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ARMENISE-HARVARD SYMPOSIUM 2014

MECHANISMS TO MOLECULES

15th Biennial Symposium
June 22-24, 2014, Poiano Resort Hotel, Lake Garda Italy

About the Symposium

L1050036The gentle brush strokes of a XVIII century oil painting seemed to make up the setting of the 15th Armenise-Harvard Symposium. A mélange of olive trees, cypresses and oleanders surrounded by green hills and reflected in the shimmering blue of the Garda Lake: in this bucolic scenario, 80 scientists met to discuss the latest advancements in cell biology.

Between June 22 and 24, the symposium held in the luxurious Poiano Resort brought together researchers working on the cutting edge of science. “Interdisciplinary” and “heterogeneity” were the main keywords: this was already pretty clear from the symposium title, “Mechanisms to Molecules”.

From single proteins activity to complete cell systems, from basic research to clinical application: the two-and-a-half-day meeting was an exciting opportunity for cross-disciplinary discussions and high-level scientific exchanges.

Twenty-one researchers, all leaders of their research groups in Italy and the United States, talked about their latest results, in many cases presenting preliminary and unpublished data. The friendly and free dialogue led to sharp debates about the most fascinating engines of our body: human cells.

The outstanding talks, very different from each other, had a common challenge: making long stories short. In fact each presented research began many years ago, when most of the cellular mechanisms discussed during the symposium were unknown.

Today, new technological tools and highly advanced microscopic techniques are making “visible” what was previously unseen; but at the same time, it is important not to lose the bigger picture.

This is what emerged from the enthralling keynote speech given by Harvard Professor Stephen Harrison, who deliberately juxtaposed the symposium name, entitling his presentation “Molecule to Mechanisms”. He outlined various ways to think of molecular activity starting from the paradigmatic example of influenza virus hemagglutinin (HA), whose binds receptor can defeat our immune response.

This started the discussion about the incredible number of interactions happening from molecular to cell mechanism level, and vice versa. The first symposium session, entitled “Mechanisms Regulating Epigenetic Regulation”, opened up the door to investigate this intriguing cellular complexity. Danesh Moazed and Stephen Buratowski talked about two fundamental ingredients of

cell regulation, chromatine and noncoding RNA. Diego Pasini exposed the activities of polycomb group proteins in proliferation and developmental control, while Giuseppe Testa broadened the field by explaining how cell reprogramming revolutionized biology over the last years.

The second session, “Mechanistic Insights from Single Molecule Analysis”, took the audience back to the molecular level. Samara Reck-Peterson and Andreas Leschziner talked about a fundamental aspect for cells’ survival, the mechanics behind molecular motors. Joseph Loparo gave a stimulating talk about DNA “intelligence”, showing how our genes strive to overcome obstacles. Sheref Mansy challenged the audience with a very novel frontier of biotechnology, the integration between artificial and natural cells.

Another important aspect is the communication amongst different cells, and this was the topic of the third session, “Mechanisms Regulating Trafficking”. Roberto Sitia explained how the quality control works within the signature pathway. Tomas Kirchhausen showed the exceptional potentialities of the modern 3D microscopy to study endocytosis. Daniela Corda and Alberto Luini gave two interesting talks about membrane trafficking and transport apparatus.

With the backdrop of the relevance of this research to medical applications, the fourth session focused on “Degradation Mechanisms”. Simona Polo talked about cell migration under normal and pathological conditions. Alfred Goldberg presented the system involved in protein degradation by proteasomes. Andrea Musacchio showed his latest results on the reconstruction of mitotic signaling, while Francesco Cecconi discussed the implication of one of the most incredible behavior of our cells, autophagy.

This led to the fifth and last session, “Transduction Mechanisms”, opened by Gianni Cesareni’s talk about the signaling mechanisms underlying the anti-tumor activity of metformin. Stephen Blacklow and Michael Eck respectively presented the signaling in the Notch cell and the control of the epidermal growth receptor. Pier Paolo di Fiore closed the session discussing the connections between cell fate determination and tumor suppression in mammary stem cells.

The task of closing the 15th Armenise-Harvard Symposium fell to Tomas Kirchhausen, who wrapped up the general take-home message of the meeting: that we are constantly dealing with a number of complex interactions, from cellular mechanisms to molecular communication. Unfolding the mysteries of these interactions is the main challenge of cell biology, fascinating and ever-growing field exploring the fundamental units of life.

Molecule to mechanisms — a case history

Stephen Harrison

Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, USA

The title of the symposium was reversed by the keynote speech: from molecule – intentionally singular – to mechanisms. With this talk, Stephen Harrison "set the tone for all the following presentations", as would have later observed Tomas Kirchhausen during the meeting closing remarks.

Harrison is one of the world's leading experts on viruses. Jeffrey Flier, President of the Armenise-Harvard Foundation and Dean of Harvard Medical School, briefly introduced Harrison's outstanding scientific activity: "He has made important contributions to structural biology, most notably by determining and analyzing structures of viruses and viral proteins, and by crystallographic analysis of protein-DNA complexes, as well as structural studies of protein-kinase switching mechanisms. His work has been widely recognized, and he has made pioneering researches to address a range of fundamental problems".

One of these problems concerns the influenza virus hemagglutinin (HA). The Harrison lab at Harvard Medical School studies how influenza virus penetrates cells by fusion of viral and endosomal membranes catalyzed by the viral hemagglutinin.

So Harrison used this virus as the case history of his presentation – a very effective way to develop the theme of the meeting at the outset.

"The hemagglutinin is a trimeric structure with three functions" he explained. "It binds the virus to its receptor, catalyzes the membrane fusion process, and has structures on the outside that can vary without compromising its two other essential functions".

In this way the virus can evolve to escape neutralization by the immune system of its host.

In the influenza, the protein hemagglutinin sticks off of the virus surface along with another protein, which is an enzyme, called the neuraminidase (NA).

Harrison focused on two particular mechanisms: HA-mediated fusion and HA antigenicity.

Membrane fusion is thermodynamically favorable, but it generally presents a high kinetic barrier. Fusion proteins lower this barrier, so they are catalysts for the merger of two bilayers; in the case of viral fusion proteins, they become "suicide" catalysts. After several experiments set up to measure fusion kinetics, Harrison and colleagues showed that for influenza virus fusion requires engagement with the target bilayer of fusion peptides from 3 or 4 neighboring HA trimmers.

"The case history lesson is summarized here" said Harrison, "That there is a contact patch between the virus and the target membrane."

This intermediate is a fundamental aspect of the fusion mechanism. But how does HA antigenicity react to this mechanism?

Antigenicity is the capacity to stimulate the production of antibodies. During the immune response, the process by which B cells produce antibodies with increased affinity for antigen is called affinity maturation.

“Antibody structures, from B-cell lineages in human responses to influenza virus vaccines, map evolution of protein interactions during antibody affinity maturation” explained Harrison.

The future challenge, he concluded, is therefore to understand the affinity maturation mechanisms well enough to design modified immunogens that might selectively elicit broad immune responses.

From molecule to mechanisms, then: the inspiring example of influenza hemagglutinin got straight to the heart of the symposium theme. Showing first of all how biology can travel between the micro and the macro level of our cells.

Gene on, gene off. In eukaryotic cells, DNA activation is often a matter of switches. But what are the biological triggers capable of unchaining gene expression? This is one of the core questions addressed by Danesh Moazed at Harvard Medical School.

His laboratory focuses on understanding the mechanisms that mediate the formation of one of the most tightly packed forms of DNA: heterochromatin, which plays a crucial role in gene silencing. For this reason, the study of heterochromatin – also called silent chromatin – helps understanding the mechanisms that keep genes in their right on or off state.

The formation of these silent domains is also responsible for the so-called epigenetic memory, which maintains cell identity during development and differentiation.

Biologists know that every single cell of our organism only turns on the subset of genes necessary to carry out particular functions. This is the reason why, for example, the genes of nerve impulse transmission remain shut off in liver cells: they are just not useful in liver cells and can interfere with liver function.

Moazed and colleagues aim at understanding how cells silence unnecessary genes to maintain their specific identities.

