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QBiC

QBiTs

Inside this issue

Talking With. . .

Shun'ichi Kuroda

The Professor at the Graduate School of Bioagricultural Sciences, Nagoya University, is an entrepreneur at heart. We talk to him about commercializing research, including his latest effort, an automated high-throughput single cell-based screening system designed in collaboration with Hiroki Ueda at QBiC.

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You give me the pictures, I'll give you the story

Devin Powell stood before 15 Riken associates and proclaimed his challenge. "Tell me about your latest paper in five sentences". A number of pens scribbled what surely had to be more than five, while the others stood frozen. The exercise was the beginning of a talk by Devin, a freelance science journalist, who had until this year been on staff at Nature, on how to talk about science to journalists. The lecture had two purposes, one to guide scientists on how best to represent their research to media and the other to explain the importance of communicating with media. Devin gave a pertinent example of when the latter is not done, explaining how some reports after Fukushima were comparing the levels of radioactivity emitted from the reactors to those emitted from cell phones, ignoring the quality and thus medical effects of the radiation and creating a false equivalency. He also provided a number of tips including the importance of simplifying ideas with metaphors and repeating statements that you want quoted. Devin finished by having two of the audience members participate in separate face-to-face mock interviews. Such interviews were even unusual for him, as Devin explained that he normally conducts interviews by phone rather than visiting the lab or meeting the researcher in person. "That's how I do 99%". ●

Talking with . . .

Shun'ichi Kuroda, the rare professor that started his career in industry.

Like many universities in Japan, Nagoya University is a mix of buildings aesthetic and archaic. The best example of the former is the Noyori Materials Science Laboratory, which was built with some of the monies Riken Director Ryoji Noyori was awarded after he won the Nobel Prize in 2001. Among the latter is the location of Shun'ichi Kuroda's laboratory, a simple, grey building that gives little inspiration from the outside. The bland exterior, however is juxtaposed by an artful interior of orange and white walls and a semi-circular partition in the middle for study.

Similarly, the man is much more colourful in person than the persona his formal e-mails give. Shun'ichi had been recommended by Group Director Hiroki Ueda, as the two had published a paper together this year on a high-throughput automated system for single cell selection. This collaboration started through Itoshi Nikaido, a former member of the Ueda lab who now runs his own at Riken and researches single cell analysis methods. The connection with Hiroki, then, was the basis of the interview.

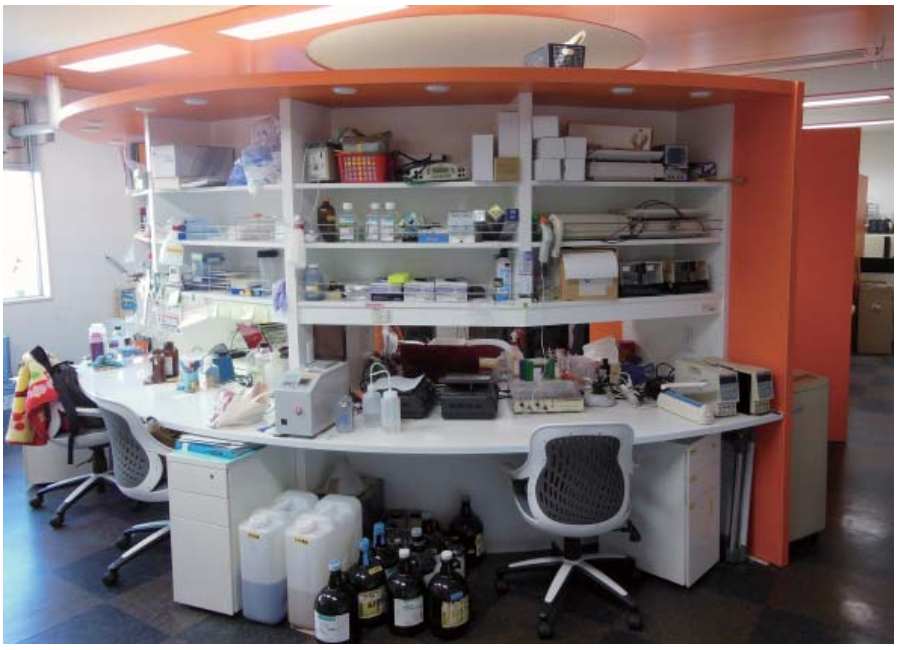
- "So how well do you know Hiroki?"
- "I have never met him".

