00:01 Sarah Crespi: Welcome to the science podcast for March 13th, 2020. I'm Sarah Crespi. First off this week, I talk with freelance science writer Christa Lesté-Lasserre about the role of scientists at the Notre-Dame Cathedral, both in the restoration of the structure and for the investigation of its past. Then we have researcher Felipe Quiroz. He's gonna talk about how our skin forms a tough barrier against the outside world and how this barrier's formation depends on phase separation. A very hot area of cell biology.

00:42 SC: First up today we have freelance science writer Christa Lesté-Lasserre. She wrote a feature this week on the scientists leaving Notre-Dame's restoration after the April 2019 fire and their use of the fire to probe the mysteries of this cathedral. Hi Christa.

01:00 Christa Lesté-Lasserre: Hi Sarah.

01:00 SC: Okay. The way you describe the scientific work that's going on at the cathedral after the fire, it can almost be broken down into these categories by material: Stone, glass, lead and wood. Each has experts, it has its own challenges and its own mysteries. All of the teams that are working together on the restoration on these different categories of materials are working together in the same organization. Can you tell us a little bit more about this group?

01:28 CL: They're all working under the same laboratory which is a part of the Minister of Culture.

01:35 SC: Yeah. Your description of it in the story was pretty amazing.

01:37 CL: Oh yeah, it's really phenomenal. It's so French. They have a wing of a 17th century castle. You have these austere iron gates that lead up to it and you just have no idea of the treasures that are hidden in that area.

01:51 SC: Let's take this material by material. First a stone. Obviously Notre-Dame is made of stone, wood and some other pieces. And I was really surprised by how dangerous getting the stone out of the building after the fire was. The researchers actually had to use robots to get the stones out.

02:11 CL: They did. You've got this vault that's teeter on collapse because they have no idea how much forces have changed. They've absorbed water, but they've also been affected by heat. So if there are some stones that have suffered some heat damage, they might crumble. At the same time, there are also some stones that are just dangling from the ceiling and they might just drop at any moment. Everything is really delicately balanced. And you've got a 30-meter drop, that's a 100 foot drop coming down.

02:40 SC: Some of these stones are actually taken back to this French chateau from this French
castle. A lot of them end up stored outside the cathedral waiting to be put back in, but they do take some back to the French castle, right?

02:54 CL: Most of them are stored in these tents that are all in front of the Cathedral and they're sorting them. It's just an immense amount of work of sorting these because they're not gonna lose anything. Anything that's about more than 5 centimeters long is gonna be put back into the Cathedral. The ones they take back to study, what they're looking for is to see different signs of heat damage. That can be seen through for example, oxidation which can change the color of the stones. But that's only a guide. But it's still a pretty good guide. According to how much heat it got you can go from red to black. And then surprisingly then to white. And when it gets to white, then you know that it's really bad 'cause it's just powder. They also are doing some testing on the water absorptions. These stones have absorbed quite a bit of water. They can gain up to 30% of their weight.

03:41 SC: The water came from trying to put the fire out?

03:44 CL: Yes, the water came from the firefighters hosing down the Cathedral. And it's taking a very, very long time for them to dry. So the fire was April the 15th and as of today it's still drying.

03:58 SC: They're still losing weight to water evaporation.

04:01 CL: Yes they're still losing weight.

04:02 SC: Oh, that's amazing. So what can they do with this information about how the stones change color? How is it helpful to know that this color change corresponds to how much heating went on with the stones?

04:13 CL: What they really need to know is the detail of which stones are likely to be damaged enough to need to be changed. Because there's some that may not have fallen but they may have been so damaged that they're not going to be able to hold up the structure correctly anymore and that this delicate balance of force is going to be upset. They also need to know how long it's gonna take for everything to dry because there's no point in testing, for example, the mortar between the stones until everything's finished drying. Because the mortar is going to continue to be affected by the changing forces.

04:46 SC: Right. Another dangerous aspect of this work is lead. Notre-Dame had a lot of lead. Tons of lead in the roof and spire. And it was melted by the fire. It was also thrown out into aerosol particles. A yellow cloud escaped and it coated everything inside the building and possibly some of the nearby neighborhoods. What do we know about the contamination in the surrounding city?