They apply a combination of approaches: from biochemistry to cell biology, from proteomics to genomics, with the common goal of identifying the factors able to mediate heterochromatin assembly and function.

Analyzing silent domains in yeast – single-celled fungi that reproduce by fission – researchers identified a special ingredient playing a central roles in the formation of repressive heterochromatin: noncoding RNAs.

“Nowadays there are a lot of ideas about what small RNAs are capable of doing, and some mechanisms are clearer than others” Moazed explained. “The way these RNAs are generated is a fundamental question, because they specify the parts of the genome that should be silenced”.

In particular, his team showed that small RNA molecules (overall, about 20 nucleotides) work through the RNA interference (RNAi) to regulate gene silencing and expression. Developing many biochemical experiments in fission yeast, they purified the RNA-Induced Transcriptional Silencing (RITS) complex, which directly links the RNAi pathway to heterochromatin assembly.

Moazed’s lab focused on two critical processes for maintenance of heterochromatin, histone methylation and siRNA amplification. These two processes were shown to be mutually dependent

and to form self-reinforcing positive feedback loops of crucial importance for maintenance of silent domains.

Such noncoding RNA-based mechanisms may be involved in regulating heterochromatin formation and gene expression in other organisms. And this is exactly what studies in other laboratories are beginning to reveal.

“Future applications? Using small RNAs to silence genes and reprogram the epigenome” he said. “It may be possible to turn-off genes at the transcriptional level, although many technical challenges have to be overcome before this can be done in systems outside simpler model organisms like yeast”.

The next step, then, is the investigation of other biological pathways that play major roles in regulating heterochromatin: in order to understand better and better the complex, fascinating switches of DNA.

Shaping the eukaryotic transcriptome with chromatin and non-coding RNA

Stephen Buratowski

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, USA

The huge dictionary building up our genome has a very complex punctuation. DNA sequences are just small strings of letters, but understanding their rules is a big challenge.

At Harvard Medical School, the Buratowski Lab studies the marks of gene expressions: in particular, the enzymes that "write" these marks, as well as the proteins that "read" them.

Using yeast as a model system, researchers analyze the mechanism of gene expression in eukaryotes, working on RNA polymerase II transcription initiation and the subsequent processing of the mRNA. Several dozen proteins are required simply to initiate transcription, and many more take actions in other processes linked to transcription. For this reason, understanding transcription means deciphering the functions of every single factor.

During the 15th Armenise-Harvard Symposium Stephen Buratowski, head of the laboratory, presented his approach to unfold the mysteries of transcription's punctuation. His research team focuses on the communication between chromatin and the transcription machinery.

They showed that the act of transcription causes major changes in the nucleosomes that package the gene. Using the yeast Saccharomyces cerevisiae, they discovered that Set1 (a type of histone methyltransferases, or HMT) protein levels are carefully calibrated to the amount of transcription occurring in the cell. Set1 is subject to degradation by the ubiquitin-proteasome system unless it is stabilized by ongoing transcription and histone methylation. Disruption of this feedback loop causes aberrant methylation patterns and gene misregulation.

This mechanism may have clinical relevance, as many leukemia and lymphomas contain translocations in the mammalian MLL1 gene, an HMT related to Set1.

Carrying out genome-wide expression screens to analyze a chromatin "reader" called Set3, Buratowski and colleagues found that the majority of genes whose expression levels changed were repressed by this factor. But what was more unexpected, they demonstrated that these effects strongly correlate with overlapping non-coding transcription. This does not mean that non-coding RNAs themselves mediate the gene expression changes; instead, the latter depend on histone methylations placed over gene promoters by overlapping transcription.

And this is exactly where clinical medicine could find new applications: "I think it is certainly worth thinking about how changes in transcription can affect diseases like cancer" Buratowski concluded. "This is because many diseases are caused by problems in either writing or reading the marks of gene expressions".

Activities of Polycomb Group Proteins in Proliferation and Developmental Control

Diego Pasini

Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

Many human diseases are caused by the loss of cellular identity. Cancer is the clearest example: in all tumors, cells acquire features that lead to abnormal growth and differentiation defects.

But how happens that cells start losing their identity? And what are the mechanisms regulating cell fate during development and differentiation?

Diego Pasini's current research at the European Institute of Oncology revolves around these questions. He aims to understand how diseases like cancer can form, maintain and develop.

During the Armenise-Harvard Symposium, Pasini explained that his lab is interested in studying these mechanisms by focusing on Polycomb Group (PcG) proteins.

PcG are a family of proteins responsible for cellular differentiation during development via transcriptional repression.

"Polycomb Group proteins are master regulators of cell development" said Pasini. "They are also essential for cellular proliferation, and play an active role in cancer formation".

The first to describe the polycomb group was geneticist Edward B. Lewis, who in 1978 observed inDrosophila that PcG was involved in the silencing of Hox gene expression.

Since then, these proteins have been the subject of intense study as it is clear that they are vital for maintenance of cell-type identity and differentiation.

"At the biochemical level, most PcG proteins form two major polycomb repressive complexes: PRC1 and PRC2" explained Pasini.

PRC1 and PRC2 repress transcription respectively by Ubiquitylating Histone H2A lysine (K) 119 and by tri-methylating (me3) Histone H3K27. Deregulation of both PRC1 and PRC2 activities is a common feature of human tumors.

To study the role of PcG proteins in regulating normal and cancer cells proliferation, Pasini and colleagues combine cell culture and in vivo studies. Their experiments demonstrated that PRCs independently regulate cellular proliferation and transformation.

So the ability of PRC1 and PRC2 to promote proliferation is a main feature that links Polycomb Group proteins activity to cancer.

For this reason, PcG inhibition has been proposed as a strategy for tumor treatment.

Joining the Zoo: Cell reprogramming and the rise of human disease models

Giuseppe Testa

Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

Have you ever thought of your body as a roving laboratory? In the very latest years, this is exactly what has happened. A true revolution occurred in cellular biology: it is called "cell reprogramming", and it allows scientists to directly study diseases in human tissues.

Cell reprogramming depends on the seminal derivation of human induced pluripotent stem cells (iPSC) from somatic cells. Born in 2007, this technique has already deeply changed the prospects not only of regenerative medicine but also, and likely in an even shorter timeframe, of our capacity to dissect the genetic contribution to human diseases.

At the European Institute of Oncology, Giuseppe Testa is working on this cutting-edge line of research. His laboratory focuses on the epigenetics of genome programming and reprogramming, in particular the mechanisms enabling lineage commitment and their aberrations in cancer and neurological diseases.

During the Armenise-Harvard Symposium, Testa gave a brilliant overview of what he called the "zoo joining": with pluripotent stem cells onboard, the menagerie of available tissue samples became potentially infinite.

This was unimaginable until the beginning of our century, when model organisms were the main resource to interrogate human disease pathogenesis. Obtaining primary samples directly from patients was very difficult; and when it happened, most of the time it was too late, post mortem or at not so meaningful stages of disease history.

Testa explained how cell reprogramming allowed, for the first time in the history of medicine, to make human genetic variation experimentally tractable through the creation of genetically matched cell lineages. On these lineages it is now possible to decipher and target disease pathogenesis, biological stand-ins or "avatars" of ourselves.

So we are the new models to work on: this means that one's disease is directly studied in vitro on one's DNA, and personalized treatments are more likely to be developed.

The Testa Lab harnesses this potential to develop physiopathologically meaningful models of both cancer and neurodevelopmental disorders.

In cancer, researchers aim at the dissection of the genomic versus epigenomic components: since tumors have them both, cell reprogramming allows to see which component is predominant in every phase of the disease.

Within neurodevelopmental disorders, they focus on a unique range of intellectual disability syndromes (including autism spectrum disorders) caused by mutations or dosage alterations in epigenetic regulators and transcription factors. After reprogramming and analyzing iPSC and their differentiated derivatives with these mutations, it is also possible to screen drugs on them at a very large scale.

These experimental settings, together with their important clinical applications, are a great example of how cellular phenomena have become an interface between molecular biology and medicine.

