Identification and Analysis of Homo sapiens and Mus Musculus *Cry 1* Gene

Shahid Raza, Hira Mubeen
Department of Biotechnology, University of South Asia, Lahore, Pakistan
Corresponding author:mianrs@yahoo.com

Abstract: Circadian clocks are dependent on multiple transcribed genes which control gene expression levels. These genes belongs to cryptochrome family and mainly includes Cry 1 and Cry 2 genes and also specialized falvoproteins. Cry1 gene was found to play an important role in maintaining energy balance under various nutritional circumstances. The circadian system is responsible for regulating a wide variety of physiological and behavioral rhythms. The analysis of these genes predicted regions of high similarity among both selected homo sapiens and mouse Cry 1 gene. The results were obtained by using BLAST tool. Phylogenetic analysis of Cry 1 gene was performed by CLUSTA W alignment software. Protein domains were analyzed by using Interpro domain repository. Therefore, the analysis through bioinformatics tools can be useful to infer 3D structure and function of proteins.

***Index Terms*:** flavorproteins, cryptochrome, BLAST, Interpro

I. INTRODUCTION

Cryptochromes belongs to class of specialized proteins called as flavoproteins that are much sensitive to blue light. Cryptochromes are mainly involved in the circadian rhythms of both plants and animals. Various number of different genes are present that code for cryptochrome proteins. Circadian clock functions to control the timing of cell division. According to previous studies, the circadian clock limits the amount of DNA damage during daytime. The circadian clock actively participates in the DNA damage mechanism. Several proteins functions in this process. Mainly, two proteins known to be involved in the circadian clock *Cryptochrome 1* and *2*. They can help in protecting the integrity of genetic information. Studies shown, these proteins evolved from light-activated enzymes that are involed in bacterial DNA repair. On the other side, mammalian cryptochromes have lost their ability to repair DNA.

Two genes are reported namely, *Cry1* and *Cry2* that code for the two cryptochrome proteins CRY1 and CRY2. In plants and insects, CRY1 plays an important role to regulate the circadian clock in a light-dependent fashion. In contrast, mammals CRY1 and CRY2 genes act as light-independent inhibitors of CLOCK-BMAL1 components of the circadian clock. Transcription factors helps Cry1 and Cry2 to associate with chromatin. They can also interact directly with DNA. Several light activated enzymes are involved in repair mechanism. Cry1 and Cry2 evolved from prokaryotic light-activated DNA repair enzymes such as photolyases.

But it was observed as they have lost the photolyase catalytic activity characteristic of their ancestral homologs [1]. The three-dimensional structures of Cry1 and Cry2 resemble those of photolyases, including the DNA binding surfaces[2-4].Therefore, the main properties suggest that Cry1 and Cry2 could retain an important role in responding to damaged DNA. Such conservation of function by divergent molecular mechanisms has been seen previously between cryptochromes derived from different species [5-7].

Cryptochrome stability is also a critical fact of circadian period length. It is reported that the direction and magnitude of the period change associated with altered expression levels of Cry1 and Cry2 seems to depend on the mechanism and context of altered [8-10]. The CRYs belong to the cryptochrome superfamily of flavoproteins. All CRYs from different species share a highly conserved core domain at the N terminus, the photolyase homology region (PHR), whereas the C-terminal tail domain (CTD) [11-12]. Despite similarity in sequence and domain structures, these flavoproteins play diverse biological roles. Bacterial photolyases can act as DNA repair enzymes upon activation [13]. In eukaryotes, the CRYs was not found to exhibit photolyase activity. Many studies predicted the role of CRYs in plants which functions to mediate phototropism, growth, and development [14-15]. Also, the *Drosophila dCry* is directly involved in the light input pathway for circadian clock entrainment [16-17]. In comparison, the mammalian CRYs are neither photolyases nor photoreceptors which mainly function as light-independent transcriptional repressors [18-19]. Similarly, mouse CRY gene was shown to exhibit repressor function in certain peripheral tissues [20-21]. The study was performed to analyze the Cry 1 gene, its homology with other variants and to predict conserved domains by using various computational biology tools.

II. MATERIAL AND METHODS

HGNC reported symbol for human cry 1 Gene

The human cry 1 gene was searched in Hugo Gene Nomenclature Committee.

