

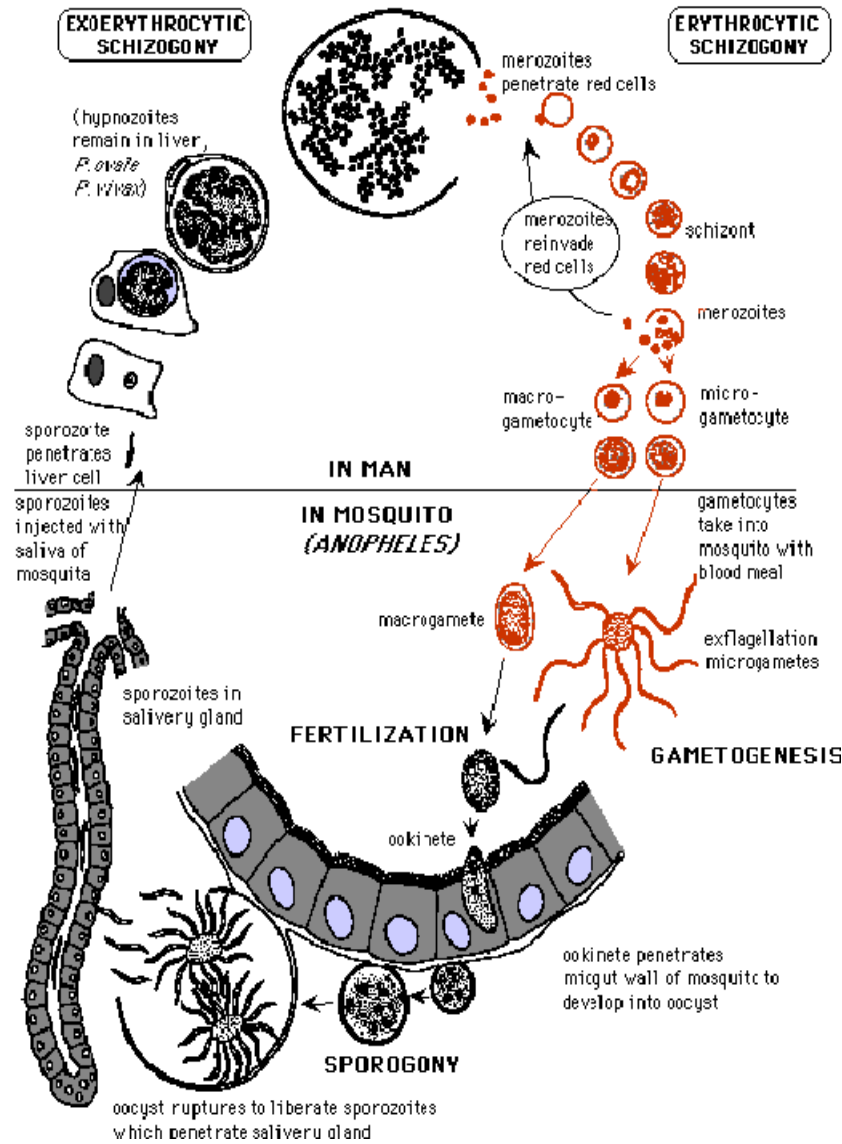
The High Mobility Group (HMG) nuclear factors of *Plasmodium falciparum*.

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Plasmodium has a highly complex life



During the pathogenic Intra-erythrocytic stage, parasites undergo rapid proliferation and reinvasion of host cells or switch to sexual differentiation.

Computational annotation of *trans*-acting elements within the genome of *Plasmodium*.

Identification of characteristic domains among eukaryotic transcription factors.

ClustalW, MultAlin

Identification of the consensus sequences.

BlastP against PlasmoDB

Annotation and control of protein sequences.

ProfileScan, PSORT

Super class	Family	Factor	ORF	Chrom.
<i>Zinc finger</i>	<i>C2H2</i>	Krox	1461	12
		Krüppel	1266	14
	<i>C3H4</i>	PHD2	205	13
		PHD bromo	1700	12
Helix turn helix	Myb	PfMyb1	414	13
		PfMyb2	914	10
		PfMyb3	2578	10
β scaffold factors with minor groove contacts	HMG	PfHMGB1	97	12
		PfHMGB2	99	8
		PfHMGB3	2284	12
		PfHMGB4	160	13

High Mobility Group (HMG) proteins.

Small nuclear « non-histone » proteins highly conserved in eukaryotes.

- HMGA: AT-hook (HMG-I/-Y)
- HMGN: Nucleosomal binding domain (HMG-14/-17)
- **HMGB: HMG-box**
 - sequence specific : SRY and SOX
 - structure specific : HMGB (HMG-1/-2).

These HMGB proteins have been also shown to be released from the eukaryotic cells and to bear cytokine activity (macrophage activating factor).

In *Plasmodium*, 4 potential HMG were annotated, all of the HMGB family.

