**Intercellular Coupling of the Cell Cycle and Circadian Clock in Adult Stem Cell Culture**Toru Matsu-ura **Introduction***This study investigates if the cell cycle (aka, cell division/mitosis) is regulated by the circadian rhythm.*The intestine is made up of many cell types, these are the key ones here:

*Stem cells*: These cells can differentiate/turn into any other cell, as needed; so, they divide.
*Progenitor cells*: These cells are partially differentiated/committed, but can still turn into a few different cells (just not as many as pure intestinal stem cells); so, they also divide.
Paneth cells: These cells are fully differentiated and have chosen their “career”/”job” in the intestinal tract (they defend against microbes and pathogens); so, they do not divide.

**Methods**
*Cultured In Vitro Study*
 - Mouse intestinal cells (enteroids) were studied.
 - Looked at various intestinal cells (stem, progenitor, Paneth cells).

*Experimental Methods*
Luciferase Bioluminescence: The researchers are able to infect the cells with a virus or some other gene manipulation where they change the genome of the cells they are targeting by inputting a gene that leads to the synthesis of a luminescent/glowing protein when the gene it is linked with is expressed. This will be explained in more detail in Figure 1.

**Results**
*Figure 1A*
*Background*: This is a schematic of the added luciferase gene (as described in the methods of these notes) wherein a green protein is expressed every time the cells undergo cell division.

Take Away: Green fluorescence describes cells dividing, undergoing the cell cycle.

*Figure 1B*
*Background*: This is the same experiment as explained in Figure 1A and the methods section of this paper, but the gene that is inserted into the cell’s genome is one that expresses a red protein when the cells activate their circadian rhythm.

Take Away: Red fluorescence describes the circadian rhythm activation.

*Figure 1C, D, E*
*Background*: These are various representations of the relationship between the red fluorescence (circadian rhythm expression) and green fluorescence (cell division) over time, in intestinal cells.

*Primary Results*:
- The circadian rhythm reaches its peak every 24 hours.
- Cell division reaches its peak around every 12 hours.

Take Away: The relationship is 2:1, for every one circadian rhythm, there are roughly two cell divisions – however, as seen by the graphs, this is not in perfect synchrony, so the researchers decide to investigate further.

*Note*: Skipping Figures 2 & 3 (and part of 4), because they relate to cell signaling pathways and finding out which one is dominant for cell division in relation to circadian rhythm; while interesting, it does not add much to the story. Ultimately, WNT signaling is most closely tied to circadian rhythmicity and cell division.

*Figure 4E&F*
*Background*: Researchers are looking at the speed by which cell cycle occurs if we split the measurements between the base of the intestinal villi (Crypt Base) and higher up in the intestinal villi (TA). The villi is a finger like projection that makes up the structure of the intestine (look it up, its better than if I can describe it).

*Primary Results*:
- There is a distinct difference in the amount of cells, depending on location, that undergo the cell cycle at varying rates.

Take Away: This may partially explain the variance in Figure 1, by showing that the location (and more specifically, the type of cell in that location) plays a role in cell division – some cells divide faster than others.

*Figure 5D&E*
*Background*: Trying to tease out the differences between the cells in the intestine, the researchers are looking at the bioluminescence of red protein (indicating more circadian rhythm) between dividing cells (stem and progenitor cells) and non-dividing cells (Paneth cells – described in the introduction of these notes).

*Primary Results*:
- Non dividing cells increase their expression of circadian rhythm.

Take Away: Only non-dividing cells, like Paneth cells, have a circadian rhythm response. This means, stem cells and progenitor cells do not react to a circadian rhythm, directly.

*Figure 5G&H*
*Background*: The researchers have replaced the media (the liquid the cells grow in) with stem cell derived media or left on the normal media (that works for non-dividing cells, as well), and tested the red fluorescent protein expression (for circadian rhythm, as previously described in methods and other figures of these notes) in a population of dividing and non-dividing cells (so, intestinal cells altogether).

*Primary Results*:
- In stem cell media only, there is a loss of circadian rhythm.

Take Away: Because we only see a loss in the 24 hour oscillations of the circadian rhythm expression with stem cell media, this implies there is an environment dependent (something in the normal media not found in the stem cell media) mechanism that triggers the circadian rhythm.

*Figure 6K*
*Background*: Researchers have ablated, or killed, the Paneth cells (non-dividing cells) and are tracking the cell division that occurs with and without this elimination of the Paneth cells.

*Primary Results*:
- Paneth cell ablation leads to elimination of cell division oscillation, but not of overall cell division.

Take Away: The Paneth cells seem to control the oscillation seen in circadian rhythms and cell division, but does not reduce the total number of cells created.

*Figure 7C*
*Background*: Researchers are now looking at organoids/enteroids (a 3D self assembled grouping of cells, almost mature enough to be intestine, but does not have the structure for it). This enteroid population of cells contains all the cells (non-dividing Paneth cells, other non-dividing cells, and dividing stem and progenitor cells), and they have knocked out the circadian rhythm gene in these cells (not just Paneth) and are comparing them to normal gene organoids/enteroids (control) for their ability to “bud”/form new pockets of cells (cell division).

*Primary Results*:
- Knocking out the circadian rhythm genes eliminated budding.

Take Away: Knocking out the circadian rhythm gene in all of these cells leads to an elimination of cell division; therefor, the circadian rhythm is necessary for cell division, directly or indirectly.

**Additional Notes**
The authors present a mechanism by which this all works – the population of non-dividing cells (like Paneth cells) in the intestine have their circadian rhythm genes activated, which allows them to secrete WNT signaling proteins, which bind stem cells and progenitor (and any other dividing cells), and induces the signal for cell division. This may all begin by serotonin from the brain binding the intestinal cells, activating the circadian gene in the first place.

**Conclusions**
Cell division depends on the circadian rhythm.

Stem cells and other dividing cells do not need the circadian rhythm directly, but likely need it indirectly via Paneth cells and other non-dividing cells which will be activated to secrete WNT signaling (not covered in notes, but is in study) every 24 hours, which binds the dividing cells, but causes stem cells to divide every 24 hours while progenitor cells divide every 12 hours – explaining the variation in cell division, when tested outright.