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Serological Relationships between Nucleus, Cytoplasm, and Cytoplasmic Products and the Concept of Complementary Molecules

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The concept of antibodies as units complementary to their antigens was originated by Breinl and Haurowitz (*2*). Pauling (*10*) has developed the idea of antibody specificity as resulting from the folding of initially stretched-out polypeptide chains over limited regions of the antigen. The molecules thus folded into specific configurations become stabilized by bonds acting between their folds, and thereafter "fit" the specific regions of the antigen. Such complementary molecules (antibodies) can subsequently combine with their antigens, since the configurations permit the very close juxtaposition of combining groups necessary for bonding. The hypothesis does not neglect the role of chemical composition in specificity, for the nature and arrangement of the residues will determine the type of folding possible at various points along the polypeptide chain. Nevertheless, chemical composition provides only the potentialities for specificity or a limited degree of specificity, the maximal degree being achieved by the folding process.

The possibility of applying the concept of complementariness to the more general problem of specificity in biological synthesis has been broached in several recent discussions (*4, 11, 13*). As Tyler (*12,* p. 13) has stated,

> Any of the macromolecular constituents synthesized in a cell would be complementary to the substances comprising the sites of synthesis. Since growth consists primarily in the formation of such substances that comprise the integral structure of the cell, we may regard the mechanism of the process of growth to be essentially analogous to that manifested in antibody formation.

The liver offers favorable material for the testing of this hypothesis, since the three broad elements in the chain of synthesis (nucleus, cytoplasm, cytoplasmic products) are readily available. If (1) nuclear constituents are the prime determiners of cytoplasmic activity, as ample evidence from classical and biochemical genetics would indicate, and (2) the cytoplasm is in turn a site of synthesis, then we might expect the complementary relationships diagramed in Fig. 1. Nuclear constituents (perhaps highly polymerized nucleohistones) serve as templates (N_1) for the synthesis of complementary cytoplasmic constituents, some of which act as templates (C_1) for the synthesis of cytoplasmic products (P_1), which in the present instance would be the serum proteins of hepatic origin. Omitting from consideration many obvious complicating factors (intermediate products, metabolic modifications, etc.), we should expect the serum products to have configurations resembling, although not perhaps exactly duplicating, the original nuclear templates. Furthermore, if antibodies contain configurations complementary to limited regions of the antigens, we may expect the general antibody-antigen relationships shown in Fig. 1. As indicated by the arrows, antinuclear bodies should react maximally with nuclear material and serum, while antiserum bodies should react maximally with nuclear and serum constituents. This is not what would be predicted on the basis of chemical composition; cytoplasm and serum certainly show a greater over-all chemical resemblance to one another than do nucleus and serum.

Rat liver nuclei and cytoplasm were separated by the Dounce method (*3*), using M/475 citric acid in the first step and distilled water at pH 5–7 thereafter. The injection of whole nuclei into rabbits indicated a low degree

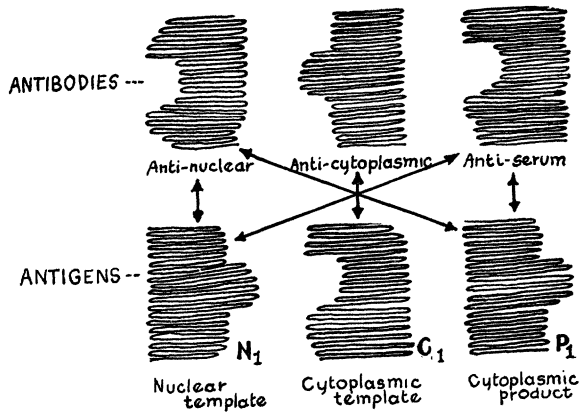

FIG. 1. Schema showing postulated complementary configurations of macromolecules of nucleus (N_1), cytoplasm (C_1), and cytoplasmic products (P_1). Portions of molecular chain represented by coiled lines. The arrows indicate expected maximal cross reactions between antibodies and antigens.

of antigenicity; hence the nuclei were treated with 10% NaCl solution to extract the nucleohistone (*9*) and make it available to the antibody-forming mechanism. All steps in the isolation and extraction procedures were carried out in the cold (6° C or below). The whole nuclei in 10% NaCl, alone or mixed with swine serum as adjuvant, were dialyzed first against water and then against physiological saline to remove excess salt and recombine the nucleic acid and histone. The products were injected intramuscularly and intraperitoneally into New Hampshire and White Leghorn fowl, and showed clear anti-

genicity. The following antigens were each injected into two birds, which were bled 8 days after the last injection:

(A) Nuclei treated as described, with 10% NaCl and dialysis.

(B) Nuclei treated as in (A) and mixed with swine serum as adjuvant.

