# People & Ideas

## Jiri Lukas: Visualizing genome integrity maintenance

Lukas studies how cells detect and deal with DNA damage throughout the cell cycle.

Although researchers devote much of their attention to genomic damage caused by environmental insults, some forms of DNA damage occur naturally, as a result of errors during replication or as an inescapable byproduct of cellular metabolism. Regardless of the source of damage, the same mechanisms are employed by cells to address both naturally occurring and environmentally induced lesions. However, the composition and scope of these mechanisms remains poorly understood.

That's a deficit Jiri Lukas would like to rectify. Having started out studying the cell cycle (1), Lukas has branched out into examining how cells sense DNA damage (2), how they respond to it (3, 4), and how inherited genomic lesions impact later cell generations (5). We took a closer look at his work and career when we called him at his new laboratory at the University of Copenhagen's Novo Nordisk Foundation Center for Protein Research.

### AT THE START

***I understand that you've recently moved to a new research institute…***

I've been living and working in Copenhagen for almost 20 years now, and my new laboratory is just two kilometers from the institute where I used to work, which was run by the Danish Cancer Society. So, the move was not very far geographically, although it is a big step in my career. Now, instead of being in charge of a group or a small department, I'm in charge of an institute with about 150 people, and in that sense it's a challenge. But my group moved with me, so our research will continue.

***Are you from Denmark originally?***

No. I was born in what used to be Czechoslovakia, in a town called Brno. That town is very important for biology and the life sciences because it is where the abbot Gregor Mendel discovered the laws of genetics. I was born not very far from Mendel's abbey.

Somehow, in this town, genetics is in the air. Kids there learn early on about genetics and biology.

***Do you have any strong memories about growing up there?***

There were a few formative experiences in my youth. The "Mendel spirit" is one. Another is that I was born into a scientific family; my mother is a biophysicist who is actually still active in the laboratory. From a very young age, I considered science and genetics as something normal, as a part of life.

I was not a very social child, though. I was the type of kid who would go into the countryside for solitude and to study what I saw around me. In fact, I still enjoy doing that kind of thing today. But as a child, I started to make notes about what I observed—not in the form of writing but by drawing what I saw. I had a very visual way of thinking. That probably informs the type of work I do now.

***So did you want to be a scientist when you grew up?***

Yes, although at first I wanted to be a zoologist. I did my PhD in veterinary science, and the step toward molecular and cellular biology came later in my career.

> "The DNA damage aspect of cell cycle regulation remains very hot at the moment."

I finished my PhD in the year that the Iron Curtain disappeared from Eastern Europe, and then we were allowed to travel. While I was in graduate school, I had met a colleague, Jiri Bartek, who actually remains a close associate of mine today. We've been working together as scientific partners for 20 years, as is obvious from all the papers we have published together. He had managed to travel abroad a bit earlier, and he helped me to arrange to spend a summer as a postdoc in Paul Nurse's laboratory in Oxford. That time in Paul's laboratory was a real shift and an irreversible change in my career.

PHOTO COURTESY OF ANNELI ENGSTRÖM

**Jiri Lukas**

My second postdoc, in Giulio Draetta's group at EMBL in Germany, was also very important. It was where I became interested in how the core cell cycle machinery—which I had first studied in Paul's laboratory—communicates with its effectors.

### MID-CYCLE

***You were looking at cyclin Ds…***

At that time, one of the few ways to block a signaling pathway in mammalian cells was to microinject either neutralizing proteins or antibodies. I was microinjecting antibodies against cyclin D, and I noticed that most cells stopped proliferating. But there was a subset of cells that continued cycling, and, when we looked at the makeup of those cells, invariably they had a mutation in the retinoblastoma gene (Rb).

The Rb pathway regulates an important cell cycle decision called a restriction point, after which cells are in a sort of "committed" phase. This was relatively unexplored at the time. Does the cell have to complete the cell cycle, even if something goes wrong? This was an incentive for us to examine the effects of DNA damage or genotoxic stress, including replication stress, on the cell cycle. When we looked at that, we noticed that cells could actually delay cell cycle progres-

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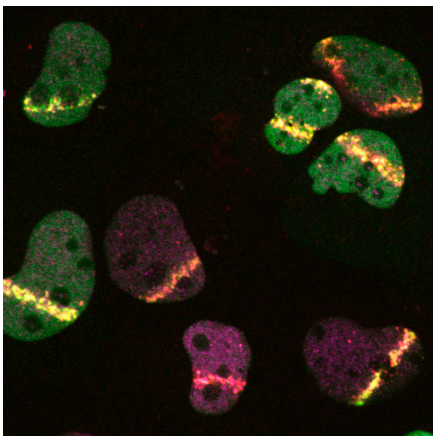

IMAGE COURTESY OF CLAUDIA LUKAS

**Repair proteins accumulate at sites of micro laser-generated DNA damage.**

sion when exposed to DNA damage, and we went on to identify a particular signaling pathway that is responsible for transiently delaying the cell cycle. The DNA damage aspect of cell cycle regulation remains very hot at the moment, and that's something we keep studying very intensively.

