There is no question that the work undertaken over the last six years has added significantly to global knowledge of food structuring and how food interacts with the human digestive system. Our discoveries will be invaluable as we move to a future where consumers become more conscious of the effects of different kinds of food on health.

Our PhD students, about half of whom have now graduated, are well equipped to deliver skills to develop the next generation of food. In this issue we feature two of our students who have just graduated, who were based at AgResearch (Grasslands, Palmerston North, and Ruakura in Hamilton). They have expressed their enthusiasm for the experience of being able to be based at a Crown Research Institute, while also being within the university’s ambit. The two cultures are quite different, but the CoRE model has allowed integration, where scientists from both organisations can work together effectively.

Early next year we plan to again hold an agrifood summit meeting, where we exhort industry leaders and government officials to develop and manage a strategy to capitalise on the burgeoning opportunities for high-value science-driven foods, particularly in the Asian economy. New Zealand has a comparative advantage in agrifood production and our science must be used to ensure the highest return on those primary products. The summit meeting is entitled “The Asian Century”. We must be mindful of the huge and rapidly growing market for protein and functional foods in Asia and how best to appeal to those consumers.

This is the last Riddet Review for 2013. We wish you all a happy and safe festive season.

Paula McCool
Communications Officer
In November, the Riddet Institute hosted Ritu Sehji, a Royal Society Endeavour Scholarship winner, and head of food technology at Diocesan School for Girls. As part of Ms Sehji’s scholarship, she is spending six months at Goodman Fielder, and also spent time visiting the Riddet Institute headquarters in Palmerston North to gain an insight into fundamental research being carried out to understand food structures.

Ms Sehji said, “Discussing the course outlines with staff at the Riddet Institute and IFNHH (Institute of Food, Nutrition and Human Health at Massey University) has encouraged me to think about the strategies and activities I could implement when I am back at school to make the transition smoother for students passionate about taking food technology at university.

“It was a pleasure to visit the Riddet Institute (Centre of Research Excellence) which is a premier centre for research that underpins development of health promoting innovative food products. Visiting the labs and facilities gave me an opportunity to look at the research that is being undertaken, resources available, career prospects and pathways available to students beyond the food technology course at school.

“My experience will enable me to be more effective and make a more positive difference in the classroom and community by understanding course expectations at university, to plan intended outcomes for student learning.”

Riddet Institute Principal Investigator Gerald Tannock, of the University of Otago, has been awarded a James Cook Research Fellowship by the Royal Society of New Zealand. The funding will allow him to concentrate for the next two years on research entitled: “A path to understanding bowel bacteria”.

A description of the research follows: “The large bowels of humans contain trillions of bacterial cells belonging to hundreds of species that form self-regulating communities known as the microbiota. These collections of bacteria have the capacity to chemically transform digestion-resistant-carbohydrates and other polymers present in the digesta. The aim of the programme is to develop ways to experiment with mixtures of bacteria that live in the human bowel. Physiological measurements of specific bacteria in pure and co-culture in laboratory microcosms will be made to determine the nutritional drivers of microbiota composition and function, especially with respect to the little studied bacterial family Lachnospiraceae. The basic science generated by this approach could be translated to technology (problem solving) with respect to the development of foods and prophylactic supplements that would contribute to sustaining life-long health. Also critical to translation of basic science to technology is the derivation and dissemination of an updated conceptual view of human bowel ecology. The proposed programme thus encompasses laboratory research and science communication and has the overall aim of providing a path to understanding bowel bacteria.”

The James Cook Research Fellowships are awarded to researchers who have the requisite qualifications and experience and are able to demonstrate that they have achieved national and international recognition in their area of scientific research.
Promotion to Associate Professor

Dr Shane Rutherfurd has been promoted to Associate Professor by Massey University, effective 1 January 2014.

Dr Rutherfurd is an Associate Investigator within the Riddet Institute and has demonstrated international science leadership at the highest levels. In particular he has achieved excellence through his research outputs and postgraduate student supervision.

He has also added to the Institute’s reputation for successful collaborations with leading international universities including Wageningen University, The University of Illinois and University of California (Davis). Dr Rutherfurd was awarded his PhD at Wageningen University, receiving the highest grading for both the dissertation and defence.

