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-Ariga *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 oligodeoxyribonucleotide 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 enconcouraging. 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

SV40 system, we repeated our reported experiments using isolated HL60 nuclei (*1*). The *myc* protein, after approximately 1 year of storage, no longer neutralized the inhibitory effect of the antibodies. Gel electrophoretic analysis showed altered properties which indicated partial degradation of these protein samples. Further purification of the polyclonal antibody to the c-*myc* recombinant protein, which was used in our previously reported experiments, reduced, but did not abolish, its inhibitory effect on DNA synthesis in this system.

These findings clearly raise the possibility that a component of the preparation other than the c-*myc* antitody inhibited DNA synthesis in the SV40 system. However, it is also possible that a deterioration of the samples during the prolonged storage has been responsible for the results of the Roche workers. In this context, it should be noted that in a more recent study antibodies to c-*myc* from another source were found to inhibit DNA replication of an autonomously replicating plasmid containing DNA sequences derived from mouse liver when they were cotransfected into HL60 cells, or in an in vitro replicating system employing HL60 nuclear extract (*3*). Another group has reported that elevated c-*myc* expression facilitates the simultaneous replication of SV40 DNA in human lymphoma cells (*4*), which can be due to indirect or direct effects on DNA replication. Thus, further work seems necessary to resolve this issue in the light of these conflicting data.

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ance of the new. An example is their offering of an adjustable-speed motor drive, based on solid-state power devices already in volume production, to counter the introduction of a sophisticated variable-speed drive based on an innovative new class of power devices. Although the newer devices were the lower cost alternative at equal volumes of production, they incurred higher initial costs because of low start-up volumes. Moreover, increasing production to sufficiently high volumes for competitiveness was threatened by the market share captured by the admittedly lower performing, but still satisfactory, Japanese product.

The close tie, or often the lack of distinction at all, between the engineers engaged in manufacturing and those engaged in development is one of the many factors that contribute to a rapid development and manufacturing cycle. This short cycle in turn fits well with the exploratory approach to marketing. The Japanese company gets the product out fast, finds out what is wrong with it, and rapidly adjusts; this differs from the U.S. method of having a long development cycle aimed at a carefully researched market that may, in fact, not be there.

The intentness with which the Japanese learn what is going on in the rest of the world is legendary. They usually seem more willing than their U.S. counterparts to learn about and profit from what others have done. They appear generally to suffer less from the psychological aspects of the NIH syndrome, which often seems to debar U.S. engineers, raised and rewarded on individual creativity, from accepting the ideas of others. Less well known is the general openness of the Japanese to discuss what they themselves are doing. Japanese engineers, in our experience, are willing to talk and are