COMBINING RNA INTERFERENCE MUTANTS AND COMPARATIVE PROTEOMICS TO IDENTIFY PROTEIN COMPONENTS AND DEPENDENCIES IN A EUKARYOTIC FLAGELLUM

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Running Title: A paraflagellar rod proteome

Eukaryotic flagella from organisms such as Trypanosoma brucei can be isolated and their protein components identified by mass spectrometry. Here we use a comparative approach utilising 2D-DIGE and iTRAQ to reveal protein components of flagellar structures via ablation by inducible RNAi mutation. By this approach we identify 20 novel components of the paraflagellar rod (PFR). Using epitope tagging we have validated a subset of these as being present within the PFR by immuno-fluorescence. Bioinformatic analysis of the PFR cohort reveals a likely calcium/ calmodulin regulatory/ signalling linkage between some of components. We have extended the RNAi mutant/comparative proteomic analysis to individual novel components of our PFR proteome showing that the approach has the power to reveal dependencies between subgroups within the cohort.

The canonical eukaryotic flagellum displays a characteristic “9+2” microtubular profile, where nine outer doublet microtubules encircle two singlet central pair microtubules; an arrangement found in organisms as diverse as trypanosomes, green algae and mammals. Although this 9+2 microtubule arrangement has been highly conserved through eukaryotic evolution there are examples where this standard layout has been modified; including the “9+0” layout of primary cilia and the “9+9+2” of many insect sperm flagella. In addition to this highly conserved 9+2 MT structure, flagella and cilia show a vast range of discrete substructures, such as the inner and outer dynein arms, nexin links, radial spokes, bipartite bridges, beak-like projections, ponticuli and other microtubule elaborations, that are essential for cilium/flagellum function. Cilia and flagella can also exhibit various extra-axonemal elaborations, and while these are often restricted to specific lineages there is evidence that some functions, such as metabolic specialisation, provided by these diverse structures are conserved (3,4). Examples of such extra-axonemal elaborations include the fibrous or rod-like structures in the flagellum of the parasite Giardia lamblia, (5), kinetoplastid protozoa (6,7) and mammalian sperm flagella, along with extra sheaths of microtubules in insect sperm flagella (8).

Several recent studies have set out to determine the protein composition of the flagellum and demonstrated the existence of both an evolutionarily conserved core of flagellum/cilium proteins and a large number of lineage-restricted components (9-13). Whilst these approaches provide an invaluable catalogue of the protein components of the
flagellum they provide only limited information on the sub-structural localisation of proteins and do not address either the likely protein-protein interactions or the function of these proteins within the flagellum. To address these issues, the protein composition of some axonemal substructures (radial spoke complexes for example 14) has been determined by direct isolation of these structures and a number of complexes have been resolved by the use of co-immunoprecipitation of indicator proteins (for example 15,16)). In addition the localisation and function of a number of flagellar proteins have been investigated by detailed analysis of mutant cell lines, (particularly of C. reinhardtii), which exhibit defined structural defects within the assembled axoneme. Early studies employed 2 Dimensional polyacrylamide gel electrophoresis (2D-PAGE) to compare the proteomic profile of purified flagella derived from C. reinhardtii mutants and wildtype cells (17-22) which showed numerous proteomic differences in the derived profiles. Available technology did not allow identification of the individual proteins within the profiles. Recent proteomic advances offer the opportunity for this identification. For instance the comparative proteomic technique iCAT has been used to identify components of the outer dynein arm (23). This technique utilises stable isotope tagging to quantify the relative concentration of proteins between two samples.

Trypanosomatids are important protozoan parasites whose flagellum is a critical organelle for their cell biology and pathogenicity. Their experimental tractability also provides opportunities for generic insights to the eukaryotic flagellum. They are responsible for a number of devastating diseases of humans and other mammals, including commercially important livestock, in some of the poorest areas of the world (24-26). All kinetoplastids build a flagellum that contains an extra-axonemal structure termed the parflagellar rod (PFR). In the case of the African trypanosome T. brucei, this consists of a complex subdomain organisation of a proximal, intermediate and distal domain as well as links to specific doublets of the axoneme and a structure known as the flagellum attachment zone (FAZ) by which the flagellum is attached to the cell body for much of its length (6,7). The PFR is required for cell motility (27,28) and serves as a scaffold for metabolic and signalling enzymes (3,29,30).

We have previously shown that the presence of this structure is essential for the survival of the mammalian bloodstream form of the parasite both in vitro (in culture) (12) and in vivo (in mice) (31) as part of a wider requirement for motility in this life cycle stage (12,32,33).

Two major protein components of the PFR (PFR1 and PFR2) have been identified (34-38) along with several minor PFR protein components (3,29,30,39-43). The availability of RNAi interference techniques in T. brucei allowed the generation of the inducible mutant cell line snl2 (44), in which RNAi mediated ablation of the PFR2 protein causes the specific loss of both the distal and intermediate PFR subdomains (Fig. 1A). After RNAi induction cells become paralysed but remain viable (44). Our laboratory (3) has previously identified two PFR-specific adenylate kinases by comparing 2 Dimensional SDS PAGE gels of purified flagella from induced and non-induced snl2 cells. These proteins cannot be incorporated into the PFR after PFR2 ablation.

The ability to ablate PFR2 and hence disable assembly of a major portion of the PFR affords an opportunity to apply advanced proteomic approaches to identify additional PFR proteins. In this present study we have used two complementary proteomic approaches, 2-dimensional fluorescence difference gel electrophoresis (2D-DIGE) (45) and isobaric tags for relative and absolute quantitation (iTRAQ – Applied Biosystems inc.), to investigate PFR+ and PFR- flagella to define 30 components of these two PFR subdomains. We have also conducted a bioinformatic analysis of amino acid motifs present in this protein cohort to gain insights into the possible functions of novel proteins and used epitope tagging approaches to confirm the PFR localisation of a test set of identified proteins. We then asked if it was possible to combine comparative proteomics with further analysis of RNAi mutant trypanosomes to provide detailed information on the individual interactions and assembly dependencies within the novel PFR components we had identified. By iterating the subtractive proteomic analysis with novel putative PFR proteins we were able to reveal the existence of distinct PFR protein dependency relationships and provide intriguing new insight into regulatory processes potentially operating within the trypanosome flagellum. Finally, this study establishes the mutant/proteomic combination as
a powerful enabling approach for revealing dependencies within sub-cohorts of the flagellar proteome.

