Clock Genes

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Synonyms

Circadian clock genes

Definition

Any of a number of genes that interact with each other to make up an auto-regulatory feedback loop, in which its activation and repression cycle takes about one day.

Characteristics

Clock genes are components of the circadian clock comparable to the cogwheels of a mechanical watch. They interact with each other in an intricate manner generating oscillations of gene expression. The underlying principle of circadian clocks is successive gene activation in the form of a cycle: the initial activation of a gene is regulated by the last one in the sequence, making up an auto-regulatory feedback loop for which one cycle takes about 24 h. This principle is illustrated in Fig. 1.

Positive elements activate the expression of negative elements, which in turn stop the activity of the positive elements. This system moves away from equili brium before returning and hence, perpetual cycling is the consequence. Although the genes involved in this mechanism can differ in various organisms, the principle illustrated in Fig. 1 is common to all of them (reviewed in [1]).

In mammals the circadian clock mechanism is made up of two interlocking, regulatory feedback loops (Fig. 2, orange and blue lines).

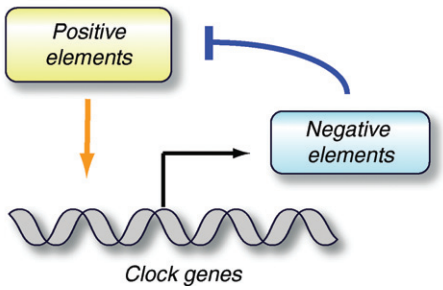
In the first loop (blue lines), two transcriptional activators ►Bmal1 (brain and muscle ARNT-like protein 1) and ►Clock (or Npas2 in neuronal tissue) form heterodimers in the cytoplasm and enter the nucleus where they bind to ►E-box sequences in the promoters of ►Period (*Per1,2*) and ►Cryptochrome (*Cry1,2*) genes contributing to the activation of their expression. In the cytoplasm various combinations of Per and Cry proteins interact with each other, enter the nucleus and inhibit the activity of Bmal1/Clock or Bmal1/Npas2 complexes. Without these complexes activating transcription of the *Per* and *Cry* genes, levels of *Per* and *Cry* transcripts and their respective protein products decline, hence *Per* and *Cry* genes shut off their own transcription (reviewed in [2]).

A second loop regulates the expression of the *Bmal1* gene (orange lines, Fig. 2). In the nucleus Bmal1/Clock or Bmal1/Npas2 heterodimers bind to E-boxes present in the promoters of genes that encode the retinoic acid-related orphan nuclear receptors ►Rev-erbα and ►Rorα, which compete for the ROR element (RORE) in the *Bmal1* promoter. Rorα activates *Bmal1* expression, while Rev-erbα represses it. As a consequence oscillations of *Bmal1* and *Rorα/Rev-erbα* are out of phase. If activation wins over expression Bmal1 protein is produced and it forms heterodimers in the cytoplasm with Clock or Npas2 depending on the tissue [3]. These heterodimers enter the nucleus and initiate the next cycle of gene activation of both loops. The regulation of Clock and *Npas2* is at present not understood.

How do Bmal1 and Clock contribute to the activation of transcription of other clock genes? It appears that transcriptional activation is facilitated by the histone acetyl transferase (HAT) activity of the Clock protein [4]. Histone acetylation promotes transcription through the modification of histones (Ac, Fig. 3) and allows opening of the condensed chromatin. This provides access to the transcriptional machinery (Fig. 3, RNA Polymerase II and general transcription factors).

The HAT activity of Clock is necessary for the transcriptional activation of the clock genes *Per* and *Cry* and therefore seems to be essential for the generation and maintenance of endogenous circadian rhythms in mammals. Transcriptional repression is mediated by several events. Per and Cry bind to the Bmal1/Clock complex. This results in loss of HAT activity of Clock by promoting Clock phosphorylation (P) and/or inducing a conformational change of Bmal1/Clock. Whether these changes induced by Per and Cry leave Bmal1/Clock bound to the E-box or cause dissociation from it is not known. In either case, loss of Clock HAT activity promotes histone deacetylation. This prevents the general transcription machinery from binding to DNA and hence transcription is repressed.

