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BASIC RESEARCH

Mechanism of Cell Cycle Asynchrony within the Animal Organism

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Abstract

Every organism is composed of multi-cellular systems. Each of these cells, from the time of birth until death, often plays a polyfunctional role. Obviously, this cycle must include periods of intense work and leisure. In other words, the organ cell masses are able to perform the asynchronous mechanism of cell cycle. The implementation of such a mechanism is regulated by the cell's gene apparatus which receives the signal from the cytosol of the functioning cell; it also performs the reverse inclusion of the cells doing the work after the rest interval. The aim of this study was to show the presence of a daily regulation of the cell apparatus of any organ, using the liver as an example. This phenomenon is the obligatory mechanism developed over the course of a long evolution and explains the lifetime of the multicellular organ system.

Key words: *hepatocyte, cell cycle, nucleus, asynchronous operation.*

Introduction

Differentiation provides a functional transition from total nucleus potency to a selective activity of certain loci and a corresponding selective repression of other loci. Due to the internal genetic differentiation, the biochemical, physiological and morphological cell differentiation, including their specialization, takes place in numerous ways. Therefore, differences that can be observed, appear in the cells of organs like the liver, pancreas, and in the cardiomyocytes and cells of the central nervous system.

Just after nuclear differentiation occurs, cytoplasmic differentiation takes place. Then, specific cell products from the various cells of the various organs appear, which influence further differentiation, the genetic system, and even systems which regulate the cells in an organ system. A study of the vast data related to the information system within the cell inspired us to study the path of individual gene loci which influence the management of the individual products.

However, the organ structure represented by specifically differentiated cells and formed in response to the information system, remains largely unexplored. Perhaps, the specialized cells of any organ (neuron, cardiomyocyte, pancreatic cells, etc.) should be evaluated as a whole, from the perspective of the genetic system, in terms of the duration of their "ability" and the ephemeral nature of their function. One cannot assume that the cells of the central nervous system, liver, pancreas are formed and operate without any control until apoptosis. Individual cases have directed us toward the study of this process, particularly the short duration of life of the lymphocytes and neutrophils, and the relatively short erythrocyte lifecycle. Studying the other organs from the standpoint of the functioning of every cell in the temporal sense becomes very difficult, as they have the same type of external morphology; also, distinguishing them on morphofunctional criteria is very difficult. For example, a study of the liver, shows hepatocytes, of the same morphological type, working up to 400 days, but performing dozens of different functions. In this study, we attempted to discover the features of genetic regulation which permit such a polyfunctional role of the cells.

The aim of this study was to show the presence of a daily regulation of the cell apparatus of any organ, using the liver as an example. This phenomenon is the obligatory mechanism developed over the course of a long evolution and explains the lifetime of the multicellular organ system.

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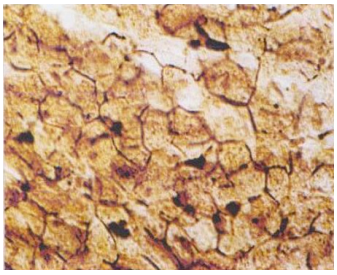
Material and Methods

To study the nature of cell activity, we chose the liver cells, because they have a characteristic high multifunctional load and a relatively long lifecycle (between 350 and 400 days)]. We studied the liver of Wistar rats. The animals were cooled in a climatic chamber «ILKA» (Germany) at -30 °C for 3 hours daily: for 5 days – 1st group, 10 days – 2nd group, 15 days – 3rd group and 30 days – 4th group, the animals of the control group were not cooled.

Each group included 10 animals. After the experiment, the animals were decapitated. The liver was fixed in Carnoy's solution for the combined reaction, where simultaneously the cell DNA was stained using DeLamater reagent, and the neutral polysaccharides in the cytoplasm were fixed and stained with fuchsin reagent. Thus, it was possible to simultaneously analyze the status of the nuclear matter within the cells and the functional processes, namely, the carbohydrate metabolism in the cytoplasm under exposure of the extreme cold stress (Fig. 1a). The reaction staining of the liver with Fast Green FCF in order to detect the histone activity within the cell nucleus was done, as also staining with gallocyanin to selectively reveal the DNA alone. Reaction to acid phosphatase was histochemically performed, according to Gomori, at pH 6.2.

