From biocontrol to cancer, probiotics and beyond

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This invited commentary covers the period 1997–2012 and has seen changes in terminology that progressed from “basic” and “applied” to “translational” research. In the context of Bioengineered, these changes map readily onto the processes of identifying microbial characteristics appropriate for specific applications, isolation of suitable cultures, strain or genome manipulation and exploitation of these or their metabolomes across a range of settings.

To a great degree, this commentary and my career reflect an engagement with molecular microbiology and the trialing of bacteria and derived constructs in applications ranging from intensive-scale crop protection to amelioration of gastrointestinal disease. This engagement began with laboratory and field evaluations of biocontrol, specifically use of pseudomonads effective against nematode and fungal plant pathogens, characterization of mechanisms mediating beneficial effects of probiotic lactobacilli and bifidobacteria and assessment of functional foods in multinational clinical trials relating to inflammatory bowel disease.

Subsequent work focused on (1) intellectual property (IP)-based medical devices for localized delivery of systemically toxic and gene cancer therapies; (2) growth of the science base supporting expansion of a multinational business including company acquisitions; (3) complementing existing inter-institutional research capabilities through development of a national industry-led collaboration; and, most recently, (4) strategic research programs at Ireland’s newest medical school.

My activities as outlined above parallel two distinct aspects of translational research: (1) involvement in knowledge-driven (commercial and research) organizations that brought together necessary resources and infrastructure and (2) availability of scale research funding from European Framework and Irish national programs.

Introduction—A Tale of Two Centers: Biocontrol at BioMerit Research Centre and Centre for Microbial Ecology

I finished my PhD in 1997 having been supervised by Fergal O’Gara and working alongside Yvan Moënne-Loccoz and David Dowling, at the BioMerit Research Centre in University College Cork, Ireland. At the time I joined this group, Fergal O’Gara and colleagues had published extensively in the field (pun intended!) of plant-microbe interactions1,2 and, most excitingly for me, had begun exploring the mechanisms mediating bacterial survival, adaptation and response to stimuli in their environment.3,4 By 1997, the team had established a brand of pragmatic molecular microbiology, deriving insight into regulation of antifungal metabolites,5,6 uptake of essential nutrients,4,7 biodegradation potential and responses to plant hosts or pathogens.8-11 My contribution related to biocontrol—in this case the introduction of beneficial bacteria to protect target crop plants from natural pests—specifically, protection of potatoes from attack by nematode12 and sugar beet from the fungus Pythium.13,14 I had isolated the bacterial strain used in most of this work and had to deal with challenges associated with it being a relatively
unfamiliar species. However, since that time, *Pseudomonas* AKA *Xanthomonas* AKA *Stenotrophomonas maltophilia* is now considered ubiquitous, the genome of our workhorse *Pseudomonas fluorescens* strain (F113) has been sequenced, and microbial interaction, as we had begun to perceive it, has evolved as quorum sensing.

Working in Fergal O’Gara’s group was a unique experience. Infamous Friday morning meetings and journal clubs were intense and sometimes intimidating affairs where, as a callow researcher, lessons regarding careful preparation were indelibly learned. Our research was at the frontier of the applied microbiology that I loved and was relevant to the scientific debates of the time. The concept of genetically modified organisms was contentious and, in a parallel and alternative approach to field testing of genetically modified crops, we were exploring potential pesticide replacement and/or reduction through use of naturally occurring or modified bacteria. In hindsight, the scale of the well-resourced team was unusual, with international visitors and opportunities for foreign travel to present at scientific conferences relatively frequent. Today, a cursory search of the European Union’s CORDIS site testifies to a research group that, in the 1990s, was competing successfully for Third and Fourth Framework funding and was developing interactions and partnerships across Europe. My work benefited considerably from the experiences of preparing some of those successful grant applications and from the subsequent funding. In particular, I worked for a time in the National Science Foundation—Centre for Microbial Ecology (NSF-CME) at Michigan State University where, working with Frans de Bruijn, I modified the *lux*-bearing transposon subsequently used in the last and most technologically sophisticated, phase of my PhD. This phase of my PhD and early post-doctorate work confirmed my interest in host-microbe interactions. I left the O’Gara group indebted to Fergal for the opportunities he provided me and knowing that my relatively uncommon experience of EU and commercial funding would be an advantage in a postdoctoral role.

