EXPERIMENTAL BIOLOGY

Spatiotemporal Organization of the Proliferative System in Small Intestinal Crypt Epithelium of Intact Mice

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We studied spatiotemporal organization of the proliferative system in small intestinal crypt epithelium of normal mice. Close relationships were found between circadian rhythms of cell proliferation and their position in the crypt. These peculiarities reflected spatiotemporal organization of the crypt epithelium. The hierarchic structure of spatiotemporal organization suggests the existence of several interrelated levels (individual cells, cell subpopulations, and cells with basal and maximum levels of proliferation within subpopulation). Each level has its proper temporal and spatial characteristics. Their interaction determines spatiotemporal organization of the proliferative system in the small intestinal crypt epithelium.

Key Words: *biological rhythm; spatial gradient; spatiotemporal organization; small intestinal crypt; proliferative system*

Study of spatiotemporal organization (STO) in various biological systems, including structural and functional systems of the organism, is a rapidly developing line of experimental and theoretical biology [1,2]. Here we assessed STO of the proliferative system in the small intestinal crypt epithelium (SICE).

MATERIALS AND METHODS

Experiments were performed on 50 male outbred albino mice weighing 16-18 g. The animals were kept at 23°C and 12:12-h light/dark regimen (daytime 8.00-20.00). The proliferative system was studied in the epithelium of distal crypts in the small intestine. Samples were taken at 4-h intervals (14.00, 18.00, 22.00, 2.00, 6.00, 10.00). In each preparation we analyzed 20 longitudinal crypt sections whose wall consisted of 25 cells. The total mitotic index (TMI) of 1000 epitheliocytes was calculated and expressed in percents. The mitotic index (MI, %) was estimated for each population of cells in the crypt wall (MI_P), subpopulation of 5 cells (MI_{SUBP}), and subpopulation of cells with maximum (MI_{max}) and basal (MI_{BAS}) levels of proliferation.

STO was studied by the chronotopobiological method. Chronobiological assay included measurements of circadian rhythms for various MI by the graphic and parametric method [3]: mesor, acrophase, active phase, passive phase, length, midpoint of active phase, absolute and relative amplitude, synchronization index (SI), diurnal mitotic pool, mitotic pool in the active phase, and relative mitotic pool in the active phase (%). Topobiological assay included estimation and graphic representation of spatial gradients for the distribution of MI_P, MI_{SUBP}, MI_{max}, and MI_{BAS} in the crypt wall. We calculated and graphically represented spatial gradients of changes in the absolute amplitude of circadian MI rhythms in the crypt wall. The goal of the chronotopobiological study was to evaluate the relationships between temporal and spatial organiza-

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tion of the proliferative system in SICE. Spatial gradients of changes in MI_P, MI_{SUBP}, MI_{max}, and MI_{BAS} in the crypt wall were estimated in each time point over a 24-h period (*i.e.*, in various phases of the circadian rhythm). The mean daily populational and subpopulational spatial gradients of MI_P and MI_{SUBP} were estimated and graphically represented. The absolute amplitude of spatial changes in MI_P (absolute amplitude of the spatial gradient for MI_P) was determined in each subpopulation (SP) of cells in the crypt wall epithelium. This index was calculated as the difference between maximum and minimum amplitudes of MI_P in the studied population. It was estimated in each time point over a 24-h period. The mean daily index was calculated. Chronotopobiological assay also included a pairwise correlation test for rhythmic (dependence of temporal changes on the topographic position of crypt cell subpopulations) and spatial changes in MI_{SUBP} at various times of day (dependence of changes on fluctuations of this index in time). Differences between spatial changes in MI_{SUBP} during the active and passive phase of circadian rhythms were evaluated. Since the curve reflecting these changes was biphasic, we determined relative values (%) of the initial rise and further decrease in MI_{SUBP} during the active and passive phase, respectively. The results recorded in the active and passive phase of circadian rhythms were expressed as means.

The data were analyzed by Student's *t* test. The differences were significant at $p \leq 0.05$.

RESULTS

In the SICE of intact mice we found monophasic circadian rhythm of TMI with acrophase at 10:00. Its active phase corresponded to the last third of the dark period and first half of the light period. Most cell populations in the crypt wall were characterized by monophasic circadian rhythms of MI (Fig. 1, *a*). These results suggest that various cell populations in the crypt wall have similar, but not synchronous rhythms of MI.

The circadian rhythm of MI_{SUBP} in the first, second, and third SP of cells in SICE was monophasic. In the fourth SP of cells this rhythm was biphasic. Acrophase of rhythms in cells of SP 1-3 and 4-5 were observed at 6.00 and 10.00, respectively. Acrophases of MI_P rhythms for cells of these SP had the same time characteristics (Fig. 1, *a*).

