

Genome metabolism of *Plasmodium falciparum* – Is there any uracil in the genome?

Lívia Marton, Petra Molnár, Richard Izrael, Ágnes Takács, István Kézsmárki, Beáta G. Vértessy



Lívia Marton, PharmD, PhD

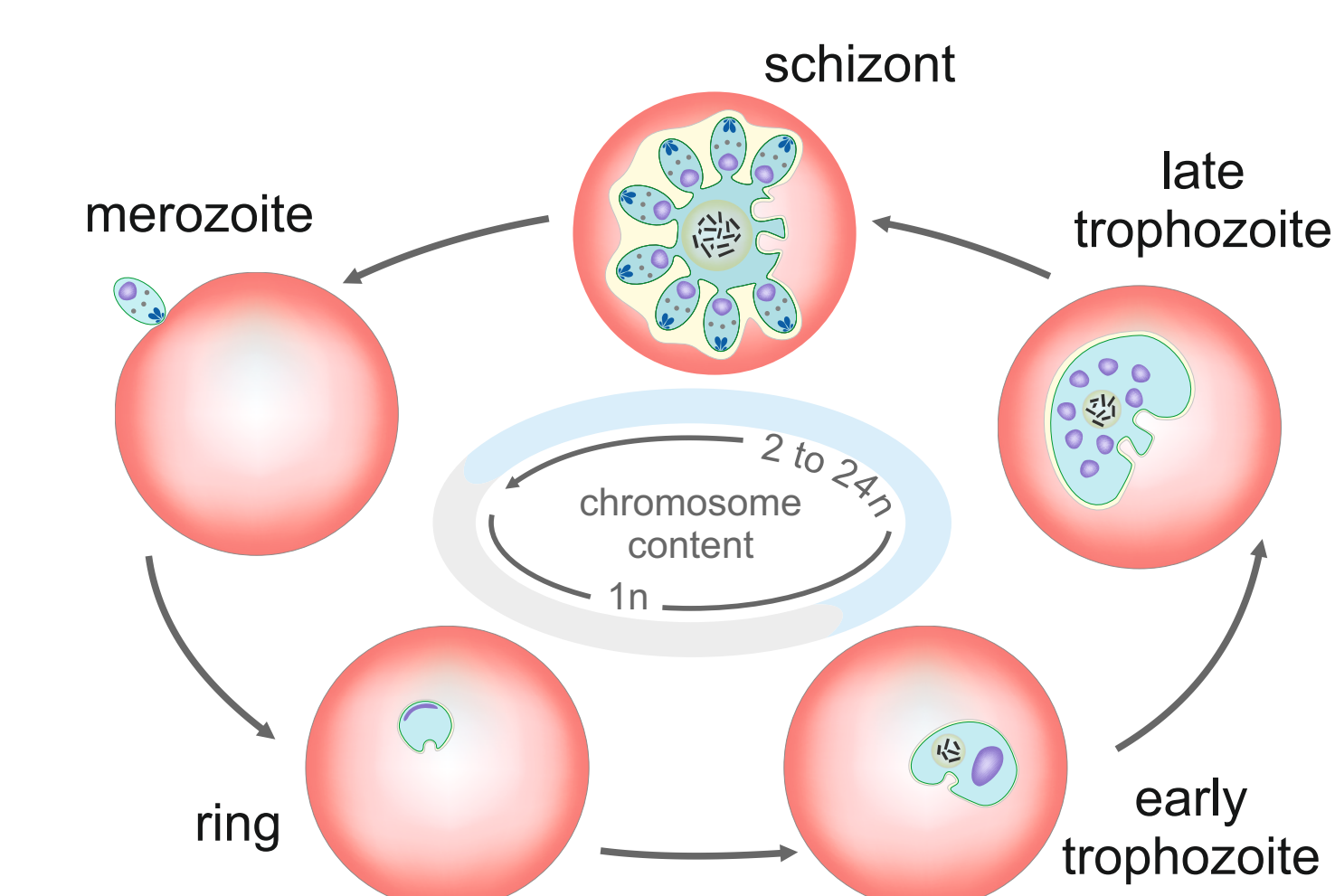
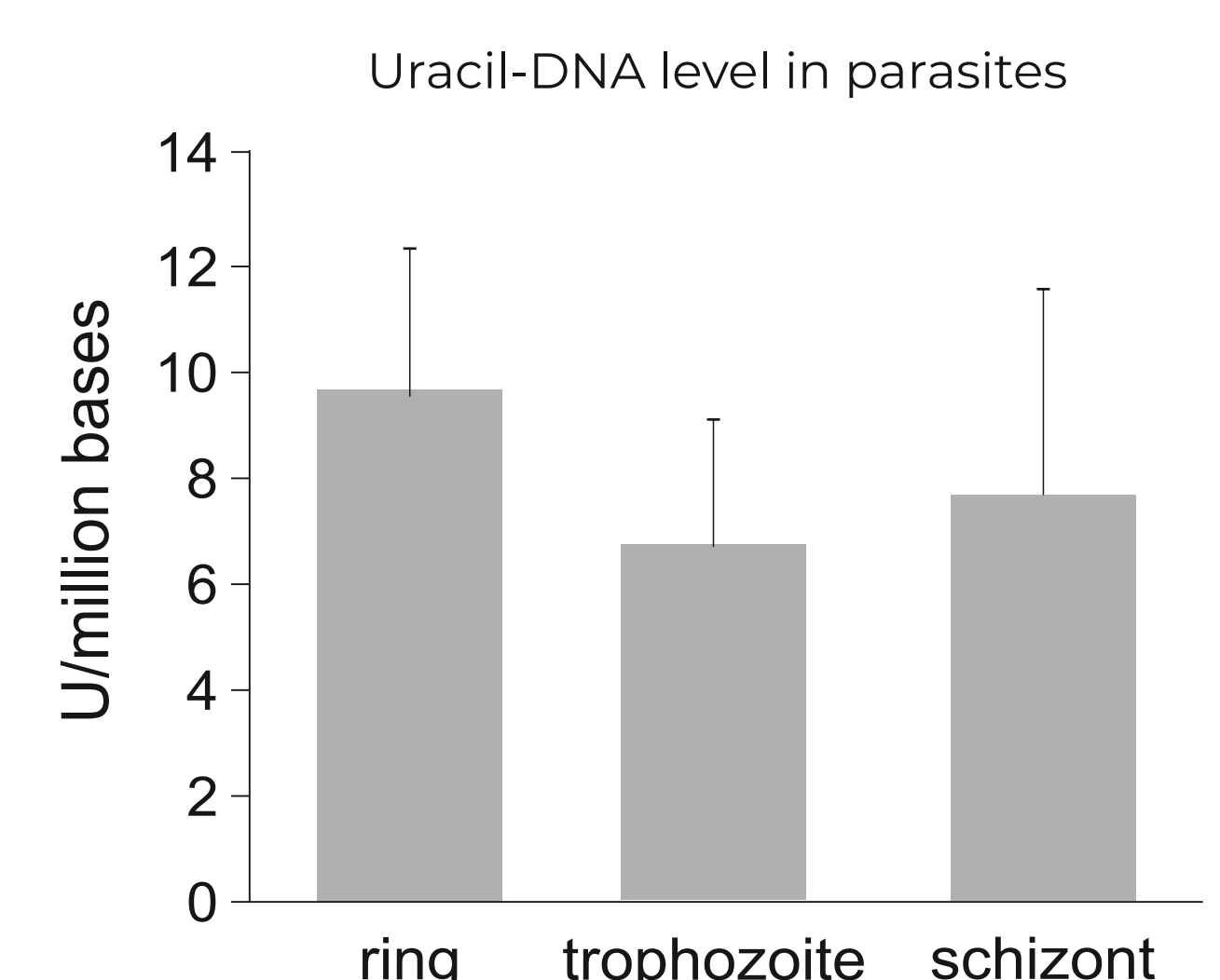
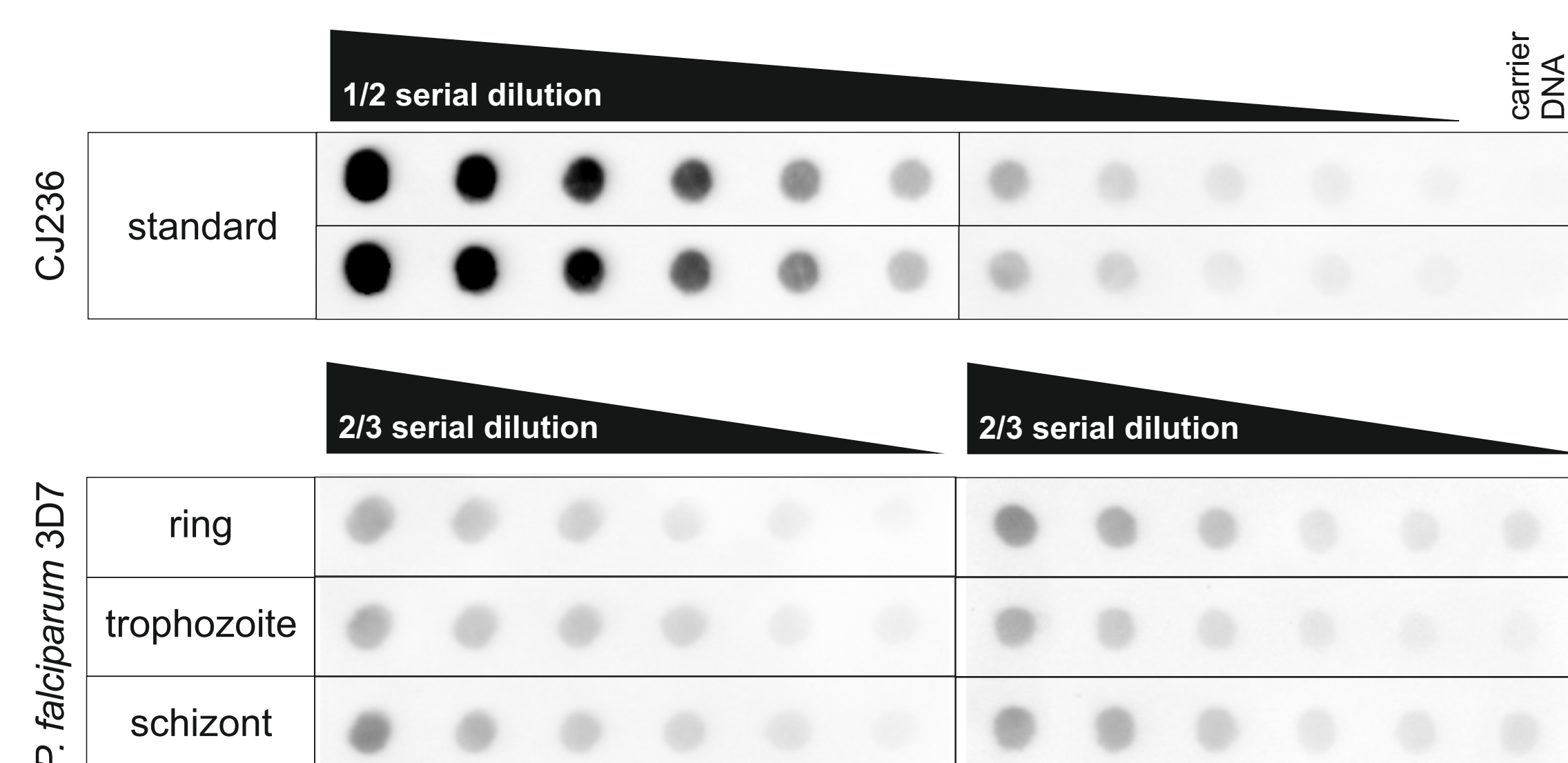
Hungarian Academy of Sciences
Research Centre for Natural Sciences
Institute of Enzymology
Genome Metabolism Research Group
BME-MTA Malaria Research Laboratory
marton.livia@ttk.mta.hu

