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DNA swivel enzyme activity in a nuclear membrane fraction

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ABSTRACT

DNA swivel (nicking-rejoining) enzyme activity has been studied in various cell fractions of a human lymphoid cell line. Swivel activity is found only in chromatin and in a nuclear membrane fraction containing DNA and possessing endogenous DNA synthesizing activity. Twenty percent of the total swivel activity and less than one percent of the total DNA are in the membrane fraction. The swivel enzyme is more tightly bound to the membrane fraction than to the chromatin fraction. These observations suggest that the swivel enzyme may be a replication factor, specifically bound to replicating DNA in the membrane fraction.

INTRODUCTION

Enzymes capable of untwisting supercoiled DNA by the nicking and rejoining of a single strand have been found in a variety of mammalian and other cells (1-5). These enzymes may provide the swivel necessary for the unwinding of parental strands during the replication of circular or very long DNA molecules. We have shown that swivel enzyme activity in cells of a human lymphoid line is high during S phase and is low or absent during G_o and G_1 (6). Thus the swivel enzyme may be a replication factor.

Mammalian swivel enzymes have been found in the nucleus (1,5). In the present work we have undertaken a more specific localization by studying the swivel activity in various cell fractions. Using low ionic strength in order to avoid elution of the swivel enzyme from DNA, the activity is found entirely in two particulate fractions: chromatin and a complex nuclear membrane fraction. Cell fractionation in physiological salt solubilizes the activity from the chromatin but not from the membrane. The nuclear membrane fraction contains 20% of the total swivel activity, tightly bound to less than 1% of the total cellular DNA. The same nuclear membrane fraction has been found to possess an RNAase-sensitive endogenous DNA synthesizing activity (7) and to contain a γ-like DNA polymerase (8). DNA synthesis in this fraction is markedly stimulated by addition of the four ribonucleoside triphosphates and by DNA-binding protein(s) isolated from the cytoplasmic fraction

(9). The presence of swivel enzyme activity in this fraction, which has many of the properties of a replication complex, supports the possibility that the swivel enzyme may be a replication factor.

MATERIALS AND METHODS

A normal human lymphocytic cell line, WiL_2, was routinely grown as described elsewhere (6). Frozen, late-log cells were thawed and fractionated into cytoplasm, chromatin, nuclear membrane and nucleoplasm. The fractionation was carried out at 0° in a physiological salt buffer as described previously (7); nuclei were sonicated for 10 sec. Alternatively, fresh, log-phase cells were fractionated by essentially the same procedure except that all saline solutions were replaced by an isotonic sucrose buffer (0.25 M sucrose, 0.05 M Tris pH 7.5, 0.025 mM KCl); after lysis of the cells for 5 min in 0.1% NP40, $MgCl_2$ was added to a concentration of 0.005 M (and this was retained in the buffer in future steps), the cells were sheared by pipetting, and the nuclei were removed by centrifugation for 5 min at 190xg; the supernatant (cytoplasm) was re-centrifuged for 5 min at 430 xg to remove a small amount of nuclear contamination.

The cell fractions were made 1 M in NaCl and then dialysed overnight at 0° against assay buffer (0.2 M NaCl, 0.001 M EDTA, 0.01 M Tris pH 7.9). The chromatin fraction was centrifuged 10 min at 16,000xg to remove particulate material just before assaying. It was shown that there was no reassociation of swivel enzyme with the particulate material when the salt concentration was lowered by dialysis, since the soluble activity was no greater if the chromatin fraction was centrifuged before dialysis. The apparent absence of reassociation with the nuclear membrane fraction is discussed in Results. The fractions were assayed either immediately after dialysis or they were frozen at -20° for 1-6 days before assaying; the activities were stable for this period.

