Les canaux ioniques dans les arythmies cardiaques

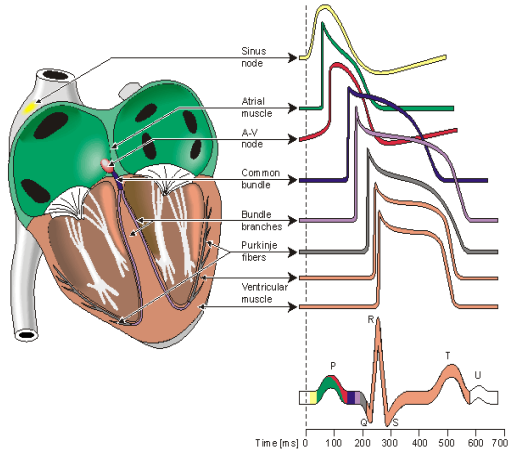
Sophie Demolombe

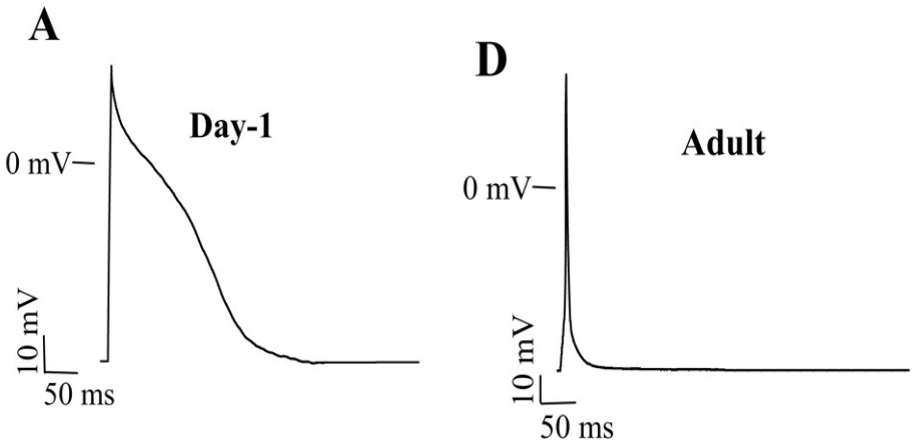
Institut de Pharmacologie Moléculaire et Cellulaire
CNRS UMR7275
Sophia-Antipolis

L'électrogenèse cardiaque

COMPLEXE ET MODULABLE

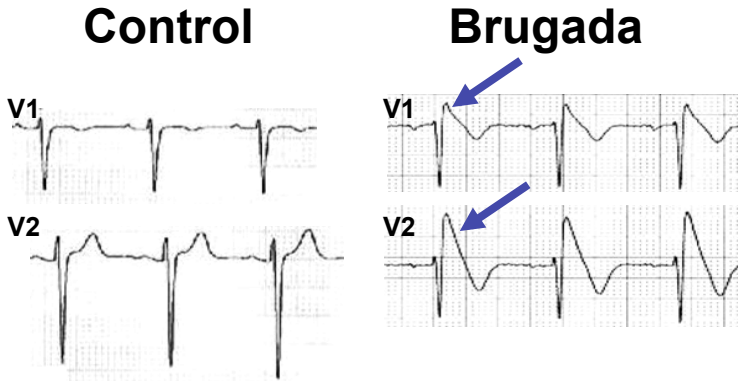
✓***Conditions physiologiques:*** *âge, hormones, …*

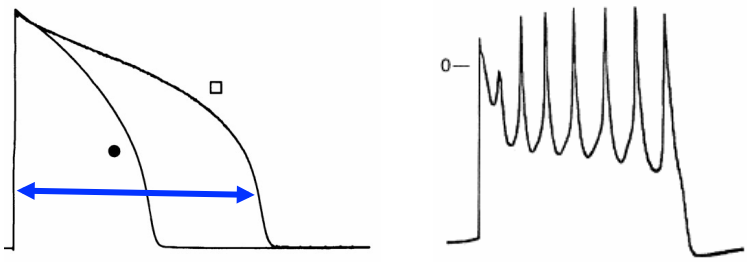




Grandy SA, et coll. *Am J Physiol Heart Circ Physiol*. 2007.

