Institut de Génétique Humaine

mRNA Regulation and Development

Martine Simonelig

Drosophila models of muscular dystrophies

The *Drosophila* model of Oculopharyngeal muscular dystrophy (OPMD)

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Nicolas Barbezier, Cédric Soler, Laurie Maynadier

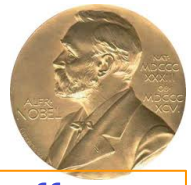
Maladies Rares et Organismes Modèles – October 8, 2012

Drosophila as a model to study human genetic diseases

- **Why does it work?**
  Genomes and molecular functions are conserved between man and *Drosophila*: 77% of genes involved in human genetic diseases have a homologue in *Drosophila*

- **Advantages of *Drosophila***
  Rapid analysis (new generation in two weeks)
  Drosophila is genetically tractable: highly sophisticated genetic tools
  Possibility of large scale screens: genetics or molecules:
  - to understand molecular mechanisms of the disease
  - to find targets for possible therapies

- **Disease models available in *Drosophila***
  Cancer, mental retardation, diabetes,
  Innate immunity: 2011 Nobel Price of Medicine in *Drosophila*: Pr Jules Hoffman

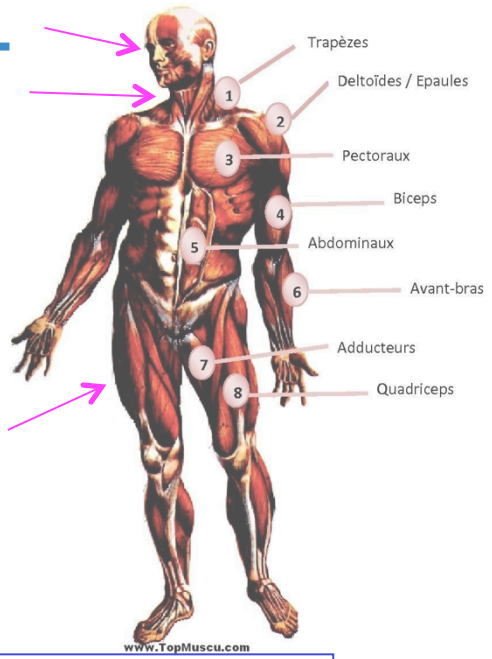


  Neurodegenerative diseases, muscular dystrophies, etc....

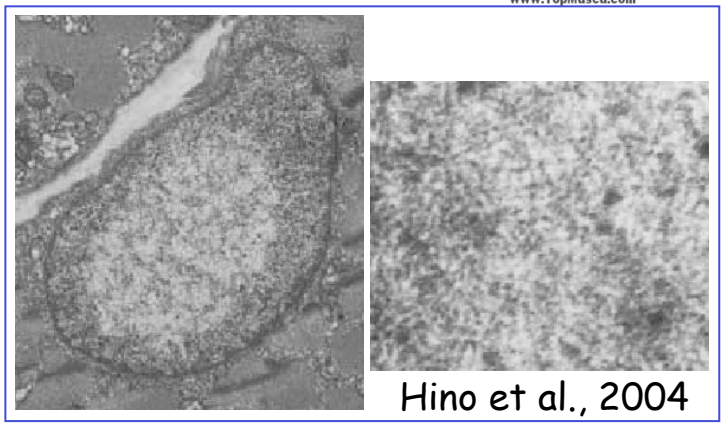
OPMD: oculopharyngeal muscular dystrophy

Autosomal dominant muscular dystrophy

- Late onset (fifth decade) and progressive weakening of muscles
  - that hold eyelids, leading to ptosis
  - involved in swallowing, leading to dysphagia
  - limb muscles



- Characterized at the ultrastructural level by nuclear inclusions of tubular filaments (8.5 nm diameter), found in muscle fiber nuclei only
  in 2% to 9% of the muscle nuclei



- OPMD is a rare disease: 1/100 000 in France
  But more common in Quebec: 1/ 1000

