



Newsletter

N°3 – August 2010

Science enlightens us with the real-life chronicles; it tells us the story of exploration, perception, discovery, creation and/or finding the ways to make it so. Science is not only unlocking the potentialities but also giving birth to a new dimension of research.

Each and every sort of scientific or creative work can contribute to mankind. Antonie van Leeuwenhoek, Louis Pasteur, Gregor Johann Mendel- all the legendary scientists have opened the enormous potentiality of research and proven their contribution to the world. But the world is not resting! It keeps rolling and rolling and thus arising new problems which demands more and more research and submerging of multidisciplinary research sectors. When areas of multi-disciplines correspond, it is likely a new dimension will arise. Thus, the emergence of collaborating research comes out. Europe always plays a significant role in such type of research collaboration and keeps contributing to our world. 'Cross-Talk' is a collaboration of research groups under the platform of 'Marie-Curie Actions'- a leading research facilitator of European Commission. Cross collaboration of research pioneers and training for a new generation of young scientists are the core objectives of Cross-Talk. The group has been focusing on the relationship between gut microbiota and human intestinal tract.

Just after the birth of a child, its gastrointestinal (GI) surfaces are rapidly colonized by waves of microbes. A mature mammal's large intestine is colonized by approximately 10^{15} bacteria which comprise more than 1000 species! So unlocking the story of this relationship would really be interesting and would definitely contribute to medical science as well as humanity. The research group has been working on different

sub-categories such as Molecular mechanisms between commensals and host, Role of the host-microbiota interplay, In-vivo validation in animal models and alternative human-derived model and so on. Cross-talk is also connected with other groups to obtain and share knowledge on recent achievements on this area of research and for future guidelines. It is in collaboration with MetaHIT for the "International Conference on Human Metagenomics" (Shenzhen, China 2010) which was a part of this loom. Moreover, it has direct communication with different companies and industries (i.e. DANONE, Nestlé, LABIP, INSERM, CIR) to pursue an effective affinity with practical research approaches. World research is a Network; scientists are communicating throughout the world via research channels which are floating through Asia, Europe, Africa, America...By this way the world is connected with a string and we should make our string stronger.

The 'News-letter' focuses the update of the research groups, recent scientific activities throughout the world, special comments and opinions of the experts, thoughts of the fellows, information of some scientific tools, applications and miscellaneous approaches.

Being one of the trainees of this project, I believe I am in the right direction under the shade of this research platform. We, the young scientists of this project, hold our hands together to serve this world and hope our tiny efforts could make this world a better place to live!

- Rejoanoul Islam

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Conference in Shenzhen, China – March 2010



Meta HIT meeting was held from 1-3 March 2010 in Shenzhen, China and 4th of March was dedicated specially for Cross Talk group. The idea of visiting the far East land of China was quite intriguing for all of us. Most of us arrived in Hong Kong and were taken to our respective hotels after clearing Immigration and Customs initially of Hong Kong and then of Shenzhen, China.



On 1st March after a grand lunch we started with a warm official welcome from the Governor of Shenzhen, followed by an introduction by Dr.Christian Desaintes of European Commission. After this we had a series of talks given by speakers invited from Scientific Institutes all over the world. The sessions were divided under the broad categories of Microbial Composition, Microbial Profiling, Data Organization and Analysis and finally Clinical studies. It was indeed great to know about the leading technologies like 'single cell sequencing' and the various ideas about analyzing the huge amount of data generated by highly efficient techniques of sequencing 16s RNA and the study of Metagenomics on the whole.

On 2nd March after dinner we visited Beijing Genomic Institute (BGI, Shenzhen) to see their highly equipped laboratories and strict lab practices and achievements.

