IS GENE EXPRESSION REGULATED BY A TOGGLE OR A DIMMER SWITCH?

DNA methylation has emerged in recent years as an important gene regulatory mechanism involved in everything from behavior to cancer. Methylation of DNA is a reversible modification that shuts down expression of genes and is particularly important during development, when genes must be turned on or off according to what is appropriate for each tissue type, and during cell division when DNA is replicated. DNA methyltransferase 1 (DNMT1) is the major maintenance enzyme responsible for DNA methylation during semi-conservative DNA replication in human cells. A research team working at the SER-CAT 22-BM-B beamline at the APS has shown that the stability, and therefore the activity, of DNMT1 is carefully regulated by two different types of modifications that respond to cellular signals to mediate the DNA methylation process. The work highlights the elegant balance of cross-talk between positive and negative signals that regulate this important enzyme and provides information that could be used to develop therapies to target the DNMT1 pathway in diseases where DNA methylation has gone awry.

The team had shown that stability of the DNMT1 protein is regulated by protein methylation of DNMT1 on the amino acid lysine 142 (Lys142) by an enzyme called SET7. When SET7 methylates DNMT1, it is tagged for destruction, reducing methylation of the genome. However, in the course of that work, they noticed something else interesting. The amino acid right next to Lys142, serine 143 (Ser143), is in the right place to be modified by another regulatory enzyme called a protein kinase that adds a phosphate group to the amino acids serine or threonine. This led the team to wonder whether this site is actually phosphorylated and how that modification affects the methylation right next door at Lys142.

Using a peptide from DNMT1 that included the amino acids from 137-146 of human DNMT1, the team established that Ser143 is phosphorylated and identified a protein kinase called AKT1 as the enzyme that makes the modification. AKT1 is known to be involved in growth and development and was identified as an oncogene, a normal gene that is involved in the development of cancer when it is overexpressed or mutated. Biochemical assays showed that if they used a DNMT1 peptide that had been phosphorylated by AKT1, it could not also be methylated by SET7. The structural basis for this was elucidated by solution of the three-dimensional structure of SET7 with the peptide from DNMT1 and the substrate for methylation, S-adenosyl-L-methionine. The crystallographic structure that was obtained at the SER-CAT beamline showed that if DNMT1 was phosphorylated on Ser143, it would set up repulsive forces that would make it impossible for SET7 to add the methyl group to Lys142 (Fig. 1). The two modifications are mutually exclusive. These data suggested that cross-talk between AKT1 and SET7 adjusts the stability of DNMT1 to regulate methylation of the genome during the cell cycle and development.

Experiments in cultured cells supported this hypothesis by showing that when AKT1 was active and expressed in high concentration, the amount of DNMT1 increased, and when SET7 was expressed in high concentration, the amount of DNMT1 decreased. The effects of activated AKT1 were not immediate, however, and appeared to be regulated by the cell cycle, suggesting that other levels of regulation are also present. When AKT1 activity was inhibited in cells, DNMT1 levels decreased and methylation of the genome was reduced. Therefore, genome methylation is controlled in a cell cycle-dependent manner that involves methylation of DNMT1 by SET7 to cause destruction of DNMT1 and phosphorylation of DNMT1 by AKT1 to cause preservation of DNMT1 by preventing methylation.

This work has clear implications in cancer, which is characterized by unregulated cell division. Data from other groups have shown that in breast cancers that have aberrant AKT1 activation, inappropriate hypermethylation is responsible for turning off a tumor suppressor gene and consequent development of cancer. The work here suggests that this could be due to the effects of AKT1 on DNMT1 stabilization, suggesting that DNA methylation could be a therapeutic target in these cancers. — *Sandy Field*

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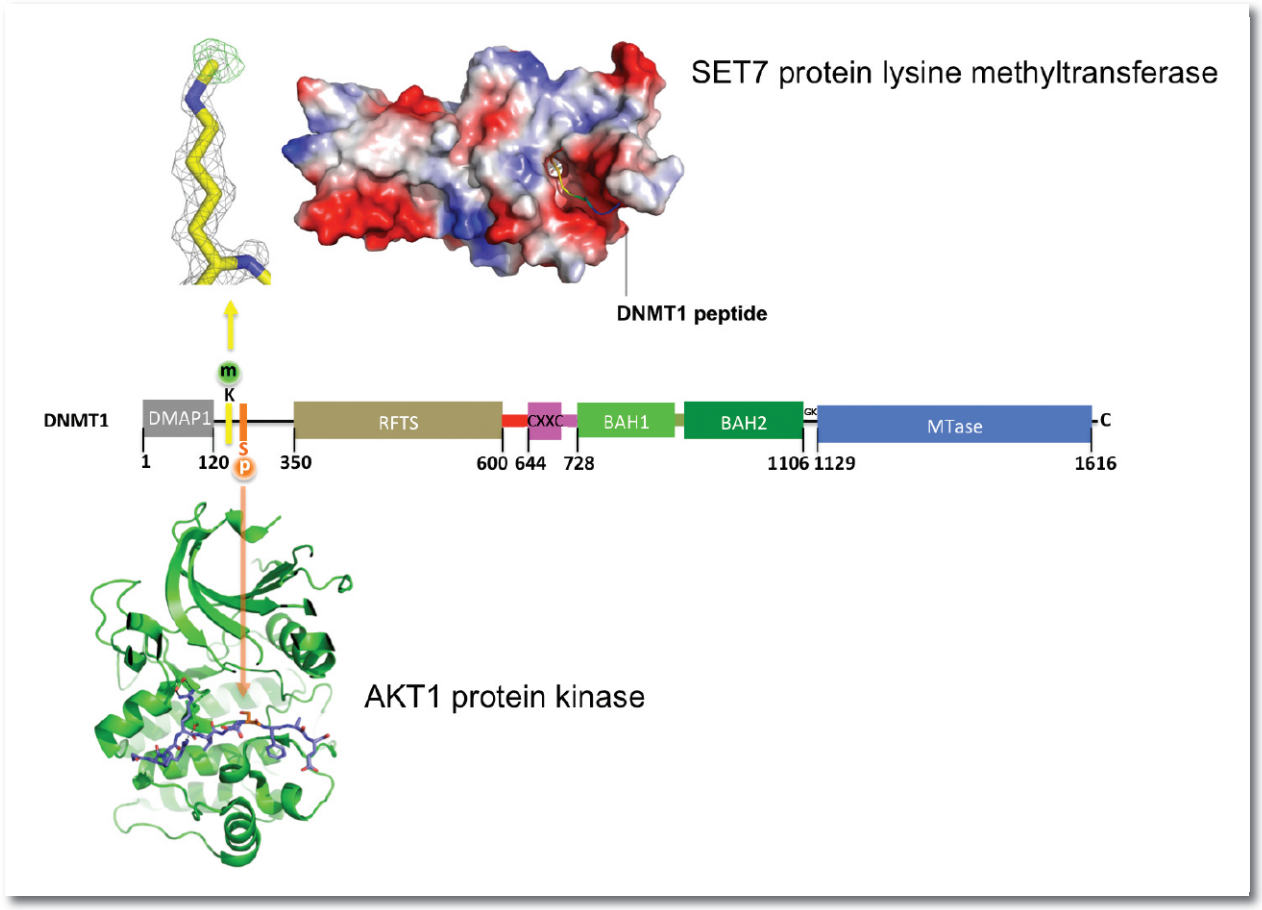

Fig. 1. Human DNA methyltransferase 1 is methylated by SET7 (top panel) and phosphorylated by AKT1 (bottom panel) between an adjacent lysine and serine (middle panel).

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