

# Cell fusion and fusogens - an interview with Benjamin Podbilewicz

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**ABSTRACT** Cell fusion is a process in which cells unite their membranes and cytoplasm. It is fundamental for sexual reproduction and embryonic development. Among the best-known cell fusion processes during animal development are fertilization, myoblast fusion, osteoclast generation, and vulva formation in *Caenorhabditis elegans*. Although it is involved in many other functions in unicellular and multicellular organisms, little is known about the mechanisms of cell fusion and the genes that code for the proteins participating in this process. Benjamin Podbilewicz has dedicated many years to understanding the processes and mechanisms of cell fusion. In this interview, he spoke to us about how he began his studies of this process, his contributions to this exciting field, his scientific ties with Ibero-America and his strategies for a well-balanced scientific/personal life.

**KEY WORDS:** *cell fusion, fusogen, Caenorhabditis elegans*

## Introduction

The development of multicellular animals has its origin in the fertilized egg. During embryonic development, the egg divides and becomes the source of all the cells in the organism. Cells limit their potential and specialize into different cell lineages. Throughout the cell differentiation process, molecular signals control cell fate. Embryos are mainly shaped by processes such as cell division, cell migration and apoptosis, while cells organize themselves into complex tissues and organs at specific times during development. Recently, the study of the cell fusion process during development has acquired great relevance since it is required from fertilization to organogenesis. Fertilization is a process that depends on cell fusion (Hernandez and Podbilewicz, 2017; Oren-Suissa and Podbilewicz, 2007). In flowering plants, there is a unique process called double fertilization, where one sperm fuses with the egg cell to form the zygote then a second sperm fuses with the central cell to form the endosperm. As is the case with other models of cell fusion, much is known about the process, but little is known about the molecules responsible for fusion and their action mechanisms (Brukman *et al.*, 2019). Benjamin Podbilewicz is a pioneer in the study of the mechanisms of cell-cell fusion in the nematode worm *C. elegans* and has made seminal contributions to the field (<https://pubmed.ncbi.nlm.nih.gov/?term=Podbilewicz+B>).

Podbilewicz grew up in Mexico where he obtained a BSc de-

gree in Chemistry, Bacteriology, and Parasitology at the Instituto Politécnico Nacional in Mexico City. He completed his PhD in Cell Biology with Ira Mellman at Yale University, USA, and his postdoctoral training with John White at the MRC-Laboratory of Molecular Biology in Cambridge, United Kingdom. Currently, he is a Professor at the Department of Biology at Technion - Israel Institute of Technology, Haifa.

In the following interview, as part of the special issue of the International Journal of Developmental Biology in Ibero-America, he told us about how his scientific interests arose in Mexico. He then describes how he became interested during his post-doctoral studies in the phenomenon of cell fusion in *C. elegans*. His contributions to the field have allowed us to better understand how the cell fusion process occurs.

## We know you were born in Israel, but you did your predoctoral studies in Mexico. What can you tell us about this story and how you started your scientific career?

My grandparents were immigrants who came to Mexico from Poland and Russia between the first and second world wars. My parents were born and grew up in Mexico and after they got mar-

*Abbreviations used in this paper:* AFF-1 anchor cell fusion failure-1; EFF-1, epithelial fusion failure-1; GFP, green fluorescent protein; PVD, neuron class of two interneurons with cell bodies situated laterally in the posterior body in *C. elegans*.

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**Fig. 1. Sydney Brenner and Benjamin at the Hebrew University of Jerusalem in 2010.**

who is famous for his work on Notch signaling in *Drosophila*. He guided me in deciding what to do in the future. I discussed with him my willingness to change fields and my top priority to pursue postdoctoral training in Europe. I knew I wanted to use a whole organism as a model system. Also, I wanted to do neuroscience or developmental biology and genetics. We discussed about *Drosophila*, zebrafish and worms as models. We also talked about different places in Europe. It was then that I sent several letters, one of which was to Sydney Brenner, Nobel laureate who established *C. elegans* as a model organism for studying development, at the MRC Laboratory of Molecular Biology (LMB) in Cambridge (Brenner,

ried, they moved to live in Israel for two years. I was born in Tel-Aviv-Yafo but grew up in Mexico since I was 11 months old. From kindergarten to high school I attended the Colegio Hebreo Tarbut in Mexico City and for my undergraduate studies I analyzed three different degree options: one at the Faculty of Sciences at UNAM, another at the Institute for Biomedical Research (also UNAM) and finally a third at the National School for Biological Sciences (ENCB) from the National Polytechnic Institute (IPN). I even attended the Basic Biomedical Research Preparatory Course at the Institute of Biomedical Research - it was an outstanding experience. Although I was accepted in the Basic Biomedical Research program, I liked the IPN more. What I liked there was the atmosphere, the teachers and my peers, it was an excellent decision. I think I had a great preparation as a bacteriologist, chemist and parasitologist. I completed my degree at the ENCB-IPN, and I did my thesis at the Center for Research and Advanced Studies (CINVESTAV) in biochemistry with Dr. Carlos Gómez-Lojero. Since my elementary school, I loved photobiology - it was one of my passions. That was why I studied photosynthesis in the cyanobacteria *Spirulina maxima* at his laboratory. There was a lot of biochemistry and studies of electron transport chains involved, I liked biophysics back then. It was an outstanding experience.

