A Conversation with Maria Elena Torres-Padilla

Interviewer: Anke Sparmann

Senior Editor, Nature Structural and Molecular Biology

Maria Elena Torres-Padilla is Director of the Institute of Epigenetics and Stem Cells at the Helmholtz Center in Munich.

Anke Sparmann: Your research focuses on transitions of cellular potency and cell fate decisions working with totipotent cells at the very early stages of mammalian development shortly after fertilization. What fascinated you about this cellular system?

Dr. Torres-Padilla: We are interested in understanding how these very early cells of the very early embryo are actually able to establish and maintain the largest plasticity that one can think of. It's quite remarkable. Everybody, at some point, was a single cell. The question is how that single cell is able to generate a new being: not only all the tissues and cells that we have in our body, but really how that single cell builds up the whole program that we call "totipotency." The system is difficult in that we have very limited materials. Obviously, we don't do experiments with humans, but we do use the mouse and other species as a model to understand these transitions. But you don't get a lot of embryos to try to understand biochemically what happens with stem cells and so on. The system is really fascinating, but it is a challenge.

Anke Sparmann: What are you seeing in these very early stages compared to when differentiation occurs?

Dr. Torres-Padilla: There've been quite a lot of things that we did not anticipate when we started to ask how this RNA regulation and chromatin architecture takes place in these early embryos. Every single hypothesis that we put out there was basically wrong. We had a lot of unexpected findings. One is that the retrotransposons, which typically occupy a very large proportion of our genome, are heavily transcribed. In our genomes and in the mouse, these transposons are around 50% of the genome. They're not normal coding genes, like for the proteins of the skin or of the liver. They're really repeats that are half of our genome. These transposons are typically known to be silenced in all your somatic cells. It has been thought that it is important to keep them silent because otherwise they can actually jump. Evolutionarily speaking, they can jump and people have thought that they can originate mutations.

Although this work was pioneered by Barbara Knowles many years ago, in the course of the last decade we have found that there's a large fraction of these transposons that are heavily transcribed. Of course, this has potential for regulation at the chromatin level, but also at the RNA level. What is the RNA that is coded by these transposons? What are these RNAs actually able to do, if anything?

Anke Sparmann: In terms of just the sheer cost of transcribing all those regions, does so much transcription cause a lot of problems for the cell?

Dr. Torres-Padilla: The question has been whether this transcription is actually functional. Is this just a side effect and a waste of energy, or is this really meaningful for the developmental process and for totipotency establishment? There again, we have found quite a few surprises. For example, we've observed recently that the LINE elements [long interspersed nuclear elements] that are very abundant—20% of the genome, roughly—seem to be actually involved in opening up the chromatin structure of the embryo. Rather than a waste of energy, it actually has become part of the developmental program. This is where it becomes interesting. How is it possible that these repeats and these remnants of viral infections that we have in the genome have been co-opted to regulate processing in development?

Anke Sparmann: In terms of the genome being much more open than in a differentiated cell, is that what's causing this transcription, or has it also other effects?

Dr. Torres-Padilla: One of the items that we have to invest quite a lot in understanding transposon function in the genome is, what is cause and what is consequence, or what is just a correlation? There are a few cases where we can really say this is actually causative. In the case of LINE, we actually tried to target transcription factors to them to manipulate their transcription indirectly. At least for a small portion of these transposons, we can probably say that their transcription is causing changes in the chromatin architecture. Whether this is because the genome is just open and is being transcribed, we still have quite a lot of work to do there. There are certain specificities that we don't really understand very well yet.

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Anke Sparmann: A recent paper that you published looking at the genome organization showed some of the dynamics that are going on at that stage of differentiation.

Dr. Torres-Padilla: Yeah, we also have been interested in understanding how the nucleus first becomes regionalized. For many years we've known that the genes that tend to be in the internal part of the nucleus in a somatic cell are more prone to activation. The transcriptional activity is higher, whereas the genes that would be a little bit more on the periphery of the nucleus tend to be silenced. The important question was when is this regionalization, which is perhaps functional, first established? That stems from observations that heterochromatin is positioned within the nucleus of the embryo in a very weird manner. It doesn't really have this typical clustering that you see in the somatic cells. Instead, it forms sort of rings around the nucleolic precursors. The question has been whether this atypical nuclear organization is actually important for development as well.

The development of new low-input protocols for looking at the genomic changes in the embryo has made for a change in the field. We teamed up with Jop Kind at the Hubrecht Institute who had set up the DamID [DNA adenine methyltransferase identification] technique to generate molecular mapping of the genome in single cells. We managed to map the regions of the genome that become organized in the nuclear periphery in proximity with nuclear lamina—what we call the lamina-associated domains, or LADs—versus those that could be in the internal regions, the inter-LADs.

There again, there were actually quite a lot of surprises. One thing that we found is that these lamina-associated domains are established very early. A few hours after fertilization the nucleus is already compartmentalized, so there's very clear LAD formation early on. At least in the mouse, 20% of the genome becomes localized in these LADs 3 or 4 h after fertilization, and it remains so for the rest of its life. These are actually regions of the genome that are constantly at the periphery. In a sense, that also could tell you that this very first cell already has kind of a "skeleton" or scaffolding for how the genome is going to be organized.

At the same time, we also found that if you look at the autosomes or at chromosomes in the mouse, we did not detect association with the lamina in the oocyte in the maternal germline before fertilization. That has quite a number of implications. First, that obviously indicates that nuclear organization is established de novo after fertilization and is not inherited. It also gives us the opportunity to try to understand how this nuclear organization is established, mechanistically speaking.

Anke Sparmann: How is that part of the genome partitioned?

Dr. Torres-Padilla: Almost 10 years ago we had done some experiments where we had tethered heterochromatin from the internal part of the nucleus to the periphery. We did those experiments trying to understand whether nuclear organization was important for heterochromatin formation or gene function. What we observed is that heterochromatin becomes derepressed. Basically, we were bringing pericentromeric heterochromatin to the periphery, and instead of having it more silenced like one would expect from what we know from somatic cells, there would be a derepression. Again, that says the embryo does seem to have a bit of a different epigenetic landscape, and that seems to be both from the activation of transposons that we were discussing before, but also the nuclear organization.

Anke Sparmann: You were talking about the female genome. How about the paternal genome?

Dr. Torres-Padilla: Unfortunately, we cannot do these kinds of DamID experiments in male germ cells because there is not really a proper laminar organization in the sperm. Also, except for protamines, their DNA basically goes into the oocyte almost naked during fertilization. It's not very clear what really happens in terms of how the nuclear envelope is formed and what the components are during fertilization. That's a process that we don't know enough about. Regardless, because there is not really a proper nuclear lamina in the sperm, we would anticipate that that organization is also de novo. Of course, we cannot rule out that there's some organization in the sperm or some information that could drive that de novo formation of LADs.

What we do see is that after fertilization when the two pronuclei are still separated the LADs are formed in both pronuclei, not only on the female one. They are slightly different in terms of genomic features in the paternal and the maternal pronuclei, but pretty much 20% of the genome goes to the periphery. Again, that's interesting because it also implies that the two parental genomes are slightly different in terms of nuclear organization, but we found that these differences are resolved by the time the embryo gets to implantation.

Anke Sparmann: It's interesting to see that these cells really are very distinct. They challenge how we think about heterochromatin.

Dr. Torres-Padilla: This is the importance of approaching questions with a very open mind. You launch a hypothesis and then you have to be quite open to see what kind of findings you're going to have, because they might be completely different to what you're used to or what you would expect, which is a cool thing about the biology that we're studying.