EXPERIMENTAL PROCEDURES

Cell culture. Procyclic T. brucei cells were cultured at 28 °C in SDM-79 medium supplemented with 10% (v/v) heat-inactivated foetal calf serum as previously described (46). For induction of RNAi doxycyclin was added to the media to a final concentration of 1 μg ml⁻¹.

Vector construction. 200-800bp from the open reading frame of the gene of interest (ORF product) and 200-300bp of sequence immediately upstream of the gene of interest (UTR product) were amplified by PCR from genomic DNA with the addition of appropriate restriction endonuclease recognition sequences (Supp methods). ORF products were inserted into p2T7-177 (47) between SpeI and XhoI sites and ORF and UTR products were inserted into pENT6 BTyYFP (48) between SpeI and BamHI sites.

Transfection. Purified linearised plasmid DNA was used to transfect logarithmically growing cultures of procyclic form T. brucei by electroporation (3x 100μs pulses of 1700V). Transfected cells were selected by the addition of 10 μg ml⁻¹ Blasticin (pENT6 BTyYFP derivatives) and/or 5 μg ml⁻¹ Phleomycin (p2T7-177 derivatives) to the growth medium.

Preparation of flagella. Procyclic form T. brucei were first treated with PEME (100mM PIPES pH6.9; 2mM EGTA; 1mM MgSO₄; 0.1mM EDTA) + 1% Nonidet P40 and then PEME + 1M NaCl in the presence of protease inhibitors, DNaseI and RNaseA. Insoluble material - consisting of components of the axoneme, PFR and a number of other flagellar associated structures but not the flagellar membrane or other soluble components such as IFT particles - was either used immediately or stored for short periods at -20°C. 1D SDS-PAGE and Western blotting were performed using standard protocols.

Analysis by DIGE. Paired protein samples were labelled with Cy Fluors for DIGE (GE Healthcare) and pooled according to the manufacturer’s protocols. IPG strips were rehydrated in the presence of the samples for 20 hours before first dimension focussing (50μA strip⁻¹ current limit; 10-500V gradient for 4 hours; 500-8000V gradient for 5 hours; 8000V hold for 6 hours). Second dimension separation was performed using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (1W gel⁻¹ for 1 hour then 13W gel⁻¹ for 4-5 hours). Spots were visualised on a Typhoon scanner (GE Healthcare) and analysed using DeCyder software (GE Healthcare). Spots of interest (criteria in main text) were excised and proteins identification was performed as below.

Tryptic digests and MALDI. 2D gel spots were excised and in-gel digested with trypsin. Briefly gel pieces were washed twice in 25 mM ammonium bicarbonate (Fluka) in 50% acetonitrile (Sigma), dehydrated with an acetonitrile wash and reduced in 10mM DTT (Fluka) for 30 mins, before being washed again and dehydrated prior to alkylation using 55mM iodoacetamide (Fluka) for 60 mins. Gel pieces were digested with 200 ng of trypsin at 37 °C overnight. Peptides were acidified using 1 μl TFA (Fluka) and extracted with a wash of 0.1% TFA in 50% acetonitrile and a wash of 0.1% TFA in 100% acetonitrile. Supernatants were pooled and dried in a SpeedVac (Thermo). Peptides were purified using a home-made C18 purification tips. Peptides were spotted using α-cyano-hydroxycinnamic matrix and analysed on an Applied Biosystems 4800 MALDI TOF TOF.

Data were searched using MASCOT (MatrixScience) against an in-house curated T. brucei database containing trypsin and human keratin. Tolerance was set at 50ppm for MS and 0.1Da for MS/MS. Carbamidomethylation of cysteine was set as a fixed modification and methionine oxidation as a variable modification. Positive identifications were accepted with a confidence interval of 99% or greater and two unique peptides.

iTRAQ and LC MALDI. iTRAQ was performed as per manufacturer’s recommendations and labelled peptides purified on an SCX cartridge (Applied Biosystems). The iTRAQ labelled peptides were fractionated by C18 RP HPLC using a Dionex U3000 nano-HPLC coupled to a Probot spotting robot. A 100 min gradient was used and fractions spotted, along with MALDI matrix, directly onto the MALDI target at 15 s intervals. The LC run was analysed on an Applied Biosystems 4800 MALDI TOF TOF mass spectrometer and data were analysed using GPS Explorer (Applied Biosystems) and MASCOT. Tolerance was set at 50ppm for MS and 0.1Da for MS/MS. Positive identifications were accepted with a confidence interval of 99% or greater and two unique peptides.
Immuno-fluorescence. Cells were settled onto glass slides and extracted by addition of 1% Nonidet P40 in PEME. Cytoskeletons were fixed in methanol and then labelled with BB2 (49) (Ty epitope) and L6B3(50) (FAZ). Labelling was visualised with 488 Fluor-conjugated α-mouse IgM (Invitrogen) or 594 fluor-conjugated α-mouse IgG1 (Invitrogen). Slides were mounted in Vectashield mounting medium with DAPI (Vector Laboratories Inc) and examined on a Zeiss Axioplan 2 microscope.

Bioinformatics. BLAST (51) alignments were performed either by using software and databases available via the Sanger Institute at GeneDB (www.genedb.org - kinetoplastids) or using an in-house BLAST programme available via NCBI (www.ncbi.nlm.nih.gov/BLAST) with genomes downloaded from JGI (www.jgi.org – C. reinhardtii) or NCBI. For reciprocal BLAST an e-value maximum of 1e-10 was used and BLAST results were processed using custom Perl scripts and Excel spreadsheets. MEME searches (52,53) were parameterised to find any number of repetitions between 6 and 300 amino acids with no limitation on the number of motifs and an entry p-value cut-off of 1e-5. WebLogo (http://weblogo.berkeley.edu/) was used to generate sequence logos (54). Hidden Markov models were generated as previously described (55).

RESULTS

Comparative proteomic analysis of the snl2 RNAi mutant cell line identifies known and putative PFR components. We have used two complementary comparative proteomic techniques, iTRAQ and DIGE, to identify proteins that are absent from flagella purified from the snl2 induced cells but present in non-induced flagellum samples.