Sequence retrieval of human cry 1 gene

Sequence of cry 1 gene was retrieved from NCBI-Genbank data repository.

Chromosomal Map Analysis

Chromosomal Map of the selected human and mouse cry 1 genes were analyzed by using Ensemble software.

Sequence Alignments

Sequences of both genes were aligned by using NCBI BLAST tool.

Homology Matching

Homology searching shows the predicted homologs of cry 1 gene.

Domain Analysis

The putative conserved domain of human cry 1 gene was found by using InterPro data repository.

Phylogenetic Analysis

The phylogenetic was performed by using T-Coffee CLUSTAL W tool.

III. RESULTS

The cry 1 gene analysis shows its various homologs in mouse and rat. The alignment results shown the region of high similarity which helps to identify various protein domains and also to study their evolutionary relationships.

HGNC reported symbol for human cry 1 Gene

The Human genome nomenclature indicates the approved symbol as cryptochrome circadian clock 1 and approved symbol as CRY1.

Chromosomal Map Analysis

Chromosomal Map of the selected human and mouse cry 1 genes were analyzed by using Ensemble software. The gene was found to be located at chromosome no 12.

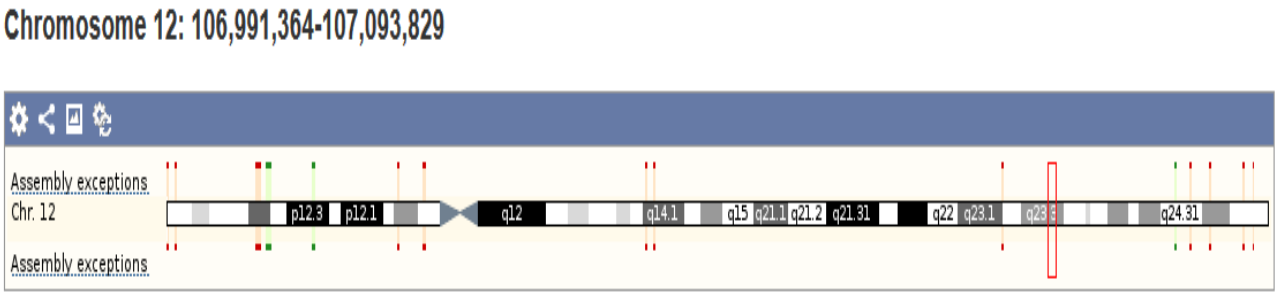

Fig 1: Chromosomal Map of Cry 1 gene.

Sequence retrieval of human cry 1 gene

Sequence of cry 1 gene was retrieved from NCBI-Genbank data repository. The accession number is gi|22539646|gb|BC030519.1| Homo sapiens cryptochrome 1 (photolyase-like), mRNA (cDNA clone MGC:40475 IMAGE:5169081), complete cds.

Sequence Alignments

Sequences of both genes were aligned by using NCBI BLAST tool. The results shown region of high similarity by matching query sequence with the subject sequence.

