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RESEARCH CONFERENCES

ESF-EMBO Symposium

Antiviral Applications of RNA Interference

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) • Spain
30 May-04 June 2010

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Co-Chairs: **Jens Kurreck** - University of Technology Berlin, DE
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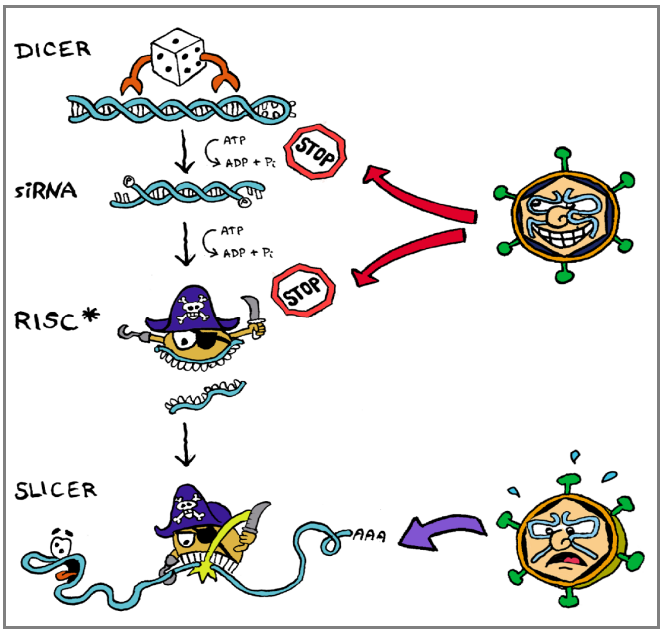

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Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

Virus infections form a major threat for humans, and most of us will vividly remember the danger of some recent emerging infections. For instance, we witnessed the SARS epidemic in 2003 and more recently the spread of a new pandemic influenza virus, which luckily turned out to be a relatively weak pathogen. Other viral pathogens like the human immunodeficiency virus (HIV-1) and the hepatitis B and C viruses (HBV and HCV) cause many million new infections per year, establishing chronic infections that eventually lead to deadly diseases when left untreated. The economic losses attributed to viruses that infect plants, including major agricultural crops, are possibly less well known to the general public.

Recent advances in the field of biology research have presented us with novel tools to attack these invading viruses, both in plants and humans, but in addition also in important lifestock such as chickens. The mechanism of RNA interference or RNAi was only recently discovered, but can be used to silence the expression of viral pathogens in a very specific manner. In fact, RNAi was first discovered in worms and plants, where it serves as an important antiviral defence mechanism. Plant viruses counter this plant defence by producing suppressor molecules that interfere with the RNAi attack. Plants in turn react by blocking these suppressors, leading to a complex, but fascinating war between the plant organism and the invading pathogen.

We now know that RNAi is an essential mechanism to control gene expression and cell differentiation in many organisms ranging from yeast to human. Viruses seem to interact tightly with the cellular RNAi mechanism in an astonishing variety of ways that were discussed at the meeting. Viruses encode molecules that reprogram the cellular RNAi machinery and cells encode RNAi molecules that affect the replication of viruses. For instance, HCV selected a major cellular RNAi molecule to facilitate its replication cycle, which forms an obvious point of attack in terms of the development of therapeutic approaches. Other viruses encode RNAi molecules that manipulate the cell cycle such that the virus can control its latency.

This ESF-EMBO meeting is unique in bringing together virologists from all fields (insects, plants, humans, animals). This common theme is the interplay between the invading viral pathogen and the cellular RNAi mechanism. Some studies addressed very basic aspects of this virus-host interplay, others focused on diverse applications: producing virus-resistant crop or lifestock, and developing a new therapy for virus infections in humans.

An ESF-EMBO meeting around the same topic was organized by us in 2008, and the chairs hope to be able to organize a third meeting in 2012.

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‘Conference Highlights’ in their communication on the scheme.

Scientific Report

Executive Summary

(2 pages max)

The meeting hosted approximately 85 participants, including 23 invited speakers. All speakers were asked to stay around for some time after their lecture in order to foster further interactions with the participants, which worked out nicely. Most participants were from academia, but a few keynote speakers from biotech industry were selected because of the exciting results obtained in the applied antiviral RNAi research. Many new and exciting scientific discoveries were presented at the meeting. The invited speakers were allowed to speak for 30 minutes. We strictly kept to the time allotted to speakers to allow at least 5 minutes of discussion per lecture. We also strictly enforced the use of microphones for the discussion session in order for everybody to actively participate. In addition, the chairs selected 14 short talks (15 + 5 minutes) from the submitted abstracts. Many of the participants contributed to the discussions, and the chairs were specifically asked to first allow young participants (not sitting in the front rows) to ask their question. We were very lucky with the weather, which allowed outdoor activities where the discussions continued over coffee or lunch.