Using iTRAQ, we analysed three independent sample pairs, each consisting of a non-induced and 72 hour RNAi induced purified flagella. Two sample pairs were analysed in a quadruplex experiment using four iTRAQ labels while the remaining sample pair was analysed in a duplex iTRAQ experiment utilising two of the available labels. In total, 239 proteins were identified in these samples of which 53% were present in our recent T. brucei flagellum proteome (12). An advantage of using a quadruplex design for two of the pairs is the ability to obtain abundance ratios between the non-induced samples of each pair. When plotted as a frequency distribution of log2 ratios this shows a near symmetrical distribution with 98% of log2 ratios falling between -1 and +1 (i.e. a less than twofold change in either direction) (Fig. 1B). A plot of log2 ratios of the two RNAi induced samples reveals a similar distribution (not shown). When average log2 ratios of the RNAi induced to non-induced samples are plotted in the same way a shoulder is observed on the distribution for values of log2 ratio less than -1 (Fig. 1C). With reference to the ratio distribution obtained by comparison of the two non-induced samples we defined our proteins of interest as those with log2 ratio of less than -1 in either iTRAQ experiment (i.e. a greater than twofold reduction after PFR2 ablation). In cases where ratios passed this test in one experiment and failed in the other, the ratio generated by the highest number of peptides was accepted. If this occurred between samples in the quadruplex iTRAQ we applied a stringent approach and did not classify the protein as of interest. The portfolio of PFR candidates generated by this approach consists of 24 proteins and results from each sample pair are plotted separately in (Fig. 1D).

We also performed a comparative analysis on the snl2 mutant using DIGE, another established proteomic technology, in three experiments using two independent paired samples (non-induced and 72 hour RNAi induced) in each experiment. The resulting gels were analysed using DeCyder software and spots were selected on the basis of a fold change in spot volume greater than two (consistent with the criteria applied to the iTRAQ results) (Fig. 2). Spots which exhibited this fold modulation were excised from the gels and subjected to tandem MS protein identification. In total 62 spots were sequenced and 36 proteins were identified. In cases where multiple identifications were forthcoming from a single spot, we again applied a conservative criterion and did not classify these as proteins of interest as we cannot be sure of the specific contribution of each protein to the reduction in spot volume. It is likely however that at least some of these excluded 20 proteins are bona fide PFR components and await further investigation for confirmation. Although one specific group of spots did show an increase in spot volume after induction, no protein IDs were forthcoming from MS/MS analysis. The observation that several spots increased in abundance as a result of PFR2 ablation may be
due to differences in post-translational modification altering the mobility of proteins in either one or both of the electrophoresis dimensions. There is no evidence of any other spots increasing in abundance or appearing as a consequence of PFR2 ablation so it is likely that the majority of the changes observed are due to the absence of proteins in the sample. In total 16 proteins were identified as PFR candidates in this screen; 10 of which were also identified by iTRAQ.

In summary these two proteomic approaches identified 30 proteins as PFR candidates (Table 1) of which twenty are novel. These novel proteins are named here as paraflagellar rod proteome component (PFC) 1 to 20. Two proteins in the dataset have existing annotations but have not previously been associated with the PFR. KMP-11 has been shown to be differentially expressed during the life cycle of several kinetoplastids and has been localised to the flagellum (56,57). Tb11.01.6300 is annotated as a PI3-related kinase by homology and our analysis of the predicted domain architecture and size of the protein are consistent with this automated annotation (see below). 15 proteins have been identified as PFR components by previous studies and eight of these proteins are present in our dataset. These are the major PFR proteins PFR1 and PFR2 (34-38), PAR1 (39,42), PFR5 (43), Tb5.20 (40), calmodulin (30) and, as mentioned above, the PFR adenylate kinases ADKA and ADKB (3).

Bioinformatic analysis of PFR proteins reveals known and novel motifs. As the PFR is an extraxonemal structure specific to trypanosomes and related protozoa it might be expected that many of the PFR proteins will be restricted to this lineage. Indeed in silico analysis using a reciprocal BLASTP methodology (Fig. 3.) revealed that 25 of the proteins identified are either restricted to T. brucei or have a corresponding gene in the Leishmania major genome but cannot be found in either the Homo sapiens or C. reinhardtii genomes. However, homologues were detected in either H. sapiens, C. reinhardtii or both for five of these proteins. In some cases, for example calmodulin, this may be as a result of other functions in the cell but it may also give an insight into conserved flagellar functions, albeit built into variable flagellar structures (4). We subsequently analysed the domain and motif architecture of proteins present in our dataset using the motif analysis tool MEME (52,53). This analysis identified numerous domains, many of which correspond to previously predicted Pfam domains (Table2). As previously reported the PFR domain (PF05149) was identified in PFR1, PFR2, PFR5 and PAR1 (43) but we also detected an additional novel occurrence of this domain in Tb5.20. A motif corresponding to the EF hand domain (PF00036) was detected in five proteins (Tb5.20, PFC1, PFC7, PFC6 and calmodulin) and one corresponding to the leucine rich repeat domain (PF00560) was present in four proteins (PFC13, PFC14, PFC2 and PFC5). As expected, motifs consistent with adenylate kinase signatures were detected for ADKA and ADKB. A Pfam analysis of the dataset also identified two IQ calcium-independent calmodulin binding motifs (PF00612) in PFC15, a Beige/BEACH domain (PF02138) in PFC10, as previously reported (43) an SH3 (PF00018) domain in PFR5 and a Phosphatidylinositol 3- and 4-kinase (PF00454) and FATC (PF02260) domain in Tb11.01.6300, consistent with the automatic PI3-related kinase annotation.

In addition to these known domains, MEME also revealed the presence of three novel motifs named here meme 1-3. Meme1 is a variable 15 amino acid motif that is present in eight proteins within this dataset (PFR1, PFR2, PAR1, PFR5, Tb5.20, calmodulin, ADKA and ADKB) (3).
in PFC4, PFC16 and PFC3 (Supp. Fig. 1D). A hidden Markov model generated from the alignment of this motif did not identify any additional proteins when used to search the *T. brucei* genome.