Molecular mechanisms leading to OPMD

OPMD is due to alanine expansion in PABPN1

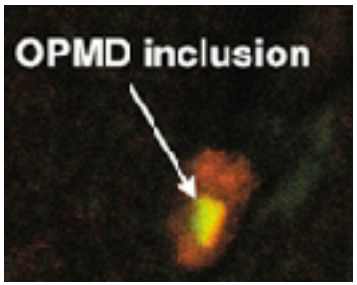
Normal PABPN1

PABPN1 in OPMD patients

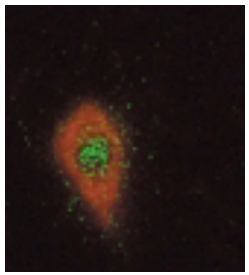
(Brais et al. 1998)

OPMD is a protein aggregation disorder

Nuclear inclusions in muscles of OPMD patients contain:
mutant PABPN1, HSP70, ubiquitin, proteasome subunits, poly(A) RNA



PABPN1
poly(A) RNA



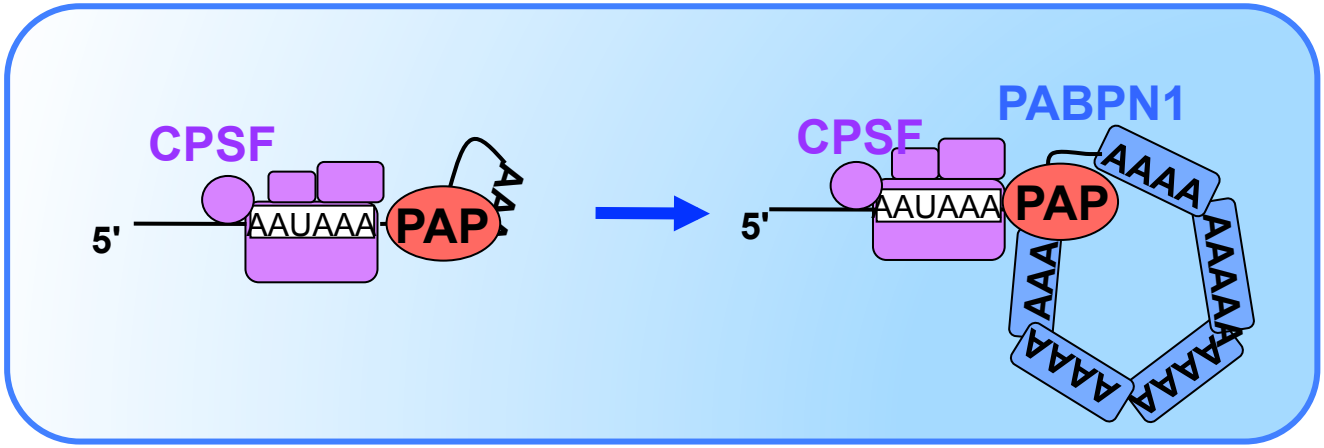
DNA
ubiquitin

(M. Carmo-Fonseca, 2000)

Extension of the alanine tract in PABPN1 leads to the formation of insoluble PABPN1 aggregates in muscle nuclei