***How do cells sense DNA damage to arrest cell cycle progression?***

DNA damage is a highly localized phenomenon, but the cell cycle affects the entire cell. One of the big discoveries in my laboratory was our finding that a tiny lesion on the chromosome can communicate with cell cycle effectors anywhere in the nucleus.

We identified the first of many molecular effectors that transduce this signaling between the local lesion and the whole nucleus: it's a protein kinase called Chk2. We found that this protein has a domain that effectively targets it to DNA breaks, where it becomes modified. This modification ejects Chk2 into the nuclear space, where it can sample the whole nuclear environment and phosphorylate its substrates. Among those substrates are important regulators of the cell cycle, which become degraded and cause a transient delay in cell cycle progression.

Then we started to think, okay, so this is just one protein we've identified. But do we really understand the full complexity of genome surveillance? So we embarked on systematically studying which proteins come to the chromosomal lesion, when, and how long they stay—all in an effort to understand the full complement of the proteins involved. For that, we had to develop many tools, including micro laser technology, an approach inspired by Thomas Cremer, who was the first to apply lasers to inflict DNA damage in cells. With this technology, we can generate damage in a very defined nuclear volume. This then allows us in real time—really within seconds of inflicting DNA damage—to see which proteins come and in what order. If we disable one of those steps, we can also see how that influences subsequent steps.

## NATURAL PROGRESSION

***Where is this approach taking you now?***

These days, we are actually trying to step aside from this artificial type of damage—away from using high doses of radiation, DNA-damaging drugs, or lasers. Instead, we're studying DNA damage that arises from natural processes, such as DNA replication or from the reactive oxygen species generated during cell metabolism. In the end, if you want to link all these mechanisms with disease, then you have to understand that the major source of genotoxic stress is actually the cell itself and its metabolic products or replication errors.

> "The key to a really groundbreaking discovery is to take the time to look at the cells."

We recently showed that some forms of DNA lesions are inheritable. For example, it was already known that there are fragile sites in the genome that often fail to be replicated in a timely manner during one cell cycle. These are converted to DNA breaks, which we showed are then transmitted to the next generation of cells. We can now track these lesions, and we are developing new systems and tools to track them through two or three complete cell cycles.

For instance, there is a protein called 53BP1 that is required to maintain genome health, but it doesn't bind directly to damaged DNA. It binds to the neighboring chromatin, to histones that are modified in areas surrounding DNA damage. Using 53BP1, we can track these modifications through successive cell generations. This allows us to ask questions about the nature and longevity of these modifications, which are an example of epigenetic memory. In this context, we're particularly interested in histone ubiquitination by the ubiquitin ligases RNF8 and RNF168.

***Do you have any advice for young scientists today?***

We really are in a good position these days because we have all these fantastic tools. Today's high-content microscope machines are so sophisticated that you can set up your screen, close it in a black box, and then get all these wonderful graphs and diagrams with standard errors. But I still think the key to a really groundbreaking discovery is to take the time to look at the cells and images in an open-minded fashion—just to be surprised by what you see. I'm a very hands-on scientist, so I like to look at every piece of data.

I have a laboratory full of good people, and my wife, Claudia, who has been working in my laboratory for many years, is a better microscopist than I ever was. So, I can still contribute to these discoveries just by looking at her data and pointing to images that on the first glance don't make much sense but that, occasionally, turn out to start a new project in the laboratory.

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2. Lukas, C., et al. 2003. *Nat. Cell Biol.* 5:255–260.
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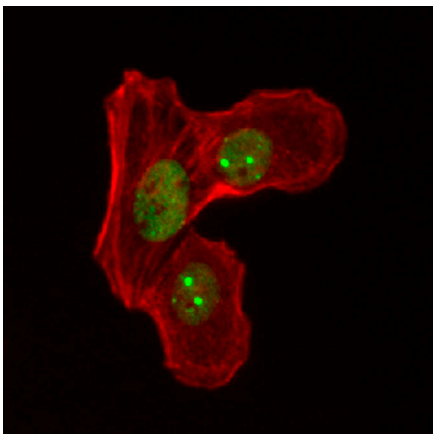

IMAGE COURTESY OF CLAUDIA LUKAS

**53BP1 (green) marks identical, inherited sites of DNA damage in a pair of daughter cells.**