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chlorobenzene into aryl radicals, for instance.

To overcome this problem, various systems have been reported that involve the use of two consecutive PET steps (see ref. 5, for example). In these approaches, a 'sacrificial' reducing agent reduces the excited catalyst molecule produced in the first step, forming a radical anion that is then excited by another photon. The resulting excited radical anion is a strong reducing agent. For instance, the excited radical anion formed from the catalyst Rhodamine 6G has an oxidation potential of −2.4 V versus SCE, which is sufficiently negative to reduce aryl bromides and aryl chlorides that have a reduction-facilitating group[6].

MacKenzie et al. now report an approach based on a salt that contains a mesitylacridinium ion (Mes-Acr+; Fig. 1). Mesitylacridinium salts have been used for almost two decades in photo-oxidation reactions[7] — when irradiated by visible light, the resulting excited species is a potent oxidant that takes an electron from a substrate and is thereby converted into an acridine radical (Mes-Acr•). The electrically neutral radical is converted back to Mes-Acr+ by an oxidant for subsequent catalytic cycles.

The authors recognized that Mes-Acr• is a relatively stable species that absorbs light mainly from two ranges of wavelengths: 350–400 nanometres and 450–550 nm. They report that, when Mes-Acr• is irradiated with light of wavelength 390 nm, it forms an excited neutral radical that acts as an extremely strong reducing agent, with a maximum oxidation potential of −3.36 V versus SCE. They propose that this large negative value is the result of charge transfer within the excited radical.

The use of an excited neutral organic radical is rare in photoredox catalysis. MacKenzie and colleagues formulated a reductive photocatalytic cycle based on Mes-Acr• using 390-nm light and a sacrificial reducing agent. This system can carry out several reduction reactions, such as the removal of tosyl groups from tosylated amine compounds (a type of reaction commonly used in organic synthesis; see Fig. 3 of the paper[1]). The researchers demonstrated that the new system is robust enough to work on scales that are useful for preparing compounds in the laboratory, by performing a detosylation reaction with 1.28 grams of a starting material.

The same approach can also be used to replace bromine or chlorine atoms with hydrogen atoms in aryl bromides and chlorides, respectively — such reactions are known as dehalogenations (see Fig. 2 of the paper[1]). This procedure is possible when various groups are present in the substrates, and it even works with 4-chloroanisole, an aryl chloride that has a reduction potential of −2.9 V versus SCE.

Another approach for the catalytic production of strongly reducing species was reported simultaneously earlier this year in two papers from different groups[8,9]. In both cases, a neutral organic molecule acts as the catalyst; this is reduced electrochemically on a cathode to produce a radical anion, which is then excited by visible light to form a strong reductant with an oxidation potential more negative than −3.0 V versus SCE. These electrophotochemical systems were used to dehalogenate electron-rich aryl chlorides, and also in a series of arylation reactions (transformations in which an aryl group is attached to another molecule).

The use of electrochemical reduction, instead of photochemical methods, to generate radicals allows catalysts to be used that do not absorb visible light. For example, naphthalene monoimide, a catalyst used in one[9] of the two papers, falls into this category and cannot undergo the initial conversion to a radical anion using visible light. By contrast, once it is transformed electrochemically into a visible-light-absorbing radical, it can enter a photocatalytic cycle.

MacKenzie and colleagues' observation of the strong reductant character of excited neutral Me-Acr• will inspire investigations into whether other molecules show similar behaviour. One can also expect increased interest in other photocatalytic approaches for the production of reductive systems[10–13]. Taking into account the highly negative oxidation potentials observed for various light-generated agents in the current work and by other research groups, we can look forward to new arylation reactions, and even to ambitious applications such as the Birch reduction[14] — a classic synthetic reaction typically performed using alkali metals.

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Developmental biology

A clock that controls human spine development

Adelaida Palla & Helen Blau

Visualization of the rhythmic oscillations of the mouse and human segmentation clocks, which are crucial to spine development, is now possible thanks to the development of sophisticated cell-culture systems. **See p.113, p.119 & p.124**

What do the flashes of a firefly and the chirpings of a cricket have in common? Both occur in a regular rhythm, which is controlled by an oscillating biological clock[1]. Another oscillating genetic clock controls the development of embryonic structures called somites, which give rise to the vertebrae that protect the spinal cord. Our knowledge of this segmentation clock stems almost entirely from research on animals[2,3], because technical and ethical considerations limit the study of human embryos in culture. Diaz-Cuadros et al.[4] (page 113) and Matsuda et al.[5] (page 124) now report a breakthrough that enables studies of the human segmentation clock in vitro. In addition, Yoshioka-Kobayashi et al.[6] (page 119) use sophisticated techniques in mice to provide insights into the mechanisms that control the mammalian segmentation clock.

