influence on PhD mental health, but one that is the most controversial to seek help for. The *Nature* survey found that, of those students who identified as having anxiety/depression, 50% did not agree that their supervisor provided 'real' supervision. The majority expressed the opinion that their supervisors did not provide 'ample support' or 'good mentorship'.

My experience on this during my PhD has been varied – I had three supervisors for my project, one primary and two secondary, and all of them had completely different approaches to supervision. I recently handed in my PhD thesis, and in the last meeting before my submission my primary supervisor asked me, 'how could I have been a better supervisor?' When he asked me this question, I was taken aback – it was a loaded question. He had always had a very busy schedule but was always willing to meet or call should I need some guidance. No PhD supervisor relationship is plain sailing all the time, just like any other professional relationship, but that doesn't mean it cannot be improved upon. In my 3-year project, we had butted heads a few times, but he had always made it clear what was important in my development as a scientist. I gave him a few suggestions, but the honest answer to his questions was that he had tried his best and I feel that our working relationship ended on a high point. I had a lot of contact with one of my secondary supervisors, and he was a great supervisor. He always had his door open to lend an ear and made sure you felt confident in where your project was going, even when things were bad.

Friends of mine, both former and current PhDs, really opened my eyes to different kinds of supervisors, ranging from supervisors who were too absent to supervisors who constantly micromanaged. Some had good working relationships with their supervisors, and some didn't at all. The way an academic's reputation is viewed means they don't always have the best interests of their PhD students in mind.

There should be greater infrastructure in place by which academics are assessed on their ability to exert a positive impact on their PhD students' development, which doesn't focus on how much funding they pull in or the frequency of their publications. Now, in practice, this may seem problematic, but an academic's career is bolstered by the work of his or her students. Therefore, their skills as supervisors should intrinsically be tied to their notoriety in their field. If PhD students are required to give in-depth feedback about their project supervision at the end of their project, likely after the viva stage, this assessment could be used when considering how an academic's career progresses.

In any case, you shouldn't be afraid to seek help and advice when you are having issues with your PhD supervisor. It may seem awkward, as sometimes you may feel there is no anonymity, especially in a small research group, but it is more important that you are getting the most out of your studentship. Make sure you take advantage of any opportunity to improve your relationship with your academic supervisor. In most cases, everyone just wants what is best for your development.

Imposter syndrome

This is probably the most famous PhD studentship buzz phrase: 'imposter syndrome'. It is the feeling that you are an imposter, imitating someone who knows more about your work than you do. I'm confident in saying that almost everyone, at some point, experiences imposter syndrome. Personally, I don't think I can recall a time during my PhD when I didn't feel like an imposter, a charlatan masquerading as a microbiologist, fooling all those around me of my competency. That sometimes doesn't go away, but rest assured that most of the time it is misplaced. You

have worked hard to be where you are now, and you have worked hard on your project. Yes, you may have a few gaps in your theoretical knowledge, but you are the expert in the work that *you* have performed. Have confidence in yourself and your work.

There are resources available all over the internet to help deal with imposter syndrome. In fact, there's even an online test that tells you just how badly you have it (http://impostortest.nickol.as/). Sharing your experiences and engaging in a dialogue with people about imposter syndrome will help not only you but also other people. It is a natural part of becoming a professional and something that all of us will face at some point.

The bottom line

Your PhD should not come at the cost of your mental health. Don't get me wrong; your PhD is going to be stressful, and nothing worth having ever comes easy, but it shouldn't cause you severe mental health issues. My PhD has been some of the best years of my life; I have made life-long friends and come out the other end a better person than when I went in. By acknowledging the seriousness of mental health and helping ourselves and each other, we can work towards eliminating its association with academia, because everyone has the right to enjoy their time as a PhD student. ■

Further reading

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- Imposter syndrome test: http://impostortest.nickol.as/
- NHS mental health helplines: https://www.nhs.uk/conditions/stress-anxiety-depression/mental-health-helplines/

Resources

- Your university will have a system in place by which you can speak to a member of staff if you feel you are experiencing poor mental health, either as a by-product of your PhD, from your personal life or both. Make sure you know who your ports of call are and make sure you utilize them.
- Online communities of graduate students exist where people share their stories surrounding mental health in academia. Reading those stories and sharing your own may offer a support network for you. The @PhD_Balance Twitter feed is an online community that discusses issues surrounding mental health and offers resources to help those struggling.
- You can seek advice and guidance on mental health from your GP. The National Health Service offers a number of mental health helplines that can be used to discuss a wide range of mental health difficulties.