Gold Medal Award for Professor R Paul Singh

Professor R. PAUL SINGH, Riddet Institute Principal Investigator, is the recipient of the ASABE 2013 Massey-Ferguson Educational Gold Medal Award in recognition of his exceptional leadership and achievements as a world-renowned educator and researcher in developing innovative curricula and educational resources in food engineering.

Singh, Distinguished Professor, Department of Biological and Agricultural Engineering, University of California, Davis, is a recognised world leader in food engineering research and education, who has helped to establish and improve food engineering programmes at universities in the US and developing countries. He has dramatically advanced the understanding of structural and functional relations in foods, transport phenomena in food processing, and quality changes in foods during distribution and storage.

At UC Davis, Singh has developed seven undergraduate courses, which include a course in heat and mass transfer in food processing. He has developed graduate courses covering cutting-edge advances such as modelling food digestion in the human stomach. Through three highly competitive USDA grants, Singh enhanced teaching materials with the establishment of online “labs” that allow students worldwide to investigate “what-if” scenarios for process variables that, because of time constraints, cannot be considered in actual lab sessions. He has developed more than one hundred animations of equipment and engineering concepts and made them available on his website, a material-rich resource that has remained the top non-commercial food engineering site on Google for many years. Singh has been a primary advisor and guider of research for 25 PhD and 53 MS students. He has provided leadership in helping to establish and make improvements in food engineering programmes at other US universities, as well as universities in numerous countries worldwide.

Singh has authored or coauthored more than 460 refereed-journal articles and technical publications, 40 book chapters and 16 books, along with related computer software and internet resources. Of his 16 books, Introduction to Food Engineering is considered a standard throughout the world and is available in four languages. He has planned and directed three international workshops on food processing with the support of the World Bank and NATO. He has held consulting assignments with the United Nations Food and Agricultural Organization, U.S. National Academy of Sciences, UNESCO, USAID and NASA, and he is in frequent demand as a speaker at international conferences and organizations.

He was elected a Fellow of ASABE in 2000 and has been a member of that organisation for 39 years. He is also a fellow of the International Academy of Food Science and Technology, the Institute of Food Technologists, and the US National Academy of Engineering.
Tim Angeli, a Riddet scholar at the University of Auckland’s Bioengineering Institute, was presented with the ‘Distinguished Young Investigator Award for the Best Clinical Presentation’, by the American Neurogastroenterology and Motility Society at their annual conference/scientific meeting in Huntington Beach, California, in September.

Mr Angeli was initially selected as one of 15 participants for a young investigator forum as part of the conference, which consisted of early-career development and mentoring from a number of international academics in the motility field, as well as research presentations from each of the young investigators. He also presented his work in one of the full-conference sessions on emerging concepts in gastrointestinal disorders. The presentation was titled: ‘High-Resolution Mapping Reveals Gastric Slow Wave Dysrhythmias in Chronic Nausea and Vomiting.’

Mr Angeli received a Riddet Student Travel Award to attend the conference and present this work.

Mr Angeli recently also gave an invited presentation at the 2nd Annual Food Structure, Digestion, and Health Conference that was held in Melbourne, Australia, in October. That presentation was titled ‘New Insights into Gastrointestinal Function through High-Resolution Bioelectrical Mapping and Multiscale Computational Modelling’.

After submitting his thesis some months ago, Mr Angeli is now waiting for the reviews and thesis defence. Originally from Michigan, USA, he plans to stay in New Zealand to continue his academic career with the gastrointestinal research group at the Auckland Bioengineering Institute as a post-doctoral fellow.

Professor Manohar Garg of the University of Newcastle, Australia, has been awarded the 2013 Nutrition Society of Australia (NSA) Fellowship, which was presented at the 37th Annual Scientific Meeting of the NSA in Brisbane, December 5-8, 2013.

The criterion for election is that “the candidate has made contributions of special merit to the scientific study of nutrition and/or its applications to people and animals.”
Riddet provides a fertile background for many PhD Fellows

The Riddet Institute CoRE provides a fertile background for many PhD Fellows to pursue high quality, cutting-edge research. It is a partnership that AgResearch is proud to be a member of and it has provided the organisation many opportunities to host high quality PhD Fellows. Drs Amy Van Wey Lovatt and Dulantha Ulluwishewa are two such examples. Both are Riddet Institute PhD Fellows who have worked at AgResearch and who were awarded their PhD degrees in September 2013. Both Amy and Dulantha developed and validated novel models that contribute to advancing our understanding of how the foods we eat interact with our gastrointestinal tract.

