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Retrospective

RETROSPECTIVE

# Laura Frontali—my life with yeast

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One sentence summary: Over 40 years of research on yeast from mitochondrial DNA to mitochondrial diseases.
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## ABSTRACT

The Author relates her life from University to recent years. It was dominated by the developing importance of yeast, from agent of industrial fermentations to eukaryotic model organism. In this frame she recalls family life , friends, teachers, collaborations.

**Keywords:** Frontali; Yeast; Retrospective

I was born in 1935 into a family with strong scientific traditions, which always assumed that my future would involve science. Nonetheless, I received a classic formation, with a special interest in Latin and Italian literature, both of which gave to me a lifelong love for several poets.

Following the suggestion by Professor T. Cooper (another yeast addict), I will try in this paper, to retrace the cultural milieu, the different scientific breakthroughs and the outstanding scientific personalities which shaped my scientific career and its connections with my personal life.

## 'IT HAS NOT ESCAPED OUR ATTENTION…'—A CHANGE IN THE SCIENTIFIC PARADIGM

Upon beginning advanced studies in chemistry and then in biology at the University of Rome La Sapienza, in the early fifties, I was immediately confronted with Watson and Crick's fundamental discovery and the change it made in the scientific paradigm. It was a shift from investigating the chemical composition of living matter to investigating fundamental biological problems through the exploitation of 'models'. The opening of this new era had an incredible impact not only on biological thinking in general, but also on the structure of the Italian Academy. The well-known understatement in the famous 1953 Nature paper 'It has not escaped our attention.…' introduced a new style of approaching scientific problems and, as a consequence, a new organisation of laboratories and academic relationships, which at the time were very rigid.

## INFECTIOUS ENTHUSIASM THAT SHAPED MY LIFE

In the University of Pavia, a group of young geneticists (Adriano Buzzati-Traverso, Luca Cavalli Sforza, Giovanni Magni, Mario Polsinelli) promoted these new approaches despite the conservatism of most Italian university professors. They organised 'Pavia courses' for young scientists and broadened their intellectual horizons by inviting international speakers. Buzzati then created the Laboratorio Italiano di Genetica e Biofisica (LIGB) in Naples, also taking advantage of the interest of several physics professors in the new biological approaches. The LIGB immediately became a mine of fundamental discussions among exceptional scientists elaborating the new science of molecular biology. Participating in these meetings was an extraordinary privilege for me: I could listen to Gamow and Crick discussing, with different approaches, a triplet-based code—Crick proposing the necessary existence of an 'adaptor RNA' and Hoagland demonstrating it. I also met Luigi Gorini and subsequent Nobel laureate Salvador Luria. The enthusiasm of that season most probably shaped my life. At the time I already had two children (Fig. 1, Top), but I could not imagine remaining at home and out of

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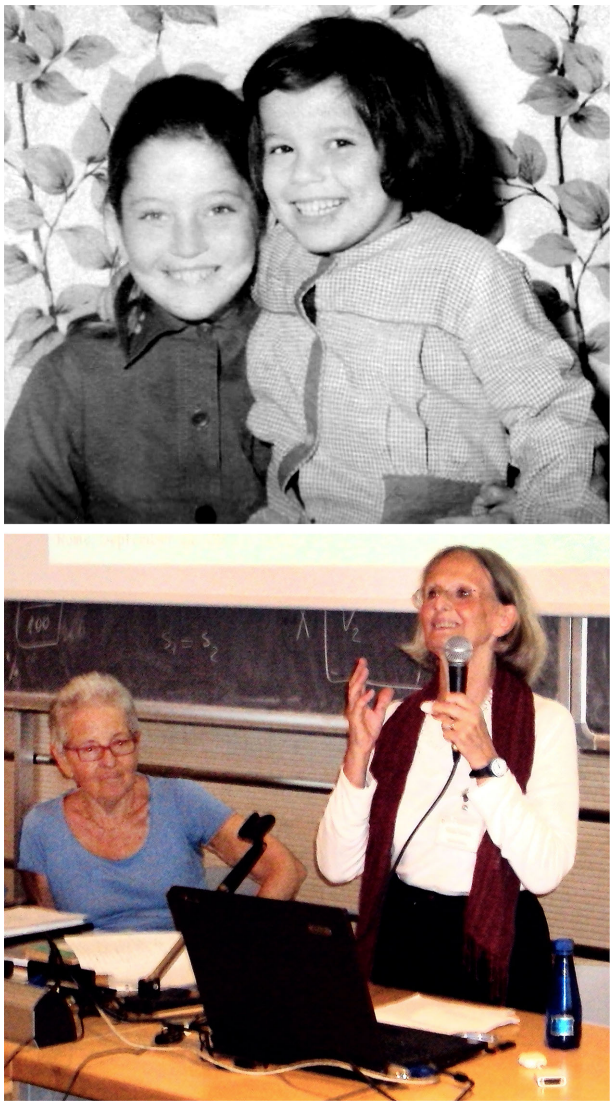