05:12 CL: We actually don't know that much about the lead contamination in the city. There was a lot of fear at the beginning. Parisians were very worried. And rightfully so, because lead toxicity could be pretty bad for children. And there are a lot of children living in Paris. But the scientists here at this laboratory have found out that the vast majority of that roof did not go up into smoke or a cloud, it melted. The melting point is 300 degrees Celsius. The evaporation point is 1700 degrees Celsius. We didn't get anywhere near that. After about 600 degrees Celsius then it created this little
micro nodules that went up into the air in aerosol. So really a small portion of the roof did go up. Quite a bit of it actually fell down into the Cathedral itself. Some did go outside, and it floated away. You can see it in the videos of this yellow cloud, that went along the Seine. But the scientists think that probably much of it did not drop down onto the ground or into the Seine River but just kept going, following the Seine further downstream. So it may not have ended up in Paris at all. We don't know where it is yet, but the researchers are looking for it.

**06:25 SC:** Right. They're gonna be testing different areas of the city trying to see if there's a signature of the Notre Dame lead there.

**06:32 CL:** They've been able to test the lead from the roof to be able to get the isotopes. This has allowed them to have an isotope signature. So when they find lead, and there's lead all over Paris, you can't just example say, "Oh, that came from Notre Dame." You don't know. But because of the isotopic signature, then you can. They're trying to test that to find out if the lead ended up in those areas.

**06:54 SC:** What about the lead levels inside the cathedral? Are they a big concern for the researchers working there now?

**07:01 CL:** Because of the high amounts of lead in the cathedral, there's a great concern with the work safety agencies, so they are requiring very, very strict procedures on lead safety. First of all, access is extremely limited, just the bare minimum people that are allowed to get in. The only access in is to go through a shower cabin. And they have to take off all their clothes. They have to wear these paper clothing, paper underwear and paper socks. You're allowed to stay for a maximum 2 1/2 hours. And the whole time that you're there you have to wear these masks that are really heavy and uncomfortable and they have breathing assistance. And it's not just the people, it's also any equipment they bring in. Anything they bring in has to be either destroyed or washed thoroughly on the way out, 'cause as they leave the cathedral, after 2 1/2 hours, they take all of their clothing off, and they go through the shower. And anything they brought with them also has to go through the shower and wash.

**07:58 SC:** With all this protective gear, all this rigorous cleanliness routine that they have to go through, it shouldn't be surprising that most of the work that they're doing right now is to get this lead out of there.

**08:08 CL:** The lead is not being removed yet, because the initial step is finding out how to get the lead off without destroying this 850-year-old monument and all of its precious art inside. And it's just an extremely expensive project to begin with, and they're trying to be as cost-efficient as possible, so cost is really an issue. They can't just go in with the most expensive kind of equipment either. And they need something that is, can be explained to the people that are going to be doing it, because the people who are cleaning it are not going to be the scientists. It's going to be a company that's gonna be brought in, and they're gonna be technicians that are gonna come in and clean. You have to be able to explain to them a protocol that can be consistent. You have to be able to say, "Wipe at a medium amount of pressure four times with this wipe." And so they're looking at ways that are preserving the surface, which means that they have to watch out for any kind of chemicals, because the chemicals could react with the surfaces. They're concerned about the stones. They don't
want to have mineral in anything that they use, because it could also react with the minerals. They
don't want to have any kind of acid that could react to the paint that's in the stained glass.

09:16 SC: Have they had any success in finding a cleaning solution that works on these surfaces, or
are they going surface-type by surface-type?

09:23 CL: They were hoping to find something that's more or less usable throughout all the kinds
of surfaces. So the teams have been working together to try to find a solution, but sometimes it
doesn't work. But one thing that they have found is that if it's a smooth surface, like the lead, or
much of the iron if the iron isn't corroded, the waxed wood surfaces and the glass, then they can just
take the cotton pad and they put de-mineralized water on that, and they just wipe it down. But then
again, it's a question of the pressure and the amount of time. So they've created a protocol using
those materials.

09:54 SC: This is such a big place, though...

09:57 CL: It is.

09:57 SC: To go over with...

