課題番号（Project number） 23

Identification of new binding partners of mammalian cryptochromes

［1］組織（Research group）
代表者（Principal Investigator (PI)）：
Ines Chaves
(Erasmus University Medical Center)
対応者（Host researcher at IDAC）：
Akira Yasui
(IDAC, Tohoku University)
分担者（Co-investigator）：

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物件費 196,000 YEN，旅費 0 YEN

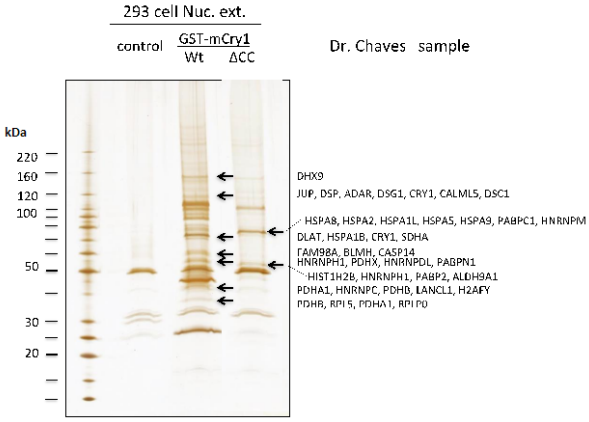
［2］研究経過 (Research setup) The current proposal is part of a multidisciplinary effort to further define the function of mCRY in circadian rhythm regulation by identifying novel binding partners. In previous studies we have done structure/function analysis of mCRY1, and have identified important functional domains (Chaves et al. 2006). One such domain is a predicted C-terminal coiled-coil domain (CC), which is required for the interaction with mPER and mBMAL1. A deletion mutant of mCRY1 lacking this domain (mCRY-dCC) is impaired in inhibiting transcription activation by the CLOCK/BMAL1 heterodimer, but the phenotype of a knock-in mouse carrying this mutation is different from the full knock-out. This is likely due to an additional function of mCRY1 outside the circadian transcription feedback-loop, which remains intact in the mutant protein. By identifying new mCRY1 binding partners we expect to further define additional functions of mammalian cryptochromes..

Host researcher, Dr. Akira Yasui, visited us in Rotterdam in September ’16 and we discussed our collaborative research. Research cost was used to determine the interacting proteins of GST-mCry1 wt and GST-mCry1 ΔCC, lacking the C-terminal sequence (see figure in (3-1)).

［3］成果（Research outcomes）

（3－1）研究成果（Results）

In order to identify novel mCRY1 binding partners, mCRY1 and mCRY1-dCC were produced and incubated with HEK cell extracts. Identification of interacting proteins was done using mass spectrometry. The focus was on unique binding partners, that specifically interacted with either mCRY1 or mCRY1-dCC. The proteins that specifically bind to mCRY1 can be subdivided in two groups: on the one hand we identified proteins belonging to the desmosome complex, and on the other hand mitochondrial proteins belonging to the Krebs cycle and complex II of the respiratory chain. These findings are very interesting and nicely correlate to other results we obtained recently.



（3－2）波及効果と発展性など（Future perspectives）

Having identified two new categories of mCRY1 binding partners, we plan to further characterise these interactions. A first step will be to determine whether mCRY1-dCC really does not interact with these proteins, suggesting that the interactions occur via the CC domain. Follow-up experiments will be performed to determine whether mCRY1 mutation or deficiency functionally affects the function of mitochondria and desmosomes.

［4］成果資料（List of Papers）

in preparation