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# Circadian Mechanisms In Alcohol Use Disorder and Tissue Injury

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## Abstract

Heavy use of alcohol can lead to addictive behaviors and to eventual alcohol related tissue damage. While increased consumption of alcohol has been attributed to various factors including level of alcohol exposure and environmental factors such as stress, data from behavioral scientists and physiological researchers is revealing roles for the circadian rhythm in mediating the development of behaviors associated with alcohol use disorder as well as the tissue damage that drives physiological disease. In this work, we compile recent work on the complex mutually influential relationship that exists between the core circadian rhythm and the pharmacodynamics of alcohol. As we do so, we highlight implications of the relationship between alcohol and common circadian mechanisms of effected organs on alcohol consumption, metabolism, toxicity, and pathology.

## Alcohol use in Modern Society

According to the Centers for Disease Control and Prevention (CDC), heavy alcohol use is constituted, in women, by a daily habit of 2 standard drinks (28g of alcohol) or by binging 4 drinks during a 2 hour period (Esser et al., 2014). In men, the standard is increased to 4 drinks daily or 5 drinks in less than 2 hours for a binge. These numbers are important because, in an alarming trend, they increasingly characterize the manner in which Americans

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consume alcohol. In 2012, a national study revealed that 8.2% of all Americans were considered heavy drinkers and 18.3% were binge drinkers, a nearly 10% increase over 2005 numbers (Dwyer-Lindgren et al., 2015). Over-consumption was highest (26%) in individuals 18–34 years of age. Characterized by the compulsive use of alcohol and anxiety associated with abstaining from alcohol, Alcohol use disorders like alcoholism and binge drinking have been linked by the World Health Organization to over 204 diseases in humans (WHO, 2014). Due in part to lost productivity, in 2010, the cost of Americans drinking to excess rose to a quarter of a trillion U.S. dollars with nearly 80% of these costs attributed to binge drinking (Control et al., 2016).

Byproducts of alcohol metabolism can result in detrimental biological effects. During alcohol metabolism by alcohol dehydrogenase and/or cytochrome P450 (CYP2E1) reactive oxygen species and other free radicals are generated that can react with critical biological molecules including amino acids, lipids, and nucleic acids to interfere with cellular mechanisms (Caro and Cederbaum, 2004). The resulting alcohol metabolism-derived oxidative stress can induce cytotoxicity, a systemic inflammatory response and damage to healthy tissues. Tissue damage resulting from heavy alcohol use, either chronically or through acute exposure, has broad medical ramifications. This is because the degree of injury, level of recovery, and possibility of clinical intervention to alcohol's effects are affected by the degree of exposure. With documented pathological impact on the brain, heart, lungs, liver, pancreas, gastrointestinal tract and skeleton, the negative health effects of alcohol is now well established (Bruha et al., 2012; Haorah et al., 2005; Kershaw et al., 2008; Keshavarzian et al., 1999; Sampson, 1998).

An intriguing observation is that only 30% of alcoholics develop organ damage like intestinal leakiness to endotoxin and alcoholic liver disease (ALD) indicating that although excessive alcohol consumption is required it is not sufficient to cause tissue injury and additional factor(s) is required for end organ damage. Compelling studies provide evidence that disrupted circadian rhythm may be one factor that promotes alcohol-induced tissue injury in a subset of alcohol consumers. A better understanding of the underlying molecular and cellular mechanisms of alcohol-induced toxicity and tissue injury are essential because it will lead to identification of novel therapeutic target(s) that should result in more targeted and effective treatment strategies to mitigate the toxic effects of alcohol and prevent and/or treat alcohol-induced end organ damage. One of the biological systems that may be the key to understanding many of effects of alcohol on behavioral and physiology is the circadian rhythm. Significant bidirectional interactions between alcohol and circadian rhythms has been known for some time (Adan, 1994; Holloway et al., 1993). These human and rodent studies have shown that circadian machinery not only influences craving and alcohol consumption, but it can also modify alcohol-induced effects on behavior, physiology and tissue injury (Adan, 1994; Holloway et al., 1993). Below, we will provide an overview of bidirectional interaction of alcohol and circadian system and provide evidence that the disruption of circadian rhythms makes alcohol consumers susceptible to alcohol-induced effects, thus potentiating alcohol-induced tissue injury, organ dysfunction and end organ damage.