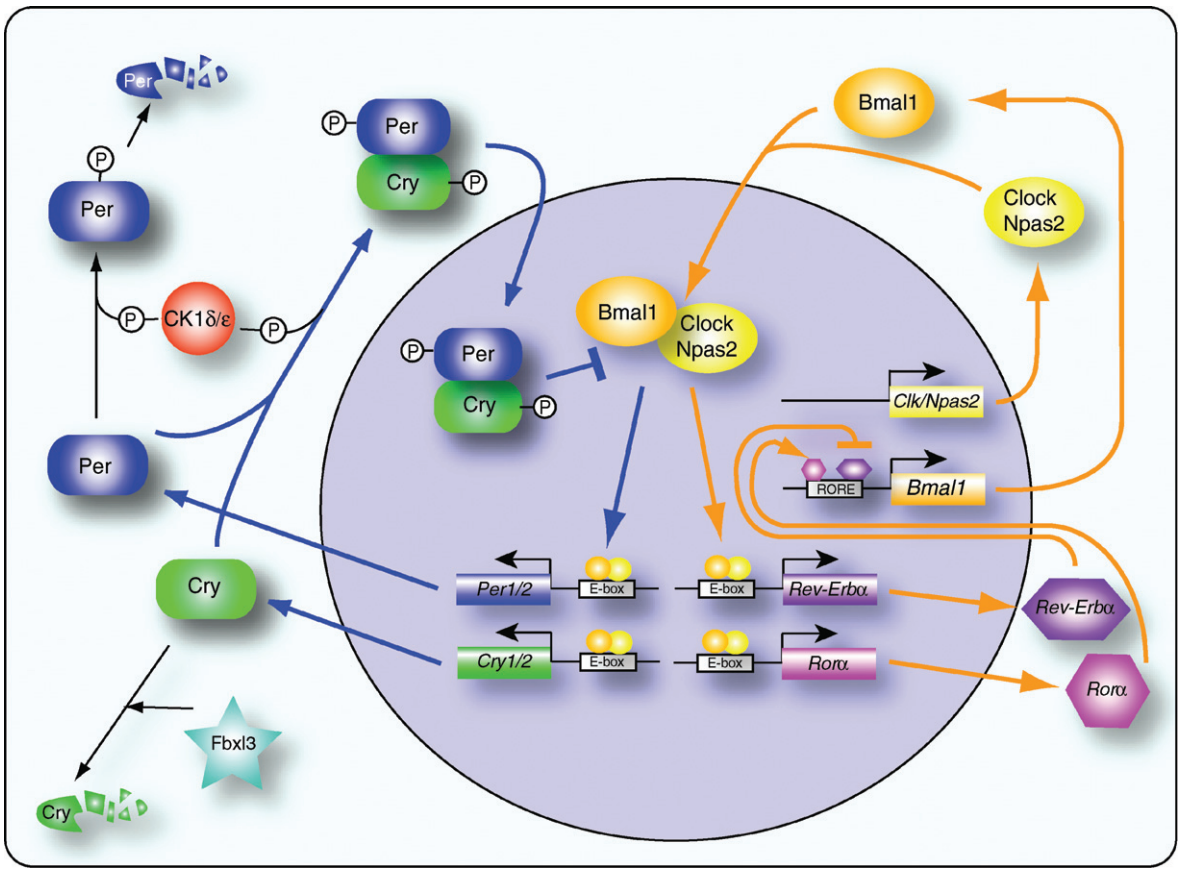
Upon degradation of Per and Cry, Clock is either dephosphorylated or degraded and resynthesized. It then interacts again with Bmal1 and acetylates histones to activate a new transcription cycle.



