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Short communication

Dramatic reorganisation of *Trichomonas* endomembranes during amoebal transformation: A possible role for G-proteins☆

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Trichomonas vaginalis is the most common non-viral sexually transmitted pathogen and is thought to represent a significant risk factor for HIV infection [1,2]. *Trichomonas* transformation from a free-swimming trophozoite to an adherent amoeba is crucial to parasite establishment in the host vagina and subsequent pathogenesis [1–4]. Amoebal transformation takes place upon binding to vaginal epithelial cells or to extracellular matrix (ECM) proteins and can be induced in vitro upon binding to ECM components laminin and fibronectin [3]. This process is believed to involve specific cell signalling events [4]. Membrane trafficking is also a key to *Trichomonas* pathogenesis; for example perforin secretion leads to host cell lysis [5]. Despite the potential importance of membrane trafficking and signalling to these clinical aspects of *Trichomonas,* little is known of the molecular machinery orchestrating these processes. Heterotrimeric G-proteins (G-proteins) are key to membrane trafficking and cell signalling in most organisms [6] and so we chose to investigate the roles of a *Trichomonas* G-protein. We have previously shown that the *T. vaginalis* G-protein alpha subunit TvGα402 locates at endomembranes in trophozoites [7].

Indirect immunofluorescence analysis (IFA) labelling of TvGα402 together with a nuclear stain (DAPI) shows TvGα402 to localise to posterior perinuclear vesicular structures (up to 2 μm in diameter) in *Trichomonas* trophozoite cells (Fig. 1A–D) [7]. There are few *Trichomonas* intracellular markers characterised but the mannose-specific lectin Concanavalin A (ConA) preferentially binds glycoproteins at the ER, Golgi and endosome/lysosomes [8] and we show ConA to partially co-localise with TvGα402 (Fig. 1E–H). ConA is likely to represent a highly specific probe for *Trichomonas* glycoproteins as only two bands were detected when a *Trichomonas* total cell extract was probed with ConA by Western blot (Supplementary Fig. S1). ER tracker labels the ER in many cell types [9,10] and in *Trichomonas* cells appears to highlight several structures, including a large oval structure similar to the nuclear membrane in size and location with respect to TvGα402. This is consistent with ER labelling. However, TvGα402 localisation is clearly fully distinct to ER tracker indicating an absence of TvGα402 at the ER (Fig. 1I–L). A partial co-localisation with ConA (Fig. 1E) suggests that TvGα402 locates at either the ER, Golgi or lysosomes; the former compartment can be eliminated due to a lack of overlap with ER tracker. Further, TvGα402 organisation appears distinct from Golgi complex morphology as in *Trichomonas* the Golgi complex is a single copy organelle located anterior to the nucleus [11], whilst TvGα402 labels structures posterior in the cell (Fig. 1A) [7]. This leads us to suggest that TvGα402 locates at endosome/lysosomes. Additionally, TvGα402 labelled vesicles share morphological similarities with *Trichomonas* endosome/lysosomes, which can grow very large (up to ∼2 μm in diameter) and are preferentially positioned posterior within the cell [12]. Immunoelectron microscopy shows *Trichomonas* to

Abbreviations: BSA, bovine serum albumin; ConA, concanavalin A; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DIC, differential interference contrast; ECM, extracellular matrix; ER, endoplasmic reticulum; IFA, indirect immunofluorescence analysis; TvGα402, *Trichomonas* G-protein alpha subunit