09:57 CL: Well that's why...

09:58 SC: Basically make-up pads.

[chuckle]

10:01 CL: But that's why they're not gonna use that everywhere. Anyway it won't work on the
stone, because the stones are too porous. Well not all the stone. Some of the stone, it won't work.
They're having to use vacuuming, and they're using quite a bit of vacuuming. It's just a Shop-Vac
with a special filter in it. They also have some other options where they're... The way they described
it to me sounded like Silly Putty. It's wet when they put it on. So they paint it on, and then it dries,
and then they can pull it off.

10:25 SC: And it takes the lead with it?

10:26 CL: And it takes the lead with it.

10:28 SC: Well, as these teams work out how to clean up and restore the cathedral, some other
scientists are using this lull, this tourist-free time to investigate some of its history to better
understand how it was built, and the world around it as it was being built, for example, the origins
of the wood and the stones and how they fit together. What do we know about where the wooden
parts of the cathedral came from?

10:54 CL: The researchers are looking at some specific details within the wood that will give them
hints. They explain to me they're kinda working like a funnel. They've got the archives that get them
a little bit of information about where this may come from, and then they narrowing it down to
different spaces. And where they are now is testing what the tree was consuming essentially through
its roots. It's a chemical analysis of what these trees had been absorbing from the substrates. I was
surprised about that. I said, "It's been 800 years or 900 years."

11:26 SC: Right.

11:26 CL: "It's not gonna be the same thing over there." And they said, "Oh, actually, yes it is,
because these are oak trees and they dig really, really deep down into the ground and at that depth
nothing's changed.

11:36 SC: One other thing you mentioned in the story that was pretty amazing to me was the idea
that some of these pieces of wood, some of these trees, might have been purposefully grown to be
made part of the cathedral.

11:47 CL: Yes, and that's a really exciting concept, isn't it? Because these trees were 100-years-old
when they were cut down, which means if somebody was planning, [chuckle] they were planning
on building a cathedral with them, then that means that it was several generations in advance that
they said, "We're going to start growing trees to build a Cathedral one day.

12:04 SC: What are some of the indications that that might have been the case?

12:08 CL: Well, they noticed that all of the beams are... They grew in a way that made them very
long and narrow and dense. They were in a dense and competitive environment. And this made
them think, maybe this is part of what they call silviculture, which is this idea of planting and
reserving a particular area of the forest for the purpose of building a monument.

saying, this cathedral was built on top of earlier religious buildings, earlier churches. And now I'm
kind of feeling lied to, because in your story, from what you write, it doesn't sound like these early
buildings have really been verified.

12:55 CL: Yes, and I was surprised about that, as well. I understood that, of course, it was built
over previous churches, but not everybody agrees with that. So looking under the cathedral would
certainly answer that question. They would like to use ground-penetrating radar to test underground.
There's no basement of Notre Dame, and there's no crypt. So this would actually be on just the
ground surface, the same surface that you walk in when you visit the cathedral. They would be
running this GPR over that surface to see what's underneath. It hasn't been approved yet, but it's
something that they would really like to do, and an added benefit of that is that if they need to
construct a new scaffolding that they want to set on the floor instead of on the walls, then they
would be able to know what's under there, and know how much force it could hold.

13:46 SC: Very interesting, you end your story with the impact of the fire on people, how traumatic
it's been for people who were there, people who watched the burning, and how this has changed the
way tourists spend time in that part of the city, how are researchers looking at that?
14:05 CL: The researchers are planning to speak to different groups of people, tourists, also people who 'til the fire were still using the cathedral as their religious home. It was still a very active church, just by being a tourist place. They want to speak to them and see how they've been affected. They're also very interested in the guides because the guides are very attached to the cathedral. They're all volunteers, and are passionate about the cathedral and these people, they can't let go of their work at Notre Dame, so they're continuing online. And they also want to see how it's affecting the tourists, they still come. People are very attached to that and it's an interesting phenomenon to see how many people across the entire planet are affected by this one monument.

14:53 SC: Alright, I'm gonna ask you the toughest question there is, Christa.

14:56 CL: Okay.

14:57 SC: How long is it gonna take to restore the cathedral?