**PFC proteins localise to the PFR** The portfolio of proteins generated in this analysis contains eight proteins previously proposed as PFR components. These initial descriptions have come from a number of kinetoplastids (3,30,38,40,42) and are supported by variable levels of evidence. Where necessary the annotation of these proteins in the *T. brucei* dataset has been inferred from bioinformatics using the TriTryp genome projects (58-60). The presence of these PFR proteins in this dataset (representing over 25% of the identifications) is comforting and shows that this RNAi mutant/comparative proteomic method is capable of identifying genuine PFR components and supports the annotation of PFR proteins previously identified only by bioinformatics. In order to validate the remaining proteins in the dataset we selected seven novel proteins that are representative of the methodologies used to identify them (PFC5 and PFC16: identified only by DIGE; PFC15 and PFC4: identified only by iTRAQ; PFC3, PFC11 and PFC14: identified by both methods) for subcellular localisation by epitope tagging and immunofluorescence microscopy. We also used this epitope tagging strategy to localise PFR2 and PAR1, proteins that have previously been shown to localise to the PFR. Transgenic cell lines were generated in which one of the endogenous copies of the gene of interest carried the in-frame coding sequence for the Ty epitope tag (49) immediately downstream of the start codon. Trypanosome cells were fixed and assayed by immuno-double labelling using antibodies against the Ty epitope and the flagellum attachment zone (FAZ) (50). In *T. brucei*, the PFR lies alongside the axoneme from a point after the flagellum exits the flagellar pocket, beyond the start-point of the FAZ, to a point beyond the region of attachment to the cell body. In all cases the tagged protein localised in a portion of the flagellum (Fig. 4A) with a labelling pattern consistent with the PFR (Fig. 4B). All proteins were distributed along the length of the flagellum as either a continuous or punctate line. Including the known PFR proteins in our dataset, we now have strong evidence for PFR localisation of 50% of the proteins identified (and 100% of those tested), suggesting that this is a robust dataset that contains a very high proportion of *bona fide* PFR proteins.

**Comparative proteomics and RNAi identifies subgroups, dependencies and interactions within the cohort of PFR proteins.** The presence of calmodulin, and the calcium and calmodulin recognition domains in the PFC proteins, is indicative of a calcium regulated system operating within the PFR. In order to investigate interactions of components within this potential calcium signalling pathway we conducted RNAi/comparative proteomic analyses using DIGE against two cryptic proteins with predicted domains involved in calcium signalling; PFC1 (EF-Hand calcium binding domain) and PFC15 (IQ-CaM binding domain). A number of spots showed volume reductions following RNAi mediated ablation of PFC1 and PFC15 and, by reference to *snl2* DIGE gels (Fig. 2), the identity of the corresponding proteins was determined to be PFC1, ADKA and ADKB. ADKA spot volumes decreased significantly as a result of RNAi against either PFC1 (log2 ratio ADKA: -0.84) or PFC15 (log2 ratio ADKA: -2.22). PFC1 spot volume was reduced as effectively by PFC15 RNAi (log2 ratio PFC1: -1.50) as it was by PFC1 RNAi (log2 ratio PFC1: -1.53), however the effect on ADKB spot volume in these RNAi backgrounds differed with a significant reduction only observed after PFC15 RNAi (PFC1 RNAi log2 ratio: -0.37, PFC15 RNAi log2 ratio: -1.25) (Fig. 5A). Although the reasons for this are not immediately clear, it may suggest a role for other proteins in this complex or transport into the flagellum/PFR as a factor. PFC15 has not been detected in DIGE analyses, possibly due to its highly basic nature (predicted pI 10.4). In order to determine the fate of PFC15 in these RNAi cell lines, we tagged one of the endogenous copies of the gene with a Ty epitope in both PFC1 and PFC15 RNAi backgrounds. RNAi induced and non-induced detergent-extracted pellets derived from each cell line were compared by Western blotting using an antibody that recognises the Ty epitope. This revealed that, as expected, Ty-PFC15 is readily detectable in non-induced samples and is not present after PFC15 RNAi. However, this analysis also showed that the Ty-PFC15 protein is not correctly assembled into the flagellum after RNAi against PFC1 (Fig. 5B). DIGE analyses using these tagged RNAi cell lines reproduced the previous result for untagged cell lines (data not shown).
not shown). Overall these results show the interdependency of PFC1 and PFC15 and suggest a possible role for calcium regulation of adenylate kinase function in the PFR. In contrast to the severe motility consequences following ablation of PFR2 and the gross reduction in the PFR structure (28) RNAi against either PFC1 or PFC15 did not obviously affect the motility of the cells under culture conditions (data not shown), as similarly reported previously following RNAi ablation of both ADKA and ADKB (3).

**DISCUSSION**

Our aim in this work was to establish a method that combines RNAi ablation of proteins of interest with cutting edge comparative proteomics techniques to generate proteomes for flagellar substructures and provide additional information about protein-protein interactions within these sub-structures. We have tested this protocol on the well characterised *T. brucei* snl2 mutant and have identified 30 proteins as components of the PFR. Furthermore, we have been able to iterate the process with novel PFR proteins to define a subset of interdependent components within the cohort. Whether the detected dependencies are due to interactions in the final PFR structure or are a result of the process of transporting proteins to the flagellum remains to be determined. There are many advantages to the use of *T. brucei* for studies of this type. Reverse genetics approaches are well advanced and the availability of a completed and well annotated genome ((58) hosted by the Sanger Institute at www.genedb.org) with a near total absence of introns greatly facilitates the construction of vectors for RNAi, over-expression and epitope tagging, as well as protein identification by mass spectrometry. In the course of this study we have been able to rapidly turn novel protein identifications into localisations and RNAi phenotypes which have allowed us to target specific cohorts of interacting proteins within the larger framework of the PFR. Most importantly for general future use, our reiteration of this RNAi mutant/proteomic approach at the level of individual proteins (PFC1 and PFC15) shows it to have high sensitivity in revealing sub-cohort protein dependencies.

**Previously identified PFR components.**

Previous studies have identified 15 proteins as PFR components in Trypanosome or *Leishmania* species, based either on interactions, localisation, bioinformatics or a combination of these approaches (3,29,30,39-43,61). Eight of these previously identified PFR proteins were identified in this screen along with an additional 20 previously hypothetical proteins and two annotated proteins that have not previously been identified as PFR components (KMP-11 and P13 related-kinase). As PFR2 was the RNAi target in the snl2 cell line it was expected that the level of PFR2 protein would be significantly reduced and this was indeed the case, both by DIGE and iTRAQ analyses. The other major component of the PFR, PFR1 is also reduced and although it is difficult to resolve these two proteins using 2D-DIGE the average log2 ratios of induced to non-induced samples detected by iTRAQ are -2.4 (PFR1) and -2.6 (PFR2) which is consistent with a near stoichiometric loss of these two proteins after RNAi. Four other proteins have previously been given the sobriquet of PFR (or PAR) (39,42,43) and of these PAR1 and PFR5 are both present in our dataset.