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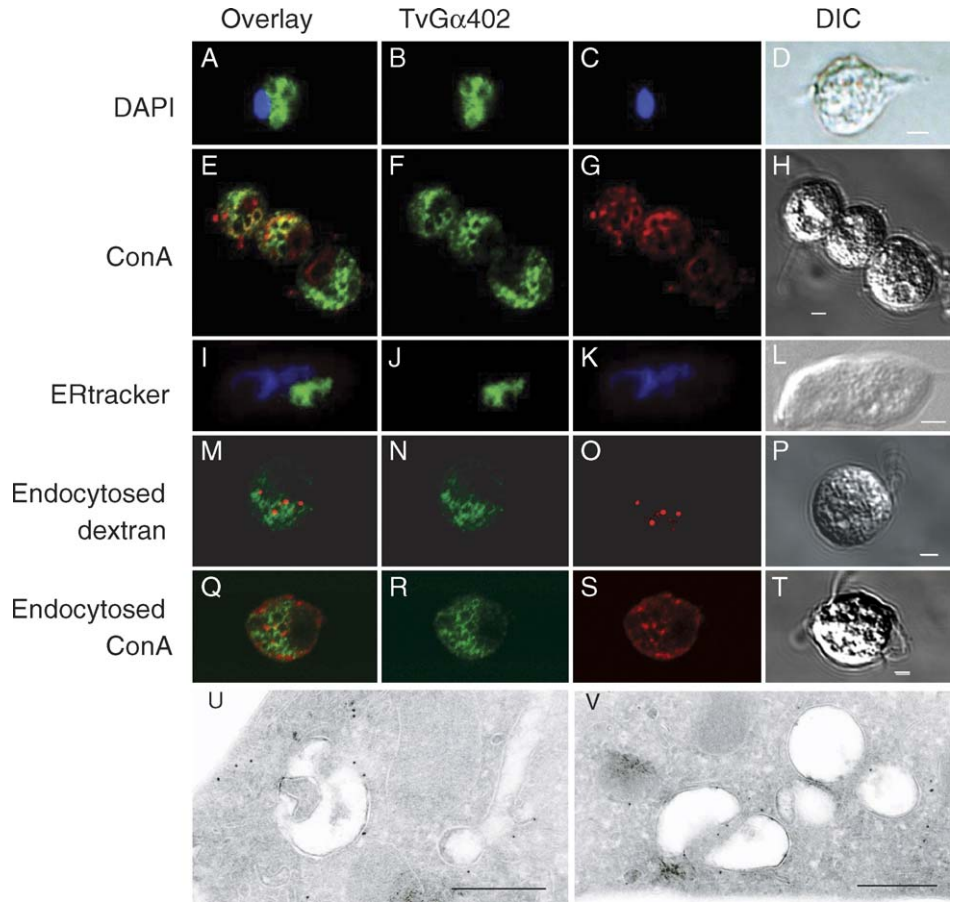

Fig. 1. Cellular localization of TvGα402 compared to different markers. TvGα402 at posterior vesicles with partial co localisation with ConA binding but distinct to ER tracker. TvGα402 vesicles are frequently linked to endocytosed dextran but not ConA. Ultrastructure shows heterogeneous location of TvGα402 including the cytoplasmic face of multivesicular body-like compartments. *Trichomonas* cells were fixed with ethanol or parformaldehyde and permeablised with Triton X-100 and labelled with anti-TvGα402 (1/5000) and DAPI (A–D) or ConA (E-H). Anti-TvGα402 antibody (M006) was detected with anti-rabbit Alexa 488. Live *Trichomonas* cells were incubated with ER tracker for 30 min and processed for IFA (I–L). *Trichomonas* endocytosis was monitored after incubation with dextran-texas red for 12 min (M–P) or ConA-biotin (Q–T) for 2 min and chased in buffer for 10 min and processed for IFA. ConA-biotin was detected with streptavidin texas red. Images (A–D) and (I–L) were captured with an epifluorescent microscope whilst the remainder were single section confocal images. Images in each row represent one cell and TvGα402 staining is shown in green in the first and second columns. Various co-stains indicated to the left of each row are shown in blue or red in the third column. The overlays of TvGα402 labelling with individual co-stains are shown in the first column. Panel (A) shows TvGα402 at posterior perinuclear vesicular structures. TvGα402 and ConA labelling (panel E) shows a partial co-localisation. In panel (I), the overlay image of TvGα402 and ER tracker clearly shows distinct labelling. Dextran labelled vesicles often appear inside TvGα402 (M–P) outlined vesicles whilst ConA labelled vesicles do not (Q–T). The bars represent 2 μm. In panels (U and V), immuno electron microscopy images of *Trichomonas* cells labelled with anti-TvGα402-protein A gold, show TvGα402 at heterogeneous vesicles. Multivesicular bodies-like compartments have membrane bound lumenal vesicles and TvGα402 location includes the cytoplasmic face of the outer limiting membrane of such compartments. TvGα402 is also found at unstructured membranes in the cytosol. The bars represent 500 nm.

have multivesicular body-like compartments, i.e. vesicles containing lumenal vesicles. TvGα402 localisation includes the cytoplasmic face of the outer limiting membrane of multivesicluar bodies-like compartements and also at unstructured membranes (Fig. 1U and V).