	Accession number PlasmoDB	HMG-box number	Length (amino-acids)
<u>PfHMGB1</u>	PFL0145c	1	97
<u>PfHMGB2</u>	MAL8P1.72	1	99
PfHMGB3	PFL0290w	2	2284
PfHMGB4	MAL13.P1.290	1	160


```

+-Famille Sry }
+-----+ } Sequence specific
! +-Famille SOX }
!
! +-----HMGB1/2-1
! +-----+
! ! ! +-UBF-4
! ! ! +-----+
! ! ! +-P.falciparum.HMG3
+-----+
! +-P.falciparum.HMG4
! !
! ! ! +Saccharomyces.NHP6A
! ! ! +-----+
! ! ! ! +Saccharomyces.NHPB
! ! ! !
! ! ! ! ! +P.vivax.HMG1
! ! ! ! ! +---+
! ! ! ! ! ! +P.knowlesi.HMG1
! ! ! ! ! +---+
! ! ! ! ! ! ! +P.berghei.HMG1
! ! ! ! ! +---+ +---+
! ! ! ! ! ! ! +P.yoelii.HMG1
! ! ! ! ! +---+ !
! ! ! ! ! ! ! +P.falciparum.HMG1
! ! ! ! ! ! !
! ! ! ! ! ! ! +---Babesia.HMG
! ! ! ! ! ! !