15:00 CL: Five years. They have to finish it within five years. That is what President Macron has decided, and everybody is working on that particular deadline. But that doesn't necessarily mean that all of the renovation will be finished. There are ways to be able to open it to visitors without it being completely renovated yet.

15:18 SC: Okay, alright, Christa, thank you so much.

15:21 CL: Thank you, Sarah. It was good to talk to you.

15:23 SC: Christa Lesté-Lasserre is a freelance science writer, based in Paris. You can find a link to her story at sciencemag.org/podcasts. Stay tuned for an interview with researcher, Felipe Quiroz, about the role of phase separation in the formation of skin.

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15:46 SC: The paper we're gonna talk about today covers a lot. We're gonna get to talk about phase separation which is a really hot area of biology right now. We're also gonna talk about how our skin gets so tough, and we're gonna talk about how phase separation is involved in that process. We're also gonna talk about a sensor for phase separation in cells, and finally we're gonna bring it all the way back to skin disease. Felipe Quiroz is here to talk us through all of these things. Hi, Felipe?

16:17 Felipe Quiroz: Hey, Sarah, how are you doing?

16:18 SC: I'm good, I'm excited. We actually haven't talked about phase separation on the podcast before.

16:23 FQ: Wow, that's crazy, how is that possible?

16:26 SC: It's kinda sad, so liquid-liquid phase separation in a cell. This is something that is
constantly being compared to oil and vinegar. Can you give us a general picture of how liquid-liquid phase separation works in a cell, and how it's kind of infiltrated all these different areas of biology?

16:45 FQ: It's a process that has been known for a long, long time, but it was only really in the last 10 years that thanks to really pioneering work of Cliff Brangwynne, and Tony Hyman, got the attention that it deserve, the idea that biomolecules within the cell have this intrinsic ability to mix or demix. The molecules go from being happy together to saying, "No. I wanna be in my own group of like type molecules," basically from their own compartments. And the surprising thing about these compartments is that they're very dynamic, they're not having lipid membranes around them, and because they're so dynamic, they essentially fuse, and behave as if they were liquids.

17:25 FQ: And so the material properties of these kinds of compartments really have received a lot of attention in the last few years because people started realizing that these unique liquid like properties would also have implications for biology.

17:37 SC: Right, I'm picturing a lot of lump right now, because they are kind of liquids, but they're separate from each other. So what are some of the processes that phase separation has been implicated in? Can you give us a few?

17:48 FQ: The most historic one, perhaps, is the nucleolus, this structure within the nucleus that is really a very, very essential part of the cell. And people knew that it didn't have membranes, but the underline sort of biophysical understanding of how that was possible or even more importantly, what the implications of that really were is something that was basically overlooked, for many, many, many years.

18:11 SC: Well, here in this paper, we're gonna talk about the role of phase separation in the formation of the layers of our skin. So let's just talk in the basic level about how our skin has different layers and how those cells get to where they need to be.

18:28 FQ: I conducted this work in the group of Elaine Fuchs who is one of the leaders in the world on skin biology, and she spent most of her career understanding how stem cells begin to detach from a specific membrane that allows them to reside at the base of the skin, and as they move outwards, towards the skin's surface, they start acquiring unique structures. Probably everyone has heard, at one point or another, that the surface of the skin is made of dead cells.

18:57 SC: Right.

18:57 FQ: And so as these stem cells detach and move toward the surface, they actually eventually undergo a really unique program of cell death in which they get rid of all their organelles. And this has been known for a long, long time because whenever you take a look, say, through electron microscopy, these cells at the surface are extremely flat, and thin and they pile up to form this layered structure that is this surface of the skin, that is actually super important, perhaps the most important in establishing the barrier properties of the skin. So the ability of the skin to prevent the loss of water from our bodies, or to prevent the entry of pathogens into our bodies, is because of this
layer of dead cells on the surface. We call them squames, just because of their scale-type morphology.

19:44 SC: That means a scale, it's Latin for scale.

19:47 FQ: Yeah, like in a fish.

19:49 SC: So, we have this very tough barrier layer made of dead cells. But they're dead on purpose, it's not like the body's throwing them away.

19:56 FQ: Exactly.

