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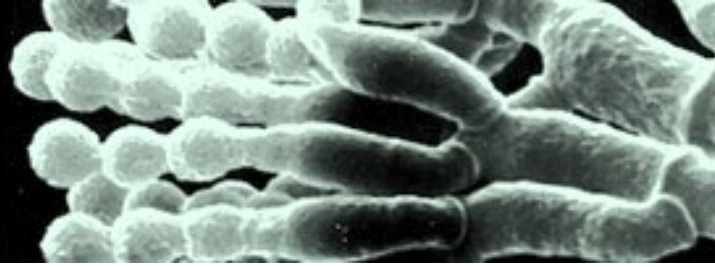

An introduction to molecular biology approaches in parasitic protozoa:

2. Molecular genetic methods for analysing gene function in protozoan parasites

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Many, but by no means all, protozoan parasites can be genetically modified in the lab (see MiSAC*matters* article '*An introduction to molecular biology approaches in parasitic protozoa: 1. Investigating gene function in protozoan parasites – general approaches and challenges'*), allowing scientists to investigate the function of genes and the proteins they encode, often in real time in living parasites. The molecular genetic techniques available to modify genes depends on the individual parasite, so this article outlines some of the more common techniques, providing case studies to demonstrate their applications. Regardless of the approach taken, it is important to have a suitable method or assay by which to monitor or detect any phenotypic changes that occur as a result of the **genetic modification**. It is also worth emphasising that genetic modification experiments are under tight legislative control, that ensures that all possible risks are assessed and mitigated prior to carrying out the experiment. It is especially important to ensure that pathogens are not likely to be made more infectious, pathogenic or toxic, or less treatable, by the proposed modification. Work with pathogens (genetically modified or not) is also only permitted to be carried out in appropriate containment laboratories that ensures they are not released into the environment and requires prior approval from a regulatory body e.g., the Health and Safety Executive in the UK.

Gene knockouts

Deleting or 'knocking out' a gene in an organism allows scientists to study the effect (or phenotype) of missing that gene, which helps them to work out what role the gene or its product usually plays. For example, if the gene encodes a protein that normally helps a parasite to invade a host cell, then knocking it out would produce a parasite that can no longer enter the host cell efficiently and cause disease. There are many different ways of generating a **gene knockout**, and crucially, the whole gene does not always need to be removed. Often, removing part of a gene or even just mutating an essential part of it, can be enough to result in a non-functional protein being expressed - a so-called 'functional gene knockout'.

Conventional gene knockout approach

Traditionally, a common method of knocking out a gene involves replacing the gene's coding sequence with a sequence encoding a protein that confers resistance to a particular drug (a **drug resistance marker**). Parasite cells are transfected with a DNA fragment comprising the drug resistance marker surrounded by the DNA sequences that usually flank the parasite gene of interest (GOI) on the parasite's chromosome (Fig. 1A). The parasite then recombines (integrates) this fragment into the gene locus when it replicates its DNA during cell division, rendering the parasite drug resistant and able to grow in medium containing the drug, while non-transfected parasites will be killed.

While this is a simple method of knocking out a gene, it does have some drawbacks. Many parasites are diploid, meaning they have two alleles of each gene, and in some parasites, there can be multiple alleles for any given gene, or multiple copies of a gene, all of which must be knocked out to generate a complete knockout, or **null mutant** cell line. However, each allele needs

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to be knocked out separately using a different drug resistance marker, usually in successive transfections, which means it can be very labour intensive to generate a null mutant, and there are only limited different drug resistance markers available for any parasite. Gene knockouts also don't work if the gene being removed is essential for parasites to grow and multiply in culture, as any knockout parasites would die before they could be analysed.

CRISPR/Cas9 knockout technology

More commonly today, the **CRISPR/Cas9 gene editing** technique is used to generate gene knockouts. This technique was discovered and developed by Emmanuelle Charpentier and Jennifer Doudna in 2012 and earned them the 2020 Nobel Prize in Chemistry. It exploits a naturally occurring defence system found in bacteria, called CRISPR that enables bacteria to enzymatically cleave the DNA of invading viruses, and allows scientists to precisely delete or modify a GOI. When used in the lab (Fig. 1B), the system involves introducing the coding sequence for a nuclease called Cas9 into the parasite, resulting in it producing the Cas9 protein. A short DNA sequence is then introduced into the parasite that results in an RNA molecule known as a single guide RNA (sgRNA) being transcribed. The sgRNA is custom designed to direct the Cas9 nuclease to a specific site in the parasite's DNA, within a GOI, which it then cleaves. The parasite will then try to repair the cut. If it is left to its own devices, the parasite uses an error-prone process known as **Non-Homologous End Joining (NHEJ)**, which often results in mutation of the gene as it is sealed back together, which can render it non-functional. Alternatively, a drug resistance cassette flanked by sequences homologous to the cut site is transfected along with the sgRNA, resulting in the drug resistance gene being recombined into the middle of the GOI (**Homology-Directed Repair (HDR)**), disrupting the gene and rendering the parasite drug resistant. This allows parasites without the gene to be isolated and studied to determine the effect of disrupting the gene (see Case Study 1). CRISPR/Cas9 technology has many advantages over conventional gene knockouts. It is much quicker to produce sgRNA molecules than the drug resistant cassettes required for conventional knockouts, and it is able to modify both alleles of a gene simultaneously. However, this methodology is still problematic if the GOI is essential for growth or multiplication, as knockout parasites will not be viable.

