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P-Glycoproteins 6 and 9 role in ivermectin resistance in the nematode model *Caenorhabditis elegans*

Blancfuney C., Garcia M., Lespine A., Alberich M.
UMR INTHERES, Université de Toulouse, INRAE, ENVT, Toulouse, France

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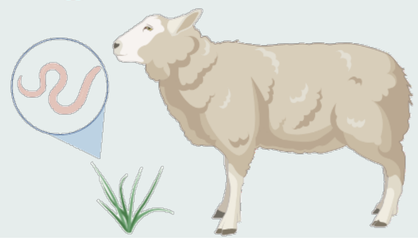


INTRODUCTION

- Ivermectin (IVM) is the **most important anthelmintic drug** of the macrocyclic lactone (ML) class and is used worldwide in veterinary medicine *(Laing et al., 2017)*.
- Intensive use has selected **drug-resistant parasite populations** globally in many animal species *(Kaplan et al, 2012)*.
- In nematodes, resistance to IVM is **polygenic** :
  - It is associated with increased expression of genes endocing detoxification proteins *(Kotze et al, 2014)*, in particular of **drug efflux transporters**, the **P-glycoproteins** (PGPs) *(Matousková et al., 2016; Whittaker et al. 2016; Ménez et al. 2016)* .
  - The nuclear hormone receptor **NHR-8** contributes to IVM tolerance and regulates the transcription of several detoxification genes.
  - Particularly, ***pgp-6*** and ***pgp-9*** appear to be regulated by NHR-8 *(Ménez et al. 2019)*.

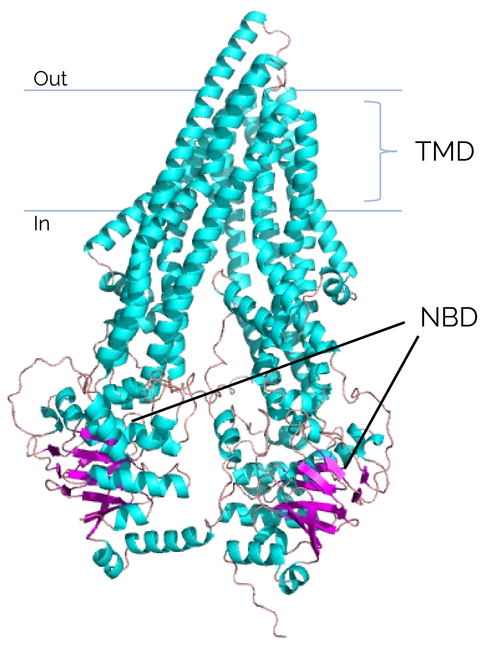


Fig 1. Predicted 3D structure of *C. elegans* PGP-6a isoform (Alpha Fold). TMD: 6 transmembrane domains; NBD: 2 nucleotide-binding domains.