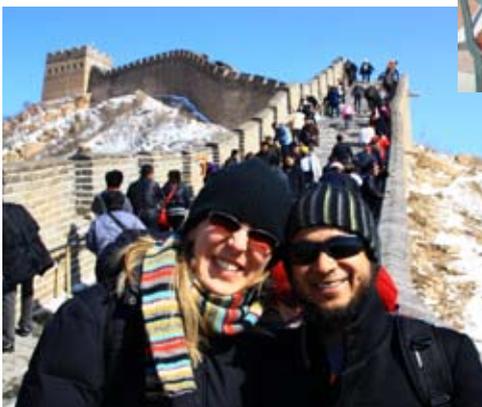
4th March we gathered the 18 Cross Talk fellows, their supervisors, researchers in the field of metagenomics and 8 young researchers who were awarded a fellowship to attend the MetaHIT meeting. We divided ourselves in groups with a supervisor and tried to analyze the MetaHIT meeting, which aspects were useful for our area of work, various impressive presentation styles and other perspectives were very helpful for most of us. We later presented each group-wise report to others and most of the fellows

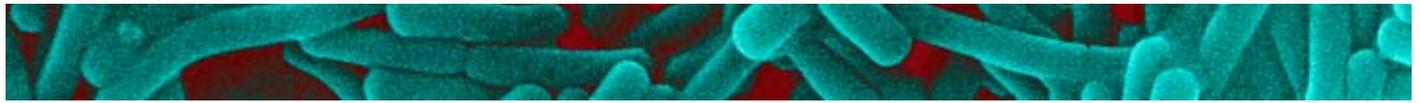
unanimously approved of talk given by Dr.Oluf Pedersen (Hagedorn Research Institute, Denmark) for the impressive presentation, Liping Zhao (Shanghai Jiao Tong U, China), Francisci Guarner (Hospital Vall d'Hebron,Spain), Janet Jansson (Lawrence Berkeley Natl.Lab,USA) for the contents. In the afternoon we also presented our posters to two groups of supervisors and later a joint decision of fellows and supervisors for the poster prize of 300 euros was given to Ms Sahar El Aidy of Wageningen University, The Netherlands.

Lastly I would like to add that BGI, Shenzhen proved a very efficient host in regards to hospitality and organization and management of the entire event.

The next meeting is in Debrecen, Hungary from August 30 to September 4,2010, where other than the routine dissemination of the various Crosstalk projects in the form of talks given by the fellows, the idea of Team Development by Jan Vaesen will be discussed, explained and analyzed. Also the bio-psycho-social approach to build resilience with PhD students is a much awaited session among many other activities and talks like the First EU auditing of Cross Talk project. So looking forward to a week full of activities in the bright and sunny summer of Hungary.

– Arunima Chatterjee.





An Interview with.... Professor Harry Flint



Professor Harry Flint was an invited speaker at the first international Metahit conference on human metagenomics held in Shenzhen last March 2010. As director of the gut division of the Rowett Institute of Nutrition and Health in Aberdeen, UK, his career has been dedicated to the study of the commensal microbiota. His talk entitled "Microbial metabolism in the gut: functional groups within the microbial community of the human colon" reported a functional study intended to evaluate changes of the composition of the microbiota under various dietary conditions. He kindly accepted to answer a few questions.

1. The Metahit consortium has just published its first paper in the journal Nature, where they reported having sequenced 3 million bacterial genes (microbiome). Could you tell us how this massive data will be analysed? Do you believe computing-assisted work will replace bench laboratory work?

Answer: This first report by Metahit is an important development for human metagenomics. It is worthwhile noting that this study could not have been interpreted without the complete sequences of reference microorganisms that the scientific community has accumulated over the past 30 years. It is clear that the larger the number of available bacterial genomes, the more information can be obtained from such studies. With the developments in new sequencing techniques such as 454 pyrosequencing and Illumina, it is getting cheaper to sequence and hopefully more bacteria genomes will be available soon. I think the computing work is important but it will never replace the bench laboratory work which is why functional approaches are also included within Metahit including the measurement of substrate breakdown activity. In addition to massive genome sequencing, Metahit also includes the single cell sequencing approach and last but not least, Metahit brings together experts in various disciplines including gastroenterology and metabolic syndrome with interest in the dynamics of microbial community analyses.