### **How and when did you decide to pursue studies in developmental biology?**

During my PhD studies at Yale University I decided to move from prokaryotes to eukaryotes and studied cell biology. After four exciting rotations on transcytosis with George Palade, on protein phosphorylation in neurons with Leonard Kaczmarek, exploring synaptogenesis with Mu-Ming Poo and endocytosis with Ira Mellman, I decided to do my PhD focusing on transferrin recycling *in vivo* and developed an *in vitro* system using permeabilized MDCK cells (Podbilewicz and Mellman, 1990). It was not until the last years of my PhD that I decided I wanted to deepen my knowledge in the fields of developmental genetics and neurosciences. One of my career advisors at Yale was Spyros Artavanis-Tsakonas,

2009). He replied in a letter saying that he no longer worked with worms (Fig. 1). However, he forwarded my letter to John White who did the first connectome of the nervous system in *C. elegans* as his doctoral work with Sydney. I had also written to Peter Lawrence who is also a prominent developmental biologist in *Drosophila* and wrote the famous book "The Making of a Fly" (Lawrence, 1992). Both invited me to come for interviews but at that time I still had not decided whether to work on *Drosophila* with Peter Lawrence or on *C. elegans* with John White. They asked me to write a research proposal, then I wrote two. The first one was about the process of cellularization during *Drosophila* early development. The second was on cell fusion in *C. elegans*. While I was reading about *C. elegans* I found that at many stages of development, the cells fuse in different tissues. The fusion was present in the skin (hypodermis; Fig. 2), the pharynx, during the formation of the vulva and uterus. The more I read I realized nothing was known about the cell fusion process. What is it good for, exactly how it happens or what are the molecular mechanisms? I started to look for cell fusion in other model organisms but nothing was known at the time. There were the beginnings of understanding of intracellular fusions and the fusion of virus envelopes to cellular membranes, but very little was known about cell fusions in any organism. It was at that moment when I fell in love with the subject. I wrote a proposal, which at first was a paragraph and grew to several pages. I sent it to Human Frontiers Science Program (HFSP) and received a postdoctoral fellowship to study cell fusion at the LMB in Cambridge. That is how I fell in love with a subject that nobody was studying at the time in *C. elegans*.

Now, when I teach my students how to write their research proposals, I tell them that mine was overambitious, to the degree that there are some issues that are yet to be explored today.

### **Clearly two people have inspired you, but can you tell us what other people have influenced your career?**

I believe that there are people at the IPN who greatly influenced my career, for example a math teacher, Professor Armando Guerra. He took interested students under his wing, and he called us "slaves".

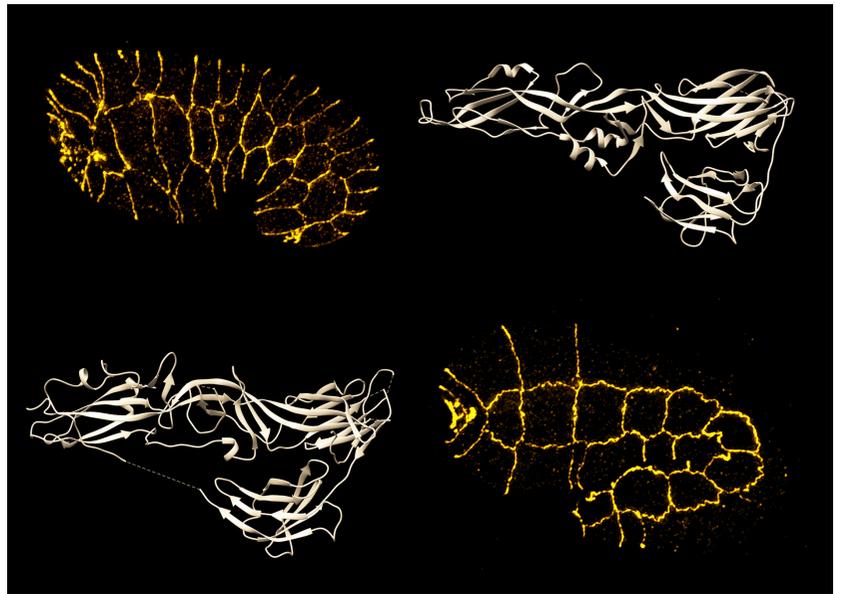
**Fig. 2. Adherens junctions of hypodermal cells of *Caenorhabditis elegans* embryos before (top left) and after (bottom right) dorsal epidermal cell-cell fusion.** Structures of protomers of EFF-1 ectodomain of *C. elegans* (bottom left; 4OJC.pdb) and E-glycoprotein from dengue virus (top right; 1OK8.pdb). The embryos and proteins are ~50,000 nm and ~10 nm long, respectively. Immunofluorescence images using structured illumination microscopy and composition by Dr. Ksenia Smurova.

We used to spend weekends talking and discussing; we even learned some computing and modeling. It had a significant influence on me. In this group, there were not only undergraduate students but also Prof. Valdemar Rodrigues, an ecologist from Brazil, and an expert in desertification. That period in my life was beautiful. I began to really understand what it was to do science.