And that was that.

Even if he had never met Hiroki, Shun'ichi's story would prove to be both interesting and unusual, as it involves shifting from industry to academics and also starting a number of small ventures. "I finished the masters course at Kyoto University and joined Takeda Pharmaceuticals. [There] I was involved in the development of hepatitis B vaccine using recombinant yeast cell. As a student I was interested in fermentation, making sake, making miso. The pharmaceutical company uses fermentation technology so I learned yeast can produce pharmaceuticals and was involved in clinical trial tests."

This led to him earning his doctorate degree in an also unusual way. In Japan, one can be accredited a degree from a university by petition. The university can deem a doctorate based on the quality of publications and other factors even if the individual was never a student during the time of research, which is how Shun'ichi earned his. "I worked at Takeda for eight years and applied to Kyoto University for a Ph.D". The transition to academia, however, needed more than just a strong publication record and Ph.D. "My work at Takeda was half designing vaccines and half analyzing protein kinase C. I collaborated with Prof. Yasutomi Nishizuka", the man who discovered protein kinase C. "He was president of Kobe University and invited me to join his lab as an assistant professor".

Shun'ichi has remained in academics since, but has been involved in a number of venture efforts, some more successful than others. One of the first is Beacle, a company that was founded in 2002 on nanoparticle technology he began developing at Takeda. Among his spinoffs, Beacle has been the toughest to make successful, partly because of how it was established. Shun'ichi explains that around that time (2001), "Prof. Morishita of Osaka University just established the first bio-venutre in Japan. Nobody encouraged commercialization." Shun'ichi consulted four other professors that each had their own relevant scientific expertise and together they founded the company.



"It is very hard to develop a company because there is no system [in Japan] and it is hard to get money".

He compares Beacle with Bone Biologics, a company that he founded with UCLA colleagues in the U.S. based on his discovery of NELL1, a bone morphogenetic protein. It was a lucky discovery, as he never intended to conduct bone-related research. At Kobe, Prof. Nishizuka wanted to find proteins that interact with protein kinase C. "I started the yeast two-hybrid system. During the course of the screening, accidently, I found the cDNA of Nell1". Shun'ichi describes the Bone Biologics experience as, "Very easy. Very easy to collect money. The company was established later than Beacle but has already started clinical trials. There is no system for developing new nano-carriers for drug delivery in Japan. The FDA already has flowcharts and guidelines. In Japan it is case by case. It is very tough for the small company to develop new nano-carriers".

The latest commercial project involving the cell sorter he published with Hiroki began when Shun'ichi spoke to Akihiko Kondo and Ikuo Fujii, who along with Shun'ichi make up the last three authors of the paper (Scientific Reports, 2013). "We were talking about the FACS cell sorting system. My interest is generating mammalian cells for new pharmaceuticals. Each cell produces different amounts of secretion. We need the best cell from 100,000 candidates. It is very hard to pick up the best cell from FACS. Based on the secretion rate or antibody affinity, we developed CS-FIA, cell-surface fluorescent immunosorbent assay, system". The three realized that they required partners to build the hardware, which resulted in a consortium with three companies. The design called for a cell array and automation, which is why they brought in the plastic company STARLITE and the electronics company Furukawa. Shun'ichi also consulted As One, a trading company, to commercialize their system.

Shun'ichi obviously enjoys translating his research into mass product, but he remains very comfortable at the university with little interest in returning to industry. "It's my dream to see my products commercialized. But I do not want to manage a company. I was deeply involved in the management at Beacle. My publication was very slow. I was like a businessman, making presentations and collecting money. It is very tough. I realized it is not very good for me. Management professionals, not me, can take care of big companies".

Shun'ichi then continues to do his basic research while always keeping an eye on business opportunities. Not all his projects require commercial potential, however, as one he hopes to solve in the near future is finally meeting Hiroki Ueda.

