Developmental Timing

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Coordination of developmental processes
I.E. Maintaining the proper relative sequence of events.

1. Regulatory hierarchies
   Early events are activated by early genes in the hierarchy. The early genes also activate genes that direct later developmental events.
   E.G. Drosophila segmentation: Genes that specify segment identity are activated by genes that produce the segmented pattern. In that way, segment identities are not specified until after the segmental pattern is achieved.

2. Reciprocal induction
   In the development of a complex organ, tissues induce back and forth to coordinate their developmental progression.
   e.g. Eye formation: The optic vesicle induces the overlying ectoderm to form a lens placode. The lens placode then signals back to induce the formation of the optic cup. Later the lens vesicle induces the formation of the retina. The retina then signals back to induce the final differentiation of the lens. In this way, the development of the two structures remains coordinated and synchronous because the lens will not proceed to the next stage until the retina is ready and vice versa.

   Kidney formation is another example where the metanephrogenic mesenchyme induces the formation of the ureteric bud which then signals back to induce proliferation of the metanephrogenic mesenchyme, which then signals back to induce branching of the ureteric bud, and so on.

3. Concentration dependence (differential affinities)
   The concentration of a determinant changes over time and different events are triggered at different concentrations. This involves differential affinities.
eg. Metamorphosis: Amphibian metamorphosis involves sweeping changes to virtually every system of the animal. The proper sequence of events is important so that the old system is not eliminated before the new one is functional. For example, it would be disastrous to eliminate gills before the lungs were functioning. It would be bad to eliminate the swimming tail before legs had developed. Metamorphosis is triggered by a hormone called T3. This hormone has a nuclear receptor which acts directly as a transcription factor. The concentration of T3 increases during metamorphosis. Early events are regulated by genes with high affinity binding sites for the T3 receptor in their promoters, and are therefore activated by low concentrations of T3 found early in the process. Late events are triggered by genes with low affinity binding sites, which are only activated by the high concentrations of T3 found late in metamorphosis.

pha-4 regulation of pharangeal gene expression is another example.

**Heterochrony**

Changes in the timing of developmental events. Heterochronic mutations cause temporal alterations in cell fate. Cell fates that are normally expressed at one time in development are attained at a different time.

Can have evolutionary consequences.

Neoteny—retention of juvenile characteristics into the adult stage, or the achievement of sexual maturity while still in the larval stage.

e.g.1 The axolotl salamander becomes sexually mature without undergoing metamorphosis. (sexually mature larval stage).
e.g.2 The coqui frog skips the tadpole stage and hatches from the egg directly as an adult frog.

**C. elegans** heterochronic genes

*C. elegans* has an invariant lineage—every worm has the same number of cells and the same pattern of cell division and cell fate. *C. elegans* has 6 stages: embryogenesis, 4 larval molts (L1-L4) and adult. At each stage, a characteristic set of cell divisions occur and cell fates are expressed. That makes it feasible to recognize mutants that acquire cell fates prematurely or too late because too few or too many cell divisions occur. Since these mutants alter the lineage, they are called *lin* mutants.

e.g. *lin-4* mutants cause a reiteration of L1 cell fates in L2 and later larval molts. Conversely, *lin-14* mutants show precocious appearance of L2 cell fates in the L1 stage. Molecular and genetic studies suggest that *lin-4* | *lin-14* | L2 cell fates. *Lin-14* encodes a nuclear protein of unknown function. The *lin-4* gene produces a non-coding RNA (i.e. it makes no protein). The RNA is about 70 nucleotides long and contains a stem-loop structure that is processed to produce a ~21 nucleotide micro-RNA. The *let-14* message contains regions within the 3’ UTR that are complementary to the *lin-4* microRNA and these are required for repression of *lin-14* by *lin-4*. *lin-14*
message levels are unaffected but protein levels are inhibited indicating that *lin-4* microRNA blocks translation.

MicroRNAs have been found in many species including mammals and plants and represent a newly discovered mode of gene regulation. In some cases, the regulation appears to act at the level of message stability rather than at the translational level.

**Somite formation**
Segmentation clock—periodicity of a molecular oscillator is converted to spatial pattern.

Periodic expression of *c-Hairy* (a transcription factor--repressor). Get expression throughout the presomitic mesoderm (PSM), then expression declines beginning in the posterior end and progressing anteriorly in a wave like manner. A band of expression remains at the posterior of the presumptive somite where the somite boundary will form.

Notch signaling is involved in somite formation. *Lunatic Fringe* is expressed in a similar temporal-spatial pattern. Fringe proteins are glycosyltransferases that inhibit Notch signaling in the PSM. Notch signaling is required for boundary formation between somites. This suggests that the periodic activation of notch signaling may result in the periodic formation of somite boundaries.