! ! ! ! ! +---+ +P.yoelii.HMG2
! +---+ ! +---+
! ! ! ! ! +---+ +P.berghei.HMG2
! ! ! ! ! ! !
! ! ! ! ! +---+ +P.falciparum.HMG2
! ! ! ! ! !
! ! ! ! ! ! +P.vivax.HMG2
! +-+ ! +---+
! ! ! ! ! +P.knowlesi.HMG2
! ! ! ! !
! ! ! ! ! +-----Arabidopsis.HMG
! ! ! ! ! +-----+
+-+ ! +---Catharanthus.HMG
! ! +-SSRP
! +-----+
! +-Drosophila.HMG
!
+---HMGB1/2-2

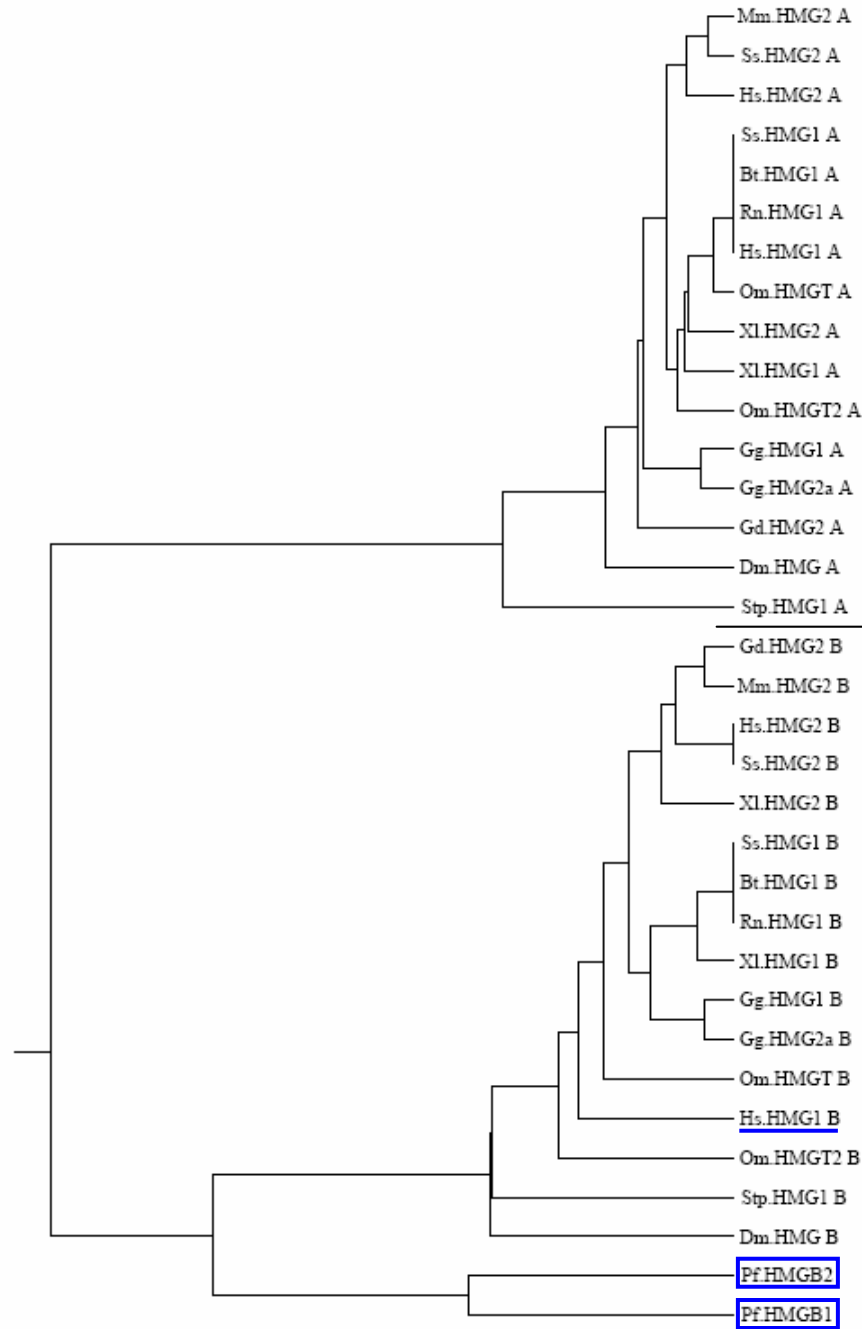
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Structure specific

Are PfHMGB1 and 2
"structure specific"
architectural factors ?

Molecular phylogenetic
tree constructed with the
« HMGB-box » domains.

Boxes A and B



Box A

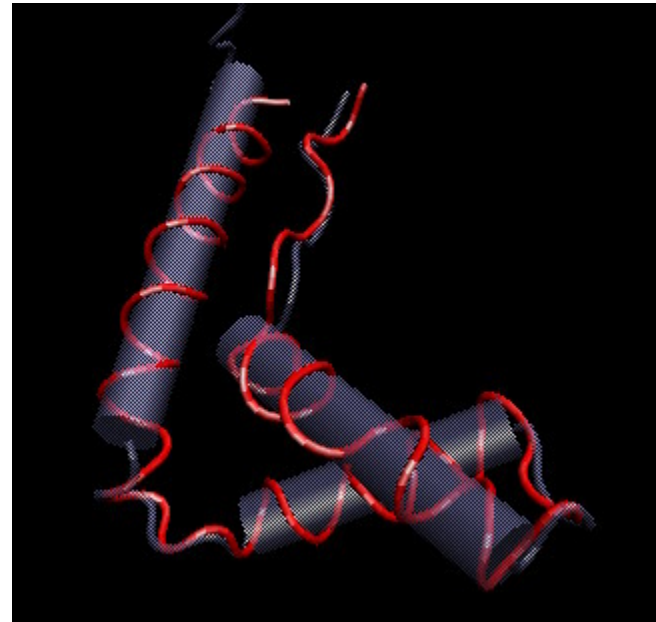
Box B

Red : PfHMGB1 (20-91)



3 alpha-helices in L-shape.

