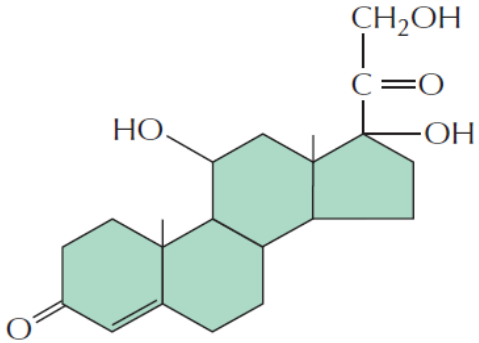
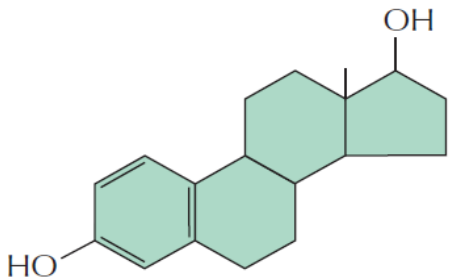
Alternative Signaling Routes in Genes Regulation

Nuclear Receptors Are Ligand-Modulated Transcription Regulators

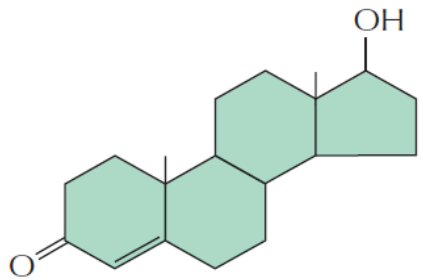
- Various small, hydrophobic signal molecules diffuse directly across the plasma membrane of target cells and bind to **intracellular receptors that are transcription regulators.**
- These signal molecules include steroid hormones, thyroid hormones, retinoids, and vitamin D.
- Although they differ greatly from one another in both chemical structure and function, they all act by a **similar mechanism**.
- They bind to their **respective intracellular receptor proteins** and alter the **ability of these proteins to control the transcription** of specific genes.



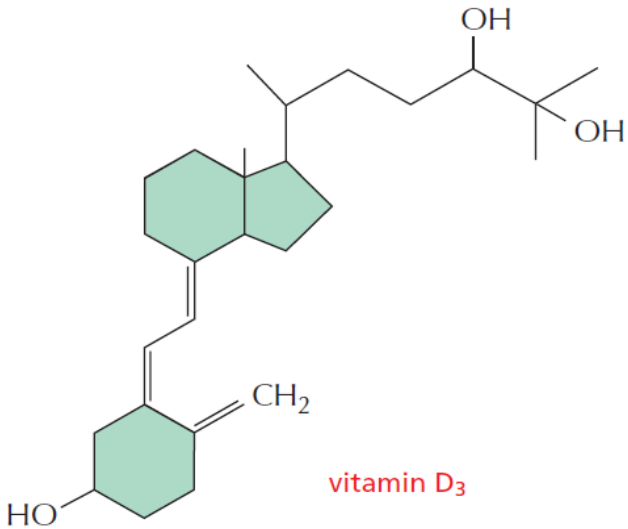
cortisol



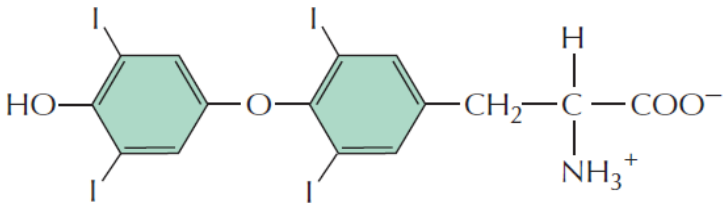
estradiol



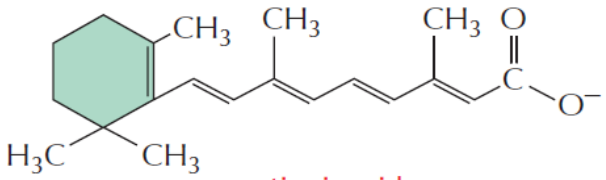
testosterone



vitamin D_3



thyroxine



retinoic acid

Nuclear Receptors Are Ligand-Modulated Transcription Regulators

- Thus, these proteins serve both as **intracellular receptors** and as **intracellular effectors** for the signal.

- The receptors are **all structurally related**, being part of the very **large nuclear receptor superfamily**.

- Many family members have been identified by DNA sequencing only, and their ligand is not yet known; they are therefore referred to as **orphan nuclear receptors**, and they make up large fractions of the nuclear receptors encoded in the genomes of humans, *Drosophila*, and the nematode *C. elegans*.

