Critique of 3 related research article topics research paper

Environment, Disaster

Posttranslational Mechanisms Regulate the Mammalian Circadian Clock

The issue of interest in these three research topics is the Circadian Clock. The first article, ‘ Posttranslational Mechanisms Regulate the Mammalian Circadian Clock’ examines the posttranslational regulation of this clock in the liver of a mouse. The study published in this article used the mPER1 and the mPER2 proteins, the BMAL1 and the CLOCK proteins to show their significance in the stabilization of phosphorylated mPER2 proteins by the mCRYs. This article shows the Circadian rhythms to be greatly influenced by the biological clocks in the mammalian bodies. The timing system in mammals for example, is shown to be organized in a hierarchical form, with the central clock situated in the anterior hypothalamus’ SCN, suprachaimatic nuclei (Lee, et al., 2001).

Materials and Methods

This research focused its attention on the liver of the mouse because it is only in this tissue that the temporal changes in the clock protein activities can be analyzed and monitored with ease in vivo. The study first used the RNase protection assay to evaluate the levels of the clock genes in the liver of the mouse. The study also had to generate specific antisera against some fragments of fusion proteins so as to characterize biochemically the clock proteins. The fusion protein fragments were derived from BMAL1, mPER1, mPER2, CKIe, and CLOCK. The mCRY1 and mCRY2 were examined by the use of some antisera that are commercially available. The researchers also made sure that each antiserum’s specificity was properly. Through the incubation

of the extracts of the liver with the antisera from the clock proteins, and by probing the immune complexes that resulted by Western Blot analysis, the study was able to examine and analyze protein interactions in vivo. To obtain a more refined view of the interaction between the binding activities of the BMAL1 and CLOCK heterodimers, the researchers combined the immunoprecipitation with the subcelllular fractionation. The nuclear extracts and the cytoplasmic were then treated with antibody and the immune complexes probed. The chromatin cross linked with the formaldehyde derived from the nuclear of the liver was Western Blotted for the purposes of examining whether the CLOCK phosphorylation had altered its capability to be bound to DNA (Lee, et al., 2001).

Methods and Results

The study made use of several experimental procedures common in the scientific field. Some of these included animals and collections, antibodies, Western Blotting, immunoprecipitation and phosphate treatment, preparation of cytoplasmic extracts and nuclear from the liver, preparation of the cross linked chromatin, quantization of the in vivo abundance of clock proteins, in vivo translation, and RNase protection assay. The study was wide and as a result uncovered numerous important attributes of the regulation of clock proteins in mammals. Some of the results for example, implicated that temporal changes in clock proteins interactions, phosphorylation, and subcelllular localization, play a very significant role in the maintaining of the mammalian Circadian Clock in a functional condition. The study also obtained results that other studies had not yet achieved and therefore,

helped in adding to the wealth of knowledge already available on the subject. An example of this new knowledge the study came up with was the realization that the mPER proteins play an important role in the body as rate limiters for the interactions between the mPER and the mCRY. This as the research found out, is an interaction very important in translocation of the complex to the nuclear (Lee, et al., 2001).

Intercellular Coupling Confers Robustness against Mutations in the SCN Circadian Clock Network

This study used bioluminescence imaging to analyze and evaluate the expression of Per2 in clock cells and tissues derived from a mutant mouse. This method was exceptionally different from the other methods other studies in the field use. Most of these researches utilize behavioral analysis and genetic perturbations to study the molecular mechanisms of the circadian clock in mammals. Just like the previous study, this study pointed out that the circadian clock system in a mammalian body is organized in a hierarchy a number of oscillators. It was because of the hierarchical nature of the clock system that this study decided to use the bioluminescence imaging technique, because other technique like behavioral analysis and genetic perturbation do not take into consideration this attribute of the circadian clock system (Liu, et al., 2007).

Materials and Methods

The study crossed Cry and Per knockout mice proteins with the homozygous reporter knockout proteins and the mPer2 Loc reporter line proteins. The researchers also measured the rhythms of the tissue autonomous mPer2Loc

proteins and compared the resulting molecular oscillations with the patterns made by the locomotor activity. In addition to this, the researchers wanted to examine and analyze the oscillators emitted by the peripheral circadian without the presence of possible intercellular synchronization. To achieve this, the group of researchers assessed the effects of deletions on the clock genes in cultures made up of primary fibroblasts that were already dissociated. They also used quantitative PCR to measure and evaluate the rhythms of mRNA of the Per2, Bmal1, and Dbp in the cultures of fibroblasts made from Cry2, Cry1, and WT (Liu, et al., 2007).

To complement the observations that the researchers had done in vivo, mathematical simulations were also used to further find out the importance of coupling. To do this, the study modified a computational clock model that was already published to be used in reproducing the behavior of the individual Per1 that was largely arithmetic, and neurons through the introduction of disturbance in terms of noise to the degradation rate of the Bma1, and also to the threshold of activation of nuclear BMA1 on the transcription of Cry1 (Liu, et al., 2007).

