In human, we follows a circadian rhythm. At midnight, cortisol⬇️, melantonin⬆️, cerebral bloodflow⬆️, blood pressure⬇️, urine production⬇️.

In Zebrafish

- Period-1 is a circadian rhythm gene: self-regulated as a negative feedback loop.
  - In Zebrafish, period-1 protein express at the highest level at the start of the light cycle
  - At around 6~9 hpf, Light induce the expression of Cry1a and 6-4 photolyase in zebrafish embryo **shown through ISH**
  - All tissue in the zebrafish can respond to the light-dark cycle, **in zebrafish mutant (masterblind mbl) without eye development, the light-dark cycle is still present**
  - Zebrafish cell lines in vitro can also be entrained to a light-dark cycle. **Cell lines exposed to L-D cycle show periodic expression of clock.** Clock is the TF promoting Per-1, Per1 inhibit Clock➡️ negative feedback
  - **Luciferase assay express short half-life fluorescent protein, allow real time expression monitoring**
  - Exposure to light pulse can alter the L-D cycle expression of circadian genes.

- Opsin involved in the light dark cycle in zebrafish
  - Gene expression tracking shows opsin, clock, and period are expressed early in zebrafish embryos
  - Opsins are expressed early in the fish brain, **shown through ISH.**
  - Especially, TMT9 is expressed in overlap with neural stem cell areas. Might be associated with NSC development

- Key regulatory genes are also expressed in a circadian rhythm:
- Several important cell-cycle regulatory genes show L-D cycle rhythm, absent in LL cycle. E.g: Cyclin-B1, Cdk1, p20, p21, NeuroD
  - Neuro-D gene shows L-D cycle rhythm, contribute to neuron differentiation**, ISH shows NeuroD expression in the retina**.
    - NeuroD downstream transcription factors driving rod and cone differentiation also shows rhythmic expression with a time delay.
  - p20 and p21 are cell cycle regulators of the G1/S-phase transition, and shows rhythmic expression, coupling light-dark cycle to cell cycle.
    - **Assessing BRDU incorporation into zebrafish cells reflects S-phase**, shows different tissues have peak BRDU intensity at different stages of LD cycle.
    - **BRDU in the gut is high when p21 is highly expressed**
    - **BRDU in the brain is concordant with p20 expression**,
      - Differential regulation roles of two cell cycle regulators in two tissues couples growth with circadian cycle. The time delay in peaking of p20 and p21 allows different timing of development, ensure nutrient availability.

- Mammalian peripheral tissue circadian rhythm: coupled to L-D cycle, but does not sense light by itself.
  - Superchiasmatic nucleus (SCN) regulates the circadian cycle in tissue through hormones/temperature/behaviour
  - Fetal circadian clock may be entrained by maternal melantonin cycle.
  - In mammals, undifferentiated embryonic stem cells does not have a circadian rhythm, however differentiated cells does, such as skin cells or mature neurons.
  - Development of a functioning circadian clock upregulates NeuroD expression, permitting progenitor cells to undergo terminal differentiation. **RNAi KD of clock gene prevent terminal differentiation of neurons.**