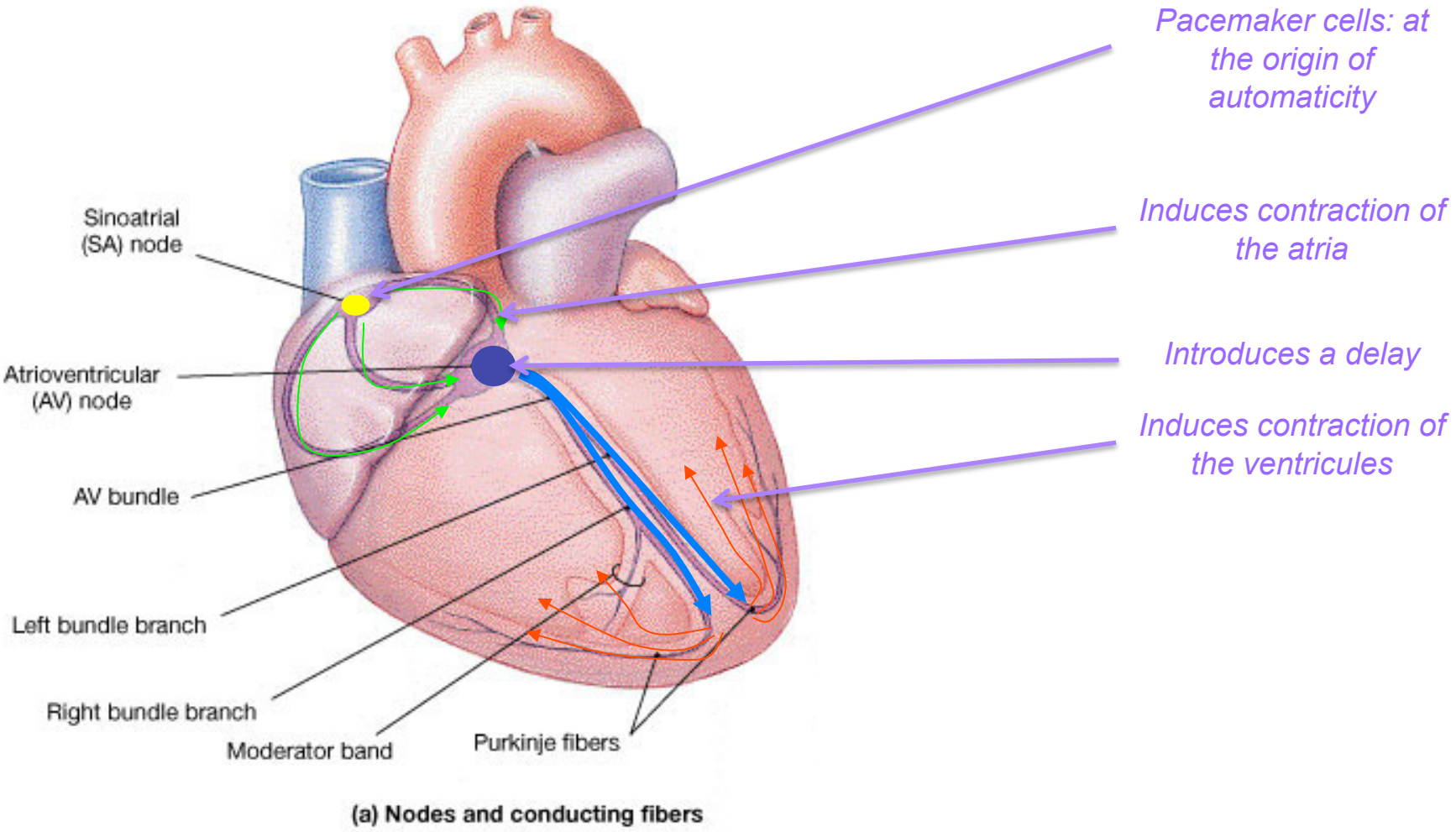
✓***Conditions pathologiques:*** *mutations, médicaments, …*





