

Is c-Myc Protein Directly Involved in DNA Replication?

G. P. Studzinski *et al.* (1) have proposed that c-myc protein is involved directly in mammalian DNA replication. This conclusion is based on their observations that polyclonal and monoclonal antibodies against c-myc protein inhibit both DNA synthesis and DNA polymerase activity in nuclei isolated from HL60 and ML-1 cells, human leukemic cell lines that contain high concentrations of c-myc protein. We have attempted to confirm and extend these observations by testing a variety of antibodies against human c-myc protein for their ability to interfere with simian virus 40 (SV40) DNA replication under conditions (in vitro) that allowed initiation of new replicons and continuation of DNA replication at the resulting forks (2). We have also measured the ability of these antibodies to inhibit DNA polymerase- α or - δ activity, or both, on DNase I-activated DNA primer templates.

Our findings (3) can be summarized as follows. (i) Antibodies to the c-myc protein supplied by G. Studzinski and R. Watt and antibodies developed independently in one of our laboratories were able to recognize human c-myc protein. However, the inhibitory activity for SV40 DNA replication and DNA polymerase activity was present only in the samples supplied by Studzinski and Watt. Therefore, the ability of antibody preparations from different laboratories to recognize c-myc protein did not correlate with their inhibitory activity. (ii) Inhibition of SV40 DNA replication and DNA polymerase activity by some c-myc antibody preparations could not be prevented by incubating the antibody preparations with an excess of c-myc protein (provided by Studzinski and Watt), in contrast with their previous report (1). (iii) The inhibitory activity against DNA synthesis was separated from the antibody activity against c-myc protein by two different methods. The antibody activity bound tightly to *Staphylococcus aureus* protein A, while the inhibitory activity did not. Second, antibodies were recovered in the flow-through during gel filtration with Sephadex G-50, while the inhibitory material remained in the column. (iv) Finally, using a protein A-Sepharose column in our laboratory, we purified monoclonal antibody B3, previously reported to be highly inhibitory of DNA synthesis (1),

from the corresponding hybridoma medium (supplied by R. Watt). B3 antibody purified in this way did not inhibit DNA polymerase activity, SV40 DNA replication, or DNA synthesis in nuclei isolated from HL60 cells; but it did react strongly with c-myc protein.

We conclude that the inhibition of DNA synthesis observed by Studzinski *et al.* (1) can be explained by contamination of their antibody preparations by an unidentified inhibitor of DNA polymerase- α or - δ , or both, instead of by a specific reaction with c-myc antibodies. Purified c-myc antibodies did not inhibit DNA synthesis at cellular or SV40 replication forks, did not inhibit DNA polymerase- α activity on a purified DNA substrate, and did not inhibit initiation of SV40 DNA replication in vitro.

While our results suggest that c-myc protein does not play a role in DNA synthesis at replication forks, they do not address the question of a role for c-myc in the initiation of cellular DNA replication. Our observations are consistent with those of Kaczmarek *et al.* (4), who found that injection of a monoclonal antibody against c-myc protein into the nuclei of cultured fibroblasts did not prevent their entry into S phase, whereas microinjection of an antibody against DNA polymerase- α did.

Note added in proof: Iguchi-Arigo *et al.* (5) have recently reported that c-myc protein binds specifically to a putative cellular origin of replication, and that plasmids that contain these sequences replicate when transfected into HL60 cells or incubated with HL60 extracts. The same laboratory has reported that c-myc could inefficiently substitute for SV40 T-antigen in initiating replication at either a functional or nonfunctional SV40 origin of replication (6). So far, we have not been able to reproduce these observations using a plasmid (pARS65, provided by H. Ariga) that contains one of these putative cellular origins or a plasmid containing the SV40 origin of replication. It has also been recently reported that a c-myc antisense oligodeoxynucleotide inhibits entry of cells into S phase (7) and that elevated c-myc expression facilitates SV40 replication in human lymphoma cells (8). These observations are consistent with the hypothesis that c-myc protein may have an indirect role in DNA replication, possibly by

regulating the level of gene products required for cell proliferation which, in turn, allows higher rates of SV40 DNA replication. In summary, a direct role of c-myc protein in DNA replication has not been demonstrated conclusively, and the function of this nuclear oncogene remains unclear.

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Response: After our report (1) of the inhibitory effect of antibodies to the bacterially expressed c-myc protein on DNA replication in nuclei isolated from human leukemic cells was published, I approached DePamphilis's group at Roche for help in further analysis of this effect. We sought this collaboration because this group had ongoing studies of SV40 replication, a system that permits initiation as well as elongation of DNA chains in vitro. We supplied the Roche group with stored samples of the antibodies and the myc protein that were used in the experiments that we reported (1), and the initial experiments were encouraging. Later it became clear that in this system the myc protein did not neutralize the replication-inhibitory effect of the antibodies and that after additional purification of the antibodies the activity inhibitory to the replication of SV40 DNA was dissociated from the immunoreactivity to c-myc. The Roche group has already published these results (2).

On learning of the results seen in the