# Modulation of the circadian clock by glucocorticoid receptor in H295R cell line

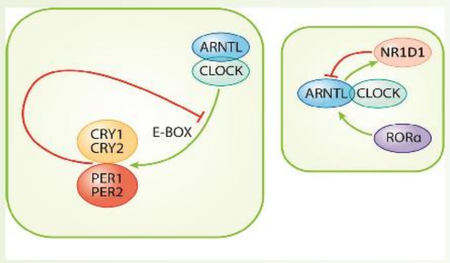
**Zsolt Nagy[1,2], Henriett Butz[3], Istvan Liko[2], Karoly Racz[1,3], Attila Patocs[2,4]**

[1]2nd Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[2]Hungarian Academy of Sciences-Semmelweis University "Lendulet" Hereditary Endocrine Tumors Research Group, Budapest, Hungary
[3] Hungarian Academy of Sciences-Semmelweis University Molecular Medicine Research Group, Budapest, Hungary
[4] Department of Laboratory Medicine Institute, Faculty of Medicine, Semmelweis University, Budapest, Hungary

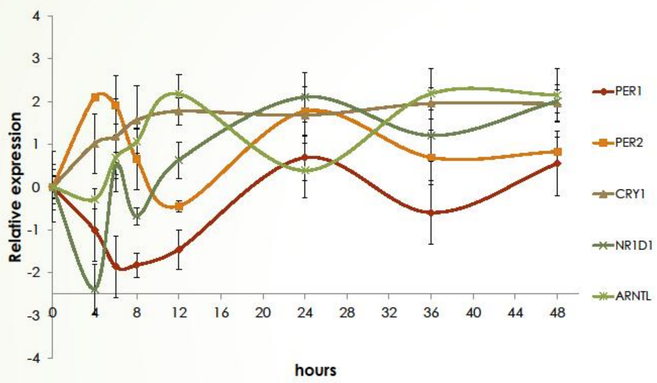
***Background:*** Peripheral clocks are set by different nervous, hormonal and metabolic stimuli and regulate the circadian expression of several genes. It has been demonstrated that circadian oscillation of gene expression can be detected in various cell lines in vitro.

***Aim:*** To explore whether a peripheral clock could be induced in human adrenocortical cell line H295R and what are the effects of glucocorticoids on this clock system.

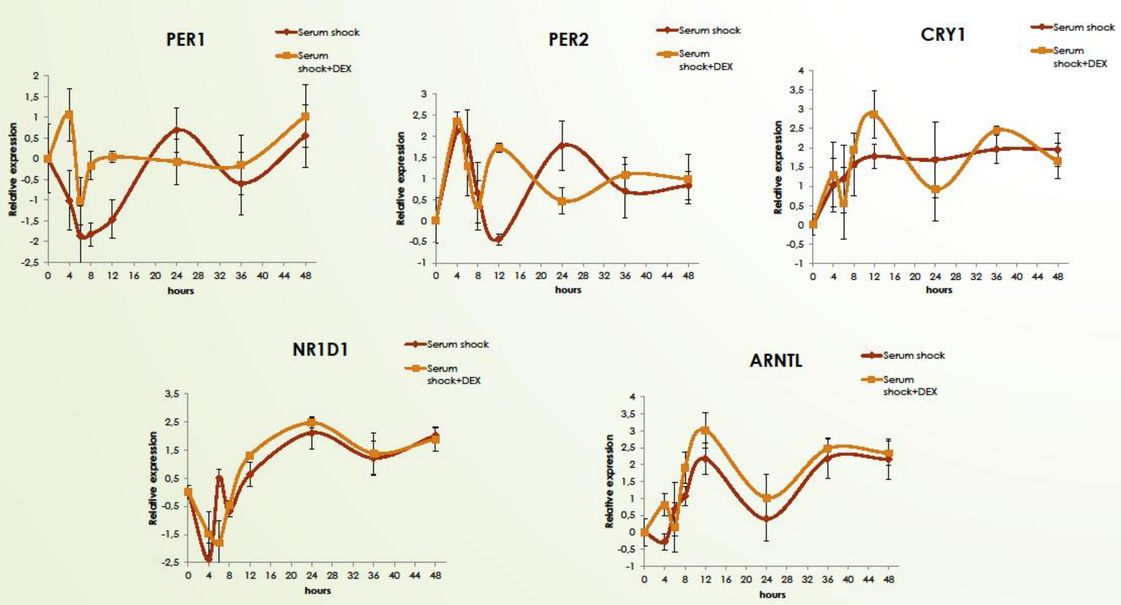
Interactive feed-back loops of the molecular circadian clock machinery