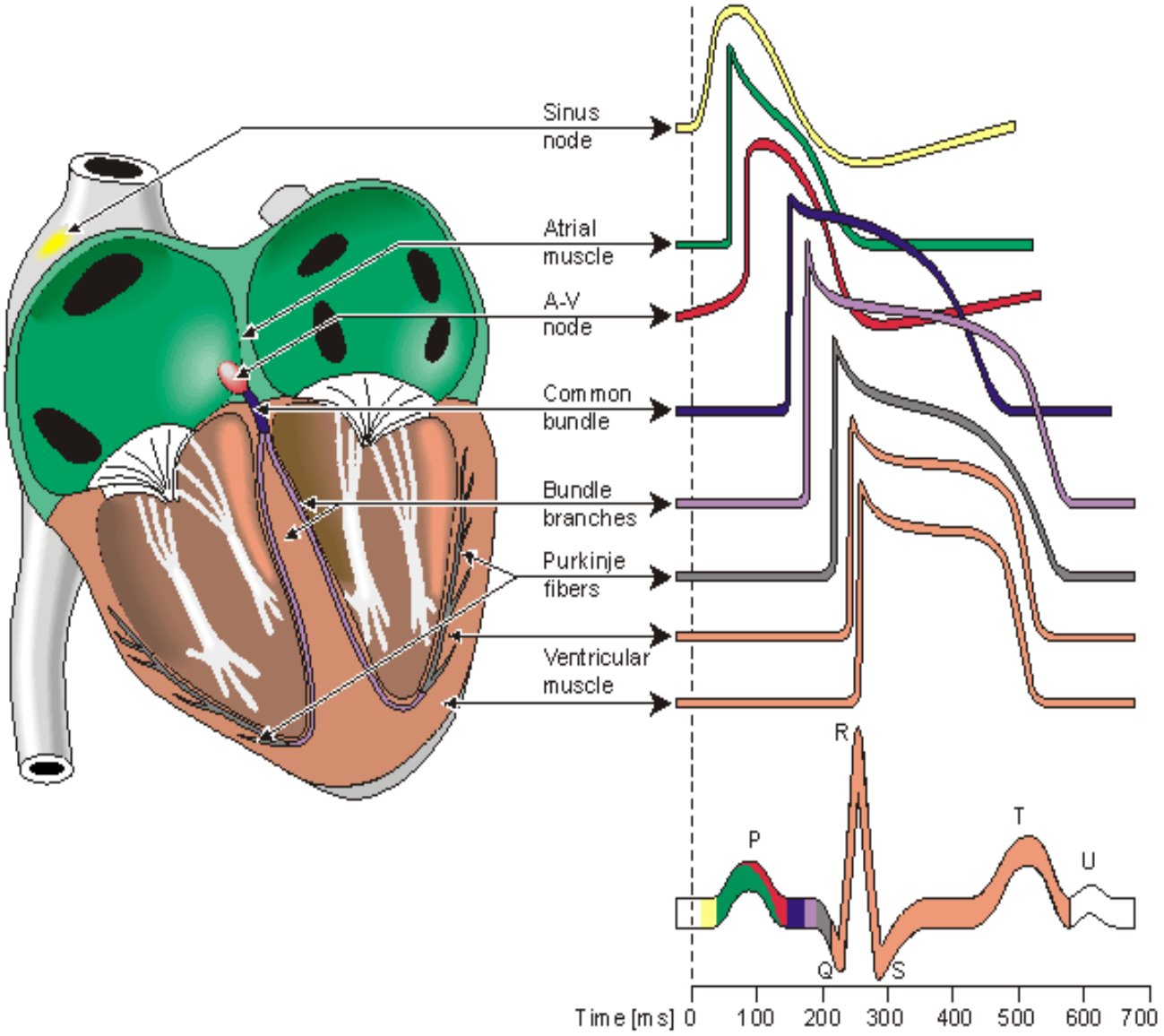
⇨ **Mort subite**

