mutation, *tau*, in the hamster. Animals with one copy of the mutated gene show a 22-hour period in locomotor activity, and animals with two mutated copies have a 20-hour periodicity[8]. Unfortunately, further molecular analysis of the *tau* locus has not been easy, owing to the lack of well-developed genetic maps in hamsters.

The mouse, however, is genetically well characterized, and a number of genes have been identified that are rapidly induced by light in its SCN[9]. All of these genes encode transcription factors that contain structural motifs such as the leucine zipper, which mediates protein–protein interactions, and the zinc finger, which mediates protein–DNA interactions. Expression of some of these genes oscillates during the light–dark cycle[2], but it is difficult to work out where these genes fit into the clock mechanism — because a clock can both measure and show time, those genes that are directly implicated in the clockwork need to be distinguished from those that mediate output signals.

In *Neurospora*, the endogenous clock is responsible for the precise circadian synchronization of cycles of asexual sporulation (conidiation), and the *frequency* (*frq*) gene seems to be a central component of the oscillator[10]. Transcription to produce *frq* messenger RNA is central to the clock, with levels of the *frq* transcript peaking in the morning. If an inducible *frq* gene is expressed at the wrong time, the conidiation rhythm is abolished; conversely, suppression of the inducible gene resets the conidiation cycle.

The *frq* messenger RNA transcript can be translated into protein from either of two initiation sequences (codons), resulting in the production of two Frq polypeptides[11]. Changes in temperature regulate the choice of codon used, setting the physiological temperature limits for rhythmicity. Immediately after synthesis, phosphate groups are added to both proteins (phosphorylation), yielding several forms of Frq[12]. It is not clear how Frq contributes to the generation of circadian rhythmicity, but it is probably at the level of transcription — indeed, Frq needs to be in the nucleus (where transcription occurs) to be active[10]. Moreover, although Frq does not belong to any well-defined class of transcription factor, it has several features that are typical of such factors: these include a putative helix–turn–helix domain through which it can bind DNA; a signal that allows it to be targeted to the nucleus (a nuclear-localization signal); and highly charged regions that could be involved in transcriptional activation (Table 1).

Molecular clocks in development

During the formation of an embryo, the events that lead to determination of the various cell types seem to be controlled by developmental timers which operate within individual cells. A remarkable example of this timekeeping is provided by the transition from rapid and symmetrical cell divisions to slow and asymmetrical divisions in embryos of the toad *Xenopus laevis*. This transition always occurs at the twelfth cleavage after fertilization, when the embryo consists of just over 2,000 cells known as blastomeres. The timing of the transition is controlled by a clock that is intrinsic to each blastomere – timing does not depend on cell–cell interactions[33]. Another example comes from precursor cells that differentiate to form oligodendrocytes, a type of non-neuronal cell found in the vertebrate nervous system. When placed individually in single wells, these precursors divide a number of times before differentiating. Two daughter cells from the same precursor undergo a synchronized number of divisions, and differentiate at the same time[34].

Developmental clocks such as these are probably distinct from those that govern circadian rhythms, although the molecular mechanisms that control proliferation and differentiation programmes are obviously intermingled with cellular oscillatory functions. For example, expression of the avian equivalent of the *Drosophila* gene *hairy* in the chick is controlled by a molecular clock that is linked to the formation of somites. *Hairy* is expressed in cyclic pulses with a periodicity of 90 minutes – exactly the time that it takes to form one somite. Movement of the pulses does not depend on cell displacement or on propagation of an activating signal, and each cell seems to have its own functional clock[35].

Because of its intrinsic free-running property, the cell division cycle must also be considered as a molecular clock. Most eukaryotic cells in culture undergo mitosis (nuclear division) with a periodicity of roughly 24 hours. Is this just coincidence, or were cells sensitive to light–dark cycles millions of years ago? If they were, what we study today as the cell cycle could represent a vestigial circadian rhythm. **P.S.-C.**

Association of clock molecules

In *Drosophila*, at least two genes are involved in clock function, and the dynamic association between their products ensures correct circadian rhythmicity. One of these, *period* (*per*), was the first clock gene ever to be isolated[13]. Nonsense mutations in *per* (which lead to an abnormally shortened Per polypeptide) result in the loss of rhythmic locomotor activity in *Drosophila*. Long-lasting phase shifts in locomotor activity are also observed when pulses of heat are given to transgenic flies bearing a heat-inducible copy of *per*. Missense mutations, which cause single amino-acid substitutions in Per, change the length of the circadian cycle[2,14].

Cyclical expression of *per* is regulated by transcription. The promoter region of the *per* gene, which is required for initiation of transcription, is sufficient to confer oscillating transcriptional regulation on another gene[14]. But production of a stable messenger RNA transcript would lead to the accumulation of this *per* messenger RNA and, hence, only minor oscillations. The trick that the *per* gene uses to ensure a strict rhythm is to have a relatively short messenger RNA half-life[15]. This is likely to be a very efficient regulatory mechanism, probably involving the rhythmic activation of factors involved in RNA processing. A mechanism of this kind exists in the dinoflagellate *Gonyaulax polyedra*, for example — circadian expression of a gene correlates with the cyclic binding of a protein to the 3′ end of the messenger RNA, regulating the stability of the transcript[16].

The second fly clock gene is *timeless* (*tim*). Mutations in *tim* have dramatic consequences on *per* (Fig. 2): the oscillatory expression is lost; phosphorylation of the Per protein is disrupted; and time-dependent nuclear transport is abolished. Conversely, cyclic expression of *tim* is altered in *per* mutants[17]. The Tim protein seems to regulate Per by interacting with it to form a Per–Tim dimer. Per contains a region called the PAS domain (Table 1), which is necessary for protein–protein interactions[18], and is now thought to be the 'signature' of this class of clock molecule. PAS domains were named after the three proteins in which this structural motif was first identified: *Drosophila* Per, the mammalian Arnt, and Sim, which is the product of the fly *single-minded* gene. These domains have since been found in several transcription factors, where they are thought to confer target-gene specificity, and they are often coupled to a DNA-binding domain (a basic helix–loop–helix or bHLH domain). *Drosophila* Per does not contain a bHLH domain, however, suggesting that it may regulate transcription without binding DNA.

Surprisingly, Tim does not seem to con-

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