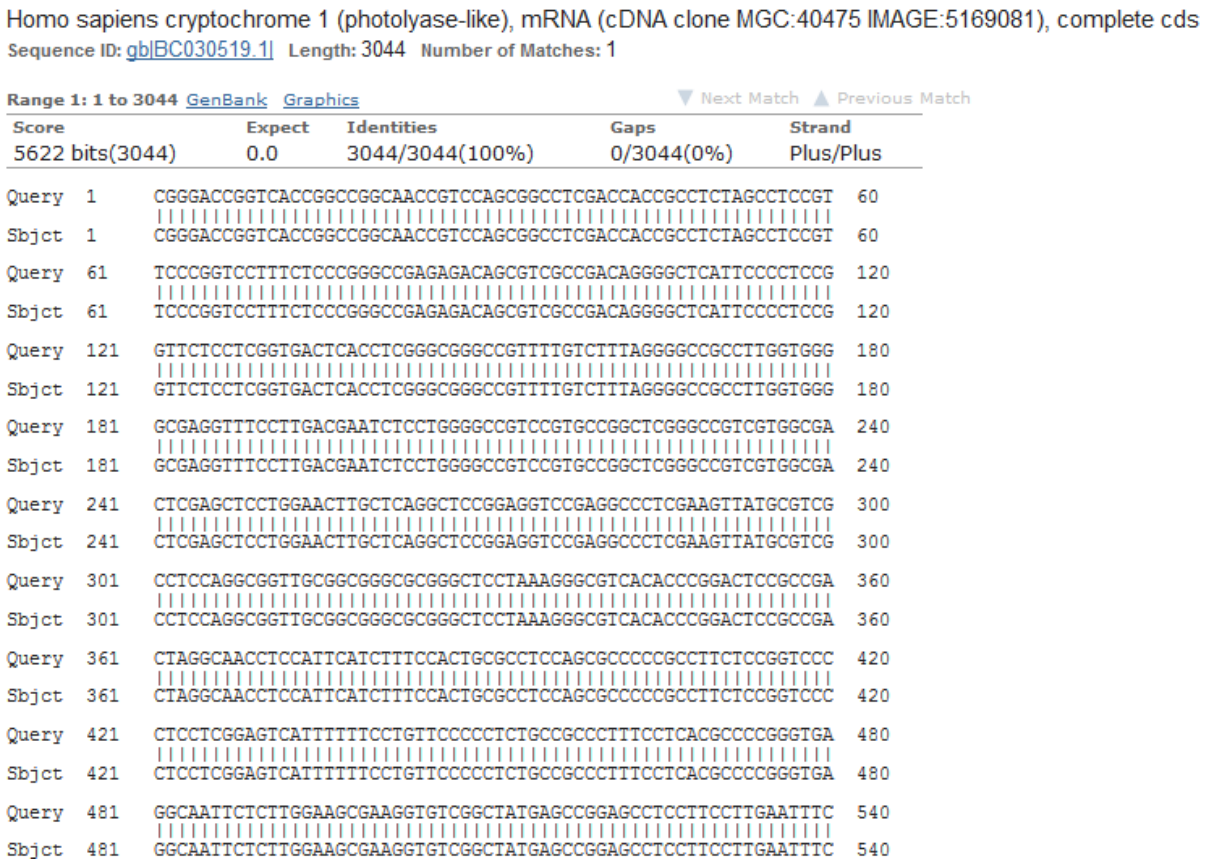

Fig 2: BLAST similarity results of selected human and mouse Cry 1 genes.

Homology Matching

Homology searching shows the predicted homologs of cry 1 gene.

Homologs

The two homologs of CRY 1 gene was found. One in mouse genome and other in Rat genome. The predicted result are shown in the table below.

Table 1: Homologs of Cry 1 Gene

Organism	Symbol	Database
Mus musculus	Cry1	MGI:1270841 C
Rattus norvegicus	Cry1	RGD:735083 D

Domain Analysis

The putative conserved domain of human cry 1 gene was found by using InterPro data repository.