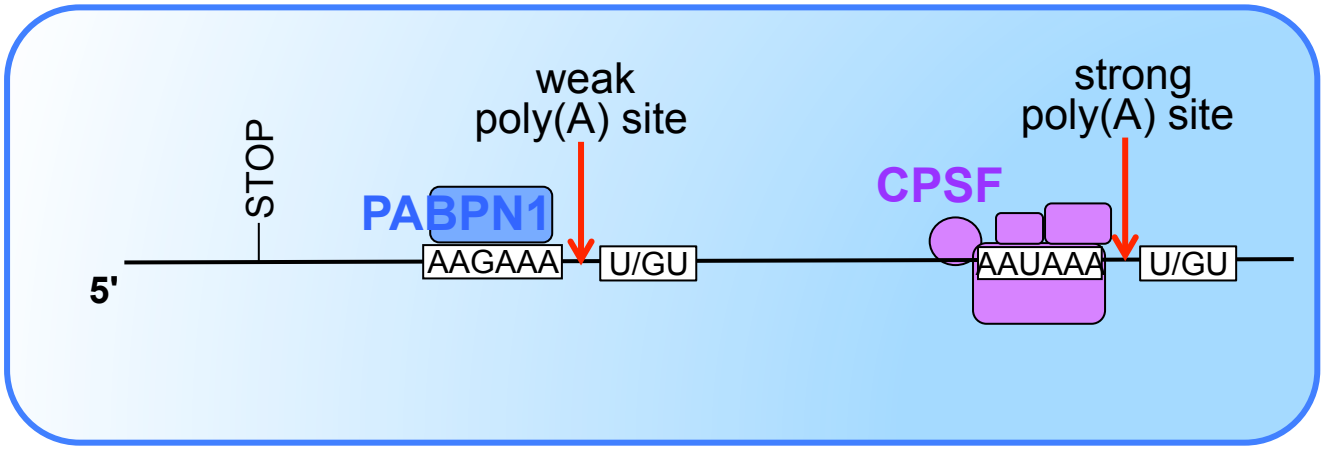
Molecular function of PABPN1

- PABPN1 is involved in nuclear polyadenylation in mammals (E. Wahle)
  This function is conserved in *Drosophila* (Benoit et al. Developmental Cell 2005)



PABPN1:
- stimulates PAP
- controls poly(A) tail length

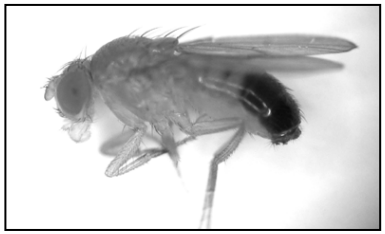
- PABPN1 prevents utilisation of weak poly(A) sites (Jenal et al. 2012, de Klerk 2012)



In OPMD mice:
- 3'UTR tend to be shorter
- Increased expression of mRNAs with short 3'UTR

The *Drosophila* model of OPMD

Expression of mammalian mutant PABPN1-17ala in *Drosophila* muscles:

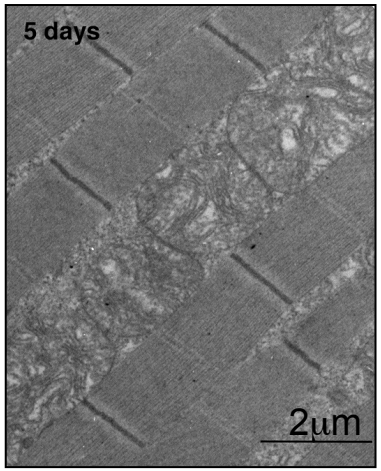


wild type

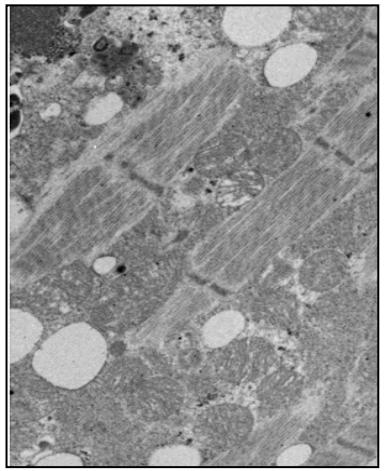
UAS-PABPN1-17ala expressed with *Mhc-Gal4* driver (specific to muscles)

PABPN1-17ala in muscles

- Progressive muscle degeneration

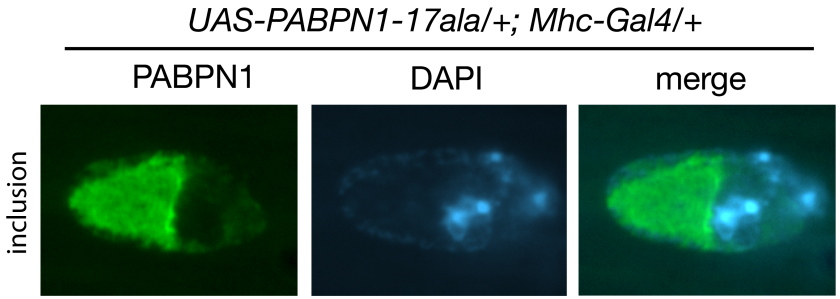


wild type



OPMD

- Formation of nuclear aggregates



Chartier et al. EMBO J. 2006

Utilisation of the *Drosophila* model of OPMD

- **Understand the pathophysiology of OPMD**

  Transcriptomic and genetic approaches to identify molecular pathways involved in the disease process

  genome-wide genetic screens to identify suppressors of OPMD phenotypes

- **Evaluation/Identification of therapeutic strategies for OPMD**
  - Anti-PABPN1 intrabodies as suppressors of OPMD in *Drosophila*
  - Identification of drugs as suppressors of OPMD in *Drosophila*