The approximately 50 posters were presented in 3 evening sessions. Participants of whom the abstract was selected for a short oral presentation were specifically asked to also bring their poster to foster further discussions. These poster sessions were well attended and triggered many lively discussions, perhaps facilitated by the free beer made possibly through company sponsoring. A 3-person poster prize committee was formed on the spot with expertise from the different fields (from plant to human). Three poster prizes, made possible by sponsoring of Oxford University Press (Nucleic Acids Research journal), were awarded on the third evening. Besides the financial award (100, 200 and 300 Euro), the first prize winner received a certificate from EMBO and a year subscription to the journal EMBO Reports.

Many young scientists were able to come to this meeting because of fellowships that were made possible by the funding raised by the chairs. In addition to the regular ESF-EMBO funds, this included financial support from the RNA Society and in particular the Spanish Ministry of Sciences. The chairs selected fellowship awardees based on: 1. A match of their research with the topic of the meeting, 2. Active participation in the meeting (poster presentation, of which some were selected for an oral presentation), 3. Facilitation of the attendance of young researchers, 4. Gender balance, and 5. When funds were limited we preferred to help European scientists.

An European COST action is currently running on the topic of “Antiviral RNAi vaccination approaches in plants”, and two of the meeting chairs (Garcia Alvarez and Berkhout) also participate in this action. We intertwined a mini COST-meeting on one of the free afternoons. For this, several additional participants came to Spain. We organized the ESF meeting such that the related plant lectures were presented in the morning and evening sessions surrounding this COST-afternoon, which allowed the COST-only participants to benefit from these lectures that are of direct relevance to them. The combined COST-ESF-EMBO action also allowed the chairs to invite more speakers because of the extra COST funds.

The free afternoons (siesta) turned out to be very productive for networking, yielding many new contacts and several new collaborations. As an example, two of the PhD students that traveled from far (Australia and South-Africa) extended their Europe trip to visit the laboratory of the chair (Berkhout) in Amsterdam to discuss the possibilities for a future post-doctoral stay.

Ben Berkhout on behalf of the co-chairs

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*
- *Assessment of the results and their potential impact on future research or applications*

The 4-day meeting was organized globally in 5 sessions: 1. RNAi mechanism and tools, 2. RNA silencing and plant virus resistance, 3. Viruses and the cellular RNAi pathway, 4. Antiviral RNAi from plants to mammals, 5. Towards therapeutics. Although some last-minute adjustments had to be made, we could roughly stick to this schedule.

There were many truly novel results that were presented, of which only a brief summary can be presented. A major challenge for RNAi applications is efficient delivery of the siRNAs into the target cells. One strategy is to deliver shRNA expression cassettes by viral vectors. **Dirk Grimm** (University of Heidelberg, Germany) reported on the use of vectors based on Adeno-associated viruses (AAV). He particularly addressed possible unwanted side effects and showed data that shRNAs can potentially lead to the formation of hepatocellular carcinoma. A bottleneck for RNAi applications is the oversaturation of the endogenous RNAi pathways. This is not only true for the exportin-5 protein as published previously, but also for the argonaute-2 protein. The use of weaker, cell type specific promoters like the liver-specific hAAT promoter reduces the unwanted side effects. Furthermore, new AAV variants generated by DNA shuffling help to optimize the cell-type specificity of the vectors. Finally, Grimm presented strategies to absorb endogenous miRNAs.

Alexander Karlas from the Max-Planck-Institute for Infection Biology (Berlin, Germany) also reported work on influenzavirus, in this case the human pathogen. His department set up a screening facility and tested two siRNA libraries targeting the druggable genome (7000 genes) or the whole genome (17.000 genes, that cover almost the complete human genome). With these libraries host cell factors were identified that are essential for influenza virus replication, and this work was published recently in Nature. The second part of his talk dealt with the interaction between influenza viruses and cellular microRNAs (miRNAs). One miRNA, miR-141, was found to be upregulated upon influenza infection and its targets and cellular functions were discussed.