**Can you tell us about the relevance of the cell fusion process at the cellular level? Why did you choose to study *C. elegans*?**

I chose *C. elegans* because we know the lineage of every cell, the precise time when cells divide and the fate that every cell will have, and all these are invariant in the wildtype. It is a fantastic model to approach many problems in biology and in particular my questions on cell fusion. The other advantage was that John White had already reconstructed the anatomy of the whole worm including its nervous system (White *et al.*, 1986). So, everything from the descriptive point of view was quite well explored and one-third of the somatic cells that are formed in *C. elegans* fuse. They are originally mononuclear cells that then form syncytia in a highly reproducible manner. That made me think this was a perfect system to use for studying the genetics of cell fusion. Ed Hedgecock, John Sulston, Sydney Brenner, and Robert Horvitz showed that cell death in *C. elegans* occurs in place and time in a predictable manner, I saw the same with cell fusion. Also, the model allows to observe what the cells do when they fuse, how they migrate and in what order fusion takes place - because there is a specific order in some organs like the vulva. It is always the same cells that are going to form syncytia; however, we discovered that in certain organs, such as the skin during embryonic or larval development, those fusions vary somewhat in their sequence. We initially studied cell fusion in a descriptive manner, by staining adherens junctions (Podbilewicz and White, 1994). We saw that before fusion, adherens junctions were apparent, but after the fusion, junctions were lost (disassembled), leaving a multinucleated cell (Fig. 2). At the same time, John White had made a TEM reconstruction of one of the most successfully studied organs in *C. elegans* through genetic screens, the vulva (the organ of copulation and egg laying) (Newman *et al.*, 1996). During my postdoc, while performing our experiments in the skin, I found that we could also follow cell

**Fig. 3. Model of vulva formation in *C. elegans* by cell fusion.** The anchor cell on top of seven vulval rings before intra-toroidal fusions and the lineage (monitor; from Sharma-Kishore *et al.*, 1999) and Benjamin Podbilewicz with a 3D model of a nine-ring vulva (from Ikea, [www.ikea.com](http://www.ikea.com)) in 2002.

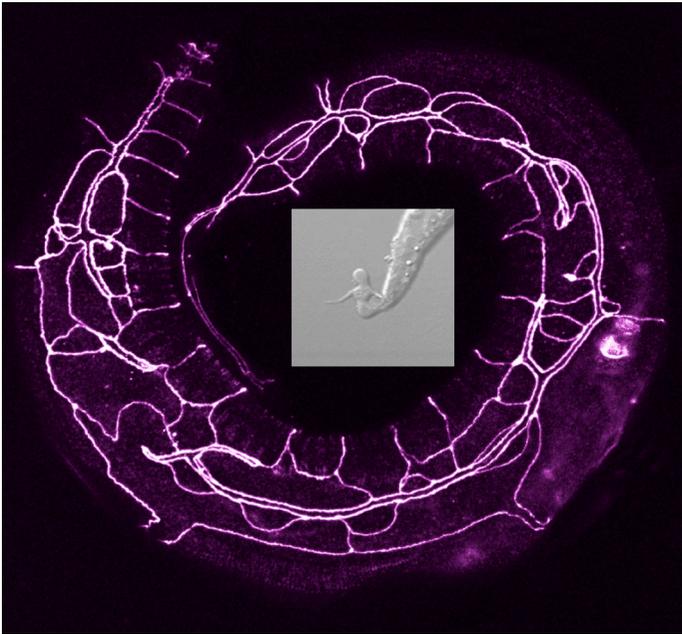


fusions inside the vulva's structure. We saw how the cells migrated to form rings placed on top of each other. Two or four cells fuse to form each ring, which is a very stable structure (Sharma-Kishore *et al.*, 1999). This was the first time we were able to observe how the fusions occur during the creation of an organ (Fig. 3). Then I established a genetic screen, looking for the genes responsible for these cell fusions. However, initially I was unable to find mutants that promoted wide cell fusion or where all fusions failed.

**Was it because fusogenic proteins are very redundant or because of the screening design?**

Now we know it was because there are just two fusogens in *C. elegans* and we were unlucky. The screens were complicated because what I did in my postdoc was done before green fluorescent proteins (GFPs). Back then, I had to perform the mutagenesis and then stain different groups to look for phenotypes. I found amazing phenotypes but the immunofluorescence screen was laborious and did not find the key genes that encode fusogens. The advantage of





**Fig. 4. Confocal image of *eff-1* mutant showing unfused hypodermal cells, abnormal migrations of unfused cells and morphological defects:** the worm is deformed, short and fat (*Dumpy*). Inset, abnormal tail of a mutant *eff-1* animal by Nomarski optics showing a unique shape instead of a pointed tail. Fluorescence microscopy by Ksenia Smurova and Nomarski by Clari Valansi.

*C. elegans* is that the primary larvae can be frozen after they hatch. The mutants from those screens are still frozen because there were some that seem important for regulating cell fusion, determining which cell fuses with which cell. They had defects in some organs or tissues but not in all. We often found mutants that showed too much fusion or that the cells fused with cells other than those they usually do. What I was looking for was a mutant in which there was no fusion. I managed to analyze 2,300 different clones or F1s. Yet, I did not find what I was looking for. A few years later, already in my laboratory here at Technion, a student in my lab, Gidi Shemer, carried out another screen. This time, he was using GFP and Eureka! We found the mutant we were looking for (Shemer *et al.*, 2000) (Fig. 4). We then cloned and characterized the *eff-1* gene, the first cell fusion gene, in collaboration with Bill Mohler and John White (Mohler *et al.*, 2002).

