products and the modification of thermal denaturation profiles and chromatographic behavior (*7*). Agents that alter DNA structure are known to elicit the functioning of bacterial repair processes (*14*). Possibly pertinent is the observation that malonaldehyde is reported to be particularly prone to react with guanine and cytidine (*7*) and that the frameshift revertants of the hisD3052 mutation observed in the present study appear to be expressed as a result of deletions of GC base-pairs (*15*).

The relationship between the present findings and human carcinogenesis or mutagenesis possibly associated with lipid peroxidation or the ingestion of a diet high in polyunsaturated fatty acids remains conjectural. In this respect it should be noted that the frameshift mutants utilized in our studies are particularly sensitive to mutagenesis by a number of polycyclic carcinogens (*16*), at least some of which are also associated with the production of lipid peroxidation during their metabolism (*5*). An additional area of possible pertinence, and the original impetus for this study, concerns the observation of chromosomal aberrations in the circulating lymphocytes of man and Chinese hamsters following inhalation of relatively low levels of ozone (*17*), an air pollutant known to be mutagenic in animals and bacteria (*18*). It is unlikely that this extremely reactive oxidant gas, which produces lung lipid peroxidation (*19*), can itself reach directly into the nucleus of circulating lymphocytes. As malonaldehyde is relatively polar it conceivably could penetrate into chromosomal material following membrane lipid peroxidation produced by ozone or other exogenous agents. In addition, a malonaldehyde-like compound has been reported to be formed during x-irradiation of DNA itself (*20*). Further study is required to ascertain the relationship of lipid peroxidation and malonaldehyde formation to human carcinogenesis and mutagenesis.

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A Serum Protein Associated with Chromatin of Cultured Fibroblasts

Abstract. *Antibodies to chromatin proteins from human and mouse fibroblasts which have been cultured for more than 25 generations with heterologous serum show specificity for a homologous α-serum protein. These results indicate that among the chromatin-associated proteins there is one (or more) which has extensive structural similarity to a serum protein. This is the first direct evidence that a serumlike protein or proteins could be chromatin associated in vivo, as has been suggested by experiments showing in vitro interaction between DNA and certain serum proteins.*

In bacteria, gene regulator proteins bind to specific sites of DNA. This suggests that in eukaryotes also, the capacity to bind DNA may be an important property of the proteins involved in genomic functions. For this reason a large number of nuclear and cytoplasmic proteins with the ability to bind to DNA have been extensively studied in multicellular organisms (*1–3*).

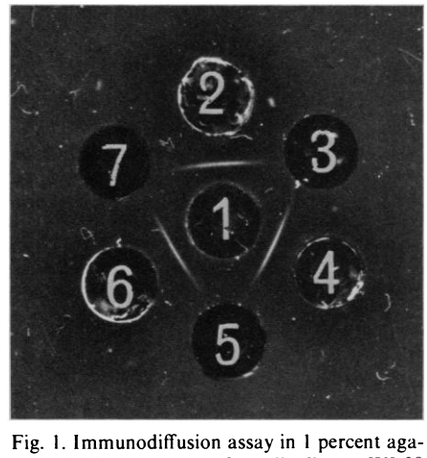

Fig. 1. Immunodiffusion assay in 1 percent agarose. *1*, Human serum; *2*, antibodies to WI-38 chromatin; *3*, mouse serum; *4*, as in *2* after adsorption by calf serum; *5*, as in *2* after adsorption by WI-38 chromatin; *6*, as in *2* after adsorption by 3T6 chromatin; *7*, preimmune serum. Chromatin and chromatin fractions were prepared as previously described (*7, 12*). Antibody adsorptions were performed mixing 1 ml of antiserum with 2 mg of DNA as chromatin in tris buffer, *p*H 8.2, 0.15*M* NaCl; or with 2 ml of serum. The antigen and antiserum mixture was incubated for 2 hours at 37°C and 16 hours at 4°C. At the end of the incubation the mixtures were centrifuged at 30,000*g* for 20 minutes. The supernatants were used for the immunodiffusion assay. The final antiserum dilution was 1 : 4. The dilution of serum used as antigen was 1 : 8.

It has been reported that mammalian serums and amniotic and cerebrospinal fluids also contain proteins capable of reacting with DNA in vitro (*4–6*). Kubinski and Javid (*5*) showed an increase in the concentration of such DNA-binding proteins in cerebrospinal fluid of patients with certain diseases of the central nervous system. More recently, Hoch *et al.* (*6*) showed that human serum contains a group of proteins with affinity for DNA and that the serum of patients with malignant disorders contains a higher concentration of such proteins. These authors suggested that these proteins could be bound to DNA in vivo and may be involved in genomic function. However, although the affinity of proteins for DNA may be related to their biological function, there is no direct evidence that serum proteins which bind to DNA in vitro are also bound to DNA in vivo.

In this report we provide evidence that serum proteins could be DNA associated in vivo. Using antibodies to chromatin proteins, we have found that a serum protein has extensive structural similarity to nonhistone chromosomal proteins.