QBiC technology transfer

QBiC also has its share of professors with the venture spirit. One example is Tsutomu Masujima, who is manufacturing nanospray tips. The tips were originally designed for proteomics studies until Tsutomu realized their potential for single-cell mass spectrometry (MS), as they are ideal for sucking the content of a cell. The tips provide a much better signal then does MALDI-TOF, which is what Tsutomu had to use for his MS experiments beforehand. In a complementary project, he is now investigating ways to automating the sucking procedure.

Tsutomu and Shun'ichi share much in common when it comes to their business experience. "I hate it", explains Tsutomu, when describing the sales and management part. Regardless, rather than partnering with other companies, his company, Humanix, has taken all the responsibility for marketing his technologies. Like any business in its early stages, it has been a slow process, but one that Tsutomu recognizes needs patience. One hindrence is that Humanix headquarters is currently located in Hiroshima, which was where Tsutomu was prior to joining QBiC and requires him to commute regularly between the two cities. He is now negotiating space inside a building that will soon have its construction begin next door the QBiC labs in Kobe.

Briefs

A number of computational methods exist for analyzing the spatio-temporal dynamics of nuclei segmentation. However, too often these methods have high computational costs and are tailored for a specific problem, which limit their application. Depending on the performance demands of the analysis, Yusuke Azuma and Shuichi Onami reports in *BMC Bioinformatics* more generic and less demanding methods as equally suitable for such studies.

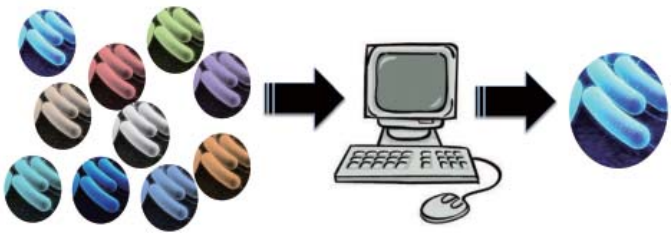
Daichi Okuno of the Laboratory for Cell Dynamics Observation in collaboration with researchers at Kyoto and Tokyo Universities has published a report that shows the effects of pressure on the rotation of F1-ATPase. By examining the rotation kinetics at several pressures the authors shows two pressure-sensitive reactions occur in the cycle, ATP binding and the other post ATP-binding. The report can be seen in *Biophysical Journal*.

In a paper with long time collaborator and now Riken CDB PI Tatsuo Shibata, Satomi Matsuoka and Masahiro Ueda report how the rate of a chemical gradient determines the accuracy of chemotaxis in the absence of any guidance cues. The authors show that spontaneous self-organization in Dictyostelium cells creates asymmetry in the distribution of signaling molecules independent of any external signal. They further demonstrate sensitivity to a shallow gradient that regulates cell polarity can be manipulated by modifying the direction of the polarity and the frequency of the self-organization. This study too can be read in *Biophysical Journal*.

Paper Highlight

New algorithm for better mutant targeting in metabolic engineering

For many, supermarkets are no longer places where one buys herbs and vegetables, but rather places where one buys the seeds to grow the herbs and vegetables at home. With time, we may see the same for cheese and beer, with the supermarket selling microorganisms that do the fermentation. Metabolic engineering is helping find ways to produce efficient bacteria for this task. It involves analyzing an exhaustive number of metabolic reactions in a microbe to find the best conditions for synthesizing a desired metabolite while not compromising the microbe's survival. However, considering the massive combinatorics, identifying which reactions to modify is not a trivial task. While a number of popular algorithms exist, their limitations maintain a constant demand for new ones. For this purpose, Chikara Furusawa, in collaboration with researchers at Osaka University, has designed FastPros, an iterative screening algorithm that is descibed in *Bioinformatics*.



In general, iterative screening has less computational costs than comprehensive screening single reaction knockouts on the production of a target metabolite. At the same time, it is not effective at screening combinations of knockouts that have a synergistic effect, as is the case when two knockouts generate higher production yields only if they occur simultaneously. FastPros identifies which simultaneous knockouts achieve production of a target metabolite by assuming biomass production maximization. The innovation in the approach comes from using the ratio of the change in the biomass production flux and the target production flux as the score

Meet the QBiC Lab. . .

