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March 23, 1971

Dr. Arthur Kornberg
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Dear Arthur,

Just a note to let you know of the progress of our work on the E. coli "32-protein" here. Nolan Sigal has about 1 mg of the pure protein and has characterized it by DNA denaturation, stoichiometry of single-stranded DNA binding, and detailed structure by electron microscopy of the complexes. Except for the fact that he has yet to detect catalysis of renaturation, and that its MW on SDS-gels is 20,000 daltons, it looks just like 32-protein so far. We have arranged for Tom (Kornberg) to test its effect on DNA polymerases I, II, and III from E. coli. For this purpose, Nolan will spend the latter part of this week at Columbia.

The procedure Nolan uses is to make a sonicated extract from 17 liters of late log cells, remove all DNA by DNAse treatment, dialyze vs. EDTA after a high speed spin (see our Nature paper on 32-protein), and then load onto a 10 ml single-stranded DNA-cellulose column in 0.05 M NaCl, 0.02 M Tris, 7.4, 0.001 M EDTA, 10% glycerol, 0.001M Mercaptoethanol, After an extensive rinse, most DNA binding proteins are eluted by addition of 1 mg/ml of Dextran-sulfate 500 (Pharmacia) to the loading buffer. After a rinse to remove dextran sulfate, the remaining proteins are eluted with 0.6 M and 2 M NaCl rinses. Eluting between 0.6 and 2M are 4 main proteins, of MW 75,70,20, and 10 thousand daltons respectively. The 20,000 protein is obtained by DEAE chromatography (elutes 0.2-3 M NaCl), followed by dialysis vs. 10% glycerol, 1 mM mercaptoethanol, 0.04 M KPO_4 pH7 where it precipitates pure. It is stored frozen in 10% glycerol, 2 mM Tris 8.1, 10 mM NaCl, 1 mM mercaptoethanol at about 1 mg/ml. It is estimated to be 0.05-0.1% of the total soluble protein.

Your student Scheckman phoned. He has probably been working with the 10,000 MW proteing which is not of 32-protein type. We don't know what it is; Nolan has that homogeneous also, and will give some to Tom to test.

It seemed to me to make much more sense for us to do the polymerase work with Tom rather than Scheckman, since Tom is not only conveniently located, but has 3 polymerases available to test.

I hope that this plan meets with your approval.

Best regards,

Bruce

Bruce Alberts

BA/ew