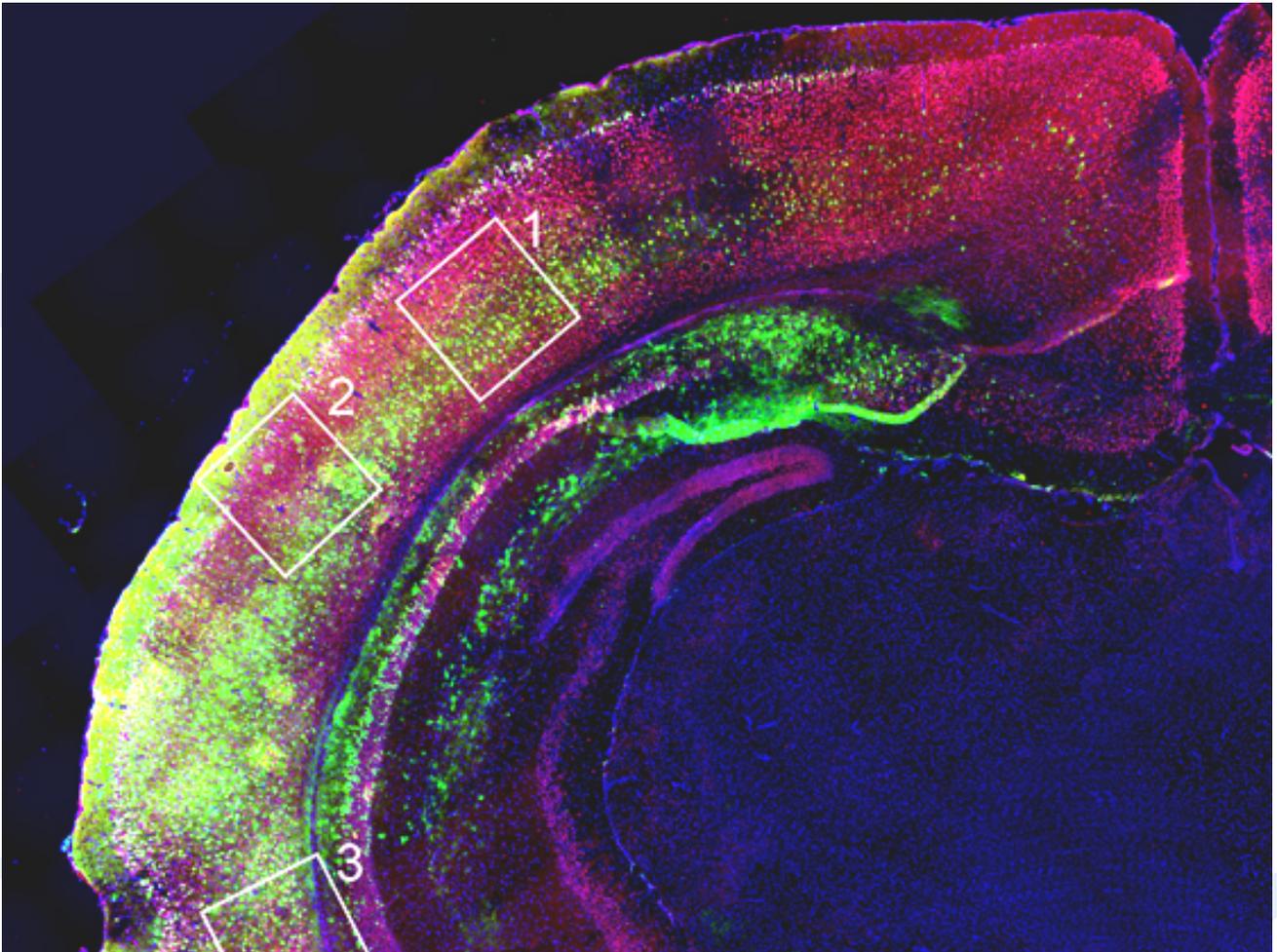


Double effort against Rett's syndrome



The trick against haploinsufficiencies: getting the "single" gene to work for two

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Although our genes normally come in pairs (one on each homologous chromosome), sometimes one of them is missing and the "surviving" one is unable to do all the work (it is referred as "haploinsufficient"): this situation can give rise to very serious diseases. So what can be done to fight these, often neurological, diseases? One possible strategy has been successfully adopted by a SISSA research team, in vitro and in vivo: using leading-edge techniques to stimulate the surviving gene to also do the work of the missing gene. The technique has so far only been applied to a specific gene, whose haploinsufficiency causes a rare disease called Rett's syndrome. The method, however, is also a general approach that can potentially be applied to all



haploinsufficiencies which, taken together, affect a considerable number of individuals worldwide, but for which no valid treatments have been developed to date. The research has been published in Scientific Reports.

