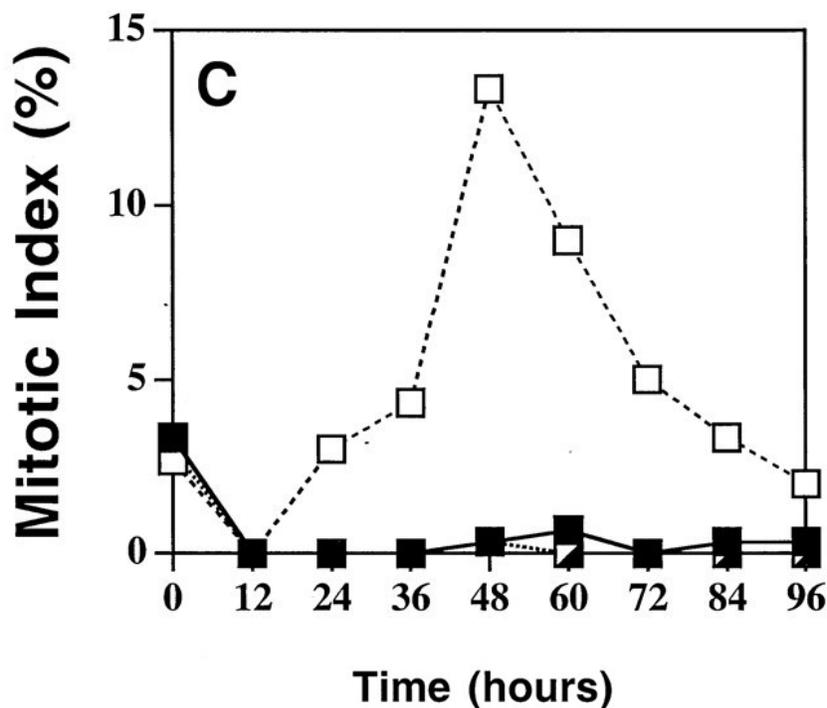




HOW TO USE THIS RESOURCE

Show the following figure and caption to your students. The accompanying Student Handout provides space below the image caption for Observations, Notes, and Questions and space next to the “Background Information” for Big Ideas, Notes, and Questions. The “Interpreting the Graph” and “Discussion Questions” sections provide additional information and suggested questions that you can use to prompt student thinking, increase engagement, or guide a class discussion about the characteristics of the graph and what it shows.

γ -radiation



Caption: Alleles of the p53 gene were selectively disrupted in a line of human cells and then monitored after exposure to DNA-damaging gamma (γ) radiation to determine what proportion of the cells entered mitosis (cell division). The shaded squares represent cells with two normal alleles of the p53 gene. The half-shaded squares represent cells with one normal and one disrupted allele of the p53 gene (note that some of the half-shaded squares are covered by the shaded squares). The unshaded squares represent cells in which both alleles of the p53 gene were disrupted. The mitotic index is the proportion of cells undergoing mitosis at a given time.

BACKGROUND INFORMATION

The p53 protein, referred to as the “Guardian of the Genome,” is a tumor suppressor that plays an important role in halting the division of cells (mitosis) that have sustained DNA damage. The p53 protein arrests the cell cycle at two different times: before DNA replication (between phases G₁ and S) and before cell division (between phases G₂ and M). Arresting the cell cycle at these points helps prevent the division of cells containing damaged DNA, which could become cancerous. The gene encoding p53 is often mutated in cancers. In fact, this gene is mutated more often than any of the other 20,000 human genes. A deeper understanding of the role of p53 in the cell cycle can therefore improve our understanding of cancers and perhaps lead to new forms of treatment.

In this study, researchers investigated the role of p53 in cell cycle regulation at the checkpoint between the G₂ and M phases. Most human cells contain two alleles of every gene, one allele inherited from the mother and the other from the father. Normally, an allele can be used to produce a protein, such as p53. However, researchers can use genetic engineering to "disrupt" an allele, making it unable to produce a properly functioning protein. In this case, the researchers genetically engineered a cell line to create two new cell lines, one in which a single allele of the *p53* gene was disrupted and the other in which both alleles were disrupted. Next, they exposed all three cell lines to DNA-damaging gamma radiation and observed how this exposure impacted cell division. They measured the impact by determining the proportion of cells undergoing mitosis, called the mitotic index, for each cell line every 12 hours for four days. The mitotic index was measured by fixing or preserving a sample of the cells and then staining them with a DNA-specific fluorescent stain (Hoechst). Using microscopy, cells containing condensed, evenly stained chromosomes were identified and counted. Condensed chromosomes are an indication that the cells were undergoing mitosis at the time they were fixed.