**Figure 1.** (Top) Laura's first two children, 1963. (Bottom) Rome 2010. Meeting 'Yeast, an evergreen model. A tribute to Piotr Slonimski', Ileana Ferrero is presenting (I am chairing).

science. Although my family supported my scientific involvement at that time, to reconcile child and household care with research and teaching duties has been 'THE' problem of my life.

Among the courses I followed for my degree in chemistry, the one given by Giorgio Tecce had an interesting title: 'Fermentations Chemistry and Industrial Microbiology', possibly derived from the work of Pasteur on ethanol production by yeast and dealing in general with the use of yeast and of its metabolic flexibility for industrial uses. Moreover at the time, the production of beet sugar was a very important activity in northern Italy and I was fascinated by the possibility of connecting microbiology with the biochemistry of fermentation and both disciplines to industrial production. I asked to be admitted into the laboratory of Prof. Tecce, where I performed my thesis on pentose metabolism in bacteria for a degree (laurea) in chemistry (Frontali and Tecce 1959). I then studied for a second 'laurea' in biology: that time my thesis was on pentose metabolism in 'petite' mutants of *Saccharomyces cerevisiae*. Tecce (thereafter the Dean of Rome University) was in close contact with the Pavia group, Buzzati Traverso and Franco Graziosi (who became director of the LIGB after Buzzati), giving me the opportunity to participate in the international courses I described above.

## YEAST ENTERS MY LIFE—DISAPPOINTED BUT RIGHT

Two further events were also very important. Perhaps by chance, I read a small book edited by Masson (Paris) in 1953: 'La formation des enzymes réspiratoirs dans la lévure'. It was the thesis that Piotr Slonimski had performed in Ephrussi's lab. The possibility of genetic studies on induced enzyme biosynthesis was a wonderful new subject connecting biochemistry to genetics. I was still working on bacteria and in particular on thermophiles (how could they live at temperatures which usually produce denaturation of double helices?). Soon, however, the interests of the lab shifted to yeast. Using CsCl density gradients in an analytical ultracentrifuge, we observed a small peak with a lower density (and hence a different base composition) compared with the high peak of nuclear DNA. The smaller peak was absent or had still lower density in 'petite' mutants (Carnevali *et al.* 1973). This was in the early seventies and mitochondrial DNA in yeast had been recently demonstrated by Mounolou and Slonimski. Our reaction on reading their paper was 2-fold: we were disappointed not to have published our observations before they did, and on the other side, we were very glad to see that our observations were authoritatively shown to be correct.

From then on, Giovanni Magni had a big influence in my scientific life. He was endowed with a sparkling intelligence and humanity. He had made important contributions to the understanding of meiosis in yeast and, importantly for me, he was interested in promoting research by young scientists. I still remember him at the poster session, during the Rochester Yeast Conference, stopping in front of my poster and asking whether I planned to continue that line of research (at that time it was mitochondrial protein synthesis and glucose repression). At my positive answer, he told me that I should become independent and that he would try to obtain a grant for me from C.N.R. And he succeeded.

I should also add that he was among the small group of yeast geneticists that founded the International Conference on Yeast Genetics and Molecular Biology and was the Italian member of its Finance and Policy Committee for many years. When leaving that Committee he asked that I be his replacement. No correspondence was involved: he simply told me (and the Committee) that he wanted to retire and proposed my name; he always had great confidence in me and I have collaborated and still collaborate with several of his former students (Pier Paolo Puglisi, Ileana Ferrero) (Fig. 1, Bottom).

## CANCEL THE COMPETITION

Meantime, I had become a full professor. I had my own laboratory and the grant Magni had obtained for me had been essential for my scientific independence and hence for receiving further grants. However, at that time my cultural position was not easy. In principle (and for the title of my discipline), I should have been interested in industrial fermenters in which 'yeasts' or 'molds' could be grown and produce useful molecules, focusing on the continuous monitoring of growth, infections and oxygen levels. In contrast, I favoured strains, genes and mutants for the same purposes. This preference was reproached to me and made advancing to a full professor of fermentations very difficult. Remarkably, a member of the commission, which had given

**Figure 2.** (Left) Gift of a Pasteur flask for Laura's 80th birthday. (Right) Laura and her family, 1990.

me the professorship, even appealed to have the competition cancelled. As a result, I tried to integrate microbiology, biochemistry, genetics and molecular biology involved in industrial production into a 'biotechnology' teaching group with yeast being the most important organism both for basic knowledge and practical purposes.