Methods and Results

Just like the previous study, this survey also utilized a number of scientific study methods to make the research feasible. Some of the se included supplemental data, animals and behavioral analysis, bioluminescence recording, data analysis, explants and cell a culture, single cell imaging and data analysis, and single cell imaging. It is through these scientific study methods that the researchers were able to come up with valid and reliable

results. Some of the results of the study included the finding that Cry1 and Per1 are very essential in the mammalian cells and tissues for the purposes of maintaining sustained rhythms. They are also required for the same function in the neurons that have been dissociated from the SCN, or the suprachiasmatic nucleus. The study also discovered that Per2 is also important in maintaining these rhythms; and that deficiencies in Cry2 and Per3 can result to defects that can last for long periods of time. The study however, found out that the interactions resulting from the oscillator networks in the SCN can be used as a form of compensation for the deficiencies in Cry1 and Per1. This can in turn preserve the very much needed sustained rhythmicity in the behavior and slices of mutant SCN. The study thus concluded that behavior cannot be used as the sole reflection or signal of clock phenotypes, with autonomy attribute (Liu, et al., 2007).

Delay in Feedback Repression by Cryptochrome 1 is Required for Circadian Clock Function

This study was launched in an attempt to add knowledge into the already limited one on the significance and the role of delay in the mammalian circadian clock feedback repression. The study therefore tries to reveal the evening time expression can be produced by a combination of night time elements, also RREs, together with its intronic enhancer and the day time elements, also known as D box, together with its Cry1 proximal promoter. The study argues that circadian clocks consist loops, autoregulatory ones, which possess the attribute of translational or transcriptional feedback repression, in which expression of the components of the clocks that is

delayed is very essential for the maintenance of the circadian rhythmicity (Ukai- Tadenuma, et al., 2011).

Materials and Methods

In this study, the researchers created a reporter construct for the purposes of examining the activities of the Cry1 promoter. This construct was P (Cry1)-Luc. In this construct, the 1. 5kbp fragment of DNA that was carrying the Cry1 promoter was conjoined to the Luc, or the Luciferase. The group also investigated the sequences of the genome of the Cry1 promoter and found the highly conserved regions, which were five in total, two of which closely resembled the sequence of the D Box, as per the analysis made by the position weight matrix. In addition to the above, the researchers also fused each promoter with a reporter gene from the Luciferase and transiently transfected them into some of the available NIH 3T3 cells. The bioluminescence's time series was also recorded in real time through the utilization of the PMT, or the photomultiplier tube (Ukai- Tadenuma, et al., 2011).

The chromatin immunoprecipitation assays, or the ChIP assays, were used to analyze the regulatory regions. This analysis was carried out so as to look for presence of the corresponding factors of transcription in vivo. The study also used the generation of intron sequence arrays harboring RREs that were mutant, including mutations such as inversion, mutation, and deletions, so as to determine the strength and reliability of the RREs of the first intron sequence of the Cry1. This was done by inserting the mutant RRE intron

sequences into a vector, P (SV40) -Luc, so as to come up with an array of introns of P (SV40) –Cry1 (Ukai- Tadenuma, et al., 2011).

Methods and Results

Just like the other two studies, this study used several scientific experimental designs, though fewer than the ones used by the previous studies. These experimental methods included preparation of the fibroblasts from the embryos' Cry1, and Cry2 from a mouse that was double knocked, real time circadian reporter assay that used NIH 3T3 cells as well as from Cry1 and Cry2 cells. The other scientific experimental procedure that the research utilized was deriving evidence and additional data from supplemental information. The study derived numerous results from the study. Some of these included the finding that coordination of the night time elements with the day time element in a mammal can result into a modulation of the length of phase delay. The experiment also found out that a significant delay of the expression of the Cry1 is important in maintenance and restoration of the rhythmicity of the circadian clock. Delayed expression was found to be an indication of slowed oscillation of the circadian system (Ukai- Tadenuma, et al., 2011).

Discussion

These three studies were conducted in a very scientific manner, something that suggests that they are both accurate and reliable to some extend. The three studies also were very effective in expanding the available knowledge on the subject of circadian clock and its system. The first study however, was especially significant as it added a new knowledge on the available

knowledge rather that just expounding on the available knowledge like the other two studies did. The third study was specifically thorough in expounding this knowledge without including any new information that the study had established. For example, Delay in Feedback Repression by Cryptochrome 1 is Required for Circadian Clock Function study acknowledge that there was indeed a gap or a need for further research on the subject as the available one was limited, but it had little to cover this gap, as it did not address the part of the issue that was absent from the published studies, but the part that was already published. A good researcher would have found this gap and contacted a study to cover this gap with new findings through research.

The three studies also were very efficient and effective in including various references and citing accordingly, other studies and texts they had used in their researches. Such documentation shows that the researches can be believed because they are based on information that has already established. The use of identified and published scientific experimental procedures, also added an attribute of reliability on the findings of these studies. All in all, the three studies were sufficiently informative and therefore, can be said to be good and reliable sources of information.

References

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