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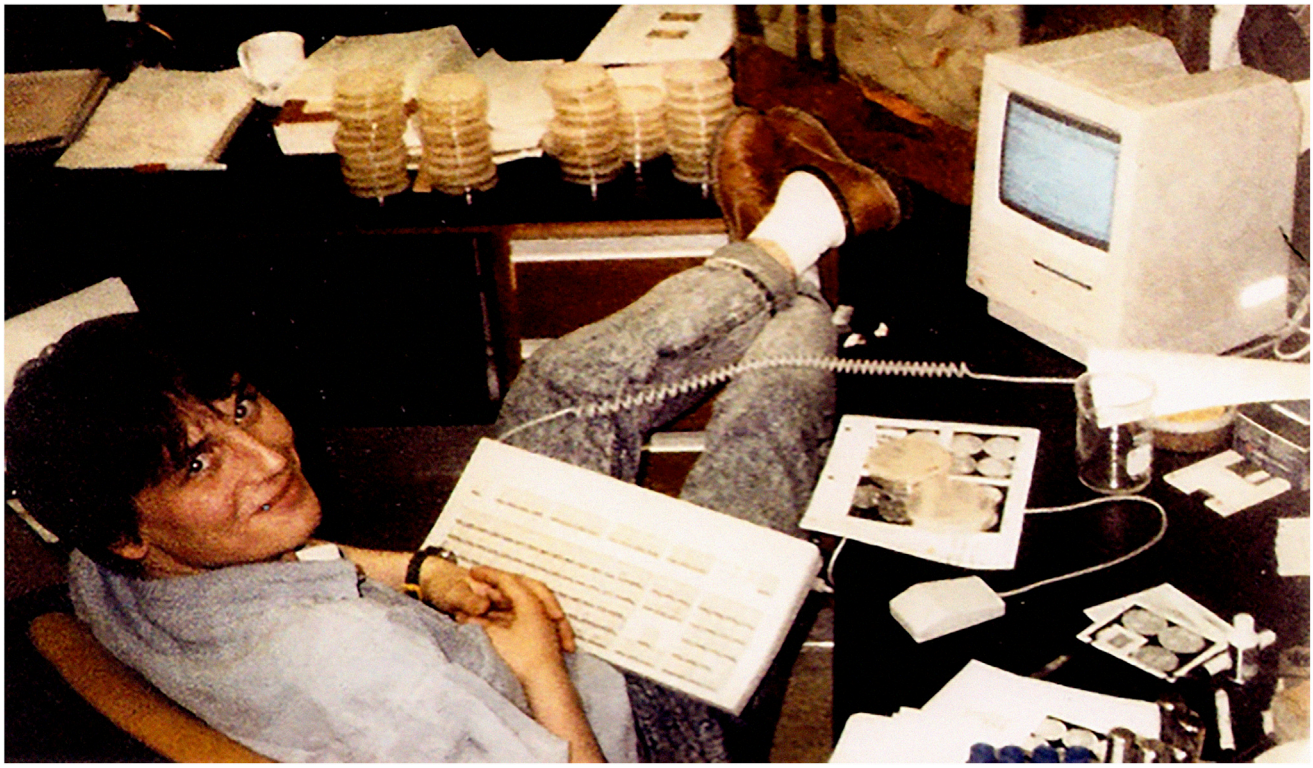

Playing Hide-and-Seek with Yeast

Marc Vidal

I like to think of the late 80s and early 90s as extremely exciting times in my career. I was working as a visiting graduate student in Rick Gaber's laboratory at Northwestern University, and some of the work I did during those years turned out to be seminal for the then-fledgling field of epigenetics. Together with other scientists, I provided long-awaited in vivo functional evidence for a model proposed by Vince Allfrey in 1964, postulating that post-translational modifications of histones, particularly acetylation and deacetylation, are crucial for transcriptional regulation.

What started as a classical yeast genetic screen to identify genes involved in potassium transport ended up with the identification of a global transcriptional repressor complex. Rpd3, one of the gene products originally named after the reduced potassium dependency phenotype of *rpd* mutants, turned out to be a homolog of mammalian histone deacetylase (HDAC), which was purified by Stuart Schreiber's laboratory in 1996, half a decade after the events in this story. A month prior to that publication, David Allis' laboratory had described how *Tetrahymena* histone acetyl transferase (HAT) is in fact an ortholog of Gcn5, another yeast protein, which had also previously been demonstrated to play a pleiotropic role in transcriptional activation.