Calmodulin has been shown to localise to the proximal and distal domains of the PFR as well as to the fibres attaching the PFR to the axoneme (30). Consistent with this, calmodulin was identified by iTRAQ as being reduced following RNAi ablation of the PFR structure in the snl2 cell line. There is evidence that calmodulin interacts directly with one of the major PFR components (30) and several novel proteins described in our analysis have pfam motifs (http://pfam.sanger.ac.uk/) predicted as calmodulin- or calcium-binding domains. In the original proteomic analysis of the snl2 mutant (3) two novel adenylate kinases were identified as PFR components. These two proteins, designated ADKA and ADKB, have an unusual N-terminal extension that is both necessary and sufficient to localise these proteins to the PFR. Interestingly, neither ADKA nor ADKB were observed by iTRAQ within our criteria but both were identified in the DIGE comparisons and are included in the final dataset on this basis. Similarly a number of proteins were detected only by iTRAQ which supports the use of both comparative methods to more fully explore the samples. The final previously known PFR component detected in this screen is the repetitive protein known as Tb5.20 (40). This protein was isolated from a cDNA library using a complex antisera raised against *T. brucei*
cytoskeletons and specific antibodies to Tb5.20 localise along the whole length of the PFR.

Seven proteins that have previously been proposed as PFR components are not in our final dataset: gamma tubulin, PAR4, PFR5, TbI2, TbI7, PDEB1 and PDEB2 (29,41-43,61). Their absence could be due to sampling variations, low protein abundance, masking by other proteins in the case of DIGE or physical properties that may be refractory to MS identification. However, such absences might indicate discreet localisation in the PFR substructures that are not ablated by RNAi against PFR2 such as the proximal domain or the links to the axoneme and FAZ (28).

**Novel PFR components.** Two proteins identified in our dataset have a pre-existing annotation but have not previously been identified as PFR components. KMP-11 is a conserved membrane protein of kinetoplastids that is mainly associated with the developmental form present in the insect vector where it has been localised to the flagellum and flagellar pocket (56,57). KMP-11 is currently exciting interest due to its immunological properties (62) and a recent examination of the KMP-11 RNAi phenotype in *T. brucei* has suggested a role for this protein in regulating basal body segregation with additional consequences for nuclear and cell division (63). Interestingly, a feature of this phenotype was the inability of cells to correctly assemble the FAZ filament in the procyclic but not the bloodstream form. A FAZ is still made in the *snl2* mutant as evidenced by the attachment of flagella to the cell body and so this suggests that KMP-11 may have a complex localisation within the PFR such that only a portion of the protein is lost after PFR2 ablation. Tb11.01.6300 is annotated in the *T. brucei* genome as a PI3-related kinase by homology and our analysis of the predicted physical properties of the protein would support this. The family of PI3-related kinases do not phosphorylate lipids but instead have a ser/thr protein kinase activity (64). We have not yet determined a function for this protein in the PFR but a number of proteins identified in this analysis migrate on 2D gels with a multi-spot pattern that suggests a role for protein phosphorylation in the PFR (Fig. 2).

We have also identified 20 proteins previously annotated as conserved hypothetical as components of the PFR and have verified seven of these by immuno-localisation at the light microscope level. We used various bioinformatic strategies as an initial screen to probe for possible functions for these novel proteins and identified a number of interesting patterns. Six PFR proteins, including previously known components and representing 20% of the dataset, have domains associated with calcium sensing and taken together with the previously published interactions of calmodulin with PFR1/2 (30) this suggests an important role for calcium regulation in the PFR. We have also identified a new domain designated meme1 that appears to be largely PFR-specific. Of the nine proteins that could be found to contain meme1, eight are in this dataset and we would predict that the ninth, which we have previously shown to be a trypanosomatid-specific component of the flagellum (12), is also present in the PFR, perhaps in one of the structures less affected by PFR2 ablation. Given that the distribution of meme1 is restricted in the genome, we would predict that this motif has a role in specific protein-protein interactions of the PFR or assembly into or transport or recruitment to this structure, similar to that already established for the N terminal extension of ADKA and ADKB (3). Finally, an intriguing domain found in this bioinformatic analysis is the Beige/BEACH pfam domain of PFC10. In humans a protein containing this domain is implicated in Chediak-Higashi syndrome, an autosomal recessive disease likely resulting from abnormalities in vesicular transport. However, to our knowledge, none of the pathology associated with this syndrome is likely to be caused as a result of flagella/cilia dysfunctions (65).

**Dependency subgroups provide clues about the role of the PFR in the regulation of flagellar motility.** In this work we have demonstrated a reciprocal dependency relationship between two novel PFR proteins; PFC1 and PFC15. These two proteins were chosen for further study as domain predictions suggested a role in a potential PFR calcium signalling network as also suggested by the localisation of calmodulin to the PFR and the finding that it interacts with the major PFR components (30). Intriguingly, we have also shown that the PFC1/PFC15 relationship involves the previously identified PFR-specific adenylate kinases ADKA and ADKB (3). Roles for calcium signalling and adenine nucleotides (in addition to the role of ATP as an energy source) in the regulation of
flagellar and dynein arm function are well established and our results may point towards these two systems being linked in the trypanosome PFR. We hypothesise that adenylate kinase function in the PFR responds to changes in calcium concentration to regulate adenine nucleotide homeostasis in the flagellar compartment. This could function to directly regulate the activity of dynein arms (66,67) or perhaps provide and/or limit substrates for calcium regulated cyclic nucleotide signalling pathways that have been described in the flagellum, and shown to influence the mode of flagellar motility (68,69). This could then provide a mechanism for calcium regulated control of flagellar waveform. Flagellar wave reversal, changes in wave form and regulation of microtubule sliding as a response to changes in calcium concentration have been described in a number of organisms, including trypanosomes (70-81). Recent work from our group demonstrated the switching from flagellar to ciliary waveform in three species closely related to *T. brucei* (82) and our unpublished observations suggest that this is also a feature of motility in *T. brucei*. Calcium regulation is an important factor in the hyperactivation of mammalian sperm which involves changes in the flagellar beat (83). Substantial evidence points to the central pair complex and radial spokes as key transducers of calcium signals to the dynein arms in *C. reinhardtii* (84) and calmodulin has been localised to both of these structures (15,16). Analysis of *C. reinhardtii* mutants suggests that the outer dynein arms control the beat frequency of the flagellum whereas the inner dynein arms are responsible for the shape of the waveform (85). In trypanosomes, however, it appears that beat frequency can be maintained in the absence of outer dynein arms, although the direction of wave propagation is reversed (33). This highlights differences in the regulation of flagellar motility between these organisms, another example being the fixed central pair position of trypanosomes compared to the rotating central pair of *Chlamydomonas*.