Conditional gene knockout

To allow the knockout of an essential gene, an extra copy of the GOI under the control of an **inducible promoter** can be inserted into the parasite first and switched on. Then the GOI can be knocked out, and once the knockout cell line has been selected, the inducible promoter is turned off. This will still be lethal for the parasite, but this **conditional knockout** allows scientists to first grow enough parasites to allow them to monitor how the parasites die, which provides clues as to the function of the gene that has been knocked out.

Case Study 1: gene knockout in Leishmania mexicana

Leishmania spp. cause a range of diseases termed the leishmaniases, ranging from disfiguring skin ulcers (caused by species such as *L. mexicana* and *L. major*) to fatal visceral disease of the liver and spleen (caused by e.g., *L. braziliensis*, *L. donovani* and *L. infantum*). While curative treatments exist for the leishmaniases, they require prompt administration, may require hospitalisation of the patient and can come with nasty side effects. Further, some parasite strains are resistant to the drugs, and treatment is less effective if the patient's immune system is compromised, e.g., due to co-infection with human immunodeficiency virus (HIV). Thus, there is a need to understand more about the parasite's biology to enable additional treatments to be developed.

The Gluenz laboratory used the CRISPR/Cas9 gene editing technique to separately knockout 100 genes in the promastigote (sand fly form) of *Leishmania mexicana* grown in culture (Beneke et al., 2019). These genes encoded proteins that had been shown to localise to the flagellum (a tail-like structure required for the parasite to swim). Fifty-six of these knockout cell lines were subsequently found to swim differently from the parental (no genes knocked out) cell line: 52 swam slower, were completely paralysed or displayed uncoordinated swimming, and four swam faster. Viewing the flagella of knockout parasites under the microscope showed that while some of these 56 knockout cell lines had normal-looking flagella, many had flagella with abnormal features. Some were shorter than normal, others were unusually curled and some had no flagella at all (Fig. 1C). This provided valuable information about the roles of flagellar proteins in building a flagellum and their importance for parasite motility in a culture flask. However, the Gluenz lab took this work a stage further, by assessing the ability of these knockout cell lines to infect *Leishmania*'s sand fly vector, *Lutzomyia longipalpis.* This showed that cell lines that were paralysed or displayed uncoordinated swimming were less able to colonise the sand fly, demonstrating the importance of flagellar motility for sand fly colonisation and the life cycle and transmission of *Leishmania*. This work also has relevance for human disease. Some of the *Leishmania* flagellar proteins studied here have human counterparts that are important for cilia to function correctly, since their mutation can result in diseases known as ciliopathies. *Leishmania* offers a simple experimental system to study these proteins, allowing the mechanisms by which their mutation causes disease to be probed.

