An Interview with Professor Gian Garriga on Asymmetric Cell Divisions: Distinct Fates of Daughter Cells

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Figure #1. Professor Gian Garriga

Dr. Gian Garriga is a Professor of Genetics, Genomics and Development in the department of Molecular and Cell Biology at the University of California, Berkeley. Professor Garriga's interest in understanding the C. elegans nervous system has led to a study into more fundamental questions of cell biology. In this interview, we talk about one such topic, asymmetric cell division, and discuss not only its molecular basis but also its role in apoptosis and stem cell differentiation.

Berkeley Scientific Journal: How did you first get involved in your field of research?

Gian Garriga: After college I was pretty sick of school so I actually did other things for many years and then sort of accidently met some people and ended up going to graduate school. I was a molecular biologist and a biochemist. As a graduate student, I studied RNA splicing. At the end of that, the original plan was to go and get a job in the industry but I thought, "Well, I could put that off."

I looked around for things to do as a post-doc and thought that it would be good fun to work on something that really wasn't understood at all. At the time, people didn't really know how the nervous system was developed. It was very different from what I had done previously so I looked at different organisms where people were studying this. And even though I didn't do genetics as a graduate student, I sort of appreciated it.

It was really a choice between doing something in drosophila or C. elegans (Caenorhabditis elegans). C. elegans were this sort of newer organism in the sense that people had only recently been studying it. And it was also very simple and people appreciated that and you could freeze it. [That's something] you couldn't do with flies and I'm not that organized so something I could freeze was better.

BSJ: And what led to this focus into cell division?

GG: That began just when I started working as a post-doc, I worked on a pair of motor neurons that innervated egg-laying muscles and stimulated hermaphrodites to C. elegans. They stimulated hermaphrodites to lay eggs. So, I started to screen for mutants that were defective in various aspects of the development of these neurons.

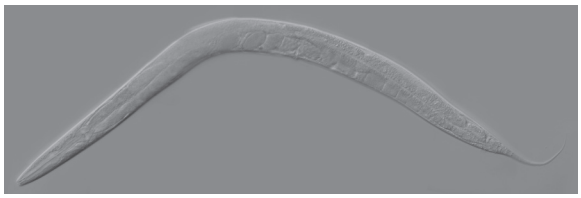

Figure #2. Caenorhabditis elegans

We identified genes that were involved in all kinds of aspects: from how the neurons were generated and how cells migrated early in their development to how they later send out axons. Just because of the genes that I found most interesting when I came to Berkeley, I focused on asymmetric cell division.

Migrating cells would polarize growth cones, which are the ends of axons that are migrating. And then these cells that divide asymmetrically give rise to different types of cells during development. It's sort of a polarity issue. Polarity drives all of these processes, so that's what we've been working on pretty much since I've been here. Which is a long time…

BSJ: Why study C. elegans specifically for this question of cell division?

GG: C. elegans is the only animal where we know it's development in detail. People in the 1970's and 1980's began to just follow the divisions of C. elegans because it's simple and transparent. You didn't need to have any sort of special methods. And you could observe the cells divide.

John Salston, who won a Nobel Prize for this, was able to follow all of the divisions in C. elegans. They're stereotyped between one organism and the next. The lineage is invariant and you can predict where any cell has been, in terms of its ancestry. You know if it's a precursor cell, it's going to divide. And if it's not, you can tell what type of cell it is: if it a neuron, if it's a muscle cell, or if it is a cell that's going to die.

One of the things we study is apoptosis. To understand how fate is specified, and understand the process of asymmetric cell division, you really have to understand this lineage and how that's generated.

BSJ: What exactly is asymmetric cell division?

GG: You can think of how you specify cells in two different ways. I'm going to pick flies as examples here. When I first came to Berkeley, there was a lot of work in Jerry Rubin's lab on fly eye development. The way that works is there's an undifferentiated epithelium as there's a morphogen that

sweeps through. And in its wake, you start to assemble these structures called ommatidia. The eye of the fly is this repeating unit of ommatidias that have a number of photoreceptor cells and support cells. You start to specify individual cells. The first one is the photoreceptor cell called R8. Through interactions of R8 and the surrounding epithelial cells, the fates of the other cells are determined. You generate these ommatidial units. If you look at the lineage that gives rise to that, any cell can be related to any other kind of cell, so there's no lineage at all.