The constrained DNA synthesis of *Plasmodium falciparum* parasites indicates the possibility of increased uracil content as a result of either oxidative deamination or thymine-replacing incorporation. We found higher levels of uracil in the different parasite stages, compared to other organisms. Based on a systematic assessment of genome and transcriptome databases, uracil-DNA repair relies on one uracil-DNA glycosylase and proceeds through the long-patch base-excision repair.

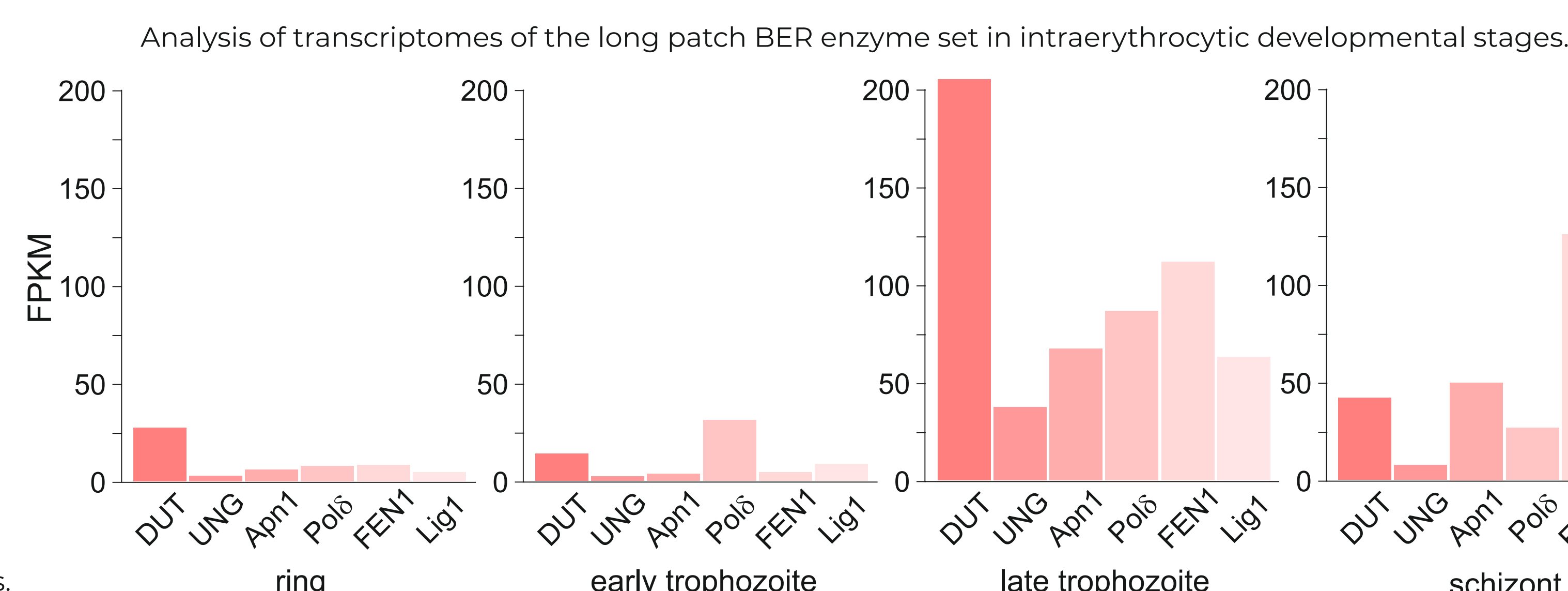
Research Highlight

DNA replication cycles within the intraerythrocytic stages proceed in an environment rich in oxidative conditions. Especially, free heme and iron during the hemozoin formation process pose notable oxidative stress, which may result in DNA modifications (eg. oxidative cytosine deamination leading to uracil and other oxidative processes). The specific AT-rich composition of genomic DNA in Plasmodia (~80% in exonic and ~90% in intronic regions¹) may also facilitate the appearance of uracil in the DNA. The AT richness of the genome may increase the possibility of uracil content, as huge amounts of thymidines are incorporated, giving more chance for the polymerase to mistake thymidines with uridines as compared to a genome with lower levels of AT content. We therefore wished to determine uracil levels in genomic DNA of *P. falciparum* during the intraerythrocytic stages. In order to assess the potential efficiency of uracil-DNA repair, we compared the existing