SV40 LARGE T ANTIGEN INTERACTS WITH THE MITOTIC CHECKPOINT PROTEIN BUB1

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INTRODUCTION. Simian virus 40 (SV40) is a member of the papovavirus family of DNA tumour viruses. The viral oncoprotein SV40 Large T antigen (LT) is sufficient for immortalization of many rodent cell types, and can also transform these cells at a low frequency (1). It carries out these functions mainly by association with host factors. Some of the critical activities of LT required for these functions map to the N-terminus of the protein comprising the pRB binding activity and the J domain - a region with homology to the DnaJ family of molecular chaperones (2).

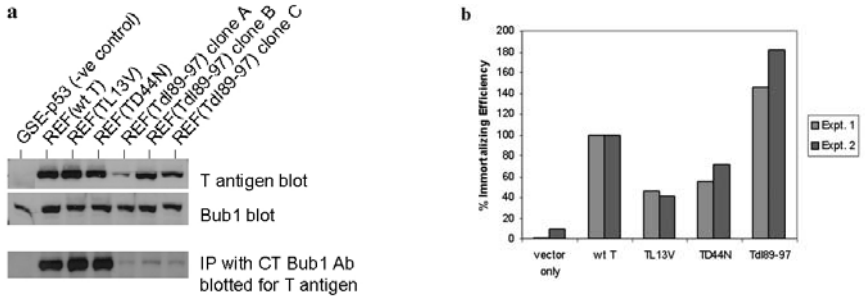
We have identified an interaction between this N-terminal region of LT and the spindle checkpoint protein Bub1 (3). Studies are ongoing to elucidate the effect of the interaction on the spindle checkpoint.

METHOD. The interaction was initially identified via a yeast two-hybrid screen using amino acids 1-136 of LT as a bait and a HeLa cDNA library as prey, and was confirmed via co-immunoprecipitation. Rat Embryo Fibroblasts (REFs) were transduced with ecotropic viruses containing different mutants of T antigen and following selection colony counts were used to determine immortalization frequency. Clones were isolated from each and used to map the interaction site of Bub1 on LT via co-immunoprecipitation. Focus assays were carried out to determine the ability of each mutant to transform Rat-1 cells. We have also generated our own monoclonal antibodies against the mouse Bub1 protein.

Antigens against the N-terminus and C-terminus of mBub1 were produced by cloning into the pET-23a(+) E. coli expression vector, incorporating a His-tag into the C-terminus of each protein to allow purification by affinity chromatography.

RESULTS. Bub1 binds to amino acids 89-97 of LT(see Fig.1a). Interaction of Bub1 with LT is not necessary for immortalization by LT (see Fig.1b), but interestingly we have shown that the interaction is required for the transformation function of LT.

Fig 1. Mapping of Bub1-LT interaction site via co-IP(a). The non-Bub1-binding (dl89-97) LT mutant retains its ability to immortalize REFs (b).



DISCUSSION. We have demonstrated that LT interacts specifically with the mitotic spindle checkpoint protein Bub1, the first report of a direct link between LT and the mitotic machinery. Interaction with Bub1 is not required for immortalization by LT but appears to be important for transformation. This, in combination with data indicating that wild-type LT compromises the spindle checkpoint, whereas dl89-97 LT does not, hints that aneuploidy and genetic instability induced by spindle checkpoint perturbation may contribute to a critical mechanism by which LT transforms cells to tumorigenicity.

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