A Conversation with Edith Heard

INTERVIEWER: GUY RIDDIHOUGH

Senior Editor, Science

Edith Heard is Chair of Epigenetics and Cellular Memory at the Collège de France and Director of the Genetics and Developmental Biology Unit at the Curie Institute.

Guy Riddihough: We are going to talk about sex chromosomes and one of the issues surrounding them that's critical for both cell development and survival. Why should we be concerned about sex chromosomes?

Dr. Heard: Sex chromosomes raise the issue of dosage compensation and getting gene dosage right. Females have two X chromosomes and males have one X and one Y. Because the sex chromosomes evolved from a pair of autosomes, the gradual degeneration of the Y chromosome carrying the testis-determining factor meant that an imbalance started to appear. The X has over a thousand genes on it. Having a double dose of many, if not all, of those genes in a female compared to a male would be an issue. A way of dealing with it had to evolve. In other organisms—fruit flies, for example—the single X chromosome is up-regulated in males. In worms, you down-regulate both X chromosomes in the female by half. Mammals evolved a way to shut down one of the two X chromosomes in females. Clearly, many different strategies evolved across organisms to deal with dosage differences when the sex chromosomes are very different. Ultimately, you need to achieve a state where, for most genes on the X, females have the same expression levels as males, although there are a few exceptions.

Guy Riddihough: Shutting down a whole chromosome is quite a molecularly spectacular event. How does that work?

Dr. Heard: The region that is necessary to trigger X inactivation had been narrowed down, but it wasn't until 1990 that a long noncoding RNA called XIST (X-inactive specific transcript) was discovered that sets off this process. It was in the target region, it was only expressed from the inactive X or the X that was going to be inactivated, and it had the capacity to be restrained in *cis* and not go off and find the other X chromosome. It's up-regulated on only one of the two Xs during development and coats the chromosome in *cis*. The genes on the chromosome that is coated by Xist are silenced very quickly over just a couple days during development. Although

poorly conserved, it's nevertheless present in all eutherians. People have only recently started to pull out the proteins that associate with XIST and we're finally starting to get a molecular handle on what it's doing to gene expression and how transcription is stopped literally in the space of just one or two cell cycles.

Guy Riddihough: XIST was one of the first examples of long noncoding RNAs. Will understanding how it works give insight into understanding other RNAs of that particular class?

Dr. Heard: I think there's a lot of wishful thinking about many other noncoding RNAs. Unlike small noncoding RNAs—the whole RNAi machinery—where mechanisms are quite well understood, the long noncoding RNA world is a vaster field because there are many noncoding RNAs that might not actually have a function. They could just be the result of promiscuous transcription. That doesn't mean that the process that makes them isn't important, but the RNA entity itself might not be a key molecule. It's very difficult to prove that a locus is acting as an RNA. It's taken 25 years of research to address XIST's functions and prove that it really is an essential RNA for this process.

Guy Riddihough: Can you put in a temporal context in human or mouse development when XIST first starts to become evident and initiate the process of shutting down an entire chromosome?

Dr. Heard: We were struck by the difference in timing when XIST starts to be expressed in different mammals. In the mouse, we see gene silencing as soon as it's expressed. As early as the two- to four-cell stage, XIST is upregulated and coats the chromosome. By the four- to eight-cell stage, most genes are silenced.

In humans, XIST is also upregulated quite early, probably between the four- to eight-cell stage. It accumulates, but it doesn't seem to be specific to one X. It happens on both Xs in the female embryo and even on the single X in the male, which seemed odd. However, even though

XIST was up-regulated, it doesn't shut down genes right away. X inactivation in humans may begin much later, probably after the blastocyst stage, which is the 64-cell stage. X inactivation and dosage compensation in humans apparently may not begin until the time of implantation.

This was a huge surprise for us because you need to dosage-compensate very early in the mouse. If you don't dosage-compensate by the time you've made your blastocyst, implantation happens but is very quickly a disaster because you need dosage compensation in the extraembryonic tissues for the embryo to develop. That clearly doesn't seem to be the case in humans. We think that although XIST probably functions in humans, the factors that allow it to silence fully might not be there yet, or maybe the chromosome is not yet competent to receive the signal that XIST provides.

We thought the timing of the whole process would be very well conserved. It isn't. We now think the molecules are well conserved but the timing is species-dependent.

Guy Riddihough: "Inactive X" is a bit of a misnomer. It's "mostly inactive X."

Dr. Heard: That's become apparent. Globally the X becomes inactivated and, in the mouse at least, only a few percent of genes escape X inactivation. Generally, the X is heterochromatic and completely silent. Some of our more recent studies in the mouse show that there are genes that can escape, but in a very tissue-specific manner. Some genes get expressed for very specific purposes in different tissues and different lineages. In humans, a lot of genes manage to either escape or override inactivation.

What's intriguing is that some of the genes that escape X inactivation, such as ATRX, produce proteins that can sometimes associate with inactive X. We're wondering if some genes escape because the females need to have a double dose, that they need to increase the protein dose of those particular genes for the process of X-inactivation to proceed because those are in fact the proteins that participate in the process.

Other genes could escape because dosage might not matter for all genes. It could just be leakiness. It could be epigenetic instability. Especially in humans, we think that a lot of genes are expressed from inactive X because the epigenetic mechanisms that normally would lock in the process are not quite as efficient, and so you get spurious expression from an otherwise silent X.

Guy Riddihough: As well as coating the entire inactive X, XIST also influences its domain structure. If you look at the active X and the inactive X, you see quite a profound difference.

