Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1985–1986, March 1994

Commentary

Inducible growth arrest: New mechanistic insights

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Cells from organisms as diverse as yeast and mammals undergo regulated growth arrest in response to adverse conditions. The integrity of the signaling pathways involved is important for maintaining viability of individual cells and, in the case of multicellular organisms, for preventing emergence of cells with severe defects in growth regulation. Mutations in the gene encoding p53 are among the most common genetic abnormalities associated with human cancer cells. Over the past few years a convincing link between the function of p53 and the activation of an inducible growth-arrest pathway has been established. More recently, experiments have unraveled important details of the molecular mechanisms involved. A brief review of these developments forms the substance of this commentary.

p53 can suppress cellular proliferation. This has been established experimentally by demonstrating that targeted overexpression of the protein suppresses the outgrowth of clones of cells that have been cotransfected with a selectable marker (1, 2). Furthermore, the growth-suppressing effect of p53 is entirely consistent with its ability to prevent transformation of cells by viral or cellular oncogenes (3, 4). Using either an inducible expression system for p53 or a conditional mutant form of the protein, p53 has been shown to suppress progression of cells through G_1/S phase of the cell cycle (5, 6). The physiological setting(s) in which p53 exerts its arresting effect were not known at the time, but it was obvious that the protein did not play an essential role in the normal regulation of the cell cycle, as mice bearing germ-line deletions of p53 exhibited normal postzygotic development (7). An important advance occurred with the demonstration that p53 plays an essential role in the induction of G_1/S arrest in response to UV or γ irradiation of cells (8, 9). p53 levels were noted to increase in response to these physical agents that damage DNA. The complement of adverse stimuli that induce p53 activity and cause growth arrest has since been expanded to include, among others, metabolic inhibitors of DNA synthesis (10, 11) and a direct assault on the integrity of the genomic DNA by restriction endonuclease (12). These results suggest that p53 induction is physiologically relevant under conditions of cellular stress and that its role is played out as an internal policeman of the cell cycle, contributing to the function of a G_1/S cell cycle checkpoint (13, 14).

In trying to understand the mechanism by which p53 induces growth arrest, certain biochemical features of the protein stand out as being potentially meaningful. (*i*) p53 possesses sequence-specific DNA-binding activity and the protein can activate the transcription of reporter genes "driven" by such binding sites (reviewed in ref. 14). (*ii*) Commonly occurring mutations in p53, found in many cancers, interfere with the sequence-specific DNA-binding activity of the protein and lead to a loss of its ability to transactivate genes under the control of p53 binding sites. The structural basis for these observations has recently been partially clarified by the demonstration that p53 interacts with specific DNA sequences through a domain roughly encompassing the central 200 amino acids of the molecule. This domain contains the regions of the molecule most conserved among various species and is the site of most of the "oncogenic" mutations in the protein (15–17). In this issue of the *Proceedings* these studies are carried one step further by showing that the mutations in p53 that most profoundly effect the sequence-specific transactivation potential of the protein also abolish its ability to serve as an inducer of growth arrest (18). In a series of "cutting and pasting" experiments the Johns Hopkins group goes on to show that, with respect to the growth-arresting phenotype, the relevant portion of p53 is also its central domain. Thus the flanking N-terminal transcriptional activation domain and C-terminal dimerization domains can be replaced by those of heterologous proteins without loss of function.