If you look in flies at other structures, sensory organs within the fly, they're generated by a particular lineage. So, a cell would divide and it will assign distinct fates each time to the two daughter cells and those divide to generate two daughter cells that have distinct fates. That process where a cell would divide to generate daughter cells that have distinct fates is asymmetric cell division.

Stem cell divisions are like that too. With a stem cell lineage, it'll generate another stem cell but also a cell that's more limited in its developmental potential. That would be considered an asymmetric cell division.

So, basically any division where you give rise to two daughter cells that have distinct fates.

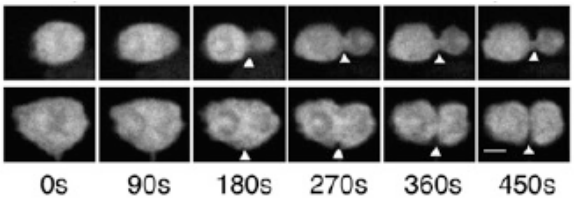

Figure #3. One type of asymmetric cell division (top

BSJ: There's this idea that specific proteins being segregated differently would lead to these distinct fates. What exactly causes that distribution?

GG: Some molecules have been identified, in drosophila and in C. elegans, as being involved in asymmetric division and encode proteins that are localized asymmetrically during the divisions. The molecules are conserved and they contain similar roles in all kinds of animals, including us. How they get distributed can really vary.

In some cases, you inherit the polarity from the cell from which it's coming. An example of that would be the drosophila neuroblast; these are cells that will divide to generate the neurons in drosophila. They come from an epithelium that's polarized and they inherit aspects of that polarity from the epithelial that they were originally. They delaminate from this epithelial layer, but they inherit the polarity of those epithelial cells. The polarity is subsequently used to generate asymmetries in division.

In other cases, the cells are polarized by cues and signals from the environment. Those signals polarize the cell and they then divide asymmetrically.

BSJ: What exactly causes the axis to align perpendicularly to where the epithelial is or wherever the separation is?

GG: If you have a cell that's dividing and you have something that is asymmetrically distributed on one side and if you want that to be inherited by one of the cells so it will adopt the fate different from its sister cell, then it's really important to align the spindle in a particular way. If you align it [parallel to the separation], both of the cells are going to inherit that asymmetrically divided protein and generate the same fate.

There's a hierarchy of molecules that are involved in this. Those molecules at the very top of the hierarchy are involved in not only controlling the distribution of fate determinants, proteins or RNA molecules, but also in controlling the orientation of the spindles so that the cell divides properly.

BSJ: Are there different types of asymmetric cell divisions?

GG: Yes! There are cell types that divide asymmetrically that are controlled by intrinsic polarity of the cell itself and there are divisions where the polarity is imparted by signaling molecules. You can even get cases where a cell divides and there is an inherent difference in the cells. They're played out by interactions either between the cell types or between the cells and the environment.

You can imagine a case where a protein is inherited by one cell type. An example of this is the Numb protein. The fate of the cells is determined by Notch signaling, but Numb interferes with notch signaling in one of the daughter cells. That Notch signal can come from the environment or sometimes even just from signaling between the two cells. So, there's a bias in the notch signaling.

The other [kind of thing that can happen is controlling the spindle by controlling the position] of the spindle where the cells that are generated are different in size. In C. elegans, we think it plays an important role in apoptosis. We don't know why but there's a good correlation between mutants that we've identified over the years that affect [the ability of the cell to survive. Some die and the cells that normally would die survive.] Divisions that are highly asymmetric, generating a much smaller cell and a much larger cell, generate cells that are going to die. The larger cell survives and the smaller cell dies. In mutants where the cells actually survive, [the position of the spindle is affected. Or at the very least, the division plane is affected.] The more symmetric the division is, the more likely it is that the cell survives.

BSJ: Is the smaller cell always fated to die?

GG: There are lots of divisions where you generate cells in different sizes where the [smaller] cells don't die. There's something different about this lineage but there's some aspect of the asymmetry of the division that is contributing to the apoptotic fate. What this is we don't know. One way of thinking about it is that you distribute molecules in the cell so that when one cell divides the smaller cell is not going to have enough something to allow it to live. And if you now make the division more symmetric it may get more molecules that control the ability to survive. So, there's a good correlation between cell size and ability to survive in the lineages where

the cells normally would die.

BSJ: In terms of apoptosis, are the triggers coming from the parent cell, maybe some apoptotic factor? Or is it that once a daughter cell doesn't have whatever resources it needs, it's conditions triggers apoptosis?

