# EXPERIMENTAL BIOLOGY

# Rhythms of Cell Division of Different Periodicity in Small Intestinal Cryptic Epithelium and Their Contribution to Circadian Rhythm Formation

**A. I. Antokhin, N. A. Zharkova, A.V. Zakharchenko, and Yu. A. Romanov**

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We used an improved method of chronobiological information processing enabling not only to detect oscillations with different frequencies, but also to determine the significance of each harmonic. This has made it possible to identify significant high-power harmonics present in the majority of cell positions in the crypt. These harmonics make the major contribution to the formation of diurnal rhythm of cell division in the crypt and hence determine spatial and temporal organization of the proliferative system in the crypt.

**Key Words:** *spatial and temporal organization; proliferation; Fourier analysis; crypt*

We previously examined the rhythms of cell division of different periodicity in the small intestinal cryptic epithelium using Fourier analysis. It was found that the intricate spatial and temporal organization consisting of rhythms with various periods; the amplitude of these rhythms considerably depended on cell localization in the crypt [3].

Here we studied biological rhythms with different frequencies and determined their contribution to the diurnal rhythm of cell division in the small intestinal crypt system in mice.

## MATERIALS AND METHODS

Experiments were performed on albino outbred male mice ($n$=40) weighing 21-23 g. The animals were kept at 23°C and 12:12-h light/dark regimen (daytime 6:00-18:00); light intensity was 250-300 lux. We studied crypt epithelium in the proximal portion of small intestine (20 longitudinally cut crypts, 25 epithelial cells). The total mitotic index per 1000 cells was calculated. To identify circahoralian oscillations in the number of mitoses, mitotic index was determined at 20-min intervals over 24 hours. For data processing, BIOGRAPH algorithm employing discrete Fourier transform was used to isolate harmonics with different frequencies and to estimate their amplitude using Fourier coefficients [1]. The new version of the algorithm allowed to check the hypothesis that calculated coefficients were normally distributed, estimate their expected values and dispersions, and to determine whether each of the calculated coefficients significantly differed from zero [1]. When the coefficients were insignificant, deviations from zero were considered to be random and the contribution of the corresponding harmonic to diurnal rhythm was considered as insignificant. High-power harmonics were regarded as essential components of the spatial and temporal organization of the prolifera-

Department of Biology, Medico-Biological Faculty, Russian State Medical University, Moscow; Joint Supercomputer Center of Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** natanyaal@mail.ru. N. A. Zharkova

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tive system in the crypt participating in the formation of the total diurnal rhythm.

## RESULTS

Significant diurnal rhythm of mitotic activity with well defined amplitude was detected in all positions of the crypt (Fig. 1).

Twelve-hour rhythm was also significantly present in the majority of positions (Fig. 2). This rhythm was insignificant and weakly expressed only in positions 3, 4, 11 and 23.

Two-hour rhythm proved to be most powerful and well presented among the high-frequency rhythms (Fig. 3). This harmonica was insignificant at 9, 17, 19 and the last four positions.

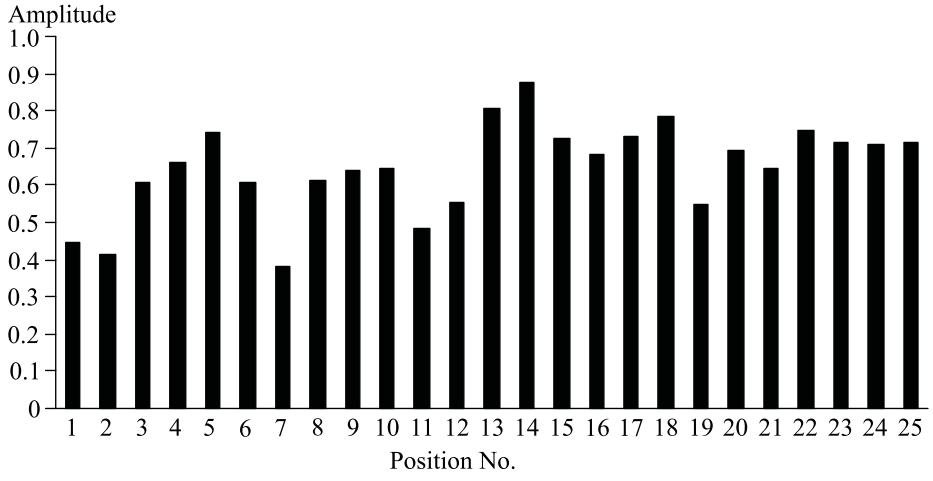


**Fig. 1.** Twenty-four-hour rhythm of mitotic activity at different positions of small intestinal crypt in mice. Here and in Fig. 2 and 3: dark and light bars correspond to significant and insignificant harmonics.

