

Positive epistasis in Kraków

Ghost Interactions

The expression of one protein can suppress or enhance the expression of another. However, even the absence of functional genes can have an effect. Ryszard Korona and Lukasz Jasnos from the Jagellonian University in Kraków found that in some cases a deleted gene in budding yeast is able to mitigate the harm caused by another deleted gene.

The term epistasis is commonly used to describe the interaction between the expression of two or more genes. Epistatic effects, however, can also appear in the context of deleterious mutations. Here, epistatic effects are regarded as “positive” when the damage caused by two paired mutations is lower than additive. Or, a gene mutation might even be able to compensate for the damage caused by the mutation in another gene.

Just imagine an unlucky fellow who suffers from complete baldness because of a particular mutant gene. What additional harm might be done to him by a second mutant gene that determines albinism? Similar effects can be found on the molecular level. A gene deletion preventing an early step along any biochemical pathway will be epistatic to a mutation that affects a step later in the same pathway.

Mutations are, of course, important for the evolution of organisms. They are the tools that finally create new species. Seen from this perspective, such epistatic effects might buffer the fitness loss caused by multiple mutations and might therefore be of adaptive value. Does evolution thus reward positive epistasis? This was the initial question that Ryszard Korona started investigating in budding yeast a couple of years ago.

The self-made man

Ryszard Korona is Associate Professor at the Department of Ecological Microbiology, situated in a new building of the Jagellonian University on the edge of Kraków. In the late nineties (the time when the yeast genome was sequenced) he became interested in the impact of mutational load on yeast populations.

Budding yeast, he felt, was the perfect model organism. Yeast cells can be haploid or diploid. The diploid form undergoes the process of sporulation, which includes meiosis and results in four haploid spores. “Because of this process you can easily separate single phenotypic traits,” Korona says. “This is probably one of the main reasons for the great success of yeast in genetics.”



However, in the beginning there were obstacles to overcome. “I had to learn the yeast genetics on my own, because there was no one in Kraków at this time who could teach me,” he remembers. Another problem was money. It was a time, when Poland was haunted by political and economical changes. So it took him some time to be able to afford the expensive yeast strains he needed.

Eventually it worked. He bought yeast strains with single gene deletions known to affect growth rate. An impairment of growth rate – this was his idea – should be an indicator of the harm caused by single or combined gene deletions to the general fitness of individuals. “When I had this idea, my first thought was that somebody else must be doing the same thing, because it was so obvious,” Korona says.

Small group – lots of work

At that time, Korona’s group consisted of him and his student Lukasz Jasnos; Polish grants did not allow for big dreams. The work in the lab was mainly done by Jasnos. And it was a lot of work. Jasnos’ first step was to mark each single gene deletion in each haploid strain. He had 758 strains with single deletions which were known to affect growth rate. He marked these by inserting the *kan* marker gene into the de-

leted gene sites in half of the strains and the *nat* marker gene in the other half. The *kan* gene provides resistance to geneticin, the *nat* gene to nourseothricin. Thus, only strains with paired deletions would survive on a combined medium. Since the aim was to pair two deletions, respectively, successful crossings immediately became visible.

Working like a machine

In a second step Jasnos crossed differently marked strains with each other. In the resulting diploid strains he induced sporulation and thus gained tetrads of haploid spores. He scanned up to forty tetrads per strain until he eventually found what he was looking for: tetrads consisting of one *kan*-marked, one *nat*-marked, one *kan/nat*-marked (the result of crossing-over during sporulation) and one unmarked spore. These tetrads, including paired deletions, were the objects of interest. Janos isolated and grew them for further investigation.

“Lukasz worked like a machine,” Korona remembers. “I don’t know how many strains he had in the end. It must have been thousands. He was very exact.”

Over the time that followed, his student assayed the tetrads of interest for maximum growth rate by measuring their optical density. As expected, he found that single and particularly paired deletions showed considerable growth defects, resulting in lower growth curves and, in some cases, even in lethality of the strains. However, the interesting finding was a different one.

Interacting ghosts

The main part of the analysis was an easy mathematical step. The growth rates of the single deletion strains from each initial tetrad were added up and compared to the summed growth rates of the “double deletion” and the “no deletion” strains. The latter turned out to be statistically higher, which indicated that the damage done to strains with paired deletions was lower than the additive damage done to the single deletion strains. Interactions between gene deletions thus buffered the negative

effects on growth rate, which would have been caused by additive gene deletions. This was exactly what epistasis means. Absent genes were able to interact with each other like ghosts.

In the following step of their investigation, Korona and Jasnos tested whether the strength of epistatic effects differed among the functional classes to which the deleted genes belonged. The functional classes of the tested gene deletions were already well known. Accordingly, the full extent of metabolic processes in the yeast cell was represented in their data set, with genes coding for organelle organization, protein modification, cell cycle and cell wall organization being only some examples. The result was surprising: Neither functional class showed stronger epistatic effects than the other. This suggested that positive gene interactions may occur in any functional context of the yeast cell.

An effect of networking

However, do epistatic interactions occur even between different functional classes? Does the deletion of a gene coding for cellular trafficking mitigate the harm done by the deletion of a gene coding for cytoskeleton organization? In fact, since Korona and Jasnos performed the initial crossings between single deletion strains at random, the observed interactions very likely concerned genes involved in different cell processes. “True epistasis is not reflecting direct interactions between gene products,” Korona states. “There is not necessarily a direct biochemical or mechanical contact between

the proteins involved.” Instead, epistasis seems to take place on the network level,



so that even deletions of genes belonging to very different functional classes may affect each other.

The End is the beginning...

When Ryszard Korona began to ask questions about the impact of gene interactions on the growth rate of budding yeast he believed that the results of his experiments would satisfy his appetite for understanding. “I thought I’d obtain the answers to my question and subsequently would have to

start something new”, he says. These answers, however, produced a whole bunch of new questions. If there is positive epistasis in the genetic network of a cell, he asks today, what are then the molecular points of its mediation? Of course, he has his own hunches. In a recently finished work, in which he replicated the epistasis experiments under four different stress conditions, he found that the buffering strength of positive epistasis depends on the stress environment. Thus, the interactions between gene deletions can be affected by external stress factors such as heat or salt. “Gene deletions, however, can be regarded as stress factors, too,” Korona says.

One of his favourite candidates for a key player’s role in the molecular mediation of the epistatic network effects is the TOR protein, an antenna-like molecule in the vacuole of the budding yeast cell. This structure is known to integrate internal and external stress signals.

... provided you have ideas

“The whole thing is ever more developing in the direction of ecology,” Korona finishes enthusiastically. “We’ll now have to integrate our knowledge about phenotypical effects with their molecular basis.”

In the meantime, Lukasz Jasnos has graduated. Very recently he obtained a PhD position in a cell biology lab in Oxsted, USA. Ryszard Korona’s group has therefore shrunk; he has only one new student. However, he has a lot of new ideas. And, as he knows, that’s the way things start.

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