OPMD European consortia

EU networks: FP5: 2002-2005 & FP6: 2006-2009

Present:

AFM eOPMD (5 partners)
Pathophysiology and therapeutic approaches in OPMD

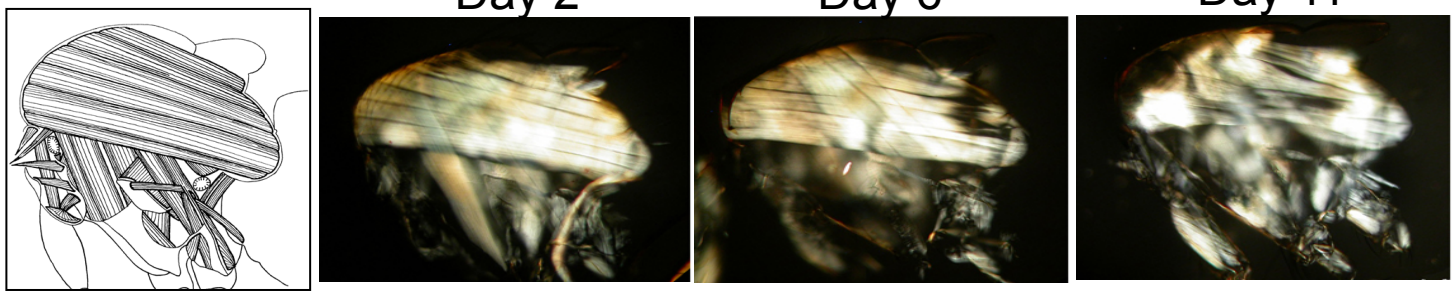
Available resources in the network:

✔ Cell models:	S. van der Maarel, V. Raz, LUMC, The Netherlands
	G. Butler-Browne, Institut de Myologie, France
✔ *Drosophila* model:	M. Simonelig, IGH, France
✔ Mouse model:	G. Dickson, RHU of London, UK
	G. Butler-Browne, Institut de Myologie, France
✔ Patient biopsies:	Pr Baziel van Engelen, Radboud University, The Netherlands
	G. Butler-Browne, Institut de Myologie, France
✔ Therapeutic tools: vectors for gene therapy	G. Dickson, RHU of London, UK

Possible validation of information from models up to patients

Transcriptomic analysis of OPMD muscles in *Drosophila*

Transcriptomic analysis of thoracic muscles in OPMD and control flies at three time points



	day 2	day 6	day 11
up-regulated genes	196	349	282
down-regulated genes	289	305	319

Identification of cellular pathways by GO term enrichment:

«Proteasome complex»