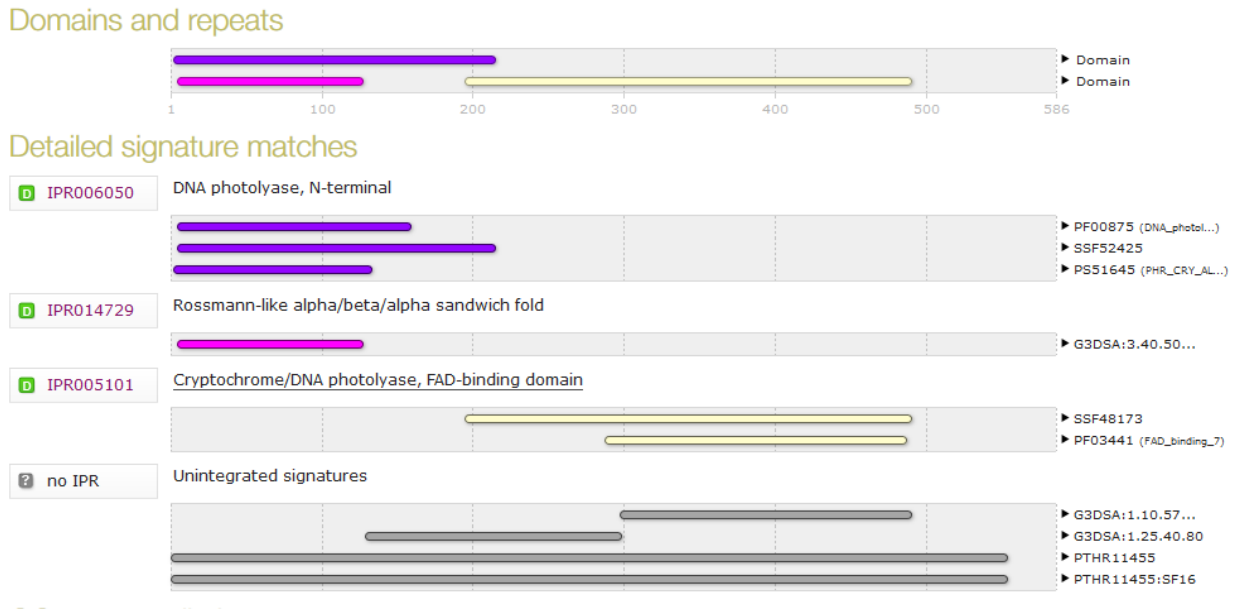

Fig 3: Interpro results for Protein domains of homo sapiens Cry 1 gene

Mouse Cry 1 gene Sequence Retrieval

The sequence for mouse cry 1 gene was obtained with accession Id gi|372099100:c85185054-85131700 Mus musculus strain C57BL/6J chromosome 10, GRCm38.p3 C57BL/6J

Mouse Cry 1 Gene Chromosomal Map Analysis

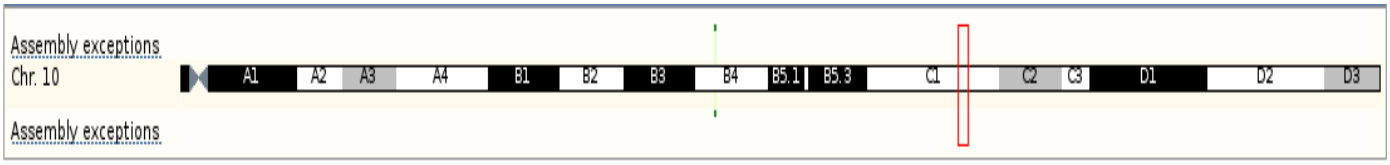

Fig 4: Chromosomal Map for mouse Cry 1 gene.

Phylogenetic Analysis

The phylogenetic analysis of both human and mouse cry 1 gene was performed by using T-Coffee CLUSTAL W software. The study predicted their evolutionary lineage with common ancestors.

Fig 5: Phylogenetic tree for human and mouse Cry 1 gene obtained through T-Coffee CLUSTAL W.

IV. Discussion and Conclusion

Cryptochromes functions as a receptor for blue and ultraviolet (UV-A) light that share sequence similarity to certain enzymes like DNA photolyases. Studies shown cryptochromes have no photolyase activity. Moreover, they are widely distributed in bacteria and eukaryotes but are not found in archaea. The first cryptochrome gene to be identified was *Arabidopsis CRY1*. Homology studies shown similarities in many plants and animals. It was observed from previous reports that animal cryptochromes act as components of the circadian clock that and as photoreceptors that mediate entrainment of the circadian clock to light. The circadian clock is important to regulate various physiological processes and circadian rhythms in different gene expression levels. Its basic composition is based on

two components: the central clock located in the brain and the peripheral clocks which is present in every tissue and organ system. Circadian rhythms allow an organism to achieve temporal homeostasis at molecular level by regulating the gene expression. Both Cry1 and Cry 2 are involved in performing different functions. The analysis of homo sapiens and mouse cry 1 gene shows similarity regions in both gene sequences. This infer the evolutionary relationships among both species. Also, the domain analysis shown many conserved regions. Hence, the identification, analysis and interpretation of these cry 1 gene and protein domain will help to study the structure, function and to overcome all related problems.