- Some mammalian nuclear receptors are regulated by **intracellular metabolites** rather than by secreted signal molecules; the **peroxisome proliferation-activated receptors (PPARs)**, for example, bind intracellular lipid metabolites and regulate the transcription of genes involved in lipid metabolism and fat-cell differentiation.

- It seems likely that the nuclear receptors for hormones evolved from such receptors for intracellular metabolites, which would help explain their intracellular location.

- Steroid hormones—which include cortisol, the steroid sex hormones, vitamin D (in vertebrates), and the molting hormone ecdysone (in insects)—are all made from **cholesterol**:

-**Cortisol** is produced in the cortex of the adrenal glands and influences the metabolism of many types of cells.
-The **steroid sex hormones** are made in the testes and ovaries and are responsible for the secondary sex characteristics that distinguish males from females.
-**Vitamin D** is synthesized in the skin in response to sunlight; after it has been converted to its active form in the liver or kidneys, it regulates **Ca^{2+} metabolism**, **promoting Ca^{2+} uptake in the gut** and **reducing its excretion in the kidneys**.
-The **thyroid hormones,** which are made from the amino acid tyrosine, act to increase the metabolic rate of many cell types,
-while the **retinoids**, such as retinoic acid, are made from vitamin A and have important roles as local mediators in vertebrate development.

- Although all of these signal molecules are relatively insoluble in water, they are made soluble for transport in the bloodstream and other extracellular fluids by binding to specific **carrier proteins**, from which they dissociate before entering a target cell.

Nuclear Receptors Are Ligand-Modulated Transcription Regulators

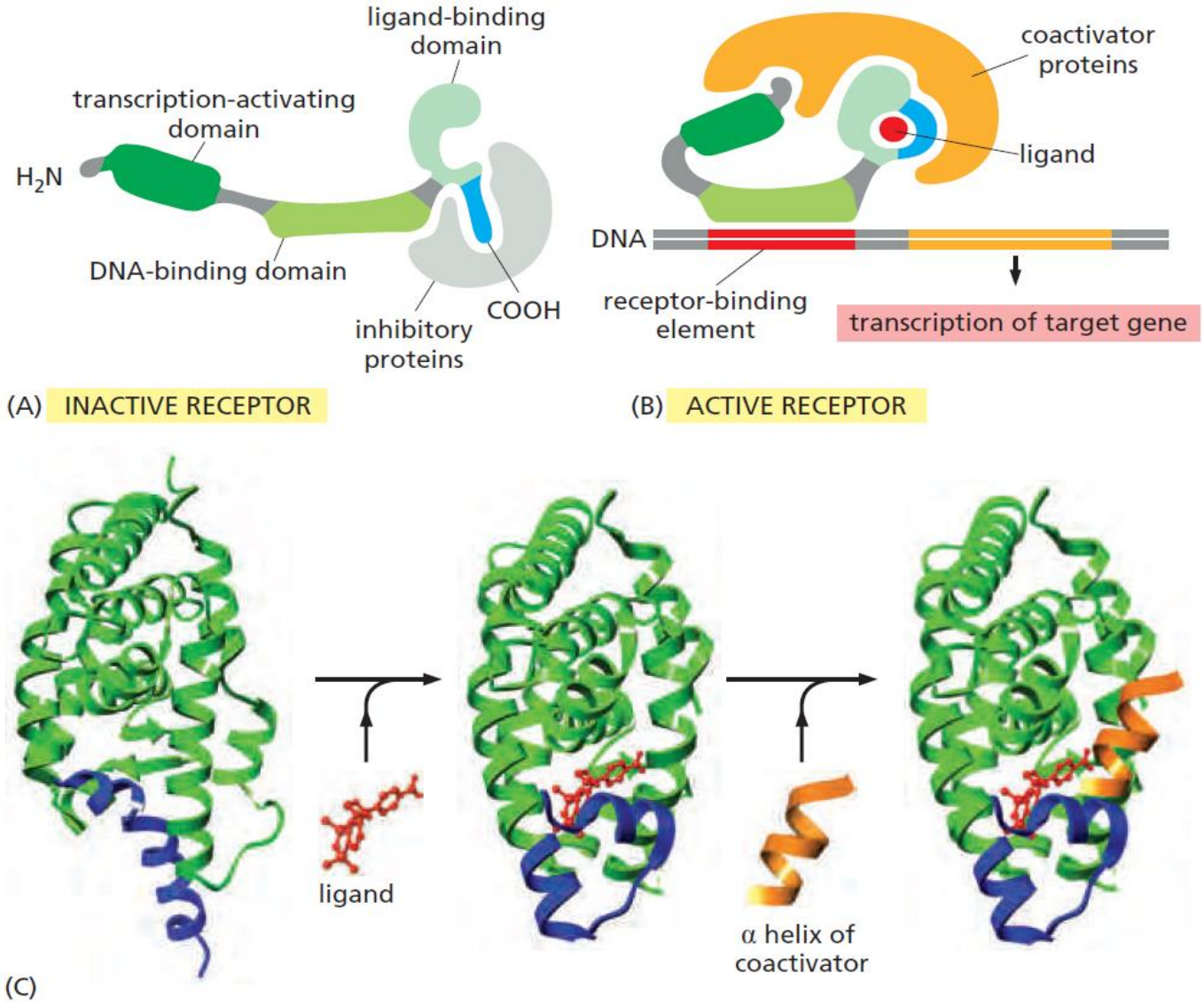
The nuclear receptors bind to **specific DNA sequences adjacent to the genes that the ligand regulates**.