Yasushi Okada and the Laboratory for Cell Polarity Regulation

We study the transport system in the cell, with a special interest in kinesin, which is essential for the building of synapses and therefore learning. As such, kinesin is a key regulator in the morphogenesis of the neuron. A single neuron has a single long axon and numerous, much shorter dendrites. This asymmetrical shape is essential for neuron function. However, a neuron is not born into this shape. Instead, a juvenile neuron has many short processes that extend and retract without any evident predisposition for becoming the axon or dendrites. The change in length comes from transported signals and materials. We are investigating the kinesin KIF5 and its role in this transport. KIF5 distributes stochastically between processes, but will eventually accumulate exclusively in the one process that eventually grows to become the axon.

Furthermore, this phenomenon occurs in the absence of any external signal. In other words, symmetry among the processes is broken spontaneously through internal dynamics. We are attempting to explain this symmetry breaking by several techniques, from live imaging microscopy to the production of animal models. One example is our design of the world's fastest super-resolution microscope. Another is our super TALEN technology. Gene edits using our method show activity levels a magnitude greater than those done with normal TALEN and can produce knockout mice in one month. A final example is methodology that can measure the protein-protein reaction kinetics in the cytoplasm at the single molecule level. We are now collaborating with computation specialists at QBiC to model our observations in the cytoplasm.

for iterative knockout screening. They call this variable the potential of the target production, or u_{target}. u_{target} acts like a shadow price such that positive values indicate conditions where the biomass production rate is positive for non-zero target production and can therefore be used to identify potential reaction knockouts for enhancement of the metabolite production while increasing the fitness of the microbe.

The authors applied FastPros to study the E. coli reaction network, which includes 625 metabolites and demonstrated that it has an accuracy comparable with the comprehensive screening algorithm OptKnock, but at far less computational cost. At the same time, FastPros proved to be far better and robust than other iterative screening algorithms like OptGene and GDLS when handling a large number of simultaneous knockouts.

Yet despite outperforming its competitors, FastPros still underperformed, as the identified knockout sets did not always lead to the desired production yield of the target metabolite. That is because, as a shadow price, u_{target} can only clarify if the flux is positive, not optimal. For this reason, FastPros was combined with OptKnock such that FastPros provided a coarse screening that significantly reduced the number of targets and computational demands of OptKnock, which could then precisely determine which knockout sets achieve optimal target production in a reasonable time.

Interestingly, FastPros found that a common reaction knockout set exists for many metabolites. Therefore, a single parent strain can be designed from which experimenters can knockout the appropriate reactions to achieve the desired production of various targets like lipids, sugars, and aromatic acids.

The authors chose to examine the usefulness of FastPros for screening reactions rather than genes only because of the completeness of the E. coli reaction network model. Chikara is quite certain that there should be little difficultly in applying the same strategies to genes. "If we can determine which reaction should be deleted, we can easily map which gene should be deleted".

Interesting People

I'll have a Duff, you have one too

The tall and stylishly dressed Takaaki, post-doc at the Laboratory for Multiscale Biosystem Dynamics, admits he regularly enjoys the taste of a good beer. That was one of his attractions to the laboratory of Hiroshi Shimizu at Osaka University, which specializes in the study of how microorganisms produce biomaterials like ethanol and where Takaaki earned his masters and Ph.D. It was also there that Takaaki devoted himself to efficient ethanol production methods by microarray and evolutionary engineering techniques. The research became a dream project when Takaaki secured a three-month internship at the Suntory Brewery, which is located nearby between Osaka and Kyoto. Suntory scientist and collaborator Yoshihiro Nakao arranged to have Takaaki study in more detail the fermentation process using advanced equipment exclusive to Suntory.

Although brief, the Suntory experience Takaaki a great deal about the research culture in which he thrives. "Many of my colleagues admitted they could not choose their own research". He was also much less free to come in and out of the lab. Working after hours, which was often required for the fermentation, required a slew of paperwork for security. He does have one lament, though. "I could always try the beers at work. That I miss".