**Blue : HMG-box B of HMG1 of
Cricetulus griseus (chinese hamster)**



High Mobility Group (HMG) proteins.

Small nuclear « non-histone » proteins highly conserved in eukaryotes.

- **HMGB: HMG-box**

- sequence specific : SRY and SOX (one box)
- structure specific : HMGB (HMG-1/-2) (box A and B)

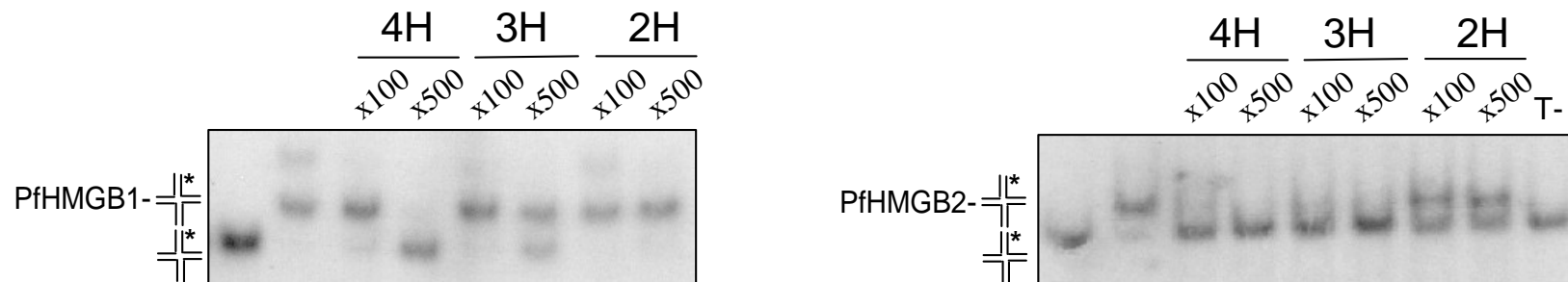
interaction with distorted DNA

bending and circularization of DNA.

In *Plasmodium*, 4 potential HMG were annotated, all of the HMGB family.

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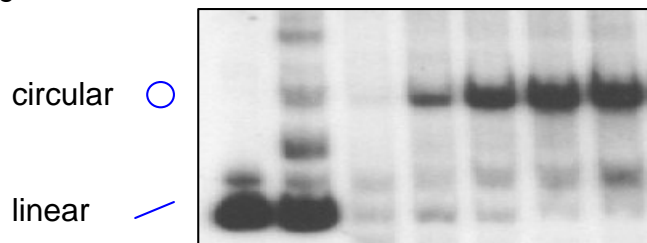
EMSA interaction with cruciform (fourway DNA junctions).



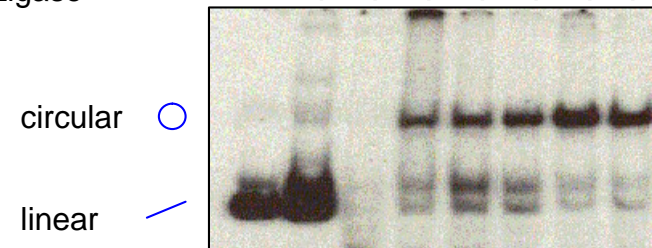
Labelled cruciform 50 femtomoles