In this story, I would like to reminisce about how a densely connected network of yeast geneticists ended up unraveling an equally dense ''interactome'' network of genetic and regulatory interactions between yeast genes, which eventually gave rise to some of the most fundamental discoveries in gene regulation. The mapping of this interactome started as early as 1964, the very year Allfrey formulated his histone regulation hypothesis. By 1996, the cumulative studies in yeast genetics were poised to provide a functional interpretation to the biochemical purification of HDAC and HAT achieved in mammalian cells. As the story unfolds, we will pull ourselves back into

The author was photographed circa 1990 in Rick Gaber's laboratory.

a pre-internet era, and describe how it felt to surf this interactome network in the absence of the sophisticated tools we have at our fingertips today.

By the time I left Belgium in January 1988 to join Rick Gaber's group, I had already worked on several yeast genetics projects with François Hilger, my PhD mentor. Although not terribly successful, these projects made me appreciate the beauty of the "hide-and-seek" games one can play with yeast. I became particularly fond of the concept of "genetic selection," which consists of designing environmental and/or genetic conditions that prevent yeast cells from growing, and then *selecting* for mutations that circumvent the growth inhibitory conditions, as a way to identify genes and functions regulating such processes.

On my first day in the lab, Rick introduced me to a set of beautiful *rpd* mutants. Prior to setting up his own research group, Rick had worked in Gerry Fink's laboratory, where he had identified a gene called *TRK1* and shown that it encodes a high-affinity transporter for potassium (K^+). Logically, *trk1Δ* cells require higher K^+ concentrations relative to their wild-type counterpart, and the hide-and-seek game of the Rpd selection scheme consisted of finding out how *trk1Δ* mutants can revert to allow growth on low K^+ concentrations.

I found that the two genes identified by recessive mutations, *RPD1* and *RPD3*, genetically mapped at two new, previously undiscovered loci. This might not sound like a big deal today. But, at that time, it was very exciting because mapping mutations felt like planting flags in the genome to claim new territories. If no other gene had ever been mapped where you found your mutations, you had discovered a new gene and perhaps a novel function. In addition, as will become clear below, genetic mapping of new genes could more often than not help establishing functional connections between seemingly unrelated genes.

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There was also a third group of dominant alleles among the *rpd* mutants, which we realized mapped very close to *TRK2*, the low-affinity K^+ transporter gene. In addition, wild-type *TRK2* was required for the effect of both *rpd1* and *rpd3* on K^+ transport and *rpd1 rpd3* double mutants did not show any synergy. In our 1990 *Genetics* paper describing the mutants, we concluded that "*RPD1* and *RPD3* might function … in a single pathway or as subunits of a single negative regulator … required for the normal expression or activity of the low affinity K^+ transporter." Figuring out whether *rpd* mutations affected *TRK2* by modulating its expression or the function of its product turned out to be of crucial importance. A set of experiments performed together with Ann Buckley, using a *TRK2::lacZ* reporter, suggested a modest, but reproducible effect on *TRK2* expression.

But the story took an unexpected turn when I decided one day to try my luck with a quick phenotypic test. As I was plating my mutants, I noticed a stack of Petri plates containing sub-lethal concentrations of cycloheximide, an inhibitor of protein synthesis, that had been poured a few days earlier by someone else for an unrelated project. To this day, I have no idea what triggered my impulse to replica plate my crosses onto these plates, but I vividly remember my excitement when I opened the incubator the next morning and realized that the *rpd* mutants grew poorly or not at all on these plates. Importantly, the phenotype was only exhibited by *rpd1* and *rpd3,* and not by *TRK2* dominant mutants. This suggested that cycloheximide hypersensitivity might not be related to *TRK2* function and that Rpd1 and Rpd3 might have a broader function and be involved in regulating other genes. From that point, I literally became obsessed with testing this possibility.

A possible global role for Rpd1 and Rpd3 was consistent with something unexpected I noticed while genetically mapping *rpd1.* It turns out that the closest gene to *rpd1* known at that time was *pho80*. To precisely place *rpd1* on the genetic map, I had to cross *rpd1* and *pho80* strains together. *PHO80* is required for the transcriptional repression of *PHO5*, a gene encoding acid phosphatase that is repressed in high phosphate concentrations. While following the *pho80* phenotype, I observed that *rpd1* and *rpd3* also increase the transcriptional levels of *PHO5* under repression conditions. Again the effect was relatively small, on the order of two-fold, but it was reproducible and significant.

