How cGAS enzyme is kept bottled up

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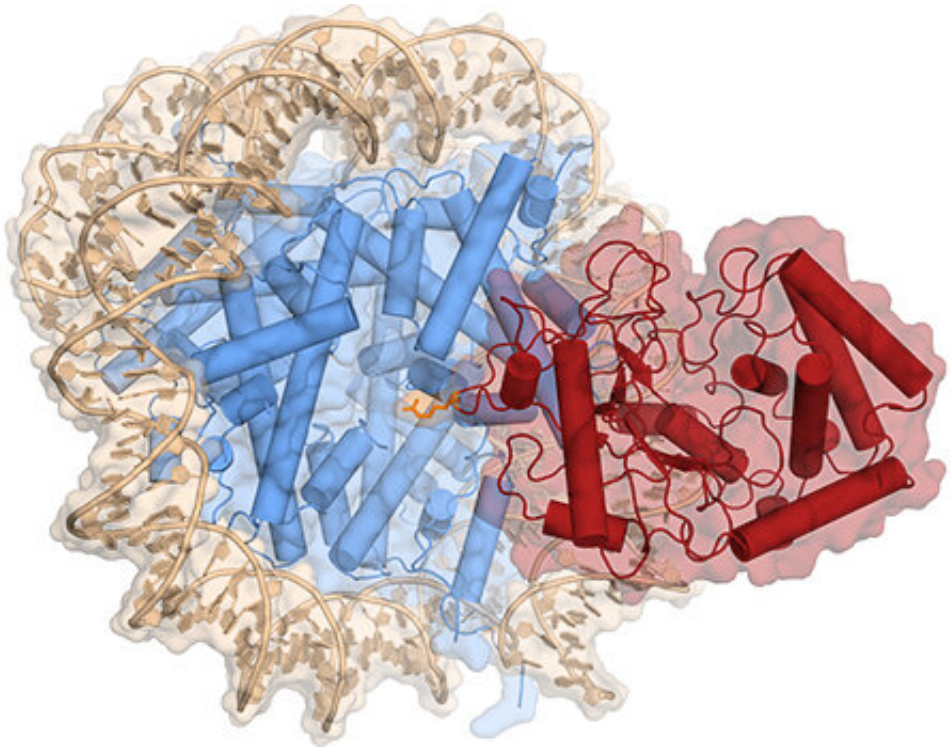

Cryo electron microscopy structure of cGAS (red) bound to the histone proteins (blue) of a nucleosome. Credit: K.-P. Hopfner

In higher organisms, detection of DNA in the cytoplasm triggers an immune reaction. The enzyme that senses "misplaced" DNA is also found in the nucleus, but nuclear DNA has no such effect. LMU researchers now report why that is so.

The bulk of the DNA in the cells of higher organisms is confined to the

nucleus, while all other organellar DNAs are restricted to defined intracellular compartments in the cytoplasm. The appearance of DNA in the soluble phase of the cytoplasm is therefore interpreted by the innate immune system as signaling the presence of intracellular pathogens—usually bacteria or viruses, although tumor cells and senescent cells can also release nuclear or mitochondrial DNA into the cytosol. Misplaced DNAs—whether nuclear, mitochondrial or extracellular in origin—elicit a strong immune reaction, which is initiated by the enzyme cGAS. Researchers had long assumed that cGAS is itself localized exclusively in the cytosol.

However, recent studies have shown that the protein is in fact preferentially found in the cell nucleus. This finding naturally raises the question of what prevents cGAS from binding to nuclear DNA and triggering an autoimmune reaction. Now a team of scientists in LMU's Gene Center, led by Professor Karl-Peter Hopfner, in collaboration with Professor Veit Hornung and his colleagues, has shown that the nature of the interaction of cGAS with the chromosomal DNA in the nucleus explains why the interaction fails to activate the innate immune system. The new findings appear in the leading journal *Nature*.

Upon binding to cytosolic DNA, cGAS synthesizes a messenger molecule which triggers an intracellular signaling cascade that results in the production of proteins that mediate an inflammatory reaction. This process is essential for the elimination of infectious pathogens. However, it is also implicated in the development of autoimmune diseases—some of which in fact involve the generation of antibodies directed against the cell's own DNA. The fact that the cGAS occurs in the nucleus therefore seems at odds with the protective function of the innate immune system, as activation of the enzyme in the nucleus itself would be expected to lead to autoimmune reactions against the nuclear DNA itself. "Curiously, recent data actually suggest that tight binding of cGAS to the DNA-protein complex found in the nucleus—which is known as chromatin—is

crucial for the prevention of DNA-based autoimmunity," says Hopfner.

In the chromatin complex, the DNA is wrapped around disk-like particles made up of proteins called core histones. The resulting "nucleosomes" are connected by "linker DNA" that is not directly associated with core histones. By means of cryo-electron microscopy, Hopfner and colleagues were able to show that cGAS binds exclusively to the protein component of chromatin, and does not interact with the DNA itself. "That was a big surprise," says joint lead author Carina de Oliveira Mann. "Moreover, its mode of binding ensures that the DNA recognition site of cGAS is occluded. As a result, the enzyme is rendered inactive in the nucleus, even when the DNA in its vicinity becomes accessible to other proteins in the course of gene activation. Paradoxically, this implies that, by trapping the enzyme in an inactive state, chromatin actually serves as a reservoir for cGAS."

In fact, cGAS is most effectively inhibited in less tightly packaged regions of the chromatin, in which most of the genes reside. "That could explain why cGAS is activated in what are known as micronuclei in the cytosol, in which chromatin is thought to be densely packed," says Hopfner. Micronuclei consist of chromosome fragments surrounded by nuclear envelope. They are the product of errors in chromosome segregation in fast-growing tumor cells or DNA damage caused by ionizing radiation. "Our study represents an important step forward in our understanding of how cGAS interacts with chromatin," says Hopfner, "and will help us to clarify the inflammatory reaction initiated by the enzyme in the context of cancers and autoimmune diseases."

More information: Sebastian Michalski et al. Structural basis for sequestration and autoinhibition of cGAS by chromatin, *Nature* (2020). DOI: 10.1038/s41586-020-2748-0