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Review

The liver of goldfish as a component of the circadian system: Integrating a network of signals

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ABSTRACT

The circadian system drives daily physiological and behavioral rhythms that allow animals to anticipate cyclic environmental changes. The discovery of the known as "clock genes", which are very well conserved through vertebrate phylogeny, highlighted the molecular mechanism of circadian oscillators functioning, based on transcription and translation cycles (~24 h) of such clock genes. Studies in goldfish have shown that the circadian system in this species is formed by a net of oscillators distributed at central and peripheral locations, as the retina, brain, gut and liver, among others. In this work we review the existing information about the hepatic oscillator in goldfish due to its relevance in metabolism, and its key role as target of a variety of humoral signals. Different input signals modify the molecular clockwork in the liver of goldfish. Among them, there are environmental cues (photocycle and feeding regime) and different encephalic and peripheral endogenous signals (orexin, ghrelin and glucocorticoids). *Per* clock genes seem to be a common target for different signals. Thus, this genes family might be important for shifting the hepatic oscillator. The physiological relevance of the crosstalking between metabolic and feeding-related hormones and the hepatic clock sets the stage for the hypothesis that these hormones could act as "internal zeitgebers" communicating oscillators in the goldfish circadian system.

1. The circadian system. The case of goldfish

Cyclic environmental changes have led to the emergence of the circadian system in all living organisms, which provides a mechanism to anticipate such external cyclic changes. In vertebrates this system consists of multiple central and peripheral oscillators that can be entrained by different environmental signals (Albrecht, 2012; Moore and Whitmore, 2014).

The molecular core of these biological clocks is based on interlocked transcriptional and post-translational auto-regulatory feedback loops of a set of genes called clock genes (Albrecht, 2012; Vatine et al., 2011). In the main loop, the positive limb is formed by the transcription factor (heterodimer) CLOCK-BMAL1 that induces the expression of *period* (*per*) and *cryptochrome* (*cry*) genes, that form in turn the negative transcriptional limb. PER and CRY proteins dimerize and inhibit CLOCK-BMAL1 transactivation with an oscillation close to 24 h (Albrecht, 2012; Vatine et al., 2011).

Several humoral and neural signals ensures the coupling of this net of oscillators that in mammals is under the control of a master clock located in the hypothalamic suprachiasmatic nucleus (Albrecht, 2012). The existence of a master clock in teleosts has not been demonstrated to date, and then, fish circadian system is considered less hierarchical than the mammalian one (Moore and Whitmore, 2014; Weger et al., 2013). The presence of clock genes in a variety of tissues from different species supports the existence of an extended net of oscillators in teleost central and peripheral locations. Clock genes have been found in zebrafish (Vatine et al., 2011), and in a variety of tissues in other fish, including the retina, brain, pituitary, liver, skin, gut and gonads (Cavallari et al., 2011; Davie et al., 2009; Martín-Robles et al., 2011; Mazurais et al., 2000; Sánchez et al., 2010; Velarde et al., 2009; Vera et al., 2013).

Location of clock genes expression in specific encephalic nuclei of several fish species have been recently reported (Moore and Whitmore, 2014; Watanabe et al., 2012; Weger et al., 2013; own data presented in the 27th Conference of European Comparative Endocrinologists), but the functioning of fish circadian system remains an elusive issue. Moreover, in fish very little information exists on the possible role of humoral signals in the coupling among clocks.

In goldfish (*Carassius auratus*), daily rhythms of clock genes have been described in the retina, hypothalamus, optic tectum, pituitary, gut, liver and head kidney (Azpeleta et al., 2012; Feliciano et al., 2011; Nisembaum et al., 2014a, 2014b; Velarde et al., 2009). A comparative orthology of clock genes (from the

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main loop) in mammals and fish is shown in Fig. 1. Online available sequences belonging to the main four families of clock genes have been used to create this figure (see the figure legend for details). In goldfish, as in other teleosts, a huge number of genes have been duplicated and some of these copies were lost during evolution in different groups (Meyer and Schartl, 1999; Vatine et al., 2011; Wang, 2008).

Considering the key role of the liver in the regulation of metabolism, the study of this organ as an oscillator (among the peripheral clocks), is crucial to understand the temporal relationship between metabolism and the circadian system. The hepatic oscillator in mammals is one of the most sensitive to different external cues, as feeding time and internal ones, as changes in circulating hormones, including glucocorticoids (Kornmann et al., 2007; Schmutz et al., 2012; Stokkan et al., 2001; Yamamoto et al., 2005). Thus, this organ seems to be an endogenous oscillator where the circadian information converges with the regulation of metabolism (Schmutz et al., 2012). This minireview shows the existing information on liver as a key peripheral oscillator in a teleost model, the goldfish.