## Circadian Rhythms

To organize biological activities of daily life, mammals have evolved a ‘master clock’ that resides above the optic chasm of the hypothalamus in the brain that functions as the primary regulator for all circadian-directed activities in the body (Klein et al., 1991). This small collection of about 10,000 cells, known as the suprachiasmatic nucleus (SCN), receives information about ambient light signals through the eye’s photosensitive receptors and the nerves of the retinohypothalamic tract, entraining the circadian system by utilizing the excitatory neurotransmitter glutamate (Kim et al., 2005; Klein et al., 1991). Through direct and indirect methods, the SCN presides over circadian clocks in cells and tissues of the brain, the gastrointestinal tract (GIT) and other organs (Buijs et al., 2001).

Behavior, the sleep cycle, food anticipation, hormone regulation, food metabolism, microbiota-gut interplay, and epithelial barrier function have been shown to be subject to circadian regulation (Malloy et al., 2012; Rosselot et al, 2016; Sadacca et al., 2011). Organ systems that are subordinate to the SCN, such as the intestine, may also use external or tissue-specific signals as temporal cues to influence circadian rhythms (Mohawk et al., 2012). These peripheral clocks can therefore have rhythms that are different from that of the master clock. An instance of this can be seen in the GIT and food consumption. Time of eating is a zeitgeber (time setter) for both the GIT and the liver, and a shifted eating schedule (e.g., consuming food at different times each day or consuming a majority of calories late in the day or at night) can result in a misaligned circadian rhythm of the digestive system from central circadian time (Shi and Zheng, 2013). The misalignment of central and peripheral clocks has been linked to metabolic dysfunction in humans and rodents (Morris et al., 2012).

## Components of the Molecular Circadian Clock

The primary driver of the molecular circadian clock that is present in virtually all cells as well as the SCN is a negative feedback mechanism involving the transcription and translation (Hut and Beersma, 2011; Tomita, 2005). The genes at the core of the circadian rhythm vary with taxonomy but in mammals, the basic clock includes genes that code for CLOCK, NPAS2, BMAL1, PER1-3, CRY1-2, ROR and REV-ERBA proteins (Bunger et al., 2000). The transcription factor CLOCK (Circadian Locomotor Output Cycles Kaput), is an important component of the circadian clock (Ko and Takahashi, 2006). Either by acting as a transcription factor, through intrinsic acetylation capacity, or through the recruitment of co-activator proteins with their own histone acetyltransferase (HAT) abilities, the CLOCK protein begins to control the circadian clock by participating in the remodeling of chromatin (Curtis et al., 2004; Doi et al., 2006). CLOCK-mediated DNA acetylation is followed by PAS-to-PAS domain binding with BMAL1 (Brain and Muscle ARNT-Like 1) to promote the removal of nucleosomes at DNA-binding sites. This is followed by the initiation of transcription of the core clock genes as well as large number of clock-controlled genes (CCGs) within the tissue transcriptome (Bozek et al., 2009; Etchegaray et al., 2003; Menet et al., 2014).

The CLOCK-BMAL1 heterodimer maintains circadian clock rhythmicity, binding to the enhancer box of core circadian genes including *Period* (*Per1, 2, & 3*) and *Cryptochrome*

(*CRY1&2*) to induce transcription (Huang et al., 2012). NPAS2 (Neuronal PAS-domain containing protein 2) has its greatest importance in the CNS where under *Clock*-deficient conditions NPAS2 can substitute for CLOCK allowing the molecular cycle of the SCN to function normally, though peripheral tissues cannot maintain circadian rhythmicity without a functioning *Clock gene* (Debruyne, 2008; DeBruyne et al., 2007). Constitutively produced, CLOCK is important to some parts of the circadian cycle, however, the cellular availability of BMAL1, which is cyclically generated appears to be a driver of circadian transcription and behavior as the mutation of this gene in mice has been shown to result in the complete loss of circadian rhythmicity (Bunger et al., 2000; Stratmann et al., 2012). Phenotypically, *Clock* mutant mice reveal the scope and the critical nature of the circadian rhythm with chronic sleep reduction, altered locomotor activity, disrupted behavioral patterns, circadian dysregulation, arrhythmic transcription of core circadian genes and CCGs, as well as various symptoms traditionally associated with metabolic syndrome (obesity, insulin resistance, hypertriglyceridemia, liver hepatomegaly, steatosis, etc.) (Turek et al., 2005).