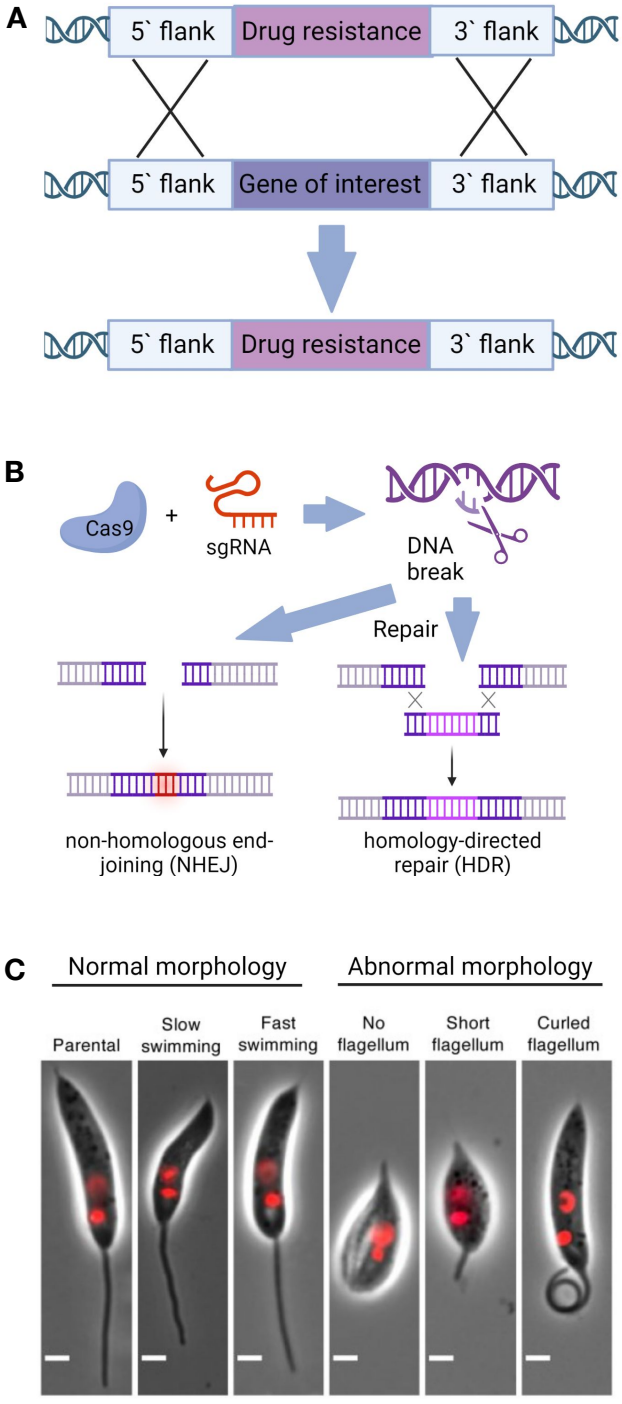

Figure 1. Generating a gene knockout.

A. Conventional gene knockout. A drug resistance cassette (top), comprising the coding sequence for a drug resistance gene (pink box) flanked by sequences (pale blue boxes) identical to those that naturally surround the parasite GOI in its chromosomal location (middle) is transfected into the parasite. Here, it is integrated into the genome in place of the GOI (mauve box). This occurs because the identical DNA sequences that flank both the drug resistance gene in the drug resistance cassette and the parasite gene on the chromosome (pale blue boxes) recombine, swapping the genes they flank (bottom). This process is not very efficient, but parasites, which are now drug resistant, can be selected from unmodified (non-drug resistant) parasites by growing them in medium containing the drug, since only drug resistant parasites will survive. For a more detailed explanation of transfection and selection, please see *'An introduction*

to molecular biology approaches in parasitic protozoa: 1. Investigating gene function in protozoan parasites – general approaches and challenges'; MiSAC*matters* Articles. Also note, that for diploid organisms, this process needs to be repeated to knockout the second copy of the GOI, using a different drug resistance gene.

B. CRISPR/Cas9 gene knockout. Parasites are genetically modified to express the Cas9 nuclease and then transfected with a DNA sequence coding for an sgRNA molecule (or with the sgRNA molecule directly). This results in a specific cut to the target GOI. The parasite will repair this cut either with NHEJ, an error-prone process, resulting in a small insertion or deletion in the gene potentially rendering its product non-functional, or, if a suitable template DNA molecule (e.g., a drug resistance cassette) is co-transfected with the sgRNA, the parasite will use HDR, again resulting in a non-functional gene product.

C. Flagellar mutants of promastigote *Leishmania mexicana*, generated by CRISPR/Cas9 knockout of genes encoding proteins that localise to the flagellum. While knockout of many flagellar genes had no obvious effects, some mutants displayed normal flagellar morphology but showed defects in swimming. Other mutants had absent, short or misshapen flagella. Red: DNA (nuclear and mitochondrial). Scale bars (white): 2 µm. Image adapted from Beneke *et al.*, 2019.

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Gene knockdown

Another alternative for studying the function of an essential gene, is **gene knockdown.** Here, the GOI is left intact, but the corresponding mRNA is depleted via **RNA interference** (or RNAi). RNAi is a defence mechanism against viruses with double stranded RNA (dsRNA) genomes, which some, but not all, parasites possess. In the lab, scientists can exploit this natural mechanism by introducing into the parasite specific dsRNA molecules complementary to a GOI, which results in mRNA for the GOI being degraded, thereby preventing the corresponding protein being made (Fig. 2A). There will still be some protein of interest (POI, already made) present in the parasite, but this may be naturally degraded by the parasite or depleted as the parasite divides in two. However, in most cases, the protein is never completely removed from the cell, and therefore this is a knockdown rather than a knockout. It should also be noted that RNAi is only possible in organisms that possess all of the genes that encode the RNAi machinery and therefore only works in a few parasite species, such as *Trypanosoma brucei* and *Leishmania braziliensis*. However, in these species, it allows the effects of knocking down GOI to be observed in real time (see Case Study 2).