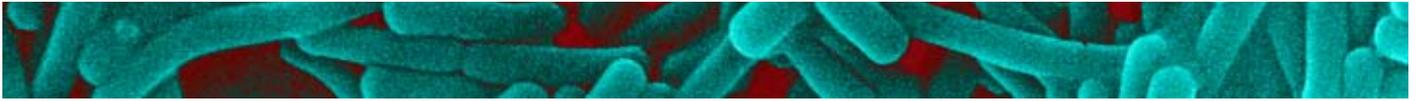
2. You have been working on the characterization of colonic bacteria which has seen many crucial advances, both in methodology and knowledge in the field of microbiology. Could you summarize this evolution, and tell us what will be the major contribution of the microbiology field applied to human health over the coming years?

Answer: When I started, knowledge of human microbiota was lacking due to technical barriers associated with culturing and maintaining anaerobic microbes *ex vivo*. However, molecular profiling techniques have been developed and have resulted in increasing knowledge of the human gut microbiota as we became able to monitor specific groups of bacteria and also how they interact with the immune system. Also, improved technical expertise in culturing and imaging techniques such as FISH added further developments to this field. Butyrate producing bacteria are one important group of beneficial bacteria and it is estimated that 75% are known. I think that the future of the discipline will be discovering diet, age-related and individual-related changes in microbial composition, together with the discovery of new probiotics and prebiotics.

3. You recently published a paper stating anaerobic butyrate-producing colonic bacteria may be used as a probiotic for the treatment of IBD. Could you tell us more about this new challenge and the mechanisms underlying this choice?

Answer: Butyrate-producing bacteria have an important role in the human intestine. In addition to influencing cellular proliferation, differentiation and apoptosis of cancer cells, they exert anti-inflammatory properties in the intestine. Hence, these anaerobic butyrate-producing colonic bacteria could be useful for certain individuals of the human population with inflammatory conditions. Much work is required to optimise formulation and delivery of such bacterial products. The commercialization could utilise encapsulation of these bacteria either alone or in combination with other probiotics to stabilize the product but this is still under research. Another probiotic option involves the use of lactate-producing bacteria (e.g. *Eubacterium hallii*) and to establish that these bacteria are safe, through genomic analysis and by testing in animal models.

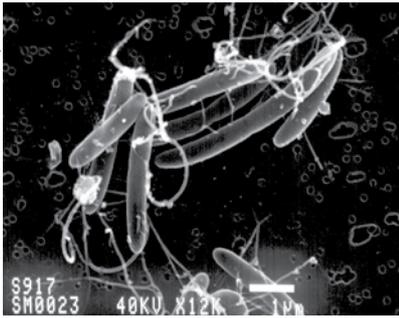




Roseburia intestinalis →
Eubacterium rectale

Faecalibacterium prausnitzii

Anaerostipes coli



4. *The population of microbes in the intestine is estimated between 500-1000 different species. What's your estimation and why? Is it possible to estimate the colonic bacteria which remains to be sequenced?*

Answer: Sincerely, I don't have a number to give you as it is dependent on how deep the sequencing goes and the number of lower abundance bacteria identified. Furthermore, transient bacteria may increase the apparent number of species. A better way is to focus on the dominant bacterial species, for example when assessing the changes in bacterial community composition induced by diet. While high resistant starch promotes a particular bacterial phenotype to proliferate, a low carbohydrate diet induces the expansion of another population of bacteria. The bacteria that remain uncultivable it is around 70% and may represent species with less frequent occurrence within the human microbiota. Their lower abundance however, does not rule out their biological importance in the human intestine. Nevertheless, this lack of knowledge of unknown, uncultivable bacteria requires more effort, time and money to be invested to progress in the field of microbiology.

5. *Do you think that other bacterial products as important as butyrate are yet to be discovered?*

Answer: Yes. For example certain dietary phenolics are recognized as having anti-inflammatory and anti-microbial properties, which bioavailability and bioactivity depends on gut microbiota. Better understanding of this relationship may help in the future to prevent intestinal diseases such as Crohn's or Colitis. It is also recognized after a lot of research that bacteria play an important part in the digestion of fibre which helps to improve ruminant digestibility. Other research in commensal bacteria that degrade mucin could clarify to what point probiotics are useful, due to their capacity to degrade this important intestinal layer that protect against pathogens and damaging agents.