#### **All these processes are preserved along the evolutionary scale. Are there any strange variants?**

When we cloned *eff-1*, we looked for homologous genes in other organisms, but we found them only in nematodes. Initially, we believed that it was probably a family that was preserved only in nematodes. Our search was done only based on primary sequence. Amir Sapir then discovered a second cell fusion gene in *C. elegans*, which is involved in specific fusions other than the EFF-1-related ones. This other gene we named *aff-1*. The two belong to the same family and share some homology (Sapir *et al.*, 2007). They are dedicated to the same purpose and have similar activities in different cells. As more genomes were sequenced, we found some relatives in some arthropods, ctenophores, also in other organisms including the cephalochordate *Amphioxus*, even in a protist (*Naegleria*), but

neither in fungi, nor in vertebrates (Avinoam *et al.*, 2011). During an extended, enjoyable and productive sabbatical in the lab of Leonid Chernomordik at NIH, Bethesda, we expressed the worm EFF-1 in insect cells and characterized the mechanisms of fusion via hemifusion in this heterologous system (Podbilewicz *et al.*, 2006).

Another group of very well-known fusogenic proteins is viral proteins. However, one of the main differences between viral proteins and ours is that the eukaryotic fusogens (EFF-1 and AFF-1) must be present in both cell membranes of the cells that will fuse, while in viruses the fusogenic protein is only found in the virus envelope, implying that the fusion mechanisms are different (Shemer *et al.*, 2004; Podbilewicz *et al.*, 2006; Sapir *et al.*, 2007). Viruses have cellular receptors but fusion itself is directed only by the viral proteins. The receptors' role is to allow the viruses to adhere to the cell, but the viral protein drives the fusion (Podbilewicz, 2014). In collaboration with Judith White we asked whether our worm fusogens can function instead of a viral fusogen and Ori Avinoam demonstrated that AFF-1 and EFF-1 can replace a viral fusogen and mediate virus-cell fusion but only if the worm fusogens are also present in the target mammalian cells (Avinoam *et al.*, 2011).

In 2004, we began a collaboration with Felix Rey, an Argentinean from the Pasteur Institute, who visited us during my sabbatical at NIH. Felix is a structural biologist, so I asked him if he might be interested in determining the structure of the EFF-1 protein. Ten years later, we published the three-dimensional (3D) crystallographic structure of EFF-1 (Perez-Vargas *et al.*, 2014). It turned out to be virtually identical to the 3D structure of fusogenic class II proteins of viruses such as dengue (Fig. 2), West Nile, Zika, chikungunya, rubella and many others that have fusogens with quite similar structures although they are not conserved at the primary sequence level (Podbilewicz, 2014). After this realization, the family grew, and we were able to find structurally similar proteins not only in nematodes (see below).

#### **Has your work on fusogenic proteins contributed in some way to the understanding of viral diseases or might it have an impact on other diseases?**

In the future, I think we may contribute to the understanding of viral diseases in general and COVID-19 in particular. For the moment, there have been and are many researchers studying viral fusion. What we know about viruses is much more than what we could say about our proteins. The good news is that three years ago, a few groups in different parts of the world, including us, found proteins related to EFF-1 and AFF-1 in plants such as *Arabidopsis*, protists and parasites, such as *Trypanosoma*, *Leishmania*, *Chlamydomonas*, and many others, that are important for sexual reproduction (Hirai *et al.*, 2008, Johnson *et al.*, 2004, Liu *et al.*, 2008). In *Chlamydomonas* and the malaria parasite *Plasmodium*, these proteins (HAP2/GCS1) were identified as necessary for gamete fusion, but it was unknown whether they were also sufficient (Liu *et al.*, 2008). We have known for many years that in *C. elegans*, EFF-1 and AFF-1 are necessary and sufficient for cell fusion (Shemer *et al.*, 2004). Recently, different groups, including ours, saw the structural homology between fertilization (HAP2/GCS1), viral and *C. elegans* fusogens (Valansi *et al.*, 2017; Pinello *et al.*, 2017; Fedry *et al.*, 2017; Fedry *et al.*, 2018). But Clari Valansi went beyond, she expressed the *Arabidopsis* candidate fusogen in a heterologous system of mammalian cells in culture, or replaced the viral proteins with cellular proteins, and in both cases there was functional conservation, further showing that they are sufficient for fusion (Valansi *et al.*, 2017).



**Fig. 5.** Podbilewicz laboratory 2014 representation of *The Anatomy Lesson of Dr. Nicolaes Tulp* by Rembrandt. The scene depicts arm and hand anatomy. From left to right: Veronika Kravtsov, Julie Grimm, Ofer Katzir, Ksenia Smurova, Boaz Gildor, Hadas Raveh-Barak, Tamar Gattegno, Clari Valansi, corpse's face Benjamin Podbilewicz (Edition by Ksenia Smurova).

So the family grew once more, since the fusion phenomena, mediated by this kind of proteins, occur not only in viruses and somatic cells, but also within plant, animal and protist species with sexual reproduction; we have named this superfamily fusexins: fusion proteins essential for sexual reproduction and exoplasmic merger of plasma membranes.

