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Nuclear Protein Matrix: Association with Newly Synthesized DNA

Abstract. *The residual structural framework of the cell nucleus, termed the nuclear protein matrix, is associated with newly synthesized DNA in regenerating rat liver. One minute after rats are injected with [^{3}H]thymidine, more than 90 percent of the total tritium in nuclear DNA is associated with the matrix DNA although this DNA comprises only 25 percent of the total nuclear DNA. In contrast, the bulk DNA, 75 percent of total nuclear DNA, contains less than 8 percent of the total labeled DNA. The percentage of total labeled DNA associated with the bulk DNA increases for 30 minutes after injection and decreases correspondingly in the matrix DNA.*

We have reported the isolation and characterization of a new structural protein matrix in mammalian nuclei (*1*). Isolated rat liver nuclei contain a residual framework structure, which maintains the spherical shape of the nucleus. When isolated, this nuclear structure extends from residual components of the nuclear envelope to the nuclear interior where it forms a distinctive internal matrix that connects to a residual nucleolar structure.

The isolated residual framework structure termed the nuclear protein matrix is essentially free of the DNA, RNA, and phospholipids of the nucleus; it accounts for approximately 12 percent of the total nuclear proteins and is composed primarily of three distinct nonhistone acidic polypeptide fractions of 60,000 to 70,000 daltons. Other physical and chemical fractionation procedures indicate that the nuclear protein matrix consists predominantly of nuclear acidic proteins and is essentially free of histone proteins. In addition, amino acid analyses of the total matrix protein fraction demonstrate a ratio of acidic to basic amino acids of 1.46.

In accord with these findings, previous investigators have observed complex residual structures in nuclei from which the chromatin had been extracted. Zbarsky and his colleagues detected residual structural components in the nucleus after treatment with 2*M* NaCl solutions (*2*). Busch and his co-workers were the first to observe a ribonucleoprotein network extending throughout the nucleus, from the nuclear membrane to the nucleolus (*3*). Busch and Smetana suggested that these nuclear elements might be involved in RNA processing and transport (*4*). The networks observed by these previous investigators were complex nuclear structures enclosed by an intact nuclear envelope. The nuclear protein matrix which we have isolated may be a residual protein component of the above structures.

We now report on the possible function for the nuclear protein matrix related to DNA synthesis. During the isolation of the protein matrix, distinct fractions of DNA are removed with each sequential extraction. Approximately 75 percent of the total nuclear DNA can be removed by reducing the magnesium chloride concentration from 5.0 m*M* to 0.2 m*M* in the nuclear suspension medium. We have termed this DNA, bulk DNA.

Electron microscopic studies reveal that the DNA remaining with the extracted nuclei (approximately 25 percent of the total nuclear DNA) is associated with the nuclear protein matrix. This total matrix DNA can be further fractionated by 2.0*M* NaCl into a soluble DNA component and a residual matrix DNA, which remains tightly associated with the nuclear protein matrix.

We have observed the temporal sequence of labeling of the above DNA fractions after a single injection of [^{3}H]thymidine into the hepatic portal vein of regenerating rat livers. Results with this regenerating rat liver system indicate that the newly synthesized DNA is associated with the matrix DNA.

Liver nuclei were isolated from male rats (Sprague-Dawley, 250 to 300 g, Charles River) (*5*). Livers were excised quickly, minced with a scalpel and homogenized at 0°C with ten strokes at 1300 rev/min in a Potter-Elvehjem apparatus with two volumes of sucrose-TM buffer (0.25*M* sucrose, 50 m*M* tris, *p*H 7.4, 5 m*M* $MgCl_2$ at 22°C). After filtration through four layers of cheesecloth, the homogenate was centrifuged at 780*g* for 10 minutes to yield a crude nuclear pellet. The pellet was resuspended in 2.2*M* sucrose TM buffer and centrifuged at 40,000*g* for 90 minutes. This purified nuclear pellet was washed twice in 0.25*M* sucrose TM buffer and subjected to the following sequence of treatments to obtain the isolated nuclear protein matrix.

Table 1. Distribution of [^{3}H]thymidine label, as a function of time after injection in nuclear DNA fractions from regenerating rat liver. Each value represents the mean ± the standard error of the mean, for three separate experiments at 20, 24, and 30 hours after partial hepatectomy. For each experiment, livers from four separate animals were pooled and DNA and radioactivity determinations were performed in triplicate. The specific activities are shown in Fig. 1A.