orthologues of mammalian base excision repair enzymes to those present in the parasite based on genome databases of *Homo sapiens* and *P. falciparum*. We also analyzed transcriptome databases of the intraerythrocytic parasite stages with regard to expression level of base excision repair enzymes.

The basis of the **dot blot based uracil detection method** is an engineered catalytically inactive uracil-DNA glycosylase, which is capable of recognizing and binding to uracils incorporated in DNA sequences². The sensitivity is equivalent to that of MS based methods, providing a limit in femtomolar concentrations. CJ236 [dut-, ung-] *E. coli* genomic DNA was used as standard. Representative dot blot images of the measurement in **ring, trophozoite and schizont** samples are shown here. The **uracil content** was determined to be **9.6±2.8; 6.7±2.4 and 7.6±3.8 uracil/million bases**, respectively. It is of interest to note that these uracil-DNA levels are significantly higher than those observed in other samples from different wild type organisms.



Changes in chromosome content in the different stages.



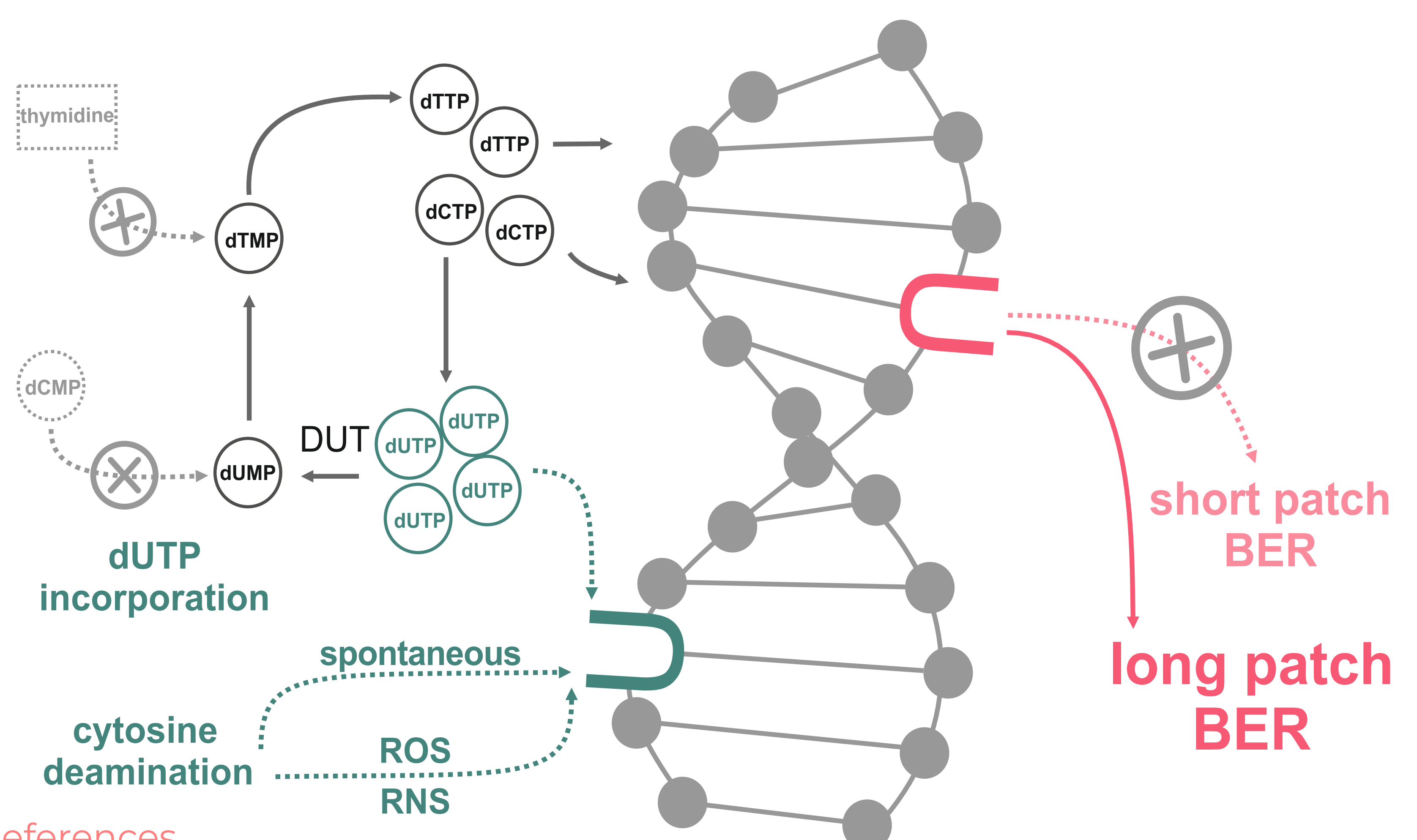
Based on the analysis of **BER** enzyme transcriptome levels of the different developmental stages, the pathway **is initiated in the late trophozoite stage**. This is in good agreement with the DNA metabolism of the parasites, as DNA synthesis starts in the late trophozoite stage followed by DNA packaging into merozoites at the end of the cycle.

