Genetic Manipulation of Kinetoplastida

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During the 1980s, many kinetoplastid genes were cloned and their function inferred from homology with genes from other organisms, location of the corresponding proteins or expression in heterologous systems. Up until 1990, before the availability of DNA transfection methodology, we could not analyze the function of kinetoplastid genes within the organisms themselves. Since then, it has become possible to create and complement mutants, to overexpress foreign proteins in the parasites, to knock out genes and even to switch off essential functions. However, these methods are not equally applicable in all parasites. Here, Christine Clayton highlights the differences and similarities between the most commonly used model organisms, and assesses the relative advantages of different approaches and parasites for different types of investigation.

The breakthrough in transfection technology started with the transient expression of reporter genes after electroporation of parasites with circular vectors[1] and has since developed to include a wide spectrum of methods for functional gene analysis.

Transient transfections

Transient transfections are very useful for rapid analysis of a variety of functions such as transcription initiation, post-transcriptional regulation and protein targeting. The minimal vector for transient gene expression in *Leishmania* consists of a circular plasmid containing a signal for *trans*-splicing of the mRNA, followed by a reporter gene[2]. Better expression can often be obtained if the reporter gene is followed by a 3′-untranslated region (3′-UTR) from a highly expressed gene. Because polyadenylation and *trans*-splicing are coupled, this 3′-UTR should be followed by a second *trans*-splicing signal (Fig. 1a). Promoters and RNA processing signals from higher eukaryotes do not function at all in trypanosomatids. Processing and expression are most efficient when 5′-segments and 3′-segments from the homologous species are used (C.E. Clayton *et al.*, unpublished). Developmental regulation of gene expression is mediated almost exclusively at the post-transcriptional level; in most examples examined so far the 3′-untranslated region of the RNA determines the RNA stability and translation efficiency. For example, the 3′-untranslated regions of the genes encoding the major surface proteins of procyclic trypanosomes (*EP1* and *EP2*, previously called *PARP* or procyclin) promote high expression in procyclic-form *Trypanosoma brucei* and very low expression in bloodstream forms[3]; that of the A2 gene of *Leishmania major* gives amastigote-specific expression[4].

Although promoterless plasmids give readily detectable expression in *Leishmania*, *Crithidia* and *Trypanosoma cruzi*, they are useless for transient assays in African trypanosomes. In these parasites, a specific transcriptional promoter is required (Fig. 1b). There is as yet no reproducible evidence for specific, strong initiation by RNA polymerase II in trypanosomatids (with the possible exception of the spliced leader loci), but RNA polymerase I promoters [*EP* and variant surface glycoprotein (*VSG*) promoters in African trypanosomes, ribosomal RNA promoters in all trypanosomatids] have proved very useful[5,6]. The RNA polymerase I promoters are species specific: hardly any activity is obtained if a vector containing a promoter from one trypanosomatid species is transfected into another[5,6] (C.E. Clayton *et al.*, unpublished). So far, nobody has obtained translatable mRNA from transcription driven by RNA polymerase III or spliced leader gene promoters.

Permanent transformation using episomes

Transient assays are subject to interexperiment variability and involve high consumption of plasmid DNA and cuvettes. Because only a small fraction of cells (often 2% or less) express the product of interest[7], it is almost impossible to study the localization of introduced gene products by electron microscopy, and difficult to obtain sufficient RNA product for quantitation or structural analysis. Therefore, the creation of permanently transformed cell lines has become the preferred route for most applications. To achieve this, a selectable marker is added to the transient transfection vector (Fig. 1c). Commonly used markers and some possible alternatives are listed in Table 1.