BMAL1 itself is a critical part of the circadian clock where it functions as a translation factor. Recent research reveals that BMAL1 associates with translational proteins in the cytoplasm to promote protein synthesis (Lipton et al., 2015). Critically the protein is rhythmically phosphorylated by kinases (mTOR-effector kinase, ribosomal S6 protein kinase 1 (S6K1)) to induce its participation in stimulate protein synthesis. Like NPAS can substitute for CLOCK, BMAL1 has been found to be conditionally dispensable with its paralog, Bmal2, becoming a viable substitute in its absence (Shi et al., 2010).

Once it is translated in the cytoplasm, PER2 protein activity within the core clock is enhanced by the CRY1 protein, which stabilizes PER2 and enhances the rate of PER2 nuclear transport (Vanselow et al., 2006). Inside the nucleus, the PER2-CRY1 heterodimer bind to the CLOCK-BMAL1 heterodimer closing the negative circadian feedback loop and inhibiting further transcription of core circadian genes. The RAR-related orphan receptors ROR-a and REV-ERB-A close another feedback loop by regulating the transcription of BMAL1. Forming the positive/negative arm respectively of the feedback cycle, nuclear receptors ROR-a and REV-ERB-A are trafficked in to the nucleus to compete against one another to either activate or repress BMAL1 transcription (Guillaumond et al., 2005). SIRTUIN1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent regulatory protein that effectively reverses the actions of CLOCK protein. SIRT1 deacetylates circadian proteins PER2 and BMAL1 to detach the CLOCK-BMAL1 dimer from E-boxes of circadian genes; it also removes acetyl groups from histones switching DNA to a closed conformation to reset the circadian cycle (Asher et al., 2008).

Dynamic trafficking of molecular clock components between the cytoplasm and the nucleus is required for proper functioning of the circadian clock making nuclear transport proteins critical to the maintenance of circadian rhythms (Herrero and Davis, 2012). Pharmacological inhibition of the nucleocytoplasmic shuttling proteins that aid in circadian protein PER2 trafficking leads to disruption of circadian regulation *in vitro* (ÖWe prop et al., 2014; Tamaru et al., 2003). A recent study analyzing the transcriptomes of 12 mouse organs found that, accounting for tissue specific variation, over 40% of gene transcription in the mouse showed rhythmic circadian expression (Zhang et al., 2014). Evaluation of circadian

dynamics reveals that manipulation or inhibition of circadian protein kinetics can disrupt function or even abolish the rhythmic expression of circadian genes Aguilar-Arnal and Sassone-Corsi, 2015; Stratmann et al., 2012; Wallach et al., 2013).

It should be noted that, in order to maintain their respective roles in temporal regulation, circadian proteins interact with partner proteins outside of the core clock. Many of these proteins have been shown to assert influence within the circadian rhythm by fine-tuning the clock through multiple post-translational modifications (phosphorylation, ubiquitination, acetylation, SUMOylation) (Gallego and Virshup, 2007). Post-translational modifiers include: casein kinases (CSNK (or CK)I-2), Glycogen synthase kinase 3 (GSK3), adenosine monophosphate-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase CAMK-1,2) mitogen activated protein kinase (MAPK1) protein phosphatases (PP1, PP2A) and others that have been shown to induce or alter circadian protein activity or binding (Reischl and Kramer, 2011; Um et al., 2011; Wang et al., 2011). Critical interacting proteins include receptors such as estrogen, peroxisome proliferator-activated receptor (PPAR), and others that link circadian rhythms to the reproductive, metabolic and other biological processes and have also been shown to exert influence the clock (Gery et al., 2007; Grimaldi et al., 2010; McElroy et al., 2009). These interactive partner proteins (e.g., CSNK1, AMPK, GSK, MAPK) could be the targets of future research and clinical interventions maximizing their influence on circadian regulation.

## Effects of Alcohol on Elements of the Clock

Alcohol metabolism by CYP2E1 is induced with high levels of alcohol. Exposure of rat intestinal tissue to high concentrations of alcohol has been shown to drive the release of oxygen radicals compared to alcohol metabolism by the alcohol dehydrogenase pathway in HEPG2 in vitro liver cell lines (Cederbaum et al., 2001; Pronko, 2002). The reactive oxygen species generated by CYP2E1 can exceed cellular defense systems resulting in oxidative stress and various pathologic consequences for several systems including the circadian rhythm (Caro and Cederbaum, 2004). Many constituent proteins of the circadian core clock (CLOCK, BMAL1, PER1-3, CRY1-2) contain redox-sensing PAS domains it has been therefore suggested the alcohol-mediated oxidative stress is likely to have an effect on the circadian clock as well as downstream effects on the various biological systems that circadian rhythms regulate.