Regulation of adenylate kinase function by calcium has previously been reported in other organisms, including in the flagellum of sea-urchin sperm (86-88) and two adenylate kinases have been localised to the fibrous sheath of mouse sperm flagella (89), a structure to which a number of intriguing parallels can be drawn to the PFR (4), suggesting that this could be a more general feature of flagellar beat regulation in other eukaryotes.

**Forward view.** Several trypanosomatids are the causative agents of devastating parasitic disease in man. In Africa, *T. brucei* species are responsible for African trypanosomiasis or sleeping sickness and in central and South America Chagas disease is the result of infection with *T. cruzi*. No vaccines are currently available and existing drug treatments are associated with high toxicity and, increasingly, drug resistance (24-26). The paraflagellar rod is a specific feature of all of these organisms and work over several years has shown that, in model systems, vaccination with PFR proteins can confer total immunity to subsequent challenge with *T. cruzi* (90) or more limited protection against *Leishmania* species (91). In this work we have presented a list of PFR proteins, many of which are conserved among trypanosomatids but which are also restricted to this lineage. Further work is needed to confirm any of these as possible vaccine candidates although recent studies on one - KMP-11 - have shown promising results (62,92).

Recent work from our group has shown that the mammalian bloodstream form of *T. brucei* is exquisitely sensitive to loss of the PFR as a result of RNAi ablation of PFR2 whereby mice are able to completely clear a normally lethal challenge by this parasite (31). RNAi mutants affecting axonemal components give a similar phenotype in the bloodstream form (12,32,33) suggesting that impaired motility is the major factor in this phenotype and not a specific effect of PFR ablation. However, the restricted evolutionary distribution of the PFR structure compared to the more conserved components of the axoneme makes this a particularly valuable result from the viewpoint of therapeutic potential.

**REFERENCES**

1. Fliegauf, M., Benzing, T., and Omran, H. (2007) *Nat Rev Mol Cell Biol* **8**, 880-893
2. Marshall, W. F. (2008) *J Cell Biol* **180**, 17-21
C. M., Tait, A., Tivey, A. R., Van Aken, S., Walker, D., Wanless, D., Wang, S., White, B., White, O., Whitehead, S., Woodward, J., Wortman, J., Adams, M. D., Embley, T. M., Gull, K., Ullu, E., Barry, J. D., Fairlamb, A. H., Operdosses, F., Barrett, B. G., Donelson, J. E., Hall, N., Fraser, C. M., Melville, S. E., and El-Sayed, N. M. (2005) Science 309, 416-422

El-Sayed, N. M., Myler, P. J., Bartholomew, D. C., Nilsson, D., Aggarwal, G., Tran, A. N., Ghedin, E., Worthey, E. A., Delcher, A. L., Blandin, G., Westenberger, S. J., Caier, E., Cerqueira, G. C., Branche, C., Haas, B., Anupama, A., Armer, E., Aslund, L., Attipo, P., Bentropi, E., Bringgaud, F., Burton, P., Cadag, E., Campbell, D. A., Carrington, M., Crabbie, J., Darban, H., da Silveira, J. F., de Jong, P., Edwards, K., Englund, P. T., Fazelinia, G., Feldbylum, T., Fere1la, M., Frasch, A. C., Gull, K., Horn, D., Hou, L., Huang, Y., Kindlund, E., Klingbeil, M., Kluge, S., Koo, H., Lacerda, D., Levin, M. J., Lorenzi, H., Louie, T., Machado, C. R., McCullough, R., McKenna, A., Mizuno, Y., Mottram, J. C., Nelson, S., Ochaya, S., Osoegawa, K., Pai, G., Parsons, M., Pente, M., Pette, M., Pette, S., Pop, M., Ramirez, J. L., Rint, J., Robertson, L., Salzberg, S. L., Sanchez, D. O., Seyler, A., Sharma, R., Shetty, J., Simpson, A. J., Sisk, E., Tammi, M. T., Tarleton, R., Teixeira, S., Van Aken, S., Vogt, C., Ward, P. N., Wickstead, B., Wortman, J., White, O., Fraser, C. M., Stuart, K. D., and Andersson, B. (2005) Science 309, 409-415

Ivens, A. C., Peacock, C. S., Worthey, E. A., Murphy, L., Aggarwal, G., Derram, M., Sisk, E., Rajandream, M. A., Adlem, E., Aert, R., Anupama, A., Apostolou, Z., Attipo, P., Bas, N., Baus, C., Beck, A., Bevery, S. M., Bianchetlin, G., Borzym, K., Bothe, G., Brusci, V. C., Collins, M., Cadag, E., Carlion, L., Clayton, C., Coulson, R.