Testa quoted what biologist Harold Kincaid called the “place holders”, processes for which we have good evidence, but whose nature is unknown. Now cell reprogramming is probably bringing this “unknown” to light.

Spatial Regulation of Molecular Motors

Samara Reck-Peterson

Department of Cell Biology, Harvard Medical School, Boston, USA

The cells that make up our body are constantly busy: they move, divide, and communicate with neighboring cells. At the same time, they need to maintain homeostasis, so that the internal cellular conditions remain stable and relatively constant. Molecular motors, which transport cellular cargos, are responsible for all of these functions. Transport occurs along two types of tracks, called actin filaments and microtubules.

All eukaryotic cells use motors for transport along actin filaments and microtubules, and damage to these transport mechanisms can lead to serious diseases. For example, neurodegenerative and neurodevelopmental diseases are known to result from defects in microtubule-based transport.

For this reason, explaining how microtubule-based intracellular transport works could represent a significant breakthrough in clinical medicine.

The Reck-Peterson Lab at Harvard Medical School is moving in this direction. “We want to understand how the motors of the cell work” explained Samara Reck-Peterson, head of the laboratory. “In particular, we are focusing on the dynein motor”.

Cytoplasmic dynein is the main motor protein driving microtubule-based intercellular transport, together with another family of motors called kinesins. Dynein motors move only towards the minus-ends of microtubules (towards the cell center), while kinesin motor move towards the plus-ends of microtubules (towards the cell periphery).

“We have over 40 kinesin genes that host diverse functions, but dynein is a little bit different” explained Reck-Peterson. “We have 15 different dyneins in the human genome and 14 of these are only found in cells that have cilia or flagella. The remaining cytoplasmic dynein motor has hundreds of cargos, many of which are still to be identified; we know that during interphase dynein has many functions including the transport of mRNAs, RNPs, proteins, and organelles. Viruses are another important cargo, they can hijack dynein to get to the center of the cell”.

To understand the molecular mechanisms underlying these functions of dynein, the Reck-Peterson Lab uses a highly interdisciplinary approach, from cell to systems biology, from biophysics to synthetic biology.

There are four genes implicated in localizing dynein to the plus-end of microtubule. In their experiments, they purified these four proteins and reconstituted in vitro the transport of dynein to the plus-end of the microtubule.

They found that two proteins – homologs of Lis1 and Clip170 – are sufficient to couple dynein to Kip2, a plus-end-directed kinesin. Kip2 transports dynein to the microtubule plus end, but not as a passive passenger: dynein resists its own plus-end-directed motion though its microtubule-binding domain.

Two others microtubule-associated proteins, homologs of Clip170 and EB1, act as processivity factors for Kip2, helping it overcome dynein’s intrinsic minus-end-directed motility.

Thus, there are four main players involved in dynein spatial regulation: two proteins that are required to couple the dynein to kinesin, and then two more proteins that make the kinesin a better motor. This reveals how a minimal system of proteins transports a molecular motor to the start of its track.

“Our main goal now is to understand how this motor works and is regulated,” said Reck-Peterson. “And if we understand that, we are a step closer to discovering why the mutations in the transport machinery cause diseases”.

Mechanisms and regulation of cytoplasmic dynein

Andres Leschziner

Dept. of Molecular and Cellular Biology, Harvard University, Boston, USA

Step after step, walking takes pretty advanced coordination abilities. Legs, muscles, nerves, brain: every single ingredient finds its role, building up the complex and harmonious action of moving.

At microscopic level, almost the same thing happens in your cells, as explained Andres Leschziner during the Armenise-Harvard Symposium. “Molecular motors are the protein machines that ‘walk’ along cytoskeletal tracks” he said. “In particular, cytoplasmic dynein is responsible for transporting most cellular cargo from the periphery towards the cell interior. Its complex motor activity is essential for many functions in eukaryotes, such as chromosome segregation and intracellular transport”.

Together with kinesins and myosins, dyneins make up the three families of molecular motors. However, dyneins are the largest and most complex group, and many of their regulating mechanisms are still unknown.

One of the biggest unsolved questions concerns Lis1, a conserved dynein regulator. Lis1 is known to keep dynein bound to microtubules, but it is not understood how it accomplishes this action.

At Harvard Medical School, the Leschziner Lab teamed up with the Reck-Peterson Lab to unfold the mysteries of dynein using 3D electron microscopy, single-molecule imaging, biochemistry and in vivoassays.

Leschziner and colleagues were particularly interested in understanding how dynein moves along microtubules, components of the cytoskeleton found throughout the cytoplasm.

A 3D structure of the dynein-Lis1 complex was obtained. This model revealed that binding of Lis1 to dynein’s AAA+ ring (which belongs to a superfamily of ring-shaped proteins) physically blocks dynein’s main mechanical element, the “linker”, from making critical interactions with the ring.

“Lis1 is a ubiquitous dynein co-factor, acting as a clutch to uncouple dynein’s cycles of ATP hydrolysis and microtubule binding and release” explained Leschziner.

So there are two different cycles involved: the first (a cycle of force-generating ATP hydrolysis) occurs in the ring-shaped AAA+ motor domain; the second (a cycle of microtubule binding and release) occurs in the microtubule binding domain, located at the end of dynein’s long “leg”.

These two cycles occur 25 nm away from each other, yet their function critically depends on their coordination.

Regulation of dynein is therefore one of the crucial aspects for the complex, articulated world of movement occurring at the cellular level. And apparently a huge responsibility falls on Lis1, playing a central role in regulating dynein’s movements.

Overcoming obstacles: Single-molecule studies of DNA repair

Joseph J. Loparo

Department of Biological Chemistry and Molecular Pharmacology; Harvard Medical School

We can think of DNA as the instruction manual packed inside our cells. If our DNA becomes damaged, then our cells may get the wrong instructions, which can lead to diseases. For this reason, when a DNA lesion occurs, our cells immediately activate their DNA repair mechanisms to fix the problem.

But there are some lesions that the repair machinery fails to find. In this case the protein machinery that copies our DNA can collide with these damaged DNA bases, stopping the DNA replication machinery in its tracks. It is then that our cells have a tough decision to make: do nothing and perhaps die or utilize an error prone DNA copying enzyme that can synthesize through the damage but could introduce disease causing mutations into our DNA.

"This is how cells overcome DNA obstacles: sometimes, it is better for them to take the risk" said Joseph Loparo, head of a laboratory at Harvard Medical School working on DNA damage tolerance and repair.

Gambling as a genetic strategy, in other words. Loparo and colleagues are working to unravel how bacterial cells choose to use these error prone enzymes: "Most bacteria have a single circular chromosome which they copy with a multi-protein machine known as the replisome, their replication machinery" he explained. "The replisome is composed of two replicative polymerase complexes which quickly and accurately copy each parental strand of DNA".

If DNA becomes damaged and the DNA repair machinery is unable to correct the lesion, this can be a block to the replisome.

"Translesion polymerases are specialized DNA polymerases capable of synthesizing over certain DNA lesions that stall the replicative DNA polymerase" said Loparo. "My lab is interested in understanding the mechanisms of this process, and how translesion polymerases are recruited to the replication machinery".

By reconstituting translesion synthesis (TLS) and observing it occur on single DNA molecules in real time, the Loparo Lab showed that the Escherichia coli b clamp, a ring-shaped molecule that encircles DNA and tethers polymerases to their substrates, can simultaneously bind two kinds of polymerases, the replicative polymerase Pol III and the error-prone translesion polymerase, Pol IV. This enables an exchange of the two polymerases and a rapid bypass of a DNA lesion which is in many ways analogous to how one maintains a spare tire in the trunk of their car.

Furthermore, they found that additional binding sites between Pol IV and b act to limit Pol IV dependent DNA synthesis under normal conditions, yet facilitates the displacement of Pol III from the DNA upon the detection of DNA damage.

These results support a new model in which interactions between polymerases and the b clamp act to both inactivate and activate error-prone polymerases. Within this regulatory network, it is like the cell is saying: "I can tell that the replication machinery is in trouble. It is worth utilizing an error prone polymerase as it is my only hope for survival".