+ recombinant PfHMGB1 400 ng
+ recombinant PfHMGB2 60 ng
Chase with cold 4H, 3H, 2H.

Ligase-mediated circularization assay.

PfHMGB1 (μM)	-	0	0	0.25	0.5	1	2
Exonuclease III	-	-	+	+	+	+	+
Ligase	-	+	+	+	+	+	+

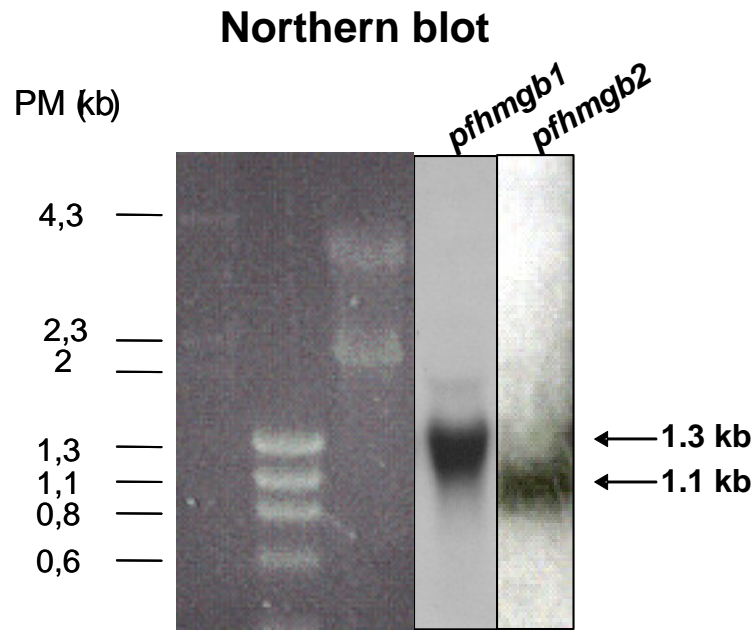


PfHMGB2 (μM)	-	0	0	3	6	25	50	100
Exonuclease III	-	-	+	+	+	+	+	+
Ligase	-	+	+	+	+	+	+	+



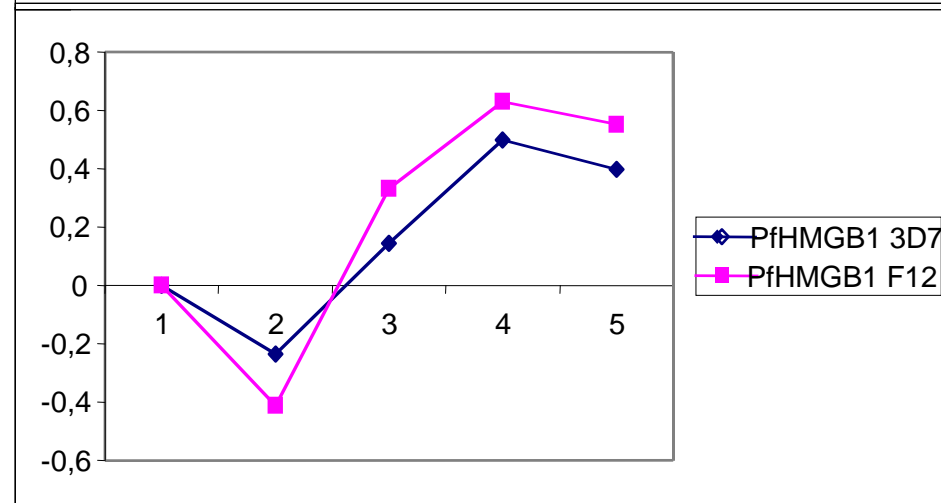
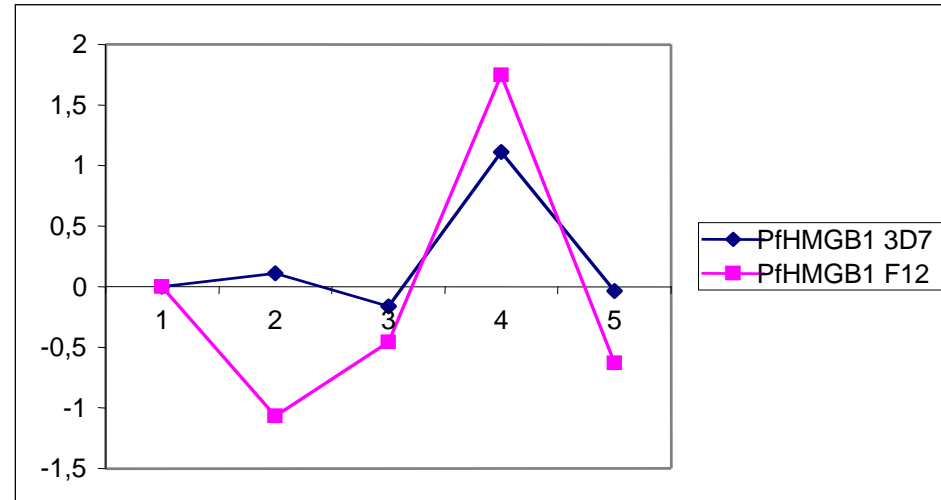
The 123 bp DNA fragment was pre-incubated with increasing concentration of PfHMGB and successively T4 DNA ligase + Exonuclease III.

Profile of the *pfhmg1* transcript during erythrocytic development.



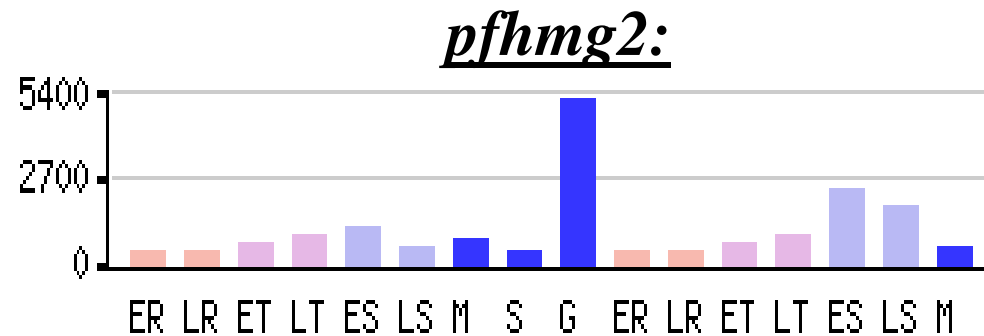
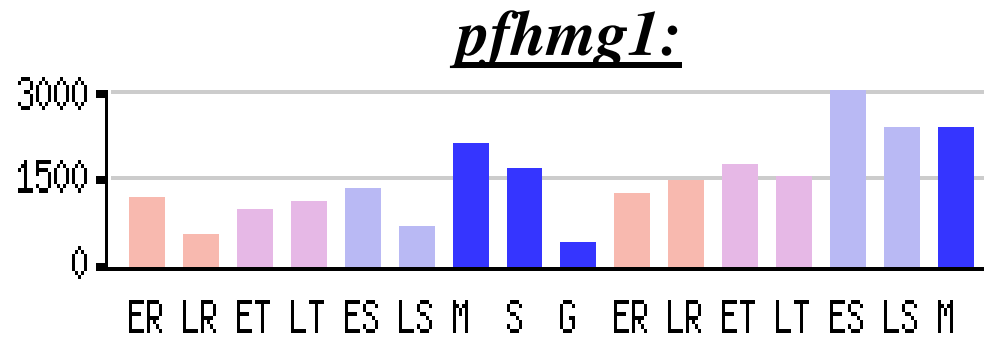
1 rings (10-14 hr)