Imagine that a colleague of yours has fallen ill and will be absent for a while from the office. What do you do? Do you go on working at your usual pace and by doing so risk a huge backlog of work that will affect the performance of the whole office, or do you roll up your sleeves and get down to it (perhaps also after your boss has motivated you by promising some benefit) by doubling your efforts and doing your absent colleague's work as well as your own? Something similar occurs with genes when their homologues are missing, a condition doctors call haploinsufficiency. When this abnormality manifests, especially when it concerns genes that have an important function in the central nervous system, it may lead to very serious diseases, such as Rett's syndrome that causes severe progressive mental retardation related to the FOXP1 gene. A group of researchers at the International School for Advanced Studies (SISSA) in Trieste, led by Antonello Mallamaci, has decided to adopt the "motivational" boss strategy by stimulating the surviving FOXP1 gene to work more to compensate for the absence of the missing gene.

"By using viral vectors to insert into neurons RNA fragments targeting the gene's regulatory sequences, we "gently" stimulated the gene to do more work, in particular nearly double", explains Mallamaci. "Note that we don't want the gene to do more than that. If it worked, say, three times as much, it could cause even worse damage". In fact, it is known that when three copies of the FOXP1 exist (one more than normal), we have West's syndrome, which is perhaps even worse as it causes a severe form of epilepsy. "It's therefore vital that the gene we stimulate does no more than about double the normal amount of work".

The method adopted by the Trieste group is a "cunning" solution to the treatment problems posed by these diseases. "Stimulating the normal gene allows us to preserve its natural endogenous regulation", explains Mallamaci. Genes in fact are not expressed everywhere and at the same intensity: to the contrary, in many body tissues they are silenced, in others their activity is time-modulated with great precision. If their regulation were to be disrupted, it is easy to imagine the chaos that this would generate. "Going back to the office worker's metaphor, it's like having an inexperienced intern do the absent worker's job: at best he won't do anything, at worst he'll mess things up. Instead, asking an experienced colleague, who's familiar with the office's processes and rhythms, to work harder, offers greater guarantees".

Multiple tests

The team ran several tests. First, *in vitro*, the scientists checked whether stimulation through promoter RNA was able to amplify gene activity only where it was needed. "FOXP1 is only active in the anterior brain and we absolutely don't want it to act elsewhere in the nervous system or the



body", explains Mallamaci. "The tests gave positive results: after stimulation, the gene continued to be expressed only in cells where it had previously been active and remained silent in tissues where it normally doesn't work. Very importantly, the activity observed increased by a factor not far from 2, i.e. that "double" expression that we were trying to achieve".

The second test, also in vitro, demonstrated that the gene's endogenous regulatory mechanisms related to the electrical activity of the neurons expressing it are not altered by stimulation with RNA: "we saw a rise in the gene's activity, but the shape of the time-activity curve was basically unchanged, a clear indication that regulation remains the same", explains Cristina Fimiani, PhD student in Functional and Structural Genomics at SISSA and co-first author of the study.

The third step was to see whether the stimulation also worked in vivo. "The test was done on healthy mice and we found that the stimulation was even more effective in vivo than in vitro," Mallamaci concludes.

"We're still at the beginning of a very long clinical process that might one day lead to treatment", he adds. "The results, though, are very clear and definitely encourage us to continue this line of research. The next steps will be in vivo tests on animal models affected by the disease".

What makes these therapies so interesting for the future? "Rett's disease is rare and affects only a small number of patients, so it doesn't attract the attention and investments of major pharmaceutical companies", concludes the scientist. "But, taken together, haploinsufficiencies affect very many people. The methodology we present in this study is therefore a test for a general method capable of fighting the large number of haploinsufficiencies affecting the nervous system, and once developed it could be easily adapted to different genes".

USEFUL LINKS:

- Information on the method: <http://lccd.sissa.it/project/rna-therapeutics-gene-haploinsufficiencies>
- Link to the original paper in Scientific Reports: www.nature.com/articles/srep39311

IMAGES:

- Rat's brain areas stimulated in the study - Credits: SISSA

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