This mechanism is also very interesting from a human health perspective, because many of the basic mechanisms of this polymerase regulation likely occur in human cells. Understanding how genetic mutations arise will lead to a better understanding of diseases like cancer.

“The next steps for us are to look at these processes in live bacteria cells, where we have all the physiological complexity” concluded Loparo. “Additionally, we are reconstituting the replication machinery in its entirety in a test tube”.

This could answer once and for all the odd, yet important question: how prone are our cells towards genetic gambling?

Integrating artificial with natural cells

Sheref Mansy

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Crossing the boundary between living and non-living, bringing artificial systems to life. Science fiction? Probably not. At the Centre for Integrative Biology (CIBIO) of the University of Trento, biochemist Sheref Mansy has taken a step forward towards making an artificial cell "breath". This would be a true revolution for traditional cellular biology, which could eventually lead to change the definition itself of life.

"A chick is a living organism, a stone is not. Everybody can clearly see the difference. But is there anything in the middle?" asked Mansy at the Armenise-Harvard symposium audience. He then presented the latest results of his research, showing a new way to address the challenge of artificial life.

The control of cellular behavior largely relies on genetic engineering, but artificial cells could be designed to better regulate cell processes through chemical communication. With his team at CIBIO, Mansy developed an artificial cell which is able to translate a chemical message into a signal that can be sensed by Escherichia coli. This could activate a cellular response otherwise impossible to be detected.

Within this system, the artificial cells work as chemical translators, sensing molecules that E. colialone cannot sense. As a consequence, the artificial cells release a molecule familiar to E. coli, thereby translating an unrecognized chemical message into a well-known one.

This allows a "dialogue" between the artificial and the natural cells, expanding the sensory capabilities of E. coli without altering the genetic content of the bacterium.

Mansy's artificial cell has a complex structure, both from engineering and biological point of views. It is built with a phospholipid vesicle containing isopropyl b-D-1-thiogalactopyranoside (IPTG), DNA, and transcription-translation machinery. The DNA template codes for a previously selected riboswitch, activating translation in response to the presence of theophylline. The theophylline riboswitch controls the synthesis of the pore forming protein a-hemolysin (aHL).

coli alone does not respond to theophylline, and IPTG does not cross the vesicle membrane of the artificial cell in the absence of the pore. And here's exactly where the artificial cells come to action: they allow E. coli to receive the chemical message, thus becoming IPTG-responsive.

"Bacteria do naturally communicate to each other" said Mansy. "Our goal was to see if a single bacterium could still communicate in case one of the cells is artificial. Apparently, the answer is yes."

These results, published in Nature Communications, are a promising basis for possible medical applications. "Our artificial cells degrade in a couple of hours: there are no long-term consequences. This implies that they could be used in biological systems – for example, to identify pollutants – without genetic intervention" explained Mansy.So far this is the first artificial, cell-like system capable of creating a communication pathway between artificial and living cells. The next step will be broadening this approach, making artificial cells able to "talk" with complex biological systems.

“If we had artificial cells that can detect all the signals of the living cells, they could also recognize the signals expressed by problem cells, like cancer” concluded the researcher.

The definition of “living cell” is thus becoming more and more foggy: but this could not be a problem, as far as we change the questions to be addressed. Just as Alan Turing did about 60 years ago, when he gave birth to the field of Artificial Intelligence.

Biogenesis and quality control of oligomeric proteins in the early secretory pathway

Roberto Sitia

Università Vita-Salute San Raffaele, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy

Presto e bene raro avviene. This typical Italian proverb, meaning that it is difficult to work fast and well at the same time, is broadly disregarded by our cells.

As Roberto Sitia explained during the Armenise-Harvard Symposium, many biological processes such as protein secretion must have high fidelity and efficiency.

At San Raffaele Scientific Institute, Sitia addresses a fundamental question in cell biology: how are the size and activity of the different compartments constantly coordinated?

In multicellular organisms, cells must promptly respond to multiple stimuli. To take the right decision, they need to continuously exchange information amongst each other and with the external world, and to unambiguously integrate the corresponding signals.

This task becomes particularly demanding during differentiation or in responses to environmental changes. Sitia and colleagues investigated the molecular mechanisms that allow cells to integrate signalling, protein quality control and sorting in the early secretory compartment.

“The early secretory pathway is emerging as a key hub, performing many difficult tasks at the same time” said Sitia. “ It ensures efficient high-quality release by the protein factory”.

The endoplasmic reticulum (ER) is a multifunctional compartment found in all eukaryotic cells. “From the ER, secretory proteins begin their journey towards their final destinations, the organelles of the exocytic and endocytic compartments, the plasma membrane or the extracellular space” Sitia explained. His research team discovered that ERp44, a multi task protein at the ER-Golgi interface, is a master regulator in this hub.

Fidelity of protein-based intracellular communication is guaranteed by quality control (QC) mechanisms located at the ER–Golgi interface, which restrict forward transport to native proteins.

And here is where ERp44 comes to action: Sitia found that it is a key regulator of protein secretion, Ca2+ signalling and redox regulation.

“If the signals conveyed by the ERp44-centered molecular hub are not working, cells suffer; but if they are too much, the cell’s function are also compromised” he said.

We are beginning to understand what makes ERp44 capable of satisfying its multiple tasks, allowing the protein factory to perform *presto e bene*.

Imaging endocytosis with high spatiotemporal resolution

Tomas Kirchhausen

Department of Cell Biology, Harvard Medical School, Boston, USA

"Seeing is believing. Biology is based on observation. But what I want to do is also measuring: this quantification can be size, can be length, can be volume, can be number of molecules, can be where are the molecules in a given moment".

Biologist Tomas Kirchhausen knows what he's talking about. His laboratory at Harvard Medical School is one of the few in the world to combine standard and 3D microscopy capable of giving rapid, high-precision three-dimensional imaging of living cells.

This cutting-edge technology is put at the service of understanding the movement of membrane proteins throughout cells. These mechanisms are of key importance for the cell's sorting machineries, and can be hijacked by toxins, viruses and bacterial pathogens.

Studying how cells can be attacked, Kirchhausen Lab aims at finding treatment for diseases depending on viral infection and pathogen invasion: from cancer to LGMD2B/Miyoshi muscular dystrophies, from Alzheimer disease to ALS (amyotrophic lateral sclerosis), as well as other neurological diseases.

In preparation to these studies, his group determined the first structure at near atomic resolution of clathrin, a protein playing a major role in the creation of coated vesicles. Clathrin has a triskelion shape composed of three clathrin heavy chains and three light chains and they form the coat surrounding the vesicles whose lattice often appears as the seem of a soccer ball.

Live-cell and single fluorescence microscopy imaging were used to "see" in three dimensions the molecular events and the intracellular compartments responsible for the formation of clathrin-coated pits and coated vesicles – a conserved "nano-machine" that generates intracellular vesicular carriers in all animals and plants.

"In our studies we have two opposite extremes" explained Kirchhausen. "The first one is the use of methods giving a complete picture, like crystallography or NMR; the second extreme is the use of fluorescence microscopy visualization methods going to a very low resolution, with a lot of dynamics".

"So the idea is to combine these two extremes to have a clearer view of the mechanism of endocytosis" he said.

Endocytosis, the process that cells use to ingest molecules (like LDL – the bad cholesterol and certain viruses) by engulfing them, was observed with the microscopy visualization techniques. They allowed sufficient temporal and spatial resolution to follow the life of a single clathrin coated pit.

"The initiation process of endocytosis is highly stochastic" continued Kirchhausen. "We observed that the clathrin adaptors, proteins that connect clathrin with the membrane surrounding the clathrin coat arrive and depart from the membrane with very low binding. When we have the adaptors in the right place, the triskelion can map this interaction, that stabilizes the structure for a few seconds and formation of the pit ensues".

The level of detail reached by Kirchhausen's observation is pioneer in quantitative biology. "Now that we have the right techniques, we can count how many molecules of a certain type are required for the different steps of endocytosis: a question that had remained unanswered for 30 years" commented Emanuele Cocucci, postdoc working in Kirchhausen Lab. "The next steps will be understanding the total number of molecules involved in endocytosis, including all the receptors. So we have to go even deeper".

