What happens when two manuscripts about the same topic arrive shortly after each other at the editorial office of the same journal? *Lab Times* looks into a recent case at *PLoS Genetics*.

Manuscript rejected, without review! First reaction: That can't be true. Then anger followed by frustration. Quite normal if one is convinced that the data is good but the editors of a journal turn round and flippantly reject many months of hard work, essentially declaring "nothing new".

Dampened enthusiasm

PLoS Genetics expressed themselves more concretely in their e-mail dated the 17th April 2011 to Inna Lermontova, Ingo Schubert and their co-authors, "While there is enthusiasm for the apparent quality and criticality of the work, there is also a consensus that the manuscript does not represent a sufficient strength of advance for a broad interest journal to do well during an extended peer review process. The key issue that came up during the internal review process is the current studies do not seem to provide a substantive insight beyond that which has emerged recently in other work on CENH3 and CENP-A like histones."

"Dubiously" rejected?

That review processes are not completely objective is a well-known fact, also acknowledged by the authors of the aforementioned manuscript, most of whom work at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. But even after this "failure", they were still convinced of the quality of their work, so they sent their works to *The Plant Journal* where it was swiftly accepted and published online on 21st July 2011 under the title "Knockdown of CENH3 in *Arabidopsis* reduces mitotic divisions and causes sterility by disturbed meiotic chromosome segregation" (*Plant J* 2011, 68(1):40-50).

Whilst the article was still under review at *The Plant Journal*, a paper by Maruthachalam Ravi *et al.* was published in *PLoS Genetics* covering the same topic and with the same conclusions as the one by Ingo Schubert, Inna Lermontova *et al.* (*PLoS Genet* 2011, 7(6):e1002121). Senior author of the competitive paper was Simon W. L. Chan of the University of California, Davis.

A closer look

Chan is not unknown amongst plant researchers. On his publication list there are a number of articles in journals in the category of *Nature, Science, Cell.* This study was published on 9th June 2011. "That was totally dubious," revolted Ingo Schubert. He was sure that unfair, preferential treatment had played a role – and was so angry that he asked us to have a closer look at this issue. And so we did.

The Ravi/Chan paper was submitted on 19th November 2010 and, after peer review, accepted on 21st April 2011. Four days earlier, Schubert and his co-authors received the rejection of their manuscript including justification. Now, the researchers from IPK had sent their manuscript to *PLoS* in March 2011. At that time, the Ravi/Chan paper was still under peer review. To Schubert, however, the *PLoS* editors explained that "principally" they had already accepted this article earlier: "... the decision to move towards publication ('acceptance in principle') for the Ravi *et al.* paper was made before Dr. Schubert's manuscript was evaluated in detail. We do strive to send letters out within a few days of editorial decisions, but that is not always possible."

Distribution battles

But why write a date under a publication if it is obviously not the right one? Is the official dating of an "accepted" only meant as an approximate indication? So what is the point if one cannot refer to it in the case of a controversy?

So much to "dating"! On to the content. In answer to our enquiry, Gregory S. Barsh and Andy Collings, chief editors and editorial manager at *PLoS Genetics*, respectively, declared, "We judged that the data and the advance reported in that work would not do well during an outside review process." Why? The editors had, after all, especially highlighted the quality of Schubert's work. So what about the "nothing new" argument now? To answer this question, we will have to look deeper into the matter.

Essentially, both manuscripts question how, in a plant cell, the separation of chromosomes during meiosis or sister chromatids during mitosis, respectively occurs.

Crucial for both cell biological processes is the formation of the kinetochores at the centromeres – the attachment sites for the fibres of the spindle apparatus dividing the chromosomes or chromatids from each other.

The "rivals" research, too

But what actually determines where centromeres form on a chromosome? It's certainly not the DNA sequence. Furthermore, it seems to be epigenetic factors, especially in the form of a certain histone, which make sure that the kinetochore proteins attach at a certain point of a chromosome. Concretely, it is a histone H3 variant called CENH3 in plants and CENP in mammals. This special histone replaces the otherwise common H3 in the nucleosome on the centromere and defines it in this way. The function of these very variable histones during mitosis, however, was mainly deciphered in baker's yeast and *Drosophila*.

During recent years, plant researchers have also made good progress. In March 2010, Ravi and Chan had already described in *Nature* that cenh3-zero mutants of *Arabidopsis thaliana* are embryonic lethal (*Nature,* 464(7288): 615-8).

Induction of GFP-marked CENH3, however, rescued the plants. But they were sterile if, in this construct, the amino-terminus was replaced by the homologous sequence of the common histone H3. The authors concluded correctly that these special mutants had a defect in their meiosis. They did not go into more detail at first, as their article was actually about the production of haploid plants.

Viable but sterile

They did, however, further investigate the reason for the observed sterility and thereby concentrated on the hypervariable amino-terminus of the protein. Accordingly, they added various GFP-marked constructs to the cenh3-zero mutants containing the original CENH3-gene, only its C-terminus, or its C-terminus plus the amino-ends of the common histone H3 or the homologous corn-CENH3-gene.