**Would you say that it is a phenomenon that is not being studied extensively, since there are not many people researching in this field?**

If we read cell biology or developmental biology textbooks and look for the mechanisms of cell fusion during fertilization there is practically nothing. Books sometimes mention cell fusion in muscles and fertilization, but neither the mechanisms nor the genes that

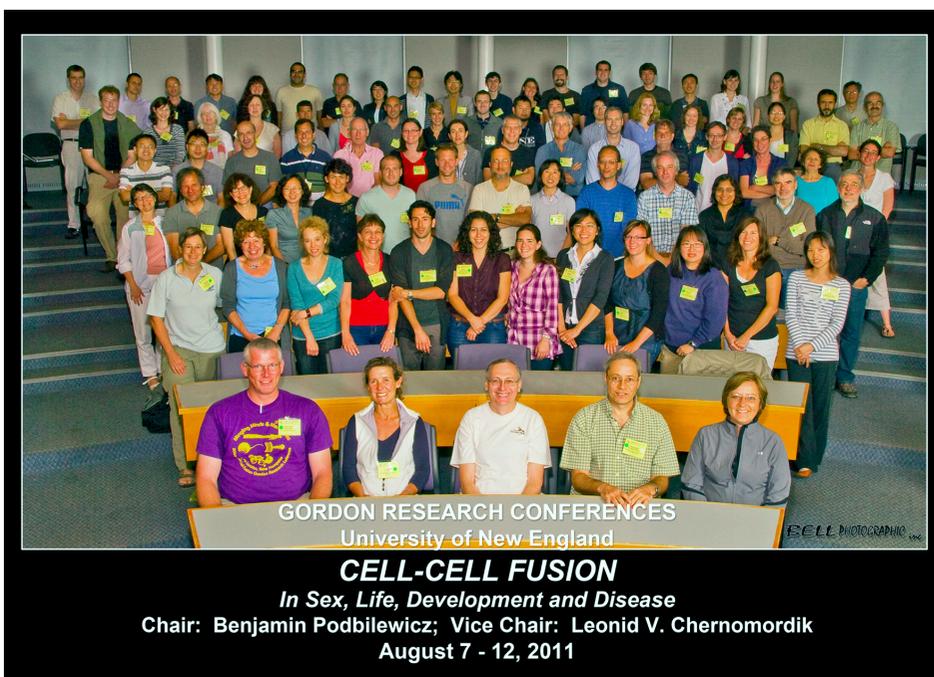
code for the proteins that participate in the event. For instance, we still do not know the genes and proteins that allow the fusion between the egg and the sperm in vertebrates. We only know proteins that participate in the formation of syncytia in the placenta, Syncytins, which interestingly are proteins of retroviral origin (Mi *et al.*, 2000). In muscles, two proteins that have an essential role in myoblast fusion were recently found in vertebrates, but other than that we still have many questions (Bi *et al.*, 2017, Gamage *et al.*, 2017). We do not even know which proteins make the cells fuse in fungi. Some researchers believe that fusogens do not exist, that fusion is something spontaneous, passive. I find this unlikely as such spontaneity has not been found and we already know the syncytins, fusexins and myomerger/myomaker as fusogens for diverse cell fusion processes.

In humans, one-third of our weight is skeletal muscle, and this is all made up of syncytia in the muscle fibers (Fig. 5). Osteoclasts are giant cells with tens or hundreds of nuclei. They are important for the regulation of bone resorption, growth and development, but we do not know how they fuse. Another tissue where there is fusion in vertebrates including humans is the lens of the eye. In this transparent tissue, epithelial cells fuse to make onion-like layers of syncytia (Bukman *et al.*, 2019). There are small capillaries that are made of a single cell layer. In zebrafish, these small capillaries originate by cell auto-fusion (Lenard *et al.*, 2015). Also, in *C. elegans*, we know many tubular structures that are formed by EFF-1- and AFF-1-mediated self-fusion (Rasmussen *et al.*, 2008; Stone *et al.*, 2009; Soulavie *et al.*, 2018). In other vertebrates or mammals, this fusion process has not been described mechanistically and fortunately our embryonic discipline is growing (Fig. 6).

**You mention in some of your articles that cell fusion and cell division are coupled events. How are the two processes coupled?**

What we have seen in *C. elegans* is that, in general, cells that are destined

**Fig. 6.** Key collaborators and lab mates over the years at the 2011 Gordon Conference. (First row) from left: Bill Mohler, Agnes Vignery, Leonid Chernomordik, Benjamin, Elena Zaitseva. (Second row) from left: Sandra Schmid, Renate Renkawitz-Pohl, Evgenia Leikina, Clari Valansi, Ori Avinoam, Meital Oren-Suissa, Karen Fridman. (Third row) from left: Judith White, Ira Mellman, Margaret Kielian, Hongmei Wang and Felix Rey (second from right). (Fifth row) from left: 1<sup>st</sup> Thomas Krey, 6<sup>th</sup> Jimena Perez-Vargas, and 7<sup>th</sup> Pablo Aguilar.



for fusion, once they fuse, they no longer divide, in fact they leave the cell cycle before fusing. Of course, there are exceptions; most significantly, in fertilization where after gamete fusion, cells begin to divide. There are also some exceptions in somatic fusions in some invertebrate organisms where cells fuse and then divide (e.g. in the intestine of leeches). But in general, in most of the examples that I know of somatic fusion, both in mammals and *C. elegans*, once cells fuse, they no longer divide (Hernandez and Podbilewicz, 2017). One of our hypotheses was that fusion was the signal for cell cycle exit, but we found that in both *eff-1* and *aff-1* mutant cells that fail to fuse do not divide. Interestingly, in the vulva any changes in the orientation of cell division, coupled with the fusion events, can determine different ring-shaped structures (Kolotuev and Podbilewicz, 2008). For instance, the number of rings in the vulva between different species of nematodes varies depending on the orientation of cell division and whether there is fusion or not (Kiontke *et al.*, 2007; Kolotuev and Podbilewicz, 2004; Shemer *et al.*, 2000). Thus, the anterior-posterior or left-right plane of cell division is coupled to short-range cell migrations and cell fusions that can change the number of rings, size and shape of the vulva.