## THE PRINCESS AND PASTEUR

I also became a member of the scientific board and then the Scientific Director of the Istituto Pasteur-Fondazione Cenci Bolognetti. This non-profit Institution was established in the heritage/will of the roman princess Cenci Bolognetti, who (to the distress of her family) left her entire fortune to Rome University for the building of a Pasteur Institute. After more than 20 years of legal debate, and a war (she had died in the thirties), the construction of such a building was out of question. However, some university laboratories in biology, medicine and pharmacy were so dedicated. Finally, this organisation was recognised as a Pasteur Institute (the only limitation was that we should not produce vaccines) and therefore a member of the 'Réseau des Instituts Pasteur'. Monod had created this network of 'Pasteur Institutes', in which the scientific directors of the more than 20 Pasteur Institutes around the world met annually to exchange results and experiences. Participating in these meetings reinforced my interest in the connection between scientific results and medical and practical problems. The love for Pasteur's works had become an important part of my scientific and teaching interests. I was fascinated by the extraordinary possibility of simultaneously finding solutions for fundamental scientific problems such as 'spontaneous generation' and at the same time for practical industrial problems such as ethanol production (Fig. 2, left). I even wrote a paper on this aspect (Fundamenta Scientiae 1981). Our Pasteur Foundation used the Princess's money to support research projects in the so-called pasteurian sciences, i.e. biochemistry, physiology, pharmacology, microbiology and genetics. We also used it to finance fellowships for young students to work either in Rome or in good foreign laboratories. Fellowships were very important both for the students and for the contacts we could establish with laboratories: Claudio Palleschi was in the lab of Don Williamson; Silvia Francisci in that of Rudolf Schweyen, while the work of Falcone and Bianchi was part of the collaboration with Hiroshi Fukuhara. Teresa Rinaldi was for many years in the laboratory of Monique Bolotin-Fukuhara, where she even obtained a 'Doctorat d'Etat' and developed work on a proteasomal mutation in yeast. The ease with which she uses an old Singer micromanipulator is probably due to Monique's influence.

## THE ST. ANTHONY EFFECT—EFFICIENCY AND UNDERSTANDING LEAD TO ADVANCEMENT

Faithful to an image of efficiency (my colleagues jokingly concluded that I was endowed with the prodigious 'St. Antony effect' consisting of ubiquity, i.e. the ability to be in two places at the same time), I accepted several positions that were offered to me. I became the Director of the Department of Cell Biology at Rome University. This appointment, however, was due not only to the St. Anthony effect, but also to my capacity to understand individuals' differing positions, to reason and thereby resolve conflicts between them. When asked by a journalist, what I thought to be the important *feminine* qualities for the participation of women in the organisation and in performing science, I responded patience and the understanding of others. I still think it is so.

## AM I THE ONLY WOMAN?

I was also the Italian member of the EMBO Council for several years. It happened like this: Piotr Slonimski proposed me for EMBO membership. However, two signatures were required on the EMBO proposal. When I suggested that Giorgio Bernardi provides the second signature, Piotr replied, 'Either Bernardi's signature or mine, you have the choice'. He suggested Ponte (Guido Pontecorvo), as he called him, instead. Ponte agreed. I was then elected to the Council by Italian EMBO members. At dinner, during my first meeting of the Council, astonished, I asked Piotr who was sitting near me: 'Piotr, am I the only woman?' I remember his answer: 'You know Laura, this is an English Club'.

As new administrative responsibilities increased, I presumed that I could successfully reconcile them with family life, but in effect they created very serious problems. My husband, who

had encouraged me so much as a very young person wishing to extend her knowledge, now resented my being a successful and very busy woman. On the other side, I felt a deep sense of culpability for not lending sufficient attention to my three children (Fig. 2, Right). I thought, as had been the case for my parents, that the most important thing was to be a good example, but the situation had changed, closer contact would have been necessary.

A further problem in those years came from my increasing distance from the bench. I soon realised that this could be very dangerous for my scientific performance. So I asked Pino Macino (a former student of mine and now a successful scientist) to teach me how to sequence (at the time it was using Maxam & Gilbert chemistry) and map transcripts. He was a very good teacher, but declined to sign the papers issued from his teaching. Nonetheless, I could now participate with my own hands to study the transcription of mitochondrial tRNA genes in yeast (Palleschi *et al.* 1984).