In the near future, temporal resolution and spatial precision of 3D microscopy could then reach higher details. But as Kirchhausen pointed out, it is crucial not to lose the bigger picture. "It's like the car traffic" he said. "We can see the global differences between the traffic of Rome and Milan, or we can count the details, like the colours of the car, the drivers, the number of passengers, and so on. Both type of quantifications are important".

Mono-ADP-ribosylation and membrane trafficking

Daniela Corda

Institute of Protein Biochemistry, National Research Council, Napoli, Italy

Between 20.000 and 25.000: this is approximately the number of genes in your genome. But if you think this quantity is high, you should probably look at your proteome. In fact the number of proteins making up the human proteome is estimated at over 1 million.

Over the last decades, scientists have discovered that single genes encode multiple proteins, and this makes the proteome far more complex than the genome.

But there is a secret ingredient which can facilitate studying this complexity: it is the process called protein post-translational modifications (PTMs). This is a fundamental step occurring after protein biosynthesis, playing a key role in determining the regulation and function of proteins and other cellular molecules.

Most often mediated by enzymatic activity, post-translational modifications can occur at any step during the "life cycle" of a protein, so understanding these processes is crucial to unfold the complexity of proteome.

At the Institute of Protein Biochemistry of the Italian National Research Council, Daniela Corda is leading pioneering research on PTMs. Among the various post-translational modifications, she is focusing on mono-ADP-ribosylation (mono-ADPR). This reaction has an important physiological role in cellular processes such as membrane traffic, immune response, DNA repair and signalling.

Corda was one of the first scientists to look at mono-ADP-ribosylation as a key mechanism in cell biology.

"For many years it has been considered a toxic reaction" she explained. "But together with other colleagues, I thought that if a toxin modifies a protein, perhaps this modification is interfering with a physiological mechanism. I thought that if a toxin modifies a protein, perhaps this modification is interfering with a physiological mechanism. This means that isolated pathological mechanisms (without their physiological counterpart) do not exist".

This intuition shook up the understanding of the protein enzymatic activity. Studying how toxins induce the ADP-ribosylation of proteins, Corda and other colleagues could identify specific cell mechanisms that were previously unknown.

In particular, the Corda laboratory studied brefeldin A (BFA), a fungal toxin causing the disassembly of the Golgi complex membranes. BFA induces the ADP-ribosylation of BARS, a protein involved in the fission of membranes at several traffic steps of the secretory and endocytic pathways.

The protein BARS was discovered 20 years ago by Daniela Corda team, and now turned out to be an essential element of the membrane fission machinery.

Selectively analyzing the brefeldin A activity, researchers identified an intermediate that covalently binds BARS, called BFA-ADP-ribose conjugate (BAC).

“BAC modifies this protein only, it is very specific” said Corda. “So if we can mimic BAC, we can inhibit a single protein, BARS. This is what we are working on: synthetizing BAC analogues, and performing virtual screenings to selects other small molecules with similar activity”.

The inhibition by small molecules of proteins known to cause a disease may eventually lead to clinical applications, in cancer as in other pathologies.

“We are testing these small molecules in vitro hoping to soon find an application in tumors such as lymphoma and breast cancer” Corda concluded.

BARS, a relatively new entry in scientific labs, is therefore shedding a new light on the cellular functions control. And this protein could soon become a pharmacological target for anticancer therapies.

Control systems of membrane transport at the interface between the endoplasmic reticulum and the Golgi.

Alberto Luini

Istituto di Biochimica delle Proteine (IBP), CNR Napoli, Italy; Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.

If you lose your balance, you will probably fall. Your cells behave pretty much the same way: they have an internal equilibrium that needs to remain constant, otherwise they "fall". This cellular balance is called homeostasis, which guarantees the cells' internal stability.

Maintaining homeostasis despite the variation of internal and external conditions is a fundamental task our cellular system needs to fulfill. So understanding this task can reveal important aspects of our cell activity.

At the Italian National Research Council, Alberto Luini and his research group study the process of homeostasis starting from the complex cell membrane transport apparatus.

Within this system, variations in membrane fluxes from the endoplasmic reticulum (ER) to the Golgi complex are balanced by opposite fluxes from the Golgi to the ER, to maintain homeostasis between the two organelles.

As Luini explained to the Armenise-Harvard Symposium audience, he and his team described a molecular device that balances transport fluxes by integrating signal transduction cascades with the transport machinery.

In particular, they found that ER-to-Golgi transport activates the KDEL receptor at the Golgi. This triggers a cascade involving Gs and adenylyl cyclase and phosphodiesterase isoforms, and then PKA activation, and results in the phosphorylation of proteins involved in retrograde traffic. This induces recycling to the ER and tends to balance transport fluxes between ER and Golgi.

Moreover, the KDEL receptor activates CREB1 and other transcription factors that up-regulate transport-related genes. In this way a Golgi-based cell-autonomous control system maintains transport homeostasis through both signaling and transcriptional networks.

Another interesting thing is that this model was obtained using very advanced microscopy including an extremely powerful technique: correlative microscopy. It was developed for the first time in Luini's laboratory, and it is used to study in vivo dynamics and ultrastructure of intracellular structures at incredible levels of detail.

Combining correlative light and electron microscopy, researchers were able to see dynamic functional assays in live cells directly with high resolution 3D morphology.

This way it became possible to observe the control systems of membrane transport integrating information on dynamics, ultrastructure and molecular composition assembly of molecular machinery. At the same time, "seeing" the engine that allows our cells to maintain their balance.

Myosin VI Bridges Ubiquitin Signaling and Cell Migration

Simona Polo

IFOM Fondazione Istituto FIRC di Oncologia Molecolare, 20139 Milan, Italy; Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy.

Human cells cannot freely travel around the body: their movement is tightly regulated and normally quite limited. But cancer cells can lose this control, travelling in the blood stream through the well-know process of metastasis. Myosin VI is a motor-protein able to "travel" along actin filaments and is involved in tumor formation and metastasis.

At the IFOM – FIRC Institute of Molecular Oncology of Milan, Simona Polo and colleagues are now investigating the control system of Myosin VI from an alternative point of view. This was the focus of the fascinating talk given by Dr. Polo during the Armenise-Harvard Symposium: Myosin VI plays an explicit role in cell migration, under both pathological and normal conditions.

Dr Polo is the leader of a research group investigating the mechanisms of regulation mediated by ubiquitin. Ubiquitin is a regulatory protein that has been "ubiquitously" found in almost all tissues; its addiction to other proteins can affect them in many ways.

"The best-known function of ubiquitin is the degradation via the proteasome, but about 10 years ago a novel function of ubiquitination in signalling was discovered" she explained. "We are studying this mechanism, and showed that Myosin IV harbours a particular ubiquitin binding domain (UBD) different from any previously described UBD".

UBDs are a collection of modular protein domains that non-covalently bind to ubiquitin; the new UBD identified by Polo's team was given the name of Myosin VI Ubiquitin Binding (MyUb) domain.

Ubiquitin can come in different flavours and they all perform different functions into the cells, most of which are still unknown.

"We found out that, differently from the vast majority of the UBDs that show no binding specificity, MyUb domains have a clear preference for K63 dimers implicating that Myosin VI and ubiquitin interaction is not for protein degradation" said Polo.

These findings prepared the ground to explain the role of Ub and MIU-MyUb domains in the physiological and pathological regulation of Myosin VI.

"It has been observed an overexpression of Myosin VI in tumor tissues" said Polo. "But we found out that this is not a generic overexpression: it is limited to the specific form able to interact with ubiquitin. We are now dissecting in details the unprecedented role played by ubiquitin in cell migration".

New insights into proteasome mechanisms in normal and disease states

Alfred Goldberg

Dept Cell Biology, Harvard Medical School, Boston, USA

"Proteasome is smarter than you think!" This exclamation crowned the conclusion of the sharp and bright presentation byAlfred Goldberg, during the third day of the Armenise-Harvard Symposium.

His laboratory at Harvard Medical School studies how proteins in our body are constantly being fabricated and then broken down into amino acids. Proteasomes take pride of place in this process: they are protein complexes whose main function is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.