To further investigate the relationship of TvGα402 to endocytosis we tracked endocytic material using the probes dextran or ConA. The endocytic marker fluorescent dextran (Mwt 10,000 daltons) is taken into many cell types by a fluid-phase route – e.g. [13]. Dextran-labelled endocytic vesicles, less than 1 μm in diameter, frequently appear within TvGα402 stained structures (Figs. 1M–P, Fig. S2 and movie 1) [7]. Thus a subset of TvGα402 positive vesicles are linked to endocytosis. This staining pattern is consistent with dextran labelling luminal vesicles of multivesicluar bodies-like compartments whilst TvGα402 labels the outer membranes, as suggested from the immunoelectron microscopy data (Fig. 1U and V).

Alternatively, ConA binds glycoproteins on the surface of live *Trichomonas* cells and allows monitoring of endocytosis of ConA-labelled glycoproteins. In contrast to dextran uptake, ConA-labelled endosomes do not appear to be associated with TvGα402 (Fig. 1Q–T). Also, the kinetics of dextran and ConA uptake are different (Supplementary Fig. S2). Thus, dextran and ConA appear to label distinct populations of vesicles. Also, not all endocytic cargo taken into the cell is passively transported within TvGα402 labelled vesicles. Thus the potential complexity of *Trichomonas* endocytosis is also demonstrated here with the active sorting of the contents of multivesicluar bodies-like compartements. Further, the recent discovery of an extremely large Rab GTPase gene family, encoding proteins key to mem-