Somites arise from a tissue called the presomitic mesoderm (PSM). During somite formation, temporally and spatially controlled oscillations in transcription yield gene-expression waves that propagate through the PSM along the embryo's head-to-tail axis. The result is a striped pattern of somites that forms the blueprint for the spine. Although the molecular components of the segmentation

clock are highly evolutionarily conserved across vertebrates, new somites form with different rhythms in each species. For instance, gene oscillations have a period of 30 minutes in zebrafish and 2 hours in mice. Oscillations have been estimated to occur every 4 to 5 hours in humans[2] – although until now they have never been directly observed.

Diaz-Cuadros et al. and Matsuda et al. set out to model the human clock using induced pluripotent stem cells (iPSCs) – cells that are generated in vitro from differentiated human cells and, similarly to embryonic stem cells, can give rise to every cell type in the body. The groups used established protocols[7–9] to convert iPSCs into PSM in vitro.

To visualize and monitor the dynamic oscillations of clock genes in the cultured PSM in real time, each group used a different 'reporter' protein. Matsuda and colleagues used a reporter in which a key segmentation-clock gene[10], Hes7, drives production of the bioluminescent enzyme luciferase. As Hes7 expression oscillates, levels of the reporter increase and decrease. Diaz-Cuadros et al. used an engineered version of Hes7 fused to a gene that encodes Achilles, which is a more rapidly generated variant of yellow fluorescent protein developed by Yoshioka-Kobayashi and colleagues. The use of Achilles enabled Diaz-Cuadros and co-workers to track fluorescent waves of Hes7 expression at the single-cell level[4] – a resolution not possible with the luciferase reporter. Analyses using both reporters provide the first definitive evidence that the human segmentation clock has a period of approximately 5 hours (Fig. 1a).

Three key signalling pathways – the Notch, Wnt and FGF pathways – act in sequential negative feedback loops to regulate oscillating gene expression during somite formation[2,3,11,12]. Diaz-Cuadros and colleagues used their culture system to investigate these pathways in detail. They confirmed the roles of these pathways in PSM cells taken from mouse embryos, and then showed that similar pathways govern segmentation in human PSM differentiated from iPSCs, with oscillations dependent on Notch signalling and another pathway, mediated by a protein called YAP. They found that FGF signalling not only determines the positions along the body axis at which oscillations stop, as previously reported[2], but also regulates the complex dynamics of the oscillations – their period, phase and amplitude.

Matsuda and colleagues used their culture protocol to study a human genetic disease, congenital spondylocostal dysostosis, in which defects in segmentation of the vertebrae lead to skeletal anomalies[13,14]. The authors generated PSM from iPSCs derived from two people with the disease, who each had mutations in a different gene of the Notch signalling pathway. Surprisingly, despite these mutations and differences in overall gene
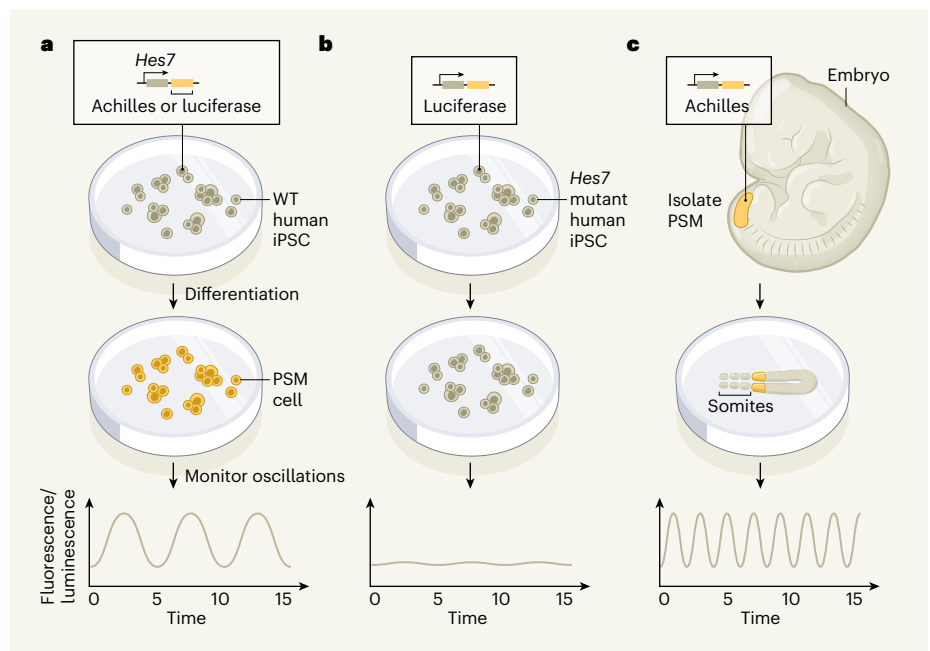

