Does Adenovirus E1B Protein Need To Interact With E4 Protein In Order To Induce Cyclin E Overexpression?

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Introduction and Hypothesis

E1B55k$^-$ (E1B$^-$) Adenovirus (Ad) has been used as oncolytic agents (1) after the assumption that its replication is p53-deficiency dependent (1,2). Further investigations disproved this theory, showing that E1B$^-$ Ads replicate in a p53-independent manner (3,5,4,6). *Sam Zhou et al.* hypothesized that E1B$^-$ Ads replicate in a CyclinE-dependent manner. Further studies showed that CyclinE (CycE) overexpression (OE) is strictly correlated with the presence of E1B protein (7,8) and that the first is required for an efficient viral replication (7), thus suggesting a new E1B function not yet discovered. Even though E1B alone has shown some activities in the cytosol (9), it mostly form a complex with E4orf6 (E4), acting as E3 ubiquitin ligase with various targets, among which p53 (9,10,11). This interaction relocalize E1B in the nucleus (9).

We hypothesize that E1B must interact with E4 in order to induce CycE OE.

Materials and Methods

Cells and viruses: A549 cell lines, transfected to express a flag-tagged CycE, will be infected with: Mock; wtAd expressing Ha-E1B and Myc-E4(positive Control); E1B$^-$/E4$^-$Ad, to determine E1B/E4-independent CycE exp levels (negative control); E1B$^-$Ad, expressing a Myc-E4 protein; E4$^-$Ad, expressing a HA-E1B protein.

Viral replication and CPE: All cell cultures will be infected with an MOI of 10 and will be arrested at h.p.i. of 12, 18, 24, 48; CPEs will also be evaluated at the mentioned time points. Quantitative Real-Time PCR will be used for viral DNA replication essay.

Immunofluorescence (IF) and FRET: monoclonal antibodies conjugated with fluorophores to visualize CycE, E1B and E4 localization; FRET will be used to visualize E1B-E4 interaction.

Western blot: monoclonal antibodies will also be used. To normalize concentration we will use an actin specific antibody.

Discussion and possible results

According to the literature, we should obtain results similar to figure 1, 2, 3.

For hypothesis confirmation, results similar to graph 1 (without the yellow section) should be obtained. In such case, E1B/E4 interaction is mandatory, and their target should be nuclear. This information will also help further investigations. In the case that E1B alone produces different CycE levels (yellow section, graph 1), it will mean that it needs the interaction with E4 just for an efficient CycE OE, and that it is not dependent by it.

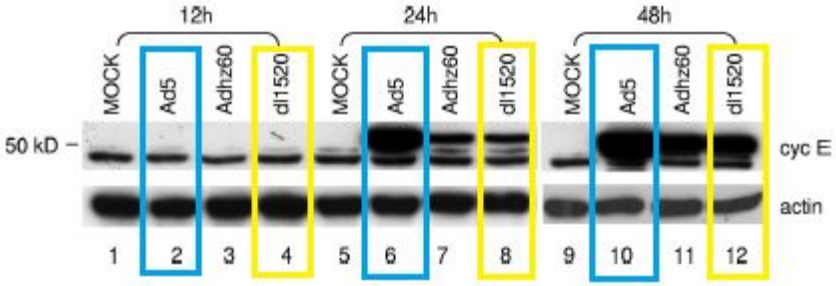


Figure 1. Western Blot showing CycE expression in 12, 24,and 48 h.p.i. in A569 cells. Ad5 is the wt virus, dl1520 is a E1B55k^{-} Ad, (Adhz60 an Ad with the entire E1B gene deleted, it does not concern our purposes). In the absence of E1B55k, Cyclin E expression is much lower, though there is some expression due to E1A sequestering pRb in order to release E2F and permit S-phase protein expression. This image shows that a Cyclin E with e different molecular weight is induced by the virus, we don't address this information since it goes beyond the purposes of this project. Image obtained from ref. 7

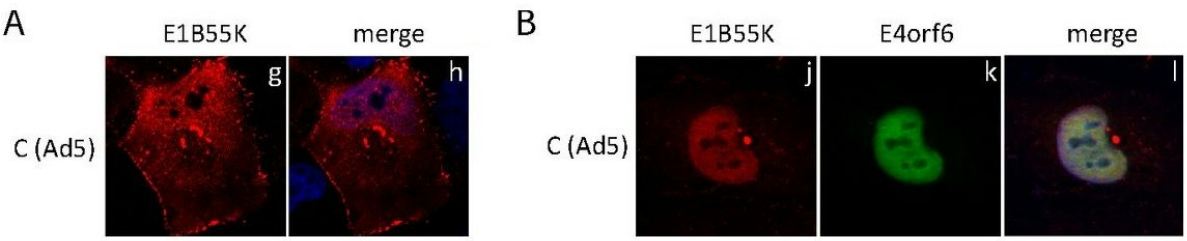


Figure 2. E1B55k localization (A) alone or (B) in the presence of E4orf6 in H1299 human non-small cell lung carcinoma cell line. In absence of E4orf6, E1B55k almost exclusively localize in the cytosol. Whether in its presence it migrates in the nucleus. Image obtained from ref. 9.