19:57 SC: It's stripping them of everything unessential, making them really tough, binding them all together, and protecting the body with them.

20:05 FQ: I always like to make the point that... And I think you said it quite right, it's done for a purpose, so that the cells at the surface actually have a very well-defined structure, so that the skin actually has very unique mechanical properties. It has to be tough, it has to flexible, but it also has to be impermeable to all of these pathogens and molecules that cannot go out of our bodies or get into our bodies.

20:31 SC: Right. So this process of the creation of the skin cells, this is where you found that phase separation was actually really important. What are the oil and vinegar in this process, [chuckle] in this scenario? Where is phase separation happening, and what are the major players?

20:49 FQ: What we found was that phase separation was playing a critical role in the step right before this major transformation. And so, when the cell goes from being a normal-looking cell, with a nucleus and all other membrane bound organelles, to becoming this flat, dead squame. The sort of star player that is driving this process is this family of proteins are known as filaggrins.


21:18 FQ: They are the ones that have evolved to the mix from the cytoplasm. So, in this context, the cytoplasm will become the oil, and then we add the vinegar, being filaggrins, and they form their own droplets, and they're highly abundant.

21:33 SC: When we say these are separating out, they're forming these droplets, what does that functionally do? What does it mean for the cell that these proteins, this family of proteins, are all getting together and separating... They're de-mixing from the cytoplasm?

21:48 FQ: That's the biggest question that not only we had as we ponder on these issues, but really one that pervades anyone thinking about phase separation in biology.

21:58 SC: Right.
It's striking to see these processes, but it's very difficult to answer the underlying questions. There are many potential explanations and, truthfully, the explanation often, I think, has multiple facets.

Right, there are a lot of things that could be happening. Just one, certain molecules could be hiding away in the droplets until the right time, till they're needed. What other options are there?

Yeah, you can imagine that you can protect things from being exposed at the wrong time. You can accumulate things that you need for later. You could concentrate reagents that you need to speed up chemical reactions. We now have begun to think more and more, and particularly in the context of our work, about just biophysical consequences. What we have in this case is these structures that are fairly large, in the order of microns, so think something that is almost as large as the nucleus, and suddenly these structures can occupy a significant portion of the cell volume. So now we have to think about, essentially crowding. So what is the consequence of that? We now have come to understand that, as we change these crowding effects, other biomolecules in the cell also change their behavior.

They're taking up space. They're taking up space, they're stretching out things, they're pushing other things together into a crowd. It's more of a physical thing than a chemical interaction.

Well, I'm saying both are at play and it depends on the scenario. In our case, in the skin, we were uniquely puzzled by the abundance of our compartments. The kind of abundance that we see for these liquids is unusual. And so in our case it was very obvious to start thinking about biophysical consequences.

What happens when you disrupt this process, when you prevent this phase separation from happening in the skin cells that you looked at?

We have this unique ability to wipe out these filaggrins from the mouse skin. And what we saw was that when we removed these filaggrins there was no longer phase separation happening. When we did that, this program in the skin of cells being able to form the surface layers of dead cells was affected. Specifically, we saw that the organelles that had to be lost were not being lost as fast. And, more importantly, that the process had lost its environmental responsiveness.

What do you mean by that?

So the skin barrier is unique, in that the skin evolves according to the environment. If you place yourself in an environmental extreme, like a very dry environment, for instance, your skin has to get better at preventing water loss. And so, how does it do that? It has to somehow couple with environmental signals. What we discovered was that phase separation is actually a unique mechanism by which the skin is environmentally responsive. And so, what we saw was that when we removed these compartments, the process of triggering this squame formation was disrupted. It was no longer coupled to specifically a signal that we discovered, which is a pH shift, interstitially in these cells.
24:56 SC: The pH varies. The deeper you go into the body, there's a change in pH?