Probiotics at Ireland’s National Food Biotechnology Centre

As an undergraduate, I had worked for a period at Chr Hansen Laboratories in the UK where John Lyne had introduced me to practical challenges of using lactic acid bacteria (LAB) in industrial production of cheese and yoghurt (I learned to detest plaque assays). However, by 1998, I was aware that LAB were being assessed for applications in clinical settings. Specifically, Fergus Shanahan had returned to Cork from Los Angeles and, with Gerry O’Sullivan, Kevin Collins, Gerald Fitzgerald and Charlie Daly, had established a screening program for LAB with potential health-enhancing properties isolated from resected human tissue. Emergence of probiotic research in the National Food Biotechnology Centre (NFBC) and later “pharmabiotics” at the Alimentary Pharmabiotic Centre, has already been comprehensively described. In 1998, I was considering a move to Switzerland when the NFBC provided an opportunity to build on my foundation of host-microbe interactions while also presenting me with the new challenge of clinical trial regulations and the complexities of IP management. I have a clear memory of my first meeting with Fergus Shanahan where he described his ambitions for probiotic research and discussed in very human terms the challenges (and the potential rewards and development opportunities) associated with realizing that vision. There was no contest. I emailed the Zurich group that day to say that I, like every loyal Corkonian, was going to stay at home.

Within a short time, we had demonstrated that selected orally-administered *Lactobacillus salivarius* strains could become established and persist in the murine intestine and, importantly, could be enumerated when excreted. We also completed a randomized controlled trial of lactobacilli, demonstrating effective delivery, transit and influence on microbial flora of healthy volunteers, and an assessment of probiotic strains in animal models of intestinal disease. In 2001, Tiina Mattila-Sandholm (then a Professor at VTT Biotechnology in Helsinki and has since had great
success at dairy giant Valio) secured European Framework funding for “PROUEALTH—The Food, GI-tract Functionality and Human Health Cluster” Gathering researchers from across Europe (64 research partners from 16 European countries in 8 projects), this initiative built on a previous EU-funded collaboration which had demonstrated nutritional functionality of probiotic foods and had proven the effects of probiotic cultures on intestinal microbiota and human health using properly controlled human clinical studies (the PROBIDEMO project). Having obtained this funding, we were then obliged to complete PROCID (Probiotics and gastrointestinal disorders—controlled trials of European Union patients) or suffer the wrath of the charismatic Finn. In actuality, the project was a tremendously educational and enjoyable experience. I have never again worked with as effective and collegiate a group. From our Cork base, the Shanahan team developed ethical approval applications, clinical trial protocols, case report documentation and product distribution and sample collection logistics for clinical teams in Ireland, Finland (Kuopio), the Netherlands (Wageningen) and Spain (Barcelona). As a result, probiotics strains, fermented to stable high numbers and distributed from Cork, were shown to persist in the colon of the Finnish ulcerative colitis patients who had consumed them, and, working with Arthe Von Wright, we were able to understand the mechanisms mediating this in vivo persistence. Subsequent work with Peter Kelly developed this considerably further, with a proteomic-MALDI-TOF analysis identifying the responsible probiotic cell wall-associated adhesins. By the end of 2001, I was part of a well-oiled team led by Fergus Shanahan that, like some others, had developed considerable expertise in microbial ecology of the healthy and diseased intestinal tract. I left this group to become General Manager of a cancer research center and, in hindsight, I now recognize the early signs of just how much potential the Irish Pharmabiotic Centre had.

Cancer Research: Mercy Hospital and BioSciences Institute

In dealing with European Framework grants, I had seen the required admin-istrative documentation evolve into extremely comprehensive consortia agree-ments that, arising from a “bang for buck” emphasis on outcomes and societal benefit, identified pre-existing knowledge and outlined models for foreground IP “exploitation.” I had also been involved as Alimentary Health Ltd (a spin-out company) partnered with multi-national industry and was familiar with the ben-efits and constraints associated with these relationships (non-disclosure agree-ments, etc.). Either because of, or due to these experiences, I was attracted when an early-stage research center dedicated solely to cancer research needed a General Manager to structure and coordinate its expansion.

The Cancer Research Centre (CRC) at the Mercy Hospital in Cork was established following successes by Gerry O’Sullivan, Kevin Collins and Fergus Shanahan in the areas of micrometastasis and, especially, high profile papers describing the apoptosis-related “Fas Counterattack,” whereby colon cancer cells resist T cell cytoxicity while themselves expressing an apoptotic death signal (ligand) to which activated T cells are inherently sensitive. By the time I was appointed, the Director, Gerry O’Sullivan, had identified novel method-ods of therapy delivery as a theme to be expanded, including genetically-enhanced bacteria. Gerry O’Sullivan was a force of nature. He passed away in early 2012 having been mentor to innumerable surgeons and scientists and a friend to many who had learned to dread any conversation that began with Gerry saying “I had an idea…” or “I went for a walk…” (which invariably meant that while walking an idea had occurred to him). His research activities had, in fact, included rescuing the tissue from which the aforementioned Cork probiotic strains had been isolated and it was as part of related work that I had met him. In 2001, the CRC was exclusively reliant on philanthropic generosity. The board, dominated by industry members, required more sustainable research fund-ing. Over the next three years, we secured competitive grants from the European Framework programs, Irish Health Research Board and Cancer Society and, crucially, with guidance from Ruth Davis (now with the Irish Higher Education Authority) we were able to access capi-tal funds from the Irish Government’s Programme for Research in Third level Institutions (PRTLI). The latter meant that the center could, to a large degree, relocate to customized laboratories in the newly constructed BioSciences Institute.

