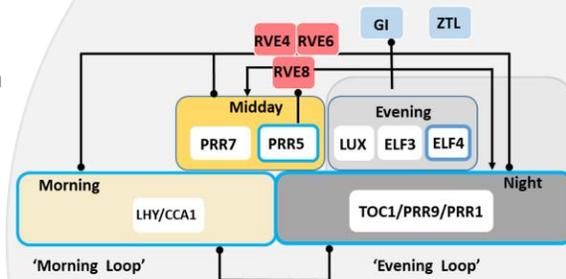


## In Optimizing Cellular Processes for Environmental Challenges Timing is Everything!

The circadian clock widely influences gene expression in turn affecting plant development and responses to the environment through its master regulators (**Figure 1**). Although several alleles of circadian clock (CC) genes are known to have been selected through breeding for crop improvement, a systematic genetic dissection of the CC through induced mutation has not been undertaken in wheat. Gene editing (GE) can create allelic variation for such genes and, with advances in directed meiotic recombination, valuable alleles can be rapidly introgressed into elite germplasm. In an AAFC-IWYP project “**Circadian Clock Editing to Increase Wheat Yield**”, John Laurie and André Laroche at Agriculture and Agri-Food Canada (AAFC), with Ian Henderson at the University of Cambridge, UK and other colleagues, are using transcriptomics to identify core CC genes. Gene editing techniques are then being used to develop mutant lines carrying allelic variation for core CC regulatory genes followed by their phenotypic characterization. At the same time, the group is aiming to manipulate meiotic recombination to facilitate the precise and rapid movement of alleles in breeding processes.



**Figure 1.** A circadian clock gene model for wheat

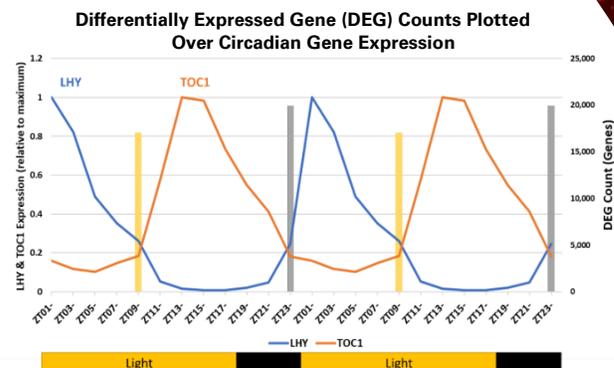
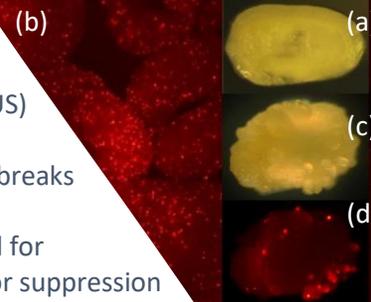
## What Solutions have been Identified?

- Characterization of the wheat transcriptome over a 24 hour-cycle (0 being the light onset time) has identified a midday (ZT9) transcriptional flux which indicates that plants have reached photosynthetic saturation and have transiently activated photorespiration. This points to a division of metabolic activities occurring on either side of this flux and coincides with the downregulation of LHY/CCA and upregulation of TOC1 genes (**Figure 2**).
- Improvement of wheat transformation by expressing BABY BOOM (BBM) and WUSCHEL (WUS) transcription factor genes allowing an accelerated recovery of transformed plants (**Figure 3**).
- Generation of wheat lines expressing dCas9-SPO11-1 to generate targeted double-stranded breaks in chromosomal DNA for stimulation of the recombination pathway.
- Characterization of lines with mutations in histone methyltransferase genes which are useful for modifying the epigenetic landscape in wheat. Epigenetic marks are thought to be required for suppression of recombination within large parts of the chromosomes.

## Anticipated Impact of this Research

- Mutations in core CC regulators will create variability in clock output for generating potential yield increases and improved stress tolerance under warmer and drier growth conditions.
- Directing double-stranded breaks and modulating histone marks is an attractive approach to target meiotic recombination and facilitate the combining of beneficial alleles in elite germplasm.

**Figure 3.** Immature embryos dissected from cv. Fielder (a). Transformation vector containing pporRFP which is easily visualized 48 hours after bombardment (b). Development of globular somatic embryos using BABY BOOM (BBM) overexpression (c & d)



**Figure 2.** The transient upregulation of differentially expressed genes at Zeitgeber time (ZT) 9 and transient downregulation at Zeitgeber time 23 coincide with shifts in LHY (late elongation hypocotyl) and TOC1 (timing of CAB expression 1) expression dominance