In retrospect

25 years of the segmentation clock gene

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The discovery of a gene that mediates periodic segmentation of the developing backbone of vertebrate embryos opened up research into how the pace of development is controlled by a molecular clock that has a species-specific rhythm.

It is a wonder to observe embryos developing autonomously under a seemingly predetermined schedule. Particularly striking is the developmental process by which structures called somites (which will differentiate into segmental body structures such as vertebrae, ribs, and skeletal muscles) increase in number in a periodic manner. Twenty-five years ago, writing in *Cell*, Palmeirim et al. reported the first molecular evidence of a gene linked to the periodicity of somite formation. The discovery paved the way to our understanding of the developmental clock—a network of genes whose expression oscillates synchronously and thereby regulates the timing of developmental events.

Somites form from an embryonic tissue called the presomitic mesoderm (PSM), which grows towards the caudal (tail) end of the embryo. As it grows, the rostral (head) end of the tissue segments repeatedly, and new segments are generated with a periodicity that is species-specific — once every 30 minutes in zebras, 90 minutes in chickens, 2 hours in mice and 5 hours in humans. Somite formation has been actively studied since the nineteenth century, and the periodicity of this process led to the hypothesis that it was controlled by a cellular oscillator dubbed the clock. However, despite researchers’ fascination with this phenomenon, the underlying mechanism remained unknown.

By the mid-1990s, many genes that regulate body segmentation had been identified in fruit flies, but most of their vertebrate equivalents — known as homologues — were found not to be involved in somite formation. By contrast, vertebrate homologues of fruit-fly genes such as *Notch*, which is involved in the development of neurons (but not in segmentation) in fruit flies, were shown to be involved in somite formation in mice. This led researchers to wonder whether segmentation arose independently in flies and vertebrates. But a gene called *hairy*, which is involved in segmentation of the body in fruit flies, caught the attention of Palmeirim and colleagues. The group reasoned that, although somite segmentation is very different in vertebrates and invertebrates, the same gene might be involved.

Indeed, when the authors examined a homologue of *hairy* in chicks, *c-hairy1*, they found...
that its transcription oscillated with a period identical to that of the chick segmentation clock. The oscillations propagated in waves up the PSM, in a dynamic pattern that could be split into three stages: broad, caudal expression during stage 1; narrower expression in the rostral part of the PSM during stage 2; and the narrowest, rostral expression during stage 3 (Fig. 1). The authors found that this dynamic expression continued in the rostral PSM in culture, even when the caudal parts of embryos were removed, indicating that such oscillations are an autonomous property of the PSM.

These ingenious experiments provided evidence of a developmental clock linked to segmentation. The clock revealed two noteworthy features of gene expression: first, that expression could oscillate with a periodicity as short as 90 minutes; and second, that oscillations could be synchronized between cells.

Palmeirim and colleagues’ groundbreaking study attracted the attention of researchers from a variety of disciplines, and led to the development of a new field, which aimed to identify the central genetic components of the clock and to understand the mechanism of synchronous oscillatory expression. During the next few years, homologues of c-hairy1 were identified in other species — Hes7 in mice (HES7 in humans), and her1 and her7 in zebrafish7. These genes all encode proteins that repress transcription, and their mutation lead to problems with segmentation. Their expression was shown to oscillate autonomously as a result of a negative feedback loop, in which the protein represses the transcription of the gene that encodes it. A 2013 study4 found that the oscillations of the mouse clock could be sped up — and more somites generated — by deleting two sequences called introns (which do not encode proteins) from Hes7. Together, these data made clear that Hes7, and her1 and her7, are the central pacemakers of the segmentation clock.

Several other genes have now been shown to have roles in the clock’s propagating-wave pattern, greatly advancing our understanding of the basic mechanism of the clock. For instance, the expression of the gene Delta (as well as its modulator Lunatic fringe in birds and mammals) oscillates under the control of Hes7 and her1 and her7 proteins. Delta is part of the Notch signalling pathway, which signals between cells to activate the transcription of genes — including those of the Hes and her families — in neighbouring cells. Thus, Delta and Lunatic fringe control the timing of Hes7 and her1 and her7 oscillations in neighbouring cells, which leads to synchronization5,6.

Although the core structure of the segmentation clock is simple, our grasp of its workings is far from complete. For example, one notable feature of the clock is that oscillations proceed stably in single, isolated cells of the PSM (implying that the clock is an intrinsic function of the cells)5,7; however, random mixtures of cells synchronize their oscillations, and self-organize along a rostral–caudal axis (implying extrinsic control)7. Notch signalling is required for the self-organizing process12, but how the cells coordinate their oscillation patterns has yet to be determined. Furthermore, we still have much to learn about downstream genes, the expression of which oscillates in different phases from those of the clock’s main components, depending on the position of the cells along the rostral–caudal axis13,14. These genes regulate the timing of segmentation — but how the differences in phase are controlled is unclear.