Case Study 2: gene knockdown in Trypanosoma brucei

In a study from the Mottram and Hammarton labs, RNAi was used to deplete all of the 190 **protein kinases** in *Trypanosoma brucei*, the causative agent of African sleeping sickness, one by one (Jones et al., 2014). Protein kinases are enzymes that add phosphate groups to proteins at serine, threonine or tyrosine residues, and are important, often critical, signalling molecules, especially during the cell division cycle. It was thought likely that many protein kinases would be essential, and that a gene knockout approach was unlikely to work. Therefore, to study their function, an inducible RNAi system, turned on by the addition of the antibiotic tetracycline to the culture medium, was used to knockdown the expression of the proteins encoded by each kinase gene. Of the 190 protein kinases, 42 were found to be required for normal parasite growth in culture, since their knockdown led to a parasite growth defect (slower growth, arrested growth or parasite death) (Fig. 2B,C). Further analyses showed that knockdown of 24 protein kinases affected the cell division cycle, with the parasites accumulating at different cell cycle stages or showing defects in the replication and/or segregation of organelles, indicating that these protein kinases were key regulators of the cell cycle. Interestingly, a subsequent study (Fernandez-Cortes et al., 2017) showed that when these trypanosome RNAi lines were grown *in vivo* in a mouse model, depletion of some protein kinases had a much bigger effect on parasite survival than when the parasites were grown in culture flasks in the lab, indicating that these kinases are important for parasites to overcome environmental stresses that they encounter within their hosts.

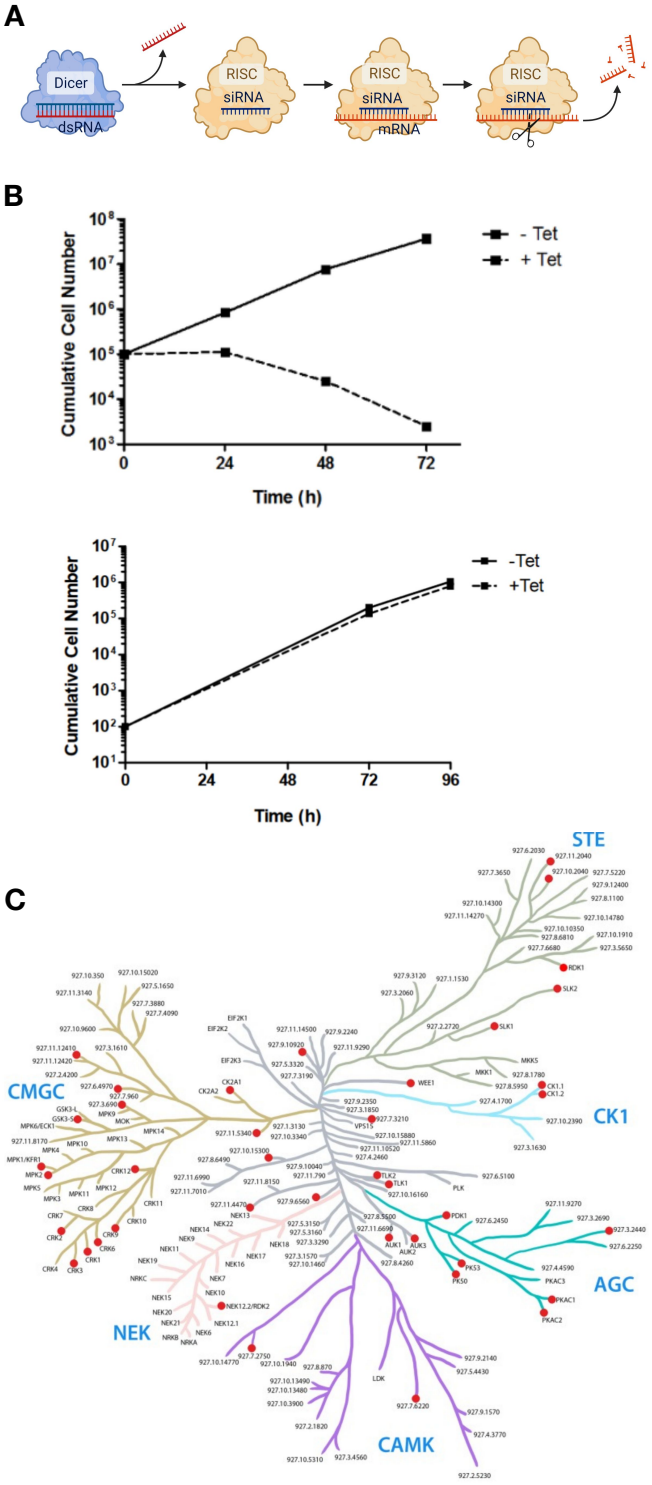

Figure 2. RNA interference (gene knockdown).