### Would you say that the cell fusion process is associated with planar cell polarity?

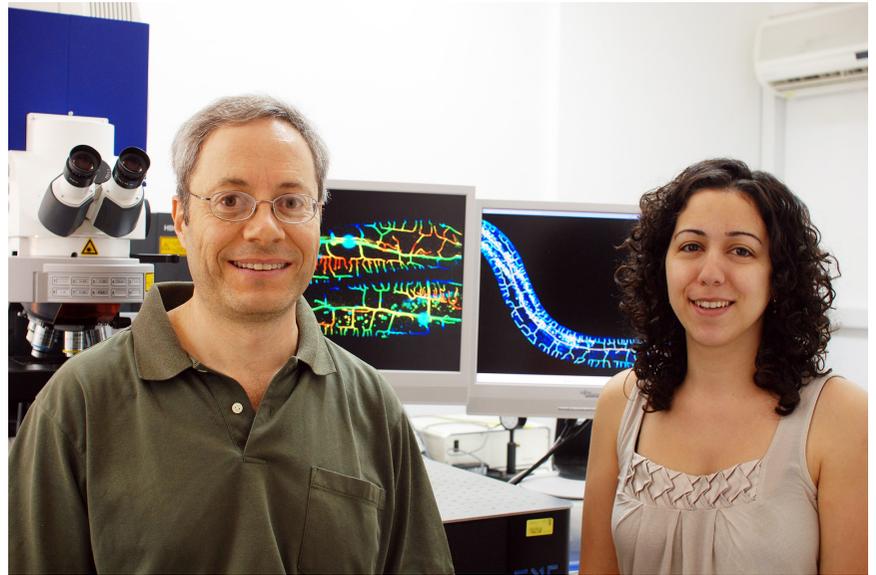
Many of the cells that fuse in *C. elegans* are polarized epithelial cells, so in that case, probably yes. It is also likely that the place where fusogen expression occurs is critical. Fusogens must be expressed at the right place and at the right time - the cell fusion events are highly regulated. There are many transcriptional repressors that inhibit the expression of fusogens and some transcription factors stimulate the expression of EFF-1 and AFF-1 at the appropriate time and the right cells (Shemer and Podbilewicz, 2002).

### During the molecular cascade of organ formation, how soon does the expression of these fusogens occur? What happens first, cell determination or cell fusion?

I think that one of the reasons we were able to find fusogens in *C. elegans* but others have failed to find them in *Drosophila*, is that in *C. elegans* epithelial cells first acquire their fate and polarity, form contacts, build tubes or structures, and it is not until the end of these pre-fusion processes that the decision to fuse or not to fuse occurs. This characteristic allowed us to uncover the fusion genes and not the genes related to the previous processes (e.g. factors that repress fusion, factors important for cell migration or cell adhesion) (Aguilar *et al.*, 2013). Also, in the muscle lineage, cell fusion does not happen before cells become myoblasts and express the typical muscle proteins.

### Does the expression of these genes also occur late in the differentiation program or is there a post-transcriptional regulation that leads to the late expression of these proteins?

What we have seen is that there is a lot of regulation at the



**Fig. 7. Meital Oren-Suissa and Benjamin.** In the background confocal maximum intensity projections of dendritic arborizations of sensory PVD neurons of *C. elegans*.

transcription level, but I am sure that there is also regulation at the level of protein translation, transport and sorting out of the proteins. Indeed, there is evidence for regulation at the level of transport and localization, as we found that endocytosis is important for downregulating the fusogen EFF-1 on the surface to avoid unwanted fusion events (Smurova and Podbilewicz, 2016, 2017).

### Do you think fusion proteins influenced the evolution of sexuality?

Fusion proteins probably originated for genetic diversification during reproduction. Single-cell prokaryotes and single-cell eukaryotes continuously exchange signals and genetic material. One of the ways to exchange large amounts of genetic material is fusion, even if this is transient. For example, in *Tetrahymena*, the gametes are fused, and afterwards separate, i.e., a transient fusion that gives rise to genetic exchange (Cole *et al.*, 2014).

### What about the influence of fusion on the emergence of multicellular organisms?

Cell fusion is essential for sexual reproduction, but I am not saying that there is only one fusion mechanism. We believe that the family of proteins that we named as *fusexins* originated early-on in eukaryotes, probably in the Last Eukaryotic Common Ancestor (Hernandez and Podbilewicz, 2017; Pinello *et al.*, 2017; Valansi *et al.*, 2017). These proteins already existed and were necessary for exchanging genetic information. They were maintained in many lineages, but perhaps in many others they were lost and other proteins took their roles. Despite looking for these proteins in vertebrates, fungi and mammals, they were not found as such. There are probably other fusexins and other families that we have not identified since the conservation is structural and not based on the sequence, so it is no small achievement to find these proteins.

On the other hand, these genes are also present in viruses, so they can also play a crucial role in promiscuously moving genes from one organism to another. Prokaryotes that fuse can use

**Fig. 8. Podbilewicz lab 2018.** From right, counter-clockwise: Benjamin, Anna Meledin, Nicolas Brukman, Sharon Ingberg, Clari Valansi, Yael Iosilevskii, Sivan Geisler-Edelbaum, Elena Matveev, Xiaohui Li.