The expression of the core PAS-domain containing circadian proteins (CLOCK, BMAL1, PER1, PER2, CRY1, and CRY2) are affected by the presence of alcohol with the expression of each protein altered in the blood of human alcoholics compared to controls (Huang et al., 2010). An *in vitro* study revealed that alcohol metabolism mediated oxidative stress induces an increase in the circadian proteins CLOCK and PER2 and this aberrant increase in circadian proteins leads to further dysfunction downstream (Davis et al., 2017). It is through this mechanism of altering circadian expression and rhythmicity that alcohol may influence a myriad of modified circadian attributes such as altered photic phase-resetting within the SCN (Ruby et al., 2009).

The disruptive effect of alcohol on the circadian rhythms in the peripheral organs may be more powerful than its effect on the central rhythm in the SCN. In a 2013 study, the SCN and liver tissue were extracted from male *Per2*$^{Luc}$ mice and PER2 protein expression was examined *ex vivo* using luminescence. Analysis of bioluminescence as revealed that in the presence of alcohol the circadian rhythm in the SCN, as measured by PER2, was not disrupted, however, in the liver, alcohol induced a significant phase advance of core circadian and clock-controlled diurnal gene expression accompanied by altered lipid metabolism indicated by hepatic steatosis (Filiano et al., 2013).

More studies demonstrate an effect of alcohol on both central and peripheral circadian rhythms. Recently published work by our group monitored the blood, urine, and activity of healthy groups of day and nightshift workers who were social alcohol drinkers before and after 7 days of daily consumption of red wine (0.5 gram/kg, 1–2 glasses of wine) (Swanson et al., 2016). After wine consumption, nightshift workers had phase-delayed blood melatonin production (a marker of central circadian rhythm) and mononuclear blood cell circadian gene expression (marker of peripheral CR), effects not observed in day shift workers consuming alcohol, demonstrating that even moderate alcohol consumption can exacerbate central and peripheral circadian rhythm disruptions in subset of subjects who already have shifted circadian rhythms. This study confirmed the hypothesis that disrupted circadian rhythms (here due to shift work) can make the drinker susceptible to alcohol's negative physiological effects.

Disruption or manipulation of the molecular circadian clock has a myriad of detrimental health effects including effects on behavior, cardiovascular disease, metabolic syndrome, and cancer, just to name a few. Comprehensive reviews of this literature are available elsewhere (Asher and Sassone-Corsi, 2015; Morris et al., 2012; Yu and Weaver, 2011), but in this review we are focusing on the effects of the manipulation of the circadian clock on alcohol consumption, alcohol metabolism, and alcohol-induced tissue injury and organ dysfunction..

## Circadian Rhythms and Alcohol Consumption

The influence of alcohol over the circadian rhythm appears to be bilateral with research revealing that the circadian clock can affect alcohol preference, consumption, and dependence and each of these factors having an effect on circadian expression. In experiments to characterize whether the circadian rhythm alters alcohol intake, the free-running periods of rats and mice genetically predisposed to prefer alcohol differ in length from those of non-preferring animals (Hofstetter et al., 2003; Rosenwasser and Fixaris, 2013; Rosenwasser et al., 2005). Likewise, circadian rhythms can be manipulated through alterations in environmental cues (i.e., light, temperature), to induce changes in alcohol intake (Spanagel et al., 2005a).

The level of expression of core clock genes have been shown to influence alcohol intake and addiction. One group found that the selective reduction of mouse CLOCK expression in the brain's ventral tegmental area (VTA) or the mutation of CLOCK result in significantly increased alcohol consumption (Ozburn et al., 2013). Multiple other studies have shown

connections between the AUD of patients and the altered expression of core clock proteins (CLOCK, BMAL1 &2, CRY1&2, PER1&2) in peripheral blood cells (Huang et al., 2010; Kovanen et al., 2010; McCarthy et al., 2013). The strongest molecular link between circadian activity and alcohol consumption may be *Per2*, with mutations *in Per2* linked to both circadian disturbance and increased alcohol intake in mice and in humans (Comasco et al., 2010; Spanagel et al., 2005b). The findings in this study have been buttressed by subsequent studies illustrating a critical involvement of *Per1* in alcohol consumption, metabolism, and addiction (Dong et al., 2011; Gamsby et al., 2013). It should be noted that although there is strong evidence suggesting that PER influences alcohol consumption there has been conflicting literature ruling out PER1-related alcohol reinforcement (Zghoul et al., 2007).