A. The RNAi mechanism. Upon introduction of specific dsRNA molecules complementary to a GOI into the parasite, an RNAse enzyme called Dicer (blue) cleaves the dsRNA into short pieces (~21-23 nucleotides in length) known as short interfering RNAs (siRNAs) and unwinds the two strands. Dicer forms a complex with other proteins known as RISC (RNA-induced silencing complex; beige), which then incorporates one strand of the siRNA (blue). This can then pair with complementary sequences in the parasite GOI's mRNA (red). This again forms a dsRNA molecule, so is cleaved by Dicer, resulting in the destruction of the mRNA and preventing any more gene product (protein) from being made.

B. In *Trypanosoma brucei*, all 190 protein kinases were knocked down, one by one, by RNAi that was induced by adding tetracycline (Tet) to the parasite culture medium (Jones *et al.*, 2014). RNAi of some kinases caused the parasites to stop multiplying and die (top graph; compare induced (+Tet) growth curve with uninduced (-Tet) growth curve), showing that these kinases were essential for the parasite to replicate in culture. In contrast, other kinases were found to be non-essential for parasite growth in culture, with the trypanosomes growing normally following RNAi induction (bottom graph).

C. Representation of the *T. brucei* protein kinases, arranged in groups based on their amino acid similarity. In all, 42 protein kinases were found to be essential for growth in culture (red circles) (Jones *et al.*, 2014).

Figure created with BioRender.com. Panels B and C adapted from Jones *et al.*, 2014.

Protein knockdown

A further approach to depleting a POI is to fuse it to a so-called **destabilisation domain** (DD) (Fig. 3A). DDs are proteins that are stabilised in the presence of a synthetic molecule or **ligand**, but rapidly degraded when the stabilising ligand is removed. By fusing the coding sequence of the DD domain to the GOI in the parasite, the resultant fusion protein will only be stable when the parasite is grown in the presence of the stabilising ligand. If the parasites are transferred to culture medium lacking the ligand, the POI is rapidly degraded, allowing the phenotype of the protein depletion to be studied. The DD technique can be used to study protein function in *Plasmodium* (causative agent of malaria), *Toxoplasma* (which causes toxoplasmosis) and *Leishmania* parasites and to date, its use has shed light on many parasite processes, including intracellular protein trafficking, organelle biogenesis, metabolism, motility, life cycle, cell division and host cell invasion and egress.

Protein knocksideways

Rather than knocking down a gene or protein, molecular biology can divert a protein from its usual location to another location in the parasite, which can prevent it from carrying out its normal functions. This is known as the '**knocksideways**' technique and is used in malarial parasites to study the normal function of a protein. It involves the use of two proteins (FRB and FKBP) that each

bind to the ligand rapamycin, as well as fluorescent marker proteins (Fig. 3B). The POI is fused to both a fluorescent protein e.g., **Green Fluorescent Protein** (GFP) to allow its localisation in the parasite to be visualised easily by fluorescent microscopy (see MiSAC*matters* Articles '*An introduction to molecular biology approaches in parasitic protozoa: 3. Visualising proteins in parasites*' for further information on fluorescent proteins) and to FKBP (POI:GFP:FKBP). In most cases, the POI still functions and localises normally in the parasite. The parasite is then genetically modified a second time so that it expresses (makes) another fusion protein comprising FRB fused to another fluorescent protein e.g., mCherry (red), which in turn is fused to a transmembrane domain that anchors the fusion protein to the plasma membrane of the parasite. When the rapamycin ligand is added to the culture medium, it is taken up by the parasite and binds to both fusion proteins. Because the mCherry:FKBP fusion protein is anchored to the plasma membrane, the POI:GFP:FRB fusion protein becomes tethered there too, and may no longer be able to carry out its normal functions, resulting in a functional inactivation of the POI, which can be studied. When applied to study the function of >30 genes of unknown function in *P. falciparum*, knocksideways revealed roles for some of these genes in blood stage development, DNA replication and mitosis (Kimmel *et al.*, 2023).