Cronin, A., Cruz, A. K., Davies, R. M., De Gaude, J., Doban, D. B., Desterho, A., Fazelinia, G., Fosker, N., Frasch, A. C., Fraser, A., Fuchs, M., Gabel, C., Goble, A., Goffau, A., Harris, D., Hertz-Fowler, C., Hilbert, H., Horn, D., Huang, Y., Klages, S., Knights, A., Kube, M., Larke, N., Litvin, L., Lord, A., Louie, T., Man, M., Masuy, D., Mathews, K., Michaeli, S., Mottram, J. C., Muller-Auer, S., Munden, H., Nelson, S., Norbertz, H., Oliver, K., O’Neil, S., Pente, M., Pohl, T. M., Price, C., Purnelle, B., Quail, M. A., Rabinowitsch, E., Reinhardt, R., Rieger, M., Rint, J., Robben, J., Robertson, L., Ruiz, J. C., Rutter, S., Saunders, D., Schafer, M., Schein, J., Schwartz, D. C., Seeger, K., Seyler, A., Sharp, S., Shiv, S., Shima, D., Squares, R., Tosato, V., Vogt, C., Volkaert, G., Wambutt, R., Warren, T., Woodward, H., Zwod, Z., Zhou, S., Zimmermann, W., Smith, D. F., Blackwell, J. M., Stuart, K. D., Barrell, B., and Myler, P. J. (2005) Science 309, 436-442

Libusova, L., Sulimenko, T., Sulimenko, V., Hozak, P., and Draber, P. (2004) J Cell Sci 117, 1-10

OCISB

LI, Z., and Wang, C. C. (2008) Eukaryot Cell 7, 194-1950

Abraham, R. T. (2004) DNA Repair (Amst) 3, 883-887

Nagle, D. L., Karim, M. A., Woolf, E. A., Holmgren, L., Bork, P., Misumi, D. J., McCraill, S. H., Dussault, B. J., Jr., Perou, C. M., Boissy, R. E., Duky, G. M., Spritz, R. A., and Moore, K. J. (1996) Nat Genet 14, 307-311

Inoue, Y., and Shingyoji, C. (2007) Cell Motil Cytoskeleton 64, 69-70

Yagi, T. (2000) Cell Struct Funct 25, 263-267

Bonomi, N. M., and Nelson, D. L. (1988) J Cell Biol 106, 1615-1623

Ono, S. K., and Stuart, K. D. (2003) Adv Second Messenger Phosphoprotein Res 23, 227-272

Naitoh, Y., and Kaneko, H. (1972) Science 176, 523-524

Hyams, J. S., and Bonis, G. G. (1978) J Cell Biol 33, 253-255

Brokaw, C. J. (1979) J Cell Biol 82, 401-411

Johnson, R. E., and Brokaw, C. J. (1979) Biophys J 25, 113-127

Okuno, M., and Brokaw, C. J. (1979) J Cell Biol 38, 105-123

Bessen, M., Fay, R. B., and Witman, G. B. (1980) J Cell Biol 86, 446-455

Cosson, M. P., Tang, W. J., and Gibbons, I. R. (1983) J Cell Biol 60, 231-249

Kamiya, R., and Witman, G. B. (1984) J Cell Biol 98, 97-107

Omoti, C. K., and Brokaw, C. J. (1985) Cell Motil 5, 53-60

Bannai, H., Yoshimura, G., Takahashi, K., and Shingyoji, C. (2000) J Cell Sci 113 (Pt 5), 831-839

Smith, E. F. (2002) Mol Biol Cell 13, 3303-3313

Holway, M. E., and McGreggor, J. L. (1976) J Exp Biol 85, 229-242

Gadella, C., Wickstead, B., and Gull, K. (2007) Cell Motil Cytoskeleton 64, 629-643

Quill, T. A., Sugden, S. A., Louri, T., Doolittle, L. K., Hammer, R. E., and Garbers, D. L. (2003) Proc Natl Acad Sci USA 100, 14699-14704

Smith, E. F., and Yang, P. (2004) Cell Motil Cytoskeleton 57, 8-17

Brokaw, C. J., and Kamiya, R. (1987) Cell Motil Cytoskeleton 8, 68-75

Notari, L., Pepe, I. M., Cugnoli, C., and Morelli, A. (2001) Biochim Biophys Acta 1504, 438-443

Notari, L., Morelli, A., and Pepe, I. M. (2003) Photochem Photobiol Sci 2, 1299-1302

Kimura, M., and Vacquier, V. D. (2007) J Biochem 142, 501-506

Cao, W., Gerton, G. L., and Moss, S. B. (2006) Mol Cell Proteomics 5, 801-810

Wrightman, R. A., Miller, M. J., Saborio, J. L., and Manning, J. E. (1995) Infect Immun 63, 122-125

Saravia, N. G., Hazbon, M. H., Osorio, Y., Valderrama, L., Walker, J., Santrich, C., Cortazar, T., Lobowitz, J. H., and Travi, B. L. (2005) Vaccine 23, 894-905

Basu, R., Bhaumik, S., Basu, J. M., Naskar, K., De, T., and Roy, S. (2005) J Immunol 174, 7160-7171

FOOTNOTES

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The abbreviations used are: RNAi, RNA interference; PFR, Paraflagellar rod; FAZ, Flagellum attachment zone; MS, Mass spectrometry; DIGE, Difference gel electrophoresis; iTRAQ, Isobaric tags for relative and absolute quantitation

FIGURE LEGENDS

Fig. 1. A. EM images (prepared as described previously (12)) of *T. brucei* snl2 RNAi non-induced and induced flagellar transverse sections shows the loss of a large part of the PFR structure. Bar 100nm. B. Frequencies (resolution 0.25) of log₂ protein abundance ratios of non-induced to non-induced samples from quadruplex iTRAQ. C. Averaged frequencies (resolution 0.25) of log₂ protein abundance ratios of induced to non-induced samples from quadruplex iTRAQ. D. log₂ protein abundance ratios of induced to non-induced samples from all iTRAQ experiments for all proteins that show at least a two-fold decrease after RNAi induction of *snl2*. α- and β-tubulin show a less than two-fold change as expected. Results of individual sample pairs are graphed separately as per key.

Fig. 2. 2D-DIGE analysis of *snl2* non-induced and induced flagella. Gels were analysed using Decyder software (GE Healthcare) and spots that show a greater than two-fold decrease in volume are marked.

Fig. 3. Domain and motif architecture of the PFR proteins identified using Pfam and Interpro databases and MEME (see text for details). Blast analysis reveals that 25 proteins out of the 30 identified in our proteome are trypanosomatid specific while the remaining five are found in either or both *Chlamydomonas* and human.

Fig. 4. A. Ty epitope tagging of endogenous loci of seven PFC proteins. All seven tagged proteins localise to the flagellum with a pattern consistent with the PFR. B. Ty epitope tagging of endogenous loci of the known PFR components PFR2 and PAR1 exemplify a PFR localisation. Green – Ty tagged protein, Magenta – FAZ, Blue – DAPI. Bar 2μm. Arrow – distal extent of the FAZ, arrowhead – start point of the Ty signal.

