Our understanding of a fatal human genetic disorder stands on the shoulders of humbler mould research

Durgadas P. Kasbekar
Fellow
and
INSA Senior Scientist,
Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India
kas@cdfd.org.in

1. Introduction. Greenberg dysplasia (GD) is a rare severe genetic condition in which the bones do not develop properly in the fetus, and is fatal before birth. Research on GD shares an under-appreciated link with research on the responses of cellular slime moulds (dictyostelids) to plant isoflavonoid phytoalexins. Dictyostelids are free-living soil amoebae that feed on bacteria that grow on decaying organic matter. Some species, e.g., *Dictyostelium discoideum*, are intensively studied by developmental biologists seeking to understand how the amoebae, in response to starvation, aggregate into multi-cellular mounds that undergo morphogenesis and form fruiting bodies, each comprising of a slender stalk that holds aloft a droplet of cells that differentiate into long-lived vegetative spores. Presumably, small fauna brushing past the droplet disperse the dictyostelid to new food sources. Phytoalexins, including pisatin of garden pea (*Pisum sativum*), are anti-fungal compounds synthesized by plants in response to fungal attack. My laboratory in the CCMB studied how dictyostelids respond to pisatin and other phytoalexins. While this was a source of justifiable pride, more often one had to justify why public money was spent on pursuing such esoterica. Soil amoebae cannot compete with dead fetuses for tax-payer sympathy and rupees. Nevertheless, highlighting the "dicty / dysplasia" link might help to strengthen the case for taking a more catholic view of "relevant" research.

The link was the enzyme sterol C-14 reductase, which catalyzes reduction of the C-14, 15 double bond in cholesterol biosynthesis. Humans have two isoforms of it. One, the lamin B receptor (LBR) protein is encoded by the LBR gene, whereas the other is encoded by the TM7SF2 gene. In 2003, mutations in LBR were reported by others to cause GD. The mutations disrupt normal cholesterol synthesis and possibly also allow potentially toxic byproducts to build up. How this disrupts bone growth and development in GD is still not known. That LBR has sterol reductase activity was reported by us in 1999. Another group pipped us to the post by reporting this in 1998, but the first hint for such activity was published in a 1994 paper from my laboratory, culminating studies initiated in 1989 when we began exploring the response of *D. discoideum* amoebae to pisatin.

2. Re-molding: From pisatin and Dictyostelium to human LBR in Neurospora.

First, pea seeds were germinated, the germlings were treated with copper sulphate, in response to the heavy metal toxicity the plant tissue made pisatin, which was extracted with organic solvents and a stock solution was made in dimethyl sulphoxide (DMSO). Dictyostelium amoebae suspended in a salt solution supplemented with 150 μg/ml pisatin were quickly lysed, but there was no lysis of amoebae suspended either in salt solution amended with a sub-lethal concentration (50 μg/ml), or just DMSO. Exposure to the sub-lethal dose made the amoebae resistant to a subsequent exposure to the higher dose whereas DMSO exposure did not. In nature cells are likely to first encounter a low concentration. Induction of pisatin-resistance, however, did not happen in a mutant blocked in wild-type sterol synthesis. Why did we think of comparing the wild type and mutant pisatin response? An answer to this question would require me to go back to still earlier PhD research, in which I had developed methods to more conveniently perform parasexual genetic analysis in Dictyostelium. No one seems to use those methods anymore, Dictyostelium parasexual genetics never took off, so I will skip it. [My friend Vidyanand Nanjundiah suggested that I say what parasexual genetics is, lest it suggest tantric practices that some readers wish they had learnt in their youth. It is eukaryotic genetics done by fusing nuclei that are not products of meiosis.]