Thomas Hohn (University of Basel, Switzerland) reported the characterization of some small RNAs from the leader region of the *Cauliflower mosaic virus* 35S RNA that could be used by the virus as a decoy strategy to counteract the antiviral RNA silencing defense of the host. Novel activities of RNA silencing suppressors targeting plant AGO1 were described by **Jozsef Burgyan** (University of Torino, Italy**)**. Whereas the tombusviral p19 downregulates AGO1 by enhancing the accumulation of miR168, which appears to inhibit translation of AGO1 mRNA, the poleroviral P0 prevents RISC formation by contributing to the degradation of AGO1, and the ipomoviral P1 mimics host-encoded GW-containing proteins and inactivates loaded RISC complexes. The results presented by **Santiago Elena** (University of Valencia, Spain) highlighted the extreme ability of plant viruses to escape from the antiviral defense mediated by specific artificial miRNAs. Even in wild type plants, in the absence of selective pressure, frequent cases of resistance-breaking mutants were observed after a few serial passages of virus progeny of an infectious cDNA of *Tobacco etch virus.*

Sebastian Pfeffer (University of Strasbourg, France) described that whereas several host miRNAs have antiviral effects, some virus-encoded miRNAs interfere with antiviral responses of the host, and he identified a cytokine and a caspase involved in apoptosis as targets of these miRNAs. A non-exclusive role in translation enhancement from the HCV IRES was reported for miR122 by

Catherine Jopling (University of Nottingham, UK). This activity requires LsM1 and AGO and could be a suitable target for development of anti-HCV therapeutics. Another discovery that could have therapeutic potential was the anti-tumor activity described by **Kuan-Teh Jeang** (NIH, Bethesda, USA) for several chemicals that inhibited miRNA action at different steps.

John Rossi (City of Hope, USA) is one of the world leading experts for the clinic development of RNAi and the first to run a clinical trial for the RNAi-based treatment of AIDS patients. While this approach is based on lentiviral delivery of shRNA expression cassettes, he now presented a new strategy for the aptamer-mediated cellular transfer of siRNAs. A siRNA targeting HIV tat/rev was coupled to an aptamer against the viral glycoprotein gp120. Only cells being infected with siRNAs present gp120 on their surface and become transfected by the construct. The strategy was found to be suitable to reduce the viral load in cell culture by several logs.

The avian flu has been a global thread in recent years. It not only affects economic processes such as the production of eggs and poultry but also is a danger for humans living together with lifestock. **Scott Tyack** (CSIRO Livestock Industries, Australia) presented efforts to generate transgenic chicken that are resistant against the avian flu. The first task was to identify efficient siRNAs against influenza. Transgenic mice expressing these shRNAs were protected against the virus. While the generation of transgenic mice is well established, this procedure is still challenging for chickens. Therefore adequate promoters and methods are currently being developed. Not only the scientific problems but also the social implications of this extremely innovative approach were discussed during the meeting.

The chairs up front discussed the possibility to write a meeting report, similar to what was organized at the first meeting. This should preferably be a participant(s) that is not connected to the organizing committee. However, we were not able to find a volunteer author, mainly because of the serious time investment this would take. One of the American participants (Fatah Kashanchi) independently took up the plan to write a mini-review on “virus-miRNA interactions”, based largely on novel findings reported at the meeting.

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

Although we have not seen the results of the questionnaire filled in by the participants, the general feeling is that this was a very exciting and successful meeting. In fact, we only received (very) positive responses from the participants, independent of their field of origin (from plant to human). This basic feeling makes us enthusiastic to go for a third meeting around this booming theme in 2012. The 3-person organizing committee is willing to organize this once more.

Several issues worth considering for a future meeting were brought up in the general discussion. We will try to satisfy on these points when allowed to organize a third meeting:
- Length of meeting: some wanted one day less (3 instead of 4 days), but most liked the current format.
- In general, one also liked the daily siesta with ample time for discussion and networking.
- Clustering of most plant talks on a single day, including the COST action in the siesta time of that day, was perhaps too much. As we may go for another joined ESF-EMBO-COST action in 2012, it seems important to spread the plant lectures a bit more.
- Overall, the plant-drosophila-chicken-human marriage was considered to be important, it is one of

the unique selling points of this meeting, we see colleagues that we otherwise would not meet.
- New topics to consider including: interplay of the RNAi and interferon mechanism in mammals, chromatin effects, more on agricultural applications (COST).
- Add a few talks outside the box to stimulate new ideas: e.g. RNA-defence mechanisms in bacteria, IFN developments, combine RNAi with other therapeutic approaches (e.g. zinc-finger proteases).
- Better advertisement of the meeting, especially in USA. The chairs were very active in this respect (email lists etc), but perhaps ESF can help in this matter.

▪ Is there a need for a foresight-type initiative?

A topic that seems ideally suited for a foresight-type initiative is translational research, how to efficiently bring novel and promising therapeutic interventions from the laboratory into the clinic.

Another topic could be nucleic acid therapeutics, which is much broader than the antiviral RNAi approaches discussed at the ESF-EMBO meeting.

Atmosphere and Infrastructure

▪ *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

General comments have been made already in the previous sections on these topics. We do not have the outcome of the questionnaire, which should be analyzed to address these issues in a neutral manner. As said, we believe that the meeting was a GREAT success.

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Date & Author:

June 21, 2010

Ben Berkhout on behalf of the co-chairs