# Research team clarifies molecular mechanisms of fungal infections

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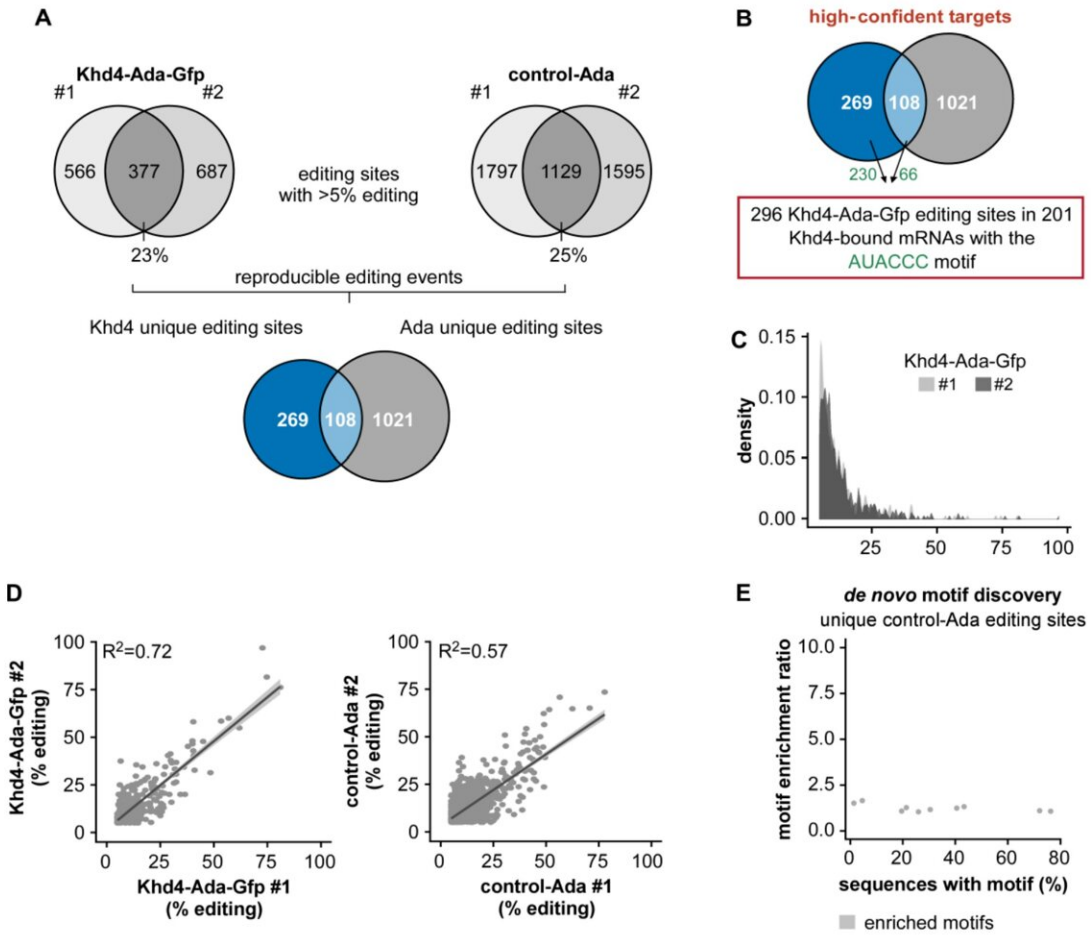