Figure 1 | Modelling embryonic segmentation in vitro. A tissue called the presomitic mesoderm (PSM) gives rise to somites – embryonic precursors of vertebrae. This process involves a 'segmentation clock' that drives rhythmic oscillations of gene expression, including that of the gene Hes7. Three groups have developed systems to analyse the clock in culture using live-cell imaging. **a**, Diaz-Cuadros et al.[4] and Matsuda et al.[5] directed wild-type (WT) human induced pluripotent stem cells (iPSCs) to become PSM cells. The iPSCs had been engineered to express a version of Hes7 that drives expression (arrow) of genes encoding the fluorescent molecule Achilles[4] or the luminescent molecule luciferase[5]. Monitoring the oscillations of these genes in PSM cells revealed that the human segmentation clock has a period of about 5 hours. **b**, Matsuda et al. performed the same experiment using iPSCs in which Hes7 is mutated, as in the skeletal disorder spondylocostal dysostosis, and found a lack of oscillations. **c**, Yoshioka-Kobayashi et al.[6] isolated the PSM from mouse embryos carrying a Hes7–Achilles reporter, and monitored oscillations, which have a 2-hour period.

expression, the authors observed normal oscillations in the PSM. By contrast, when the authors produced PSM from cells genetically engineered to carry a Hes7 mutation that had previously been identified as a cause of spondylocostal dysostosis[15], they observed a dramatic loss of oscillations (Fig. 1b). This work highlights the potential of using iPSC-derived PSM to determine the relative roles of various clock components in development.

It is known that, although individual PSM cells show autonomous oscillations, Notch signalling between cell neighbours synchronizes these oscillations[1,16] to produce gene-expression waves at the population level. Yoshioka-Kobayashi et al. set out to examine this role for Notch signalling in detail. The authors engineered mice to carry a Hes7–Achilles reporter, and to lack a protein called Lunatic fringe that modulates Notch signalling. They then isolated the entire PSM from embryos that lacked Lunatic fringe and from controls that did not, and made use of optogenetics, a light-triggered gene-expression system, to visualize somite development in culture by tracking Hes7 oscillations over time (Fig. 1c). Although the autonomous oscillations of single PSM cells were unaffected by loss of Lunatic fringe, the researchers observed oscillation defects at the population level.

Notch signalling involves the release of the protein DLL1 from one cell and its binding by Notch receptors on another. This interaction triggers a downstream signalling cascade in the receiving cell that causes increases in the expression of various genes, including Hes1 (ref. 17). This sender–receiver system can be modulated using a genetically engineered optogenetic variant of the Dll1 gene that is expressed in response to stimulation by light[18]. The authors stimulated Dll1, and compared how long it took for neighbouring cells to exhibit Hes1 upregulation in mice lacking Lunatic fringe with the time it took in controls. The study revealed that Lunatic fringe controls population-level oscillations by regulating the timing and amplitude of the signal-sending and signal-receiving process in adjacent cells. This work underscores the intricate role of Notch components in the cell–cell interactions that control clock oscillations.