An even more rewarding connection was provided by a talk I heard from Randy Strich, then a postdoctoral fellow in Shelly Esposito's laboratory. He was describing the isolation of unscheduled

meiosis or "*ume*" mutants based on the derepression of *SPO13*, a gene required for sporulation and normally tightly repressed in wild-type mitotic cells. At some point, he mentioned in passing that one of his mutants, *ume4*, mapped somewhere in the vicinity of *rpd1*. The connection was almost too beautiful to be true. I jumped to the podium at the end of his talk, and in a couple of minutes, we arranged how we were going to test whether *rpd1* and *ume4* mutations might reside in the same gene. The answer as we both expected was positive, which triggered an extremely fruitful collaboration.

The cloning and sequencing of *RPD1,* which altogether took close to a year and a half of hard work, provided yet another essential connection. Remember, this was before the yeast genome sequencing project, and it could often take years for two teams to realize they were working on the same gene. To speed things up, Mark Goebl at Indiana University had organized an informal database of unpublished yeast sequences. The system was simple. People would send him their sequences, he would compile them, and when matches were found between sequences obtained in different laboratories, he would inform the relevant parties. Lo and behold, Rick got a breath-taking call one day. Mark had spotted that *RPD1* was identical to *SIN3*, a gene being sequenced in David Stillman's laboratory at the University of Utah.

Sin3 mutations had originally come out of screens independently performed in the laboratories of Ira Herskowitz and Kim Nasmyth, who were searching for *SWI5*-independent expression of an *HO::lacZ* fusion. *HO* is an endonuclease that is tightly repressed at the transcriptional level by a complex mechanism involving cell-cycle regulation and mother/daughter identity. It took us relatively little time to demonstrate the allelism between *rpd1* and *sin3* mutations, but since David published his *SIN3* sequence about a year before we published our findings on *RPD1*, the name Sin3 is the most commonly used for the corresponding protein.

Randy Strich and I proceeded to demonstrate using a battery of *lacZ* fusions to other structural genes, as well as direct RNA level measurements, that *sin3* mutations affect both transcriptional repression and activation of more than a dozen unrelated genes involved in functions as different as ion transport, acid phosphatase metabolism, cell-type regulation, and cell differentiation. In all cases tested, the phenotypes were nearly identical in *rpd3* mutants. This was the support we needed to demonstrate that Rpd gene products were indeed global regulators of transcription.

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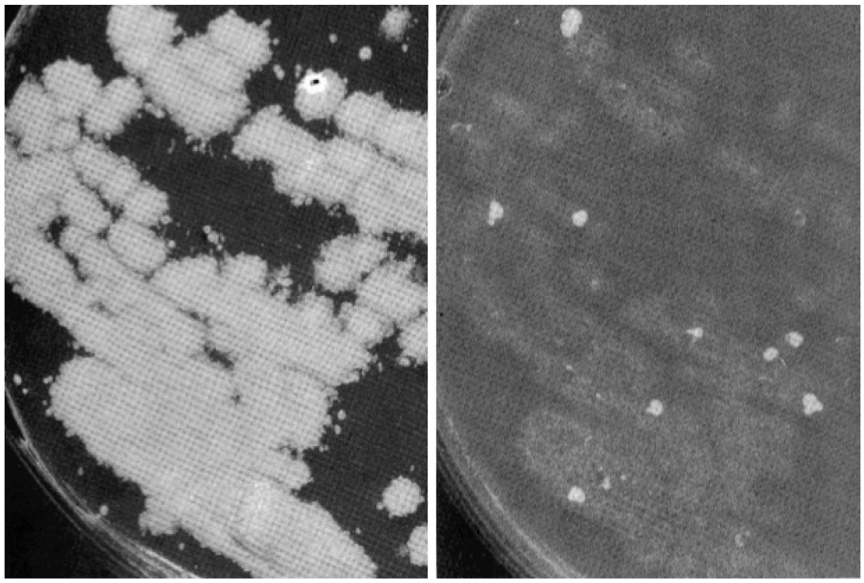
However, the cloning and sequencing of the *RPD3* gene failed to reveal anything informative. The translated Rpd3 protein sequence was novel and had no homology to any protein known at the time. I remember being very excited about having discovered a novel gene, but also being very depressed about not being able to find any domain or signature that could help us determine the biochemical function of the Rpd3 product. But of course in retrospect, it didn't matter at all since the Rpd3 sequence ended up being the key link that identified an in vivo role for HDAC.