The DNA swivel activity of each cell fraction was determined by measuring its ability to untwist supercoiled SV40 DNA I. The reaction was carried out for 5 min at 37° in the assay buffer mentioned above, as described previously (6); higher concentrations of EDTA did not affect the results. The amount of SV40 DNA I untwisted was determined by agarose gel electrophoresis (6). This technique shows the conversion of supercoiled DNA I to partially uncoiled intermediates (10,11) and totally uncoiled DNA. However, since there are two totally uncoiled forms of DNA, the nicked form II and the closed circular form I_o, which band together by this method, we also carried out electrophoresis in the presence of ethidium bromide. In the latter method, forms II and I_o band separately. The results, similar to those illustrated in our earlier work (6), demonstrate the absence of nuclease activity since no form II is produced.

To determine the amount of DNA in each cell fraction, a culture was labeled for one generation with (^{14}C)thymidine (0.25 μCi/ml, 49 mCi/mM), fractionated as described above, and aliquots of each fraction were acid-precipitated, washed, oxidized in an Intertechnique Oxymat and counted in a scintillation counter.

RESULTS AND DISCUSSION

Cell Fractionation. Human lymphoid cells in late log phase were fractionated into cytoplasm, chromatin, nucleoplasm and nuclear membrane. The cells were

lysed with NP40 and separated into nuclear and cytoplasmic fractions. The nuclei were lysed by treatment with NP40 + DOC followed by very mild sonication. The chromatin fraction was then removed by low-speed centrifugation, and the supernatant was fractionated by higher-speed centrifugation into a soluble nuclear fraction, the nucleoplasm, and a particulate nuclear membrane fraction. The nuclear membrane fraction has been shown to contain membrane, DNA, RNA, DNA polymerase, RNA polymerase activity, and possibly other factors, and it possesses endogenous, RNAase-sensitive DNA synthesizing activity (7,8,9).

The fractionation procedure was carried out originally in an isotonic salt buffer (7). We have repeated this and have also utilized an isotonic sucrose buffer of low ionic strength, in order to avoid dissociation of the swivel enzyme from DNA.

Swivel Activities of Cell Fractions. Each cell fraction was treated with 1 M NaCl to release any bound swivel enzyme, then dialysed against the assay buffer and assayed for swivel activity. The procedures are reported elsewhere (6) and are discussed in Methods. Assays of each fraction were carried out at a series of concentrations in order to find the measurable range. Data for cell fractions prepared in low salt, and their conversion to relative activities, are given in Table I.

The relative activities of the cell fractions obtained by fractionation in low salt or in physiological salt buffers are compared in Table II. In low salt preparations the DNA swivel activity is found entirely in particulate fractions of the nucleus; there is no activity in the cytoplasm or nucleoplasm. But when the cells are fractionated in a buffer containing 0.15 M NaCl, it is clear that essentially all the chromatin-bound activity is eluted early in the fractionation procedure from the nucleus into the cytoplasm fraction. This appears to rule out the possibility that the swivel enzyme in the chromatin fraction might be a form of the histone F1 (12), which would not be dissociated from DNA by 0.15 M NaCl.

While the swivel enzyme activity is eluted from chromatin, the activity in the nuclear membrane fraction remains stably bound. This indicates that the swivel enzyme is more tightly bound to the membrane fraction than to chromatin. The stable binding of DNA swivel activity to the nuclear membrane fraction in the presence of 0.15 M NaCl is emphasized by the lack of activity in the nucleoplasmic fraction, which is the supernatant from which the membrane fraction is separated. Moreover, we found that the nuclear membrane fraction had little or no swivel activity (as assayed in buffer containing 0.2 M NaCl) unless it was first treated with 1 M NaCl to solubilize the enzyme. Thus, the swivel enzyme remains bound to the membrane fraction in 0.2 M NaCl and is not active on exogenous DNA while it is membrane-bound. Once released by higher salt, however, reduction of the salt concentration to 0.2 M does not appear to result in reassociation of the enzyme with the membrane fraction.