Having recently passed his viva, Dr Jack Caudwell is a medical engineer and scientific illustrator based in Leeds, UK. Born and raised in Nottingham, Jack moved to Leeds in 2012 to start his undergraduate studies, which saw him spend a year studying biochemistry abroad at the University of Massachusetts as part of his International Masters in Chemistry. After graduating in 2016, he started a PhD in medical research towards the treatment of bacterial biofilms. Since getting his PhD, Jack has been working as a medical engineer in electrophysiology whilst spending his spare time doing scientific illustration. Email: jackacaudwell@gmail.com

In memoriam

The Biochemical Society offers its condolences to the friends, family and colleagues of all those they have lost recently.

Professor Sir Jack E. Baldwin, FRS

I was very sad to receive an email on 8th January 2020 with the news that Professor Sir Jack E. Baldwin, FRS (or 'JEB' as he liked to be known) had passed away that previous weekend.

I first learnt of 'JEB' as a third-year undergraduate at the University of Leicester, when I encountered 'Baldwin's rules of ring closure'. Shortly afterwards, I was applying for jobs including a post at Beecham's Pharmaceuticals. During the interview, it was suggested that I should undertake a DPhil within the JEB group on a joint project. Off I went to Oxford for the interview and met JEB; we spent around 2 hours discussing the project and other chemistry topics, and I joined the JEB group in the September of that year.

JEB was a larger-than-life character, who was well known within the chemistry community for his work on β-lactam antibiotic biosynthesis and the enzymes involved. He would occasionally be seen riding his Harley-Davidson motorcycle or walking his very large dogs and was very much living up to his reputation from the 'Cutting Edge' programme shown on Central TV the previous year. This was a very exciting time to be in the JEB group, exploring 'cutting-edge' science and meeting many other scientists who went on to become academics. I remember my Oxford days with great fondness. The experiences I had there were first rate and set me up for my long career in chemical biology.

JEB will be greatly missed by colleagues around the globe.

Matthew D Lloyd (University of Bath, UK)

Professor Arne Holmgren

Dr Arne Holmgren is widely acknowledged as one of the leading figures in the field of redox biology. He is particularly known for his seminal work on the characterization of the thioredoxin family and the discovery, naming and characterization of the glutaredoxin family. He is recognized as a genuine redox pioneer, who helped establish the reduction and oxidation pathways that occur in, and are essential to, all living cells.

Dr Holmgren worked at the Karolinska Institutet throughout his career, starting as an assistant professor and progressing through to professor, Chairman of Biochemistry and Director of the Medical Nobel Institute for Biochemistry. In 2008, Dr Holmgren became a senior professor, but he remained highly active in the field and led a vibrant research group until late in 2019.

In addition to his many scientific achievements, Arne Holmgren was a member of the Nobel Assembly at the Karolinska Institutet and the Swedish Royal Academy of Sciences. He was a recipient of a number of prizes and awards and mentored many prominent scientists in the field of redox biochemistry.

Michael Davies (University of Copenhagen, Denmark; President, Society for Free Radical Research Europe)

Peter Francois Zagalsky

Peter Francois Zagalsky passed away at the start of 2020. He was a member of the Biochemical Society and was well known for his pioneering work on *carotenoproteins*. A lecturer at Regent's College (now Regent's University London), and later Royal Holloway, he was passionate about his research work and wrote many important papers – many focused on the lobster coloration protein crustacyanin, which paved the way for new discoveries in this field. His work is continued by Professor Naomi Chayen at Imperial College. He is survived by his daughter Alexandra. He will be missed by friends and family.

We were also very sad to hear of the passing of Professor James Barber of Imperial College London and Professor David Hogness of Stanford University. Further details will be included in later issues of the magazine.