Time after injection (min)	Percent of total DNA	Percent of total label	Ratios	
			Specific activity to total nuclear DNA specific activity	Specific activity to bulk DNA specific activity
		Bulk DNA		
1	74.8 ± 3.6	7.6 ± 2.3	0.05 ± 0.01	1.0
5	72.0 ± 1.2	19.4 ± 2.4	0.19 ± 0.02	1.0
10	74.3 ± 2.3	35.6 ± 4.6	0.36 ± 0.05	1.0
30	75.0 ± 1.1	67.2 ± 3.1	0.78 ± 0.05	1.0
		Total matrix DNA		
1	25.2 ± 3.6	92.4 ± 2.3	3.50 ± 1.1	46.7 ± 14.8
5	28.0 ± 1.2	80.6 ± 2.4	2.06 ± 0.32	11.4 ± 2.6
10	25.7 ± 2.3	64.4 ± 4.5	1.97 ± 0.46	5.37 ± 0.58
30	25.0 ± 1.1	32.8 ± 3.1	1.24 ± 0.09	1.49 ± 0.23
		High-salt (2.0M NaCl) soluble matrix DNA		
1	23.6 ± 3.4	82.2 ± 3.3	3.73 ± 0.72	44.7 ± 14.4
5	26.5 ± 1.7	74.6 ± 2.3	2.41 ± 0.16	11.1 ± 2.4
10	23.2 ± 2.8	58.2 ± 4.9	1.99 ± 0.45	5.42 ± 0.58
30	23.7 ± 0.9	31.6 ± 3.2	1.25 ± 0.11	1.51 ± 0.22
		Residual matrix DNA		
1	1.7 ± 0.3	9.8 ± 1.3	5.6 ± 1.9	77.6 ± 2.8
5	1.5 ± 0.4	6.0 ± 0.6	2.6 ± 0.8	14.9 ± 5.3
10	2.6 ± 0.5	6.2 ± 1.7	2.0 ± 0.7	5.61 ± 1.5
30	1.2 ± 0.3	1.2 ± 0.7	1.0 ± 0.3	0.97 ± 0.2

The following series of extractions were carried out at 0°C, and each solution contained 10 m*M* tris buffer, *p*H 7.4. (i) Three extractions with 0.2 m*M* $MgCl_2$ for 10 minutes followed by centrifugation at 780*g* for 20 minutes. Subjecting nuclei to this low magnesium environment resulted in the extraction of approximately 75 percent of the total nuclear DNA (bulk DNA). The DNA remaining with the extracted nuclei represents the total matrix DNA. (ii) Three extractions with 2*M* NaCl containing 0.2 m*M* $MgCl_2$ for 10 minutes followed by centrifugation at 780*g* for 40 minutes. This procedure removed about 90 percent of the total matrix DNA. (iii) One percent Triton X-100 in 5 m*M* $MgCl_2$ for 10 minutes followed by centrifugation at 780*g* for 20 minutes removed over 95 percent of the total phospholipid of the nucleus but removed no DNA. (iv) The residual matrix DNA was removed by enzymatic digestion of nucleic acids with 200 μg of electrophoretically purified pancreatic deoxyribonuclease 1 (Worthington) and 200 μg of pancreatic ribonuclease (Worthington) per milliliter containing 5 m*M* $MgCl_2$ for 1 hour at 22°C. Digestion was terminated by centrifuging the mixture at 780*g* for 20 minutes and washing the pellet twice in 10 m*M* tris buffer (*p*H 7.4) containing 5 m*M* $MgCl_2$. More than 99.9 percent of the total nuclear DNA was removed by these extractions. The remaining residual nucleus is termed the nuclear protein matrix.

Partial hepatectomy was performed by the surgical removal of two-thirds of the liver. All operations were performed between 9 and 12 a.m. The animals were housed under controlled conditions of light and dark and were compared to sham-operated control animals. The first wave of DNA synthesis in the regenerating liver began approximately 16 hours after partial hepatectomy and reached a peak after 24 hours. Each animal received 200 μc of [methyl-^{3}H]thymidine (50 c/mmole) in 0.2 ml of sterile water injected directly into the hepatic portal vein. After the indicated times, livers were removed rapidly and homogenized immediately at 0°C.

The specific activities of DNA in the various fractions were plotted as a function of time after a single injection. The specific activity of matrix DNA is higher than that of the total nuclear DNA. In contrast, the bulk DNA, representing more than 75 percent of the total nuclear DNA, had a very low specific activity for the first 10 minutes and then progressively increased. These experiments were begun 20 hours after partial hepatectomy of the animals (Fig. 1A).