Antibodies to chromatin proteins were obtained by injecting rabbits with chromatin preparations from 3T6 mouse or WI-38 human fibroblasts as previously reported (*7*). In the immunodiffusion assay we observed only a single precipitin line between each of our antiserums (five preparations for 3T6 chromatin and eight preparations

for WI-38 chromatin) and freshly prepared immunogens. This is in agreement with an earlier report by Messineo (*8*) on immunodiffusion studies of leukocyte chromatin and its antibodies. Such antibodies have a stronger specificity for nonhistone chromatin proteins than for histones, and practically none for DNA (*7*).

Antibodies to WI-38 human fibroblast chromatin, in the immunodiffusion assay, react with human serum showing a pattern of partial identity with the chromatin precipitation line. No reaction was observed with heterologous serums. The precipitation with human serum was not detected after antibody adsorption by human serum but not by calf serum (Fig. 1). A similar inhibition of the precipitation reaction between human serum and antibodies to WI-38 human fibroblast chromatin was also observed after antibody adsorption by WI-38 chromatin but not by 3T6 chromatin. In order to establish to which chromatin fraction such antibody adsorption was due, WI-38 chromatin proteins were purified and fractionated into histone and nonhistone chromosomal proteins and antibody adsorbed by these two separate fractions. Precipitation inhibition was observed only when nonhistone chromosomal proteins were used (Table 1).

Antibodies to 3T6 mouse fibroblast chromatin showed specificity to mouse serum, and inhibition of the reaction was obtained only with 3T6 chromatin, 3T6 nonhistone chromosomal proteins, or homologous serum.

In all cases, precipitation lines obtained from the reaction between antichromatin antibodies and homologous serums gave negative results when tested by the Feulgen-Formazan color reaction for the identification of nucleic acids (*9*). During immunoelectrophoresis the chromatinlike serum proteins moved in the α-region, giving a single precipitin arc in both human and mouse serum (Fig. 2).

These present results show that in at least two different species, antibodies to fibroblast chromatin react with a homologous serum protein. The pattern of partial identity obtained between chromatin and homologous serum indicate that, as expected, not all the antibodies induced in rabbits by chromatin react with serum proteins (*10, 11*). Therefore, these experiments indicate that some of the determinants present on nonhistone chromosomal proteins are also present on a homologous serum protein or proteins. The presence of only a single arc in the immunoelectrophoretic assay (Fig. 2) indicates that these common determinants are very likely to be localized in only a single serum protein. In conclusion, these results indicate

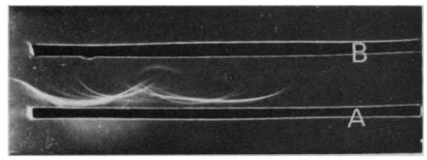

Fig. 2. Immunoelectrophoresis of mouse serum in 1 percent agarose. (A) Rabbit antiserum to total mouse serum; (B) rabbit antiserum to 3T6 mouse fibroblast chromatin.

that among nonhistone chromosomal proteins at least one has extensive structural similarity to a serum protein (*10*).

The purity of chromatin preparations used to induce antibodies in rabbits may be the most critical step in this study. We can exclude any contamination from homologous serum, since the human and mouse fibroblasts were cultured for more than 20 generations in calf serum. Also, it has been established that cytoplasmic contaminations in chromatin, prepared as described, are negligible (*12*). However, minimal amounts of such contamination having high immunogenic activity are difficult to rule out. In the context of this report, chromatin must be considered as an operational definition.

The biological significance of this protein in the nucleus has not yet been established, but we can include this protein in the heterogeneous group of nonhistone chromosomal proteins. Recent studies have suggested that nonhistone chromosomal proteins may be responsible for the regulation of differential gene expression and cellular proliferation (*2, 13*). However, in addition to regulating the transcription of specific genome loci, the nonhistone chromosomal proteins include a large number of enzymes and proteins involved in determining the structure of the chromatin.

Table 1. Immunodiffusion assay of antichromatin antibodies. Inhibition of the precipitation reaction between human serum and antibodies to WI-38 chromatin (A) or mouse serum and antibodies to 3T6 chromatin (B) after adsorption of antiserums by different immunoadsorbents. For the procedures, see Fig. 1. Abbreviations and symbols: NHCP, nonhistone chromosomal proteins; plus sign (+), reaction of precipitation; minus sign (–), absence of precipitation reactions.

Immunoadsorbents	A	B
Human serum	–	+
Mouse serum	+	–
Calf serum	+	+
WI-38 chromatin	–	+
3T6 chromatin	+	–
WI-38 NHCP	–	+
3T6 NHCP	+	–
WI-38 histones	+	
3T6 histones		+

At present, it is not known whether this protein can migrate from the serum to the cell nucleus and vice versa. However, it is accepted that mammalian cells take up macromolecules even though little is known about this process (*14*). Kolodny (*15*) reported that in cultures of 3T3 cells, even histones and nonhistone chromosomal protein can move from cell to cell. Michl and Spurna (*16*) recently showed that cultures of human diploid LEP 19 cells take up into the nuclei a serum α-globulin which has been shown to have mitogenic activity in cultured mammalian cells.

All these observations suggest that more consideration must be given to the possibility of a direct action of extracellular factors on the genomic functions.

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