MI_{max} and MI_{BAS} underwent monophasic circadian changes in the first, second, third, and fifth SP. These changes in the fourth SP were biphasic. Acrophases of rhythms for MI_{max} and MI_{BAS} were observed at 10.00 (Fig. 1, *a*, *b*).

We studied the spatial gradient of mesor for MI_P in SICE. This index was characterized by wave-like changes in a direction from the bottom to the cervix of crypts. Maximum values were observed at 5-cell intervals (fifth, tenth, fifteenth, and twentieth cells, Fig. 2). Therefore, a complete cycle of changes in mesor for MI_P in the crypt wall proceeded within the segment consisting of 5 epithelial cells. Discrete units

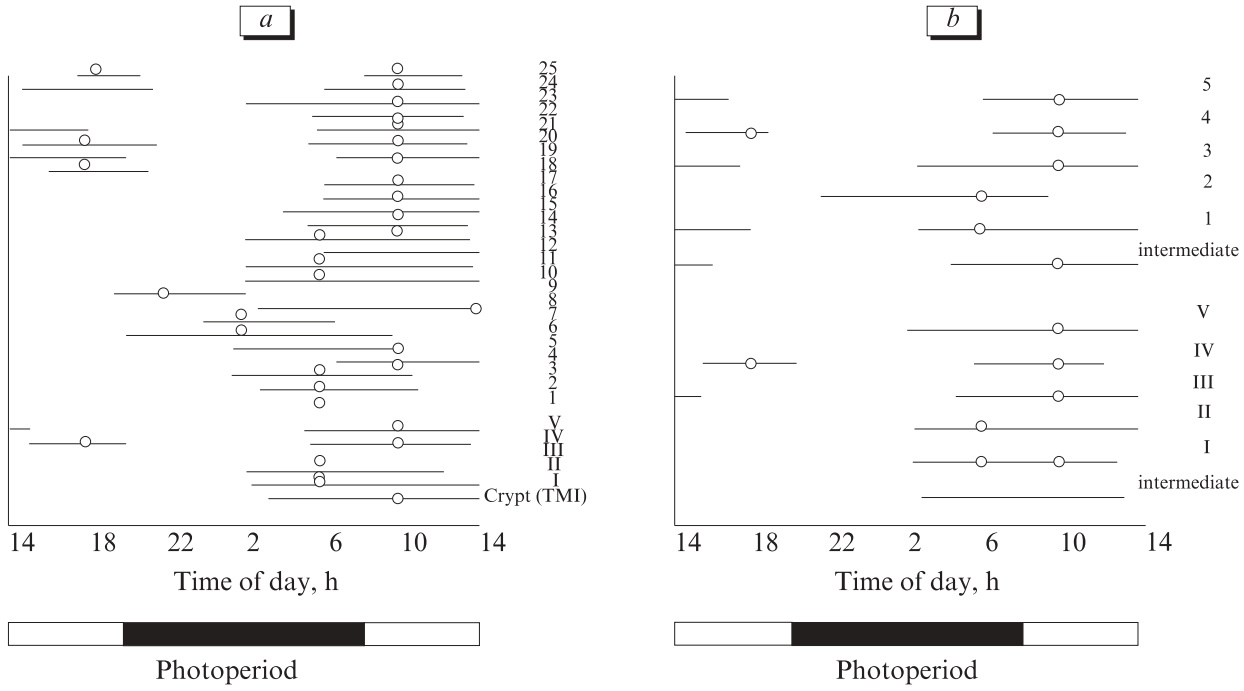

Fig. 1. Phasograms of circadian rhythms of the total mitotic index (TMI) and mitotic indexes for cell subpopulations, individual cells (1-25, *a*), and subpopulations with maximum (*1-5*) and basal levels of proliferation (*I-V*, *b*) in the small intestinal crypt epithelium of mice. Circles: acrophases of rhythm.

of changes in proliferative activity were set in SICE. These SP were positioned along the crypt axis and included 5 epithelial cells. There were cells with high and low levels of basal mitotic activity (MI_{max} and MI_{BAS}).