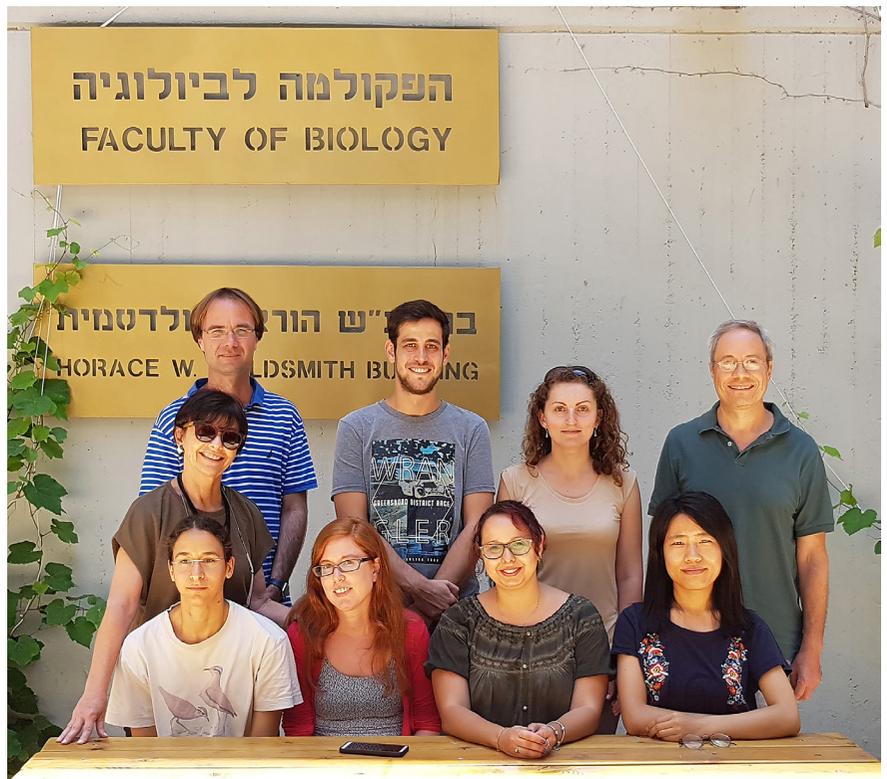
proteins from this family and can also pass them to phages. I think early on in evolution, it was an advantage to have proteins that could fuse the membranes and cross this barrier between individual cells. In multicellular organisms, fusexins are very deep-rooted and fusion may have influenced the emergence of multicellular organisms. We have discovered that too much fusion kills, because when we overexpress our fusogens in a *C. elegans* embryo, it dies with hyperfusion phenotype (Smurova and Podbilewicz, 2016).

**Neural plasticity may be an extreme refinement of fusion in *C. elegans*. How do these proteins participate in dendrite fusion?**

Over 100 years ago, there was a great debate between Santiago Ramón y Cajal and Camillo Golgi. They shared the Nobel Prize in 1906 but had a different understanding of the nervous system. Golgi believed that, based on

his staining, the nervous system was a syncytium. In contrast, Ramón y Cajal established that neuronal cells are connected by synapses. I think they never agreed, but now everyone agrees that Cajal was right and that the neurons are not fused. In *C. elegans*, we know that if the axons are cut with a laser, certain neurons and certain axons will not have Wallerian degeneration and axonal regrowth. Instead, axon fragments will fuse and grow again. *eff-1* mutant animals fail to undergo axonal fusion and other genes help EFF-1 to do its expert repair by fusion (Oren-Suissa *et al.*, 2017; Yaniv *et al.*, 2012).

At the same time, Meital Oren-Suissa was studying other neurons with large dendritic trees with a beautiful repetitive pattern like candelabra with many candles (menorahs), similar to Purkinje cells (Fig. 7). To study how this pattern is generated, she decided to analyze genes that could affect that structural organization of these complex and reproducible dendritic arborizations. We observed that the *eff-1* mutations resulted in disorganization and hyperbranching of the dendritic trees (Oren-Suissa *et al.*, 2010). Later we found that EFF-1 has a new role independent of fusion, which is to retract the dendritic branches. If we cut the dendrites, they also fuse, but the fusion is mediated by AFF-1 instead of EFF-1 (Inberg *et al.*, 2019, Kravtsov *et al.*, 2017, Oren-Suissa *et al.*, 2010). As far as I know, *C. elegans* is the only system where neurons are definitively known to fuse. Some anecdotal reports suggest that neurons in vertebrates can fuse but these lack sufficient experimental evidence. There are some



reports in other invertebrates (Hoy *et al.*, 1967) but little is known about these processes and maybe it is not real neuronal fusion. In summary, although there are many anecdotal reports in mammals and invertebrates of axonal fusions and fusions of neurons with stem cells, today the only species where it has been seen that neurons can fuse is *C. elegans* (Inberg *et al.*, 2019). Interestingly, when the same experiment is carried out in *Drosophila* cutting a dendrite, it does not fuse. Instead, the distal part degrades, and a dendrite grows back in a process also seen in vertebrates. This also happens in many neurons of *C. elegans*. This line of research has great potential for understanding neuronal regeneration in humans after a stroke or a severe injury to the spinal cord. It is a unique and



**Fig. 9. Amos and Benjamin Podbilewicz, Cerro Valleccitos (5,450 m), Andes, Argentina (February, 2020).**

curious system, neurons can regenerate, yet this regenerative capacity decreases with age. When worms get older, three or four days into adulthood, the ability to regenerate is impaired. Nevertheless, if we ectopically express *AFF-1* in neurons, then neurons can fuse again. Thus, aging affects the worm's regenerative capacity by fusion (Kravtsov *et al.*, 2017).