Dr. Heard: XIST is a multitasking molecule that shuts genes down but also changes the way the X is organized. My lab showed that one of the first things that happens to the X when XIST coats it is that it becomes compartmentalized. Repetitive sequences seem to be inside the XIST-coated part and the genes seem to be outside of it and then somehow genes get reeled in.

Using chromosome conformation capture, the lab of Wouter de Laat showed that XIST can alter the overall

organization of the X chromosome. If you take XIST away in a somatic cell, the conformation of the inactive X changes back to the conformation of the active X, yet the genes stay silent. XIST has the capacity to reorganize the X chromosome independent of its capacity to shut down genes. There's one part of XIST, the A-repeat region, that seems to be essential for shutting genes down and reeling them into the silent compartment, and the factors that bind to that have recently been identified. There are other parts of XIST that seem to be important for changing chromosome shape and yet other parts that seem to be important for bringing in chromatin factors. You have this huge RNA, 17,000 nucleotides long, and different bits of it are used in different ways to deal with the process of X inactivation.

Guy Riddihough: X inactivation was discovered in 1961, and it's been 25 years since XIST was discovered. That suggests how difficult it might be for people working on long noncoding RNAs to tease out their functions. Is XIST particularly complicated because the process in which it's involved is so profound in terms of the whole chromosome?

Dr. Heard: I don't think XIST is more complicated than other noncoding RNAs. I think the more you look at a process or a molecule and not understand how it works, the more complicated it seems. As soon as we work out which bits of XIST are doing what and what factors are helping it, it will suddenly seem much simpler. I think other noncoding RNAs that have a function are going to be much the same. I think what's tough is working with RNA. Working with proteins and DNA has become relatively simple. RNA is much more challenging. I don't think the X-inactivation field is slow or XIST is more challenging. We just have an example of how long it takes to sort out what a noncoding RNA really does.

Guy Riddihough: It's not the only noncoding RNA that functions there. There's another one involved.

Dr. Heard: There are several in the X-inactivation center. Xist has an antisense transcript called Tsix that regulates the Xist locus itself. Although you could call it a noncoding RNA, I think most people would agree that the way this antisense transcript functions is mainly through the act of transcription. So, the Xist noncoding RNA is regulated by an antisense transcription unit that we think acts as a sort of "rheostat" to allow Xist to be monoallelically regulated.

What's interesting is that this antisense transcription unit seems to be mouse-specific. It's not present in humans or other mammals. We think it's present in the mouse because mouse X-inactivation has to happen very quickly. Mouse development is very rapid and there was probably a need to evolve this antisense "rheostat" to get it right very quickly. In humans and other mammals, the inactivation process may be a bit slower. It could be that X inactivation can afford to be a bit sloppier in these species and it doesn't really matter, because during development you can counterselect the cells that don't do it. We still don't really know.

In human or rabbit embryos where X inactivation happens later, sometimes both Xs start to up-regulate XIST in the female, and even to silence both X chromosomes. Somehow, they can then backtrack on one X. In those cases, you don't have Tsix and that could be why XIST is biallelically expressed initially so efficiently. Somehow, it has to sort itself out and only one of the two Xs will carry on expressing XIST and continue with X inactivation. I think there's a lot going on at the mouse Xist locus that is rodent-specific because the process has to be so quick and efficient.

Guy Riddihough: Does this complicate things for you? Ultimately, you really want to know what happens in humans.

Dr. Heard: I think humans are very interesting, but I also think it's interesting to look across species. As Theo Dobzhansky famously said, "Nothing in biology makes sense except in the light of evolution." Obviously, we want to know how it works in humans. Personally, I think human embryonic stem cells are quite challenging to work with. It's not clear how well they really reflect what happens in human embryos.

I think we can learn a lot by looking at this process in other mammals. Although there are differences, the final outcome is the same. You need to shut down most genes on one of the two X chromosomes and you're using a non-coding RNA to do it. The way you regulate that noncoding RNA or the degree to which you silence the genes might vary a little bit, but basically we can still tease out the essential factors and elements as we go from one species to another. That's why we work on the mouse, we work on humans, we also work a bit on rabbits. We don't restrict ourselves to the humanized version of X inactivation.

Guy Riddihough: I want to finish off by asking about something completely different. What's it like doing science in France?

Dr. Heard: Science in France is actually pretty exciting. There's a very strong epigenetics community, not just in Paris, but other places in France. I've been there since 1990 and it's become more exciting to be working there over the years. There's a pace of doing science that's quite different than the pace you find in the States. It doesn't seem as if less is achieved. It's just done in a slightly different way. It's as stimulating to do science in France as it is in the States. I spent one year at Cold Spring Harbor so I saw quite well what it's like here. It's exciting in a different way.

Guy Riddihough: The funding is good?

Dr. Heard: As in the States, funding in general in France and in Europe is a bit of a challenge right now. I don't think France is doing particularly well. We're lucky to have the ERC System in Europe. The support in France for basic research is becoming more difficult to get. I and others are actively trying to promote support for basic research. One of the biggest advantages in the French system is that there's a certain stability. They have permanent positions, so scientists can actually take risks, do long-term experiments that don't necessarily pay off quickly. That really is an advantage when you're working on a challenging process such as X inactivation. You can perform experiments that might take several years to interpret and sort out.

The other thing that's possible is that you can have a family, you can have kids. In France, one is very well supported. In general, women in science are very successful in France because there's excellent child care and schools, and child care is subsidized. It's actually very well looked upon to be working full-time, even as a mother. All in all, I think science in France is not too bad.

Cold Spring Harbor Symposia on Quantitative Biology

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Cold Spring Harb Symp Quant Biol 2015 80: 324-326

Access the most recent version at doi:[10.1101/sqb.2015.80.029975](https://doi.org/10.1101/sqb.2015.80.029975)

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