To account for the higher level of uracil compared to other normal wild type organisms, the **balance between processes leading to uracil presence in DNA and its removal** need to be considered.

There are **two mechanisms** by which uracil can arise in DNA. On the one hand, if cellular dUTP levels are high as compared to dTTP levels, polymerases can readily incorporate dUMP moieties into DNA. The enzyme family of dUTPases are responsible for keeping dUTP levels at a low value to prevent **thymine-replacing incorporations**³. Although thymine-replacing uracil incorporation into *P. falciparum* genomic DNA may be **enhanced by the exceptionally high AT content** of the parasite genome.

Another possibility for uracil to appear in DNA is the **oxidative deamination of cytosine**, resulting in a G:U pair instead of G:C. Without repair, this will lead to the formation of an AT pair. The deamination of cytosine is considered one of the most frequent DNA mutations, with a rate of 100 to 500 U/cell/day⁴. In case of *Plasmodium falciparum*, the **detoxifying process resulting in the formation of hemozoin** crystals gives rise to the **formation of oxidative agents**.

Uracil removal from DNA requires the **base-excision repair** process. In mammalian cells, both short- and long-patch BER pathways are present for the repair of base excisions, but the **parasites rely only on the long-patch repair**⁵. Moreover, from the different families of uracil-DNA glycosylases, ***P. falciparum* contains only the single UNG protein**, further limiting the capacity of parasites to remove uracil from the DNA.



References

- 1 Gardner MJ et al. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 419, 498–511.
- 2 Róna G et al. (2016) Detection of uracil within DNA using a sensitive labeling method for in vitro and cellular applications. *Nucleic Acids Res.* 44, 1–13.
- 3 Vértessy, BG. & Toth J (2009) Keeping Uracil Out of DNA: Physiological Role, Structure and Catalytic Mechanism of dUTPases. *Acc Chem Res.* 42, 97–106.
- 4 Krokan HE et al. (2002) Uracil in DNA - Occurrence, consequences and repair. *Oncogene* 21, 8935–8948.
- 5 Haltiwanger BM et al. (2000) DNA base excision repair in human malaria parasites is predominantly by a long-patch pathway. *Biochemistry* 39, 763–772.

Acknowledgement

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