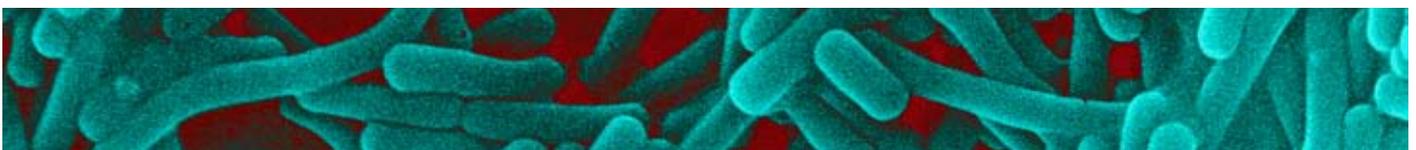
6. *The Cross-talk project has emerged to train future researchers specialized on relations host-microbiota, with connections between industry and academic areas. Do you think that this project is relevant for future products and therapeutics important to human health?*

Answer: Of course it is relevant. There are already bioindustries producing microbial products including probiotics, which have their own research capability and require strong researchers to work and progress in this field. I think the opportunities will increase due to the demands of the human population to have both a healthier lifestyle and better therapies for preventing and managing diseases. Also the prebiotics are important in the earlier stage of life as they promote the growth and/or activity of commensal bacteria or of a bacteria limited in number in the colon. CT project is for sure an important opportunity for further progress in the field and for the development of potential therapies for IBD (Inflammatory Bowel Disease). Advances in genomics will also allow new bacteria and bacterial genes to be identified whose functions remain to be explored.



probiotic *Lactobacillus*

– Vanessa Ferraria and Edouard Monnais



Crohn's - a genetic, environmental, bacterial and viral disease

Inflammatory bowel disease (IBD) in humans, such as Crohn's disease (CD) and ulcerative colitis, is a chronic inflammatory disorder of largely unknown cause in a genetically predisposed host. Crohn's disease is thought to be an autoimmune disease, in which the body's immune system attacks the gastrointestinal tract, causing inflammation; it is characterized by ulceration and inflammation occurring in the distal part of the small intestine, and pathological changes in the colon. It primarily causes abdominal pain, diarrhoea (which may be bloody if inflammation is at its worst), vomiting, or weight loss, but may also causes complications outside of the gastrointestinal tract such as skin rashes, arthritis, inflammation of the eye, tiredness, and lack of concentration.

Many factors have been implemented to play an important role in the development of the disease, so far however none of them could be identified as a driving force of the disease. Some researches claimed it is the change in the bacterial flora in the gut, some other suggested Crohn's disease to be an environmental disease caused by stress. Some other tried to find specific genes that could be responsible for the imbalance of the immune responses of the intestine. In this article I would like to summarize the research done so far on CD with a special attention to the latest discovery of Cadwell *et al.*, published in Cell in June 2010, showing how complicated this pathology really is.

Crohn's disease as a genetic disease

Some research has indicated that Crohn's disease may have a genetic link. The disease runs in families and those with a sibling with the disease are 30 times more likely to develop it than the general population. Also research done on mono- and dizygotic twins indicate that link.

In the last ten years many researches were trying to find the genetic causes of common diseases. Genome-wide association studies (GWAS) are designed to identify a plethora of common sequence variants of genes and their regulatory regions that predispose an individual to developing a particular disease. One of the diseases for which GWAS have been spectacularly successful is Crohn's disease. Changes in over 30 loci have been associated with increased risk of Crohn's disease. Among them we can find the genes involved in the immunity like NOD2 or receptor for interleukin 23 - a pro-inflammatory cytokine. Yet, these changes individually or even in combination confer limited risk. In recent years, the most interesting is a gene nominated Atg16L1. Atg16L1 which

is involved in autophagy – a process of “self-eating” of the cell. Autophagy is used by cells in order to get rid of old organelles and proteins; it can also serve as a way to defend against pathogens that managed to get in to the cell. Mice which lack the Atg16L1 gene displayed gut inflammation. In particular one type of the intestinal cells was affected, paneth cells. Paneth cells contain granules packed with antimicrobial peptides. When Atg16L1 gene was deleted paneth cells produced less antimicrobial substances, but on the other hand secreted more pro-inflammatory signals. This way, the pathogenic bacteria couldn't be killed and the whole intestine got inflamed. Even though promising, the gene is mutated in 50% of European population and only very few of the carriers are affected by Crohn's. Therefore the malfunctioning of Atg16L1 cannot in itself explain the pathology.