The smart proteasome Goldberg referred to is the 26S proteasome, the major site for protein degradation in mammalian cells. In recent years, inhibitors of its 20S peptidase activities (e.g. bortezomib) have greatly advanced the treatment of multiple myeloma.

Moreover, the processing of ubiquitin (a small regulatory protein found in almost all tissues of eukaryotic organisms) conjugates by 26S proteasome's 19S regulatory particle involves many enzymatic steps that may be targets for drug development.

So the study of 26S proteasome has immediate application also in clinical medicine, and this is one of the reasons why Alfred Goldberg took it as the "main character" of his research and his talk. Here this character became the protagonist of four short stories: four surprising mechanisms controlling proteasome function.

"The first story is that the proteasome is tightly regulated" he explained. "We now think we understood the mechanism, the overall linkage between the ubiquitin chain and the breaking down of the proteins: the regulation that occurs in that process".

In fact Goldberd Lab discovered that when mammalian 26S proteasomes are inhibited, the ubiquitin-receptor subunit, Rpn13, becomes polyubiquitinated by a 26S-associated ubiquitin ligase. This modification prevents binding of ubiquitinated substrates, and presumably evolved to prevent build-up of conjugates when proteasome function is stalled.

And here we come to the second story. "The surprise is that proteasome triggers unexpected responses" Goldberg told. "When the proteasome is inhibited, it has a mechanism saying: 'don't give me anymore ubiquinated proteins!' It's a very advanced self-regulated mechanism".

Proteasome regulation concerns the third story as well: "We found that when the proteasome is partially inhibited, even just a little bit, the signals of production of new proteasomes evolve very specifically. This is a very smart structure that knows how to get the cell to compensate possible problems".

The fourth story is about proteasome and disease. "Proteasome is really affected in common neurodegenerative diseases: this had been suggested many times, but with no clear evidences" said Goldberg. "We have used new techniques to show in mouse model disease of frontotemporal dementia, which is very close to Alzheimer disease because has mutations in Tau gene".

In this case the proteasomes are defected: this is tightly bound with disease development in mice. “Our hypothesis is that in all tmajor neurodegenerative diseases there is a progressive failure to degrade the ubiquinated conjugates, and this condition is associated to the accumulation of tau and phospho-tau” concluded Goldberg.

Connecting these four stories could get the proteasome mechanism to work better, improving Neurodegenerative diseases therapies. Taking at the same time advantage of the proteasome’s unexpected smartness.

Towards in vitro reconstitution of spindle checkpoint signaling

Andrea Musacchio

Department of Mechanistic Cell Biology, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany

The distribution of the parental genome to two daughter cells during mitosis and meiosis is the essence of gene inheritance and therefore of life itself. Not surprisingly, therefore, this process involves what is probably the most complex ensemble of cellular molecular machinery and provides an astounding example of the ability of biological matter to self-organize.

The mitotic spindle, a structure made of microtubules, molecular motors and their regulators, hosts a crucially large fraction of the machinery of cell division. This bipolar, elongated, and remarkably dynamic structure defines the division plane of the mother cell, and devotes itself to the capture of chromosomes, to their clustering in the middle plane, and to their subsequent segregation to the daughter cells. Thus, the mitotic spindle is crucial to ensure that the daughter cells inherit exactly the same number and type of chromosomes, therefore guaranteeing cellular and organismal viability.

Despite the importance of the spindle for cell division, the exact molecular basis of its function remains poorly understood. Trying to unfold this mystery is the main goal of Andrea Musacchio at the Max-Planck-Institute of Molecular Physiology in Dortmund, Germany. Musacchio leads a research team focused on complex protein scaffolds known as kinetochores. Kinetochores are large proteinaceous structures built on the centromere region of chromosomes. Their primary function is to provide a site of attachment of chromosomes to the mitotic spindle.

Kinetochores play a second, subtler function that is however crucial for accurate cell division: they control a feedback mechanism known as mitotic checkpoint, or spindle assembly checkpoint (SAC). The function of this checkpoint is to prevent separation of the duplicated chromosomes – the sister chromatids – until they have properly attached to the spindle apparatus. Dysfunction of the SAC may result in incorrect partitioning of the sister chromatids to the daughter cells, creating a pathological cell condition known as aneuploidy. Such condition strongly correlates with tumorigenesis, supporting the speculation that checkpoint dysfunction may be – among others – a prominent cause of transformation.

Over the last years, Musacchio's laboratory has made significant contributions to the field: "We reconstituted several kinetochore sub-complexes in the inner and the outer kinetochore, the regions of the kinetochore implicated in centromere binding and microtubule binding and checkpoint control, respectively", said the researcher.

Indeed, biochemical reconstitution, coupled with medium- or high-resolution structural investigations is playing a leading role in developing an understanding of kinetochore organization. For instance, Musacchio and colleagues were recently able for the first time to gain a detailed view of a crucial kinetochore sub-complex made of four subunits, the CENP-HIKM complex.

The next challenge is explaining the relationship between kinetochores and checkpoint control: this is what Musacchio called the "third decade of checkpoint studies", after the discovery of the main checkpoint components (1991-2000) and the investigation of their interactions (2001-2010). To address this challenge, his research group is approaching the reconstitution in vitro of spindle

checkpoint signalling. This effort follows a traditional path of "reductionist" studies that interpret the emergence of complex biological functions as a result of specific interactions of the macromolecular building blocks that populate cells.

The scale of ambition implicit in Musacchio's plans, however, transcends that of most current efforts. "The word 'towards' is very important: we're still not there" pointed out Musacchio. "But we believe that it is possible to reconstitute the catalytic apparatus of the checkpoint on reconstituted kinetochores". In vitro reconstitution of this complex biological structure will revolutionize the understanding of chromosomes division during mitosis, shedding a new light on one of the most exciting examples of self-organization in living matter.

The autophagy signaling network in the coordination of a cell's response

Francesco Cecconi

Unit of Cell Stress and Survival, Danish Cancer Society Research Center, 2100 Copenhagen, Denmark; Department of Biology, University of Rome Tor Vergata, Rome, Italy

The world "cannibalism" usually conjures up images of primitive and brutal practices. But deep down, we are all cannibals: our cells are constantly eating themselves, discarding of their redundant molecular garbage through a process known as autophagy.

Deriving from a Greek term meaning "self eating", autophagy is most of the times a surviving mechanism, allowing cells to "recycle" their biological waste. And to many scientists' surprise, recent discoveries have shown that faulty autophagy mechanisms contribute to the development of various diseases.

Francesco Cecconi's laboratory is committed to unraveling the upstream regulation of autophagy and elucidating the role it plays in three different pathological conditions: neurodegeneration, autoimmunity and cancer.

The latter in particular is still object of discussion: "In cancer there is a sort of controversy going on about the role of autophagy" explained Cecconi. "If we knock-out an autophagy gene, then we get accumulations of toxic compounds that autophagy can't help getting rid of. For example, damaged tissues can overproduce ROS, which can damage DNA causing chromosome instability and cancer. In this case, mutations in the autophagy genes set are responsible for cancerogenesis".

On the other side, autophagy can have the opposite function, being even detrimental for the patient. "If a tumor originates from a completely different set of mutations and we have the autophagy system perfectly working within the cells, then the autophagy is helping cancer cells to survive".

There is then in cancer a dual function of autophagy: depending on the tumor origin, autophagy should either be pushed to work or blocked.

In this context, Cecconi's lab has identified a novel protein called Ambra1 (Activating Molecule in Beclin 1-Regulated Autophagy), playing multiple roles as a scaffold factor in autophagy control.

"We are studying the function of Ambra1 in cell cycle regulation and its implications in tumor insurgence" said Cecconi.

Together with his research team, he developed an experiment to understand the role of Ambra1 in cancer. The results could shed a new light on the link between autophagy and tumor development.

"I hope I convinced you about the importance of this protein" concluded Cecconi to the Armenise-Harvard audience, and he probably did.

The signaling mechanisms underlying the anti-tumor activity of metformin

Gianni Cesareni

Department of Biology, University of Rome Tor Vergata, Rome Italy

Fighting cancer with a well-known medicine. A few years ago, this hypothesis dominated many scientific magazines, as a new possible frontier for tumor treatment.