2 early (20-22 hr)
3 late (26-28 hr) trophozoites,
4 early (32-34 hr)
5 late (40-42 hr) schizonts.

qRT-PCR



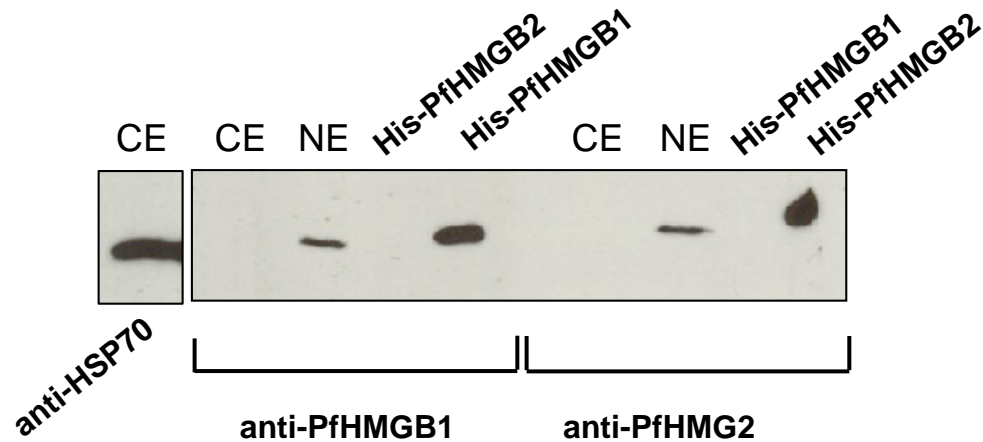
Microarray

Expression profile of *pfhmg1* and *pfhmg2* transcript during *Plasmodium* cycle.



PfHMGB1 and PfHMGB2 are present in the nuclear extracts.

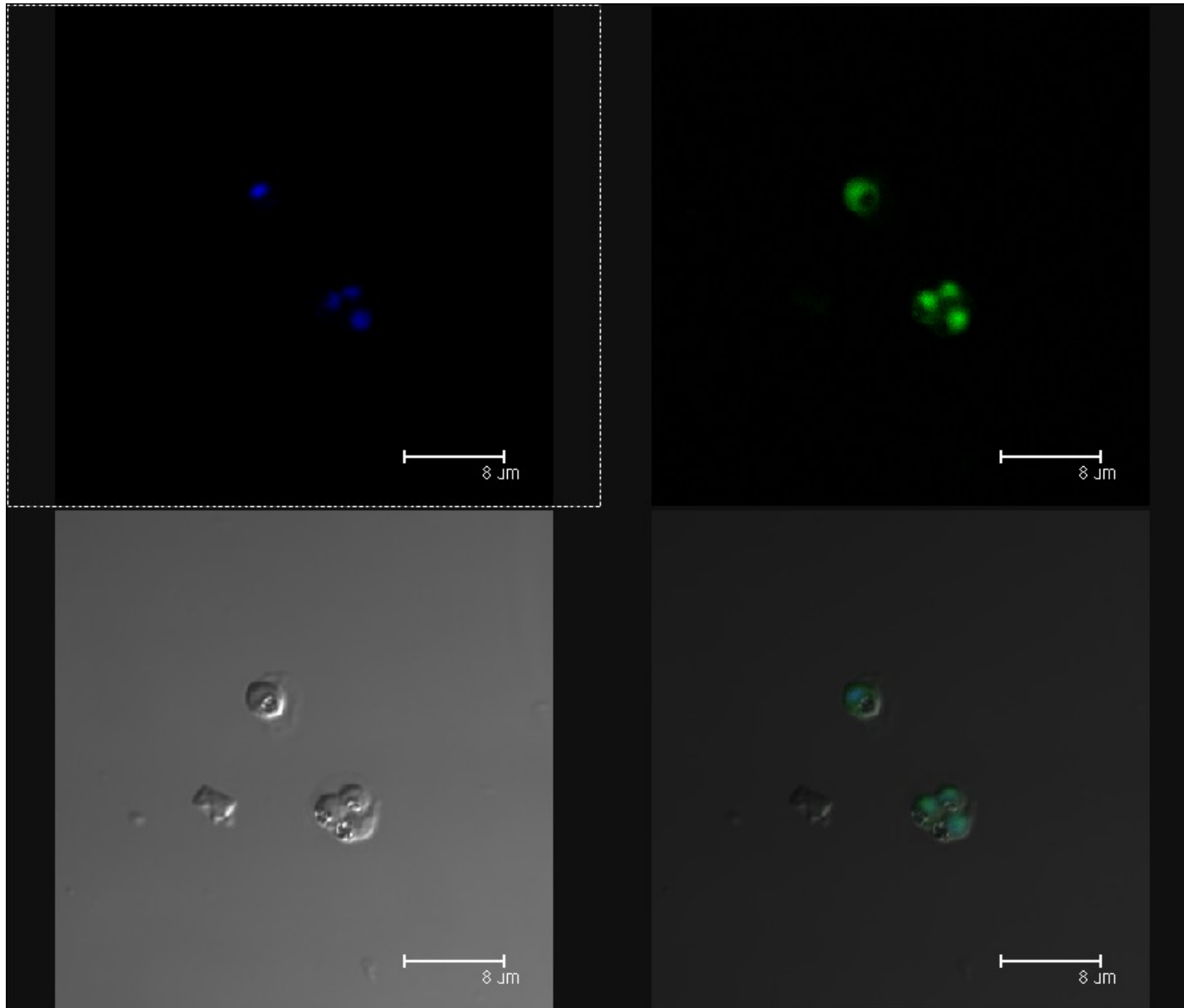
Western blot



- Expression of re-PfHMGB1 and PfHMGB2,
- Production and purification of the re-proteins
- Production of the of antibodies, etc.

- 3D7 nuclear extracts of trophozoites,
- no cross reaction was observed between the two antibodies.

PfHMGB-1 localization



Dapi

anti-PfHMGB1

DIC

(Differential interference contrast)

merge

Hsp70, cytoplasmic control



