

Advantage of a baculovirus expression system for protein-protein interaction studies. Evaluation of the involvement of posttranslational modifications

Józefa Węsierska-Gądek¹, Jacek Wojciechowski¹ and Gerald Schmid^{1,2}

Cell Cycle Regulation Group, Institute of Cancer Research, Medical University of Vienna, Vienna, AUSTRIA, LMU Klinikum Grosshadern; Klinische Forschung Chirurgie, Muenchen, Germany

We recently observed the interaction between poly(ADP-ribose) polymerase-1 (PARP-1) and the product of the tumor suppressor gene p53 [1-4]. However, more extensive studies on both proteins, especially those on characterization of their domains involved in the interaction were difficult due to very low expression levels of the latter in mammalian cells. Therefore, we generated recombinant proteins for such studies.

To clarify which domains of human PARP-1 and of human wild-type (wt) p53 were involved in this protein-protein interaction, we generated baculoviral constructs encoding full length or distinct functional domains of both proteins. Full length PARP-1 was simultaneously coexpressed in insect cells with full length wt p53 protein or its distinct truncated fragments and *vice versa*. Reciprocal immunoprecipitation of Sf9 cell lysates revealed that the central and carboxy-terminal fragments of p53 were sufficient to confer binding to PARP-1, whereas the amino-terminal part harbouring the transactivation functional domain was dispensable. On the other hand, the amino-terminal and central fragments of PARP-1 were necessary for complex formation with p53 protein [5]. Since the most important features of p53 protein are regulated by phosphorylation, we addressed the question whether its phosphorylation is essential for binding between the two proteins. Baculovirally expressed wt p53 was post-translationally modified. At least six distinct p53 isomers were resolved by immunoblotting following two-dimensional separation of baculovirally expressed wt p53 protein. Using specific phospho-serine antibodies, we identified phosphorylation of baculovirally expressed p53 protein at five distinct sites. To define the role of p53 phosphorylation, pull-down assays using untreated and dephosphorylated p53 protein were performed [6]. Dephosphorylated p53 failed to bind PARP-1 indicating that complex formation between both proteins was regulated by phosphorylation of p53 [6]. The marked phosphorylation of p53 at Ser392 observed in unstressed cells suggests that the phosphorylated carboxy-terminal part of p53 undergoes complex formation with PARP-1 resulting in masking of the NES and thereby preventing its export. The functional significance of the interaction between both proteins was investigated at two different conditions: inactivation of PARP-1 and overexpression of PARP-1. Our results unequivocally show that the presence of PARP-1 regulates the basal expression of wt p53 in unstressed cells.

Thus, the baculovirally expressed recombinant proteins offer an advantage in comparison to conventional proteins generated in bacteria.

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