In the end, the constructs with the manipulated amino-terminus could not completely complement the mutation. The plants were viable but sterile, if GFP was attached to the modified N-terminus (of *Arabidopsis* histone H3 or corn CENH3). Cell-biologically, the authors documented that in the meiotic cells the chromosomes of the mainly infertile plants did not arrange correctly and that, therefore, the kinetochores at the chromosomes couldn't be pulled efficiently by the fibres of the spindle apparatus.

Mostly lost

Furthermore, the researchers from California looked at the dynamic of the CENH3-proteins and found that the mutated variants, connecting correctly at the centromeres of the mitosis-chromosomes,

were also present in pre-meiotic chromosomes but they could not be found any more during meiosis – they had mostly been lost.

In addition, they followed the same processes in normal, haploid microspores, which *Arabidopsis* forms with low frequency (19%). These have to go through a further mitosis after the meiosis – and the mutants were always able to do that. At the centromeres mutated CENH3-proteins were found with the same frequency as wildtype proteins in wildtype plants. From this, the researchers concluded the existence of two pathways to load the kinetochores.

Two methods, one result

And now to the work at Gatersleben. To circumvent the lethality of homozygous T-DNA-insertion mutants, Schubert *et al.* produced RNAi-knockdown mutants. A large part of the article is about the description of these plants, which accordingly still express CENH3 in small amounts. In somatic cells of the root tip the kinetochore proteins attached correctly at the centromeres. Still, the mutants stayed all in all too small and formed fewer cells than wild type plants. In addition, they were partially sterile and underwent strange forms of meiosis.

The Germans transformed the knockdown plants with EYFP-CENH3 constructs (EYFP = enhanced yellow fluorescent protein) and documented – for the first time in a whole organism – that meiosis, and therefore also the ability to reproduce, was impaired in ripening pollen, if the plant produced only small amounts of endogenic CENH3-protein and, at the same time, the amino-terminus of the new-induced CENH3 was either missing completely or changed. On the other hand, transgenic plants with high endogenic CENH3 levels were more or less normal.

Antibody tests with anti-GFP and anti-CENH3 showed both types of CENH3-proteins to be present at the centromeres in the root tips during the whole mitosis and the interphase – whereas during meiosis only the endogenic CENH3 was found.

Using completely different methods, both groups came to the conclusion that CENH3 has different roles during mitosis and meiosis – and that the N-terminus is absolutely necessary for meiosis to proceed correctly.

Why not side-by-side?

Surely, one could have published both articles side-by-side. At least Schubert would certainly have favoured that. He wrote to *PLoS Genetics* that scientific fairness would have required publishing both articles together in the same issue instead of not even passing his to reviewers to be checked. But that, according to *PLoS Genetics*, was "never an option".

Moreover, Schubert received the following answer to his complaint: "We do not agree with your contention that the manuscripts are identical. In our view, the overlap between the two manuscripts is based primarily on the last section of your work in which it is shown that the N-terminus of CENH3 is important for meiotic but not mitotic loading onto the centromere. However, we feel, respectfully, that the Chan paper goes farther in both additional experiments and biologic conclusions that can be drawn."

Indeed, comparing both articles, it is apparent that the article by the German group concentrates, at first, very much on the pure description of the mutants, before mentioning the actually interesting fact – the influence of CENH3 on meiosis. And even then, the authors don't go too deep into the matter.

In contrast, the Americans tried to elucidate the cell biological mechanism for the different behaviour of the same molecule in meiosis and mitosis – even if, in the end, they only succeeded to a degree. At least they discussed the possible cell biological reasons for the observed effects on the basis of available literature more thoroughly then Schubert and his colleagues. In addition, the article by Chan *et al.* seems more pleasant and is an easier read (but this, certainly, should not be an argument for or against a publication).

Extremely unlucky

Nevertheless, the final, shattering criticism from the *PLoS* editors that the studies of Lermontova *et al.* "... do not seem to provide a substantive insight beyond that which has emerged recently in other work on CENH3 and CENP-A like histones..." is so untenable. And that the article had never even been good enough for review is not at all comprehensible.

After all, at the time the researchers from Gatersleben submitted their work, nobody had ever published that CENH3 played a different role during meiosis to that during mitosis. Schubert *et al.* couldn't have known that Ravi, Chan and colleagues had, of all things, submitted their article at the same time. That was extremely unlucky! But to be fair, the *PLoS* editors should have explained the situation immediately and clearly to the team of authors from Gatersleben.

Whether the *PLoS* editors have – for whatever reason – somewhat dubiously favoured the US team can hereby neither be proven nor excluded. Schubert, in any case, still believes this.

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A German version of this article first appeared at Laborjournal *online on February 2nd, 2012.*

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Unfair treatment or just bad luck?
Ingo Schubert's paper didn't make it to the finish line at *PLoS Genetics*.