An interview with a *Forbes* 30 Under 30 researcher: Andy Tay Kah Ping

Andy Kah Ping Tay, 29, is an independent Brunel Fellow at Imperial College in London. He will be starting his lab in mid-2020. Named as one of the scientists in the *Forbes* '30 Under 30' list (USA/Canada/Global) of 2019, his research seeks to understand how biomechanical forces impact cells in our bodies. His research has given insight to treat chronic pain, which affects millions of people around the world. *The Biochemist* spoke to Andy Tay.

Hi Andy, can you introduce yourself for our readers and tell us a little bit about yourself?

I grew up in Singapore and completed my bachelor's degree in the Department of Biomedical Engineering at the National University of Singapore. My mentor, Professor Lim Chwee Teck, inspired me to do a PhD.

At Univeristy of California, Los Angeles where I did my PhD, my mentor, Dino Di Carlo, was extremely nurturing and encouraged me to explore my research interests. That was when I integrated my expertise in biomaterials and biomechanics to tackle the issue of chronic pain through an unconventional biophysical lens.

Interested in neuroscience, physiology, magneto-genetics and microfluidics – that is a mixed bunch, what drew you to the molecular biosciences?

I see molecular biosciences as an extremely important tool to understand about the systems I am working on and to demonstrate that my technologies work. For instance, in proving that my magnetic gels can be applied towards pain modulation, I made use of molecular biology techniques like sequencing and immunolabelling.

Can you tell us about your current research? The magnetic gel you helped to develop for the treatment of chronic pain sounds very interesting. Can you tell us more about it?

In all cells, there are proteins called ion channels in the cell membrane. Some of these ion channels are open, and they close in response to mechanical forces. Because of their sensitivity to forces, they are referred to as mechano-sensitive channels (MSCs). Some prominent MSCs are those in the PIEZO and TRPV families. MSCs help us to sense pain (which is important for survival); but when there are too many of them on neurons in our spinal cord, the neurons become too sensitive to forces, leading to chronic pain.

In a previous work (Tay and Di Carlo, *Nano Letter*, 2017), I discovered that neuronal networks exhibit a phenomenon called 'mechanical homeostasis'. We found that when neurons are continually stimulated with mechanical forces, they reduce their expression of MSCs possibly to restore homeostasis and reduce overactivity.

This motivated my colleagues and me to create biocompatible magnetic hydrogels that can be used to stimulate neurons for potential pain relief. The hydrogel can also be programmed to have different biochemical and biophysical properties to match the different bodily regions we intend to inject them at.

What do you think will be the biggest breakthrough or discovery in the next 5 years?

There are many I can think of. I feel that with advances such as bioinformatics and artificial intelligence, we can make greater use of big data to analyse the effects of biophysical forces on cells and generate safer and more interesting biomaterials.

Specific to the problem of chronic pain, many patients also suffer injuries to their spinal cords and their neurons do not regenerate. Interestingly, it has been reported recently (Song et al., *Cell*, 2019) that the overexpression of MSCs also reduces neural regeneration. I also found in my work that when we stimulated neurons with magnetic hydrogels, their MSC numbers went down and they grew better. In the next 5 years, I am confident we can better understand the connections between MSC expression, chronic pain and neural regeneration. This will be a great leap forward for regenerative medicine.

How did it feel being named as one of the *Forbes* 30 Under 30?

I am still very surprised to be chosen because there are so many outstanding young scientists that I know. I feel very honoured! Being named a *Forbes* 30 Under 30 has brought me attention from the media and companies. I see this award as a great platform for me to share my work and emphasize the importance of scientists working with different stakeholders to communicate science to society and translate technologies to benefit patients.

What is your view on the pressures faced by and the opportunities afforded for younger researchers?

I think young researchers face an increasing number of expectations at work, but there are insufficient resources from their institutions to support their learning and careers. As an *eLife* Early Career Advisory Group Member, I am working with my team members to equip young researchers with skills such as science writing, leadership and mental wellness that are not typically taught to researchers. I would strongly encourage young researchers to check out the *eLife* ambassador programme to learn more. Here's the link: https://elifesciences.org/inside-elife/2a8f1672/elife-ambassadors-an-invitation-to-take-part-in-2019

What has been the greatest challenge in your career so far?