Some of the receptors, such as those for **cortisol**, are located primarily in the cytosol and enter the nucleus only after ligand binding; others, such as the **thyroid** and **retinoid** receptors, are bound to DNA in the nucleus even in the absence of ligand.

In either case, the inactive receptors are usually bound to inhibitory protein complexes.

Ligand binding alters the conformation of the receptor protein, causing the inhibitory complex to dissociate, while also causing the receptor to bind **coactivator proteins** that stimulate gene transcription.

In other cases, however, ligand binding to a nuclear receptor inhibits transcription: some thyroid hormone receptors, for example, act as transcriptional activators in the absence of their hormone and become **transcriptional repressors** when hormone binds.



Circadian Clocks Contain Negative Feedback Loops That Control Gene Expression

- We now turn to gene regulation by a more **global environmental signal**: the cycle of **light and darkness** that results from the Earth's rotation.

- Life on Earth evolved in the presence of a daily cycle of day and night, and many present-day organisms (ranging from archaea to plants and humans) possess an **internal rhythm** **that dictates different behaviors at different times of day**.

- These behaviors range from the cyclical change in **metabolic enzyme activities** of a bacterium to the **elaborate sleep–wake cycles** of humans.

- The **internal oscillators** that control such diurnal rhythms are called **circadian clocks**.

- Having a circadian clock enables an organism to anticipate the regular daily changes in its environment and take appropriate action in advance.

- Of course, the internal clock cannot be perfectly accurate, and so it must be capable of being reset by **external cues** such as the light of day.

- Thus, circadian clocks **keep running** even when the environmental cues (changes in light and dark) are removed, but the period of this free-running rhythm is generally a little less or more than 24 hours.

- External signals indicating the **time of day** cause small adjustments in the running of the clock, so as to keep the organism in **synchrony with its environment.**

- Following more drastic shifts, circadian cycles become gradually reset by the new cycle of light and dark, as anyone who has experienced **jet lag** can attest.

Circadian Clocks Contain Negative Feedback Loops That Control Gene Expression

- We might expect that the circadian clock would be a **complex multicellular device**, with different groups of cells responsible for different parts of the oscillation mechanism.
- Remarkably, in almost all multicellular organisms, including humans, the **timekeepers** are individual cells.
- Thus, a clock that operates in each member of a specialized group of brain cells (the SCN cells in the suprachiasmatic nucleus of the hypothalamus) controls our diurnal cycles of **sleeping and waking**, **body temperature**, and **hormone release**.
- Even if these cells are removed from the brain and dispersed in a culture dish, they will continue to oscillate individually, showing **a cyclic pattern of gene expression with a period of approximately 24 hours**.
- In the intact body, the SCN cells receive neural cues from the retina, entraining the SCN cells to the daily cycle of light and dark; they also send information about the time of day to another brain area, the pineal gland, which relays the **time signal** to the rest of the body by releasing the **hormone melatonin** in time with the clock.
- **Although the SCN cells have a central role as timekeepers in mammals**, almost all the other cells in the mammalian body have an internal circadian rhythm, which has the ability to reset in response to light.
- Similarly, in *Drosophila*, many different types of cells have a similar circadian clock, which continues to cycle when they have been dissected away from the rest of the fly and can be reset by externally imposed light and dark cycles.
- The working of circadian clocks, therefore, is a **fundamental problem** in cell biology.

Circadian Clocks Contain Negative Feedback Loops That Control Gene Expression

- Although we do not yet understand all the details, studies in a wide variety of organisms have revealed the basic principles and molecular components.

- The key principle is that circadian clocks generally depend on **negative feedback loops**.

- **Oscillations in the activity of an intracellular signaling protein can occur if that protein inhibits its own activity with a long delay**.