The promising medicine was metformin, the most frequently prescribed drug for type 2 diabetes patients. These findings immediately triggered several clinical trials. But the mechanisms underlying the anti-tumor activity of metformin weren't fully clarified, and its role as a cancer suppressor is still under examination.

At the University of Rome, Tor Vergata, Gianni Cesareni used a new approach to address these questions. Together with his research group, he decided to look at the behavior of a cancer cell as a system and not as a simple linear combination of its parts. These relatively new approach is commonly referred to as systems biology, as explained during the 15th Armenise-Harvard Symposium.

"We look at the cell in its entirety, aiming at describing how metformin perturbs the physiological processes of the cancer cell thereby preventing its proliferation" he explained. "This is a new approach in modern biology, aiming to describe the cell as a system and not simply as the sum of its parts".

In order to work out metformin's role in cancer, the Cesareni Lab applied a combination of high content multi-parametric analysis with logic modeling and simulation techniques. The objective was to map cell perturbations on complex logic networks.

In this way they obtained a predictive cell-specific logic model, later applied to functionally characterize the molecular mechanism underlying the anti-cancer activity of metformin.

"We analyzed how signaling networks are rewired in breast cancer cells upon metformin treatment" said Cesareni. "First, we monitored the activation of about 20 key signaling proteins in cancer cells treated with a variety of perturbations before and after metformin treatment. Next, we built two cell specific network models of metformin for treated and untreated cells".

The results were extremely interesting: the experiments showed that metformin rewires the signaling networks, modifying different connections between signaling proteins.

"We found that metformin changes the structure of the networks underlying some cell pathways" explained the researcher. "So the cells become more sensitive to topic treatments and less prone to proliferation".

To sum up, metformin doesn't kill the tumor cells, but make them weaker. And this is certainly quite a good starting point.

Mechanistic events regulating notch signal transduction

Stephen C. Blacklow

Harvard Medical School, Boston, MA, USA; Dana Farber Cancer Institute, Boston, USA

This story begins in 1917, when Thomas Hunt Morgan identified alleles of a gene responsible for notching of the wings in fruit flies. Subsequent work, led in large part by Spyros Artavanis-Tsakonas of Harvard Medical School, revealed that the gene responsible for this phenotype, called Notch, is a highly conserved receptor in a signal transduction pathway that control numerous cell fate decisions in organisms ranging from flies to humans. Whereas normal Notch signaling makes it possible for adjacent cells to communicate effectively with each other, dysregulated Notch signaling often as a result of mutations in the Notch receptors or their ligands, contributes to a variety of human diseases, including neurodegeneration and cancer.

Thus, understanding how normal and aberrant Notch signaling takes place has important implications for clinical medicine. At Harvard Medical School, this is one task of the Blacklow Lab: to understand the molecular logic of cell-surface receptors such as Notch proteins, which are highly relevant to human physiology and disease.

“A major focus of the laboratory is to understand how normal notch signaling takes place. This knowledge will also yield important insights into the effects of aberrant Notch signaling in cancer and other diseases” said Stephen Blacklow, head of the laboratory.

His efforts are mainly directed toward understanding how activation is induced by ligands and how notch cooperates with other factors to regulate target gene transcription.

“One central unanswered question in signaling is how the binding site for ligands can communicate with a regulatory switch that is 600 Angstroms (a long distance in molecular terms) away, and how ligand stimulation normally relieves autoinhibition” said Blacklow.

One important clue to answering this question is that signal-sending cells rely on endocytosis of the ligands to deliver the signal to the Notch receptors on the signal-receiving cells. Two possibilities might explain this dependence: one is that ligand endocytosis exerts mechanical force to pull on the Notch receptor in order to expose a metalloprotease cleavage site to activating proteolysis, and the second is that ligands rely on endocytosis for an “activating” modification that renders them competent for signaling. In other words, endocytosis and recycling of ligand may be required for conversion of ligands from a latent state into an active form.

The laboratory is currently working on distinguishing between these two possibilities. “It is possible to substitute the normal ligand-receptor interaction with a synthetic system, which retains the dependence on ligand endocytosis in sending cells, and on the activation switch in receiving cells,” said Blacklow. “So the working model that can be tested in the synthetic system is that endocytosis is actually supplying the force that opens the regulatory switch”.

Mechanism of inhibition of the epidermal growth factor receptor by Mig6

Michael J. Eck

Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School; Cancer Biology, Dana-Farber Cancer Institute, Boston, USA

Lung cancer is the leading killer in the world among tumors. According to the World Health Organization 1,59 million of deaths for lung cancer were registered in 2012.

Although lung cancer is closely linked to smoking, every year it affects tens of thousands of people who never smoked.

In recent years, scientists continued to unravel genetic factors involved in cancer development. And for lung cancer in nonsmokers, there is an increased likelihood of finding a somatic mutation of the epidermal growth factor receptor (EGFR). EGFR is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands.

At Harvard Medical School, Michael Eck is working on the structure of signaling complexes that underlie cancer, with a particular focus on lung cancer-derived mutations in the epidermal growth factor receptor. His lab's structural approaches are also used to facilitate development of anti-cancer drugs.

"About 15% of lung cancers are caused by EGFR mutations" explained Eck during the Armenise-Harvard Symposium. "In particular, somatic mutations in EGFR are a major cause of non-small cell lung cancer".

He presented the latest results of his laboratory: the discovery of a quite unexpected mechanism of inhibition of EGFR by an endogenous regulator, Mig6.

Mig6 (Mitogen-induced gene 6, also called RALT) is a feedback inhibitor of EGFR family members that acts by directly binding activated EGFR, inhibiting its catalytic activity and directing its internalization and degradation.

Mig6 is probably a tumor suppressor: this means that its loss leads to tumor formation. In particular, focal deletions spanning its chromosomal locus occur in GBM (Glioblastoma multiforme) and lung cancer.

His studies revealed that Mig6 is actually a "mechanism-based" inhibitor of EGFR. "EGFR is trapped in the act of phosphorylating Mig6" explained Eck. "Once the EGFR kinase phosphorylates Mig6, it is effectively irreversibly inhibited".

These findings may lead to new therapeutic strategies for lung and other cancers caused by mutant EGFR.

Connecting the machineries of cell fate determination and tumor suppression in mammary stem cells

Pier Paolo Di Fiore

Fondazione IFOM-Istituto FIRC di Oncologia Molecolare, Milan Italy

It is hard to imagine that stem cells, the most "immaculate" biological existing material, could lead to cancer. But according to a recent theory, "stem-like" cancer cells are responsible for the generation of tumors and for sustaining tumor growth.

This model predicts the existence of cancer stem cells (CSCs) with properties characteristically associated to normal stem cells, such as self-renewal, multipotency and quiescence.

At the Institute of Molecular Oncology Foundation, Pier Paolo Di Fiore is exploring this challenging stem-cell theory of cancer. He is investigating the molecular mechanisms governing the maintenance of the stem cell compartment in normal tissues, and how these mechanisms are subverted in cancer.

During the Armenise-Harvard Symposium closing talk, he explained how is lab is addressing this study performing both basic and translational cancer research.

"The existence of cancer stem cells has been proved for a number of cancer types, including breast cancer" he said. "However, the breast stem cell compartment remains poorly characterized due to the lack of reliable techniques for their identification and isolation. We have developed a new technique to specifically label and purify breast stem cells from mammary gland tissue".

This technique exploits both the propensity of breast stem cells to generate mammospheres (3D clusters of cells) in suspension culture, and the relative quiescence of stem cells compared to other breast cell types, when propagated in vitro.

Di Fiore and colleagues are using purified normal and cancer stem cells to isolate a "stemness" signature from which they can extract diagnostic, prognostic and therapeutic markers that can then be evaluated for testing in clinical trials.

In this context, a crucial role is played by the mechanisms of asymmetric cell division. In particular, researchers are investigating whether, and how, Numb and endocytosis-based mechanisms are involved in the regulation of asymmetric cell division of human breast stem cells.