Together, the current studies provide a remarkable demonstration that simple iPSC culture systems can be used for in-depth analysis of the oscillatory gene expression associated with somite segmentation at single-cell resolution. However, they also have limitations. For instance, Diaz-Cuadros et al. and Matsuda et al. did not observe final stages of somite development and vertebra

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More broadly, gene-regulatory networks are highly conserved between mammals, regardless of the animals' size or whether they are bipedal or quadrupedal. This is in stark contrast to the species-specific timing of gene oscillations, which is fundamental to body-plan development. What causes these crucial differences in timing remains an enigma — but one that can now begin to be unravelled.

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Marine conservation

Predators on track for ocean protection

Ana M. M. Sequeira

Satellite tracking of marine predators in the Southern Ocean has revealed key ecological areas under disproportionate pressure from human activities. These results show the value of tracking data for informing conservation efforts. **See p.87**

Even the most remote marine ecosystems on Earth — such as those at high latitudes, including in the Southern Ocean around Antarctica — can no longer be considered pristine[1]. The effects of humans on marine ecosystems now have a global footprint[2–4], and mitigation of associated threats requires knowledge of the areas of particular ecological and biological significance. Such areas sustain the healthy functioning of marine ecosystems and should therefore be protected. On page 87, Hindell et al.[5] report analyses of tracking data for marine species that reveal these key areas in the Southern Ocean.

The waters of the Southern Ocean encircle the Earth through the Drake Passage, the ocean region between the tip of South America and Antarctica. Because of this passage, the Southern Ocean has a key role in global climate and ocean circulation[6]. This ocean is also home to a unique range of marine fauna, including many charismatic predators, such as penguins (Fig. 1) and seals, as well as the precious Antarctic krill (*Euphausia superba*). These krill are at the base of the marine food web, and,

alongside species of toothfish (*Dissostichus eleginoides* and *Dissostichus mawsoni*), are the target of the largest fishing industries in the Southern Ocean[7,8]. The fisheries compete with animals for food resources, and fishing activities along with the pressures from

> **"Tracking data are increasingly being used to inform conservation policy around the world."**

climate change are raising concerns about the possibility of ecosystem collapses there[8,9].

The Commission for the Conservation of Antarctic Marine Living Resources is the main management body for the Southern Ocean, and is tasked with ensuring the preservation of this ecosystem. To succeed, the commission needs to take precautionary steps, including the establishment of more and better-designed marine reserves as has been suggested[8], and sites for these should be chosen on the basis of

knowledge of the whereabouts of ecologically significant marine areas[10]. However, accurately defining these areas in a highly dynamic, changing environment is challenging.

Monitoring predators at the top of a marine food web can help with this task. Such predators migrate within and between ecosystems, and can be used as indicator species[11] — those able to provide information on the status of an ecosystem or habitat if alterations occur in their movement patterns, behaviour or reproductive success. In particular, tracking top predators can assist with identifying the areas that they use most, which can be considered as regions of great ecosystem importance, not only for the predators but also for a wide range of other species[11]. Indeed, tracking data are increasingly being used to inform conservation policy around the world[12], and have been used to quantify the extent of spatial overlaps between species and fishing activities globally[3].

Hindell et al. report analyses of tracking data from 4,060 individuals of 17 species of marine predators (seabirds and mammals), and suggest a way to use such data to predict key ecological regions in the Southern Ocean. Tracking data were collected between 1991 and 2016 using electronic tags attached to the animals. These tags provided location estimates (obtained using satellite information or other methods) as the animals migrated. The authors used some of these data (for 2,823 individuals) to develop predictive models to identify crucial habitats in the Antarctic region for all of the predator species combined. These integrated results provide a spatially defined assessment of areas of high biodiversity that includes species across multiple levels of the food chain (termed trophic levels) in the Southern Ocean.

Defining a single, integrated result from such varied data sets and from so many species is a complex undertaking. Predators in the Southern Ocean include a large range of species from across different taxonomic groups. These include species living in the Antarctic region and species residing immediately north of it (in the sub-Antarctic), all with different diets and patterns of movement. The authors used a series of data-processing steps to generate a value they termed 'habitat importance', which they predicted using data across all of these species together (assemblage-level maps). To do this, Hindell and colleagues first mapped habitat importance for the species living in the Antarctic separately from those living in the sub-Antarctic, and then selected the maximum habitat-suitability values in those two maps to generate an overall assemblage-level map for all of the predator species combined.

Finally, the authors defined the regions in the top 10% of their calculated habitat importance value as the areas of the most ecological significance in the Southern Ocean. This final