Crohn's as a bacterial disease

A variety of pathogenic bacteria were initially suspected of being causative agents of Crohn's disease. However, most health care professionals now believe that a variety of microorganisms are taking advantage of their host's weakened mucosal layer and inability to clear bacteria from the intestinal walls, both symptoms of the disease. Some studies have suggested that Mycobacterium plays a role in Crohn's disease. Other studies have linked specific strains of Escherichia coli to the disease. Still, this relationship between specific types of bacteria and Crohn's disease remains unclear.

During the last decade many new methods have been developed in order to study the composition of the microbiota in the gut, like metagenomics – a big scale analysis of bacterial genomes. Large multi-national cooperatives have been established in order to define the

core microbiome that is common to all healthy humans. Meta-HIT, one of such projects was set up in order to produce a catalogue of bacterial genes that humans carry in their intestine and find difference between healthy individuals and those with inflammatory bowel disease and diabetes. Crohn's disease patients were characterized by clear difference in the structure of the microbiome, mostly by a reduction in total number of bacteria and their diversity. That was further confirmed by many studies with the use of animal models. Composition of bacterial flora and presence or absence of specific species that may influence the development of the intestinal inflammation. Again however, nobody was able to directly link a specific change with Crohn's disease conclusively. If bacteria are important for the pathology it is very possible that there need to be specific conditions allowing the disbalance, like for example infection or genetic susceptibility.

Crohn's as an environmental disease

Diet is believed to be linked to its higher prevalence of the disease in industrialized parts of the world. It has been shown that people eating more animal products like milk or meat have a higher risk of developing the disease. On the other hand a diet rich in vegetable proteins has the protective effect. Also smoking has been shown to increase the risk of the return of active disease, or “flares”.

Another hint of the environmental cause of the CD comes from studies done on animals. Mice fed with some chemicals that irritate the gastrointestinal tract also develop colitis. One of the most commonly used chemicals is dextran sodium sulfate (DSS) which in lower concentrations can cause a persistent inflammation in the intestine however with distinct features characteristic of Crohn's.

Crohn's as a viral disease

This possibility is the less studied one. However, the concept that viral infections trigger disease in individuals with certain genetic backgrounds is broadly recognized. In animal models, autoimmune diseases can be influenced by the presence of viruses. There are also cases of various viruses causing gastrointestinal inflammation. One of them is noroviruses - RNA viruses responsible for the majority of epidemic nonbacterial gastroenteritis in humans. The mouse variant of this virus - MNV is prevalent in mouse facilities around the world and can persist for months after initial infection.

New idea: Crohn's - a genetic, environmental, bacterial and viral disease

The new idea that Crohn's disease is actually caused by all four factors mentioned before arose by accident. One of the groups involved in this study has previously published a paper on the mice having a mutation in the autophagy gene *Atg16L1*. These mice displayed striking abnormalities in the Paneth cells and had a general inflammatory phenotype. When these mice were transferred into a new, enhanced barrier facility, the intestinal problems completely disappeared. The scientists started to wonder about what could be present

in the first animal house and absent in the other that could trigger the disease. One of the candidates was previously discovered virus from the family of noroviruses - MNV. As expected it could be found in the standard barrier facility but not in that of enhanced barrier. When mice from "clean" facility were infected with MNV they started to show the abnormal intestinal architecture again. Then the group went one step further. They have used previously described a chemical agent DSS in order to induce a general intestinal inflammation. Mice with mutated *Atg16L1*, both infected with the virus and not, together with the control mice were affected by the chemical insult. However, only the mice carrying the mutation and being infected with MNV developed a disease very similar to that seen in Crohn's patients manifested as blunting of intestinal villi. As I mentioned before, there is long postulated role of bacteria in the development of Crohn's disease. To test, if this is also the case in their particular mouse model Cadwell et al., used antibiotics in order to get rid of the big part of commensal microbiota inhabiting the intestine. After the use of drugs mutant mice infected with MNV could no longer respond to DSS treatment in the previously observed way. They did develop inflammation but it was no longer similar to that observed in Crohn's patients.