## SEQUENCING THE YEAST GENOME

The European decision to sequence yeast Chromosome III and then the entire yeast genome was a turning point in my scientific life from several points of view. First, our lab was involved, along with many others, in an important international enterprise in which goals, interests and methodologies were shared. It also gave, in my view, a molecular basis to our knowledge of structure and the evolution of a eukaryotic organism. And, finally, it revealed the extraordinary conservation of many genes and, in particular, those involved in human disease. In the subsequent Functional Analysis project, we grouped with Piotr and that occasioned the important development of scientific and human contacts described below and which greatly influenced our subsequent research.

## MEETINGS LEADING TO LIFELONG COLLABORATIONS

In 1975—1976, we had two important meetings. One in Bari starting the series of Bari meetings and another at the Genetics Institute in Munich. The Bari meetings were organised by Cecilia Saccone and A.M. Kroon and focused on the organisation and expression of the mitochondrial genome. These meetings generated not only important contacts among scientists (Fig. 3, left) but also fierce discussions on yeast mitochondrial genome, introns and split genes. I met Hiroshi Fukuhara in both meetings. He invited me to Orsay as a visiting professor and we started an extraordinary 30-year collaboration. Hiroshi was Chief of Laboratory in the Biology Section of the Fondation Curie-Institut du Radium. He had been living in France for several decades, but he never wanted to relinquish his Japanese citizenship. Indeed, he was deeply Japanese despite being issued from an unconventional family. In his youth, he had participated in courses of the martial arts and how to serve tea, but he read Dante in French (in the beautiful translation by Jaqueline Risset) and then in Italian. He was also enthusiastic about Dante's readings by Benigni. He had long been a member of Slonimski's laboratory (Piotr once told me regretfully 'He was my right arm..'), but then he became a victim of one of the conflicts which, unfortunately, were not infrequent in that lab. Incidentally, I should say that several of my best friends came from the lab in Gif: besides Hiroshi, his wife Monique Bolotin, Giovanna Carignani and Jacqueline Verdière (Fig. 3, Right).

With Hiroshi, we first used deletion 'petite' mutants to localise mitochondrial tRNA genes in yeast mitochondrial genome (Wesolowski *et al.* 1981). We then collaborated in a survey of mtDNA in several yeast species. In *Kluyveromyces* species, we found a 2 micron-like plasmid, having the same flip-flop characteristics as occurs in *Saccharomyces cerevisiae*, but with a completely different base composition (Falcone *et al.* 1986). We then decided to study the molecular biology of *K. lactis*: a 'petite' negative yeast, which lacked glucose repression. *Kluyveromyces lactis* was a good producer of heterologous proteins and Hiroshi suggested filing for a patent. I would never have thought of this possibility. However, I recognised the Japanese aspect of this proposal and a successful application was filed and awarded. The long collaboration with Hiroshi was an extraordinary example of a relationship in which curiosity-driven discussions resulted in long-term experimental decisions, in practical utilisation (e.g. heterologous protein production in *K. lactis*), and in the possibility for science to approach general human problems. Student exchanges between our laboratories were also frequent and fruitful. Claudio Falcone was the first of my students who went to Hiroshi's lab followed by Michele Bianchi. Meanwhile, Micheline Wesolowski came for several months into my lab. All of them

**Figure 3.** (Left) 1980 Bari Meeting: Laura and Nancy Martin, competitor and friend. (Right) In New York early eighties at MOM: Hiroshi Fukuhara, Giovanna Carignani, Chris Herbert and Laura Frontali.

**Figure 4.** (Top) Laura, Monique Bolotin-Fukuhara and Robert Martin, 2010 at the 'Yeast, An Evergreen Model meeting'. (Bottom) Laura and Piotr (with pipe) 2001 in Prague.

became Hiroshi's friends as well as friends and collaborators of Micheline.

## MUTATION BY TUNGSTEN BULLETS

Another very important collaboration, which still continues, is with Hiroshi's wife Monique Bolotin. Some years ago, Monique had a very good idea. Tom Fox had described the possibility of transforming yeast mitochondria by shooting into them tungsten bullets coated with DNA from 'petites' bearing the desired information. Through this *biolistic* procedure, yeast became the only organism in which mitochondrial transformation was possible. Monique proposed using the procedure to introduce into yeast mitochondrial tRNA genes the same mutations that produce very serious mitochondrial illnesses in humans (Fig. 4, Top). These mutations were obtained in her lab, and we verified the corresponding effects in yeast. We then tried rescuing those effects by overexpressing yeast mitochondrial protein synthesis factors (Chris Herbert helped us with a gift of several plasmids). We succeeded! My lab is still pursuing this strategy and trying to extend it to human cybrids. An important part of this study was done in collaboration with Bob and Zosia Lightowlers. Discussions with Monique, Silvia Francisci, me and all of the students in our labs were precious and still continue.