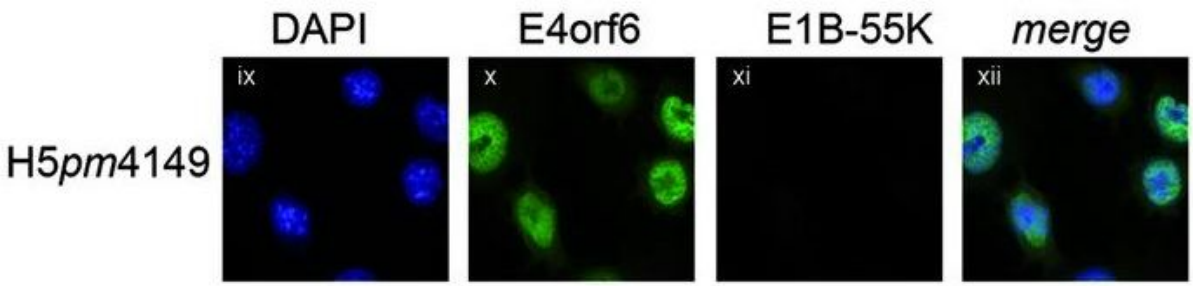
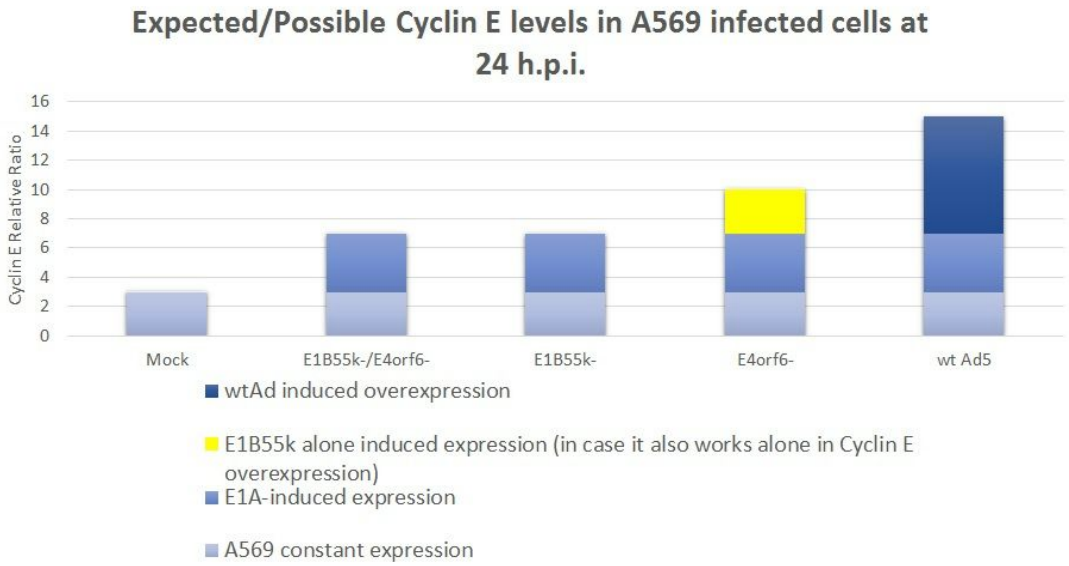


Figure 3. E4orf6 nuclear localization in absence of E1B55k, in H1299 human non-small cell lung carcinoma cell line at 24 h.p.i.. Image obtained from ref. 12.



Graph 1. Aspected and possible concentration expressions among different virus infections. If, in the absence of E4orf6, CycE expression is higher than E1A-induced level (4th column, yellow part) it would mean that E1B55k works also alone (regarding CycE), presumably in the cytosol, and therefore it's not dependent on E4orf6. .

Abbreviations Legend: E1B55k is referred as E1B. E1B55k$^-$ is refered as E1B$^-$. Cyclin E is referred as CycE. Overexpression is referred as OE. E4orf6 is referred as E4. E4orf6$^-$ is referred as E4$^-$. Expression is referred as exp. Immunofluorescence is referred as IF.

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