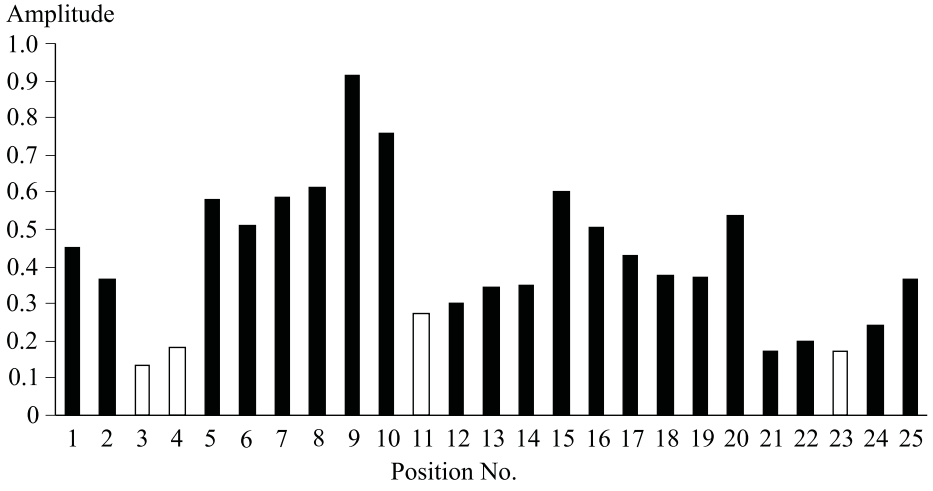


**Fig. 2.** Twelve-hour rhythm of mitotic activity at different positions of small intestinal crypt in mice.

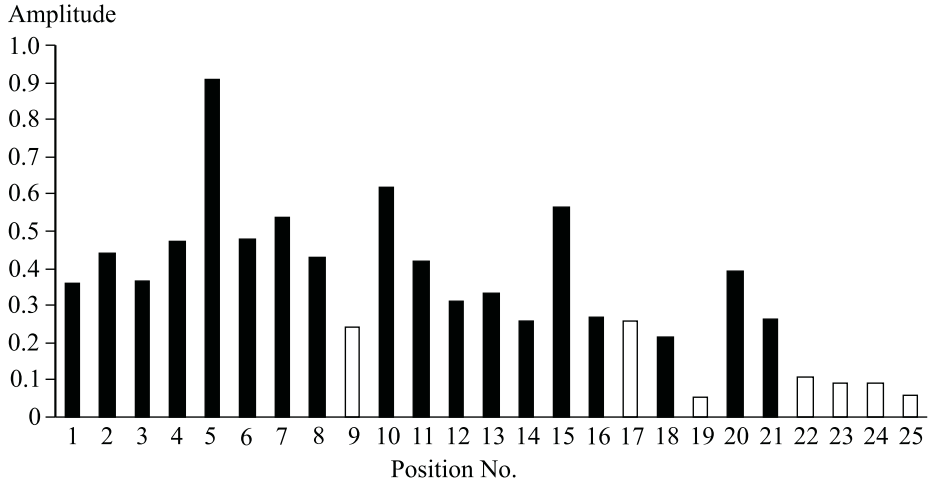


**Fig. 3.** Two-hour rhythm of mitotic activity at different positions of small intestinal crypt in mice.

All the rest harmonics usually had low power and were rarely seen, being randomly scattered throughout the crypt.

Rhythms with a period of 24 and 12 h are usually considered as exogenous, most likely reflecting the adaptation of organisms to environmental conditions. Photoperiod, feeding regime, and other environmental factors drive these rhythms [2].

High-frequency rhythms are usually regarded as the result of oscillatory processes such as negative feedback self-regulation of the system [2]. The results of our study suggest that the main (most pronounced) self-oscillation process in the proliferative system of the crypt has a period of 2 h. There is literary evidence that many important intracellular processes exhibit rhytmicity with period of 2 h (for example, 2-h rhythm of protein synthesis rate [4]).

Thus, we revealed an intricate spatial and temporal organization in the proliferative system of small intestinal crypt. Significance computation of the calculated Fourier coefficients allows us to determine the contribution of different harmonics in the formation of diurnal rhythm of cell proliferation. Two-hour period rhythm is the major rhythm among high frequency rhythms.

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