INTERPRETING THE GRAPH

The figure shows the mitotic index, or the proportion of cells undergoing mitosis (*y*-axis), over time (*x*-axis) after cells were exposed to gamma radiation. The figure includes data on a cell line (shaded squares) containing two normal alleles of the *p53* gene, as well as two genetically engineered versions of this cell line. The genetically engineered cell lines have either one normal and one disrupted allele of the *p53* gene (half-shaded squares) or two disrupted alleles of the *p53* gene (unshaded squares).

The results show that cells with two normal alleles of *p53* will not enter mitosis for at least 96 hours after exposure to DNA-damaging gamma radiation. This important delay gives the cells time to either correct DNA damage or undergo apoptosis (cell death). The cell line with one normal and one disrupted *p53* allele displayed the same trend as the cell line with two normal *p53* alleles after irradiation. This suggests that cells will behave in the typical fashion for this trait when they have at least one functioning allele of the *p53* gene.

In contrast to these two cell lines, a much higher fraction of cells from the cell line with two disrupted alleles of the *p53* gene (unshaded squares) entered mitosis after irradiation. This result suggests that p53 plays an important regulatory role in the cell cycle, preventing damaged cells from proliferating. p53 must be activating a "checkpoint" that blocks cells from entering mitosis. The cell line with two disrupted alleles has no normal *p53* alleles, so it cannot produce p53. These cells thus begin to enter mitosis around 24 to 48 hours post-irradiation, because the p53 protein is not available to initiate the process that halts mitosis.

Notice that most of the cells with both alleles of the *p53* gene disrupted (unshaded squares) didn't enter mitosis until 48 hours after exposure to gamma radiation. Considering that a complete human cell cycle is typically around 24 hours, this delay in the onset of mitosis could suggest that other proteins involved in the cell cycle may also contribute to the delay or prevention of mitosis after DNA damage even in the absence of p53.

Teacher Tip: Prompt your students to explain the parts of the graph as applicable:

- Graph type: Line graph
- X-axis: Number of hours after cells were exposed to gamma radiation
- Y-axis: Mitotic index (the proportion of cells undergoing mitosis)

DISCUSSION QUESTIONS

- What trends do you notice in this graph?
- Which cell line(s) have a properly functioning p53 protein?
- How many normal alleles of the *p53* gene do cells need to function properly? Use evidence from the figure to support your claim.
- Predict what might happen at a cellular level and at an individual level if a person has one normal and one mutated allele of the *p53* gene. What if they have two mutated alleles of *p53*?

- Based on the figure, what role does the p53 protein play in cell division? Why might a cell need to stop dividing?
- Why was gamma radiation used in this experiment?
- In the cell line that entered mitosis, why do you think there was a delay before the onset of mitosis after exposure to gamma radiation?
- In the cell line that entered mitosis, why do you think that only a fraction of the cells did so instead of 100%?
- At what point in time were the highest percentage of cells without the *p53* gene undergoing mitosis? Compare this with the length of a typical human cell cycle: 24 hours. What could be responsible for this difference in the onset of mitosis?
- How would you test whether the slight increase in the mitotic index at 60 hours in the cells with two normal alleles of the *p53* gene is statistically significant?
- How would you design a follow-up experiment to determine whether other proteins in addition to p53 play a regulatory role in cell division after DNA damage?

KEY TERMS

allele, cancer, cell cycle, gene knockout, genetic engineering, mitosis, tumor suppressor

SOURCE

Figure 2c from:

Bunz, F. et al. "Requirement for p53 and p21 to sustain G2 Arrest after DNA damage." *Science* 282, 5393 (1998): 1497-1501. <https://doi.org/10.1126/science.282.5393.1497>.

An annotated version of this article is provided by the Science in the Classroom resource "[Arrested development: When cells make mistakes.](#)"

AUTHOR

Natalie Dutrow, PhD, Judge Memorial Catholic High School, UT

Edited by Bert Vogelstein, MD, Johns Hopkins Medical School; Paul Beardsley, PhD, Bridget Conneely, and Jessica Johnson, HHMI