The brain, circadian rhythms, and clock genes

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Every day we experience profound changes in our mental and physical condition as body and brain alternate between states of high activity during the waking day and recuperation, rest, and repair during night time sleep. These cycles are not a passive response to the world around us: they are pre-adapted, driven by an internal clock. We know this because when human volunteers are held in experimental isolation and deprived of any temporal or social cues, they still show daily cycles of sleep and wakefulness, in core body temperatures, and urinary output (fig 1).[1 2] As with all biological processes, the clock driving these cycles is slightly imperfect, therefore the measurable rhythms free run with periods of slightly less than or greater than one solar day, hence circadian (approximately a day). Notwithstanding this inaccuracy, the circadian clock is extremely robust. It is capable of continuing for several months and with a reproducibility to within a few minutes per cycle.

Summary points

- Circadian timekeeping is a fundamental property of all higher forms of life
- In mammals the principal circadian mechanism lies in the individual neurones of the suprachiasmatic nuclei
- Comparative studies of the clock in mammals and fruit flies have provided a model of autoregulatory feedback to explain its basic properties
- The genes encoding this feedback loop, and how they and their protein products respond to synchronising cues, are being characterised
- This opens the way for an understanding of how genes regulate a basic aspect of behaviour and what are suitable targets for intervention when this timing mechanism breaks down

The clock in our brain: the suprachiasmatic nuclei

In humans and other mammals the primary body clock is located in the suprachiasmatic nuclei, a cluster of around 10 000 neurones located on either side of the midline above the optic chiasma, about 3 cm behind the eyes.[3 4] If these nuclei are destroyed, either experimentally in animals or as a result of disease in humans—for example, compression by expanding pituitary tumours—the ability to express any overt circadian rhythms is destroyed. The temporal programme of behaviour and physiology is scrambled.

In experimental animals with such ablation, central grafting of neonatal hypothalamic tissue containing the suprachiasmatic nuclei can restore circadian patterning to the activity-rest cycle. Not only is this compelling evidence that the clock is an autonomous property of the suprachiasmatic nuclei, it is also an excellent example of the restoration of function by neural grafting. Also, when neonatal suprachiasmatic nucleic tissue is dissociated and held in vitro, the individual neurones show robust circadian rhythms of electrical firing, each of them with a slightly different period from its neighbours as they free run in the culture dish.[5] The circadian mechanism is therefore autonomous in cells, and the clock is so powerful that the rhythms of a single neurone can be recorded continuously for several weeks with only the slightest deviation from 24 hours.

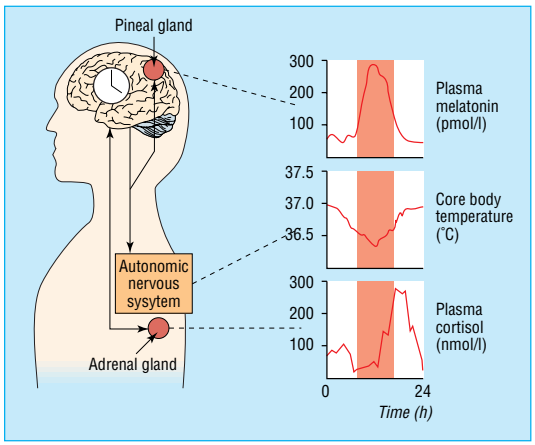

Fig 1 Most aspects of physiology and behaviour are governed by a central clock mechanism in the hypothalamus. The clock acts on neural and endocrine pathways to regulate individual circadian rhythms so that internal state varies predictably over 24 hours. This enables adaption to daily and seasonal environment and enhances efficiency by separating anabolic and catabolic processes in time

Circadian clock molecules in flies

The expression of circadian timing in individual neurones shows that it is not an emergent property of a neural circuitry or system but an integral feature of the biochemistry of the cells. An early indication that the machinery is specified genetically came from studies of the circadian rhythms of cortisol secretion in twins, but as in so many other biomedical fields, the real impetus to molecular genetic analysis of the clock has come from fruit flies (Drosophila spp). By analysing the circadian patterns of activity and emergence from the pupal case of mutant flies, several genes have been identified that encode essential elements of the clock.[6 7] Mutations of these genes can either speed up or slow down the clock, giving flies with days of 20 or 28 hours. Alternatively, mutations can destroy altogether the ability of a fly to be rhythmic.