I have moved a lot in my career. So far, I have worked in the USA, UK, Germany, Australia, Japan and Singapore, and with each move, I had a specific goal in mind: learn from the best in a field I am interested in. A challenge I face is starting almost from ground zero, including acquiring new knowledge in an unfamiliar field and making new friends. While this can be nerve racking, it also keeps me excited.

You are in London working at Imperial, how are you enjoying London? What is your favourite thing about the UK?

I love London. It has so much to offer and I don't think one can ever get bored of it. I like the reading culture in London a lot and since coming here, I have been spending more time reading books of all kinds.

You were previously shortlisted as a finalist for the Biochemical Society's Science Communication Competition. What did you write about and do you have a passion for communicating science?

I wrote about the effects of space on physiology and the edited article was eventually published by *The Conversation* together with the World Economic Forum Agenda. You can read it here: https://theconversation.com/life-on-earth-is-used-to-gravity-so-what-happens-to-our-cells-and-tissues-in-space-73041

I think it is extremely important for scientists to communicate with society because the impact of one's research is more than publications and citations. Besides writing, I also try to be creative in how I perform science outreach. For instance, I volunteered as a tour guide during my PhD, showing visitors how wildly cancer cells grew to highlight why cancer is so deadly. I also designed a toy kit with the Stanford Design School to educate children fighting cancer about immunotherapy. I think no scientific topic is too hard to explain, and we can be more inclusive in how we communicate science.

Do you have any advice for anyone interested in pursuing a career in the molecular biosciences?

I would advise my students to approach science with an open mind about where they will end up with a degree in sciences in the future. Many students are inspired by their professors and hope to become professors after their PhD and postdoc training, but that is not the only way or even the best way they can impact society. There are many career options such as in the industry and as consultants, writers and policymakers. I think keeping one's options open and exploring different options should be encouraged. ■

A day in the life of a Senior Scientist

Dr Cristina Gutierrez-Caballero is a molecular and cellular biologist. She graduated with a BSc in Biology from the University of Salamanca (Spain), where she subsequently completed her PhD studying proteins involved in cell division and their role in cancer, aging and longevity. After her PhD, Dr Gutierrez-Caballero continued her research in cell division at the University of Warwick as a postdoctoral researcher, funded by Cancer Research UK. More recently, her interests have transitioned towards translational work and drug discovery, and in 2017, she started a position as a Senior Scientist in Exonate Ltd, an early-stage biopharmaceutical company that develops novel drugs to treat age-related macular degeneration (AMD). Lorenza Giannella (Training Manager, Biochemical Society) spoke with her about her work.

How did you get into science?

I have been always interested in science. When I was a child, I wanted to be an astronomer or a biologist, and because I was not very keen to complete a physics degree in order to become an astronomer, I opted for biology BSc. The advantage of doing a general biology degree is that it includes a broad range of subjects, including zoology, ecology, genetics, biochemistry and so on. Hence, you have the opportunity to decide which topic attracts you the most as your knowledge expands. In my case, I found that I loved genetics, and molecular and cellular biology, so I chose a PhD project in which I could use genome engineering to develop mice models to study the role of uncharacterized proteins in cell division and cancer.

Can you describe a typical day?

A typical day in my current role would include designing and executing experiments, analysing data, contrasting information in the literature and, on 1 or 2 days per week, presenting and discussing the results with the head manager, CSO and colleagues. In general terms, the experiments I typically carry out investigate the effect of the compounds we are developing in human cell lines. I run a considerable range of experiments; therefore each day can be very different from the other.