In all trypanosomatids, apart from the salivarian species, stable transformants can be selected easily after transformation of the cells with circular plasmids. Usually, these multimerize to form bigger circles[8]; only *Crithidia fasciculata* retains monomeric plasmids of under 10 kb[9]. Expression of genes from episomes can be enhanced by increasing the concentration of selective drug, resulting in amplification of the plasmid copy number in the cells (see for example Refs 4,10). The mechanism of transcriptional initiation in such episomes is obscure. For at least one example there was evidence for transcription of both DNA strands; strand preference might be determined by terminators[11]. In *T. cruzi*, expression from episomes can be enhanced if a ribosomal RNA promoter is present[12]. In general, circular episomes are unstable in the absence of drug pressure, but stable recombinants are formed if the circles integrate into the genome.

Transfection of *T. brucei* with circular plasmids (with or without promoters) very seldom yields drug-resistant parasites. In the rare exceptions, the DNA has either built an episome containing multiple tandem repeats of the plasmid[13,14] or the plasmid has recombined into the genome. Two groups have selected for sequences capable of promoting maintenance of episomes in *T. brucei* (reviewed in Ref. 15). Although stability-promoting sequences were found, there was no evidence that they served as origins of replication, and the presence of a *EP* promoter in the plasmids appeared to be obligatory. Episomal vectors and their uses are reviewed in Ref. 16.

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Permanent transformation by integration

To promote integration of DNA into the genome, the input vector is linearized by restriction enzyme digestion within sequences identical to the proposed integration site[17]. In the examples shown (Fig. 1c,d) the region upstream of the first open reading frame is used for targeting. Non-homologous recombination of introduced DNA has not been reported. If several genomic targets are present, those of highest homology with the input plasmid are preferred[18]. In most cases it is advantageous to clone transfectants (on agarose or in microtiter plates) directly during the selection procedure, to ensure survival of transformants showing mildly deleterious phenotypes that result in slowed growth.

If a plasmid is targeted into a region of the DNA that is transcribed by RNA polymerase II, the whole integrated sequence is transcribed by read-through from upstream. To obtain a higher level of transcription that is reliably dependent on an added polymerase I promoter, the plasmid must be integrated into a silent region of the genome, such as the ribosomal RNA spacer[14] or the spliced leader array spacer[19] (Fig. 1e). In *T. brucei*, transcription of a reporter gene by RNA polymerase I results in 10–50 times higher expression than read-through transcription by RNA polymerase II[14,19]. If the expression obtained is not adequate, it might be useful to look first at the region 3′ of the gene to be expressed; as already mentioned, different 3′-regions can give very different levels of expression according to the life cycle stage. An additional order of magnitude increase in transcription can be obtained by replacing the trypanosome promoter with the bacteriophage T7 promoter, and transfecting the construct into transgenic parasites that stably express bacteriophage T7 polymerase[7,20]. Although the T3 promoter and polymerase can also be used, the expression levels are lower than those seen with T7, and we have found that they decline upon prolonged passage[7,19].