The proteins encoded by these genes are components of a self sustaining negative feedback loop, which is now thought to form the driving oscillation of the timing system.[8] Period (Per) and Timeless

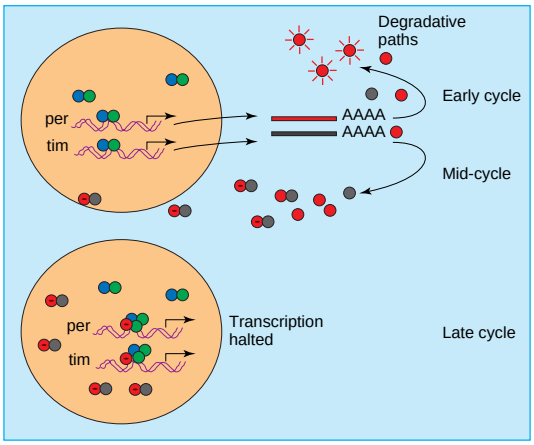

Fig 2 Circadian clock molecules in drosophila. Early cycle: Expression of the clock genes period (per) and timeless (tim) is stimulated by the factors Bmal and Clock (blue/green circles). As cytoplasmic concentrations of per mRNA increase, Period protein (Per) is produced (red circles). Initially it is unstable and degraded. Mid-cycle: As the concentrations of Per and Timeless (Tim) proteins increase, the proteins form heterodimers (red and grey clusters), which enter the nucleus and suppress the expression of per and tim genes. Late cycle: Transcription of the genes is halted. With time Per and Tim proteins are inactivated, and without mRNA no new proteins can be produced. Consequently, Bmal and Clock are able to exert their stimulatory actions and the cycle begins again after about 24 hours

(Tim) proteins move around the cell, their abundance and location defining circadian time (fig 2). The genes encoding these proteins (per and tim respectively) are active in the early part of the night, producing mRNA; proteins start to accumulate later in the night. Initially the proteins are rapidly degraded within the cytoplasm, especially Per. However, the Per protein has a specialised binding site, which enables it to associate with Tim as heterodimers. These dimers are much more resistant to degradation, and in the act of association, surfaces of the protein that hold Per in the cytoplasm are obscured and the dimers become able to enter the nucleus. This is a key event because these clock proteins have another property—they can control the activity of various genes. Expression of the per and tim genes is suppressed by their own dimerised protein products, closing the feedback loop. As a result, once the dimers gain access to the nucleus, the clock genes are turned off and no new clock mRNA or protein is synthesised. After a lag the existing proteins in the nucleus start to be broken down and the genes are released from inhibition to become active again and reinitiate the cycle. Because of the long lags between gene activation and turn off, the whole sequence takes about 24 hours and is self sustaining.

Circadian clock molecules in mammals

Studies in mammals have advanced our understanding of the clock mechanism in two ways.

Firstly, the human and mouse equivalents of the drosophila per gene have now been identified,[7 8 9] and studies showing the presence of mammalian tim are likely to be published in the next few months. The parallels between the fly and mammalian forms of the genes show that evolution has conserved not only the property of circadian timing but also its molecular basis, indicating how deeply the clock is entrenched in our make up.

Secondly, molecular genetic approaches have revealed another pair of essential clock parts. What the feedback model of drosophila does not explain is why removal of inhibition would be followed by gene switch on—in other words, what positive factors are responsible for activating per and tim when the heterodimer proteins are inactivated? These positive factors have now been identified as the Clock and Bmal proteins: positive transcriptional regulators which act together to stimulate the per and tim genes.[10] Mutations of clock in mice and of the equivalent genes in drosophila ablate circadian rhythmicity, probably because the per and tim genes need this positive drive to trigger a new cycle. In its absence the clock is unwound.

As might be expected, the mammalian per genes, clock, and bmal are all expressed in the suprachiasmatic nuclei, but whereas the genes encoding Clock and Bmal are turned on permanently, expression of the per gene is rhythmic, being highest in the middle of the day and suppressed at later stages of the cycle. The assumption is that the inactivation reflects the negative feedback of Per (and possibly Tim) proteins antagonising the positive drive from Clock and Bmal, just as it does in drosophila.