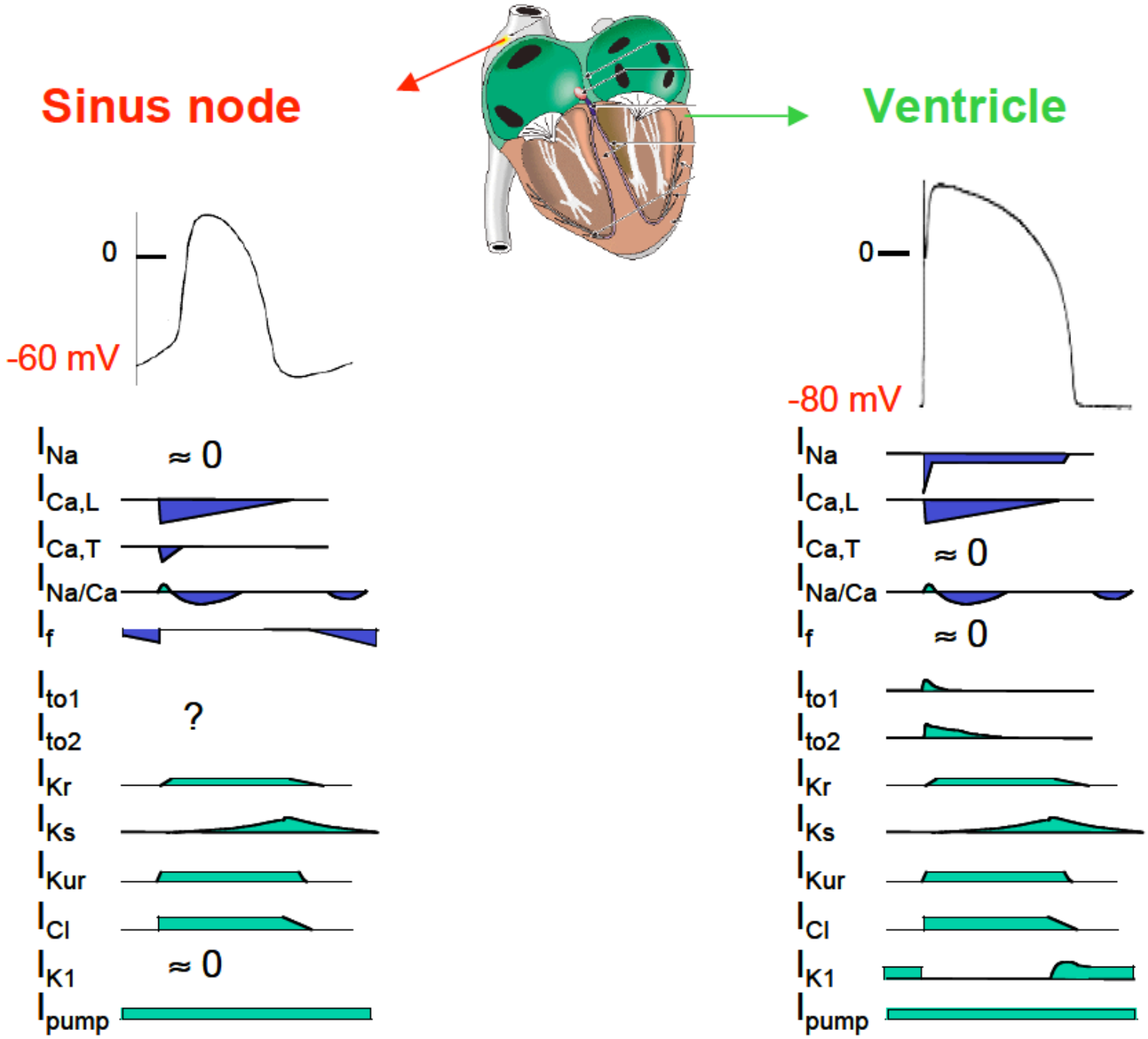
CONDUCTION DU SIGNAL ELECTRIQUE DANS LE COEUR