GG: We don't know! All we do know is this correlation and we don't understand this mechanism that drives that correlation. Apoptosis in C. elegans is pretty well understood so we really understand how cells execute this [conserved] apoptotic pathway. We know in certain cases that the apoptotic fate is controlled by transcriptional regulation of the most upstream apoptotic gene. So, if you turn that on, you kill the cell.

But there's this additional thing going on where there has to be a control of the positioning of the furrow and that contributes to the apoptotic fate. Normally the furrow would be in the middle of the cell. But these cells go through the effort to displace this furrow. Most of the molecules that we're studying that we originally thought were involved with apoptosis, we think are really involved in controlling asymmetry of division in terms of furrow position.

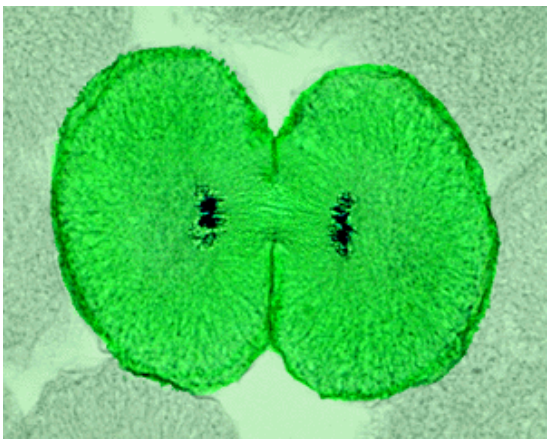

Figure #4. A cleavage furrow in the middle of the cell

BSJ: What are aggresomes and how does asymmetric inheritance of those influence apoptosis?

GG: Nobody knows why cells die in C. elegans. In some other cases it's pretty clear why you would kill a cell… During limb development in mammals, the cells between the digits die and if that fails [to happen], you get fused digits. There are places in our brain that 50% of cells generated die and that's a little less clear why you would do that. In C. elegans there's a few cases where you would understand why cells would die! There are some cells that are sexually dimorphic, so they're used in the hermaphrodite and killed off in the male or vice versa. The other places where you have these lineages that are repeated on the anterior or posterior axis and you may need some cells near the middle of the animal but not outside so you'd kill those off. But for the most part in a hermaphrodite there are 131 cells that die and we don't know why.

So, there's been speculation on one idea that these were pseudo genes, which are thought to have no function and are found all over the place in genomes. So this idea was called pseudo-cell hypothesis. It's kind of an evolutionary argument: if you allow a cell to die, it can drift in function and if you allow it to survive most of the time, it wont have a new function and will be the equivalent of a pseudo gene or pseudo cell. But in some cases it may acquire new functions that would have adaptive value.

We were asked to review some papers that we thought were interesting and might be related to apoptosis. We came across this paper in PloS Biology where they were overexpressing this Huntington protein with amino acid repeats that cause abnormal folding and cause aggregates. They were just expressing these aggregates in tissue culture cells which people have done and they saw that these Huntington protein aggregates would sort-of overwhelm the ability of the proteasome to degrade it. And when you do that, there's this mechanism where they form these aggresomes. [Aggresomes] are then transported back along the microtubules to the microtubule-organizing center are dealt with there. This paper went one step further and watched what happened when cells divide. So you duplicate those organizing centers to generate centrosomes and they found that one of the cells always inherited the aggresome asymmetrically. They went on to express these in drosophila neuroblasts that generate asymmetric division, and they found they were asymmetrically distributed to the stem cell that actually died before the neurons.

So, the idea was that this was a mechanism to put these aggregates of proteins into the longest living cells. They even looked at the intestines of people with a neurodegenerative disease. In intestines, there are these asymmetric stem cell divisions and there they found, even with no pathology there, these cells divide and generate another stem cell and then a cell with more limited developmental potential. They saw that [the cells with limited developmental potential inherited the aggresomes].

So, the idea is that you protect the cells from these aggregated proteins by distributing them to the cells that'll be around less. We saw that and proposed that maybe that's what's going on in C. elegans: the cells that die are trashcans. I still think that's a really good idea but I haven't convinced anyone to test this by misexpressing these proteins high enough to produce aggresomes; this is just an idea.

BSJ: Which comes first… this distribution of aggresomes that then triggers apoptosis or apoptotic signals that then attract the aggresomes?