Table I. DNA Swivel Activity in Cell Fractions Isolated in Low Salt

Cell Fraction	Concentration (cells per assay)[a]	Activity (% of Supercoiled DNA Untwisted)	Relative Swivel Activity[b]	Activity per unit weight of DNA[c]
Cytoplasm	$84x10^4$	0 ±5	<1	
Chromatin	$5.6x10^4$	30	100	1
Nuclear Membrane	$40x10^4$	53	25	28
Nucleoplasm	$40x10^4$	0	<2	

Cells were fractionated in a sucrose buffer containing 0.05 M Tris, 0.005 M $MgCl_2$ and 0.025 mM KCl; cell fractions were treated with 1 M NaCl and dialyzed against the assay buffer before carrying out the reaction (see Methods). Activity was determined by measuring the percent of supercoiled SV40 DNA I untwisted during the reaction, as observed subsequently by gel electrophoresis. The values given are the average of 2-3 determinations. There is an uncertainty of about 5% in the activity results.

a) The concentration of each cell fraction in the reaction mixture is given in terms of the number of cells from which it was extracted.

b) Relative activities correspond to the activities of the different fractions at a fixed cell concentration; the fixed concentration ($19x10^4$) was arbitrarily chosen so that the fraction with the highest activity has a relative activity of 100. Calculation of relative activities from the data in the table is validated by the finding that activity is proportional to conentration.

c) The activity per unit weight of DNA was determined from the relative swivel activity and the fraction of the total DNA in each cell fraction (see text).

By labeling the cells with (^{14}C)thymidine before fractionation it was shown that the nuclear membrane fraction contains about 0.9% of the total DNA and the chromatin contains essentially all the remainder. However, since the nuclear membrane fraction contains 20% of the total swivel activity and the chromatin contains 80%, the swivel enzyme activity per unit weight of DNA is therefore 28 times greater in the nuclear membrane fraction than in the chromatin fraction (Table II). This, together with the strength of the binding, indicates that the swivel enzyme in the nuclear membrane fraction is probably specific rather than randomly or artifactually bound.

We conclude that in mammalian cells a significant proportion of the DNA swivel enzyme activity appears to be specifically bound to a nuclear membrane fraction which possesses endogenous DNA synthetic activity and has many of the properties expected for a native DNA replication system. This is consistent with the possibility that the swivel enzyme may be a replication factor. It also suggests that replication may take place, at least in part, on the nuclear membrane, although this remains a controversial question (13, 14). Whether or not the nuclear membrane is the locus of replication *in vivo*, it appears from our work and the biochemical studies of others (cited in refs. 13 and 14) that the membrane as obtained *in vitro* carries with it replicating DNA (13,14) and other parts of the putative replication system

Table II. Relative DNA Swivel Activities of Cell Fractions Isolated at Different Salt Concentrations

Cell Fraction	Relative Swivel Activity	
	Low Salt	Physiological Salt
Cytoplasm	<1	100
Chromatin	100	<2
Nuclear Membrane	25	25
Nucleoplasm	<2	<1

Cells were fractionated in either a low salt buffer (0.25 M sucrose, 0.05 M Tris pH 7.5, 0.005 M $MgCl_2$, 0.025 mM KCl) or a physiological salt buffer (0.15 M NaCl, 0.01 M Tris pH 7.8, 0.001 M $MgCl_2$) (7). Cell fractions were treated with 1 M NaCl and dialysed against assay buffer, and their relative activities determined as discussed in Table I and Methods.

(7,8,9), as well as the DNA swivel enzyme, presumably in the form of a replication complex.

The portion of the cellular swivel enzyme activity that is not in the membrane fraction is weakly bound to chromatin. Although it is possible that the chromatin-bound enzyme represents a different species, it seems more likely to us that it is adventitiously bound to inactive sites, while the more tightly-bound enzyme in the nuclear membrane fraction may be part of a functional complex.

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