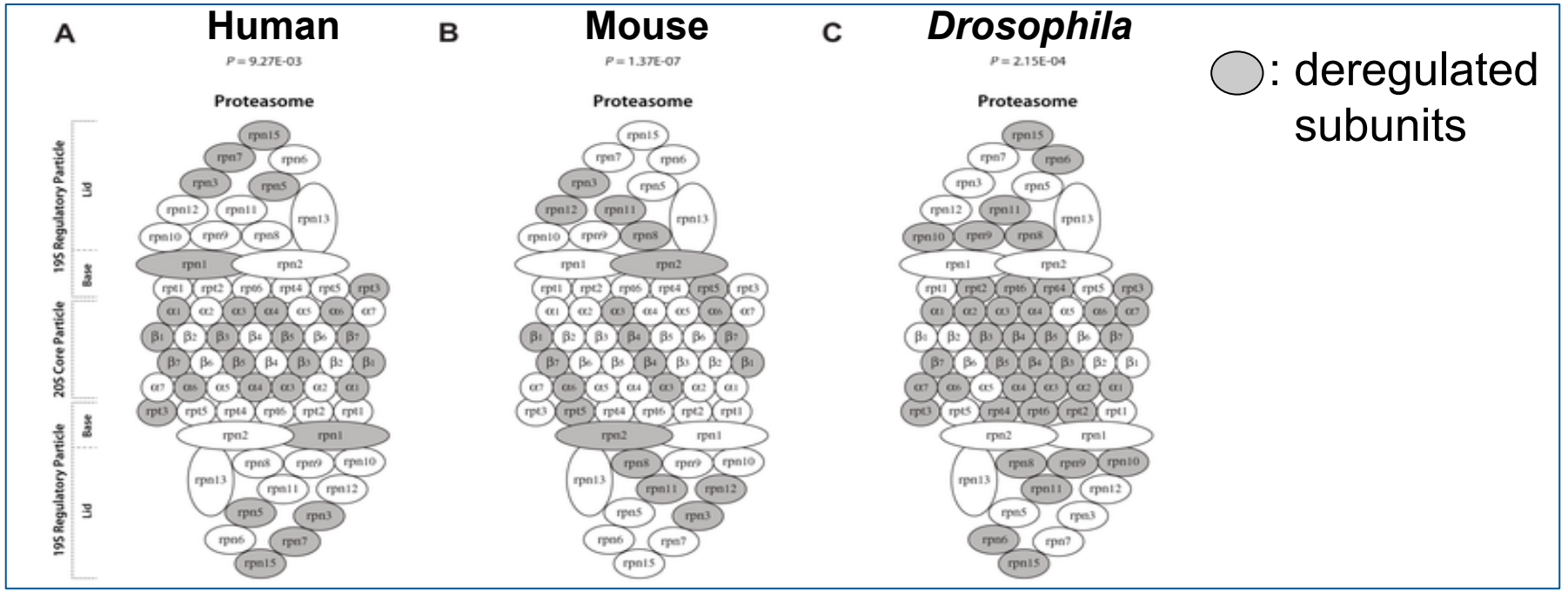
«Mitochondrion»

Chartier et al. HMG 2009

Consistent deregulation of the Ubiquitin-Proteasome System (UPS) in OPMD across species

KEGG Pathways	Human	Mouse	*Drosophila*
Ubiquitin mediated proteolysis	1.52 E-03	8.25 E-08	2.03 E-03
Proteasome	9.27 E-03	1.37 E-07	2.15 E-04

Silvere van der Maarel
Vered Raz



Anvar et al. Skeletal Muscle 2011

Identification of molecular pathways involved in OPMD using genetic screens in the *Drosophila* model

- Genome-wide genetic screen using large genomic deletions (deficiencies)
  Screen on larval lethality

Early expression of PABPN1-17ala in embryonic muscle (mesoderm) induces a larval lethality:

OPMD larvae
Dead

5% to 15% of pupae

OPMD larvae + deletion or mutation

Rescue of larval lethality:
up to 35% of pupae

- Specific genetic screen: for regulators of mRNA metabolism and RNA binding proteins (17 genes tested)
  Screen in adults, on wing position defects

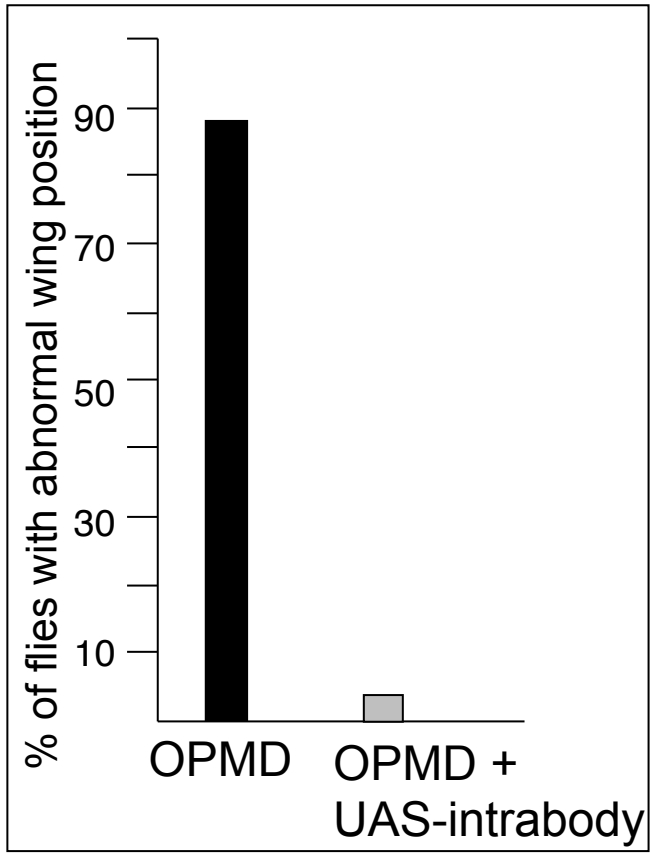
Results of genetic screens: pathways involved in OPMD