Dapi

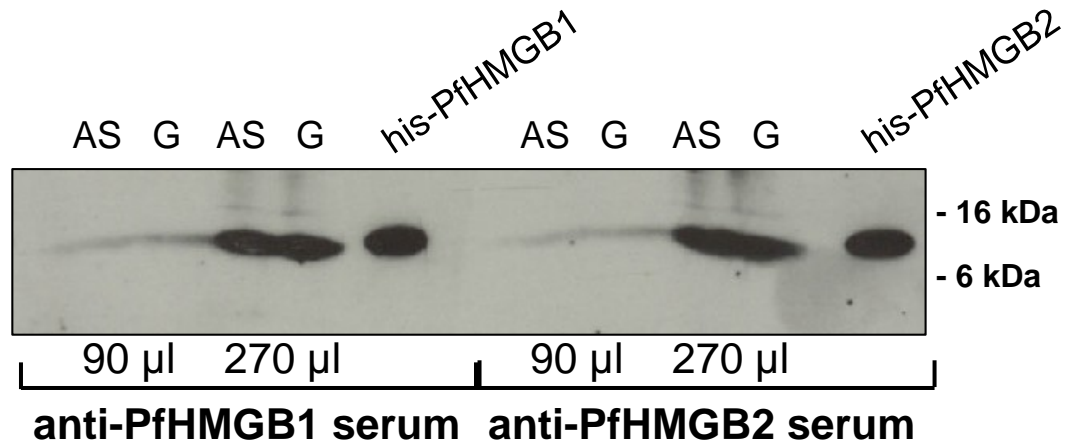
anti-PfHSP

DIC

(Differential
interference contrast)

merge

PfHMGB1 and PfHMGB2 are present in 3D7 culture supernatant.

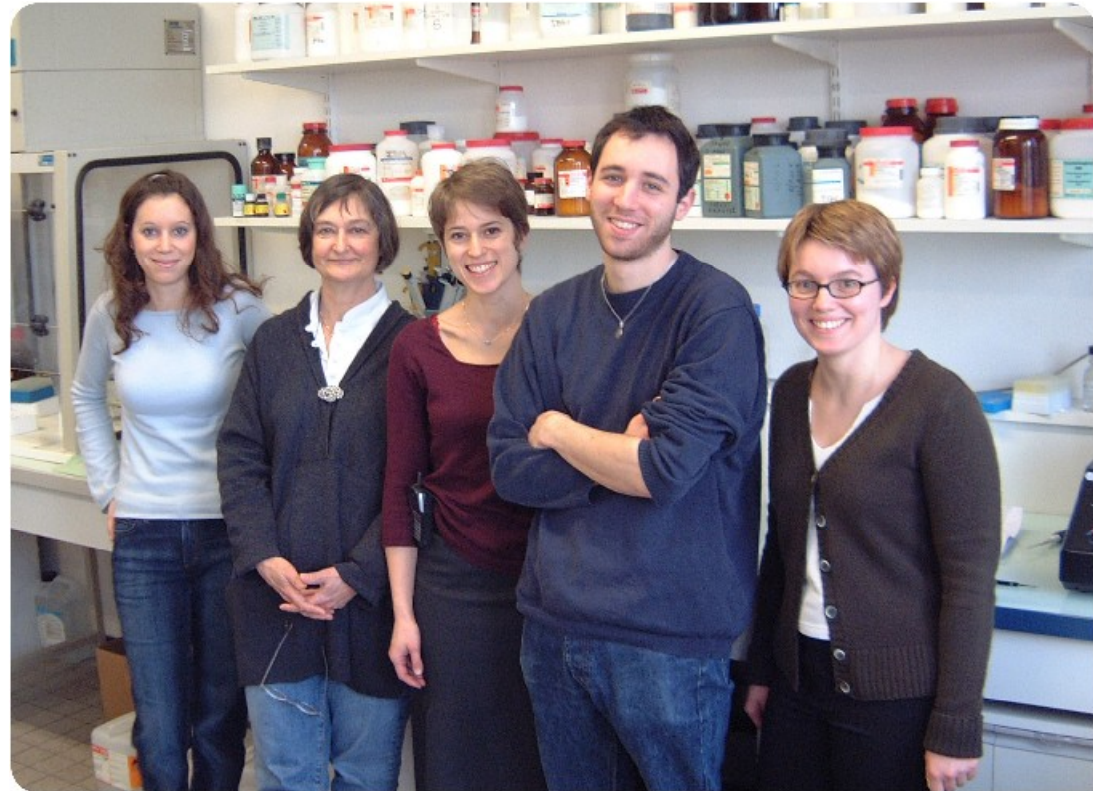


AS supernatant of enriched asexual stages,
S supernatant of enriched sexual stages.

Conclusion and perspectives.

- PfHMGB1 and PfHMGB2 are genuine architectural factors that might modulate the remodelling of the nucleosomes and therefore modulate the transcription.
- We have to identify the potential partners of the factors involved in the protein complexes and their localization on the gene promoters.
- For the function of these factors, we intend to inactivate the messengers alone or in association as was done for the *pfmyb1* transcript.