In mutants defective in Notch signaling, the periodic expression of *Hairy* and *Lunatic Fringe* is disrupted—there is a static “salt and pepper” expression pattern. This suggests that Notch signaling is required for the clock and may be part of the molecular oscillator. Consistent with this, several genes that are activated by Notch signaling show the same periodic oscillations as *c-Hairy*. These include *Lunatic Fringe* and a transcription factor called *HES7* (Hairy and Enhancer of Split family). HES7 inhibits its own expression and the expression Lunatic Fringe. This creates a negative feedback loop.

As the Lunatic Fringe protein turns over, Notch signaling becomes active. This activates the expression of *Lunatic Fringe* which then shuts off Notch signaling again. Notch signaling also activates expression of the transcriptional repressor *HES7*. This inhibits its own expression and the expression of *Lunatic Fringe*. As the HES7 and Lunatic Fringe proteins turn over, Notch signaling becomes active again and starts another cycle of the oscillation.

**Seasonal Cues in Life Cycles**
The life cycles of many organisms are tied into seasonal cycles. Photoperiod is the most reliable seasonal cue in most temperate climates. Diapause is a dormant period in insects. In temperate zone insects, it allows winter survival and is induced by photoperiod. Diapause occurs at different stages in different species. In larval diapause, it is caused by low PTTH, resulting in low ecdysone. Diapause is broken by cold. The brain produces PTTH which then induces a molt.

Many plants are photoperiod sensitive and have specific requirements for flowering.

Photoperiod responses are intimately tied to the Circadian Clock. Organisms somehow compare the day night cycle to their internal clock.

**Circadian Clocks**

Circadian rhythms affect many behavioral and physiological processes. Circadian rhythms have three important characteristics
1. They show a periodic cycle of about 24 hours.
2. They can be entrained by environmental stimuli (zeitgebers), most commonly light but sometimes temperature.
3. They show continued periodicity for several cycles if the organism is moved to a constant environment such as constant light.

At the center of circadian rhythms in mammals and Drosophila is the periodic transcription of two genes, *period* and *timeless*. Active transcription of *per* and *tim* occurs during the day and at night, transcription is repressed.

Transcription is activated by two proteins, CLOCK and CYCLE. CLK and CYC are Pas domain transcription factors that form a heterodimer and bind to the promoter of the *per* and *tim* genes to activate transcription.

The PER and TIM proteins also form a heterodimer. During the day, when there is active *per* and *tim* transcription, the PER and TIM proteins accumulate in the cytoplasm of the cell. At night, PER/TIM enters the nucleus and inhibits CLK/CYC, thereby shutting off transcription of the *per* and *tim* genes.

When lights come on, TIM is actively degraded, releasing inhibition of CKL/CYC and allowing transcription of *per* and *tim*.

The *cyc* gene also shows cyclic transcription. It is transcribed at night and turned off in the day. CLK/CYC represses *cyc* transcription during the day (probably indirectly by activating the putative repressor *vrille*). At night, when PER/TIM enters the nucleus and inhibits CLK/CYC, this releases the repression of *cyc* which then becomes transcribed and begins to accumulate.

In plants and Drosophila, light signals feed into the circadian clock through the blue light photoreceptor. This is a protein called CRYPTOCHROME. Mutants in the cry gene are
impaired in their ability to entrain circadian rhythms if the periodicity of light/dark cycles is changed. In Drosophila, CRY shows light dependent binding to TIM and PER. CRY + light blocks the action of PER/TIM, thereby relieving CLK/CYC inhibition and allowing transcription of \textit{per} and \textit{tim}.

Putting the whole regulatory pathway together, it looks like this:

\[
\text{CRY} + \text{light} \quad \longrightarrow \quad \text{PER/TIM} \quad \longrightarrow \quad \text{CLK/CYC} \quad \longrightarrow \quad \text{per and tim} \quad \longrightarrow \quad \text{cyc}
\]

In mammals, CRY has a more complex and still poorly understood role in controlling circadian rhythms. Mice with mutant \textit{cry} genes are arrhythmic; that is they have no clock. Under a normal light-dark cycle, they behave normally. If the cycle is changed, animals normally require a period of adjustment to adapt their endogenous rhythms to the new cycle (the cause of jet lag when traveling). The \textit{cry} mutant mice instantly adapt to the new cycle. Therefore, in mammals CRY does not just function to entrain the clock but it is required for the function of the clock itself. In addition, human CRY has been shown to bind to PER, CLK and CYC in addition to TIM and binding is not light dependent.

Cycling of this clock feeds back out to control gene transcription and other physiological and behavioral phenomena. I.e. the CLK/CYC and PER/TIM proteins regulate other genes besides themselves.