NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Preparatory Assignment for

Is p53 a Smoking Gun?

How Mutational Signatures Forced Big Tobacco to Change

by

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Read about p53, then complete the "Test Yourself" question below. Bring your answers to class.

p53 is a protein that is critical for keeping our genome (DNA) intact and free of damage, including free of potentially harmful mutations. In fact, p53 is commonly known as the "guardian of the genome." Our DNA is exposed to many damaging agents all the time. Some of these come from the environment (various chemicals, radiation), and some are even produced by normal cellular activities (so-called free radicals). If DNA damage is not repaired, new mutations can arise, some of which can promote the development of cancer. A mutation is a change in the DNA sequence; a change in the order of the A, T, G and C nucleotides that make up DNA. Mutations can also be introduced as a result of uncorrected errors that occur during DNA replication. Depending on the location and nature of mutations, they can alter (or abolish) the activity of proteins encoded by genes. In some cases, this can lead to the development of cancer. Thanks to the activity of p53, very little damage is sustained. This is why p53 is also often referred to as a "tumor suppressor."

You might wonder how p53 can do this. Does it act as a "shield" or "force field" to prevent damaging chemicals or radiation from even reaching DNA? Not quite. When DNA damage occurs, several proteins are able to detect the damage. These proteins can then activate p53, so that it can help with repairing the damage.

p53 is a type of protein known as a transcription factor. Transcription factors are important initiators of the process of gene expression. This is the process by which the information encoded in genes is used to build the corresponding proteins. Transcription factors help determine which genes are expressed (and therefore which proteins are produced), in which cells, at which times. They do so by binding to specific DNA sequences and recruiting important players (other proteins and enzymes) involved in gene expression.

When p53 becomes activated, it can help express genes that encode proteins that play several key roles with respect to detecting and repairing DNA damage. One of these is a protein that can temporarily pause the progression of a cell through its cycle of cell division. This means that the cell will not continue to divide, giving it time to repair the damaged DNA. Once the DNA has been repaired, the cell can continue to divide, but with intact DNA. Another protein produced as result of p53 activity is a protein that can help repair DNA. Finally, if the damage is so extensive that it cannot be repaired, p53 activity can cause the cell to die through a specific death pathway known as apoptosis. This prevents a cell with irreparable DNA damage from continuing to replicate and pass possibly dangerous mutations to its daughter cells.

In order to do its job as a transcription factor, p53 needs to bind DNA at specific regions just ahead of each gene that it is helping to express. Once it has bound to the DNA at the proper location, it needs to help start the process of transcription. In order to do this, it binds to proteins that "loosen" up the DNA in that region; this allows the transcription machinery to access the gene. It also helps bind to and bring in some of the proteins that are part of the transcription machinery.

In order to accomplish all of this, the p53 protein has several regions, or "domains" that contribute to these specific functions. These domains are shown as the regions A, B, C and D in Figure 1 (see nexpt page). Instead of going in the order A–D, let's consider how these domains work in relation to how p53 does its job.

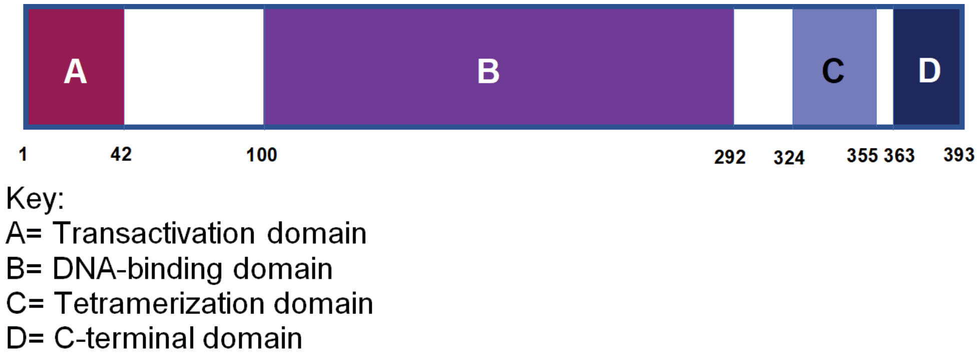

Figure 1. Domains of p53. The numbers correspond to the amino acids within the protein. For example, the DNA-binding domain includes amino acids 100–292 in the 393 amino acid protein. [Adapted from Pecorino (2016) and Tafvizi et al. (2011).]

The Tetramerization Domain (region "C"): p53 does not work as a single protein; it must form a physical complex of four p53 molecules. This complex is known as a tetramer. The four p53 molecules bind to one another using their tetramerization domains.

The DNA Binding Domain (region "B"): Once p53 tetramers have formed, they need to bind DNA at specific regions near the specific genes regulated by p53 to turn on the genes' expression. This requires the DNA-binding domain. Altering even one amino acid in the DNA binding domain can prevent p53 from binding to DNA.

The C-Terminal Domain (region "D"): While the DNA binding domain is the domain that actually binds to DNA, there is strong evidence that the this C-terminal domain (region "D") assists with this process. The C-terminal domain helps to "scan" DNA, to find the appropriate binding sites, and then helps stabilize the binding of the p53 tetramer to DNA.

The Transactivation Domain (region "A"): Once bound to DNA, the transactivation domain binds to (and brings in) other proteins necessary to start the process of transcription. Some of these proteins help "loosen up" the DNA so that the transcription machinery can access the genes to be expressed. Some of these proteins help bring in the enzyme that will actually synthesize the RNA.

Test Yourself

Define or describe the significance of these key terms and concepts related to p53:

- DNA sequence
- Mutation
- Gene Expression
- Transcription
- Transcription Factor
- DNA-binding domain of p53
- Tetramerization domain of p53
- C-terminal domain of p53
- Transactivation domain of p53
- DNA repair
- Pause/delay in cell growth