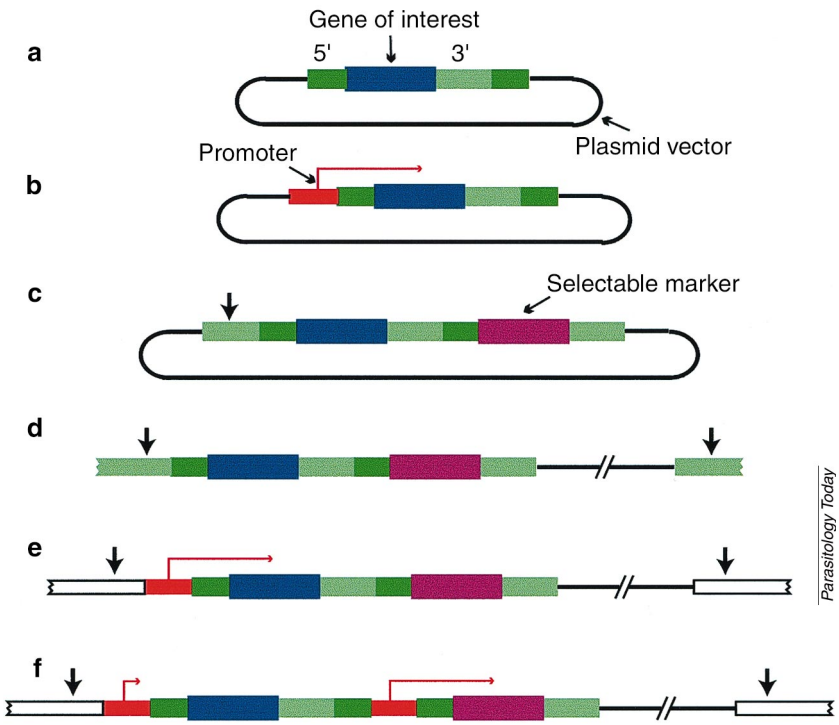

Fig. 1. A selection of transfection vectors. Crucial elements are not to scale and are color coded: promoter, red with red arrows indicating the direction of transcription; 5′-region, dark green; reporter gene or gene of interest, blue; 3′-region, light green; selectable marker, purple; silent intergenic region, white. The plasmid backbone is shown as a black line. Types of vector, with examples, are: simple promoterless transient transfection vector (a); transient vector with promoter (b); selectable promoterless expression vector (c). The arrow indicates a site of cleavage that can be used to generate linear plasmid suitable for homologous genomic integration; selectable vector (shown in c) integrated into the genome after cleavage at the site indicated by the arrows (d); promoter-bearing expression vector integrated into a silent portion of the genome (e); plasmid with two promoters integrated into a silent portion of the genome (f).

Table 1. Markers available for selecting permanently transformed trypanosomes and *Leishmania*[a]

Selectable marker	Selection	Drug target	Mode of resistance	Refs
Neomycin phosphotransferase (NEO)	G418	Ribosome	Enzymatic inactivation of drug	23, 46–48
Hygromycin phosphotransferase (HYG)	Hygromycin	Ribosome translocation	Enzymatic inactivation of drug	23, 49, 50
Bleomycin resistance protein (BLE)	Phleomycin, zeocin	DNA (causes breakage)	Binding (sequestration) of drug	51, 52
Puromycin acetyltransferase (PAC)	Puromycin	Protein synthesis	Enzymatic inactivation of drug	25, 51
Streptothricin acetyltransferase (SAT1)	Nouseothricin	Ribosome	Enzymatic inactivation of drug	53, 54
GARP (surface protein)	Immunofluorescent stain, antibody panning	–	–	55
Green fluorescent protein	Cell sorter could be used	–	–	56
Ornithine decarboxylase	Complementation of auxotrophic mutant			43
Thymidine kinase (as a negative marker only)	Bromodeoxyuridine (BrdU)	DNA synthesis	Selects against the gene, because BrdU is incorporated into DNA	33, 57, 58

[a] Note that the levels of drug needed to kill different organisms, or different life-cycle stages of the same parasite, can vary by at least an order of magnitude. In addition, some selectable markers work only in some parasites or stages. All markers have been used in *Leishmania major* promastigotes and in *Trypanosoma brucei* procyclic and bloodstream forms, with the exception of *PAC* and *SAT1*, which have been tried unsuccessfully in bloodstream forms. A new marker, encoding resistance to blasticidin, is currently being tested.

