Nucleic acids undergo naturally occurring chemical modifications. Over 100 different modifications have been described and every position in the purine and pyrimidine bases can be modified; often the sugar is also modified\(^1\). Despite recent progress, the mechanism for the biosynthesis of most modifications is not fully understood, owing, in part, to the difficulty associated with reconstituting enzyme activity \textit{in vitro}. Whereas some modifications can be efficiently formed with purified components, others may require more intricate pathways\(^2\). A model for modification interdependence, in which one modification is a prerequisite for another, potentially explains a major hindrance in reconstituting enzymatic activity \textit{in vitro}\(^3\). This model was prompted by the earlier discovery of tRNA cytosine-to-uridine editing in eukaryotes, a reaction that has not been recapitulated \textit{in vitro} and the mechanism of which remains unknown. Here we show that cytosine 32 in the anticodon loop of \textit{Trypanosoma brucei} tRNA\(^{\text{Thr}}\) is methylated to 3-methylcytosine (m\(^3\)C) as a pre-requisite for C-to-U deamination. Formation of m\(^3\)C \textit{in vitro} requires the presence of both the \textit{T. brucei} m\(^3\)C methyltransferase TRM140 and the deaminase ADAT2/3. Once formed, m\(^3\)C is deaminated to 3-methyluridine (m\(^3\)U) by the same set of enzymes. ADAT2/3 is a highly mutagenic enzyme\(^4\), but we also show that when co-expressed with the methyltransferase its mutagenicity is kept in check. This helps to explain how \textit{T. brucei} escapes ‘wholesale deamination’\(^5\) of its genome while harbouring both enzymes in the nucleus. This observation has implications for the control of another mutagenic deaminase, human AID, and provides a rationale for its regulation.