Two tandem and independent sub-activation domains in the amino terminus of p53 require the adaptor complex for activity

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The ability of p53 to function as a tumor suppressor is linked to its function as a transcriptional activator, since p53 mutants that do not transactivate are unable to suppress tumor cell growth. Previous studies identified an activation domain in the amino terminal 40 residues of the protein, a region that binds to several general transcription factors and to some oncogene products. For example, mdm-2, a cellular oncoprotein, binds to this region and represses p53 transactivation. Here we describe a new activation domain within the amino terminus of p53 that maps between amino acids 40–83, and whose residues trp-53 and phe-54 are critical for function both in yeast and in mammalian cells. In vivo studies in yeast show that the new activation subdomain, unlike the previously described, is mdm-2 independent. Both p53 activation subdomains (1–40 and 40–83) require the yeast adaptor complex ADA2/ADA3/GCN5 for transcriptional activation. Moreover, since activation by p53 requires GCN5's enzymatic histone acetyltransferase domain, p53 may regulate gene expression by influencing chromatin modification.

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Introduction

The tumor suppressor protein p53 (for a review, see Donehower and Bradley, 1993, Ko and Prives, 1996. Levine, 1993 and references therein) is inactivated in more than half of all human tumors (Greenblatt et al., 1994). p53 is a sequence-specific transcription factor that suppresses oncogenic transformation (Eliyahu et al., 1989. Finlay et al., 1989) and induces cell cycle arrest (Leonardo et al., 1994) or programmed cell death (Clarke et al., 1993, Lowe et al., 1993a,b) in response to DNA damage.

Three distinct domains have been identified within p53. the acidic aminoterminus is an activation domain (Fields and Jang. 1990. Funk et al., 1992: Raycroft et al., 1990). the hydrophobic central region contains a sequence-specific DNA-binding domain (Bargonetti et al., 1993. Halazonetis et al., 1993. Wang et al., 1993). and the basic carboxyterminus comprises an oligomerization domain (Clore et al., 1994, Jeffrey et al., 1995, Lee et al., 1994. Sakamoto et al., 1994. Sturzbecher et al., 1992) and a region that regulates DNA binding affinity (Halazonetis and Kandil, 1993. Hupp et al.,

1992. Waterman et al., 1995) Hot-spots for p53 mutations in tumors (Hollstein et al., 1991. Levine et al., 1991) are found predominantly in the central DNA-binding domain.

In addition, p53 can act as a transcriptional repressor, to down-regulate promoters lacking wild type p53 binding sites (Ginsberg et al., 1991 Santhanam et al., 1991. Subler et al., 1992) The amino terminal transactivation domain of p53 also is required for its inhibitory effects on transcription (Sang et al., 1994; Subler et al., 1994)

Tumor suppression and transcriptional activation are strongly correlated functions of p53 (Farmer et al., 1992. Fields and Jang, 1990; Funk et al., 1992) Mutations or deletions in the aminoterminus of p53 coordinately abolish transactivation and tumor suppressor functions. Tumor-derived p53 mutants fail to activate transcription of wild type p53 responsive genes Furthermore. mdm-2, a cellular oncoprotein which is overexpressed in 30–40% of human sarcomas (Oliner et al., 1992), owes its oncogenic ability to its ability to conceal the transactivation domain of p53 (Brown et al., 1993. Momand et al., 1992. Oliner et al., 1992). Biochemical (Lin et al., 1994) and crystallographic analyses (Kussie et al., 1996) of mdm-2·p53 binding show that similar residues in p53 are critical for both mdm-2 binding and transactivation (Lin et al., 1994).

p53 transactivation may operate through direct contacts with components of the general transcription machinery. For example. interactions have been demonstrated between the p53 amino terminus and TATA-binding protein (TBP) (Horikoshi et al., 1995. Liu et al., 1993). TFIIH (the p62 (Xiao et al., 1994). ERCC2 and ERCC3 (Wang et al., 1995) components), as well as the coactivators TBP associated factors (including Drosophila TAFII40. TAFII60 (Thut et al., 1995) and human TAFII31 (Lu and Levine, 1995) It is unclear whether these interactions are sufficient for transactivation in vivo. or whether additional factors are required.

Although the activation domain of p53 has been mapped to residues 1–40 (Unger et al., 1992), adjacent sequences contribute to overall p53 activity (Chang et al., 1995) The region encompassing residues 1–90 of the protein is remarkably acidic and its primary structure is generally similar to other activation domains. For example. the potent activator of herpes simplex virus. VP16. contains an activation domain between residues 413–490. and. like p53. has overall negative charge with bulky hydrophobic amino acids grouped in clusters. The negative charge contributes to overall potency. but the hydrophobic residues are essential for transactivation (Cress and Triezenberg

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