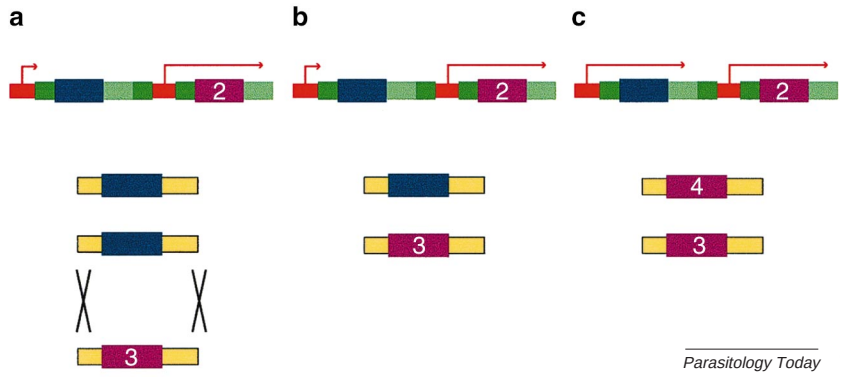

Fig. 2. Creation of an inducible knockout. Color codes are as in Fig. 1. The selectable markers are numbered. The starting trypanosomes contain a *tet* repressor gene linked to one selectable marker (in our system, usually phleomycin resistance) (a). A second selectable marker (marker number 2, in our system hygromycin resistance) is linked to the inducible gene. To knock out the first genomic copy we use a third marker (G418 or neomycin resistance) (b). The second copy can either be knocked out using a fourth marker (marker 4 in the diagram) (c) or by increasing the G418 concentration to obtain a loss of heterozygosity.

Artificial chromosomes

Artificial chromosome vectors are available for both *Leishmania* and *T. brucei*[21,22] (S. Beverley, pers. commun.). These have to be linearized within a segment of telomere sequence before transfection. A problem with artificial chromosomes is that – at least in *T. brucei* – they have a tendency to acquire additional segments of DNA of unknown origin. This might depend on the sequences present in the input plasmid, as results of one study suggested that the minimum size for a stable linear chromosome was about 50 kb[21] whereas, in another study, 15 kb minichromosomes were obtained that were stable in the presence of selection[22]. Such constructs could be used, for example, to study chromosome segregation and structure.

Abolishing expression of genes

Knockouts. If a gene is not essential, it can be replaced by homologous recombination. For a complete gene deletion, untranslated 5′- and 3′-segments from the gene to be targeted are placed on either side of a selectable marker. The plasmid is cleaved at the boundaries of these sequences and the resulting fragment effects a knockout by gene replacement. (It is also possible to inactivate genes by deleting just part of the coding region, but this can produce deceptive results if fragments of the corresponding protein can still be made.)

Because kinetoplastids are diploid, a complete knockout requires two rounds of deletion, usually using different selectable markers (see for example Ref. 23). Alternatively, cultivation of the hemizygous knockout cells in increasing amounts of selective drug can result in selection of cells bearing two copies of the selectable marker, but no longer carrying the targeted gene[24,25]. This 'loss of heterozygosity' effect can arise either by a second homologous recombination or simply as an accident of chromosome segregation. Although this method is attractive in principle, and can be extremely useful if the number of selectable markers is limited, it is extremely laborious, time-consuming and unreliable in practice.

If a homozygous gene knockout is deleterious, clones with the knockout genotype might not be obtained. This is readily seen when a cell with a hemizygous knockout is transfected with a second selectable marker. In theory, either the first marker is replaced by the second, or the essential gene is replaced and the cells die. In practice, doubly drug-resistant cells arise at low frequency, but these retain a copy of the wild-type locus. The plasmid might integrate either upstream or downstream of the targeted gene or in another position with sequence similarity to other parts of the plasmid (such as 3′-regions). Alternatively, the plasmid might be preserved as an episome – or the cells might become polyploid, containing both knockout chromosomes as well as a copy that retains the essential gene[24,26,27].

Sometimes a homozygous gene knockout has no effects on parasite growth *in vitro*, but gives a marked attenuation in virulence *in vivo* (see for example Ref. 28). Indeed, such effects can sometimes be seen as a result of a hemizygous knockout[29]. Because alterations in biological properties frequently arise as a consequence of prolonged *in vitro* cultivation or cloning, it is always essential to perform the positive control of re-transfecting the mutant with the gene that had been deleted.

