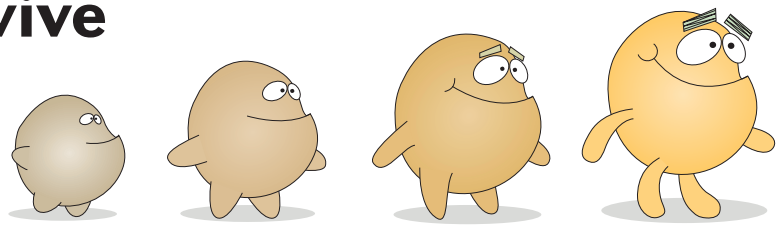
GENOME WATCH

Evolve and survive

Alena Pance



This month's Genome Watch explores how *in vitro* directed evolution can be used to identify the target of a drug for the treatment of Chagas disease, which is caused by *Trypanosoma cruzi*.

Directed evolution is an experimental strategy that is used to mimic natural selection to generate a library of biomolecule variants. The most commonly used methods to generate diversity are random mutagenesis, which is applied when no structural or functional information exists, and focused mutagenesis, which directs the changes to residues or regions suspected to be involved in protein function[1].

A recent study[2] used a modified strain of yeast that lacks 16 ABC transporter export pumps (ABC16-monster) and is thus more sensitive to cytotoxic drugs, to perform directed evolution. Random mutagenesis was induced in this strain by sublethal doses of a number of compounds from the malaria box from the Medicines for Malaria Venture. Although these compounds have activity against *Plasmodium falciparum*, some of them are also known to affect other kinetoplastids, including *Trypanosoma cruzi*[3]. The yeast clones that survived treatment with the selected compounds were subjected to whole-genome sequencing to identify the mutations responsible for resistance, and single nucleotide variants (SNVs) for each compound were detected following comparison with the parental reference genome.

One of the compounds tested, MMV001239, which is known to be effective against the intracellular stages of *T. cruzi*, exhibited potent activity against the modified yeast in the directed evolution experiments. Four clones that were resistant to this compound were isolated. A surprisingly low number of SNVs were detected in the evolved clones and only four of these consisted of non-synonymous substitutions, which affected either *ERG11* or *ERG25*. These genes encode enzymes involved in the biosynthesis of ergosterol, which is an essential structural component of the plasma membrane of protozoans that has a role similar to that of cholesterol in mammalian cells. Reproducing the SNPs in *ERG11* in the parental yeast strain using CRISPR–Cas9 caused the same resistance to MMV001239 as observed in the evolved clones, confirming that *ERG11* is the target of MMV001239.

The *T. cruzi* homologue of yeast ERG11 is Cyp51, which has a high degree of homology including one of the amino acids that was shown to be mutated in the resistant yeast clones. The authors showed that MMV001239 binds directly to this highly conserved region of Cyp51 and inhibits the sterol pathway. Finally, the mutations observed in ERG11 are in close proximity to the active site of the enzyme and predicted to inhibit binding of the drug without affecting ligand binding. Therefore, MMV001239 possibly inhibits enzyme function by binding close to the active site of the protein.

Directed evolution has also led to the identification of the target of the synthetic antimalarial drug KAE609 (also known as cipargamin or NITD609). This spiroindolone is a promising new drug that has been shown to effectively clear infections by *Plasmodium vivax* as well as *P. falciparum*, and to block transmission. The drug target was first identified by treating *P. falciparum* with sublethal doses of KAE609, which caused a high frequency of mutations in *ATP4* (which encodes a plasma membrane P-type ATPase) in the surviving parasites[4]. However, as the structure and function of this protein are unknown, a recent study also used the ABC16-monster yeast strain to perform directed evolution to understand the mechanism of action of KAE609. The authors identified the binding site of the drug and suggest that KAE609 interferes with the ATPase activity[5].

There is an urgent need to find new drugs for a range of infectious diseases to overcome the frequent emergence of resistance and treat vulnerable patients without side effects. Although thousands of compounds are being discovered that are biologically active against human pathogens, it is important to identify their targets and mechanisms quickly to establish a compound as a new potential drug. It is also a major advantage to repurpose known compounds for the treatment of diseases they might not have been designed for; this is exemplified by MMV001239, which having been proposed for the treatment of malaria is now proving very effective for the treatment of Chagas disease. The strategy of directed evolution can achieve both of these objectives swiftly and effectively to move drug discovery forwards at a much faster pace.

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Competing interests statement
The author declares no competing financial interests.