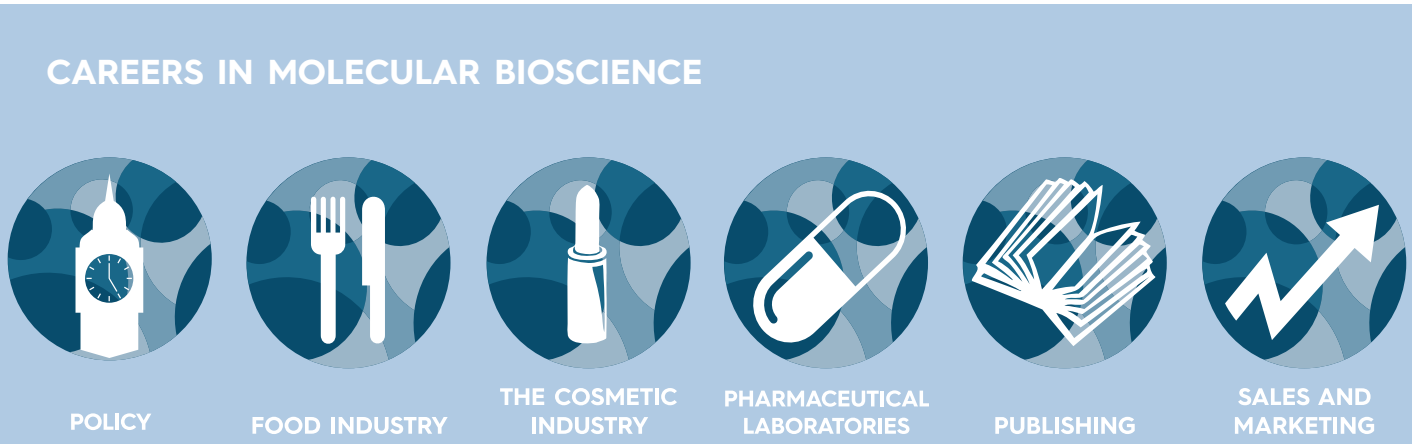
What inspires you about your job?

During my research in academia, I was inspired by the opportunity to contribute knowledge to the understanding of how cells divide and how this process could go wrong. Also, just simple things such as being able to see the chromosomes or to record and visualize the division of a cell still thrills me (even after doing it for more than a decade!).

Working in drug development in a biopharmaceutical company, I am inspired by the possibility of contributing to improvements in the life of patients.

What has been the greatest challenge in your career so far?

Probably, moving to another country on my own and starting my journey as a postdoc in a new lab with a new project and in another language, which I did not fully master



right away. Luckily, my supervisor was patient and helpful, which made the transition easier; I don't regret it at all. The move gave me an opportunity to improve my CV, meet lots of people in my and other fields, experience another culture and broaden my outlook. I really encourage anyone who is interested in doing a postdoc to consider doing it abroad.

What is your advice for someone who would like to move from academia to industry?

If you are a PhD student considering moving into industry in the future, I would advise you just to do it after the PhD, as there is no need to carry out a postdoctoral position first; many available positions in industry are for recent PhD graduates. Of course, you can also move after a postdoc; but if you are sure you want to leave academia, it would be better to invest those years in acquiring experience in industry.

The most important tip when looking for a job in industry is to adapt your CV to the industry format, which is very different from the academic one. You should create a profile on a professional social media platform (i.e., LinkedIn) and start building up connections. ■

Job Profile – Senior Scientist

A Senior Scientist works in a laboratory on a specific project. The job can be based in a university, hospital, research institute or industry. Senior Scientists plan and conduct experiments, and analyse results, either with a specific end use, such as developing a new product or a commercial application, or to broaden scientific understanding.