Inducible expression. Both over- and underexpression of a gene product can be lethal. In yeast, strategies to study such phenotypes often depend on the existence of the haploid phase, or on the use of RNA polymerase II promoters that are controlled by environmental signals: properties that are absent in trypanosomatids. The usefulness of temperature-sensitive mutant proteins is compromised in trypanosomatids by the fact that temperature changes can play a role in signaling life cycle differentiation.

Therefore, we have developed a system using the bacterial *tet* repressor in *T. brucei*. In *Escherichia coli*, this repressor acts by binding to a specific operator sequence situated very close to the start site of transcription, thus inhibiting initiation. Upon addition of tetracycline, the repressor binds the antibiotic, changes conformation and is released from the operator. Our inducible construct is similar to that shown in Fig. 1e; it contains (from left to right) a region for targeting into a silent portion of the genome (the rRNA spacer), an *EP* locus promoter bearing two copies of the *tet* operator just downstream of the transcription initiation site, a 5′-region, the gene of interest followed by a 3′-region, then a selectable marker surrounded by appropriate 5′- and 3′-regions. This construct is linearized within the targeting region and transfected into transgenic trypanosomes that constitutively express the *tet* repressor. Transformants are selected in the presence of tetracycline, to keep the promoter switched on and ensure expression of the selectable marker. When tetracycline is washed out, transcription is switched off. Levels of gene expression can be regulated over four orders of magnitude by varying the amount of tetracycline in the medium[30].

It will be impossible to select transformants in the presence of tetracycline if expression of the upstream gene is lethal to the cells. Therefore, we developed vectors containing a second, constitutive promoter immediately upstream of the selectable marker (Fig. 1f)[31]. At the same time, we optimized the vector for higher or lower induced and repressed expression in different life cycle stages by changing the sequence 3′ to the upstream gene[31].

The use of this system to create an inducible knockout is shown in Fig. 2. Cells expressing the *tet* repressor are transfected with an inducible vector expressing the gene under study (blue) (Fig. 2a). We knock out the first endogenous genomic copy of the gene by homologous replacement (Fig. 2b). Now the inducible gene is turned on by addition of tetracycline. Then we knock out the second gene, either via loss of heterozygosity or by using another marker. In the resulting cells (Fig. 2c), all expression of the gene of interest is under tetracycline control.

For example, we have recently created *T. brucei* containing a single, inducible copy of the gene encoding trypanothione reductase (TRYR), an enzyme thought to be important in maintaining the redox balance in kinetoplastids, and a promising drug target. These cells grow normally in the presence of tetracycline, but after tetracycline withdrawal the level of TRYR is reduced to 1% of normal within three days. Cell growth stops and the parasites become hypersensitive to oxidative stress (S. Krieger *et al.*, unpublished). By adjusting the level of tetracycline we were able to determine the minimal level of trypanothione reductase required for normal growth. Importantly, expression of this gene can also be controlled in mice. To obtain an infection, it is essential to put doxycycline (a tetracycline derivative) in the animals' drinking water, to induce expression of TRYR in the parasite (S. Krieger *et al.*, unpublished).

A second example concerns a glycosomal membrane protein, TbPEX11, which is the homologue of a yeast gene that is required for division of peroxisomes. (Glycosomes are evolutionarily related to peroxisomes.) After the addition of tetracycline to trypanosomes containing an inducible *PEX11* gene, the cells became filled with long tubular glycosomes and stopped growing. Withdrawal of tetracycline from an inducible knockout line resulted in a gradual decline of TbPEX11 protein levels to 10% or less of normal. The resulting cells had no growth defect but had fewer, larger glycosomes than the wild type[25].