Three other fortuitous events had occurred by end-1990. First, I was awarded Rockefeller Foundation's Biotechnology Career Fellowship to visit Hans VanEtten's laboratory in the University of Arizona, and use their radioactively-labeled pisatin to test whether the inducible resistance depended on a pisatin-degrading enzyme. It did not. Second, Robin Holliday (1932-2014), of Holliday Junction fame, visited the CCMB and advised me to explore similar phenomena in fungi. He even sent me a *Ustilago maydis* culture to get me started. In parallel, I had written to the Fungal Genetics Stock Center, USA, and received from them wild type and sterol mutant strains of *Neurospora crassa*. Our Dictyostelium results had prompted me to include the mutants in my request for strains. In the very first experiment we found the Neurospora mutants were sensitive to concentrations of pisatin that were non-inhibitory to the wild type. Third, Marc Orbach had joined as a new faculty in VanEtten's Department. Orbach had done his PhD in Charlie Yanofsky's Stanford laboratory, where he (and Matt Sachs) had constructed and used a Neurospora genome library. Orbach also knew the Stanford Neurospora geneticist, David Perkins, very well. On my second visit to Arizona in 1992, Orbach taught me to do research with Neurospora, I looked up Perkins in Stanford, and by selecting for cosmids that complemented the pisatin-sensitive mutant phenotype I cloned the two sterol-biosynthesis genes. In three months I had become a Neurospora geneticist.

K. G. Papavinasasundaram, then a post-doctoral scientist in my CCMB laboratory, sequenced one of the clones. BLAST alignment revealed, unsurprisingly, its homology with the just sequenced yeast sterol C-14 reductase gene. But most unexpectedly, it also revealed a comparable homology with the C-terminal transmembrane ~400 residues of chicken LBR.

Sometimes coming in second is lucky. The LBR sequence was probably uploaded into the database subsequent to the yeast sequence upload. My student Prakash Arumugam showed the C-terminal domain of human LBR complemented the Neurospora mutant's pisatin-sensitive phenotype, and thus established its sterol reductase activity. Again we came in second (this was the 1999 paper). Two decades later, such obsessing over "first/second" seems childish.

3. TM7SF2 remains enigmatic. Recall that TM7SF2 is the second human sterol C-14 reductase isoform. In 2008, mice knocked out for its gene were found to be apparently healthy, although there may be an impaired response of liver cells to proliferative stress. Thus, in mouse the Lbr protein apparently compensates for Tm7sf2 loss. A 2020 paper has reported the two isoforms have a negative relationship in different human tissues, and only one of them tends to be predominantly expressed in each tissue. The authors further speculate that LBR might be the constitutively active C14-sterol reductase, whereas TM7SF2 might be tunable and adjust to the local demands for cholesterol. Prakash Arumugam found that the human TM7SF2 protein did not complement either the Neurospora or yeast mutants. Possibly, TM7SF2 contains residues that prevent it from functioning in these heterologous systems. For example, its residues might be modified in human cells before its enzyme activity can be turned on. It might be possible to identify such residues by testing LBR/TM7SF2 chimeras for ability to complement in Neurospora or yeast. Interestingly, since 1998 when both isoforms became first known, 221 papers were published on LBR versus only 29 on TM7SF2.

4. Conclusions. With a "medically-relevant" result in the bag, I let my newly learnt Neurospora Genetics do the walking (rewording the famous Yellow Pages slogan). But these rambles over the past more than two decades are not discussed here.

The Dictyotelium pisatin response studies were not pursued much further. Except that we found the amoebae did not aggregate when starved in its presence. Amoebae are great foragers of bacteria, and pisatin might be the plant's way to recruit them to "disinfect" root lesions and protect itself from potentially pathogenic bacteria. Alternatively, the pisatin might signal to the amoebae that a nearby plant is in trouble, and the trouble might soon lead to a windfall of bacteria growing on the decaying organic matter. Either possibility would represent a novel plant-pathogen interaction.

My objective in narrating the dicty / dysplasia story was to record a particular and personal example of a medically relevant discovery that came "out of left field", and show that interesting research in genetics can be carried out without necessarily appealing to human health and disease. Medically relevant research is too important to be left only to those purporting to do it.

Acknowledgements. Vidyanand Nanjundiah, a fellow Dictyostelium researcher, offered useful comments on an earlier version of the article. K. G. Papavinasasundaram, Prakash

Arumugam, and Marc Orbach helped me in different ways to evolve into a Neurospora geneticist. The dicty / dysplasia story can scarcely be told without invoking the memory of Hans VanEtten, David Perkins, and Robin Holliday.