The distribution of total nuclear label in matrix and bulk DNA was compared at 20, 24, or 30 hours after partial hepatectomy (Fig. 1B). For the first 20 minutes after injection the matrix DNA contained most of the label; after that with increasing time, the label appeared progressively in the bulk DNA fraction.

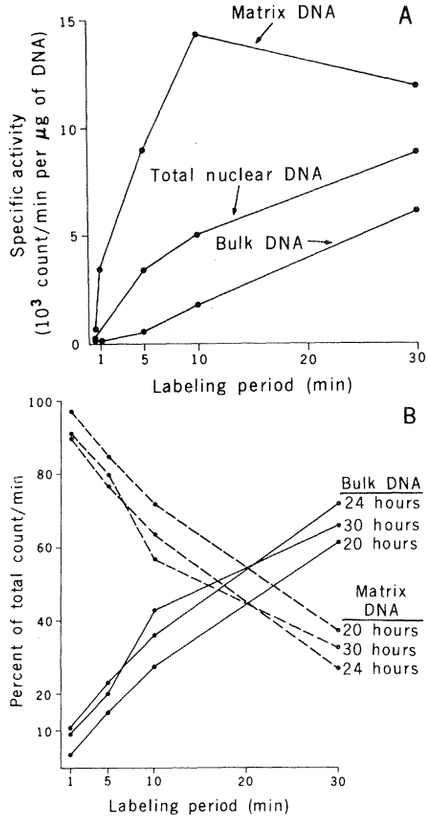

Fig. 1. (A) Specific activity of nuclear DNA (20 hours; regenerating liver). Total nuclear DNA was fractionated into bulk DNA and matrix DNA. The specific activities were determined for the two DNA fractions and for total nuclear DNA. DNA was isolated (*14*) and assayed in triplicate by the Burton modification of the diphenylamine reaction (*15*). Radioactivity was determined in triplicate with approximately 100 μg of DNA in each sample. (B) Distribution of total label in nuclear DNA (20, 24, and 30 hours; regenerating liver).

The absolute values for the distribution of nuclear label in the various DNA fractions as a function of time are indicated in Table 1. Thus, it appears that the DNA associated with the matrix is enriched in newly synthesized DNA in comparison to bulk DNA. During the first 10 minutes the fraction with the highest specific activity was the residual matrix DNA.

Over the range of 1 to 10 minutes, the percentage of total nuclear label in the matrix DNA fraction decreased with time in a linear fashion (Fig. 1B), while the specific activity of the matrix DNA was still increasing (Fig. 1A). This suggests sequential transport of labeled matrix DNA to the bulk DNA fraction. These temporal labeling patterns of the matrix DNA and bulk DNA are inversely related and further support a precursor-product relationship (Fig. 1B). Other interpretations are possible and might include differences in the relative rates of synthesis and turnover of the two fractions with increasing time.

We have observed a similar nuclear protein matrix isolated from nuclei of several mammalian tissues. The processing of nuclei from different tissues requires special considerations, and it is important to emphasize that the procedures described in our report apply only to rat liver. Endogenous nuclease activity may be an important factor in the release of specific DNA components during the extraction procedures (*6*).

A number of investigators have studied kinetic properties of DNA synthesis in the regenerating rat liver (*7*), and others have identified specific DNA fractions enriched in newly replicated DNA in regenerating liver as well as in other cell systems (*8*). The structural localization of these newly replicated DNA components, however, remains unclear. Although the nuclear envelope has been suggested as a site for the localization of newly replicated DNA (*9*), some of the biochemical findings are conflicting (*10, 11*). Moreover, electron microscopic autoradiographic studies with [^{3}H]thymidine as precursor have demonstrated the distribution of the radioactive grains throughout the nuclear interior (*11, 12*). More recently, Fakan and Hancock (*13*) using exponentially dividing mouse cells (P815) in culture have shown that after short pulses of [^{3}H]thymidine (30 seconds), the label was preferentially localized at the borders between condensed chromatin and interchromatinic areas. Our electron microscopic studies of the nuclear protein matrix suggest that this residual framework structure is derived primarily from the interchromatinic structures of the nucleus.