Unfortunately, the usefulness of this system is compromised by the intrinsic adaptability of trypanosomes, whose mutation rate has been estimated at 10^{-6} per cell per generation[32]. Mutations in either operator or repressor – or translocation to a different locus – could lead to loss of control of expression. When we have cells with an induced deleterious phenotype, a small minority always escapes control after about five days: expression of an essential product is switched back on (S. Krieger *et al.*, unpublished), or expression of a lethal product is switched off[33]. When we shut off expression of an essential gene, in theory, the extent of depletion should be determined by the rate at which transcription is shut off, the half-life of the mRNA and protein and the rate of cell division. On the other side of the equation is the rate at which mutations or epigenetic effects restore expression. We suspect that the most important variables in the rate of shut-off are the stabilities of RNA and protein. This could explain why it is possible to deplete TRYR to background levels, whereas the very stable PEX11 protein is never reduced to below 10%.

In bloodstream trypanosomes, *EP* promoter vectors give only moderate overexpression. To achieve higher inducible expression, Wirtz *et al.* replaced the inducible *EP* promoter with a T7 promoter carrying an operator sequence. Because of the very high activity of the T7 promoter, it was now necessary to increase the amount of repressor present to suppress background expression. To attain more reliable, higher expression of the repressor, an attenuated T7 promoter (with 10% of normal T7 promoter activity) was inserted upstream of the *tet* repressor gene. Cells expressing T7 polymerase can now be taken as a starting point and, if desired, the repressor-bearing construct can be engineered to knock out one allele of the gene to be studied[20,34].

Trypanosoma brucei is currently the organism of choice when inducible gene expression is required. However, the *T. brucei tet* system has been adapted recently for use in *Leishmania donovani* (S. Yan, P.J. Myler and K. Stuart, pers. commun.) and the degree of control attainable, about 100-fold, is more than adequate for many purposes.

Developmental regulation of gene expression can also be obtained by placing a 3′-untranslated region from a regulated gene downstream of the gene one wishes to regulate. For instance, the 3′-untranslated region of an amastigote-specific gene from *L. donovani* was used to obtain preferential expression of a deleterious gene, a cytosolic 3′-nucleotidase, in amastigotes. Promastigotes transfected with the episomal construct showed little or no growth inhibition, but the cells grew poorly or not at all when they differentiated into amastigotes[4].

As mentioned above, our ability to examine the function of essential genes is currently limited by the facility of mutation in trypanosomatids. Site-specific recombination could provide a solution to this problem. The *cre* recombinase of P1 phage mediates highly specific recombination at *lox* recognition sites, such that a piece of DNA flanked by *lox* sites can be excised from the genome as a circular DNA molecule. Transient expression of *cre* recombinase in cells that contain only one copy of an essential gene flanked by *lox* sites should result in complete loss of the gene from the genome[35]. In addition, this methodology allows the repeated use of a single selectable marker, which could be very useful if the number of available markers is limited.

Dominant-negative strategies. If a gene is present at more than two homologous loci, or present as tandem repeats that include other interspersed genes, knockouts become difficult or impossible. Dominant-negative strategies are very attractive in this situation.

If a protein is active as a multimer, the activity of wild-type subunits might be suppressed by oligomerization with suitable mutant subunits. Tovar *et al.* expressed, in *T. cruzi*, a mutant version of TRYR that formed inactive dimers with wild-type subunits[36]. The level of TRYR activity was reduced to 14% of wild-type levels. Although the cells grew normally in axenic culture, they had a reduced capacity to survive in activated macrophages.

Another possibility is the expression of antisense RNA. Results using this technology have so far been variable. Expression of antisense *TRYR* RNA from episomes in *T. cruzi* reduced TRYR levels very little[37], and selection for a higher copy number of the antisense episome yielded only episomes in which the antisense gene had inverted to the 'sense' orientation. This result suggests that the antisense strategy might have actually worked, but because TRYR is essential there was strong selection for cells in which the antisense effect was bypassed. However, Zhang and Matlashewski[38] succeeded in reducing levels of expression of the amastigote-specific A2 protein of *L. donovani* using a very similar antisense strategy, and showed that the resulting parasites had impaired virulence for mice. In both experiments described, the antisense RNA was expressed from a promoterless episome. The 'direction' of the RNAs produced was therefore defined in relation to the surrounding RNA processing signals and the orientation of the linked selectable marker. Thus, some 'sense' RNA could arise by transcription of the other strand of the episome. Northern blots yielded no trace of the antisense transcript, but a marked decrease of the endogenous (sense) transcript, suggesting that antisense transcription had caused specific RNA degradation.