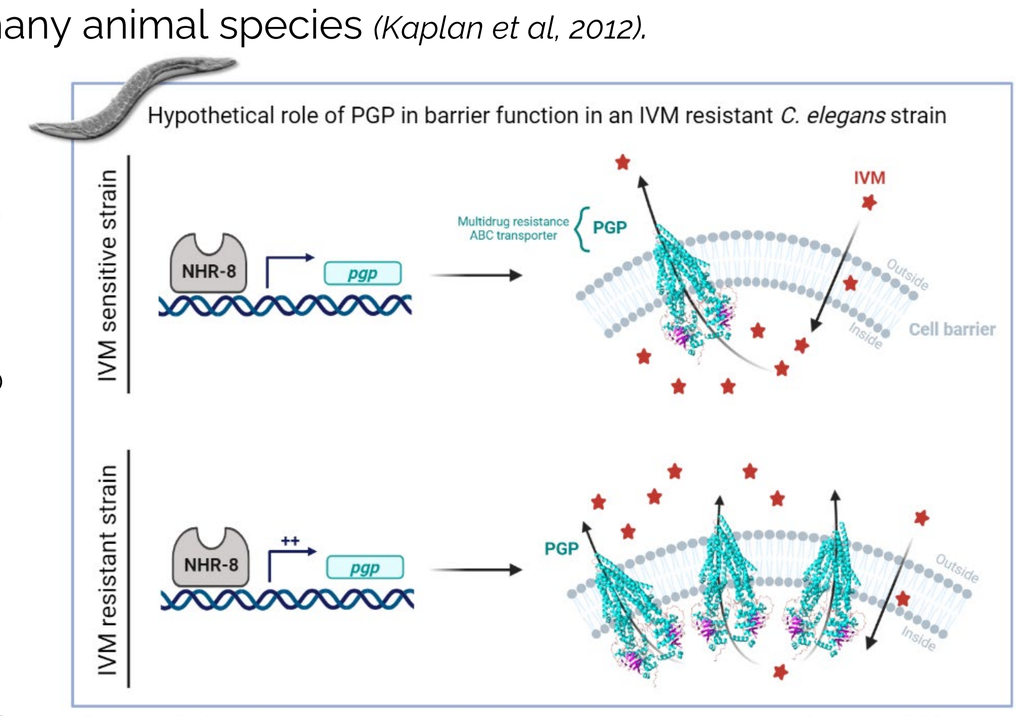


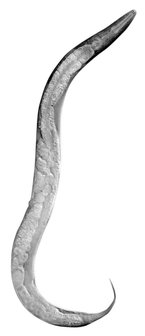
Fig 2. NHR-8 up-regulates several *pgp* expression (particularly *pgp-6* and *9*). PGP allow IVM efflux, preventing the drug from reaching its target (glutamate-gated chloride channels).

OBJECTIVE: Determining the putative role of individual PGP-6 and 9 in IVM resistance

MATERIAL & METHODS

Original *C. elegans* strains

- IVM sensitive: Wild-type N2 Bristol (N2B)
- IVM resistant: IVR10 (obtained by step-wise IVM exposure *(James et al., 2009; Ménez et al., 2016)*).
- Knock-out (KO) strains on IVR10 background: CRISPR-Cas 9 (CRISPR-sdm transgenesis method, Invivo Biosystems):
  - *nhr-8* KO : IVR10$^{nhr\text{-}8\text{-}/\text{-}}$
  - *pgp-6* KO: IVR10$^{pgp\text{-}6\text{-}/\text{-}}$
  - *pgp-9* KO: IVR10$^{pgp\text{-}9\text{-}/\text{-}}$

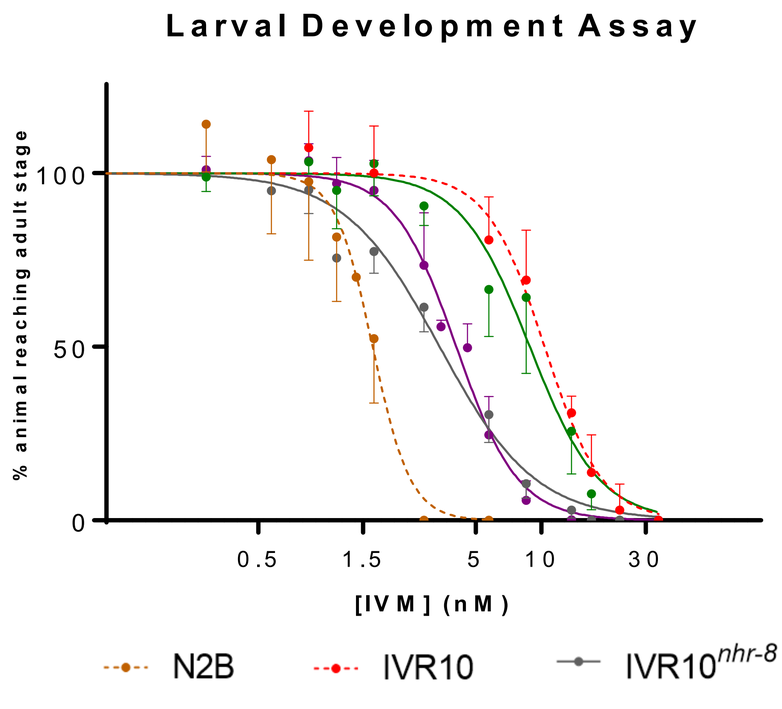


Genotype verification: PCR and electrophoresis for the deleted sequence (*nhr-8*, *pgp-6* or *9*) and the residual one.