Amy's PhD focused on how nutrients are released from food particles during digestion in the gastrointestinal tract. Specifically, Amy developed and validated mathematical models that describe (1) soluble particle loss during gastric digestion at various pH levels, (2) the growth of bacteria in monoculture from the degradation of sugars and the resulting by-products, (3) preferential degradation of sugars by bacteria, (4) nutrient competition and cross-feeding between two bacterial populations and (5) nutrient transport within bacterial biofilms. Amy's thesis was judged by her examiners as an original and comprehensive body of work that made a significant contribution to our knowledge in the field. As a result, Amy's thesis made the Dean of Graduate Research School's 'List of Exceptional Theses'. Amy, an Earle Food PhD Fellow, was mentored by Professor Warren McNabb, Dr Paul Shorten, Dr Nicole Roy, Dr Adrian Cookson, Dr Tanya Sobolova and Professor Paul Moughan.

Dulantha's PhD study focused on exploring the cellular and molecular interactions between anaerobic intestinal bacteria and human intestinal epithelial cells. Dulantha developed and validated a novel experimental chamber to co-culture aerobic human intestinal epithelial cells and anaerobic bacteria, mimicking the gastrointestinal microenvironment. This model will allow researchers to directly measure bacterial-epithelial interactions and gain new insights into a major area of physiology. Along the way, Dulantha received awards for his ability to communicate his research. He was the runner-up for the Massey University 3MT competition in 2011 and won the best poster at the 3rd TNO Beneficial Microbes Conference (which highlighted advances in understanding how endogenous bacteria influence health and disease), The Netherlands in 2012. Dulantha's work provided the preliminary data that enabled Dr Rachel Anderson to secure a Marsden Fast-Start grant. Dulantha was mentored by Professor Warren McNabb, Dr Nicole Roy and Dr Rachel Anderson, Professor Jerry Wells and Professor Paul Moughan.

Amy’s and Dulantha’s PhD journeys epitomise the dedication, resilience and excellence that is needed to be successful in science. The Riddet Institute CoRE funding enabled them to have the opportunity to shine and to demonstrate the talent they clearly both have.

Mathematician explores the large bowel

Amy Van Wey Lovatt has just graduated with her PhD from Massey University. She looks back on her PhD project and says she thoroughly enjoyed it.

“I came out to New Zealand from the USA, where I was lecturing at a community college in Oregon. The financial crisis meant that jobs in education were under threat and there was a lot of insecurity. I had always wanted to live in a foreign country so applied to do PhD study in New Zealand.”

For her PhD project, Dr Van Wey Lovatt was engaged in mathematical modelling of the human digestion process – part of the Riddet Institute’s wider work investigating what happens to food inside the gastrointestinal tract. Her work described how nutrient loss occurs during digestion and how intestinal bacteria break down food particles.

“I first looked at the way the stomach
Mysteries of the human intestine unravelled

The human intestine has an estimated surface area of 200-400 m², which is continuously exposed to large amounts of food, chemicals, bacteria, and potentially harmful antigens. Because of this exposure, it has an important barrier function in addition to its role in food digestion and nutrient absorption. A well regulated barrier is essential for health because it prevents unwanted compounds from entering the body, and controls activation of the immune system.

Dr Dulantha Ulluwishewa, who graduated at the end of November 2013, studied the intestinal barrier. His PhD was funded by AgResearch as part of the Riddet Institute Centre of Research Excellence. In particular he focused on commensal bacteria, of which there are approximately 100 trillion (collectively referred to as the microbiota) residing in the intestine. These bacteria interact with the human intestinal cells at the molecular level, and influence various aspects of intestinal barrier function.

The majority of published research investigating intestinal host-microbe interactions focuses on oxygen-tolerant bacterial species; however, over 90% of commensal bacteria are obligate anaerobes, meaning that they cannot survive in the presence of oxygen. This represents a significant knowledge gap and, according to Dr Ulluwishewa, “This knowledge gap is due to the technical difficulty in co-culturing oxygen-requiring human intestinal cells with oxygen-intolerant obligate anaerobic bacteria.”