Responsibilities

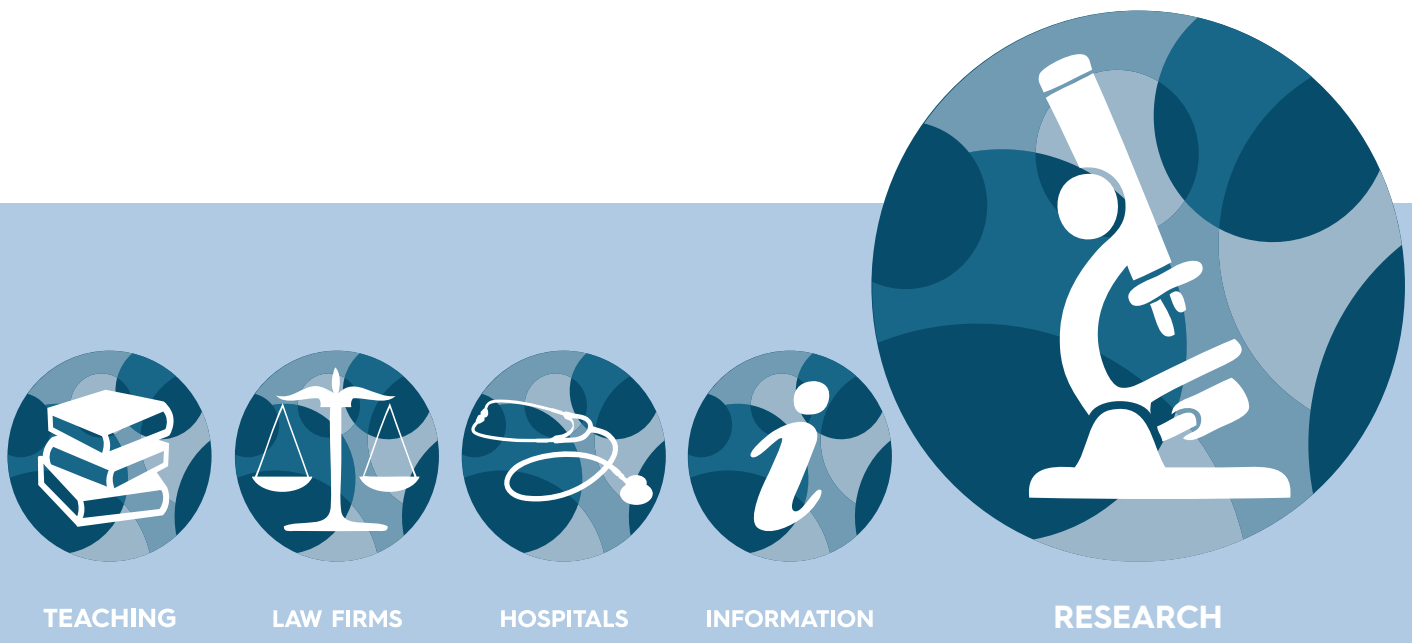
Senior Scientists will often manage a project; they will plan the project and experiments, conduct the experiments, analyse results and write reports. They may also supervise junior members of the team and ensure smooth running of the lab. The role requires the individual to keep abreast of the current research in their field and to present their work at relevant conferences or meetings, as well as write journal articles.

Qualifications and key skills

A PhD in a related discipline or BSc plus research experience is usually required for the role. Specific to the role will also be the required technical skills and/or research experience in the relevant field. Senior positions may require project management and/or leadership skills. General key skills include an ability to work independently and a self-driven approach to working, a flexible and adaptable attitude and good problem-solving skills. Good oral and written scientific communication skills are required for writing scientific documents and presenting at conferences.

Salary and career development

Salaries for Senior Scientists can depend heavily on their qualifications, experience and location, ranging from £29,000 to above £40,000. Career progression can be towards the managerial side or a Principal Scientist role.



Upcoming Online Training Courses

Practical Python for beginners: a Biochemist's guide
4 May 2020, Online
This course aims to help researchers at any career stage learn the core skills that underlie the application of Python to complex, real-world research problems. 'Practical Python for beginners: a Biochemist's guide' was developed by an experienced team of Python trainers and early career researchers with first-hand experience of learning Python for biochemical problems.

Using technology for effective teaching in HE
22 June 2020, Online (FREE for members)
From early career to experienced lecturers, this course is aimed at anyone interested in embedding relevant technology in their practice to improve students' learning in Higher Education. Completing this course will also help colleagues working towards various levels of fellowship of the HEA.

Introduction to Public Engagement and Science Policy
12 October 2020, Online (FREE for members)
Communicating beyond the lab requires a different set of skills. This online course explores how the molecular biosciences can contribute to UK policy and shares tools that can be used to engage the public effectively.

Scientific Meeting Public Event
Medal Lecture Training Events and Courses
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For more information:
www.biochemistry.org/Events

Meeting Reports

Coronavirus (COVID-19): a message from the Biochemical Society

The Biochemical Society has been monitoring developments and advice from the UK Government and World Health Organization (WHO) since the emergence of the novel coronavirus (SARS-CoV-2) in January.

Regretfully, the Biochemical Society has taken the decision to postpone or cancel its forthcoming scientific meetings and face-to-face training events. This difficult decision has been made by the Society's Council of Trustees in light of the developing situation both within the UK and worldwide. Our online training courses will continue as programmed.

We're publishing information regarding specific events on their respective online pages and will continue to be in touch with speakers, sponsors and registered delegates to arrange full refunds.

