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Roles of Calcium in the Entrainment of Mammalian Circadian Clock Gene, *per1*

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Although there are substantial evidences that glutamate or NMDA mimics the effects of light on the mammalian circadian clock *in vivo*, the molecular mechanism by which clock is entrained to light/dark cycle is unknown mainly due to the lack of *in vitro* model. The present study is designed to set up an *in vitro* model for the photic entrainment of circadian clock gene expression. High concentration of serum induced circadian expression of hPer1 mRNA, a major component of circadian clock, in human neuroblastoma SK-N-SH cells. SK-N-SH cells expressed NMDAR1 and NMDAR2B mRNA and NMDAR1 receptor proteins. Glutamate (100 mM) induced nuclear translocation of mPER1 proteins. However, NMDA was unable to modulate hPer1 mRNA levels when treated at valley (6 hr) or peak (12 hr) of hPer1 oscillation curve. Interestingly, NMDA (100 mM) increased intracellular calcium level. In addition, ionophore A23187 (2 mM) significantly increased hPer1 mRNA levels. This result indicates that extracelluar calcium influx appears to be critical in the regulation of hPer1 expression *in vitro* in SK-N-SH neuroblastoma cells.

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SRp30c Interacts with Tra2a in the GnRH RNA Splicing

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Recently, we found that Tra2a binds to the exonic splicing enhancer located in the exon4 (ESE4), which mediates the enhanced RNA splicing. Although Tra2a is an important splicing factor, it may not work alone, but may need some other serine/arginine-rich(SR) protein(s) in enhancing GnRH RNA splicing. In an attempt to isolate putative SR protein(s) which interacts with Tra2a, we screened the mouse GT1 cDNA library by yeast two hybrid system using Tra2a as a bait. We isolated one of the SR proteins, mouse SRp30c which is about 30 kDa in molecular weight. Sequence analysis indicates that mouse SRp30c has a short C-terminal RS domain and two N-terminal RNA recognition motifs, which is mostly identical to human SRp30c. In order to verify the interactions between Tra2a and Srp30c, several criteria were employed. First, SRp30c and Tra2a were co-localized in the nucleus when transfected into CHO cells. Second, SRp30c was expressed in the Baculovirus system and purified. In vitro GST pull-down assay revealed that Tra2a and SRp30c were co-precipitated. These data clearly indicate the interaction between Tra2a and SRp30c. We are in progress of elucidation of the functional role of SRp30c in GnRH RNA splicing.

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Functional Importance of Exonic Splicing Enhancers in Excision of the First Intron of the GnRH Primary Transcripts as Revealed in Hypogonadal (hpg) Mice

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Recently we demonstrated that exonic splicing enhancers (ESEs) located in the gonadotropin-releasing hormone (GnRH) exons 3 and 4, were involved in enhanced excision of the first intron (intron A) of the GnRH gene. To elucidate the functional importance of the ESEs in excision of the intron A from the GnRH primary transcripts, we employed hypogonadal (hpg) mutant mice. Hpg mice lack the ability to synthesize the GnRH peptide due to a deletion of two exons (exons 3 and 4) in the GnRH gene. Intron A excision rate of GnRH pre-mRNA was examined by ribonuclease protection assay and competitive reverse transcription-polymerase chain reaction (RT-PCR) with three RNA samples from the preoptic area (POA) where the majority of GnRH-synthesizing cell bodies and GnRH mRNA are concentrated, olfactory bulb (OB), and cerebral cortex (CTX) derived from the adult hpg (hpg/hpg), heterozygous (hpg/+), and normal (+/+) mice. Intron A excision rate in the POA of hpg mice was severely lower than that of normal and heterozygous mice but similar to that in other tissues such as CTX and olfactory bulb. In contrast, the level of GnRH pre-mRNA containing intron A in hpg mice was not significantly different from that of heterozygous and normal mice. It appears that ESEs are important for excision of intron A of the GnRH primary transcripts.

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First Intron Excision of Gonadotropin-Releasing Hormone Pre-mRNA during Postnatal Development of Normal Mice

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Gonadotropin-Releasing Hormone (GnRH) is a hypothalamic decapeptide that plays a pivotal role in the neuroendocrine regulation of pituitary functions, and GnRH gene is consisted of four exons and three introns. We recently found that intron A excision appears a rate limiting step for GnRH pre-mRNA splicing. In the present study we explored whether the splicing rate of GnRH pre-mRNA is developmentally regulated. The preoptic area (POA) where the majority of GnRH synthesizing cell bodies and GnRH mRNA are concentrated and cerebral cortex (CTX) as a control were removed from mice at the age of 1 to 7 weeks. GnRH pre-mRNA splicing rate was examined by competitive reverse transcription-polymerase chain reaction (RT-PCR) using a variety of primer sets. An intron A excision rate of GnRH pre-mRNA in the POA was altered during mouse postnatal development: The intron A excision rate in the POA was significantly increased in 3 week-old mice and further increased until adulthood. In contrast, in the CTX, intron A excision rate was extremely low, drastically decreased in 3 week-old mice, and remained at low levels until adulthood. Intron B excision rate in the POA was not significantly changed during development. GnRH mRNA levels in the POA were gradually increased during development as similarly as the intron A excision rate, but pre-mRNA containing intron A (1A23) levels was not significantly changed. It suggests that the alteration in GnRH mRNA levels during development roughly parallel with changes in intron A excision rate. Collectively, the present study indicates that intron A excision rate is developmentally regulated and involved in the function of GnRH neurons.