fighting, the habenula seemingly can induce differential motor responses. This is concordant with recent studies describing the habenula as a hub integrating multiple sensory cues (*11*) to produce an adapted motor response. How this signal is relayed at the molecular and neuronal level is not yet known. Multiple modulatory neurotransmitters and peptides are associated with aggressive behavior (*10*, *12*), but their diversity in the habenula and IPN is most likely underappreciated. The dissection of neuromodulatory systems using innovative genome-editing technologies may reveal the full circuitry governing social defeat in vertebrates.

Zebrafish has emerged as a powerful system to study social interactions (*13*), and there are many social behaviors in this animal to unravel (14). For example, the recent observation of social preference in juvenile zebrafish (*15*), at stages when whole-brain imaging is feasible, opens new paths of investigation into the sensory cues and the genes and circuits mediating these early social interactions. It should be possible to find out why some individuals are reluctant to go toward others when the majority are attracted by conspecifics.

Although zebrafish have a short generation time and are easy to maintain in captivity, the bottleneck often lies in the fine description and quantitative analysis of complex behaviors. The study of Chou *et al.* illustrates how the genetic manipulation of pathways governing innate behaviors in zebrafish can inspire investigations in other model organisms by providing new working hypotheses and brain regions to target. The next step will be to combine whole-brain imaging, optogenetic manipulations, and CRISPR-mediated genome editing to unravel the complete circuits underlying social behaviors in vertebrates. ■

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ILLUSTRATION: P. HUEY/*SCIENCE*

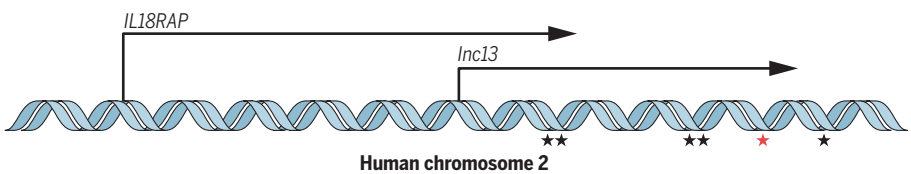
RNA

A lncRNA links genomic variation with celiac disease

A long noncoding RNA is associated with an intestinal autoimmune disorder

By **Maite Huarte**

The majority of human single-nucleotide polymorphisms (SNPs) associated with increased disease risk map to noncoding regions of the genome. The nucleotide variations therefore cannot be directly related to changes in the function of proteins. Indeed, SNPs frequently localize to DNA regulatory elements such as enhancers or promoters, or within intergenic regions that are transcribed to produce long noncoding RNAs (lncRNAs). LncRNAs are RNA molecules longer than 200 nucleotides that do not encode proteins; in many instances, they regulate gene expression through diverse mechanisms. On page 91 of this issue, Castellanos-Rubio *et al.* (*1*) report that a relationship between the function of a lncRNA and the SNPs within its locus underlies celiac disease, an autoimmune disorder that causes intolerance to gluten.

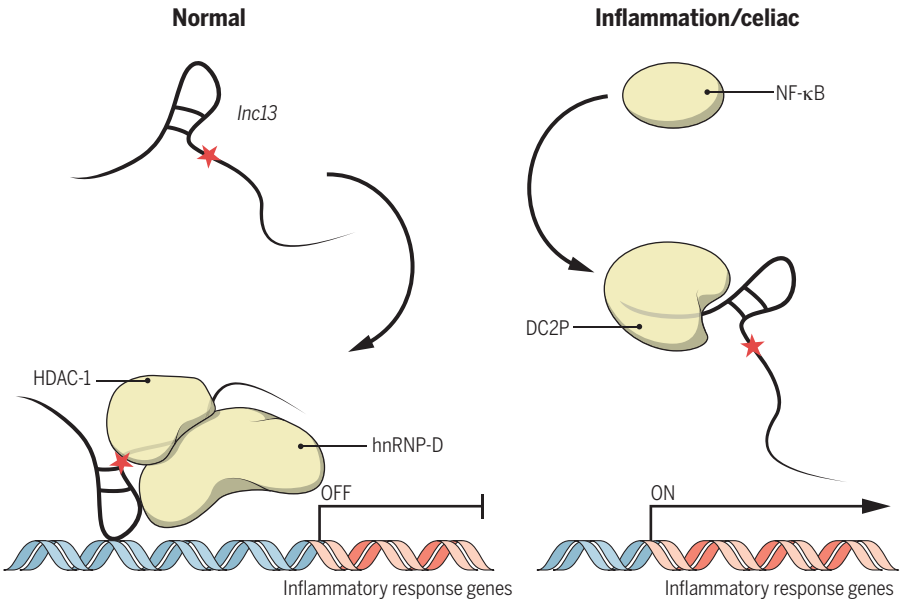


Location overlap. The genomic loci of *IL18RAP* and *lnc13* overlap in human chromosome 2. SNPs associated with celiac disease are indicated with blue stars. A red star shows the specific SNP, rs917997.

Genome-wide association studies revealed that six SNPs that are linked to increased risk of suffering from gluten intolerance form a haploblock (closely associated SNPs) located in chromosome 2 (*2*). One of the SNPs (rs917997) is positioned 1.5 kb downstream of interleukin-18 receptor accessory protein (IL18RAP), a gene that has been associated with susceptibility to celiac disease and other autoimmune diseases (*2*–*4*). In the mouse genome, a previously annotated lncRNA, *lnc13*, is transcribed in this position. Castellanos-Rubio *et al.* identified a lncRNA in the equivalent position of the human genome and showed that both human and murine lnc13 regulate the expression of genes of the inflammatory response. Despite limited DNA sequence conservation, the function and mechanism of lnc13 is conserved between mouse and human.