One group analyzed the expression of core circadian genes in men to determine whether there is a connection between the circadian rhythm and alcohol use disorder (AUD) (Ando et al., 2010). A negative relationship was found to exist between alcohol consumption and levels of *Per2.* The results confirm the findings in other mouse studies identifying a relationship between PER2 and alcoholism (Huang et al., 2010; McCarthy et al., 2013; Sarkar, 2012; Sjoholm et al., 2010). Notably, there was also an inverse relationship between alcohol use *and Bmal1 gene* expression in blood cells linking another core circadian constituent to AUD and alcohol-induced suggesting impairment of circadian clock at a critical point in the cycle. The core clock is multifaceted and dynamic with contrasting differences across organ systems so further study on the effect of other components on alcohol consumption is required.

## Circadian Rhythms and Alcohol Metabolism

While alcohol elimination was traditionally considered a zero-order process, it has become increasingly clear that the breakdown of alcohol is guided by the time-of-day-dependent oscillations of the circadian rhythm much like other aspects of metabolism (Cederbaum, 2012). Many of the enzymes responsible for alcohol metabolism (CYP2E1, ADH3, 4) have been observed to have a high Michaelis constant (Km) having a lower alcohol binding affinity and requiring a higher alcohol concentration to reach ½ of their maximum reaction rate (Vmax) (Holford, 1987; Ramchandani et al., 2001). These enzymes also have a diurnal variations in their activity levels (Baraldo, 2008; Gachon and Firsov, 2011) Taken together these mean concentration and time-dependent variations in alcohol metabolism that are influenced by elements of the circadian rhythm.

Circadian clocks regulate metabolism through the timed production of enzymes and the modulation of pathway specific endocrine factors (Bailey et al., 2014). This regulation includes the production of protective antioxidant enzymes necessary for the biological amelioration of certain re-dox states which have been linked to activity by of the CLOCK BMAL1 heterodimer (Kondratov et al., 2006, 2009). Oscillations in transcription and protein activity of ROS detoxifiers superoxide dismutase’s (SOD), catalase, glutathione peroxidase (GPx), glutathione (GSH), GSH-s-transferase, and glutamyl cysteine ligase (GCL) have been reported in mice and rats (Fonzo et al., 2009; Xu et al., 2012). These data indicate

possible circadian-dependent susceptibilities to alcohol that need to be further characterized in future studies.

Circadian proteins CLOCK and BMAL1 are also associated with increased expression of *Cyp2e1*1. *Cyp2e1* mRNA was found to be increased in the livers of alcohol-exposed mice *Per2 and Cry1* were linked to levels of CYP2E1 protein expression (Forsyth et al., 2013; Matsunaga et al., 2008) (Matsunaga et al., 2008). There appears to be a mutually influential relationship between the circadian system and the proteins it regulates because, in another mouse study, the siRNA knockdown of *Cyp2e1* prevented the alcohol-mediated induction of CLOCK and PER2 proteins (Forsyth et al., 2013). Studies also reveal daily fluctuations in NAD+, a cofactor for CYP2E1-mediated alcohol metabolism, influencing circadian gene expression and alcohol metabolism through histone acetylation and methylation. In this way, alcohol induced depletion of NAD+ inhibits the deacetylase activity of SIRT1 (Etchegaray et al., 2003; Sahar et al., 2011; Thompson et al., 2015).

Studies into SIRT1 function have shown that inhibition by NAD+ depletion could lead to alterations in the rhythmic conversion of chromatin that permits binding of the CLOCK/BMAL1 heterodimer effectively changing the pace of the circadian cycle and likely the expression of circadian regulated enzymes and cofactors like NAD+ and CYP2E1 (Belden and Dunlap, 2008; Ripperger and Schibler, 2006). Using these direct (enzyme expression) and indirect (cofactor cycling) mechanisms the circadian rhythm asserts influence on the rate of alcohol metabolism, and by extension, the production of the damaging oxidative radicals that are produced through the breakdown of alcohol. Focusing attention on these time-dependent changes may allow researchers to highlight the amount of alcohol induced tissue damage that occur throughout the day and adjust the strength of interventions accordingly.