- Since, we identified the 2 factors in the supernatant of the infected erythrocytes, we are going to analyse if the PfHMGB factors behave as the mammalian ones. They might play a role in the cerebral malaria.

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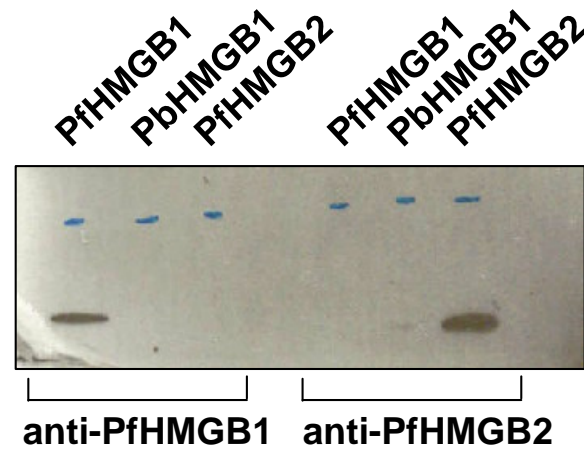


**INSERM U511, Pitié-Salpêtrière,
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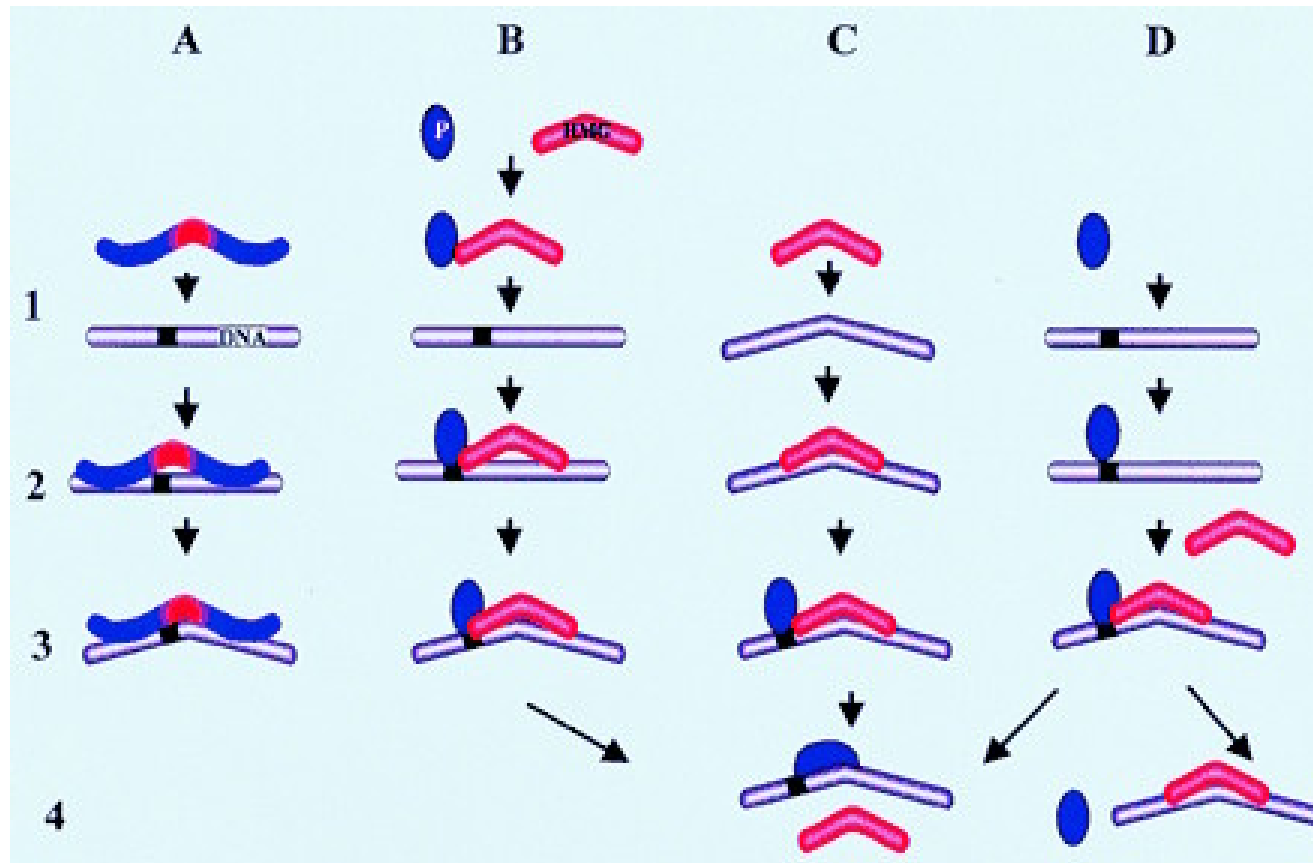
FIN de la présentation.

Absence of cross reaction between

- the two *P. falciparum* proteins and
- with the murine PbHMGB1 protein.



Multiple modes of sequence recognition and DNA bending.



According to Michael Bustin, Mol, Cell Biol, 1999.