After a rather painful attempt with another journal considered more prestigious, we ended up publishing these findings in two back-to-back papers in *MCB* in 1991. But even at *MCB* whose review process was well known to be professional and fair, it wasn't so easy to get the papers accepted. I recall that one reviewer was arguing that two-fold increases, as observed for most genes derepressed by *sin3* and *rpd3*, could only be relevant to someone's *salary*, and would not likely correspond to anything important in biology.

The next question that we focused on was how Sin3 and Rpd3 might target the appropriate genes under various conditions to mediate their repression. We started by looking at the *TRK2* dominant mutations, and to our surprise, found that they were localized at the *TRK2* promoter, in a site similar to an upstream repressing sequence called *URS1*, originally identified by Terry Cooper's laboratory in the 1980s using a mutation located upstream of the *CAR1* gene. *CAR1* encodes arginase, the enzyme mediating the first step of the arginine catabolism pathway, and remarkably, this brings our story back to Belgium, my home country.

In the early 1960s, Marcelle Grenson and Jean-Marie Wiame's laboratory in Brussels had set out to understand the regulation of arginine biosynthesis and catabolism. In a genetic selection first



Yeast cells carrying a gene deletion of the high-affinity K^+ transporter encoded by *TRK1* grow normally on plates containing high K^+ concentrations (left), but poorly if at all on low K^+ concentrations (right). Yeast colonies growing on the right are *rpd* suppressors of the *trk1Δ* phenotype.

described in a meeting report of the *Société belge de Biochimie* in 1964, which the reader will remember, corresponds to the very year that Allfrey suggested his model, they described mutants affected in the repression of arginine biosynthesis. Oddly, these mutants also turned out to be affected in their ability to *activate CAR1* under conditions triggering arginine catabolism, and this provided another hide-and-seek situation to select for mutations that would derepress *CAR1*. This is precisely how the original *URS1* dominant mutation in *CAR1* was found, in addition to recessive mutations in three separate *trans*-acting genes, two of which were later found by Evelyne Dubois and Francine Messenguy to be identical to *SIN3* and *RPD3*.

This was a breathtaking loop of life for me. *SIN3* and *RPD3* were like my "babies" in graduate school, and when everything was said and done about them, it turned out that they had first seen the light of day in the 1970s, in my home country, just a few miles away from the place where I was born.

In the immediate aftermath of our findings on Sin3 and Rpd3, Randy Strich and Shelly Esposito, together with Evelyne Dubois and Francine Messenguy, demonstrated that the third gene identified in the Brussels mutant hunts was identical to *UME6*, another gene required for repression of *SPO13* in mitotic cells. Importantly, it was demonstrated that Ume6 binds the *URS1* site. This in turn strongly suggested that Ume6 recruits Sin3 and Rpd3 to their target promoters, at least at the *SPO13* and *CAR1* promoters, which immediately suggested a mechanism of specific recruitment to the appropriate promoters.

Our two 1991 *MCB* papers were soon followed by a publication in *EMBO J* by Tassos Georgakopoulos and George Thireos. While studying the *GCN5* gene, which had been identified among general control non-derepressed or "*gcn*" mutants, Tassos and George made two important observations. First, Gcn5 was required for the ability of Gcn4, a well-known DNA binding transcription factor, to activate transcription in vivo. Second, Gcn5 also performed similar co-activation functions with other unrelated DNA binding transcriptional activators. The conclusion to be drawn from this was that Gcn5, too, was likely a global pleiotropic factor involved in transcriptional regulation.

In summary, starting in Brussels in 1964, the year Allfrey proposed his model of epigenetic regulation, yeast experiments intertwined in the most elaborate way possible led to a very dense network of molecular and genetic interactions almost as if, in retrospect at least, someone had

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planned every detail of the story from its very beginning. The Rpd3 and Gcn5 connections established by the Schreiber and Allis papers were literally a bombshell in the field of transcription, providing the basis for the so-called epigenetic revolution of the last two decades. They are and should be celebrated as such. But as one will immediately notice reading or rereading them, both papers could make their point without having to show any result on transcription per se. And that's what our genetic network contributed.