In their study Cadwell and colleagues define a complex inflammatory disease in mice that depends on an interaction between a specific virus and a single host disease susceptibility gene, that can be modulated by other environmental factors and the presence of intestinal flora. Their results are very important for the development of treatments of Crohn's disease and show the scientific community that when studying a disease a broad spectrum of possible mechanisms should be taken into account. Their study is a great example of multidisciplinary research that is needed in today's world to solve the most difficult mysteries of diseases like Crohn's.

– Agata Korecka

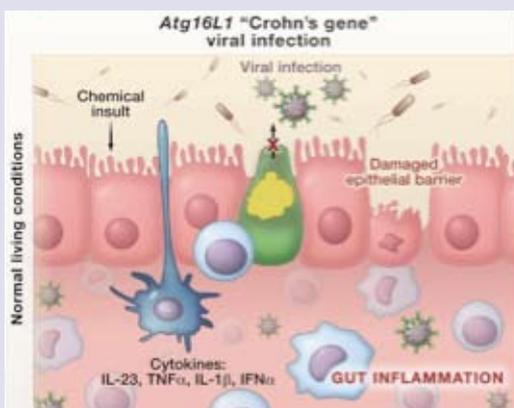
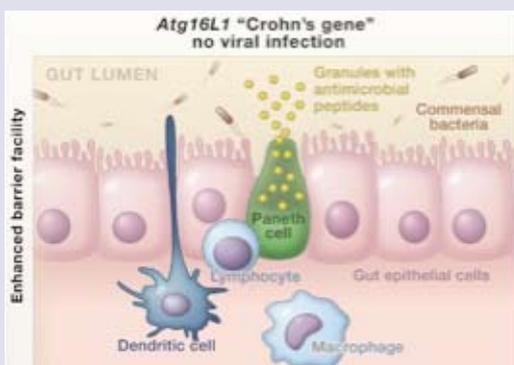
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Qin et al., A human gut microbial gene catalogue established by metagenomic sequencing. 2010 Nature 464, 59-65



Environmental and Genetic Factors Required for Crohn's like Disease in Mice.