The fine regulation of lnc13 expression is probably crucial for its proper function. It is transcribed from the same strand of DNA as IL18RAP, and its 5′ end overlaps with the 3′ end of IL18RAP (see the first figure). However, despite this overlap, both genes have independent promoters and are independently regulated. IL18RAP expression is induced in the intestinal epithelium of celiac patients and in lipopolysaccharide (LPS)–stimulated macrophages (a treatment that mimics the inflammatory response). However, under these conditions, the expression of lnc13 is diminished. Reduction in the amount of lnc13 is a posttranscriptional event dependent on the signaling pathway controlled by the transcription factor nuclear factor kappa B (NF-κB). Indeed, despite not being translated, lnc13 is highly stable in nonstimulated macrophages. By contrast, in LPS-activated macrophages, NF-κB induces the expression of Dcp2, a negative regulator of the stability of capped RNAs. Dcp2 thereby keeps lnc13 amounts low (see the second figure). Thus, the co-inherited IL18RAP and lnc13 are transcribed from the same locus and take part in the same biological process. However, their regulation is independent, in part because of the posttranscriptional control of lnc13.

The expression levels of lnc13 correlate negatively with the expression of several genes of the inflammatory response, some of which are up-regulated in celiac disease patients' biopsies. In fact, lnc13 inhibits the expression of these genes in cells where the inflammatory response is inactive. Similar to several other characterized lncRNAs, lnc13 is preferentially present in the nucleus



Gluten triggers inflammation. The action of *lnc13* in a normal macrophage or a macrophage with an active inflammatory response is shown. A decrease in available *lnc13* (due to its increased association with DC2P) is linked to celiac disease. *lnc13* function is impaired by the change in RNA sequence caused by a risk-related SNP, rs917997 (red star).

of the cells, and through binding to chromatin factors, it mediates gene silencing (*5*). In particular, lnc13 forms a complex with heterogeneous nuclear ribonucleoprotein D (hnRNPD) and the histone deacetylase HDAC-1. In nonstimulated macrophages, this lnc13-containing repressor complex is localized to the promoters of pro-inflammatory genes, keeping them transcriptionally silent. In agreement with this model, Castellanos-Rubio *et al.* have shown that lnc13, hnRNPD, and HDAC-1 are associated to those gene promoters. However, it remains unknown whether these are the only targets of lnc13, or whether it regulates the expression of additional genes of the inflammatory response, or even of other cellular pathways.

The activity of lnc13 seems to depend on the interaction with the RNA binding protein hnRNPD. hnRNPD is an abundant protein that functions at different levels of RNA metabolism and is also involved in transcriptional regulation. hnRNPD binds with high affinity to RNA molecules that contain AU-rich (A, adenine; U, uracil) elements (AREs) (*6*). Interestingly, the interaction between lnc13 and hnRNPD is affected by the sequence variation of the SNP rs917997. The interaction is substantially decreased when the genomic sequence variant is the disease-associated "TT" instead of the wild-type "CC" (T, thymine; C, cytosine). However, it is not clear whether the nucleotides corresponding to the allelic variants of lnc13 are part of an ARE. Thus, the nucleotide change may be affecting RNA structure rather than being localized to the protein-RNA interface. This not only highlights the relevance of RNA structure, but also shows how minimal changes in RNA sequence may have a functional impact.

The study by Castellanos-Rubio *et al.* demonstrates that lncRNAs play an active role in celiac disease and represents an exciting example of how a disease-associated SNP can directly affect the function of a lncRNA. It may be that other SNPs associated with risk of disease act in a similar manner. Unfortunately, it is still not possible to predict how long RNA molecules fold, and whether and how they interact with RNA binding proteins or genomic regions. At present, only individual detailed experimental analyses can deliver that information. Such studies will hopefully help us to infer the molecular principles that govern the function of lncRNAs and allow the design of lncRNA-targeting therapies. ■

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CHEMICAL SYNTHESIS

Going with the flow

A desktop machine allows on-demand medicine production

By **Rainer E. Martin**

Imagine it is the middle of the night. A severe snow storm has hit the region, and your 3-year-old's fever is rising. You suspect a serious infection and cannot wait until the next morning to go to the pharmacy, yet the roads are impassable. No problem—you were recently granted access to a prototype machine no larger than a kitchen microwave that allows the user to synthesize their pharmaceutical of choice. You start up a smartphone app that has access to the family medical history and other relevant parameters, such as allergies and body weight. A few minutes later, you have consulted an emergency pediatrician through the app, inserted the relevant capsule, and pressed the start button. After a short wait, a single, personalized dose of the necessary antibiotic is ready for use. All this may sound like science fiction, but the report by Adamo *et al.* on page 61 of this issue (*1*) proves that this scenario might become reality in the not-too-distant future.

The authors have created an on-demand synthesis platform that can produce thousands of formulated, ready-to-use liquid drug doses per day. Currently, the end user can choose from four key drugs, but the drug catalog can be extended. The fully integrated machine is roughly the size of a refrigerator and contains individual synthesis, purification, and formulation modules. Once a drug is selected, the required synthesis steps are conducted automatically by guiding starting materials and reagents through a flow reactor network that is established within the machine for the selected drug. Complex, laborious, and time-consuming steps such as phase separation, precipitations, and crystallizations, which are required to get to the purified pharmaceutical, are all conducted in a fully automated fashion.

Since the early days of Justus von Liebig (1803 to 1873), the art of organic synthesis

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ILLUSTRATION: P. HUEY/*SCIENCE*

A lncRNA links genomic variation with celiac disease

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