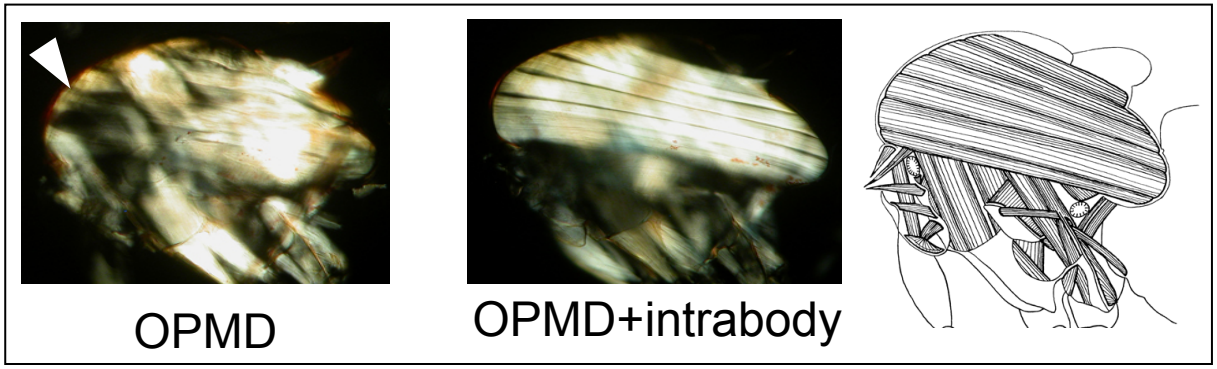
- 43 suppressors/enhancer identified
  37/43 have one or several homologues in man

- Major pathways involved in OPMD:
  Hsp70: protein chaperone
  Ubiquitin-proteasome system
  Mitochondrion
  mRNA processing/Regulation of poly(A) tail length

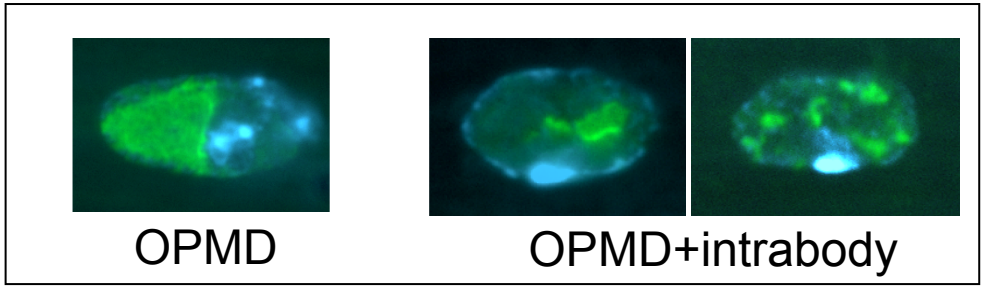
Therapeutic approach 1: anti-PABPN1 intrabody

- Expression of anti-PABPN1 intrabody in muscles suppresses OPMD-like phenotypes in *Drosophila*