Khd4-Ada-Gfp editing sites are highly reproducible. (A) Overview of the hyperTRIBE workflow. Reproducible editing sites overlapped between the two independent biological replicates of the Khd4-Ada-Gfp (blue) and control-Ada (gray) data were compared with each other (#1,2- replicates 1 and 2). Only editing events with 5% editing were used for the analysis. (B) The genes

corresponding to the Khd4-Ada-Gfp-specific editing sites (blue) were subsequently analyzed for the presence of the AUACCC binding motif and were defined as high-confident Khd4-bound mRNAs (red). High-confident editing sites from each respective category are indicated in green. (C) Density plot of percentage editing events in Khd4-Ada-Gfp. (D) Scatter plots comparing the correlation of editing levels between two replicates (rep#1,2- replicate 1 and 2) of Khd4-Ada-Gfp and control-Ada. ($R^2$- coefficient of determination). (E) De novo motif discovery analysis on sequences carrying unique controlAda editing sites. Scatter plot comparing the enrichment ratio relative to the background (gray; random genome background) with the percentage of tested sequences containing the enriched motifs. Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2301731120

Fungal infections pose a threat to humans, animals and plants, and can have serious consequences. Together with colleagues from Frankfurt/Main and Aachen, a research team from Heinrich Heine University Düsseldorf (HHU) has now clarified an important mechanism in how such infections are regulated at molecular level. In *Proceedings of the National Academy of Sciences* (*PNAS*), they describe how this discovery may lead to the development of new antifungal agents.

As pathogenic agents, fungi can cause serious diseases in humans, animals and plants. In humans, the skin is frequently affected, for example by the largely harmless condition athlete's foot. However, particularly in the case of a weakened immune system, internal organs may also be affected—for example, the lung disease aspergillosis is caused by molds of the Aspergillus family.

In crop plants, diseases caused by fungi can do significant damage—well-known examples include ergot, which attacks rye and is highly poisonous to humans, or corn smut, for which the fungus Ustilago maydis is responsible.

In order to develop new defense strategies to protect humans, animals and plants, it is important to understand how the infections are regulated at molecular level, above all at DNA and RNA level. However, knowledge about the RNA regulation of fungal pathogens in particular is currently still limited.

In collaboration with research groups from Frankfurt and Aachen, the working group headed by Professor Dr. Michael Feldbrügge from the Institute of Microbiology at HHU applied an effective RNA marking technique for fungi, which functions in the living organism ("in vivo"). The researchers found out how an important RNA-binding protein (for short: RBP) called Khd4 regulates the growth of infectious hyphae—the filament-like form of the fungi, only this form triggers an infection.

An important factor for the growth of infectious hyphae is membrane trafficking: a recycling process that enables the exchange of material between the fungus and its environment by using organelles such as vacuoles.

Determining the stability of information-transmitting mRNAs was an important aspect in the published work. By nature, RNA is not very stable and actively degrades. The protein amount is regulated via the mRNA degradation.

Professor Feldbrügge says, "For the first time, we have discovered a new regulatory concept for infections: A single RBP controls the polar growth of infectious hyphae by determining the stability of mRNAs, which in turn regulate membrane trafficking. This opens up points of attack for developing new fungicides, which use RBPs as new targets for fighting fungi."

The research work was conducted in close collaboration between various facilities at HHU and with external partners. The lead author and Ph.D.

student Srimeenakshi Sankaranarayanan and Dr. Carl Haag, primarily focused on comparing plant and human pathogenic fungi. The RNAs were sequenced at the Biological Medical Research Centre (BMFZ) at HHU. The cooperation partner, Dr. Kathi Zarnack from the Goethe University Frankfurt am Main, completed the bioinformatic analysis in the project.

"One important aspect was the mathematical modeling, in which the groups working on the theoretical and experimental aspects were closely integrated," says Professor Feldbrügge.

"This form of cooperation is part of the fundamental concept of our Collaborative Research Centre 'MibiNet', which was launched in 2023. The contribution of Professor Dr. Anna Matuszyńska from Aachen University of Technology (RWTH) was key to the success of the project."

**More information:** Srimeenakshi Sankaranarayanan et al, The mRNA stability factor Khd4 defines a specific mRNA regulon for membrane trafficking in the pathogen Ustilago maydis, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2301731120

Provided by Heinrich-Heine University Duesseldorf