I also would like to mention my life-long contact with Mario Polsinelli. He was a member of the Pavia group and became Professor of Genetics in Florence. But, before that, he was a student in an Oenological Institute. His interest in yeast and wine strains has continued throughout his life and led to his fruitful collaboration with Bob Mortimer. His laboratory in Florence was open: anyone with a research problem to discuss was welcome and sure to find an interested listener, as I did several times concerning *Kluyveromyces lactis* plasmids and glucose repression. These discussions also renewed my interest in the connection between yeast studies and production.

## PIOTR—CONNECTING DIVERSE DATA IN ORGINAL WAYS

The years 1975–1976 were also the same years that my contacts with Piotr Slonimski were progressing. For years I had listened with increasing admiration to his talks (and to his participation in the fierce discussions that occurred during international meetings). These debates had led to the explanation that long and short mitochondrial genomes in yeast derived from differing numbers of non-coding or differently coding sequences. What I found so admirable was Piotr's extraordinary capacity to connect different data and to interpret them in an original way. He and his lab proposed the existence of split genes, of introns and of maturases well before their recognition by sequencing.

As previously mentioned, we formed a group with Piotr for the European project on genome functional analysis. We had previously collaborated on the evolution of mitochondrial genomes, but the collaboration now became much more important and involved several young students. Piotr often came to Rome to discuss the experiments in detail. We published several papers in which Piotr's name appears with those of obscure students (Bianchi *et al.* 1999, 2004). Reciprocally, my students were very fond of him and when he died, they honoured him with a meeting held in Rome University 'Yeast, an evergreen model. A tribute to Piotr Slonimski'.

Piotr liked and encouraged our starting interest in studying yeast as a model for human pathologies and the possibility of correcting them in yeast by overexpressing mitochondrial factors (Feuerman *et al.* 2003). He thought and told me that using yeast as a model for human mitochondrial diseases was exactly the kind of approach where the use of yeast genetics was invaluable. In Fig. 4, Bottom, he appears in the final photo of a satellite meeting of the Yeast Conference in Prague in 2001. The meeting (Yeast as a model of mitochondria related human disorders) was held near the Conference building in a former Police Station. Piotr was especially pleased and amused to use a Police building for science, perhaps from an earlier contact with one (See Piotr Slonimski—The Warrior Pope, FEMS Yeast Research 16:fow004).

## APPRECIATING THE BEAUTY OF ROME IN A FACTORY

Piotr liked Rome very much: he was deeply interested in the paleochristian churches I showed him and in museums, even the less celebrated ones. I still remember my asking him rather

**Figure 5.** Laura with Rome in the background.

timidly if he would not be interested in hearing a rather obscure Opera composer (Cilea): his answer was enthusiastic: 'J'adore'. We shared a deep appreciation, not only for the beauty of Rome monuments (Fig. 5) but in particular for the mixture of different styles (ancient roman, medieval, baroque…) contributing to their beauty. I remember his enthusiasm for a museum (Centrale Montemartini, poorly known at that time) which was housed in the twentieth century electric central of Rome, indeed a very beautiful example of industrial archeology, in which many Roman and Greek statues had been placed. The white of those statues had a wonderful effect against the dark colour of the turbines.

I must add that a very important part of my friendship with Piotr took place in the last years of his life. I visited him every time I was in Paris for my collaboration with Hiroshi and then with Monique Bolotin. For years, there had been aspects in his interactions with other scientists, especially his students (of whom he sometimes failed to see the reasons) that I did not like. But I always admired his culture and his deep interest in many aspects of human activity beyond science: art, music, history and politics. Moreover, with the diminishing of his power, the loosening of his very tight relationship with the success and importance of his results, I finally deeply appreciated his capacity to understand—with intelligence, detachment and irony—the development of science and an individual's relationship with it. This was a very important aspect of his personality: the mixture of passion and irony with which he made science worthy of being done. So I close with the phrase that Bernard Dujon used to conclude Piotr's commemoration after his death. 'Piotr Slonimski considered Science either as a play or as an art, and he was a great artist of basic research'.

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