This decision has not been taken lightly and has been made in the interests of public health. We value the time and effort that has gone into organizing these events and are keen to reschedule affected events for 2021.

Please get in touch with our Conferences and Events Team if you have any questions or require any more information: conferences@biochemistry.org

You can also visit our website for more information on how we're responding to ongoing developments: biochemistry.org

5th N8 biophysical and biochemical symposium: Emerging techniques

10 January 2020, University of York, UK

This symposium series brings together academics from the N8 universities (Lancaster, Liverpool, Manchester, Sheffield, Leeds, York, Durham and Newcastle) with the aim of encouraging collaboration and sharing of expertise and equipment.

A broad range of techniques were discussed at the meeting, including protein engineering for the development of new materials, and nanosensors and design of smaller molecules that could be used to quantify metal binding or target specific proteins for degradation within the cell. Additional techniques showcased included phosphoproteomics by mass spectrometry, neutron scattering for investigating bacterial membrane structure and single-molecule Förster resonance energy transfer (FRET) for accurate distance measurements within protein complexes.

With an emphasis on collaborative research, the symposium included talks from early career researchers as well as experienced professors, with poster and flash presentation prizes awarded. Overall, the symposium highlighted some of the approaches that will drive new discoveries in biophysics and biochemistry in the coming years, and hopefully inspired participants to incorporate them into their own work.

Alex Heyam (University of York, UK)

1st International meeting on cilia, flagella and centrosomes

27–29 November 2019, Paris, France

With approximately 200 participants from all over Europe and as far as Asia, 23 talks and 62 posters were presented, containing much unpublished research and reflecting a dynamic field utilizing a diversity of techniques, model approaches and organisms with a strong foundation in cell biology, development and evolution. Sessions covered cilia and centrosome structure and trafficking, motile cilia and flagella, and ciliary and intraflagellar transport in signalling and development. Clinical aspects of ciliopathies and their underlying molecular mechanisms were also discussed.

New structural imaging including electron cryotomography, super-resolution and ultrastructure expansion microscopy continue to propel the field. The importance of cilia-coordinated motility and flow was illustrated, with new molecular level understanding for the functions and development of various organisms. Different modes of cilia molecular sub-assemblies were illuminated, with new

insights into the ciliary vesicular and intraflagellar trafficking mechanisms involved in cell signalling, migration and developmental functions in health and disease. The keynote speakers added important historical perspective, and notable founders of cilia biology, Peter and Birgit Satir, were honorary invitees. The meeting was co-organized by the French and British cilia and centrosome communities.

Hannah Mitchison (University College London, UK)
Paraskevi Goggolidou (University of Wolverhampton, UK)
Barbara Tanos (Brunel University London)
Nathalie Delgehyr (IBENS, Paris, France)
Sigolène Meilhac (Imagine Institute and Institut Pasteur, Paris, France)
Sylvie Schneider-Maunoury (IBPS, Paris, France)

2019 South Coast RNA meeting

18 December 2019, University of Surrey, UK

At this meeting, RNA specialists from the South Coast of England were offered the rare opportunity to gather and report on their recent studies. Talks covered a wide range of the RNA biology field, from describing innovative RNA arrays with promising outlooks in synthetic biology and drug discovery to regulatory stories challenging the accepted dogmas of RNA function.