## Circadian Rhythms and Alcohol Toxicity

Chronic alcohol consumption has been shown in patients to induce damage in multiple tissue types in the body from the heart muscle (i.e., cardiomyopathy) to peripheral nerves (i.e., peripheral neuropathy) to the brain that reveals impeded neuronal growth, nutritional deficits, and shrinking white and gray matter under MRI with long-term heavy drinking (Ammendola et al., 2000; Haorah et al., 2005; Piano and Phillips, 2014; Rosenbloom et al., 2003) Currently however, most extensive research characterizing the role of circadian mechanisms in the development of tissue damage is takes place in the area of the liver and intestine; studies into the role of circadian mechanisms in the alcohol-induced damage seen in other organs would be valuable. Current evidence suggests that disruption of circadian rhythms either induced by alcohol, by genetic manipulation, or environmentally (e.g., alterations in light:dark cycles) is critical to increase the susceptibility of the colon and liver to alcohol-induced tissue injury and play a direct role in the severity of their alcohol induced pathology (Keshavarzian et al., 1999; Swanson et al., 2011).

Studies in mice demonstrate that disruption of circadian rhythms promote alcohol-induced effects on the GIT and liver. Wild-type C57BL6/J mice subjected to weekly circadian disruption induced via once weekly alterations in the light:dark cycle (i.e., 12:12hr inversion) have more intestinal hyperpermeability, endotoxemia, and liver steatosis

compared to non-circadian rhythm disrupted controls (Summa et al., 2013). Similar results were observed in diurnally arrhythmic mice harboring a mutation in the *Clock* gene (i.e., $Clock^{\Delta 19}$) (Summa et al., 2013). Indeed, both environmental and genetic circadian disruptions promote alcohol-induced effects on intestinal tight junctions. These disruptions allow the transfer of pro-inflammatory bacterial byproducts across the intestinal barrier, a critical step required for inflammation and liver pathology. Analysis of intestinal epithelial cells (i.e., Caco-2 cell model) reveals that disruption of the molecular circadian clock is vital for alcohol-induced effects on intestinal permeability. Specifically, siRNA knockdown of *Clock* or *Per2* prevents the alcohol-mediated increase in intestinal permeability that is necessary for the development and progression through the stages of ALD (Steatosis, Fibrosis, Cirrhosis, then Liver Failure) (Forsyth et al., 2013; Swanson et al., 2011). These data point to a critical importance of an intact and proper functioning circadian clock in the intestine since disruption of the clock promotes alcohol-induced pathology.

Studies in humans are equally as compelling as those in mice. Several epidemiological and clinical studies suggest that disruptions to circadian homeostasis makes organs such as the liver and intestine more susceptible to alcohol toxicity. Consistent with binge studies in mice, multiple studies in human alcoholics have shown altered circadian gene expression that is delayed from their wake-sleep routine and blunted compared to healthy controls (Danel et al., 2003; Huang et al., 2010). These alcohol-mediated changes are usually attributed to an AUD and also shown to interfere with the ability of the person to limit consumption (Kovanen et al., 2010).

Reminiscent of mouse results these studies also reveal alcohol induced physiological changes as a result of circadian disruption. Healthy participants in one study with a reliable drinking pattern, working either a night or a day shift in nursing, filled out questionnaires and submitted blood and urine for analysis throughout the study. This work, published by our group, shows that participants working the night shift showed increases in alcohol induced intestinal barrier dysfunction, endotoxemia and elevated pro-inflammatory cytokine IL-6 compared to day shift workers without circadian rhythm disruption (Swanson et al., 2016). More work is necessary to establish the causal link between disrupted circadian rhythms and worsening of alcohol-induced pathology. Further investigation could include interventional studies to determine if the normalization of disrupted circadian rhythms by circadian-directed intervention (e.g., chronotherapeutics, pharmaceutical drugs, prebiotics, probiotics, etc) can prevent/mitigate alcohol-induced end organ damage like gut leakiness and steatohepatitis in man.