Results

The hepatic lobule is composed of radially divergent plates from the central vein [1]. Bile ducts pass between the plates and begin with the bile capillaries. The liver, first of all, is the organ



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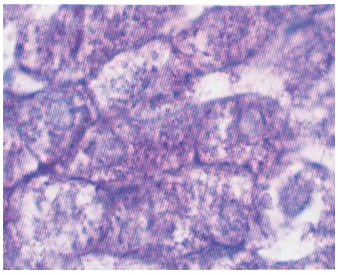

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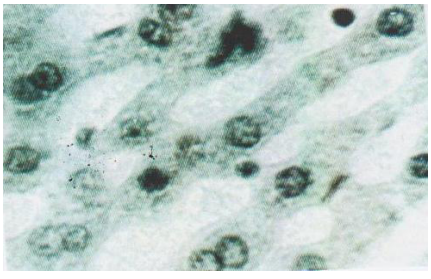

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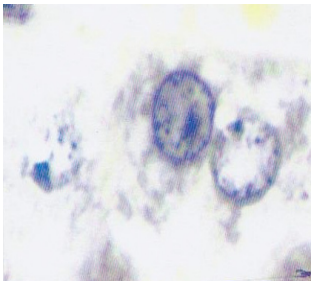
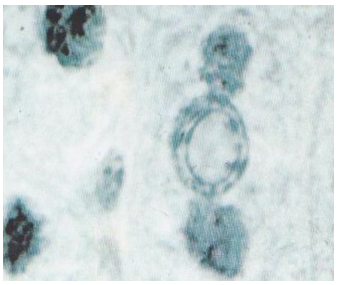
Figure 1

a - *General view of the hepatocytes stained by Van Duino. Magnification×100;*
b - *Formation of the bile capillaries. Acid phosphatase, pH 6.2. Magnification 90;*
c - *The general structure of liver lobules. Binuclear cells among the hepatocytes. Gallocyanin×40.*

capable of converting the cholesterol into the primary bile acids. This is clearly seen when the liver slices are processed using Gomori's method to detect the acid phosphatase activity at pH 6.2 (Fig. 1b) [2].

The liver cells are not morphologically different from each other. However, these cells possess a unique feature, in that almost 23-25% of them contain two nuclei (Fig.1c). Among the binuclear liver cells, between 8 and 10% cells are in the asynchronous functional state. One of the nuclei is stained densely, while the other nucleus showed signs of working, with an active nucleolus. This nucleus was always lighter than the first (Fig.1d) and stained intensely with gallocyanin or Fast Green FCF. The latter indicates that the DNA is strongly linked to the histones and that the DNA strand is inactive – in other words, the nucleus is resting. Therefore, it is obvious that the nuclear matter of the overtired working cell receives a signal from the information cell center (part of the genome), which induces the nucleosome tail (histone-1) and closes the free DNA chain; thus, the nucleus becomes temporarily functionally "dumb." At this time, the other nucleus of the binuclear hepatocytes works more powerfully.





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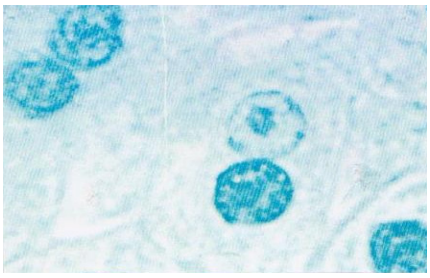
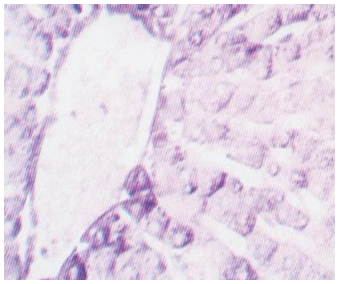


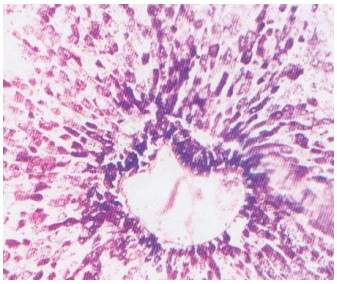
f

Figure 1

d, e - Hepatocytes with asynchronously working nuclei: "intensively working nucleus" (↑). Gallocyanin × *1000;*
f - Two hepatocytes. In one hepatocyte both nuclei are inactive, while in the other hepatocyte, only one nucleus is working intensively (↑). Fast Green FCF×*1000.*

Asynchronous operation of the nuclei in the binuclear cells is well observed after gallocyanin or Fast Green staining (Fig. 1 e-f). The functional load of the organ distinctly detects those cells with asynchronously working nuclei which stained intensely and were detected to produce substances (e.g. polysaccharides)





b a

Figure 2

a - *The general plan of the liver lobule after 5 days of cooling. The variety in the polysaccharide content of the hepatocytes is seen. Stained by Van Duino. Magnification×40;*
b - *After the 30-day cooling period, some hepatocytes are found to contain many polysaccharides. Stained by Van Duino. Magnification×400;*

(Fig.2). We conducted the experiment using cold stress conditions to ensure that the cells which are able to work in cycles and under heavy loads receive a signal from the genetic center; this is most evident among the cells which possess the two nuclei received during evolution.