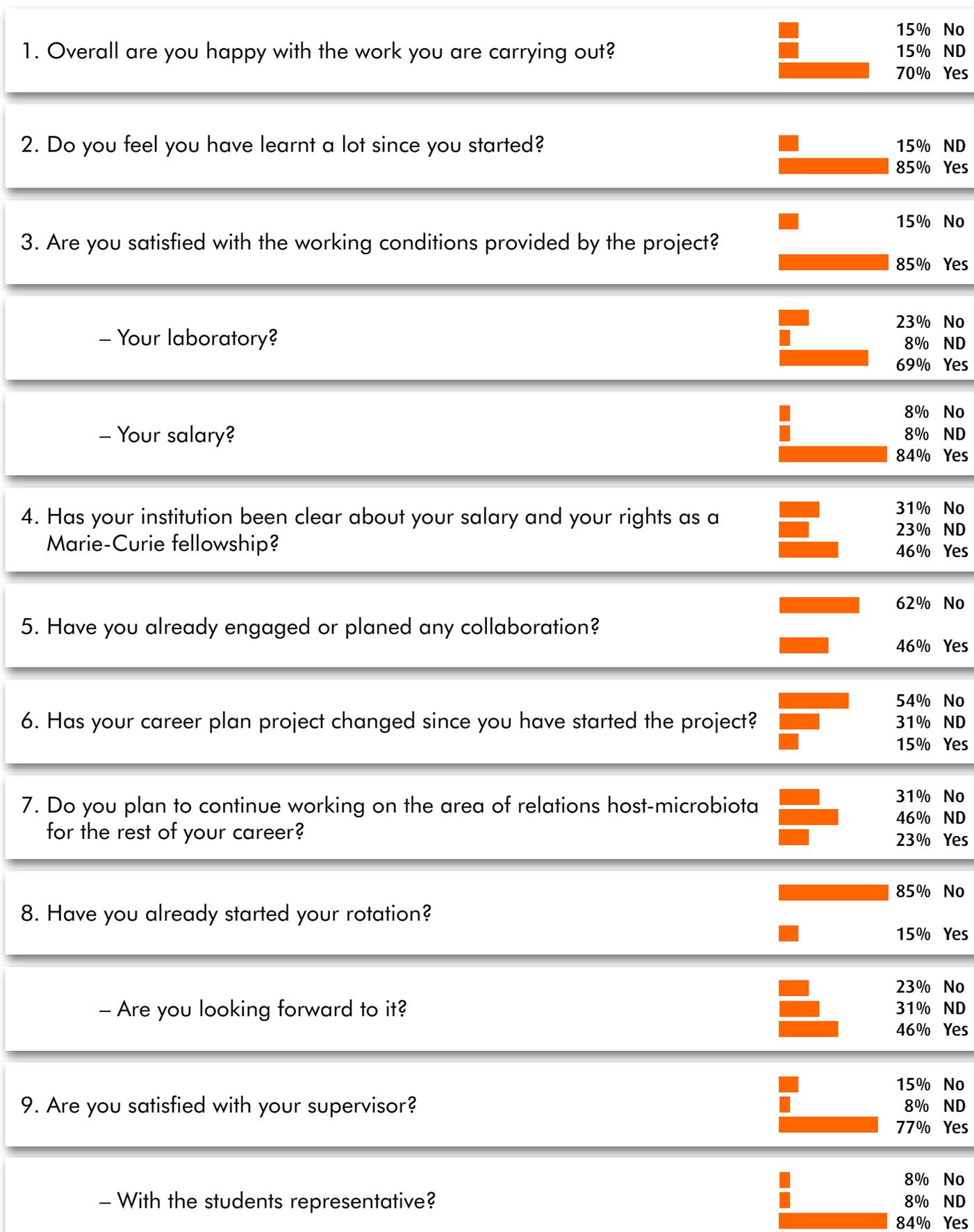
TOP – Mouse model of Crohn's disease in which the mouse *Atg16L1* ortholog is disrupted, the Paneth cells have a normal phenotype when the mice are raised in virus-free enhanced barrier facilities.

BOTTOM – However, in conventional living conditions these mutant mice are infected with norovirus and develop similar abnormalities in the Paneth cells as those observed in patients with Crohn's disease. This pathology also depends on the interactions of commensal bacteria, the gut innate immune system, and the production of proinflammatory cytokines.

Source: John A. Todd, Cell 141, June 25, 2010

Cross Talk students survey

Cross Talk is already halfway through. How do the student fellows feel themselves, in their lab, within the project, and about their professional future? (ND - not determined.)



For the majority of students, it can be said that students are satisfied and happy with their ongoing work. Students have had the experience of a new country, culture and society, as well as gratifying interest for their work, altogether meaning the project has already been rewarding. Although very clear when introduced, financial issues ruling fellowships have been sometimes much less well perceived when explained and translated by the host institutes, causing confusion and resulting in stress with possible impact on the working relationship. Let's hope that time and perseverance will help everybody to understand each other. Already a third of the students have engaged in a collaboration either within the

project or outside. Cross talk can also boast 25% of students are already set for a career in the field of host-microbiota relationships, while 31% assume their unknown future, and the rest is still waiting to be persuaded. A small number (15%) have already started their rotation period in another lab, whereas the rest is half-divided as to whether they look forward to it. Finally it is worth noting that supervisory board and students representatives are considered and appreciated by the majority, it reflects a kind of general well-being of both students and projects. Let's keep it up!!