So far, attempts to obtain effects by inducible expression of antisense RNAs in *T. brucei* have failed completely. For example, we were able to express inducibly a tenfold excess of antisense *TRYR* RNA, with no effect on the levels of sense *TRYR* mRNA and TRYR protein (S. Krieger, PhD Thesis, Universität Heidelberg, 1998). Similarly, Bastin *et al*.[39] saw no effect whatsoever when they inducibly expressed an excess of antisense RNA to the paraflagellar rod protein PFRA. In the latter case, however, one rare transformant was obtained that showed a marked reduction of *PFRA* mRNA and protein. In this transformant, the antisense construct had integrated into the *PFRA* locus itself; the phenotype was not tetracycline dependent, suggesting that transcription of the antisense construct was no longer dependent on the inducible promoter.

The mechanisms whereby the anti-PFRA construct downregulates PFRA RNA levels – in particular the levels of transcripts originating from the unaltered second, homologous allele – are obscure, but one possible explanation of all the 'antisense' observations is that the active species is nuclear double-stranded RNA. This could be produced from promoterless episomes or from transcription of sense and antisense genes in close proximity on the chromosome. By contrast, when inducible constructs are used, no sense transcript is made from the construct and the antisense RNAs might be efficiently exported to the cytosol without encountering a sense molecule. Double-stranded RNAs are known to mediate efficient and specific suppression of gene expression in *Caenorhabditis elegans*[40] and recent results suggest that a similar phenomenon occurs in trypanosomes[41].

Cloning by complementation

The facility with which *Leishmania* can be transformed by large episomes, combined with its energetic growth on agar plates, makes this species the organism of choice when wishing to clone genes by complementation. To find the enzymes responsible for synthesis of surface lipophosphoglycan in *Leishmania,* for example, the parasites were mutagenized and selected for defects in adherence to lectin. The genes responsible for the defects could be cloned by transfecting the mutant with a *Leishmania* genomic cosmid library and selecting for the recovery of lipophosphoglycan synthesis[42]. Similar approaches should be possible with the other trypanosomatids[43,44].

Because trypanosomatids are diploid, it is difficult to identify recessive mutations. A possible way to circumvent this problem might be to generate cell lines with partial chromosomal deletions. These would be haploid for a segment of one specific chromosome. So long as haploidy had no deleterious effects, a set of such partial monosomic cells could be subjected to mutagenesis to find recessive mutants (N. Dilmac and J.H. LeBowitz, unpublished).

The use of complementation to identify the genes that are defective in a mutant has already been described. Chemically mutagenized cells might harbor a large number of different mutations, which can significantly complicate analyses of complex phenomena such as differentiation. Recently, an alternative strategy for the creation of mutants was described that simultaneously allows tentative identification of the mutant gene without recourse to complementation. Gueiros-Filho and Beverley transfected into *L. major* plasmids encoding the mariner transposase and a cassette containing a modified mariner element[45]. The mariner element could jump into the *Leishmania* genome, potentially interrupting *Leishmania* genes and creating mutations that are tagged by the presence of the mariner sequences. This approach would clearly be particularly powerful in combination with a panel of partially monosomic lines.