- Reduction of the size of nuclear inclusions

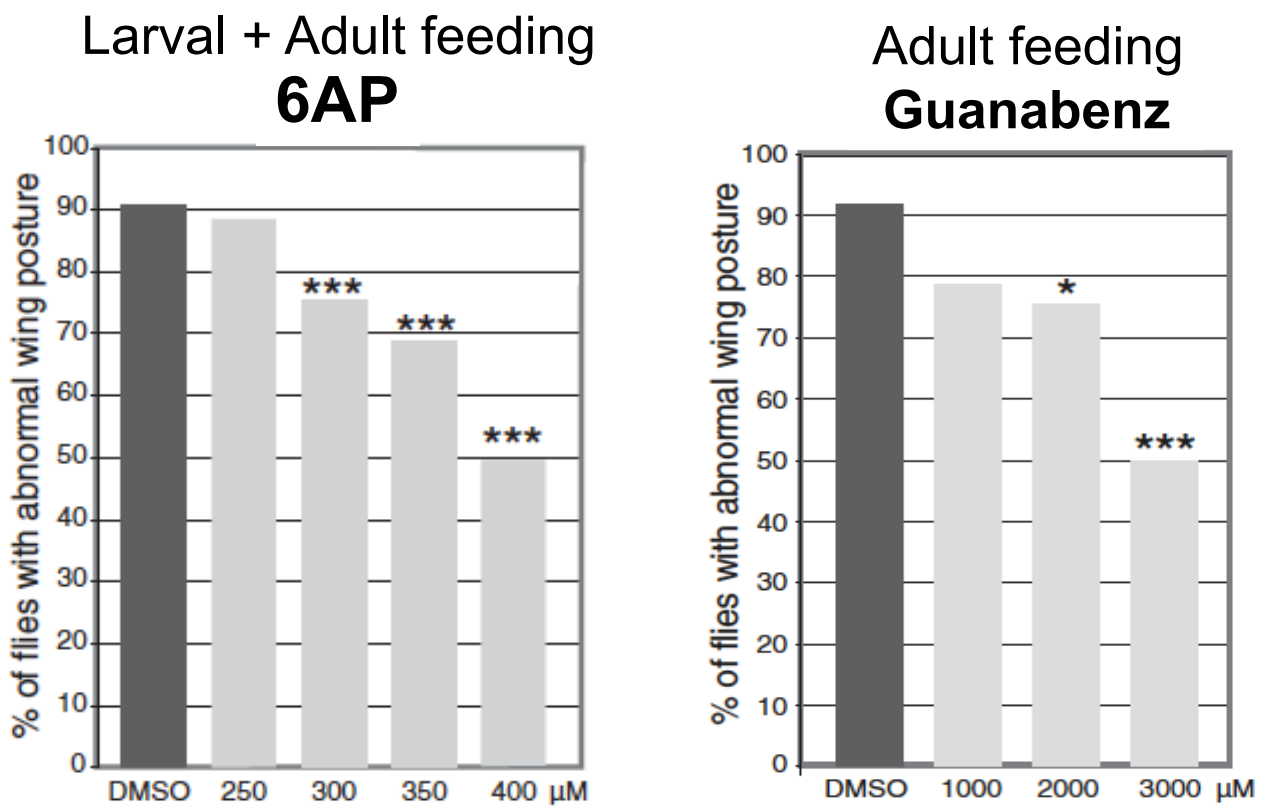


Chartier et al. Human Molecular Genetics 2009
Collaboration: S. van der Maarel

Proof of principle that the anti-PABPN1 intrabody has a therapeutic potential *in vivo*

Therapeutic approach 2: chemical compounds

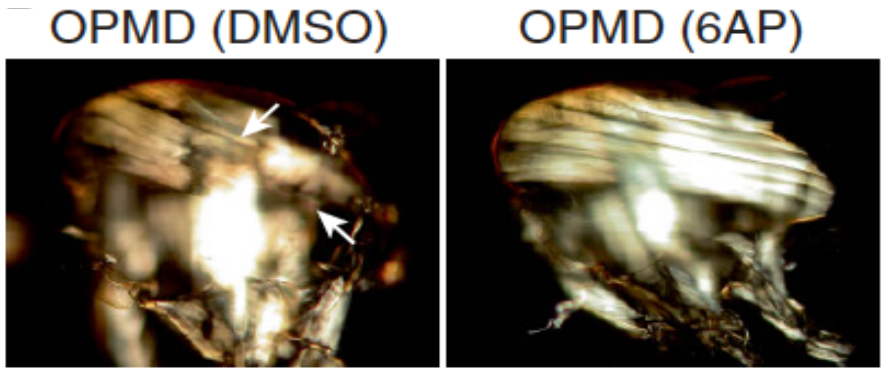
Anti-prion drugs 6AP (6-aminophenanthridine) and Guanabenz decrease OPMD-like phenotypes in *Drosophila*