"Numb is a cell fate determinant that by asymmetrically partitioning at mitosis controls binary cell fate decisions" explained Di Fiore. "In human breast cancers, there is frequent loss of Numb expression, due to its exaggerated ubiquitination and ensuing degradation".

This Numb loss causes alterations in two major downstream pathways. On the one hand, lack of Numb allows for unchecked signaling activity of the Notch receptor. On the other, lack of Numb causes attenuation of the p53 signaling pathway. Tumors displaying loss-of-Numb expression are addicted to this event and to its molecular consequences.

Di Fiore claimed that this leads to a first important conclusion, which also constituted the "take-home message" of his talk: "When you have high Numb, you have high p53; when you have low Numb, you have low p53".

This suggests a double role of Numb: it is probably a tumor suppressor, and its action causes decreased p53 activity in breast cancers. Therefore, Numb controls both an oncogenic pathway and a tumor suppressor pathway, and this may lead to new promising clinical applications to fight cancer stem cells.

the giovanni ARMENISE HARVARD foundation

Armenise-Harvard Symposium and young researchers: the Career Development Award

“My father believed, and so do I, that superb results most often come from collaboration. The Foundation intends to continue looking for ways to enhance conversation and cooperative work on both sides of the Atlantic”.

With these words the Armenise-Harvard Foundation’s Chairman, Count Giampiero Auletta Armenise, gave a tribute to his late father, founder Count Giovanni Auletta Armenise, on the last day of the symposium. He greatly summarized the Foundation’s mission: establishing a multidisciplinary-based science research.

The 15th Armenise-Harvard Symposium fully reflected this vision. In the end, everyone agreed it had been an exciting meeting, with a strong interdisciplinary approach.

Along with the scientific sessions, over 30 scientists presented posters. This gave group leaders and younger researchers the chance to talk over their work, finding new collaboration opportunities.

This is the spirit of the whole Foundation, best expressed by its most forward-looking grant program: the Career Development Award (CDA). Since 2001, the Armenise-Harvard Foundation has funded the development of talented young scientists, establishing collaborative relationships between Italian researchers and Harvard Medical School.

In the last 14 years, 20 scientists have moved to Italy from elsewhere around the world, supported by the Foundation. They set up their own labs all over the country, from Palermo to Trento.

The 15th Armenise-Harvard Symposium witnessed the success of this funding program. 8 Career Development Awardees attended the meeting: Vincenzo Costanzo, Federico Forneris, Claudia Lodovichi, Marie Laure Baudet, Rosella Visintin, Tiziana Bonaldi, Stefano Gustincich and Sheref Mansy, who also was one of the speakers. All of them are developing a promising career in science.

“This is one of the few opportunities to independently work in Italy on a specific research program” commented Vincenzo Costanzo, winner of the 2013 CDA grant. He recently moved to the Vertebrate Genome Stability, IFOM Istituto FIRC di Oncologia Molecolare in Milan, where he is working on the role of DNA damage response factors in vertebrate DNA replication.

“We study how DNA repairs itself, and why tumor cells are not capable of fulfilling this task” he explained. “Cells are like planes: every single mechanism is related to many others. If one of these mechanisms is damaged, a DNA damage response is activated. We want to understand how to selectively intervene on tumor cells, which defects in the specific genes of the DNA damage response”.

To this end, Vincenzo Costanzo is applying a multidisciplinary approach – the same vision broadly promoted during the 15th Armenise-Harvard Symposium.

As Count Giampiero Auletta Armenise stated: “Our founders believed in looking at problems from multiple angles, and the Foundation will continue to pursue this goal”.

Glossary

Actin: a globular multi-functional protein found that forms microfilaments. It is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Actin participates in many important cellular processes, including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signalling, and the establishment and maintenance of cell junctions and cell shape.

Autophagy: an evolutionarily conserved catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. Although originally classified as a type of programmed cell death, autophagy is more widely viewed as a basic cell survival mechanism to combat environmental stressors

Chromatin: a complex of macromolecules found in cells, consisting of DNA, protein and RNA. Its primary functions are: to package DNA into a smaller volume to fit in the cell; to reinforce the DNA macromolecule to allow mitosis; to prevent DNA damage; 4to control gene expression and DNA replication.

Clathrin: a protein that plays a major role in the formation of coated vesicles. It forms a triskelion shape composed of three clathrin heavy chains and three light chains.

Dynein: a motor protein converting the chemical energy contained in ATP into the mechanical energy of movement. It transports various cellular cargo by "walking" along cytoskeletal microtubules towards the minus-end of the microtubule, which is usually oriented towards the cell center.

Endocytosis: an energy-using process by which cells absorb molecules (such as proteins) by engulfing them.

Epidermal growth factor receptor: the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands.

Heterochromatin: a tightly packed form of DNA, which comes in different varieties. These varieties lie on a continuum between the two extremes of constitutive and facultative heterochromatin. Both play a role in the expression of genes, where constitutive heterochromatin can affect the genes near them (position-effect variegation) and where facultative heterochromatin is the result of genes that are silenced through a mechanism such as histone deacetylation or piRNA through RNAi.

Histone: a highly alkaline protein found in eukaryotic cell nuclei that packages and orders the DNA into structural units called nucleosomes. Histones are the chief protein components of chromatin, acting as spools around which DNA winds, and play a role in gene regulation.

Histone methylation: a process by which methyl groups are transferred to amino acids of histone proteins of chromosomes. Depending on the target site, methylation can modify histones so that different portions of chromatin are activated or inactivated. This process is critical for the regulation of gene expression that allows different cells to express different portions of the genome.

Histone methyltransferases (HMT): histone-modifying enzymes that catalyze the transfer of one, two, or three methyl groups to lysine and arginine residues of histone proteins.

Kinesin: a protein belonging to a class of motor proteins found in eukaryotic cells. Kinesins move along microtubule filaments, and are powered by the hydrolysis of ATP. Most kinesins walk towards the plus end of a microtubule, which, in most cells, entails transporting cargo from the centre of the cell towards the periphery.

Messenger RNA (mRNA): a large family of RNA molecules that convey genetic information from DNA to the ribosome, where they specify the amino acid sequence of the protein products of gene expression.

Metformin: an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.

Microtubule: a component of the cytoskeleton, found throughout the cytoplasm. Microtubules are formed by the polymerization of a dimer of two globular proteins, alpha and beta tubulin; they are involved in maintaining the structure of the cell and, together with microfilaments and intermediate filaments, they form the cytoskeleton.

Myosin: a protein of ATP-dependent motor family, best known for its role in muscle contraction and its involvement in a wide range of other eukaryotic motility processes. Myosins are responsible for actin-based motility.

Notch-1: a human gene encoding a member of the Notch family. Notch family members play a role in a variety of developmental processes by controlling cell fate decisions.

Notch proteins: a family of transmembrane proteins with repeated extracellular EGF domains and the notch (or DSL) domains. These proteins are involved in lateral inhibition in embryogenesis.

Notch signaling pathway: a highly conserved cell signaling system present in most multicellular organisms. Notch signaling promotes proliferative signaling during neurogenesis, and its activity is inhibited by Numb to promote neural differentiation.

Numb: a protein that in humans is encoded by the NUMB gene. It plays a role in the determination of cell fates during development.

Phosphorylation: the addition of a phosphate (PO_4^{3-}) group to a protein or other organic molecule. Phosphorylation turns many protein enzymes on and off, thereby altering their function and activity. Protein phosphorylation is one type of post-translational modification.

Proteasomes: protein complexes inside all eukaryotes and archaea, and in some bacteria. In eukaryotes, they are located in the nucleus and the cytoplasm. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.

Reactive oxygen species (ROS): chemically reactive molecules containing oxygen. ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis.

RNA interference (RNAi): a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules.

RNA-induced transcriptional silencing (RITS): a form of RNA interference by which short RNA molecules – such as small interfering RNA (siRNA) – trigger the downregulation of transcription of a particular gene or genomic region.

Yeast: single-celled fungi that reproduce by budding. Yeast size can vary greatly depending on the species, typically measuring 3–4 μm in diameter, although some yeasts can reach over 40 μm.