On analyzing the liver of the rats cooled for 5 days, it is evident that the polysaccharide reserves in the cell cytoplasm are greatly utilized (Fig.2a,b). However, some of the cells continue to work hard, and among them are a large percentage of cells with asynchronously working nuclei. Even during the prolonged

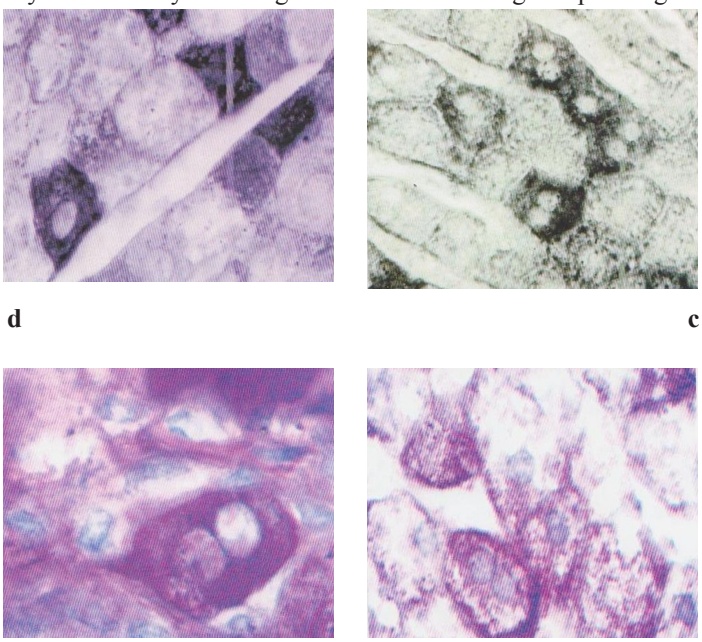


Figure 2

c - *Despite the long cooling time (30 days) some hepatocyte groups continue to intensively synthesize the polysaccharides. The binuclear cells predominate among the hepatocytes. Gallocyanin×1000;*

d, e, f - *The liver cells function actively despite the unfavorable life conditions (cooling at -30 °C for 30 days). Asynchronously working nuclei are observed in the binuclear cells. Stained by Van Duino. Magnification×1000.*

cooling period (30 days), when there is an almost complete utilization of the cells' energy reserves, a small fraction of the cells continues to maintain their efficiency. Significant among these cells are those with asynchronously working nuclei (Fig. 2c-f).

The data obtained suggest that the cellular systems in the specifically constructed organs of a multicellular organism usually perform polyfunctional work. They are enabled and disabled from the working state at different times. Morphofunctional uniformity of cells does not allow the working and resting cell groups be registered at any given time. In some organs, this problem can be studied more easily. Therefore, it is understood that the liver, through evolution, acquired the ability to include up to 25% hepatocytes with two nuclei. This permits this organ to perform a high workload due to the asynchronous type of nuclei in the binuclear cells, despite their relatively short lifecycle. Such data clearly raises the question about the location of the loci that control the cell workload during their daily lifecycle.

Conclusion

The cells of each tissue system operate with varying degrees of intensity during different periods of life. Therefore, the leukocytes freely circulating in the blood have a small supply of the vital potency. Their lifetime is limited to 9-10 days. The cells of the gastrointestinal tract lining work under a heavy load. The enterocyte lifecycle is 72 hours. In other organs, this is much more difficult to define. However, it is clear that the replacement of neurons in the brain was not observed. They leave their "official position" only with the death of the whole organism.

Each animal, during its lifecycle, has periods of hard work interspersed with rest in the form of sleep. In the tissues of multicellular organisms, regulating the cell cycle is operated through the nuclear-cytoplasmic unit. In fact, 8-10% of the binuclear liver cells have an asynchronous operating status of the nuclei at every moment. The "overwork" of the working cell leads to the formation a cytosolic signal, which enters into the nuclear matter, and as a result the tail portion of the nucleosome closes the free chain of DNA and the nucleus becomes temporarily functionally "mute." At the same time, another nucleus of the binuclear hepatocyte works, taking on more workload.

Asynchronous operations of the nuclei in the binuclear cells are well observed after staining with gallocyanin or Fast Green. Staining with the Fast Green specifically stains only the histone proteins: one nucleus is brightly stained while the other nucleus remains light, in the asynchronously working cell.

Obviously, this phenomenon follows a consistent pattern of functioning in the multicellular systems of any living organism. However, it is very difficult to ascertain the operating status of the cell and its state of rest in the tissues comprising only mononuclear cells (e.g. respiratory epithelium, intestinal epithelium, urinary tract, etc.). In such tissue systems, it is necessary to identify other ways to control the asynchronous cycle resulting from intercellular signals. For every cell, there is a time to live and a time to die. When the work is completed, programmed cell death (apoptosis) occurs.

References

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