We compared values of mesor for MI_{max} in various SP. Mesor in the second SP was 1.4 times higher than in the first SP. Mesor in the third and fourth SP were 1.4 and 3.3 times lower than in the second and third SP, respectively. MI_{BAS} in the second SP was 2.5 times higher than in the first SP. MI_{BAS} in the third and fourth SP were 1.2 and 2 times lower than in the second and third SP, respectively (Fig. 2). These results indicate that proliferative activity of cells in SICE decreased in a direction from the bottom to the cervix, which is consistent with published data [4-7]. However, we revealed fluctuations in the proliferative spatial gradient. Similar gradients are typical of the spatial distribution of MI_{max} and MI_{BAS} in various times of day. It should be emphasized that the ratio between individual peaks of MI_{max} and MI_{BAS} was different in the active and passive phase of circadian rhythms of mitotic activity in SICE. MI_{max} in the second SP was higher than in the first SP during both phases of the rhythm. MI_{max} in the third SP was lower than in the second SP. It was especially pronounced in the passive phase. Moreover, MI_{max} in the fourth SP was lower than in the third SP. In the passive phase the observed differences were less significant than in the active phase. MI_{BAS} in the second SP was higher than in the first SP during the passive phase. In the second SP we revealed opposite changes. MI_{BAS} in the third and fourth SP increased similarly during both phases of the rhythm (Table 1). Spatial changes in MI_{max} and MI_{BAS} differed in various phases of the circadian rhythm.

The absolute amplitude of circadian MI_P rhythms underwent spatial fluctuations. Similarly to spatial changes in MI_P, they had maxima at the intervals of 5 crypt epithelial cells.

The diurnal mitotic pool in SICE was high (206.4%). Therefore, all cells underwent division over a 24-h interval. These findings confirm the hypothesis that SICE is a rapidly renewing cell system. The intensity of division differed in various SP. It was maximum in the second SP, but minimum in the fifth SP (diurnal mitotic pools 374.4 and 52.8%, respectively). Cells constituting these populations divided over 6 and 48 h. Mesor and diurnal pool of MI_{SUBP} in the second SP were 1.8 times higher than in the first SP. These indexes in the third, fourth, and fifth SP were 1.4, 2.5, and 2.2 times lower than in the second, third, and fourth SP, respectively (Fig. 3). Proliferative activity increased in the first portions of the crypt, but progressively decreased in a direction from the bottom to the cervix. Most significant differences in the relative mitotic pool were observed in the active phase. The data indicate that this phase of the circadian MI_{SUBP} rhythm play a role in proliferation. The second and fourth SP differed in the relative mitotic pool by 1.4 times. However, this index was practically similar in other SP. Despite between-SP differences in the diur-

TABLE 1. Changes of MI_{max} and MI_{BAS} in Subpopulations of Crypt Epithelial Cells from Intact Mice in the Active and Passive Phase of Circadian TMI Rhythm

Phase, subpopulation		Time of day						Mean
		14	18	22	2	6	10	
MI_{max}								
Active	2	157				151	122	143
	3	69				66	82	72
	4	24				23	31	26
Passive	2		208	133	112			151
	3		56	78	43			59
	4		79	20	42			47
MI_{BAS}								
Active	2	136				217	204	186
	3	113				84	118	105
	4	39				38	58	45
Passive	2		186	599	321			369
	3		98	30	56			61
	4		65	56	38			53

Note. Changes of MI in SP relative to previous SP.

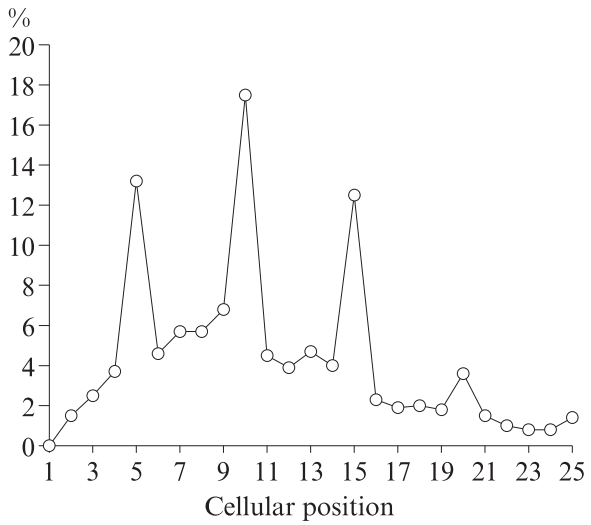

Fig. 2. Spatial changes in mesor for the mitotic index in individual cells of the crypt wall epithelium in mouse small intestine (spatial gradient).