## Conclusion

As a small, interactive molecule with almost unfettered access to every tissue alcohol has been linked to much pathology. Abstinence is the most effective means to prevent alcohol pathology; however, the promotion of abstinence through educational programs is difficult to achieve because of the craving that accompanies alcohol addiction. Circadian rhythms are linked to alcohol craving/addiction as well as alcohol consumption, therefore circadian-directed interventions such as light adaptation stimuli, chronobiotics (e.g. melatonin), dietary intake (the time of meal consumption and probiotics) or drugs targeting the circadian

proteins may be opportunities to address alcohol addiction and increase adherence to alcohol abstinence. Observations of differential susceptibility to alcohol-mediated organ damage indicate that AUD is a requirement but insufficient to disease development and the circadian rhythm appears to be a critical factor.

The studies provided offer compelling evidence that the interplay between alcohol and the proteins of the circadian rhythm is key to understanding alcohol-induced pathologies. Again, this indicates the potential utility of circadian-directed interventions to normalize circadian misalignment a promising intervention to prevent and treat alcohol pathology. Further studies will be necessary to determine the ability of circadian-directed therapies to mitigate alcohol-mediated pathology. The ability of an intervention to override alcohol induced transcription, translation, transport and/or the activity of circadian components will be paramount. It may be necessary to investigate therapies that can redirect the reactive oxidative species associated with alcohol or adjuvants that can mediate downstream effects of this metabolic process.

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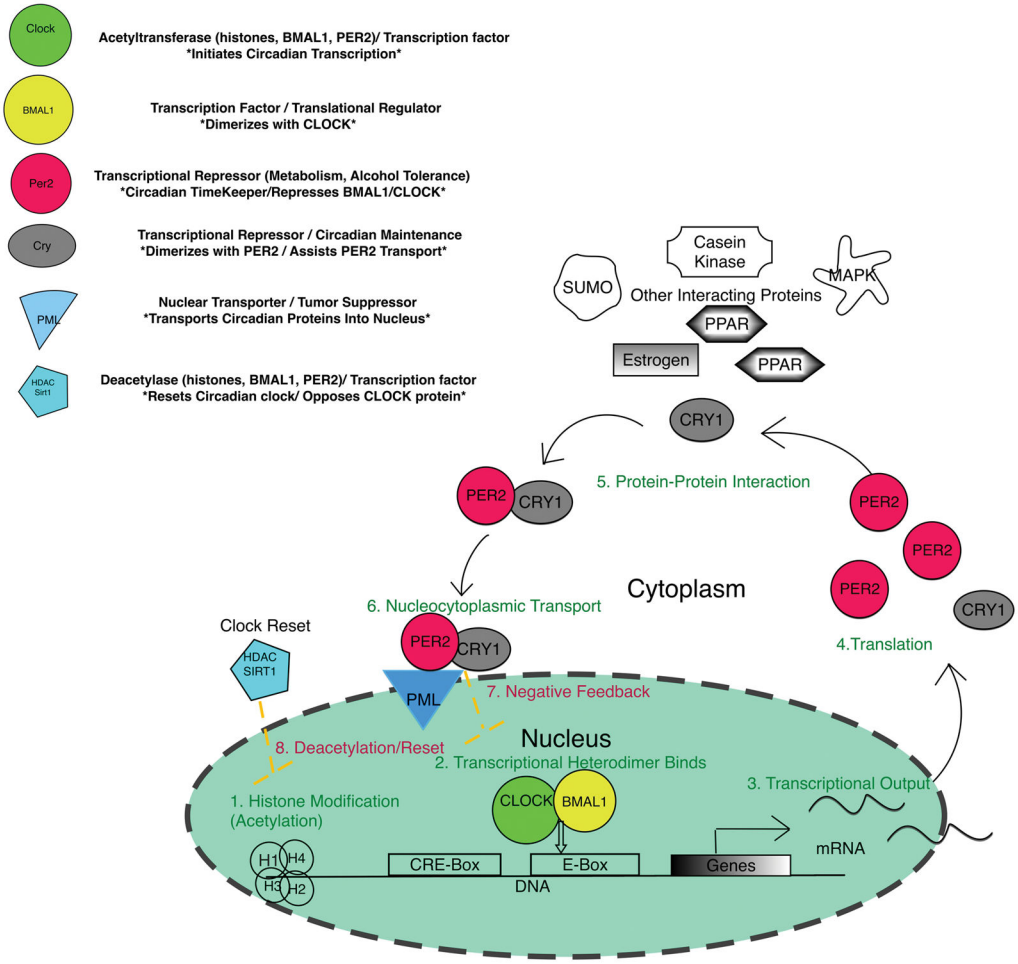


**Figure 1.**

Main Protein Components of Core Circadian Clock

The core circadian clock is a transcriptional-translational feedback loop that is modulated Circadian rhythms are generated by intracellular transcriptional-transcriptional feedback loops that temporally synchronizes or separates biological processes through interaction, modification, and trafficking of proteins.