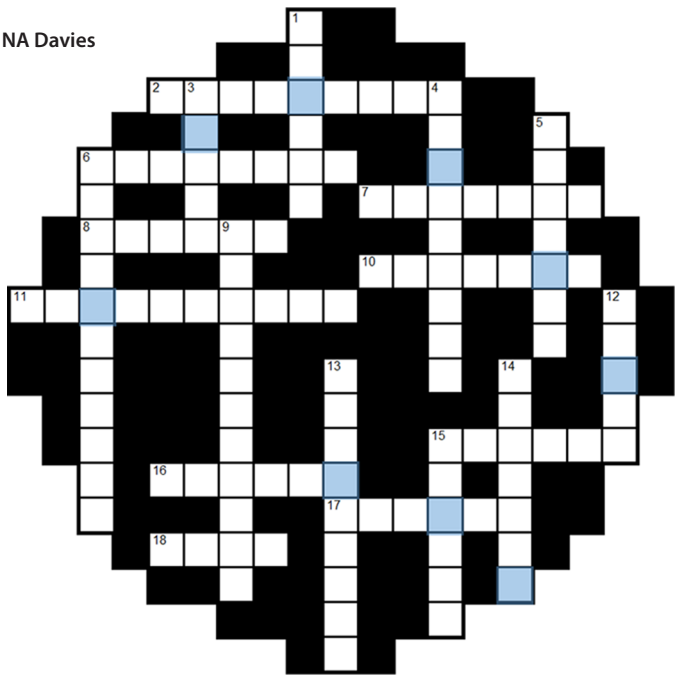
Speakers covered a novel appreciation of how RNA-binding proteins can perform specialized functions through non-canonical RNA-binding domains and their roles in metabolic pathways. The role of liquid-phase translation in cellular processes was also discussed, as well as the role of P-bodies as inhibitors of RNA degradation factors under stress and novel paracrine signalling events driving stress granule assembly.

Finally, the audience were reminded of the importance of quantitatively understanding the process of translation, with a talk on how the elongation rate plays a role in regulating translation under stress. Overall, this was a successful meeting, with plenty of networking opportunities, and will run again in 2020 at the University of Sussex.

Nicolas Locker (University of Surrey, UK)
Valentina Iadevaia (University of Surrey, UK)

Prize Crossword

NA Davies



Across

2. Periodic physiological events recurring throughout a 24 hour day (9)
6. Nerve tissue mass (8)
7. Weariness (7)
8. Uniform procedure with regular pattern (6)
10. Internal endocrine secretion (7)
11. Environmental cue, time giver (10)
15. Gland producing 13 Down (6)
16. Innermost coating of the posterior eyeball (6)
17. Physical sensation that leads to drinking (6)
18. Nourishment (4)

Down

1. To add condiments (6)
3. Radiant energy (5)
4. Active at night (9)
5. Opposite to 4 Down (7)
6. The process by which development begins (11)
9. State of dormancy in cold climates (11)
12. Alternately rising and falling (5)
13. Hormone regulating the sleep-wake cycle (9)
14. Pertaining to motion (7)
15. Portion of time (6)

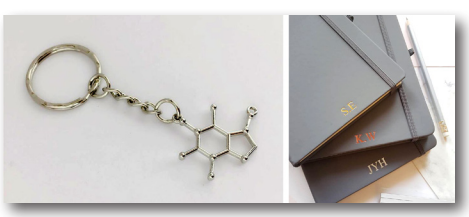
Solutions to the crossword featured in the December 2019 issue are: **Across:** 3. Cone snail, 7. Aristotle, 9. Aposematic, 10. Cytotoxin, 11. Nociceptor, 12. Scorpion, 14. Gila, 16. Analgesia, 17. Antivenom. **Down:** 1. Funnel web, 2. Neurotoxin, 4. Pesticide, 5. Thrombosis, 6. Myotoxin, 8. Tarantula, 13. Cobra, 15. Venom, 16. Ant.

Crossword Competition

Win

This issue's crossword prize is a caffeine keychain and a personalized grey A5 lined notebook. Simply email the missing word, made up from letters in the highlighted boxes to editorial@portlandpress.com, by Tuesday 23 June 2020.

Please include the words 'April crossword competition' in the email subject line. The winner will be announced in the August 2020 issue.



Congratulations to the winner of the December competition:

Liisa Lutter, University of Kent (UK)

The missing word from the December issue's competition was THERAPEUTIC. Liisa received two enamel pins from Science on a Postcard.

Terms and conditions: only one entry per person, closing date Tuesday 23 June 2020. The winner will be drawn independently at random from the correct entries received. The winner will receive a caffeine keychain and a personalized grey A5 lined notebook. No cash alternative available. No employee, agent, affiliate, officer or director of Portland Press Limited or the Biochemical Society is eligible to enter. The winner will be notified by email within 7 days of the draw. The name of the winner will be announced in the August issue of *The Biochemist*. The promoter accepts no responsibility for lost or delayed entries. Promoter: Biochemical Society, 5th Floor, 90 High Holborn, London, WC1V 6LJ; do not send entries to this address.

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