New avenues of research into the segmentation clock opened up in 2018, when organoid technology (which enables the 3D culturing of complex tissues in vitro) began to enable easier analysis of somite development15. Organoid-based analyses of the clock revealed that the species specificity in developmental pace is due to differential biochemical reaction speeds for the synthesis and degradation of HES7 (ref. 16).

Our knowledge of the segmentation clock has provided insights into gene regulation that extend beyond what might have been predicted in 1997. For instance, it has long been known that environmental stressors (such as low oxygen concentration in the mother’s blood) affect pregnancy; we now understand that this is in part because of effects on clock-gene expression that can lead to vertebral disorders8,9.

Mathematical theorists have created models that have provided surprising insights — for example, demonstrating that the oscillations in gene expression are robust even when 90% of protein synthesis is blocked10. Going forward, an open question is whether biological systems other than somite segmentation have similar molecular clocks. Oscillatory gene expression occurs in many cell types — for example, it has a role in regulating the proliferation of neural stem cells and muscle progenitor cells11. Animals also exhibit ultradian rhythms (those with a period shorter than that of the circadian rhythm), which are involved in various activities, such as hormone secretion and regulation of body temperature. The groundwork laid by the research into the segmentation clock will be essential in helping biologists to understand the significance of these rhythmic events.

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Astronomy

Shock waves spark blazing light from black holes

Lea Marcotulli

Radiation from a jet of ultrafast particles powered by a supermassive black hole suggests that the particles are accelerated by shock waves propagating along the jet, making them shine with the brightness of 100 billion Suns. See p.677

Most of the 200 billion galaxies in the Universe are centred around enormous black holes that can weigh as much as one billion Suns. Many of these black holes are dormant, but some are still growing, devouring gas from their surroundings and releasing vast amounts of radiation. Even fewer of these active supermassive black holes are capable of launching powerful jets from their cores — ultrafast streams of particles that shine brightly, and can travel distances of up to 100 times the size of their own galaxy. But what provides the initial kick that enables these particles to release so much energy? On page 677, Liodakis et al.1 report that the push comes from shock waves that are generated naturally when the rapid particle outflow encounters slower material moving along the jet.

In the early 1960s, a new class of astronomical object known as the quasi-stellar radio source, or quasar, was revealed. As the name suggests, early observations noted that these objects looked like stars. However, something was not quite right: they radiated very brightly at radio frequencies, and their optical spectra contained strange emission lines2 that are not associated with ‘normal’ stars. It was soon realized that these sources were not stars at all: they were gigantic black holes in the middle of galaxies that were millions, or even billions, of parsecs away from Earth3.

The revelation that these black holes could launch such energetic jets from their cores came in the decades that followed, as radio astronomy advanced and the first satellites dedicated to observing emissions in X-ray and γ-ray frequencies were launched. These jets can be thought of as cones, in which charged particles are accelerated close to the speed of light, and release huge amounts of energy in the form of radiation. If a jet is oriented towards Earth, the generating quasar is known as a blazar, and light emanating from the jet can be seen at all possible wavelengths — from radio waves all the way up to γ-rays.

Since the discovery of these jets, much effort has been devoted to understanding how they form. One commonly used technique, known as polarimetry, is based on the wave-like nature of light. In simple terms, polarimetry measures the extent to which the light waves emanating from a source oscillate in the same direction. If the waves all oscillate in random directions — as with light from an electric bulb — the light is not polarized. If, instead, the waves all oscillate in a specific direction, then the level of polarization is high. For example, the light from a computer screen is strongly polarized in a horizontal direction, and this is why it can’t be seen through some polarized sunglasses, which are designed to filter out horizontal oscillations.

The physical process that makes an astrophysical source shine can be determined by looking at the polarization of the light it emits, because different processes result in different levels of polarization. Polarimetry measurements4 have led to an understanding that the light radiating from the jets emitted by active supermassive black holes — from radio frequencies all the way up to X-ray frequencies — is produced by electrons through emission known as synchrotron radiation. This radiation is generated when the path of charged particles travelling close to the speed of light is bent by a magnetic field. As the particles change direction, they lose energy in the form of light, and the light is polarized.

This much has been known for several decades, but our understanding of what makes these particles start radiating away once they have been funnelled into jets has been incomplete. What was needed was an instrument capable of measuring polarization at X-ray frequencies. The Imaging X-ray Polarimetry Explorer, launched in December 2021, has provided just such an instrument6. One of the mission’s first targets was a very bright blazar known as Markarian 501, which lies a mere 140 megaparsecs away from Earth. This is the first blazar ever observed through the lens of an X-ray polarimeter, and the results reported by Liodakis et al. are dazzling.

1 used measurements of the polarization of X-rays coming from one of these jets to determine that the particles were initially accelerated by a shock wave moving out along the jet. Particles lose energy when they move through a shock wave, resulting in the generation of highly polarized X-rays, which were measured by the authors. After moving past the shock, the radiation emitted by the particles becomes progressively less polarized.