25:00 FQ: And it's been known for a long time, that the surface of the skin is acidic. What we didn't know is how this extracellular pH on the skin surface translated to intracellular pH changes, because these droplets are within the cell. So, we deployed some pH reporters that allowed us to, essentially for the first time, see how, as cells approach the surface, their intracellular pH changed very rapidly. And it's specifically at the exact time when the cells have to transform from these normal looking cells into these squames. Now, these was coupled to this liquid-like droplets. The liquid-like droplets we showed are uniquely evolved to be pH responsive. And so, if the droplets were present when the pH shifts, these squames were formed. But when we depleted the droplets the pH shift no longer actuated the process. So it really showed a link between the dynamics of those liquids, their pH responsiveness, and the ability to form skin in an environmentally trigger manner.

26:09 SC: One thing we definitely need to touch on before I let you go, is the fact that part of this paper involves the development of a sensor for phase separation. So can you talk a little bit about how that works?

26:20 FQ: We've gotten really good as a field in looking at phase separation in artificial systems, but we really just wanted to, for the first time, be able to do these experiments in the actual tissue. The question for us was really, is this actually happening in the skin? So what we were able to do was create this new strategy where we introduced into these transgenic mice, fluorescent proteins that by themselves do not undergo phase separation, but they're designed to sense if anyone in their surroundings is undergoing phase separation. When these mice stem cells started differentiating, the phase separation sensor is reporting that nothing is happening, that's what we see, but when these cells first express the protein, these filaggrins, these sensors become co-opted to engage in interactions with those filaggrins. And in doing so allows us to visualize the location and the material properties of those emerging droplets. The beauty of the approach is that we don't have to modify filaggrins. We knew that whenever we modified those endogenous proteins, say to have fluorescence through fusion with a fluorescent protein, we know that bio-physical properties are being affected.

27:36 SC: You could end up modifying them so they don't form droplets anymore.

27:39 FQ: Exactly, or you could enhance the formation of those droplets and, perhaps more difficult to understand, you could change the material properties of those droplets.

27:49 SC: So they're stickier to each other or?

27:51 FQ: Yeah, they're no longer liquids, they become solids. So really having tools to nondisruptively assess phase separation, I think that's something that we need to move forward as a field with.

28:01 SC: Let's circle back to disease now. Is there any indication that the proteins that you do know are involved in the droplets are defective in certain disease states?
28:12 FQ: Yes. That's actually kind of what drove us throughout the process. This was a lengthy project, but the reason why we're so committed is because we knew mutations in these proteins are the strongest predictor of skin barrier disorders, particularly atopic dermatitis, and the most severe forms of atopic dermatitis. People have been very excited about this for over a decade, but the function of this family of proteins has been completely unknown. But no one ever thought, to our knowledge at least, that the functional state of the proteins had to do with these granules. So the granules themselves have been known forever.

28:51 SC: Right, so you can look at a microscope, you can see them, you don't know it's in them, you know this proteins really important according to the genetics of the disease.

29:00 FQ: Exactly.

29:00 SC: And now you know, you put them together.

29:02 FQ: It's really crazy because the genetics of it developed over the last 10 years, but once the studies started to pile up, I mean it was very clear this protein was essentially perfectly link with the disease, but without a good understanding of the underlying biology and what it was doing, basically I think we really haven't been able to make progress in terms of linking potential therapies to the genetic defects. And now that we understand what these proteins are doing, it opens up the window for thinking about, "Okay, well, we know this is defective, it is because of the phase separation process, what can we do about it?" How can we target now phase separation as a way to treat disorders? It's not an easy question to answer, but now we have a handle and a new direction and that's quite exciting to us.

29:50 SC: Alright, thank you so much Felipe.

29:53 FQ: Thank you for talking to us.

29:53 SC: Felipe Quiroz is an Assistant Professor in the Department of Biomedical Engineering at the Georgia Institute of Technology and Emory University. You can find a link to his paper at sciencemag.org/podcast.

[music]

30:07 SC: And that concludes this edition of the Science Podcast. If you have any comments or suggestions for the show, write to us at sciencepodcast@aaas.org. You can listen to the show on the Science website. That's sciencemag.org/podcast. There you'll find links to the research and news discussed in the episode and of course you can subscribe to the show on Overcast, Stitcher, Spotify, Pandora, and many other places. This show was edited and produced by Sarah Crespi with production help from Podigy, Meagan Cantwell, and Joel Goldberg. Jeffrey Cook composed the music. On behalf of Science Magazine and it's publisher AAAS, thanks for joining us.