References

1. Ozturk N, Song SH, Ozgur S, Selby CP, Morrison L, Partch C, Zhong D, Sancar A. Structure and function of animal cryptochromes. Cold Spring Harbor Symposia on Quantitative Biology. 2007;72:119–131. doi: 10.1101/sqb.2007.72.015.
2. Maul MJ, Barends TR, Glas AF, Cryle MJ, Domratcheva T, Schneider S, Schlichting I, Carell T. Crystal structure and mechanism of a DNA (6-4) photolyase. Angewandte Chemie. 2008;47:10076–10080. doi: 10.1002/anie.200804268.
3. Czarna A, Berndt A, Singh HR, Grudziecki A, Ladurner AG, Timinszky G, Kramer A, Wolf E. Structures of *Drosophila cryptochrome* and mouse cryptochrome1 provide insight into circadian function. Cell. 2013;153:1394–1405. doi: 10.1016/j.cell.2013.05.011.
4. Xing W, Busino L, Hinds TR, Marionni ST, Saifee NH, Bush MF, Pagano M, Zheng N. SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. Nature. 2013;496:64–68. doi: 10.1038/nature11964.
5. Yuan Q, Metterville D, Briscoe AD, Reppert SM. Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. Molecular Biology and Evolution. 2007;24:948–955. doi: 10.1093/molbev/msm011.
6. Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB, Evans RM. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science. 2009;326:437–440. doi: 10.1126/science.1172156.
7. Kim YM, Choi J, Lee HY, Lee GW, Lee YH, Choi D. dbCRY: a Web-based comparative and evolutionary genomics platform for blue-light receptors. Database. 2014;2014:bau037. doi: 10.1093/database/bau037.
8. Vitaterna MH, Selby CP, Todo T, Niwa H, Thompson C, Fruechte EM, Hitomi K, Thresher RJ, Ishikawa T, Miyazaki J, Takahashi JS, Sancar A. Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. Proceedings of the National Academy of Sciences of USA. 1999;96:12114–12119. doi: 10.1073/pnas.96.21.12114.
9. Hirota T, Lee JW, St John PC, Sawa M, Iwaisako K, Noguchi T, Pongsawakul PY, Sonntag T, Welsh DK, Brenner DA, Doyle FJ, III, Schultz PG, Kay SA. Identification of small molecule activators of cryptochrome. Science. 2012;337:1094–1097. doi: 10.1126/science.1223710.
10. St John PC, Hirota T, Kay SA, Doyle FJ., III Spatiotemporal separation of PER and CRY posttranslational regulation in the mammalian circadian clock. Proceedings of the National Academy of Sciences of USA. 2014;111:2040–2045. doi: 10.1073/pnas.1323618111.
11. Lin C., Todo T. (2005) The cryptochromes. Genome Biol. 6, 220.
12. Oztürk N., Song S. H., Ozgür S., Selby C. P., Morrison L., Partch C., Zhong D., Sancar A. (2007) Structure and function of animal cryptochromes. Cold Spring Harbor Symp. Quant. Biol. 72, 119–131.
13. Sancar A. (2004) Regulation of the mammalian circadian clock by cryptochrome. J. Biol. Chem. 279, 34079–34082.
14. Ahmad M., Cashmore A. R. (1993) *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366, 162–166.
15. Cashmore A. R. (2003) Cryptochromes. Enabling plants and animals to determine circadian time. Cell 114, 537–543.
16. Emery P., So W. V., Kaneko M., Hall J. C., Rosbash M. (1998) CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669–679.
17. Stanewsky R., Kaneko M., Emery P., Beretta B., Wager-Smith K., Kay S. A., Rosbash M., Hall J. C. (1998) The *cryb* mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. Cell 95, 681–692.
18. Kume K., Zylka M. J., Sriram S., Shearman L. P., Weaver D. R., Jin X., Maywood E. S., Hastings M. H., Reppert S. M. (1999) mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.
19. Griffin E. A., Jr., Staknis D., Weitz C. J. (1999) Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. Science 286, 768–771.
20. Krishnan B., Levine J. D., Lynch M. K., Dowse H. B., Funes P., Hall J. C., Hardin P. E., Dryer S. E. (2001) A new role for cryptochrome in a *Drosophila* circadian oscillator. Nature 411, 313–317.
21. Collins B., Mazzoni E. O., Stanewsky R., Blau J. (2006) *Drosophila* CRYPTOCHROME is a circadian transcriptional repressor. Curr. Biol. 16, 441–449.