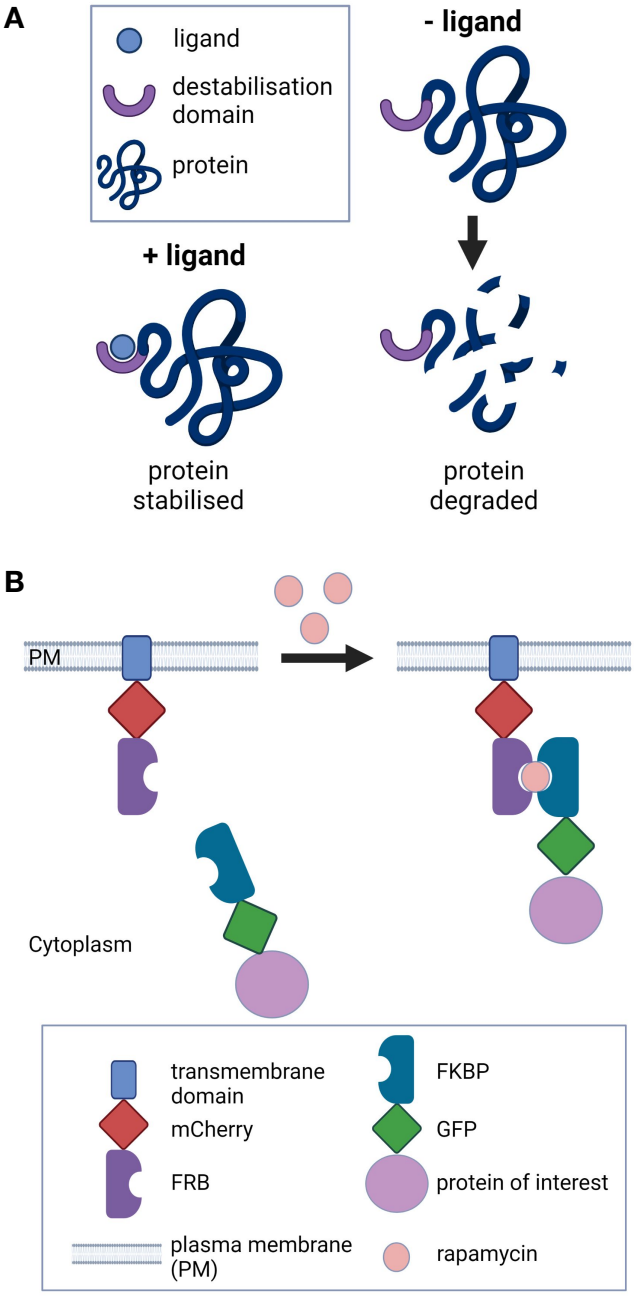

Figure 3. Protein knockdown and inactivation

A. Fusing a destabilisation domain (purple; e.g., a mutated version of the human protein FKBP12) to a POI (dark blue) results in it being degraded unless the stabilising ligand (light blue circle; e.g., Shield-1) is present. Adding the ligand to the culture medium results in it being taken up by the parasite and the protein is stabilised. Moving the parasites to medium lacking the stabilising ligand quickly destabilises the protein and it is degraded, allowing scientists to see the consequences in real time.

B. Schematic of the knocksideways technique. The POI is fused to a green fluorescent protein (GFP) to allow it to be visualised and to the protein FKBP. Another protein, FRB, is fused to a red fluorescent protein (mCherry) and to a transmembrane domain, which tethers it to the parasite's plasma membrane (PM). When the ligand rapamycin is added, it binds the FRB and FKBP proteins together at the plasma membrane, which often renders the POI non-functional.

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Protein overexpression

Finally, it is also possible to genetically modify cells so that they produce increased amounts of a POI (**overexpression**). Commonly, specially adapted promoters are used for this that can be switched on by adding a particular chemical to the culture medium. Such inducible promoters are placed upstream of a copy of the GOI, and when turned on, make the cell produce more of the corresponding protein than usual (Fig. 4A). This can help scientists work out what the protein's function is. Sometimes, too much protein changes something in the cell or can be toxic to the parasite, especially if the protein is an enzyme, and this can provide clues as to the protein's normal function. For example, if overexpressing a protein is found to result in the parasite swimming faster (Fig. 4B), that protein might normally promote parasite motility. Alternatively, if overexpressing a protein deregulates the parasite cell division cycle because the parasite enters mitosis (where division of the nucleus occurs) too early, that protein might normally regulate mitosis. It is also possible to modify the overexpressed protein. Mutating the gene (and therefore the encoded protein) may cause disruption to the parasite if the mutant protein is non-functional (e.g., if its catalytic site is disrupted; Fig. 4B) and outcompetes the native protein (e.g., by binding in place of the native protein in protein complexes and rendering them non-functional too). It is also possible to add fluorescent or epitope tags (see MiSAC*matters* Articles ' *An introduction to molecular biology approaches in parasitic protozoa: 3. Visualising proteins in parasites*' for further information) to the protein to help to visualise it within the parasite (Fig. 4C), and to isolate the protein and its binding partners from the cell.