In summary, the nuclear protein matrix which forms a residual framework extending from the nuclear envelope to the residual nucleolus, is associated with a fraction of nuclear DNA which is labeled rapidly during DNA synthesis in the regenerating rat liver nucleus. The characteristics of matrix DNA and the specific localization of the replicating sites within this fraction have not been resolved. Nevertheless, it appears that the nuclear matrix and the DNA associated with this structure may have an important role in the initiation and replication of DNA.

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Ocean Thermal Gradient Hydraulic Power Plant

Abstract. *Solar energy stored in the oceans may be used to generate power by exploiting thermal gradients. A proposed open-cycle system uses low-pressure steam to elevate water, which is then run through a hydraulic turbine to generate power. The device is analogous to an air lift pump.*

For the last few years, a voluminous literature on energy-related problems has shown one thing clearly: for the way of life we enjoy to continue phenomenal progress will be needed in power generation methods. Reallocation and minor reductions in the use of oil, coal, and nuclear fission can do little more than give us time for using our ingenuity—if we hurry. Only fusion and solar power promise long-time solutions, and if we are responsible enough to look ahead for the probable life of the earth, only solar power remains. One possible method of utilizing solar energy for power generation is discussed in this report.

In the last century, the French physicist D'Arsonval suggested use of a refrigerant as the working fluid in a machine using a closed Rankine cycle to exploit the thermal differences between the cold currents emanating from the polar regions and the warm currents, such as the Gulf Stream and the Japanese Current, emanating from tropical seas. Another French investigator, Claude, foresaw the extreme difficulties of heat transfer with the small temperature difference available (typically 22°C or less) and attempted to develop an open-cycle machine in the late 1920's (*1*). His efforts resulted in thermodynamic successes but ocean engineering disasters. His main difficulties were mechanical problems with the draft tube used to bring cold ocean water from ocean depths to the condenser at the surface. With 45 years additional ocean engineering experience, we are in a position to do better in this area. The problems of heat transfer with a low temperature difference, while better understood, are no less difficult than in Claude's time.

On the basis of Claude's work and some more recent studies of materials and ocean engineering, it would now be practical to build an open-cycle system using low-pressure steam such as Claude attempted. However, for the kind of power generation needed today it would be necessary to use low-pressure steam turbines perhaps hundreds of feet in diameter—far larger than anything built to date.

Recent work (*2*) supported by the National Science Foundation (and now under the new Energy Research and Development Administration) promises not only feasibility but perhaps reasonable costs in a closed-cycle system such as suggested by D'Arsonval. The projected size of the necessary heat transfer equipment is so large, and its fouling so poorly known, that the overall potential for development is far from proved. A simpler concept, preferably of the open-cycle type but avoiding the very low pressure steam turbine used by Claude, would provide a more reasonable engineering approach to early development. Such a concept is discussed here.

This concept would retain the simplicity and other advantages of the open cycle proposed by Claude and at the same time avoid the requirement for gargantuan turbines. I propose introducing the warm surface water through a restriction in the lower end of a vertical pipe. The resulting cavitation would provide the necessary nucleation for the formation of steam bubbles, which in a two-phase mixture in the vertical pipe would provide the gravity head to elevate the water well above the ocean's free surface. This would be done inside a pressure hull of the power plant, which could be sited on an island, such as Guam, or on an anchored floating platform. Theoretically, the maximum head could be very great—hundreds of feet. I suggest calling this vertical tube assembly the "steam lift water pump," as it is a steam-driven counterpart of the well-known air lift pump.

After the steam-water mixture exited from the upper end of the pump it would be separated, and the steam would be condensed in some variation of a barometric condenser. This simple device was used by Claude and was well known in power generation before the advent of the surface condenser. Loss of the condensate would be of no consequence since it is not recirculated as in a closed-cycle plant. The water would leave the power plant through a conventional hydraulic turbine under gravity flow into the ocean at a depth commensurate with its temperature, which would be reduced from the surface inlet temperature by evaporation and mixing with cold subsurface water.

This system would avoid most of the problems associated with the closed cycle. However, because of the height of the power plant above the ocean's surface, it could become unstable in tropical storms. For this reason, even if it were otherwise practicable, the very large tower would probably not be used on a floating platform, but only in a land-based power plant. For floating power plants there appears to be a more practical approach, involving a lower overall height. The steam lift water pump would be reduced in height, perhaps to a few tens of feet or less. At the upper end of this lift pump the steam-water mixture would be deflected horizontally and the remaining available energy imparted to the water by the steam. The steam would be condensed just ahead of a nozzle deliv-