Although we are searching for fusogens in vertebrates, there is no evidence for the presence of fusion proteins in mammalian or vertebrate neurons. There is not enough experimental evidence of neuronal fusion in the cases where it is reported to exist. (A recent elegant report has shown very convincingly that inner hair cells in the mouse cochlea fuse. This is probably the first confirmed case of neuronal fusion in vertebrates (Jean *et al.*, 2020)).

### Regarding these fusogenic events, what are the big questions that remain unresolved?

I believe that identifying the mechanisms of the fusogenic process remains unresolved (Brukman *et al.*, 2019). Although we have identified the proteins, it does not mean that we know how they work. Thus, only a few laboratories and ourselves (Fig. 8) are studying the fusion mechanisms of proteins in the superfamily that we have identified, as well as the two candidate fusogens of mammalian muscles recently identified, Myomaker and Myomerger. Fusion also takes place in the mammalian placenta. However, in this organ, the proteins (Syncytins) are closely related to those from retroviruses such as HIV, the AIDS virus and they were acquired by placental animals recently in evolution. For that reason, many researchers believed that they work as if they were viral proteins. They interact with receptors that are different from viral receptors and their expression is highly regulated, there is a tissue-specific route that determines which cells fuse or not (Brukman *et al.*, 2019). Some researchers believe that other cellular fusogens involved in fusion of somatic cells (*EFF-1* *AFF-1*) originated in viruses and this could also be the case for sexual fusogens (*GCS1/HAP2*) from plants and protists. Alternatively, viruses may have taken the genes encoding for fusogens from early eukaryotes or even from prokaryotes.

On the other hand, in vertebrates, we do not even know which fusion proteins are involved in fertilization. Additionally, there are groups of organisms such as fungi that fuse during mating, but their fusion proteins are unknown. For decades geneticists have tried to find the fusion proteins in yeast such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and in other fungi without success.

### If you had not decided to dedicate yourself to science, what other activity would you have preferred?

When I was deliberating what to study, I was hesitating between biology and architecture. I like architecture, sculpture too, perhaps I would have gone for the arts or architecture, which is also an art. I still like it! I do not know, maybe someday I will start a second career. When a student, postdoctoral fellow, or a lab member finishes their work in the lab, I make for them a sculpture related to



**Fig. 10. Eugenia del Pino and Benjamin Tabgha, Northern Sea of Galilee, Israel.** From *Darwin to Evo-Devo: A symposium in honor of the 150th anniversary of Darwin's "The Origin of Species," Haifa, 23-24 November 2009.*

their work as a gift.

I like to hike! When I lived in Mexico, I was in a mountaineering club called Citlaltepeltl of Mexico. I climbed many volcanoes, including Popocatepetl, Citlaltepeltl and Iztaccihuatl. I like birdwatching, walking and hiking (Fig. 9). I enjoy singing since I was 15 years old, when I started singing in a choir in Mexico, called Convivium Musicum. All along since then I was singing in choirs in the United States, England, and now in Israel. Before I used to sing in bigger choirs, now I sing in the Haifa Madrigal chamber choir: contemporary, renaissance, classic, romantic and baroque music.

### If you had the opportunity to interview a scientist, dead or alive, who would it be, and what would you ask?

That is a difficult question. I would ask Ramón y Cajal how the concept of the synapse first came to him. I have read his books, but my question would be how the light came up, the flash of genius.

### Is there anything else beyond what we have asked you that you would like to talk about?

For me, the laboratory environment is significant, I'd like everyone to get along well, and I try to promote a vivid scientific environment with people asking fundamental questions. I also love to interact with the students, postdocs, and colleagues. I enjoy discussing scientific problems, not necessarily issues that I am working on. Something beautiful in science is that we have a vocabulary and curiosity that we use to communicate, ask questions, and begin to find answers. We can be here talking about Mexico and Israel and be interested in the history of science and how ideas developed, etcetera.

### Do you have a relationship with people in Ibero-America?

Yes, in 2018 I participated in the 13<sup>th</sup> Congress of the Mexican Society for Developmental Biology in Chautla, Mexico and in February 2020, I attended the 2nd Latin American Congress on *C.*

*C. elegans* in Rosario (the birthplace of Messi and el Che) and I gave seminars in Mendoza and Buenos Aires. My son came with me and we spent a week hiking in the Andes, it was a fantastic experience and we got to Cerro Vallecitos (5,450 m) with wonderful views of the Aconcagua (Fig. 9). Maybe Rosa Navarro and I will organize the 4th Latin American Congress on *C. elegans* in Mexico. I have collaborations or interactions with people from Ibero-American origin in different parts of the world (Fig. 10) and I have active and exciting collaborations with colleagues in Argentina (Pablo Aguilar), Uruguay (Héctor Romero and Martín Graña) and Mexico (Rosa Navarro). I had a Mexican postdoctoral researcher who is now in Guadalajara (Jorge Verdín) and currently Dr. Nicolas Brukman, from Buenos Aires, is pioneering work on sperm-egg fusion in mouse in my lab. For over two decades my lab manager and friend has been Clari Valansi and she is also from Buenos Aires. I have advised scientists from Mexico, and I try to go to Mexico once or twice a year and give a talk each time. I gave a lecture a few years ago at the Instituto de Investigaciones Biomédicas UNAM and was fortunate to give a talk in Spanish at the Instituto de Fisiología Celular at UNAM on February 28, 2020, this was my last *in vivo* seminar with a full house in the times of COVID-19.

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