- In *Drosophila* and many other animals, including humans, the heart of the circadian clock is a delayed negative feedback loop based on transcription regulators: **accumulation of certain gene products switches off the transcription of their own genes**, but with a delay, so that the cell oscillates between a state in which the products are present and transcription is switched off, and one in which the products are absent and transcription is switched on.

- The negative feedback underlying circadian rhythms does not have to be based on transcription regulators.

- In some cell types, the circadian clock is constructed of proteins that govern their own activities through **post-translational mechanisms**.

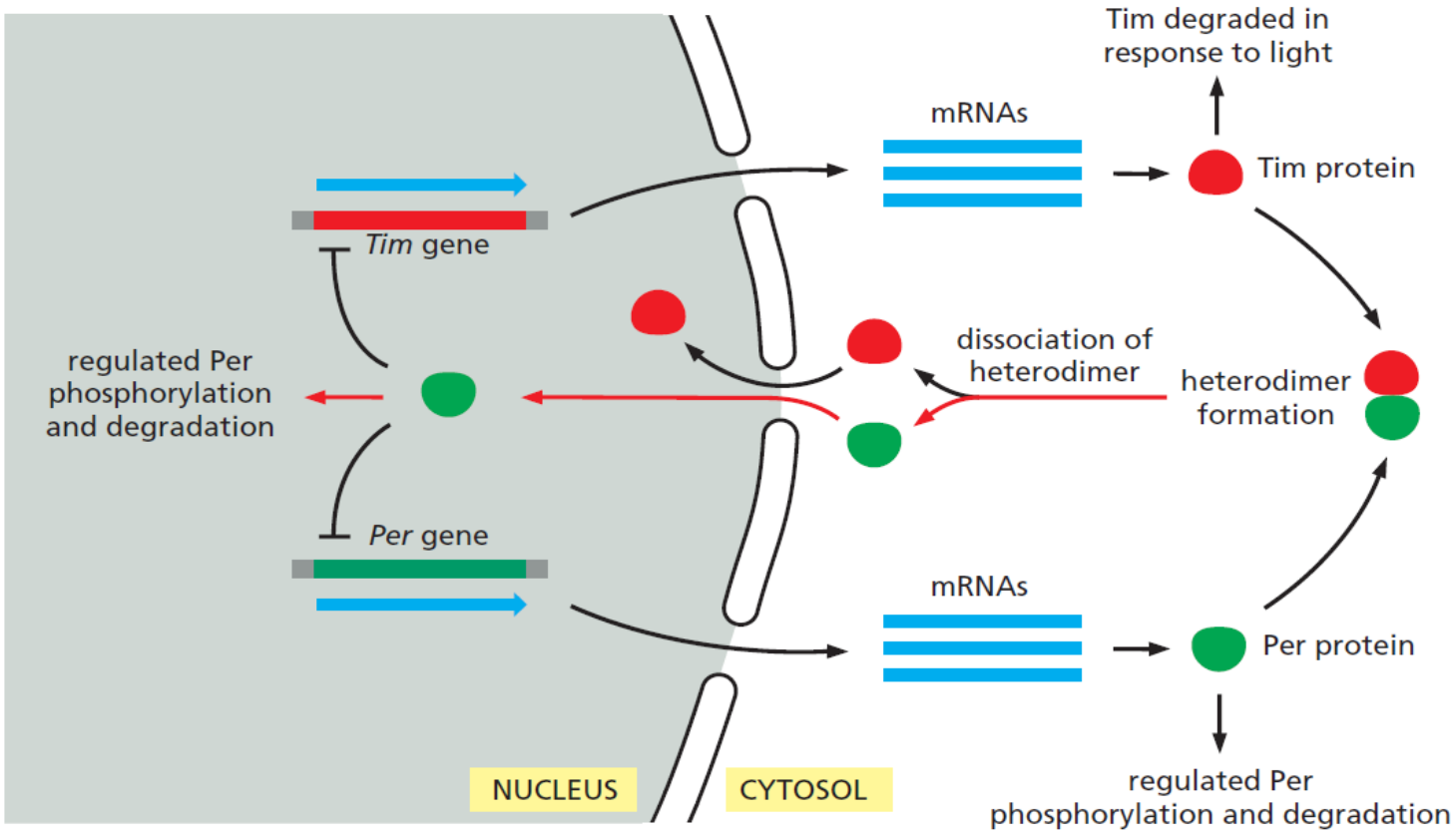
Simplified outline of the mechanism of the circadian clock in *Drosophila* cells

A central feature of the clock is the periodic accumulation and decay of two transcription regulatory proteins, **Tim** (short for timeless, based on the phenotype of a gene mutation) and **Per** (short for period).

