A 2A peptide-based system for *in situ* gene tagging that preserves cis-acting regulatory elements in trypanosomatids

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Abstract

Conventional approaches for *in situ* gene tagging in trypanosomes replace endogenous untranslated regions (UTRs). Such replacements alter normal gene expression levels since regulatory elements within the endogenous UTR are disrupted. Using a self-cleaving viral 2A peptide, we provide a new system for epitope-tagging that retains native UTRs. The system uses CRISPR/Cas9 to knock-in PCR-generated 2A peptide cassettes containing a drug selectable marker and a fluorescent tag flanked by ~40 bp homology arms to the target site. We validate our system by fusing mNeonGreen (mNG) or mScarlet (mSc) to three proteins: ESAG3 (E3), cytosolic Hsp70, and ESAG7 (E7). PCR and whole genome sequencing showed single-copy in-frame integration events only at the target loci, with full length endogenous UTRs intact. We show localisation of mNG-tagged E3, and N- and C- terminal tagging of cytosolic Hsp70 with mNG and mSc, respectively. By tagging one subunit (E7) of the transferrin receptor, an essential protein for iron uptake and survival, we demonstrate that our approach maintains normal expression, regulation in response to an external stimulus iron, and does not interfere with transferrin binding. Comparative western blot analyses of all 2A-tagged proteins generated in this work provide evidence for high cleavage efficiency of the 2A peptide derived from *Thosea asigna* in bloodstream trypanosomes. Our 2A tagging system is applicable to all Kinetoplastids amenable to CRISPR/Cas9 gene editing which rely on polycistronic transcription and is useful for studying post-transcriptional and post-translational regulation of a given gene within the framework of a single experiment.