Dr Ulluwishewa’s research focussed on the development of an apical anaerobic model of the human intestinal barrier that allows human intestinal cells to be co-cultured with obligate anaerobes. Using a custom built dual-environment co-culture chamber he grew human intestinal cells in an environment without oxygen. These cells received oxygen for survival from a separate compartment of the chamber, mimicking the cells in the intestine which receive oxygen and nutrients from the underlying capillaries.

His research showed that the intestinal cells remained viable and maintained an intact barrier in the apical anaerobic model. He found that over time the expression of genes within the intestinal cells changed, allowing the cells to adapt to the lower supply of oxygen. Having established that the apical anaerobic model was suitable for its intended application, he demonstrated its usefulness using the obligate anaerobe Faecalibacterium prausnitzii.

F. prausnitzii is usually an abundant member of the human commensal intestinal microbiota, but has substantially lower prevalence in patients with intestinal disorders. Previous studies have suggested that this bacterium has a protective role in the intestine, and that the bioactive compounds responsible for this protective effect are produced only by live bacteria. However, being an obligate anaerobe, F. prausnitzii cannot survive for more than two minutes under conventional conditions. Dr Ulluwishewa discovered that the survival of F. prausnitzii improved greatly in the apical anaerobic model. He compared the effects of both live and dead F. prausnitzii on intestinal cells, and showed that only the live bacterium was able to alter the permeability of the intestinal barrier. Furthermore, live F. prausnitzii cells were able to alter the gene expression of intestinal cells in a manner than reduced inflammation in the intestine more than dead F. prausnitzii cells.

Most conventional models of the intestine do not allow live commensal obligate anaerobes to be co-cultured with viable intestinal cells. However, it is clear from this research that live and dead bacteria can have very different effects on the intestinal cells. This illustrates the importance of the apical anaerobic model – because the bacteria stay alive in this model, it is likely to give more accurate insights into their role in intestinal barrier regulation. Dr Ulluwishewa’s model will assist in the development of strategies to improve intestinal barrier function using food.

Dr Van Wey Lovatt’s model of how particles degrade food particles, the food/human interaction. I then looked at how bacteria in the large intestine break down food particles. So there is a three-way dynamic involving food, bacteria and the host (or human body)."

“I also did some lab work on the differences between the digestion of carrot and cheese and the role of acidity in the stomach in digesting these different particles.”

“Most nutrients are absorbed in the small intestine, but some particles (mainly plant-derived material) go through to the large intestine. These particles are left over to be broken down by bacteria.”

Dr Van Wey Lovatt’s model of how particles are broken down by acids is the first accurate model of its type, and clearly shows the reasons why the mechanism works as it does. Through a modelling approach, she was also able to show that nutrients within bacterial biofilms (slime-covered communities of bacteria attached to a surface) move at different rates within the biofilm.

“I discovered that the rate of nutrient transfer in biofilms is slower than people assumed and is highly dependent upon the distribution of the bacteria.”

During her study she was based at AgResearch in Ruakura, near Hamilton. She is a strong advocate of the Centre of Research Excellence model that allowed her to work with people in a Crown research institute, and also interact with other students and supervisors at a university.

“As a student within AgResearch, I was treated as a colleague by staff. It was quite good being outside the university environment on a day-to-day basis, but still connected with it.”

Dr Van Wey Lovatt had six supervisors: Drs Warren McNabb, Paul Shorten, Nicole Roy, Adrian Cookson (all AgResearch), Tanya Soboleva (MPI) and Distinguished Professor Paul Moughan (Massey University and Co-Director of Riddet Institute).

As for the future, she is optimistic. “Of course I want to stay in New Zealand, not least because I met and married my husband during the course of my PhD and he is a Kiwi.”
Shenshen Zhang, a PhD student from Nanchang University in China, has recently joined the Riddet Institute for a 12-month internship. Her PhD in China is exploring the biological activities of polysaccharides extracted from natural sources, with a focus on their immune-supporting and anti-cancer activities. While in New Zealand, she will work on a related project, providing her with an opportunity to develop new skills and use new methods. Shenshen will be working on a natural extract developed by Alpha Group, a New Zealand company with expertise in supplements based on natural medicine and supported by science.

The Riddet Institute has secured $200,000 in funding from MBIE over two years to develop probiotic foods for India.