(C) Defatted nuclei (using cold alcohol-ether, 1:1) suspended in 0.9% saline.

(D) Rat serum.

(E) Liver cytoplasm in 0.9% saline.

(F) Liver cytoplasmic fraction insoluble in 0.9% saline but soluble in 10% saline.

Antisera against these antigens were tested by layering against dilutions of the following test antigens:

(A_1) Nuclear fraction soluble in 10% saline.

(C_1) The 10% saline-soluble fraction from defatted nuclei.

(EF_1) Cytoplasmic fraction soluble in 10% saline. The antisera were diluted 1:1 with 20% NaCl solution, since the test antigen dilutions were prepared with 10% NaCl.

Table 1 shows the cross reactions obtained. Antinuclear sera reacted with all the test antigens, giving titers which conform on the whole with expectations derived from the concept of complementariness as here applied. Despite the low concentration of the nuclear test antigen (3.0

TABLE 1

CROSS REACTIONS OF ANTINUCLEAR AND ANTICYTOPLASMIC ANTIGENS AND ANTISERA*

Test antigens† mg/ml‡	Antisera versus: (A) Nuclei	(B) Nuclei + swine serum	(C) Defatted nuclei	(D) Rat serum	(E) Whole cytoplasm‖	(F) 0.9% Insoluble cytoplasm
A_1 Nuclear extract 3.0 mg	100	40,000	10	10	100	10
C_1 Defatted nuclear extract 14.6 mg	undiluted	10	10	20,000	10	0
D_1 Rat serum§	1000	40,000	0	40,000	0	0
EF_1 Cytoplasm‖ 23.5 mg	10	1000	100	10	100	100

TABLE 2

ABSORPTION OF ANTINUCLEAR SERA WITH CYTOPLASM AND RAT SERUM*

Test antigens† mg/ml‡	Unabsorbed antisera¶ (A) Nuclei	(B) Nuclei + swine serum	(C) Defatted nuclei	Absorbed antisera (A) Nuclei	(B) Nuclei + swine serum	(C) Defatted nuclei
A_1 Nuclear extract	100	1000	10	1000	1000	10
C_1 Defatted nuclear extract	1000	1000	1000	1000	1000	1000
D_1 Rat serum§	1000	40,000	0	0	0	0
EF_1 Cytoplasm‖	10	1000	100	0	0	0

Note: *Footnotes are applicable to all four tables.* Controls, not given in tables, were normal fowl serum layered against test antigens and antisera layered against 10% saline.

* Titers given as reciprocals.

† Fraction soluble in 10% saline.

‡ Solids in mg/ml of undiluted antigen; values given in Table 1 apply to corresponding test antigens in all tables.

§ Rat serum diluted 1:1 with 20% NaCl solution.

‖ Pooled cytoplasm from second and subsequent steps of Dounce's method.

¶ Unabsorbed antisera diluted with 10% saline to correspond to dilution of absorbed antisera, except in Table 4, in which 0.9% saline was used for dilution in hemolysis experiments; thereafter the salt concentration was increased to 10% for the test antigens.

TABLE 3

ABSORPTION OF ANTICYTOPLASMIC SERA WITH 10% SOLUBLE NUCLEAR EXTRACTS*

Test antigens† mg/ml‡	Unabsorbed antisera¶ (E) Whole cytoplasm	(F) 0.9% Insoluble cytoplasm	Antisera absorbed with: Nuclei, 10% NaCl fraction (E) Whole cytoplasm	(E) Whole cytoplasm	Defatted nuclei 10% NaCl fraction (F) 0.9% Insoluble cytoplasm	(F) 0.9% Insoluble cytoplasm
A_1 Nuclear extract	100	10	0	0	0	0
C_1 Defatted nuclear extract	10	0	0	0	0	0
D_1 Rat serum§	0	0	0	0	0	0
EF_1 Cytoplasm‖	100	100	100	100	100	100

TABLE 4

ABSORPTION OF ANTISERA WITH CHICKEN RED BLOOD CELLS*

Test antigens† mg/ml‡	Antisera versus: (A) Nuclei	(B) Nuclei + swine serum	(C) Defatted nuclei	(D) Rat serum	(E) Whole cytoplasm‖	(F) 0.9% Insoluble cytoplasm
	Unabsorbed antisera¶					
A_1 Nuclear extract	100	100	1000	10	100	10
C_1 Defatted nuclear extract	100	100	100	100	10	undil.
Hemolysis**	+	+	?	+	+	+
	Antisera absorbed with red cells					
A_1 Nuclear extract	100	100	10	100	100	10
C_1 Defatted nuclear extract	100	100††	100	100	10	10
Hemolysis**	0	0	0	0	0	0

** Suspensions of red blood cells tested: 0.2, 0.33, 0.5, 1.0, and 2.0%.

†† Titer possibly higher, since this was highest dilution tested.

mg/ml) and the high concentration of cytoplasmic antigen (23.5 mg/ml), the titer for the latter was low. The presence of swine serum (which probably formed conjugates with dissociated nucleic acid [5]), increased the titers generally but did not change the basic relationships.