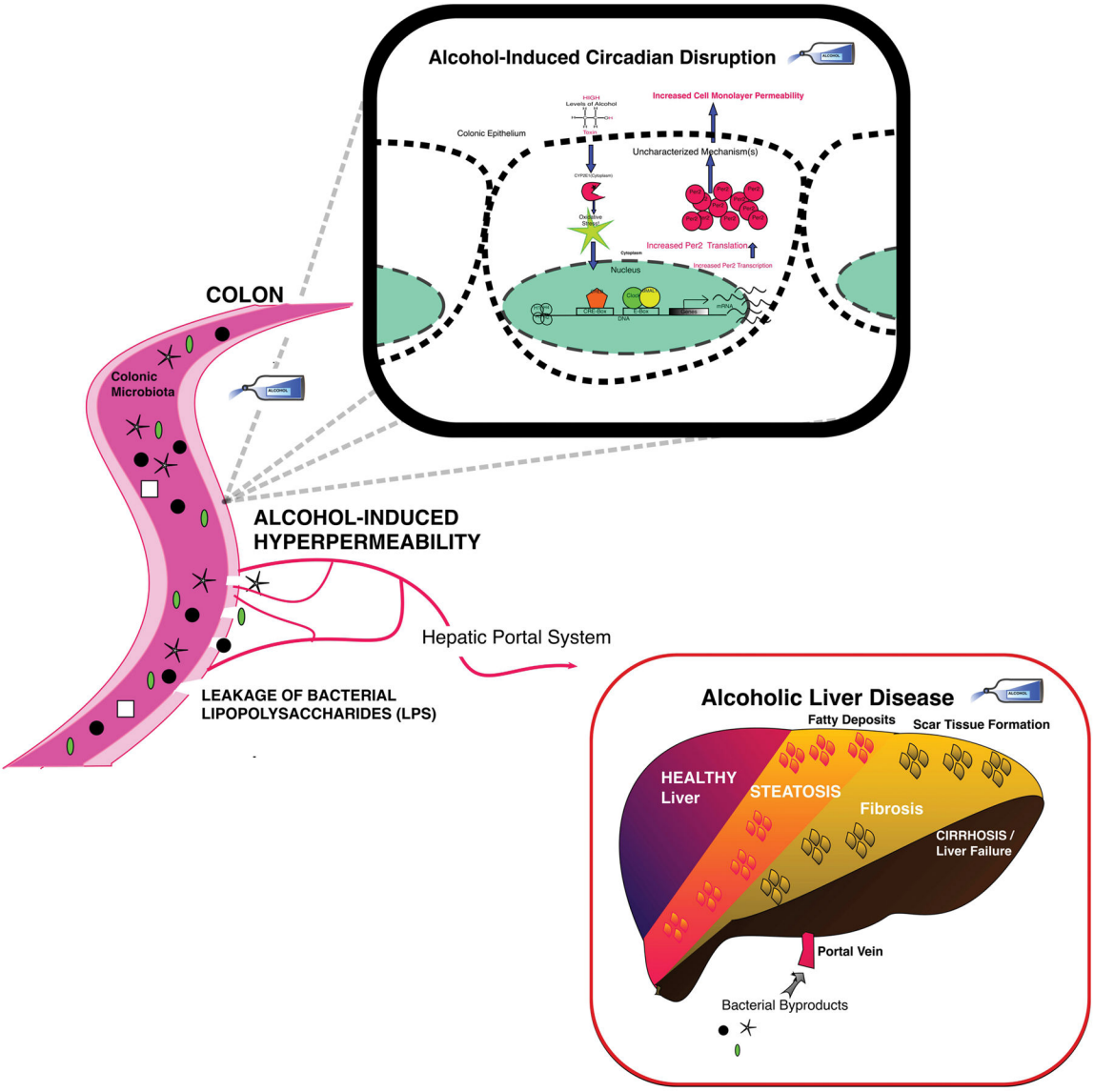


**Figure 2.**

Role of Core Circadian Clock in Alcohol-Induced Pathology: Alcohol mediates disruption of circadian rhythm mechanism(s) inducing the increased intestinal permeability that is critical to the development and progression of alcoholic liver disease.