Riddet Institute Co-Director Distinguished Professor Harjinder Singh said, “Our aim is to develop probiotic products containing bacterial strains of Indian origin with clinically-proven health benefits that are shelf stable under the climate conditions of India. We will be working with the highly prestigious National Dairy Research Institute (NDRI) in Karnal, India and so will further strengthen our existing relationship.”

Probiotics are defined as live micro-organisms that confer a health benefit on their host. Probiotic bacteria have been successfully applied in the treatment and prevention of diverse gastro-intestinal diseases and for improving immunity. In many parts of India poor drinking water quality and other environmental factors compromise the immune and digestive health of many segments of the population, especially infants in the rural sectors.

While the market for probiotic products in developed countries is well established, it is at a nascent stage in India primarily because of two major limitations. Evidence of inherent differences in the gut microflora of the Indian population has been suggested to occur stressful the need for selecting bacterial strains of indigenous origin with clinically-proven health benefits specifically tested on the Indian population. The other barrier to market growth is the tropical climate and poor cold chain distribution in most Indian regions that restrain probiotic fortification to shelf stable foods making survival of the temperature-, and humidity-sensitive, probiotics a major technical challenge.
Dr Thierry Astruc and Dr Véronique Santé-Lhoutellier, internationally recognised for their work on meat biochemistry, visited the Riddet Institute in late November.

The project will develop tools and prototype processes and products that will allow the meat industry to produce new types of meat products.

Dr Mike Boland, from the Riddet Institute, said: “We are investigating the effects of ‘new to New Zealand’ mild processing technologies, including high pressure processing (HPP), and sous-vide cooking, as well as conventional cooking, and their combinations on meat microstructure and protein digestion.

“We have a particular interest in exploring HPP and the sous-vide cooking method, and combinations thereof, for enhancing the texture and protein digestion of meat foods,” he said.

Sous-vide cooking takes place in vacuum sealed plastic pouches or trays at lower than normal cooking temperatures for extended times. By manipulating these processes, it should be possible to target specific proteins in the meat to improve tenderness and digestibility.

“Cooking of meat at high temperatures can result in protein oxidation through the generation of oxygen free radicals, which can lead to oxidation of amino acids, leading to altered meat protein digestibility after cooking,” said Dr Boland.

“The sous-vide and HPP meat products are an interesting alternative to diversify the range of ready-to-eat products developed from New Zealand meat, by adding value to low-value cuts of meat.”

The complexities of meat microstructure and its role during gastro-intestinal digestion of meat protein will be investigated by combining the complementary strengths of the New Zealand and French teams, which are among only a few groups working in this area globally.
Crisis leads to new study path

When Jane Mullaney’s husband died of bladder cancer at age 49, his death was to have a profound impact on the direction of her future university studies.

Ms Mullaney was completing a Master’s degree at Massey University at the time, in microbiology. As she had very good grades, her supervisor, Professor Bernd Rehm, encouraged her to study further and to look for a PhD scholarship. While she was interested in pure microbiology, the impact of her husband’s death prompted her to think more about using science to promote health, and in particular, what effect food might have on the human body.

When a Riddet Institute PhD scholarship was offered, Ms Mullaney took the opportunity to step out of pure science into the world of food and nutrition research. “It was an area I knew nothing about, but events had jolted me into an awareness of illness and whether I could do anything to contribute to finding ways of helping,” she said.

Ms Mullaney’s PhD project examined the biotransformation of glucosinolates, from a bacterial perspective. Glucosinolates are compounds containing nitrogen and sulphur found in brassica plants, such as broccoli.

“I suppose if I was to put the results of my PhD research into one sentence, I would say that probiotics, along with the bioactives from broccoli, increase the levels of protective enzymes in the bladder.”

Her research examined the way glucosinolates convert into bioactive compounds, which in turn stimulate a host response involving detoxification pathways. Conversion of glucosinolates is catalysed by the enzyme myrosinase, which is co-produced by the plant. Myrosinase activity can be reduced or lost during storage of vegetables and is often inactivated by cooking. Bacteria, too, are capable of carrying out a myrosinase-like activity on glucosinolates.

Ms Mullaney says there is an association between the consumption of cruciferous vegetables and reduced cancer of the colon, bladder and bowel. And while this is just an association, I have demonstrated that bladder tissue was affected by beneficial bacteria and glucosinolates alone, or together, which suggests that both exert a protective effect. This was measured by elevated quinone reductase, a biomarker for cancer chemoprevention.”