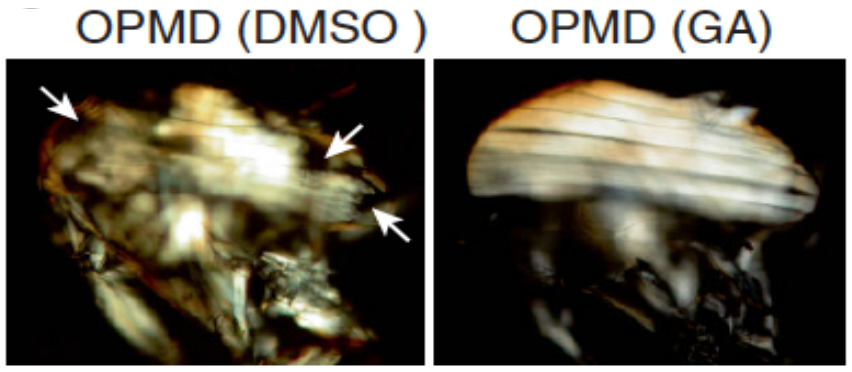
Therapeutic approach 2: chemical compounds

- 6AP and Guanabenz reduce muscle degeneration

6AP



Guanabenz



- 6AP and Guanabenz reduce the PABPN1 aggregation load

6AP

Nuclear Inclusion surface area	
Control (DMSO)	692 ± 303 n=58
6AP	464 ± 279 n=105

Guanabenz

Nuclear Inclusion surface area	
Control (DMSO)	555 ± 258 n=48
Guanabenz	289 ± 230 n=96

Barbezier et al. EMBO Molecular Medicine 2011
Collaboration: Marc Blondel

Therapeutic approach 2: chemical compounds

Guanabenz, from yeast to *Drosophila* and mouse...

Therapeutic potential of Guanabenz for OPMD:

✔ Guanabenz has a positive effect in the *Drosophila* model of OPMD

✔ Guanabenz is already used in medicine as a treatment against hypertension, without major side-effects.

In progress: Test of Guanabenz in the mouse model of OPMD by our collaborators: G. Dickson, RHU London

Conclusions

Molecular mechanisms of OPMD

Through genetic screens:

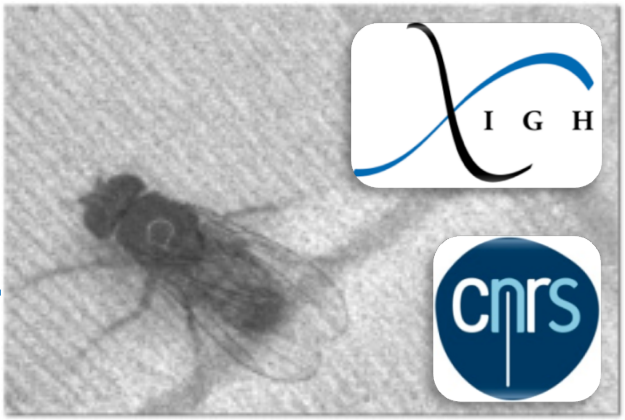
- Identification of several pathways potentially involved in OPMD
- mRNA poly(A) tail regulation has a major role in OPMD
- Fonctional validation of the ubiquitin-proteasome pathway, in progress

Potential therapeutic strategies for OPMD

- Proof of principle that the anti-PABPN1 intrabody has a therapeutic potential *in vivo*
- Identification of Guanabenz, a compound used in medicine as beneficial for OPMD in the *Drosophila* model

mRNA Regulation and Development

Institut de Génétique Humaine, Montpellier



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Yannick Bidet
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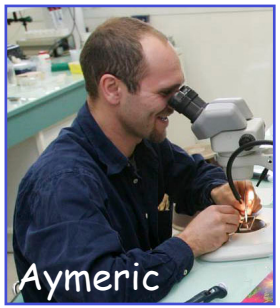


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Collaborations

AFM eOPMD Projet Stratégique

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