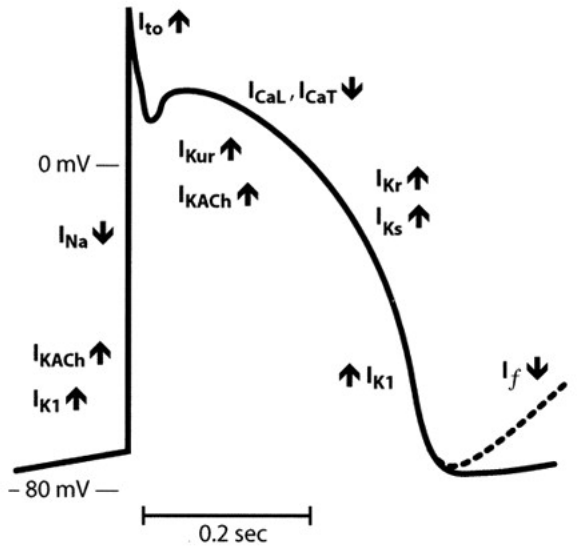
(a) Nodes and conducting fibers

CONDUCTION DU SIGNAL ELECTRIQUE DANS LE COEUR

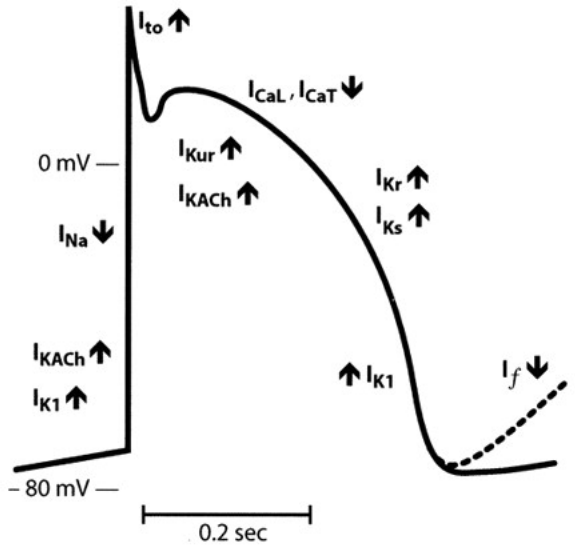



Sinus node
Ventricle
0
-60 mV
0
-80 mV
I_{Na}
$I_{Ca,L}$
$I_{Ca,T}$
$I_{Na/Ca}$
I_f
I_{to1}
I_{to2}
I_{Kr}
I_{Ks}
I_{Kur}
I_{Cl}
I_{K1}
I_{pump}
≈ 0
?
≈ 0
≈ 0
≈ 0

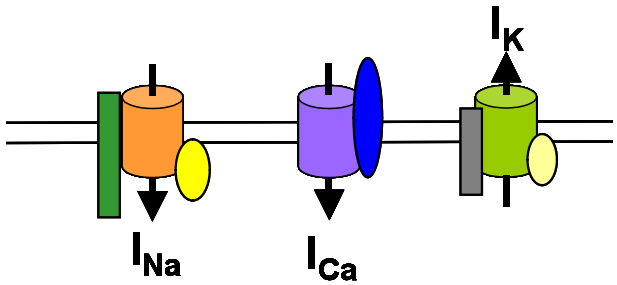
1/ La fonction électrique résulte de l'activité de nombreux courants



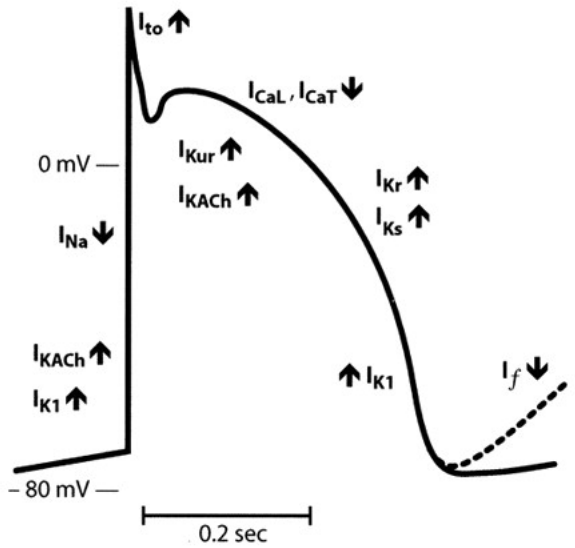
1/ La fonction électrique résulte de l'activité de nombreux courants



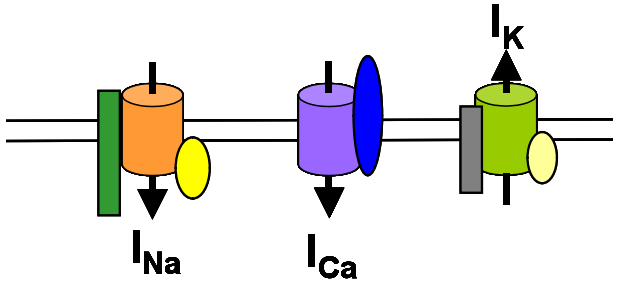
2/ Un courant est généré par une famille de canaux ioniques et sous-unités auxiliaires