GG: Right, I would predict that you would generate aggregates of proteins under certain situations that are bad for the cells and that something evolved to get rid of that. But there are plenty of places that are important developmentally to have apoptosis so apoptosis could've evolved independently and then gotten used [for these other purposes].

BSJ: How do you approach these questions and what methods do you use to study how and why these things

happen?

GG: We study the "how". Our fundamental approach has been genetic. [And the genetic approach] has been an incredibly important approach in general. The C. elegans have been the workhorses for this and for a lot of what we know about the mechanisms for development in invertebrates. Or, at least, the molecules that are involved really came from studies in C. elegans, drosophila and yeast. So, genetics is an approach to understanding a biological problem.

What geneticists do is that they screen for mutants that are defective in the process that they're interested in. Then, from the mutant phenotypes, they first try to figure out how the mutation effects function and if the mutation recessive or dominant. If it's recessive, has it partially or completely lost the function of the gene? If it's dominant, how has it messed up the function of the gene? [Then, what the gene normally does is inferred by how the gene function is affected by the mutation.] If your screen works well, you'll have a number of genes involved in the process.

You move on from there [by looking at] which molecules were encoded by the genes and what those molecules are doing in terms of cell biology. So, sometimes you hit molecules that you have no idea what they're doing biochemically and those are the hard ones. But those maybe are the more interesting ones!

So, that's kind of an initial approach into the problem. There are lots of people who figured out apoptosis in C. elegans, and that is what they did. They looked for mutants that were defective in apoptosis. Randy Schekman studied secretion and the initial thing that was done was a screen for temperature sensitive mutants that are defective in the ability of yeast to secrete proteins. So, you can just go through the list of the different processes that people have studied.

BSJ: What about newer techniques based on RNAi (RNA interference) and genome wide studies?

GG: So, we have done RNAi screens but there are qualifications associated with it. Sometimes it doesn't work (due to off target effects) and, actually, it doesn't work particularly well in neurons. In C. elegans, though, they tend to be quite specific and there are not too many off target effects compared to other organisms. And it's really easy to do in C. elegans. So, yes, it's a valid approach but sometimes it doesn't work or you get very, very weak effects. That is, you don't knock down the function… Some genes are really sensitive to dosage effects so if you just reduce them by 70-80% and generate a phenotype. But there are other genes where you need to get rid of 90% of the function to see a phenotype. So these would be more impervious to RNAi.

But there has been resurgence in screen approaches just using genetics because of the ability to quickly identify mutations using whole genome sequencing. It used to be that it would take a huge amount of time to find the mutations… now it's much easier!

BSJ: In regards to the various proteins involved in asymmetric cell division, what do we know about the model right now in terms of where the known players fit in?

GG: We've identified a lot of components… There are the Wnt signaling pathways involved in many different aspects including migration and asymmetric division. The Wnt signal seems to polarize the cells and that's actually understood very well in C. elegans. What we found is that the Wnt additionally controls the positioning of the furrow (the cleavage furrow). How the Wnt controls that is not understood but there are these protein kinase pathways and membrane trafficking pathways that we've identified. The idea here would be that these are regulating components of the Wnt signaling pathway.

The other thing that we're interested in came from this understanding that there are two mechanisms controlling the asymmetry in cell divisions: Firstly, the spindle can move and that determines the position of the furrow. In other cells, the spindle eventually moves but the furrow forms first. There are a couple of molecules we've identified in our screens that control one of these types of divisions without controlling the other. We're interested in developing the model for how these mechanisms work. Most of the molecules we've identified are involved in both, so there are some shared pathways but we do have some molecules that are specific!

This brings up a very interesting question of why the cell is undergoing apoptosis and why there would be two different mechanisms to generate this asymmetry for apoptosis. But we can continue working on the "how" of the system but the "why" eludes us.

BSJ: And how does these models translate to vertebrate and human biology? Would you expect it to be analogous to some extent?

GG: It's always hard to say for sure but all the molecules we've identified have homologs. These don't necessarily have to contribute to apoptosis… We know of one case whereby the gene isn't only controlling the apoptotic pathway but also other divisions that are asymmetric. The idea is for these basic cell biology principles to be conserved [through evolution].

BSJ: And perhaps even more broadly, where do you see this field and your research going in the near future?

GG: I would like to figure out how this polarity is established and how the asymmetric division is executed before I retire!

Image Sources

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