– Vanessa Ferraria and Edouard Monnais

LinkedIn – a networking tool for professionals

Have you ever wondered if you can use Facebook as a tool for professional networking? You probably could, but what for, if there is something much better – LinkedIn. LinkedIn is a business-oriented social networking site. It works very similarly to Facebook, Hi5 etc. The only exception is that your contacts need to be correlated with you on professional basis– colleagues, co-workers, supervisors, CEOs, both present and past.

LinkedIn was created in 2002 by Reid Hoffman and in 2010 reached over 70 million registered users from over 200 countries. Half of the users live in United States, but there are growing Asian and European communities. Every second a new member is registered. Each user can invite people that he/she knows – they will create a first-degree connection. However, your network consists not only of your connection, but also of your connections' connections and the people they know. Thanks to that you gain access to trusted professionals even if you do not

know them in person. LinkedIn offers a wide range of opportunities for registered users. It can be used to find jobs, business opportunities and people. The site gives you the possibility to recommend people you know and they can recommend you, which may be important when you look for a job. Users can follow various companies and get notification about a new job opportunity. Employers on the other hand can list jobs and search for potential candidates. LinkedIn also allows users to research companies, like for example by giving statistics concerning the most

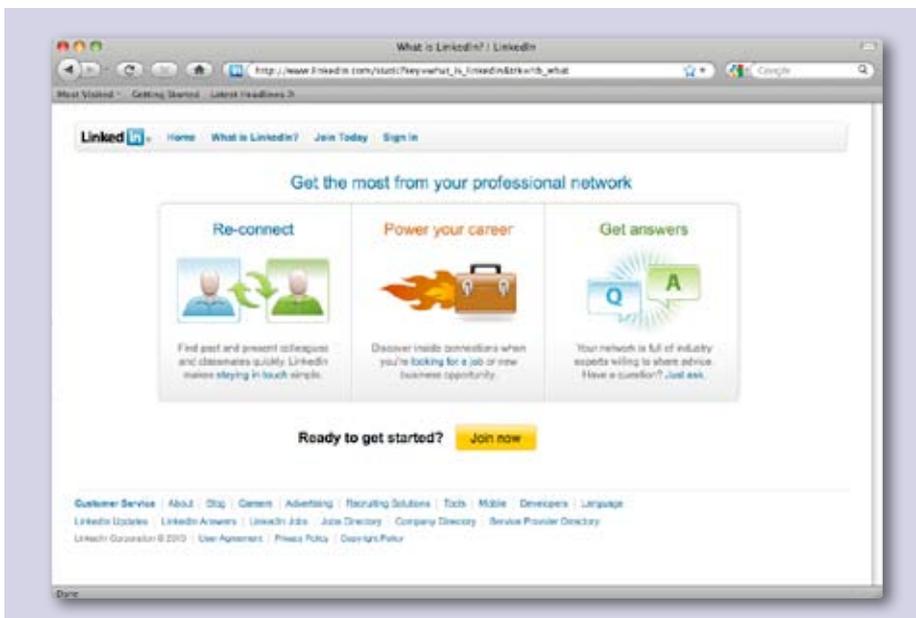
common positions or the male to female employees' ratio. Two more features are worth mentioning – LinkedIn Answers and LinkedIn Groups. The first one allows you to ask questions and get answers from specialist in a given field. The latter allows establishing new business relationships by joining the alumni, industry, academia and other relevant groups.

LinkedIn is a great tool to reconnect with your previous supervisors, co-workers and mates. Gives you opportunity to discuss with likeminded professionals and discover inside connections that can help you find a job. It is worth spending some time to create your profile correctly – this way you manage the information about you that is publicly available and make it easier for your future employer to know what kind of person you are even before he sees you in person. Remember that today's employers like to "google" the candidates – the more professional information they can find the better for you.

I strongly recommend LinkedIn as a great networking tool. The more people join it the more power it will have. And on the other hand it is always good to keep in touch with the professors and colleagues we meet during our scientific journey around the world. Join now at: www.linkedin.com.

– Agata Korecka

Sources: www.linkedin.com, Wikipedia.org





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