It was at Hiroshi's lab where Takaaki met Chikara Furusawa, who recruited Takaaki to QBiC as a post-doctorate. Although both come from quantitative science backgrounds, Chikara from physics and Takaaki from engineering, Takaaki's current research only needs relatively simple math and a much stronger biology background. Takaaki is currently investigating evolution and adaptation in E. coli by observing the genes responsible for ethanol tolerance. According to Takaaki, although some labs have reported gene mutations that affect ethanol tolerance, little is known about the relationship between the genotypes and phenotypes. His goal is to understand how E. coli can adapt to ethanol stress by changing their genotype and internal state. However, because as many as 100 gene mutations seem to change during the evolution, looking at all possible combinations is an impossible task. "I gave up", he explains. His strategy now is to analyze groups of genes (3-5) in hopes of narrowing down the one or few key genes responsible for ethanol tolerance.

His approach uses microarrays to conduct large-scale analysis of E. coli evolution in hopes of measuring the internal state, like gene expression patterns, of a cell. "Evolutionary experiments are very frustrating. I have to do the same experiments everyday at the same time – Christmas, New Year's. I am now trying to automate the system so I don't have to work everyday. But that too is not working well, so I am in everyday working on the automation". Nevertheless, Takaaki views this work as an important first step in understanding the E. coli evolution process, which nicely complements the studies using E-Cell done by the Takahashi lab. The two groups are now discussing possible collaborations.

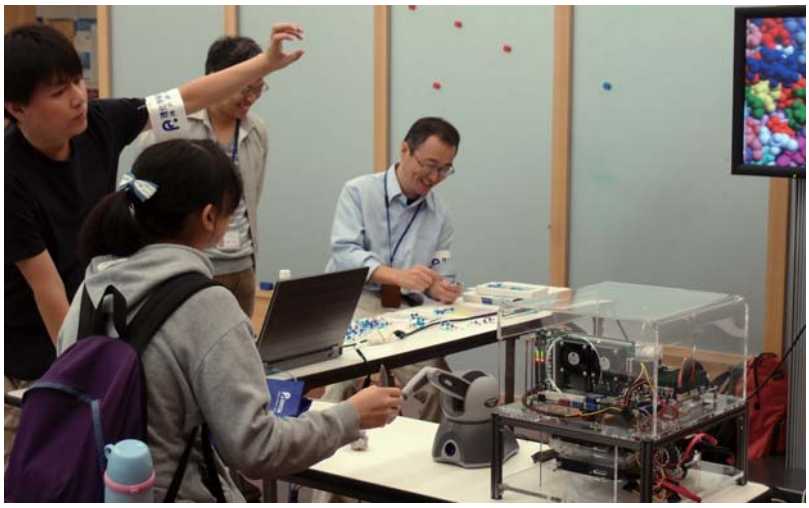
As years of research have past, Takaaki is realizing that ethanol need not be a major part of his science career anymore. Pondering whether in his next lab he will keep ethanol at the heart of his research using new techniques or use the same techniques to study other systems, Takaaki prefers the latter. "I get my ethanol from drinking. It need not be at work too".

A National Honour

Toshio Yanagida walked into his weekly meeting on October 25th to a proud applause. As fond as the QBiC staff is of its director, never have they expressed such admiration. However, this was no normal meeting. Earlier that day it had been announced that Toshio had been one of 15 people named this year as a bunka-kourousha (文化功労者; Person of Cultural Merit), one of Japan's most prestigious awards given annually to individuals for their contributions to Japanese culture. (For our Canadian readers, think Order of Canada; for our non-Canadian readers, think…Order of Canada). Winners come from all walks of life such as kabuki performers, sumo wrestlers, novelists and on rare occasion even foreigners. In a normal year, about half the recipients come from the sciences including those who would later win Nobel Prizes like Riken's Ryoji Noyori and Susumu Tomegawa. The award includes a visit to the Imperial Palace and dinner with the Emperor. Although the award recognizes Japanese culture, interestingly only the women wore traditional kimonos, while the men were ironically dressed in black suits. Moreover, the meal was what Toshio describes as "French cuisine. Simple French cuisine".