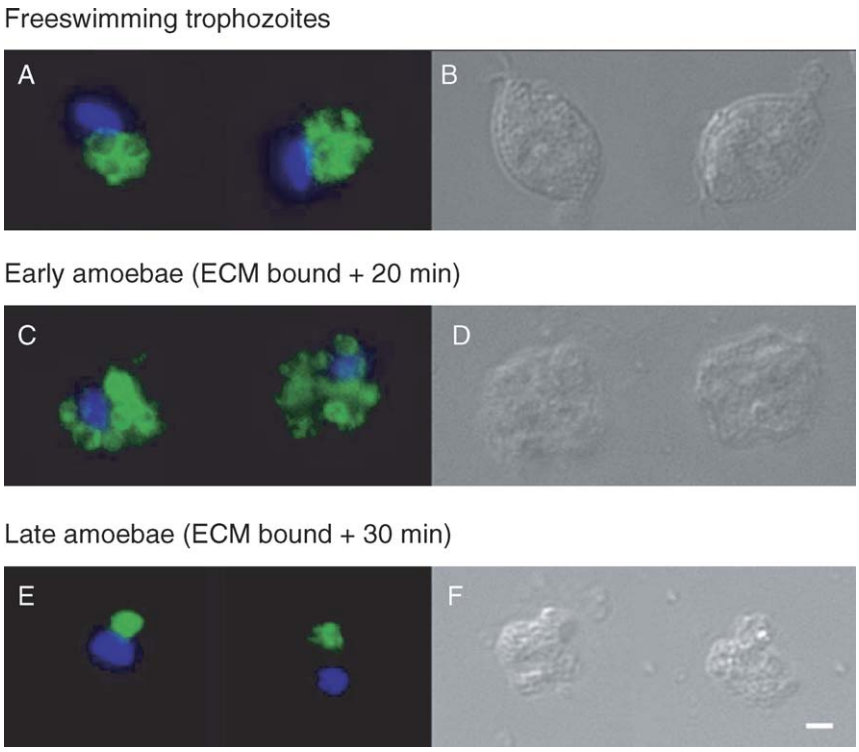

Fig. 2. TvGα402 location is dramatically altered with trophozoite to amoeba transformation. The cellular location of TvGα402 is compared in trophozoite cells (A and B) to amoeba after incubation with ECM proteins for 20 or 30 min. Within 20 min of contact with ECM proteins *Trichomonas* start to transform into early amoeboid forms (C and D). After 30 min, late amoebae cells show a reduced size and pseudopodia are often apparent (E and F). Trophozoites were fixed and adhered to slides with poly-l-lysine whilst amoebae adhered via ECM proteins for indicated times and then fixed and processed for IFA. Panels A, C and E show overlay images of TvGα402 (green) and DAPI stained the nucleus (blue). Panels B, D and F show the respective corresponding DIC images. TvGα402 labelled structures change from a perinuclear posterior location in the trophozoite (A), to occupying most of the cell in the early amoeba (C). In the late amoeba TvGα402 is found at the cell periphery in reduced numbers and often in pseudopodia (E and F). TvGα402 labelling appears brighter and more condensed at vesicles in the amoebal stages. The bar represents 2 μm.

brane trafficking in model systems, also strongly suggests an exceedingly complex endomembrane system in *Trichomonas* [14].

Binding to ECM proteins fibronectin and laminin induces differentiation of *Trichomonas* trophozoites into an amoebae form [3]. After 20 min of contact with ECM proteins the cellular morphology of *T. vaginalis* clearly reflects the differentiation process as cells in transition show an irregular outline under differential interference contrast (DIC) that is distinct from the trophozoite (compare Fig. 2B and D), indicating remodelling of the cell surface and overall cell shape. During this process TvGα402 labelled structures differentiate into well-defined vesicles, which proliferate in number (from ∼3 to >10 per cell) and dramatically occupy most of the cell volume (Fig. 2C and Movie 2). The TvGα402 labelled vesicles appear with a similar morphology in terms of shape and size as in the trophozoite, suggesting the labelling of endosomal/lysosomal compartments into early differentiation. Strikingly, TvGα402 labelling appears brighter and more distinct at vesicles during the differentiation to the amoeba (comparing Fig. 2A and C and Movies 1 and 2). Semi-quantitative RT-PCR analyses shows a dramatic increase in TvGα402 mRNA in amoebae bound to ECM proteins compared to trophozoites (Fig. S3 [19]). It is also possible that the fraction of TvGα402 in the trophozoite that is observed as diffuse staining by immunofluorescence (Fig. 2A) and likely at cytoplasmic membranes by electron microscopy (Fig. 1U and V), may relocate to vesicle membranes during the amoebal transformation together with membrane reorganisation (Fig. 2C). Therefore the increased TvGα402 signal from trophozoite to amoeba detected by immunofluorescence is likely due to the combined effect of a burst in TvGα402 protein synthesis and the possible relocation of the original pool of protein together with endomembrane reorganisation. After 30 min of contact with ECM proteins, in *Trichomonas* "late" amoebae, TvGα402 labelled vesicles often relocate to a single focus towards the cell periphery, often at pseudopodia (Fig. 2E and F).

The present data demonstrates dramatic changes occurring in the *Trichomonas* endomembrane system that accompany amoeba transformation following contact with ECM proteins. Significantly these studies also highlight at least two developmental phases in the amoeboid transformation, an early phase involving an increase in TvGα402-labelled vesicle numbers and a later phase where these are reduced. At present the significance of the realignment of TvGα402-labelled membranes is not clear but the relocation of vesicles towards the cell surface suggests a role in secretion. In better-characterised systems G-proteins have a well-established role at multivesicluar bodies in the secretion of exosomes [15,16]. As *Trichomonas* stores HIV particles at multivesicluar bodies-like compartements [17] reminiscent of TvGα402-positive compartments, it is possible that TvGα402

plays an important role in the release of harboured HIV upon contact to host tissue and contributes to the well established increased risk of HIV infection for *Trichomonas* infected people [1,17,18]. Further study of the functions of TvGα402 and its associated organelles is clearly of importance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molbiopara.2006.02.022.

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