The above observations are easily explained by a model in which p53 serves as a sequence-specific DNA-binding transcriptional activator and it is the products of its downstream target genes that carry out the growth-arresting program. The importance of mediators of p53 action (as opposed, for example, to a direct effect of p53 on the DNA replication machinery) is supported by the fact that p53-mediated G_1/S arrest in irradiated cells persists for many hours after levels of the protein have returned to normal (12). Until recently, the few genes shown to be inducible by p53 failed to provide the missing link between p53 induction and regulation of cellular growth. This changed dramatically with the identification of WAF1, a gene directly inducible by p53 (19). WAF1, also known as Cip1 and p21, encodes a protein that turns out to be a potent inhibitor of a variety of cyclin-dependent kinases active in G_1/S (20, 21). To the extent that the kinase activity of these cyclin-dependent kinases drives cells into S phase, the identification of an inhibitor as a target gene for p53 lends strong support to the model spelled out above. Another mediator of inducible growth arrest also appears to utilize similar mechanisms; the cytokine transforming growth factor β (TGF-β) induces growth arrest in epithelial cells. This arrest is associated with the appearance of an activity that antagonizes the cyclin E/cdk2 kinase, which is active in late G_1 (22). The parallel between the two different arresting pathways suggests a functionally conserved and physiologically meaningful common mechanism of action.

WAF1 may not be the only mediator of the growth-arresting properties of p53. The growth arrest- and DNA damage-inducible gene (GADD45) previously identified as a direct target gene for p53 (23) has recently been shown to have growth-suppressing properties of its own (24). GADD45 has been found to act synergistically with another arresting protein GADD153 (or CHOP) in inducing growth arrest (24, 25). GADD153 is also inducible by DNA damage; however, in contrast to GADD45 this induction is independent of p53, being observed, for example, in HeLa cells, which have little active p53 (26). These findings suggest the existence of overlapping and synergistic pathways for the induction of G_1/S arrest in response to stressful events; some of these pathways are p53-dependent and others may be p53-independent.

The pathway by which p53 is induced in response to DNA damage is poorly understood. Some biochemical and genetic clues as to how this process might come about have been uncovered, however. (*i*) Activation of p53 is associated with an increase in the protein half-life

(27). (*ii*) A variety of *in vitro* allosteric modifiers can alter the DNA-binding activity of the protein (28). (*iii*) p53 is phosphorylated on multiple sites *in vivo*. (*iv*) In certain rare genetic disorders such as ataxia telangiectasia and Bloom syndrome, potentially significant defects in the activation of p53 by DNA damage have been uncovered (12, 23). One hypothetical mechanism of activation of p53 would assume the existence of a relevant kinase (or kinases), similar to the recently identified DUN1 kinase that controls DNA damage response in yeast (29). Phosphorylation of p53, or a protein that regulates the function of p53, by this putative kinase would, by inducing an allosteric modification in p53, alter the half-life and DNA-binding properties of the protein, setting in motion the chain of events that leads to cell cycle arrest. Cells from patients with ataxia telangiectasia and Bloom syndrome would be predicted to suffer from the inability to execute any one of the above steps leading to p53 activation.

The identity of the upstream activators of p53 is not the only remaining mystery surrounding the protein. Events downstream of p53 are still incompletely understood. Specifically, the molecular mechanisms by which loss of a cell cycle checkpoint contributes to cellular transformation are not known. In certain tumor cells, specific defects in regulatory molecules other than p53 that participate in regulated growth arrest have been uncovered. Examples are the loss of the *RB* gene in retinoblastoma and other tumors and the more recent identification of the CHOP (GADD153) gene product as a participant in a DNA damage-inducible cell cycle checkpoint (24, 25), that may be deranged in certain rare tumors (30, 31). In the case of p53, the observation that loss-of-function mutations are associated with an increase in chromosomal instability (10, 11) raises the specter of a "mutator mutation" phenotype, in which the cells acquire the propensity to accumulate mutations at an unusually high rate. The observation that p53-deficient cells fail to undergo programmed cell death in response to certain stimuli (12, 32, 33) suggests a complementary mechanism in which clones of cells that would otherwise be eliminated by programmed cell death persist and perhaps serve as precursors for a more transformed phenotype. The relationship between the growth-arresting properties of p53 and its role in apoptosis remains to be clarified.

The study of p53 is an example of reductionist biology at its best. By focusing on one important molecule, a whole field of research has been established that has provided us with many important insights into how cell growth is regulated. If the rapid pace of progress in the study of p53 persists, we should very soon have answers to many of the questions raised above and, more importantly, we are sure to have many new and equally interesting questions for study in the future.

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