It was not the first time Toshio had met the Emperor. In 1998, Toshio was awarded the onshishou (恩賜賞) for his academic accomplishments, which also resulted in a royal visit. The previous encounter with the Emperor may have calmed his nerves in his second meeting, but not his confusion. "I understand why I won before. But I have no idea why they selected me for culture".

RIKEN Open Houses



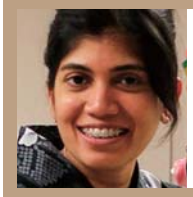
On September 28 and October 19, Riken Yokohama and Kobe hosted open houses for the public to partake in science experiments. Because QBiC has laboratories at both sites, it participated on both days. Takanori Kigawa's team prepared a family-orientated display of his NMR work at Yokohama. His team built a 1.5-meter wide compartment made of cardboard and plastic balls to model the living cell in which children (and adults) could "float" on organelles inside the cytosol. Shuichi Onami, Atsuo Kawahara, and Makoto Taiji and their respective groups had exhibits at the Kobe site, which included opportunities for visitors to observe different fluorescent images of a living cell or manipulate real-time molecular simulations on MDGRAPE. Chikara Furusawa also participated by discussing with the public during an open seminar just how difficult it really is to give a simple definition of life.

A headstart on overeating for the holidays

November 21st was a day of food and drink at the QBiC Osaka site, where members enjoyed a special tea break and then ended the day with a wine party. Both QBiC sites have a daily tea break in the afternoon, but on one day each month money is pooled to buy some treats. This time, the Science Communications Team promised a more festive mood by baking the German Christmas cake, stollen. Along with the traditional flavour rich in dried fruits, several experimental stollen were prepared including chocolate-raisin, coconut-rosemary, and lavender-cranberry. Several researchers regretted their late arrival, as no cake remained at the end.

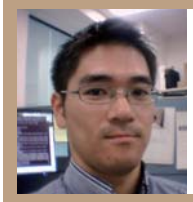
Members congregated again in the evening for a more bubbly affair. Every three months, labs rotate in hosting a wine night, with the Furusawa and Taniguchi labs taking responsibility this time. The teams chose Beaujolais in accordance with Beaujolais Nouveau Day, although it is unlikely participants would have cared had another wine been selected. Teetotalers also had reason to come for the abundance of food.

Newcomers at QBiC



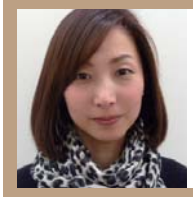
Me: Brit David
Lab: Team Watanabe
Hobbies: Reading, movies
Cheers: HANSHIN TIGERS

Me: Kazunari Mouri
Lab: Team Okada
Hobbies: Tropical fish and turtle hobbyist, bicycle riding
Cheers: anyone but the Giants



Me: Tadashi Ando
Lab: Team Sugita
Hobbies: Playing with my daughter, Reading books
Cheers: Chiba Lotte Marines

Me: Akiko Kaneyama
Lab: Team Ueda
Hobbies:Visiting spas, Baking breads
Cheers: HANSHIN TIGERS



Me: Eri Noda
Lab: Team Furusawa
Hobbies: Sweets
Cheers: HANSHIN TIGERS

Me: Aki Sakai
Lab: Team Furusawa
Hobbies: Manga, Games
Cheers: HANSHIN TIGERS

THE CHOW DOWN

Wafu Swordfish

Have a recipe you want to share, contact us at: **qbits@riken.jp**

Ingredients

swordfish 4 pieces
sliced ginger 20g
shiokouji 3 tablespoons (salted rice malt)

sauce:
soy sauce 3 tablespoons
sake 3 tablespoons
sweet sake 3 tablespoons
sugar 2 tablespoons
water 100cc

1. Cut the swordfish into 5-6 pieces
2. Marinade the swordfish in shiokouji and let rest in the refrigerator (30 min-1 hr)
3. Pour hot water over the swordfish in a colander
4. Mix and boil the sauce ingredients in a pan
5. Put the swordfish and the sliced ginger in the pan and cover the fish with foil
6. Cook on medium heat (about 10 min) and cool to room temperature
7. Again cook on medium heat (about 5 min)
8. Just before serving, heat again and add the sauce

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