Phenotypic tests to assess drug efficacy

- Larval Development Assay (LDA): IVM potency to inhibit *C. elegans* development from L1 stage to adulthood.
- Worm Microtracker (WMi): IVM effect on worm motility after drug exposure

RESULTS



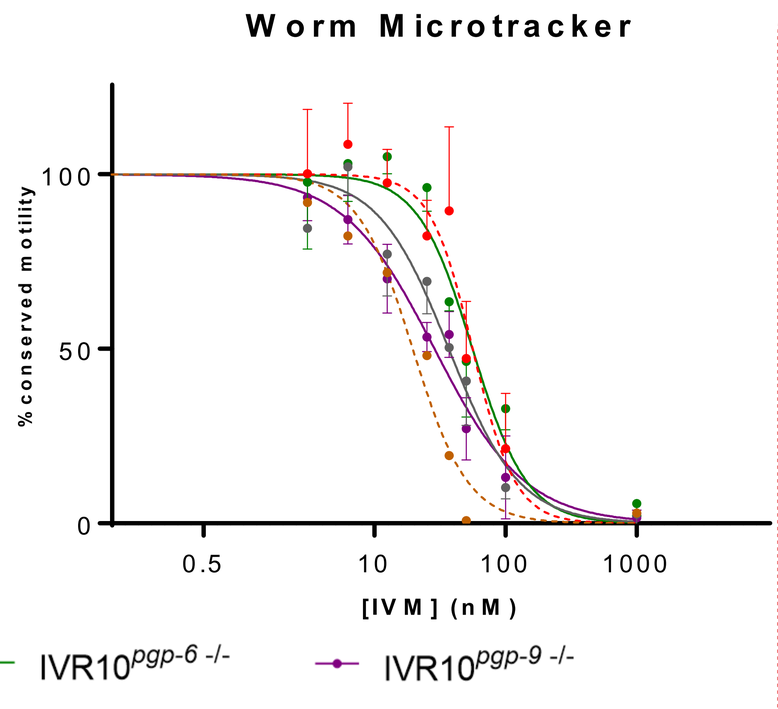


Table 1. Effect of n*hr-8*, *pgp-6* and *9* deletion in IVR10 strains on IVM tolerance. IVM IC50 (half maximal inhibitory concentration). RF (Resistance Factor) is the fold resistance relative to N2B. ****p<0.0001; ***p<0.001; *p<0.05 vs IVR10 (Anova). Data are mean ± S.D. from 3-7 independant experiments. [1]WMi IC50 of N2B and RF_{WMi} are preliminary results (WMi for N2B, n=1).

	N2B	IVR10	IVR10$^{nhr\text{-}8\text{-}/\text{-}}$	IVR10$^{pgp\text{-}6\text{-}/\text{-}}$	IVR10$^{pgp\text{-}9\text{-}/\text{-}}$
$IC50_{LDA}$ (nM)	1.6 ±0.2 (****)	10.5±1.8	3.2±0.2 (****)	9.4±2.7	4.0±0.6 (****)
RF_{LDA}	1.0	6.7	2.1	6.0	2.5
$IC50_{WMi}$ (nM)	19.6[1]	57.9±17.7	36.1 ±7.7	59.5±11.1	29.1±4.2(*)
RF_{WMi}	1.0	2.9	1.8	3.0	1.5

CONCLUSIONS

- **PGP-9 plays a key role in IVM resistance**.
- *pgp-6* deletion did not restore IVM sensitivity in IVR10.
- *nhr-8* deletion restored IVM sensitivity in IVR10 to a level close to N2B *(Ménez et al., 2019)*.
- Motility tests allowed discrimination between sensitive and resistant strains, and confirmed PGP-6 and 9 implication (or lack of) in restoring IVM sensitivity.

PERSPECTIVES

- Studying structure/function of PGP-6 and PGP-9 and interactions with IVM.
- Investigating other PGPs and cytochrome (i.e., drug metabolism) for their individual and combined implication.

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Clara Blancfuney, INRAE
clara.blancfuney@inrae.fr
UMR 1436 - INTHERES
Tél. : + 33 (0)5 82 06 63 21

180, chemin de Tournefeuille,
31 027 Toulouse Cedex 3
FRANCE