Ms Mullaney says that the stresses of doing a PhD are underestimated by many students. “You have to be really passionate to keep going. It sucks the life out of you. You cannot do it in isolation. I found the support of the people I worked with kept me going and the opportunity to work with experienced scientists and new technologies at Plant & Food Research, AgResearch and Massey University (all Riddet Institute partners) was a strong motivator.

The next step in Ms Mullaney’s career path will be in Australia.

“Naturally, I was passionate about what I was doing, and other events just reinforced the way my career was heading. My PhD took me three years and over that time I remarried, and had other family worries associated with various illnesses. Last year my father died, diagnosed with dementia just months before, just as I was finishing my thesis. My PhD oral exam was delayed when one of my examiners got appendicitis. All these events, and other major health issues my husband experienced after a fall from a roof, reinforced my passion, which was to find out what promotes human health.”

In June Ms Mullaney was interviewed for a post-doctoral position with the University of Queensland’s Diamantina Institute (UQDI), which is partnered with the Translational Research Institute combining several research institutes dedicated to discovery and development of treatments and therapies to improve health for people worldwide. UQDI conduct research on autoimmune diseases and cancer. “My work on gut microbiota was a real fit with what they were looking for which was to study the link between genes, microbes and the autoimmune disease type I diabetes. UQDI has world-class bioinformatics, proteomics and high throughput gene sequencing facilities. It is a great opportunity.”

Ms Mullaney took up her role on November 4 but says that she is committed to returning to New Zealand. “This is my country and I want to put back something in return for all the first-class training I have been lucky enough to have had.”
Scholar returns home to assistant lectureship

Riddet PhD scholar Charith Hettiarachchi has completed the requirements for his PhD at the University of Auckland and will graduate in May next year. He has returned to Sri Lanka, where he has a position as an Assistant Lecturer at the University of Peradeniya, the university where he gained his Bachelor’s degree.

His PhD project looked at what happens when milk protein nanofibrils meet carbohydrates from plant cell walls. The complex interactions between proteins and carbohydrates are very important contributors to the different structures in foods.

Mr Hettiarachchi discovered that under certain conditions, some proteins form structures called nanofibrils, very small fibres or filaments that are measured on the scale of billionths of a metre. There has been limited investigation of how nanofibrils and carbohydrates interact and he, with others, decided to explore these using β-lactoglobulin (the main protein in the whey fraction of cow’s milk) and pectin (the carbohydrate which gives many plant cell walls their structure). Pectin is also widely used in the food industry for gelling, thickening and stabilisation.

Results of his research showed that the type of complex formed is dependent on both the negative charge density and the distribution of these negative charges within the pectin molecules.

The group Mr Hettiarachchi worked with is now considering how the structures of the different complexes may be used, both in food and non-food systems.

Whey protein strongly linked to satiety

Riddet Institute scholar Dr Sylvia Chung Chun Lam graduated from Massey University at the end of November. Dr Chung’s work investigated whey protein and satiety in humans and her findings supported the use of dairy whey protein as part of a diet to stimulate satiety.

Dr Chung said that knowledge of satiety is important for the treatment and management of obesity. For her PhD she demonstrated that adult humans’ consumption of dairy whey protein resulted in lower subsequent food intake and reduced appetite when compared to consumption of carbohydrate. Moreover, her study of peripheral hormones and metabolites showed that amino acids and pancreatic polypeptide hormone may be important mediators.

Dr Chung also sought to uncover the underlying reasons for the stronger satiating effect of whey protein. The intakes of individual whey protein components and a dietary mixture of free amino acids simulating the amino acid composition of whey protein afforded the same satiety effect as whey protein. This suggests that the action of a single amino acid or group of amino acids may be the causative factor.
Continuing the dialogue

*the Asian century*

A CONTRIBUTIOAN TO A NATIONAL AGRI-FOOD STRATEGY FOR NEW ZEALAND

The Riddet Institute summit meeting will discuss the considerable potential of the agri-food sector in this, the Asian century. Leading influencers will discuss this opportunity.

Wednesday 19th February
Wellington

Enquiries to Ansley Te Hiwi, a.tehiwi@massey.ac.nz

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