But unravelling the workings of the clock does not stop there—the most recent gene to be identified in flies, double-time, encodes a kinase enzyme thought to be responsible for phosphorylation of Per protein.[11] Mutation of double-time in flies speeds up the clock because without phosphorylation the breakdown of Per protein in the cytoplasm is attenuated. This allows Per concentrations to rise faster, thereby shortening the lag between gene activation and the entry of heterodimer proteins into the nucleus. This illustrates the important point that the integrity and speed of the core oscillation of the clock depends on several intracellular events—that is, the clock's biochemical context. Many factors independent of the core oscillation might be manipulated to affect circadian timing, and these, rather than the core oscillator itself, may be more suitable targets for both experimental and therapeutic purposes.

EYE OF SCIENCE/SPL

Scanning electron micrograph of a mutant fruit fly with leg antennae


PHOTONICA

Synchronising body time

How can internal time based on the Per-Tim loop be synchronised to the outside world? In flies light seems to destabilise the heterodimers by breaking down Tim.[7] In mice, however, light acts through the retina and direct neural pathways to the suprachiasmatic nuclei to stimulate per gene expression. Perturbations by light can advance or delay the clock, depending on the stage at which a new pulse of Per is injected into the ongoing cycle. Under normal circumstances this ensures that small daily adjustments to the clock around dawn and dusk are sufficient to keep it tightly synchronised to the environment.[1 2] In addition, our habits may affect the clock independently of light because recent work has shown that applied schedules of physical activity can alter circadian period. Although the neural pathways mediating these non-photic effects are being mapped, how arousal might affect the behaviour of clock molecules in the suprachiasmatic nuclei is not known. Nevertheless, these findings have important therapeutic considerations in situations where the clock is desynchronised—for example, in jet lag, shift work, and particular forms of depression.[12]

Firstly, resetting can proceed only at a rate of around 1-2 hours a day, so it may well take the clock the best part of a week to adapt to a reversed shift pattern. While the readjustment occurs, the unfortunate person may be expected to perform demanding mental and physical tasks at a time when the clock is driving reaction times and mental performance to their nocturnal nadir.[13]

Secondly, the recognition of the potency of non-photic resetting stimuli adds to the potential range of compounds that might be convenient "chronotherapeutics"—that is, compounds useful for regularising clock function. The pineal hormone melatonin is one such compound.[12] Synthetic derivatives are now being tested clinically and may prove to be useful as alternatives to benzodiazepines in managing sleep disorders.

How many circadian clocks are there?

Recent work in drosophila has shown that when they are excised and cultured in isolation many tissues continue to express circadian patterns in their biochemistry, some tissues being directly photoresponsive.[14] A fly's body therefore consists of a series of independent clocks, which must in life be synchronised by endocrine, neural, and other linkages. Until recently, the view for higher vertebrates was that the principal clock structures are the lateral eyes, the pineal organ, and the suprachiasmatic nuclei, with the suprachiasmatic nuclei being predominant in mammals.[3 4] However, when mammalian cell lines were first deprived of serum and then exposed to a high concentration of serum with all of its rich soup of signalling factors, the cells in culture very quickly turned on a large number of genes, among them mammalian per.[9] This wave of gene expression then subsided, a typical response to serum. On continued sampling, however, the investigators found that after one, two, and even three circadian periods after serum stimulation, the cultures spontaneously turned on per and some other genes.[9] This showed that cultures of immortalised cell lines, which had been held in the laboratory for 25 years, had the capacity to express endogenous free running circadian cycles.

This landmark study opens up an enormous range of opportunities and questions, not the least of which is whether every cell in our body has the potential to be a circadian clock. If so, how do they talk to each other?

A longer term view of biological time?