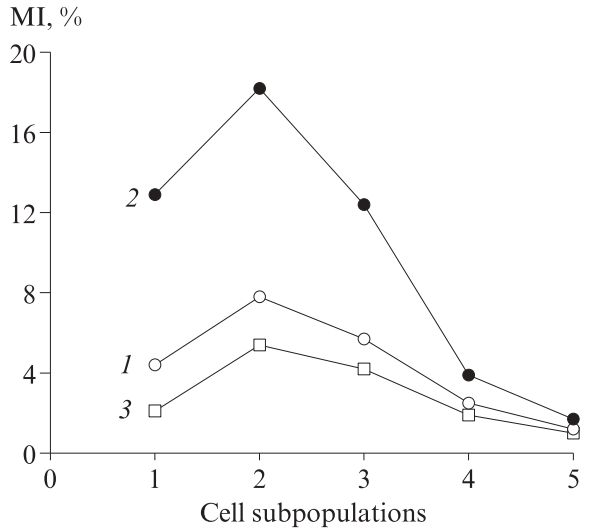

Fig. 3. Spatial changes in mesor for mitotic indexes of cell subpopulations (*1*) and subpopulations with maximum (*2*) and basal levels of proliferation (*3*) in the small intestinal crypt epithelium of mice (spatial gradient).

nal mitotic pool, the active phase plays an important role in the maintenance of proliferative activity.

Mesor for the absolute amplitude of the spatial gradient of MI_p and diurnal mitotic pool underwent similar changes. Mesor in the second SP was 8% higher than in the first SP. In other SP this index decreased. Mesor in the fourth SP was 5.1-fold lower than in the second SP. The mean value of mesor in the active phase was higher than in the passive phase by 1.7 times.

In both phases of the circadian rhythm mesor for MI_{max} 3.4-fold surpassed that for MI_{BAS}.

The ratio of differences between maximum and minimum MI_{SUBP} in the ascending and descending portions of the spatial gradient curve practically did not differ in the active and passive phase of circadian rhythms. However, in the active phase absolute differences in the ascending and descending portions of the curve were less pronounced than in the passive phase (by 21 and 39%, respectively). Therefore, changes in MI_{SUBP} that accompany the increase and decrease of this index in the spatial gradient were balanced with each other in both phases of the circadian rhythm. The relative spatial distribution of proliferating cells in various parts of this gradient remained practically unchanged in various phases of the circadian MI_{SUBP} rhythm. However, the difference between maximum and minimum values of MI_{max} in the descending portion of the spatial gradient curve in the active phase was 2.5 times higher than in the passive phase. These differences in the ascending portion of the curve were practically similar in both phases of the circadian rhythm. The mean ratio of differences between maximum and minimum values of MI_{max} in the ascending and descending portions of the spatial gradient curve in the passive phase 2-fold surpassed that in the active phase. Differences between maximum and minimum values of MI_{BAS} in the ascending and descending portions of the spatial gradient curve in the passive phase were higher than in the active phase by 2.0 and 1.5 times, respectively. The mean ratio of differences between maximum and minimum values of MI_{BAS} in the ascending and descending portions of the spatial gradient curve in the passive phase 2.5-fold surpassed that in the active phase. As differentiated from MI_{SUBP}, the spatial distribution of proliferating cells determining MI_{max} and MI_{BAS} was imbalanced in various parts of the spatial gradient. It was related to differences in phases of the rhythm in the descending and ascending portions of the spatial gradient curve, respectively. An opposite imbalance in the distribution of dividing cells in various portions of the spatial gradient curve during the active and passive phase probably equilibrates this index in various portions of the spatial gradient curve for MI_{SUBP} determined by cells with maximum and minimum levels of proliferation.

A positive correlation between temporal rhythmic changes in MI_{SUBP} was most significant in adjacent SP (r=0.72). Less pronounced relationships were revealed between SP topographically divided by 1 (r=0.56), 2 (r=0.37) and 3 SP (r=0.30). The coefficient of correlation between rhythmic changes in MI_{SUBP} decreased with an increase in the distance between SP. Therefore, rhythmic changes in this index depended on its spatial position in the crypt wall.

The coefficient of correlation between spatial (gradient) changes of MI_{SUBP} in various times of day was high in the active and passive phase of circadian rhythms (0.99 and 0.92, respectively). Our results are consis-

tent with changes in the spatial gradient over a 24-h period. We studied changes in MI_{SUBP} accompanying the increase or decrease of this index in the spatial gradient during both phases of the circadian rhythm. During the active phase MI_{SUBP} increased less significantly than during the passive phase. At the same time, the decrease of MI_{SUBP} in the active phase surpassed that in the passive phase by 100%. Our findings indicate that spatial and temporal changes in proliferative activity of SP were interrelated. The observed changes depended on the phase of circadian rhythms.

We conclude that the proliferative system of SICE in intact mice is characterized by STO.

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