The mRNAs encoding these proteins rise gradually during the day and are translated in the cytosol, where the two proteins associate to form a heterodimer.

After a time delay, the heterodimer dissociates and Tim and Per are transported into the nucleus, where **Per represses the Tim and Per genes**, resulting in negative feedback that causes the levels of Tim and Per to fall.

In addition to this transcriptional feedback, the clock depends on numerous other proteins:
For example, the **controlled degradation of Per** indicated in the diagram imposes delays in the accumulation of Tim and Per, which are crucial to the functioning of the clock.

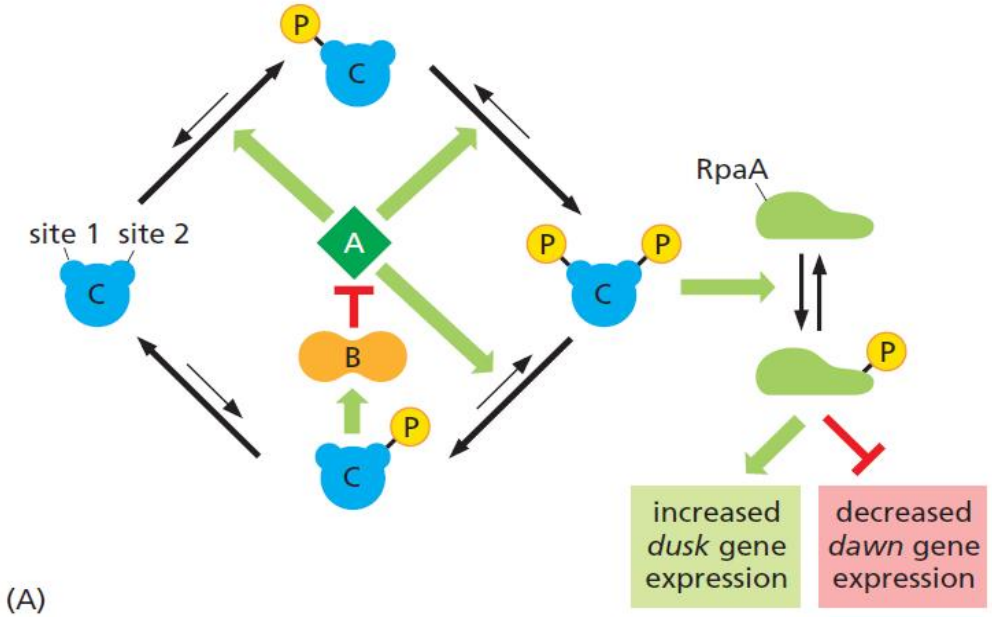


Steps at which specific delays are imposed are shown in red.

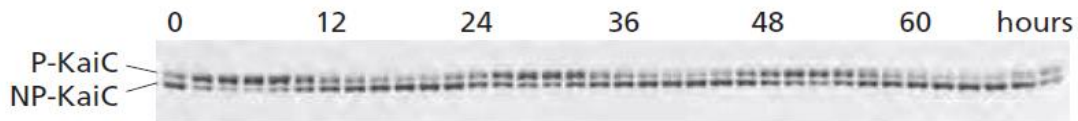
Entrainment (or resetting) of the clock occurs in response to new light–dark cycles.

Although most *Drosophila* cells do not have true photoreceptors, light is sensed by intracellular flavoproteins, also called **cryptochromes**.

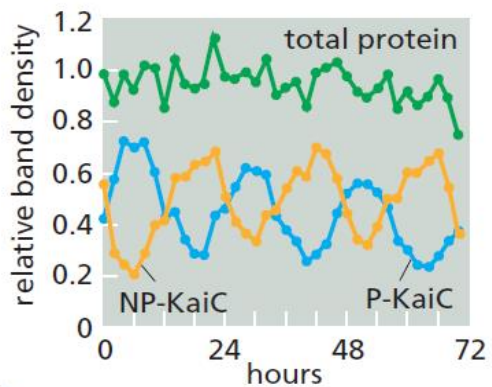
In the presence of light, these proteins associate with the Tim protein and cause its degradation, thereby resetting the clock.


P
C
site 1
site 2
C
A
T
B
P
C
P
P
C
RpaA
P
increased
dusk gene
expression
decreased
dawn gene
expression

(A)


0
12
24
36
48
60
hours
P-KaiC
NP-KaiC

(B)


1.2
1.0
0.8
0.6
0.4
0.2
0
relative band density
total protein
NP-KaiC
P-KaiC
0
24
48
72
hours

(C)