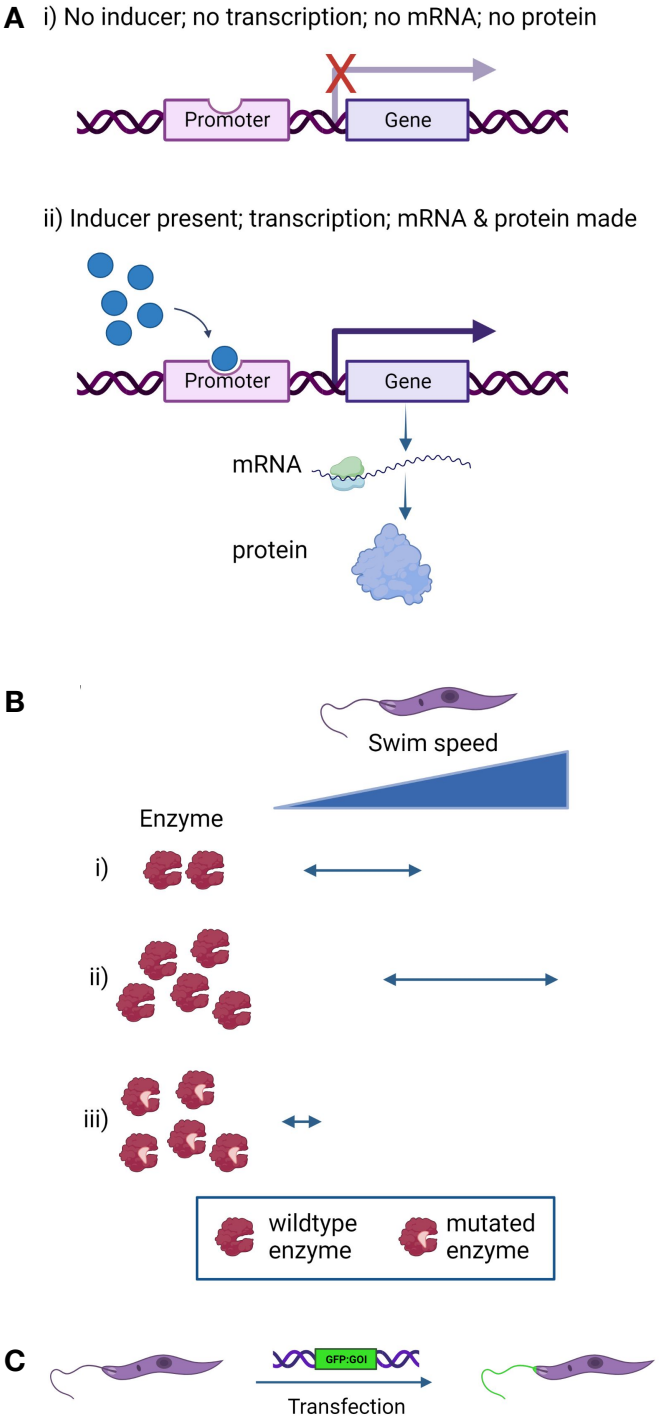

Figure 4. Protein overexpression.

A. An inducible system is often used to overexpress proteins. A gene encoding the POI is inserted into the parasite, by transfecting the parasite with DNA that either integrates (recombines) into the parasite's genome or remains in the cytoplasm (if the DNA is present on a circular piece of DNA called a plasmid). The expression of the gene is controlled by an inducible promoter located just upstream of the gene's coding sequence. The promoter contains a binding site for a chemical inducer and is only active when the inducer is bound to it. By adding the inducer to the culture medium (which allows it to be taken up by the parasite), scientists can control when the promoter is active and when the gene is expressed. Usually, the inducible promoter is a stronger promoter than the gene's native promoter, and this results in the encoded protein being expressed (produced) at higher levels than normal (overexpression).

B. Hypothetical example of how overexpression can be used to determine the function of a POI. In this case, the protein (red) is an enzyme, and when overexpressed, the parasite (in this case, *Leishmania*) swims faster (ii) than the wildtype parasite does (i). This suggests the enzyme plays a role in motility. If the enzyme is mutated (pink region) so that it is no longer active (iii), the parasite swims slower than the wildtype parasite, further indicating that the enzyme is important for motility. Blue triangle indicates that swim speed increases from left to right of the figure. Double-headed arrows indicate swim speed range for each parasite line.

C. Example of fluorescent tagging. A GOI is fused to the coding sequence for a fluorescent protein (in this case green fluorescent protein (GFP)). DNA containing this gene fusion is transfected into the parasite, allowing the gene to be expressed, producing a fluorescent version of the GOI, which can be detected by fluorescence microscopy. Following on from the hypothetical example in (B), if the enzyme important for motility was tagged with GFP, it might be found to localise to the parasite's flagellum.

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Glossary

Conditional knockout – a technique used to knockout a gene that is thought to be essential, which involves inserting an extra copy of the gene under inducible control and turning it on before deleting the native gene copies. The extra gene copy is then switched off allowing the knockout phenotype to be observed.

CRISPR/Cas9 gene editing – a natural anti-viral defence system from bacteria that has been harnessed by scientists to provide a customisable lab tool to edit an organism's genome in a specific way e.g., by changing individual DNA bases, deleting genes or inserting DNA sequences.

Destabilisation domain (DD) – a protein that is unstable except in the presence of a chemical termed a ligand. By fusing a DD to a POI, the POI is only stable in the presence of the ligand. Removing the ligand allows researchers to observe the effects of losing the protein.