2. Environmental synchronizers of the hepatic clock

In goldfish, the daily light–dark cycle and feeding schedule are able to synchronize two classic outputs (overt rhythms) of the circadian system, the daily locomotor activity and food anticipatory activity rhythms (Aranda et al., 2001; Feliciano et al., 2011). These two synchronizers also drive clock genes rhythms in the liver of this teleost (Fig. 2) with similar acrophases as reported in the brain (Azpeleta et al., 2012; Velarde et al., 2009). When only one signal (LD cycle or feeding time) is present, *Per1a* rhythms in the liver keep acrophases, but their amplitudes decrease (Fig. 2B and C; Feliciano et al., 2011; Tinoco et al., 2014), suggesting that both environmental signals works together in the sustaining of the molecular clockwork in the liver. The amplitude of *Per1a* rhythm is higher in the hepatic oscillator than in the brain (Feliciano et al., 2011; Tinoco et al., 2014). In fact, the liver is highly sensitive to the feeding schedule in goldfish, as reported in mammals (Kornmann et al., 2007; Stokkan et al., 2001), since one meal might drive clock genes rhythms in this metabolic organ (Feliciano et al., 2011).

3. Hormonal signals as inputs of the hepatic oscillator

Besides the environmental cyclic changes that synchronizes the hepatic oscillator in goldfish, it is reported that clock genes in the goldfish liver could be regulated by different food-related peptides involved in energy balance (as ghrelin and orexin; Nisembaum et al., 2014a,b), and by glucocorticoid hormones (own data presented at the XIII Congress of the European Biological Rhythms Society). These data are summarized in the Fig. 3.

Recent studies suggest a relationship between ghrelin and the circadian system in mammals. Ghrelin would act as a promoting factor in the generation of the food anticipatory activity, and as a regulator of clock genes expression (LeSauter et al., 2009; Yannielli et al., 2007). In goldfish liver, ghrelin induces the expression of three genes from the Period family (Nisembaum et al., 2014a; Fig. 3). This effect seems to be mediated by specific receptors (GHS-R1a1 and GHS-R1a2) highly expressed in the liver (Kaiya et al., 2010), since *Per* induction is blocked by a ghrelin antagonist (Nisembaum et al., 2014a). Ghrelin also induces the hypothalamic expression of *gPer1a* and *gPer3* at 1-h post-injection (Nisembaum et al., 2014a), showing that this hormone is able to act at different levels of the circadian system, and it is a potential candidate as a connecting signal among oscillators.

Orexin is another feeding related peptide also involved in the sleep-wakefulness rhythm. The presence of this neuropeptide in the mammalian suprachiasmatic nucleus (Deurveilher and

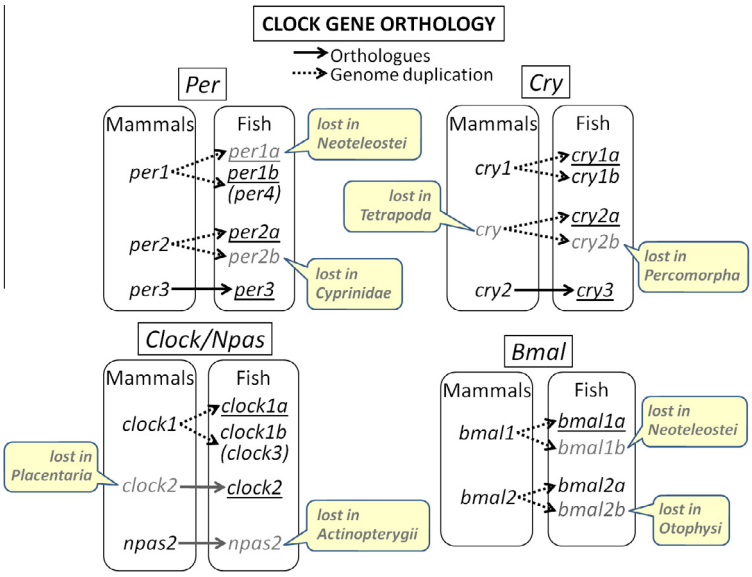

Fig. 1. Comparative orthology of clock genes in mammals and fish. The figure resumes the current information available on the presence of clock genes (Per, Cry, Bmal and Clock/Npas2 families) in teleosts and mammals, and suggests the possible gene orthology. Continuous black arrows indicate orthology. Discontinuous arrows indicate teleost-specific genome duplication (3R hypothesis). The underlined genes have been partially cloned in goldfish. Genes in grey have been lost in the groups indicated in ballons. Parentheses () indicate alternative names for some genes. Genes in black and not underlined have not been cloned in goldfish yet, but their presence is presumed. Databases GeneBank and Ensembl were used as main source of published sequences. Tools used were: blast (included in GeneBank and Ensembl web sites) for searching homologs, and ClustalX ver. 2.1 (NJ method) in order to verify orthology. When the nucleotide sequence was the only available, it was translated with the help of Wise2 tool (www.ebi.ac.uk/Tools/Wise2/advanced.html). Fish taxons referred in the balloons include the species with the more complete knowledge of their genome: Cyprinidae (*Danio rerio, Carassius auratus, Cyprinus carpio, Pimephales promelas*) Otophysi (*Astyanax mexicanus, Ictalurus punctatus* plus *Cyprinidae*). Percomorpha (*Xiphophorus maculatus, Oryzias latipes, Gasterosteus aculeatus, Poecilia formosa, Oreochromis niloticus, Maylandia zebra, Pundamilia nyererei, Neolamprologus brichardi, Haplochromis burtoni, Takifugu rubripes, Tetraodon nigroviridis*). Neoteleostei (*Gadus morhua* plus *Percomorpha*). Actinopterygii (*Lepisosteus oculatus, Salmo salar, Oncorhynchus mykiss, Esox lucius* plus *Otophysi* and *Neoteleostei*).