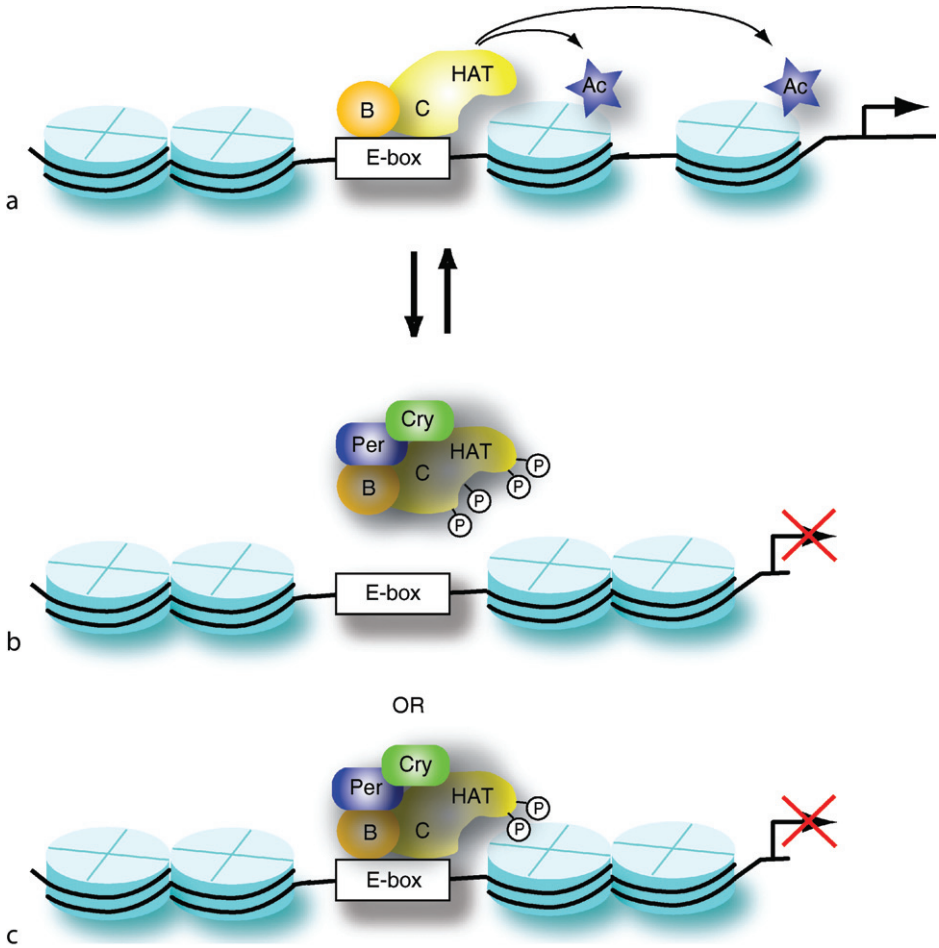
Clock Genes. Figure 1 General principle of the transcriptional autoregulatory feedback-loop. This principle underlies the clock mechanism in organisms that have a circadian clock.

Clock gene expression regulated exclusively by transcriptional processes would run into equilibrium and no oscillation of gene expression would be observed. Transport of clock proteins from the cytoplasm into the nucleus as well as posttranscriptional processes are additional levels of regulation of the clock mechanism for generating oscillations of approximately 24 h. Per and Cry proteins interact with each other which prevents rapid degradation of these proteins and enables them to enter the nucleus. Mutation of interaction sites in either Per or Cry protein disturbs the nuclear and cytoplasmic localization with consequences on the clock oscillator (reviewed in [2]).

Phosphorylation and dephosphorylation of proteins is a widely used mechanism to regulate protein stability, activity, and structure in many biological processes such as signal transduction. In the generation of mammalian circadian rhythms phosphorylation and dephosphorylation of Per proteins plays a critical role in determination of period length. For example, casein kinase 1 ε or δ (CK1ε/δ, Fig. 2) phosphorylates Per2

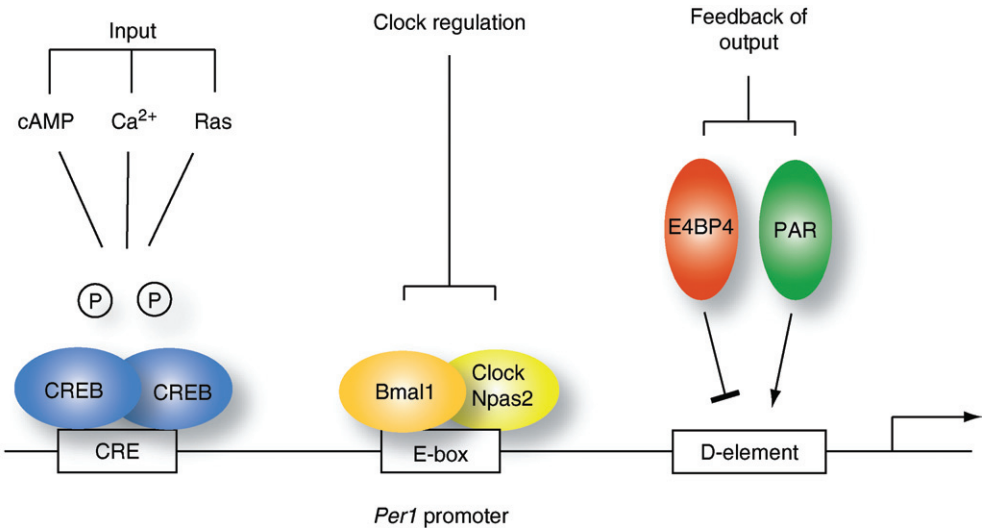


Clock Genes. Figure 2 Hypothetical clock mechanism in mammals. Note the two loops (*blue lines* and *orange lines*) converging on the transcriptional activators Bmal1 and Clock/Npas2. Besides transcription, nuclear import of clock factors and posttranslational modification of these factors (such as phosphorylation, circled P in diagram) seem to play an important role in the regulation of the feedback-loop. For details see text.