Drug resistance marker – a DNA sequence encoding a protein that confers resistance to a drug.

Gene knockout – a cell line where all copies of a particular gene have been deleted. It is not always necessary to delete the whole gene – deleting or interrupting part of the gene or mutating the gene can result in the protein product being non-functional. This is known as a functional gene knockout.

Gene knockdown – a term applied to molecular biology techniques, such as RNAi, that result in a gene's expression being reduced, leading to a reduction in the amount of corresponding protein in the cell. This differs from a gene knockout because the gene itself is still present.

Genetic modification – changing the genetic makeup of an organism by modifying its DNA. This can be achieved by introducing new DNA or by removing or altering some of its existing DNA.

Green fluorescent protein (GFP) – a protein that naturally fluoresces green when excited by light in the blue to ultraviolet range. GFP was originally purified from the jellyfish *Aequorea victoria* and has since been mutated so that it fluoresces different colours; additional fluorescent proteins have also been discovered e.g., mCherry which fluoresces red. By fusing POIs to GFP, it allows researchers to directly visualise their POI in real time under a fluorescence microscope. The scientists Osamu Shimomura, Martin Chalfie and Roger Tsien shared the 2008 Nobel Prize in Chemistry for their work in discovering and developing GFP.

Homology-directed repair (*HDR*) – error-free repair of a double stranded DNA break, often by homologous recombination, that requires a homologous DNA template to be present.

Inducible promoter – a promoter (a region of DNA that controls when a gene is expressed) that can be turned on by the addition of a chemical. The appropriate chemical is added to the parasite growth medium, and is taken up by the parasite, resulting in the promoter (and gene downstream of it) being turned on. Removal of the chemical inducer will result in the promoter being turned off.

Knocksideways – a molecular biology technique where a POI is artificially tethered to the parasite's plasma membrane. The protein is still present, but not at its usual location and may not be able to carry out its normal functions, so this can resemble a functional gene knockout.

Knocksideways – a molecular biology technique where a POI is artificially tethered to the parasite's plasma membrane. The protein is still present, but not at its usual location and may not be able to carry out its normal functions, so this can resemble a functional gene knockout.

Ligand – a chemical molecule that binds to a protein, and in the case of a destabilisation domain, stabilises it.

Non-homologous end joining (NHEJ) – a pathway that repairs double stranded breaks in DNA. Unlike homology-directed repair, this directly joins the broken ends of DNA together without the need for a homologous DNA template. However, NHEJ can sometimes repair the DNA inaccurately, leading to insertions or deletions of bases at the join.

Null mutant – a cell line where all copies of a particular gene have been deleted.

Overexpression – a molecular biology technique which results in the cell producing more of a specific protein than usual, often in response to adding a chemical inducer to the culture medium.

Protein kinase – an enzyme that transfers a phosphate group from the molecule adenosine tri-phosphate (ATP) to either itself or another protein (its substrate) in the cell. The addition of a phosphate group often alters the activity and sometimes the localisation or function of the protein it is transferred to, and hence, protein kinases are important cell signalling molecules.

RNA interference (RNAi) – a gene knockdown molecular biology technique that leads to degradation (destruction) of a specific GOI's messenger RNA (mRNA), and in turn, a reduction in the amount of corresponding protein that is made.

References

Beneke, T., Demay, F., Hookway, E., Ashman, N., Jeffery, H., Smith, J. *et al.* (2019) Genetic dissection of a Leishmania flagellar proteome demonstrates requirement for directional motility in sand fly infections PLoS Pathog **15**, e1007828 https://doi.org/10.1371/journal.ppat.1007828

Fernandez-Cortes, F., Serafim, T. D., Wilkes, J. M., Jones, N. G., Ritchie, R., McCulloch, R. *et al.* (2017) RNAi screening identifies Trypanosoma brucei stress response protein kinases required for survival in the mouse Sci Rep **7**, 6156 10.1038/s41598-017-06501-8 https://doi.org/10.1038/s41598-017-06501-8

Jones, N. G., Thomas, E. B., Brown, E., Dickens, N. J., Hammarton, T. C., and Mottram, J. C. (2014) Regulators of Trypanosoma brucei cell cycle progression and differentiation identified using a kinome-wide RNAi screen PLoS Pathog **10**, e1003886 10.1371/journal.ppat.1003886 https://doi.org/10.1371/journal.ppat.1003886

Kimmel, J., Schmitt, M., Sinner, A., Jansen, P., Mainye, S., Ramon-Zamorano, G. *et al.* (2023) Gene-by-gene screen of the unknown proteins encoded on Plasmodium falciparum chromosome 3 Cell Syst **14**, 9-23 e27 10.1016/j.cels.2022.12.001 https://doi.org/10.1016/j.cels.2022.12.001

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