Future directions

Reverse genetic methodology is essential to make use of the megabases of sequence information being produced by the trypanosomatid sequencing projects. The availability of homologous sequences from the different trypanosomatid species will enable us to take advantage of their respective advantages. Particularly where the properties under study are common to all trypanosomatids, it might be beneficial to use a variety of different parasites for specific purposes. Examples are glycosome biogenesis and function, RNA processing mechanisms and many aspects of intermediary metabolism. Some *Crithidia* lines can grow on defined media, so will be extremely useful for studies of intermediary metabolism and substrate transport – but at present the transfection efficiency of *Crithidia* is much lower than that of *Leishmania*, making complementation approaches very difficult. *Leishmania* species are the organism of choice for mutation and complementation approaches, but inducible expression is better developed in the salivarian trypanosomes. *Crithidia* and *Leptomonas* grow to much higher densities than the mammalian pathogens, in cheap broth, so are ideal for purification of large quantities of macromolecules.

Sometimes, however, properties are species specific – it is no more possible to study antigenic variation in *Leishmania* than it is to study lipophosphoglycan synthesis or survival in the lysosome in African trypanosomes. Nevertheless, transfection of genes from one species to another might enable us to determine which genes are responsible for a particular species-specific property.

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Focus

Schistosomiasis of the Female Genital Tract: Public Health Aspects

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In this paper Gabriele Poggensee, Hermann Feldmeier and Ingela Krantz discuss the public health relevance of female genital schistosomiasis (FGS). Some of the stated hypotheses are supported only by clinical observations and/or circumstantial evidence as valid epidemiological and immunological data of this disease entity are still very scanty. Morbidity caused by the presence of schistosome eggs in the lower and upper genital tract have been almost completely neglected during the past two decades. This has been acknowledged by the WHO and, in 1997, the Gender Task Force of the WHO's Tropical Disease Research Programme (TDR) included FGS in a list of scientific areas that deserve high research priority.

Female genital schistosomiasis (FGS), as we define it, is characterized by the presence of schistosome eggs/worms in the upper or lower genital tract. Such a disease manifestation was reported for the first time in 1899 when Madden[1] observed a tumorous growth that consisted of numerous egg granulomas in the vagina of a young woman from Egypt. Since then, schistosome ova and adults have been detected throughout the female genital tract, from the vulva to the ovaries, by pathologists from many countries where urinary schistosomiasis is endemic[2]. Clinically apparent vulval, vaginal and/or cervical schistosomiasis has been reported from both endemic and non-endemic areas. This has led the Gender Task Force of the World Health Organization Tropical Disease Research Programme (WHO-TDR) to include FGS in a list of scientific areas that deserve high research priority[3].

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The pathophysiological basis of FGS

Copulating adult worms have been found in histological sections of the vulva, cervix, uterus and fallopian tubes[4–7]. The specific vasculature of the small pelvis enables adult worms to migrate and can cause schistosome eggs to be transferred to the genital organs. Anastomoses between the different venous plexuses of the small pelvis, the veins of which are almost without valves and allow blood to flow in either direction, as well as portosystemic anastomoses, present a network of routes for the migration of adult worms and/or embolization of eggs (Fig. 1). Adaptive vascular changes in puberty and changes in the direction of the venous blood flow during pregnancy increase the chance of ectopic localization. As early as 1953, Gelfand and Ross[8] remarked that: 'if a patient has urinary bilharziasis, the probability is that she will have ova of *Schistosoma haematobium* in some portion of the genital tract'.

The prevalence of FGS

Until recently, the occurrence of FGS was inferred exclusively from postmortem studies and histopathological data from endemic areas identifying schistosome eggs in the genital tissue. In postmortem studies, the observed frequencies were 7–100% for lesions in the lower reproductive tract and 2–83% for lesions in the upper reproductive tract[2].

Four population-based studies have now been performed to determine the point prevalence of FGS of the lower reproductive tract[9–12]. In these studies, the diagnosis of FGS has been achieved by the detection of eggs in the biopsies of genital tissue. Although the study design was different, these studies showed that FGS is a common manifestation in *S. haematobium* infection, with a prevalence rate ranging from 30% to 75% (Table 1). In the most recent and extensive Tanzanian study, nearly half of the female population of childbearing age living in the study village have been screened for the presence

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