By this time, core research themes had matured. While apoptotic-related work remained important, industry-commissioned work (IP-driven) and European funding led to development of innovative medical devices (based on blood cell disruption by ultrasound or induction of tumor cell porosity via microneedles and/or electroporation) for gene and systemically-toxic drug delivery for cancer treatment. Much of this came about because of collaborations with colleagues in the Irish National Microelectronics Research Centre (now Tyndall National Institute), the UK (Les Russell and John Preston), France (Ilais Mir and Michel Marty), Denmark (Julie Gehl) and Italy (Ruggero Cadossi). By mid-2004, we had progressed innova-tions through laboratory and animal testing, had developed proprietary IP and had begun clinical trials in pallia-tive patients. Promising results pointed to further growth potential but the commercial world beckoned.

Beyond Research

Glanbia is a mid-sized multinational business that, in 2004, had expanded its strat-egy to include bioactives. This expansion involved establishing two core innovation centers (in Ireland and the USA) in addition to localization/product development teams in Nigeria, China, Germany and the UK. Known best as a producer of con-sumers dairy products and as a business technology (2B) supplier of cheese and dairy-derived ingredients, the Nutritionals
businesses were tasked with enhancing revenues by diversifying from commodity products. As Director of Research for the Nutritional EMEA businesses I had responsibility for technical due diligences associated with company acquisitions, licensing and acquisition of novel products and technologies, IP management and, in many cases, proof of concept studies (often clinical trials) and business development related to new high margin boilerplate delivering anti-hyperpertensive, anti-hypercholesterolemia, anti-inflammatory, body composition and human and animal performance benefits. For confidentiality reasons I am unable to describe these activities and, so, will instead concentrate on two aspects of the role that particularly interested me, (1) challenges of open innovation and (2) establishment of Food For Health Ireland.

Open innovation has been promoted as a process by which commercial groups "can and should use external ideas as well as internal ideas and internal and external paths to market, as the firms look to advance their technology." Although collaboration, particularly between universities and companies, predates this concept, open innovation has proved a remarkably successful source of new products and technologies for many companies. Of these, Procter and Gamble’s (P&G) Connect and Develop™ (C&D) corporate innovation platform is probably the best known and, based on the success and growth of C&D, many companies and inventors/innovators have been attracted by the potential benefits of this new way of working. Today, C&D and open innovation are associated with short-termism and inadvertent slowing of the pace of innovation, which is evident when internal R&D capability is reduced in companies and research spending is linked to immediate rather than sustained profit (i.e., incremental development rather than breakthrough research).

As research champion in Glanbia, I began to see many examples of how open innovation could be suitable for large resource-enabled multinational companies (MNC), but ill-suited to small and mid-size companies. To my mind, open innovation assumes incorrectly that the partners involved (innovators and industry) possess the capabilities and capacities necessary to: (2) identify and appropriately protect innovations (IP); (3) possess the capabilities and capacities necessary to: (4) communicate the characteristics/benefits of the innovations to potential buyers (innovators and industry); (5) understand what is being offered by innovators (industry); (6) recognize the regulatory requirements, market potential and level of investment required to pilot and scale-up manufacture (innovators and industry); (7) understand that innovations may occasionally be sourced from larger MNCs when the scale of the opportunity or market position, despite investment and development, no longer fits implementation of their strategy and they wish to divest (industry).

Simply put, it was clear to me and counterparts in competitor companies and Irish Government agencies that, for “Ireland Inc” to exploit the considerable research infrastructure of Irish research institutes and universities, collaborative funding and sharing of expertise would be required.

Following extremely lengthy dialog between the companies involved (Glanbia, Dairygold, Kerry, Carbery) mediated by Enterprise Ireland (Paul Roben and John Mulvihill) and review of work-plans submitted by academic researchers, Food for Health Ireland (www.fhi.ie) was established in 2009. As this was an industry-led research initiative to accelerate mining and testing of milk-derived bioactives for eventual commercial use, considerable emphasis was placed on avoiding challenges associated with State Aid rules, provision of opportunity for additional industry involvement and appropriate management for commercializing any foreground IP that might result from the funding. Direct plus overhead contributions to the research institutes, combined with industry investment, provided more than €20 million in research support and, in 2009, represented the largest investment made by Enterprise Ireland in an R&D consortium since its launch in 1998. At the time of writing this initiative continues and second round funding is anticipated.

Conclusions

In late 2009 I was appointed Chair of Research at the Graduate Entry Medical School at the University of Limerick, Ireland. We have, since then, recruited professional researchers across the principal medical and surgical specialities and have established the Centre for Interventions in
Infection, Inflammation and Immunity—
known as 4i (www.4i.ie)—to provide the type of supports detailed above to col-
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