Abstract Recent research on the circadian system of the zebrafish is reviewed. This teleost has become an attractive model system because of its advantages for genetic analyses. Circadian rhythms of zebrafish behavior, visual system function, and pineal melatonin synthesis have been described, and behavioral and pineal rhythms are being used to identify and characterize clock mutants. Zebrafish heart, kidney, and embryonic cell lines contain circadian oscillators and phototransduction mechanisms for entrainment, suggesting that circadian pacemaking functions may be distributed throughout the animal. Studies of circadian system development in zebrafish have found that a molecular circadian oscillation in unfertilized oocytes persists through embryonic development with its phase intact, but that the pacemakers that drive rhythms of melatonin synthesis and behavior require environmental entraining signals late in development for initial synchronization. Zebrafish homologs of several of the core clock genes identified in other animals have been cloned. Transcripts for most of these are rhythmically expressed in multiple tissues. The interactions of clock gene products are for the most part similar to their interactions in mammals, although there are some potentially interesting differences.

Keywords Circadian rhythm · Clock gene · Development · Peripheral oscillator · Teleost

Introduction

Clock mechanisms in zebrafish are of interest both from a comparative standpoint and as a model system with unique experimental advantages. Teleost circadian systems have received relatively little attention in the past, with the exception of numerous studies of physiological rhythms of the pineal and retina, and a few studies demonstrating circadian control of behavior (cf. Cahill 2001). Whereas the zebrafish cannot really represent all of this large and diverse class of vertebrates, it does serve as one example for comparative analyses. Comparative issues aside, a major reason for the recent increase in attention to the zebrafish circadian system is its potential as a model system for the genetic analysis of clock mechanisms. Genetic approaches have been tremendously successful in elucidating circadian clock mechanisms in other model systems (cf. Allada et al. 2001; Loros and Dunlap 2001; Young and Kay 2001; Stanewsky 2002; Okamura et al. 2002). The zebrafish has become a major model system for mutational analysis of embryonic development, and this has led to development of the genetic and genomic technologies and resources required to identify mutant genes and determine their functions. These include methods for mutagenesis and transgenesis and the accumulation of rapidly growing genomic information. Point mutations can be induced easily and efficiently in the zebrafish germ line with chemical mutagens (Mullins et al. 1994; Solnica-Krezel et al. 1994), and insertional mutagenesis has also been successful (Amsterdam et al. 1999). A high-resolution meiotic map of the genome, based on simple sequence length polymorphisms (SSLPs), has been generated, enabling linkage mapping of mutations (Shimoda et al. 1999). Two zebrafish-mammalian radiation hybrid panels have been produced (Kwok et al. 1999; Chevrette et al. 2000) and used to generate gene maps that are anchored with SSLPs from the meiotic map (Geisler et al. 1999; Hukreide et al. 2001). These radiation hybrid maps, together with meiotic maps of zebrafish genes (Woods et al. 2000), facilitate cloning of mutant genes by suggest-
ling candidate genes that map to the same location. Together, these resources now make it possible positionally to clone genes identified by mutations; this will become easier with the completion of the zebrafish genome sequence, an effort that is currently under way. There have also been major advances in technology for the production of transgenic zebrafish, with the achievement of high rates of germ line transmission and tissue-specific transgene expression, and this will be instrumental in the analysis of gene function (Higashijima et al. 1997; Jessen et al. 1998).

Published studies of the zebrafish circadian system to date have focused on the characterization of behavioral and physiological rhythms, on the tissue distribution of circadian oscillators and photoreceptors, on the development of the circadian system, and on the expression patterns and functions of zebrafish homologs of known clock genes. These studies, reviewed here, not only provide information that will be critical for the genetic analysis of clock mechanisms, but have also revealed some important new principles about vertebrate circadian system organization.

Behavioral and physiological circadian rhythms in zebrafish

Circadian rhythms of locomotor activity, visual system function, and pineal melatonin synthesis have been described. My laboratory has examined circadian regulation of locomotor activity in adult and larval zebrafish (Hurd et al. 1998; Cahill et al. 1998). The activity of adults housed individually in small (50 ml) recording chambers was measured with infrared motion detectors (Hurd et al. 1998). There was considerable variability in the activity patterns measured in this way, and only about 70% of the fish showed strong activity rhythms, but some basic features of the circadian system were discernible. When maintained in a light:dark cycle, zebrafish were most active during the daytime. When the fish were kept in constant conditions, circadian rhythmicity in locomotor activity was observed for up to 10 days. The period of the freerunning locomotor rhythm was temperature-compensated, varying little over the range of 18–28.5°C, although it was longer in constant darkness (~25 h) than in constant dim light (~24.4 h). In some activity records, two rhythmic components with different circadian periods were observed, suggesting the regulation of activity by more than one circadian oscillator that can run independently.

The locomotor rhythms of larval zebrafish (5–18 days old), measured by a computerized video image analysis system, have proven to be much more robust and reliable (Cahill et al. 1998). The system that we use can simultaneously track the movements of up to 150 animals housed individually in 0.8-ml wells for a week in constant conditions. The activity records of over 95% of larval zebrafish display statistically significant circadian rhythmicity in this system, and satisfactory phase and period estimates can typically be determined from 85%–90% of the animals. Larval zebrafish, like the adults, are most active during the subjective day, and the average freerunning period under constant infrared light is 25.5 h. We use these behavioral rhythms as an assay in a screen for chemically-induced semidominant mutations that alter the period of the rhythm. We have identified mutations that shorten or lengthen the period of the behavioral rhythm by 0.5–1.5 h in heterozygotes and 1.0–2.5 hours in homozygotes. Figure 1 shows actograms from a wild-type zebrafish with a typical 25 h period and of a mutant with a period shorter than 24 h. We are now in the process of mapping the mutations with the goal of cloning the mutated genes.

Behavioral, physiological and molecular studies have revealed that several functional aspects of the visual system are regulated by the circadian system in zebrafish. Using a behavioral assay, Li and Dowling (1998) have shown that the threshold light intensity for detection of a visual stimulus is ~2 log units higher during the night than during the day (i.e. the visual system is most sensitive during the day). This is true for the dark-adapted responses of both rods and cones. The sensitivity rhythm persists for a few days in constant conditions, indicating that it is under the control of a circadian clock. The rhythm dampens to a state of high sensitivity in prolonged constant conditions, a finding that has led to the