
9. Summary

The step from G₁- to S-Phase will be initiated by E2F activating E2F-dependent genes. As a result of γ -irradiation double strand breaks of DNA exist. To combine broken dsDNA-breaks cellcycle progression has to be stopped and transcriptional activity of E2F has to be blocked. Before beginning of this Ph D work an interaction has been identified roughly of E2F3 as well as E2F1 and Ku70. DNA-PK, which is involved in the repair of DNA double strand breaks, and the recombination of immunoglobulin genes, has recently been shown to be involved in cell cycle control.

The specificity of the interaction of E2F1 marked box with Ku70 could be confirmed in vitro by using GST pull-down assays and by surface plasmon resonance (SPR). In vitro translated Ku70 or Ku80 protein do not associate with E2F1 GST-fusion proteins lacking the marked box. Inversely, the E2F1 marked box alone interacts with Ku70 as well as Ku80. Only the carboxy terminal GST-fusion mutant of E2F1 including the marked box acts as a useful deletion mutant to isolate Ku70 and Ku80 from Raji nuclei extracts. SPR results consider wtE2F1 as a direct interaction partner of Ku70. There was no significant affinity of E2F1 deletion mutants, lacking the marked box, to Ku70. Moreover, the presence of the pocket protein domain in the absence of the marked box does not show any interaction to Ku70, demonstrating that the pocket protein domain is unnecessary for the protein-protein interaction.

We tested the regulatory function of E2F1 in complex within the cell cycle, whether there is a potential functional mechanism of this protein complex. The results identified a significant reduction of E2F transactivation activity after transient cotransfection and γ -irradiation of Ku70 and Ku80. It can be postulated that the modulation of E2F activity by DNA-PK is a key element in blocking the G₁/S phase transition in the cell cycle after DNA damage, thereby allowing DNA repair before replication.