Fig. 5. A. 2D-DIGE analysis of PFC1 and PFC15 non-induced and induced flagella. Gels were analysed using Decyder software (GE Healthcare) which was used to generate 3D representations of the spots that show a change in volume after induction. In both RNAi environments significant reductions in volume were seen for spots corresponding to PFC1 and ADKA. An equally significant volume decrease was observed for ADKB after PFC15 RNAi but this was not observed after PFC1 RNAi. B. Western blot showing the absence of Ty epitope tagged PFC15 from the detergent resistant fraction following RNAi against PFC15 and against PFC1. Ponceau stained membrane is shown as a loading control.

Supplementary Fig. 1. Alignment of the three novel motifs identified by MEME. A. Alignment of meme1, a 15 amino acid motif, present in 8 of the 30 proteins of our proteome. Both alignment and logo analysis display the most conserved residue. B. The distribution of expectation values resulting from a search of the *T. brucei* predicted proteome using a hidden Markov model generated from the meme1 alignment demonstrates the specificity of the domain. C. Alignment of meme2, an 11 amino acid repeat present 53 times in PFC9 and 12 times in Tb5.20. D. Alignment of meme3, a 21 amino acid repeat present in three PFR proteins. Dark blue – identity, turquoise – functional similarity.
Table 1. Summary of PFR candidates identified in this analysis. Paraflagellar rod proteome components (PFC) and known PFR proteins are identified by a reduction in protein abundance following inducible RNAi against PFR2. Accession numbers relate to the *T. brucei* genome project (www.genedb.org). Relative abundance of proteins is shown as a log₂ of the ratio of spot volumes (DIGE) or peak areas of reporter ions (iTRAQ) between RNAi induced and non-induced samples.

| Accession Number | Name            | DIGE peptides for identification | Log₂ average Induced/Noninduced DIGE ratio | iTRAQ Quadruplex peptides | iTRAQ Duplex peptides | Log₂ average Induced/Noninduced ratio |
|------------------|-----------------|----------------------------------|--------------------------------------------|---------------------------|----------------------|--------------------------------------|
| Tb09.211.4513    | KMP-11          |                                  | -1.06                                      |                           |                      |                                      |
| Tb10.26.0680     | PFC16           | 7                                | -2.79                                      |                           |                      |                                      |
| Tb10.389.0100    | PFC20           | 2                                | -1.61                                      |                           |                      |                                      |
| Tb10.61.1260     | PFC15           | 6                                | -1.47                                      |                           |                      |                                      |
| Tb10.6k15.0140   | PFC19           | 16                               | -1.42                                      | 3                         | 2                    | -1.24                                |
| Tb10.6k15.0810   | PFC14           | 27                               | -2.24                                      | 7                         | 2                    | -1.56                                |
| Tb10.6k15.1510   | PFC18           |                                   |                                            |                           |                      |                                      |
| Tb11.01.3000     | PFC17           | 4                                | -1.38                                      | 3                         |                      | -1.57                                |
| Tb11.01.4623     | Calmodulin      |                                  |                                            |                           |                      | -1.34                                |
| Tb11.01.5100     | Par1            | 38                               | -2.78                                      | 8                         | 6                    | -1.75                                |
| Tb11.01.6300     | P13-related kinase |                                | 2                                           |                           |                      | -1.52                                |
| Tb11.01.6510     | PFC9            | 3                                | -1.88                                      |                           |                      |                                      |
| Tb11.01.6740     | Tb5.20          | 4                                | -1.94                                      |                           |                      |                                      |
| Tb11.02.2350     | PFC12           | 2                                | -1.83                                      |                           |                      |                                      |
| Tb927.2.2160     | PFC11           | 24                               | -1.11                                      | 3                         | 2                    | -1.47                                |
| Tb927.2.3660     | PFC10           | 2                                |                                            |                           |                      | -1.30                                |
| Tb927.2.4330     | PFR5            | 3                                | -1.42                                      |                           |                      |                                      |
| Tb927.2.5660     | ADKA            | 15                               | -3.38                                      | 8                         | 6                    | -1.63                                |
| Tb927.2.950      | PFC13           | 2                                |                                            |                           |                      | -1.31                                |
| Tb927.3.3750     | PFC7            | 8                                | -2.84                                      | 2                         |                      | -2.25                                |
| Tb927.3.3770     | PFC6            | 20                               | -1.84                                      | 2                         |                      | -0.66                                |
| Tb927.3.4290     | PFR1            | 35                               | -2.72                                      | 28                        | 30                   | -2.53                                |
| Tb927.6.3670     | PFC8            | 2                                |                                            |                           |                      | -1.51                                |
| Tb927.6.4140     | PFC4            | 2                                |                                            |                           |                      | -2.51                                |
| Tb927.7.1920     | PFC5            | 14                               | -1.77                                      |                           |                      |                                      |
| Tb927.8.1550     | PFC3            | 33                               | -2.13                                      | 9                         | 8                    | -1.38                                |
| Tb927.8.3790     | PFC2            | 9                                | -2.63                                      |                           |                      | -2.45                                |
| Tb927.8.4970     | PFR2            | 38                               | -2.72                                      | 27                        | 32                   | -2.38                                |
| Tb927.8.6660     | PFC1            | 14                               | -1                                         | 9                         | 2                    | -1.90                                |
| Tb10.70.7330     | ADKB            | 13                               | -2.12                                      |                           |                      |                                      |
| Homologue in L. major | LmjF29.1760 | LmjF16.1425 | LmjF27.1850 | LmjF32.1910 | LmjF09.1320 | LmjF30.2850 | LmjF07.0310 | LmjF36.4230 | LmjF32.2390 | LmjF24.1560 | LmjF29.1170 | LmjF09.0920 | LmjF10.0180 | LmjF21.1250 | LmjF19.0520 | LmjF35.2210 | LmjF36.4780 | LmjF32.1460 | LmjF36.5870 | LmjF02.0310 |
|----------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Homologue in H. sapiens | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          |
| Homologue in C. reinhardtii | X         | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          | X          |
Combining RNA interference mutants and comparative proteomics to identify protein components and dependencies in a eukaryotic flagellum
Neil Portman, Sylvain Lacomble, Benjamin Thomas, Paul G. McKean and Keith Gull

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