Clock Genes. Figure 3 Diagram depicting the histone acetyltransferase (HAT) activity of Clock (*yellow*). (a) Acetylation (Ac, *blue stars*) of histones (*light blue discs*) near the promoter region of target genes greatly facilitates transcription by RNA polymerase II. Upon binding of Per/Cry, Clock is phosphorylated which either leads to detachment of the complex from the E-box (b) or simply inactivates HAT activity (c) leading to inactivation of transcription (*red crosses*).

protein at different sites. If predominantly amino-terminal sites are phosphorylated, Per2 protein will be degraded. However, if sites in the second part of the Per2 protein sequence are phosphorylated, Per2 is stabilized and can interact with Cry proteins to enter the nucleus and interfere with the Bmal1/Clock or Bmal1/Npas2 complexes (Fig. 2) (reviewed in [5]). Interestingly, mutations in the CK1ε as well as in sites of Per2 that are important for CK1ε binding and phosphorylation cause alterations in period length. Patients with a specific form of familial advanced sleep phase syndrome have the mutation S662G in their PER2 protein, leading to a loss of binding of casein kinase 1ε/δ (CKIε/δ) and hypo-phosphorylation of PER2 [6]. This leads to a shortened period length of the circadian clock and hence these patients have an accelerated clock. As a consequence they display a 4-h advance of the daily sleep-wake rhythm. *In vitro* studies and mouse genetics revealed that alteration of the serine at position 662 in mouse Per2 recapitulates the finding in humans. Furthermore a change from S to D, mimicking by its constitutive phosphorylation and allowing constitutive binding of CKIδ, increased phosphorylation of Per2 leading to a longer period length (reviewed in [5]). This indicates the importance of regulation of clock proteins to specify period length. Therefore it is not unexpected that Cry also is regulated by CK1ε/δ Furthermore, Cry abundance is regulated by its interaction with Fbxl3, a subunit of one of more than 70 mammalian ubiquitin ligase complexes that recognizes targets for degradation by the proteasome, a multisubunit molecular protein shredding machine (reviewed in [7]). Upon binding of Cry to Fbxl3 it becomes ubiquitinated and is degraded by the proteasome. It appears that circadian oscillations



Clock Genes. Figure 4 Regulatory elements in the promoter of the clock gene *Per1*. Besides regulation by clock factors through E-boxes, activation of the input pathway for example by light leads to changes in various signaling pathways converging on the CRE-element. Also clock-controlled genes can feed back and influence clock gene expression by binding to D-elements either activating (PAR leucine zipper transcription factors such as Dbp) or inhibiting (E4BP4) transcription.

are tuned by a delicate ratio of Per and Cry proteins, whose levels are regulated by phosphorylation and ubiquitination. If their relative abundance is changed, alterations in the clock oscillator are the consequence [8].

The circadian clock is not only a timekeeper. To serve as a predictor of recurring events in nature it needs to have the potential to adapt to changes in lighting and feeding conditions. Therefore clock genes not only respond to regulators of the clock mechanism described in Fig. 2, but also to signaling pathways that connect the organisms biochemical organization with timed events in the environment (reviewed in [9]). Signals such as light stimulate cellular changes in calcium and cAMP levels which lead to phosphorylation of the cAMP responsive element binding protein (CREB) that homodimerizes and binds to the cAMP responsive element (CRE) in the promoter of some clock genes such as *Per1* (Fig. 4).

This causes fast induction of transcription of this gene leading to an adjustment of the circadian clock. Transcription factors, such as E4BP4 and Dbp (PAR leucine zipper transcription factor) are regulated by nutritional cues and the clock (see clock-controlled genes). Through binding to the D-element in the promoter of clock genes such as *Per1* they either stimulate (Dbp) or repress (E4BP4) transcription [10]. In this way, the metabolic state of an organism is reported back to the clock (Fig. 4, feedback of output). Hence, clock genes are not only generating a circadian rhythm but also integrate the metabolic state of the organism and information from the environment.

References

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