The daily clock is crucial for longer term processes in many animals. Migration, hibernation, fattening, and fur growth are all adaptations to winter, while the annual rut of large animals and the summer population explosion of smaller ones are all cued, months in advance, by the change in day length. The circadian clock is central to this effect because the signal it gives out changes its shape to reflect the longer nights of winter.[2] As a result, the nocturnal peak of melatonin secretion by the pineal gland, which is tightly controlled by the suprachiasmatic nuclei, provides an internal endocrine calendar. A lengthening melatonin signal from night to night indicates the season is moving through autumn to winter, while progressive shortening means the worst of winter may soon be over.[15]

So the daily clock provides an endocrine calendar, but is it important for humans? There are certainly reports of seasonal changes in mood, especially winter depression with atypical features of increased appetite and amelioration by bright artificial lighting,[12] but for most of us our physiology remains almost immune to season. However, recent studies have shown that the photoperiodic timing system may be latent in our bodies, especially for sleep.[16] Human subjects held in isolation under a summer-like long day length have a single consolidated nocturnal sleep bout, a sharply defined core body temperature minimum, and a short melatonin signal. When the nights are prolonged, the melatonin profile lengthens, sleep breaks into two components at the beginning and end of the night, with an intervening interval of quiet wakefulness, and the nocturnal nadir of the core body temperature rhythm either broadens or shifts phase towards one or other sleep interval. This vestigial seasonal reorganisation of the circadian temporal programme is probably not of great importance to most of us living a modern life. However, the subjective descriptions of the quiet

wakefulness, with the mind hovering back and forth between dream-filled sleep and conscious awareness suggest a deep psychological resonance with season which may underlie seasonal changes in normal and disordered mood. Perhaps we do all have a primitive need, driven by our clock, to turn down the lights, put another log on the fire, and sit back and rest, waiting for the winter to pass.

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Neurogenetic determinism and the new euphenics

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As we approach the end of what in the United States has been termed the decade of the brain, and with a complete map of the human genome in sight, it may be time to try to re-evaluate what the vast increase in molecular knowledge of brain processes has achieved. Certainly there has been no shortage of claims. The abnormal genes and their protein products associated with neurodegenerative diseases such as Huntington's chorea have been identified. Genetic risk factors for Alzheimer's disease are known, and the molecular processes which culminate in the devastating neuronal death and malfunction that are responsible for the disease are subject to intense investigation. However, for neither of these conditions has the new genetic knowledge brought—yet—any effective treatment or prevention. Of course it may come, although not, as many of the advocates of the new genetics once promised, as a result of genetic engineering, but rather because the increased biochemical understanding that follows from genetic information may help in constructing more precisely targeted drugs. Indeed, the best pointer to neuroprotection against Alzheimer's disease has come from epidemiological evidence of the protective effect of hormone replacement therapy in older women rather than from molecular studies.

Summary points

Genes have been identified for several neurodegenerative diseases, but so far this has not led to effective treatment

The tendency to view the complexities of human behaviour as genetically determined has important consequences

One is the construction of new diseases—for example, disruptive children are diagnosed as having attention deficit hyperactivity disorder and are prescribed methyphenidate hydrochloride

Another is that social problems such as violence and alcoholism can be regarded as neurogenetically determined; solutions are then seen as lying in molecular research rather than in reshaping society

Genes for all reasons

But when we move beyond the terrain of relative diagnostic certainty represented by such traditional neurological disorders, things become much murkier. Gene markers, if not genes, associated with conditions such as schizophrenia or manic depression have been proclaimed, amid great ballyhoo, only to be quietly withdrawn as non-replicable. The trouble is that as each old claim disappears into the mists, newer and even more extravagent ones appear. Genes, it is said, are responsible for such diverse features of human conduct as sexual orientation; poor behaviour in school; alcoholism; drug addiction; violence; risk taking; criminal, antisocial, and impulsive behaviour; political anti-authoritarianism; religiosity; tendency to midlife divorce; and even compulsive shopping. Major funding programmes are under way, mainly in the United States but also in the United Kingdom and elsewhere in Europe, to identify such genes, presumably with a view to either screening for and aborting fetuses which show the potential for such undesirable characteristics or generating drugs which will alleviate the condition, turn gays into straights, or radicals into conservatives.

The universalistic claims made for selective serotonin reuptake inhibitors such as fluoxetine (Prozac) are by now very familiar; it is as if all too many of us have too little fluoxetine in the brain without regular recourse to the drug. Less well known is the case of methylphenidate hydrochloride (Ritalin), an amphetamine-like drug now apparently prescribed for anything up to 10% of all American children—mainly boys between the ages of 8 and 13[1]—but coming soon to a general practice near you. Some 40 000 prescrip-