Extracts of defatted nuclei gave titers similar to those obtained with whole nuclei except in the case of serum for which titers were low or completely negative. This, together with the titers shown by most of the antisera for defatted nuclear extracts, suggests that nuclear lipids are antigenically active. The situation is, however, by no means clear-cut, since antiserum (D) reacted to unusually high titer with defatted nuclear extract. Strict comparison between antisera (A) and (C) is not warranted, since the defatted nuclei were not treated with 10% saline.

Antirat serum showed highest titers with defatted nuclei (C_1) and serum (D_1). The low titer with whole nuclear extract does not conform to expectations unless we consider the low concentration of this test antigen.

Anticytoplasmic sera (E, F) showed little or no activity for serum (D_1) but gave unexpected high titers with nuclear extract (A_1). These results suggest a high degree of resemblance between nuclear and cytoplasmic constituents; in fact, the titers were as high as those obtained with antinuclear sera (A), and recall the numerous observations indicating the passage of nuclear constituents into the cytoplasm (cf. 8).

On the basis of complementariness (as well as the general chemical and enzymatic differences between nucleus and cytoplasm), we might expect antigenic specificity for both nucleus and cytoplasm. The results of absorption experiments are shown in Tables 2 and 3. Antinuclear sera absorbed with serum and cytoplasm retain essentially the same titers for nuclear extracts (Table 2). Anticytoplasmic sera absorbed with either whole nuclear or defatted nuclear extract also retain their anticytoplasmic activity (Table 3). Serum apparently also shows antigenic specificity distinct from that of the nucleus and cytoplasm, since anticytoplasm reacts very poorly or not at all with serum. However, this point was not tested directly. We should expect to find antiserum bodies which are nonreactive with liver cell constituents, since serum constituents are not exclusively of hepatic origin (e.g., pituitary factors, gonadal hormones, etc.).

Nuclear, cytoplasmic, and serum specificity were further demonstrated by absorption with chicken red blood cells (Table 4). The antisera showed hemolytic activity shortly after they were obtained. The hemolytic activity was lost after storage for seven weeks in the cold and was not renewable by the addition of fresh guinea pig serum. Although this indicated the absence of hemolysins, the antisera were absorbed with a 4% suspension of fowl red blood cells. As shown in Table 4, the titers for nuclei, cytoplasm, and serum were little affected, with the exception of the antidefatted nuclear serum (C).

We believe that nuclear specificity may have a significant bearing on theories of embryonic differentiation. If one could obtain all the products synthesized by liver cytoplasm, then on the basis of complementariness the cell products (P_1 in Fig. 1) should be able to combine with all antinuclear bodies. Our closest approach to an experiment like this was the absorption of antinuclear sera with both liver cytoplasm and serum (Table 2). This presumably provides all the products synthesized by liver cytoplasm, whether they retain an intracellular position or are extruded into the serum, with the possible exception of fibrinogen and other proteins possibly removed with the blood clot. The fact that such absorption left antibody activity specific for the nucleus suggests that some constituents of the liver nucleus are not concerned in synthesis. From the point of view of cellular differentiation it will be of interest to ascertain whether such nucleus-specific antibodies also occur in nuclei from other tissue, and if so whether or not they are identical.

Earlier work on the antigenicity of nuclear constituents (reviewed in 6, 14) was based on extracts of whole tissues rather than on isolated nuclei, and in all probability involved higher degrees of denaturation and degradation than is produced by more recent methods. The results were conflicting both as to the antigenicity and specificity of nuclear constituents. The more recent work of Maculla (7) was also done on extracts of whole tissues, but some of her results (particularly on liver fractions) are in good agreement with corresponding portions of our data. Results with other organs (e.g., lung and kidney) did not accord with those for liver. Arnesen *et al.* (1) did not find evidence of specificity as between nuclei and various cytoplasmic granules of the spleen. However, they utilized the complement fixation method and *whole* nuclei, which made only the nuclear surface available for antibody combination.

Perhaps the essential phenomena brought out by the present study are: (1) on the basis of antibody titers certain constituents of serum have a greater resemblance to certain nuclear constituents than to cytoplasmic constituents; (2) similarly, nuclear constituents of the liver resemble serum more than they do liver cytoplasm; and (3) the nucleus and cytoplasm contain constituents (or determinants) distinct from one another, and from those in serum and on the surface of red blood cells of the same species.

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