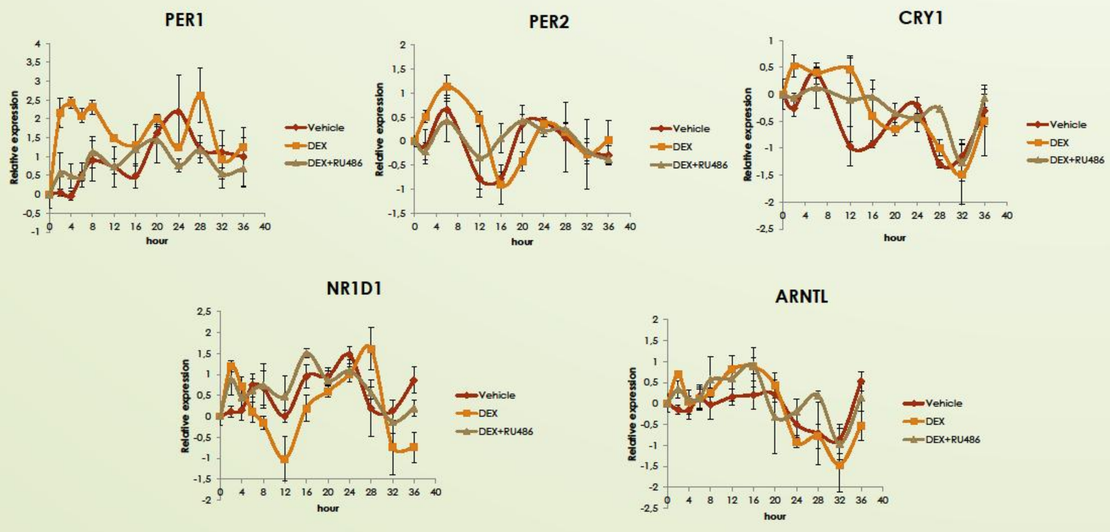

Rhythmic expression of clock genes in H295R cell line upon serum shock treatment




Dex modifies the pattern of serum shock induced clock genes




Dex induces the expression of PER1, PER2, CRY1 and suppresses NR1D1 in a glucocortiocid receptor dependent manner in H295R cell line




***Methods:***

H295R human adrenocortical cell line was studied.

- For serum shock experiments cells were serum starved for 24 h and incubated with 30% Nu Serum for 2h then returned to normal medium with either 0.04% ethanol or 100nmol dexamethasone (Dex).
- For Dex experiments cells were serum starved for 24h, maintained in serum free medium and treated with 0.04% ethanol or 100nmol Dex with or without 1μmol RU486.
- Cells were harvested at the indicated time points. All experiments were carried out in triplicate.
- Total RNA was isolated with miRNeasy Mini Kit (Qiagen) and transcribed with Invitrogen Superscript VILO reverse transcriptase (LifeTechnologies).
- RT-PCR was carried out using predesigned TaqMan Gene Expression Assays. PER1, PER2, CRY1, ARNTL, NR1D1, NR3C1, StAR, POMC, CRH and actin expressions were measured by qRT-PCR on 7500 Fast Real-Time PCR system.

***Results:***

- After synchronization of cells the **rhythmic oscillation of clock genes *PER1*, *PER2*, *NR1D1*, and *ARNTL*** was observed.
- **Glucocorticoid** treatment **induced** a rapid respond of **PER1 in a glucocorticoid receptor (GR)-dependent manner.** Continuous glucocorticoid stimulation caused elevation of PER1 and altered its rhythm.
- **Glucocorticoid** treatment **induced** the expression of **PER2 and delayed its phase**, **increased** expression of **CRY1** and, after 6 hours, it **suppressed** expression of **NR1D1**.
- Administration of a glucocorticoid receptor antagonist, **RU486 disrupted the circadian oscillation of clock genes** and prevented the acute changes in PER1, PER2 and CRY1 levels.
- These alterations occurred independently from ACTH or CRH.

**Conclusions: Our data demonstrated that a peripheral clock system is present in human adrenocortical cell line and periodic oscillation of clock genes are influenced by glucocorticoids.**

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