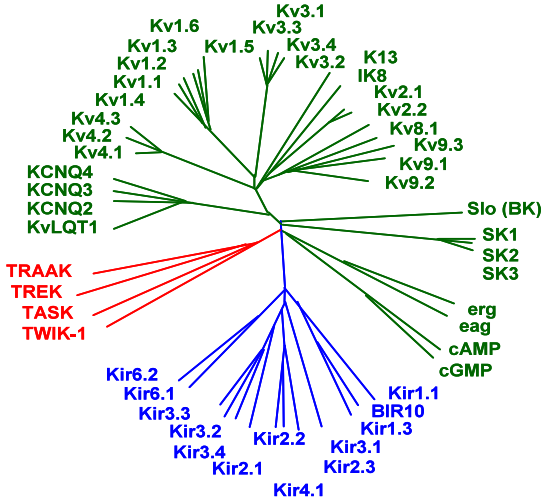
1/ La fonction électrique résulte de l'activité de nombreux courants



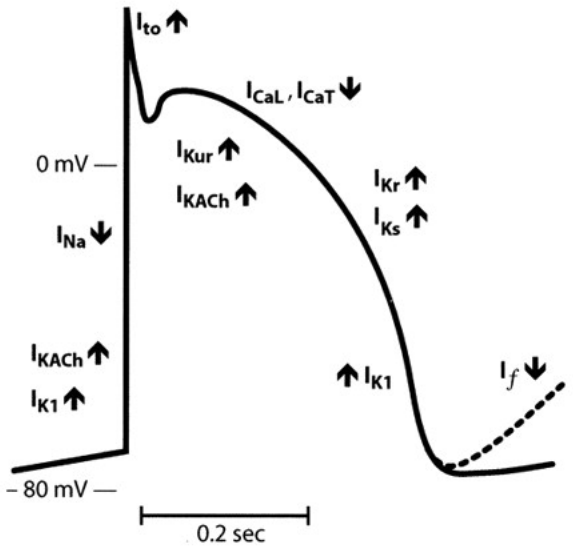
2/ Un courant est généré par une famille de canaux ioniques et sous-unités auxiliaires



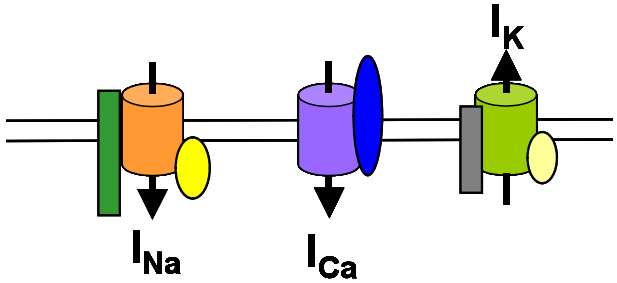
3/ Une famille de canaux est codée par de nombreux gènes



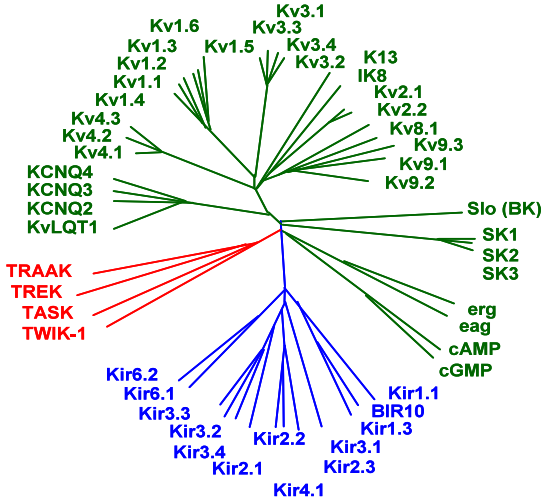
1/ La fonction électrique résulte de l'activité de nombreux courants



2/ Un courant est généré par une famille de canaux ioniques et sous-unités auxiliaires



3/ Une famille de canaux est codée par de nombreux gènes

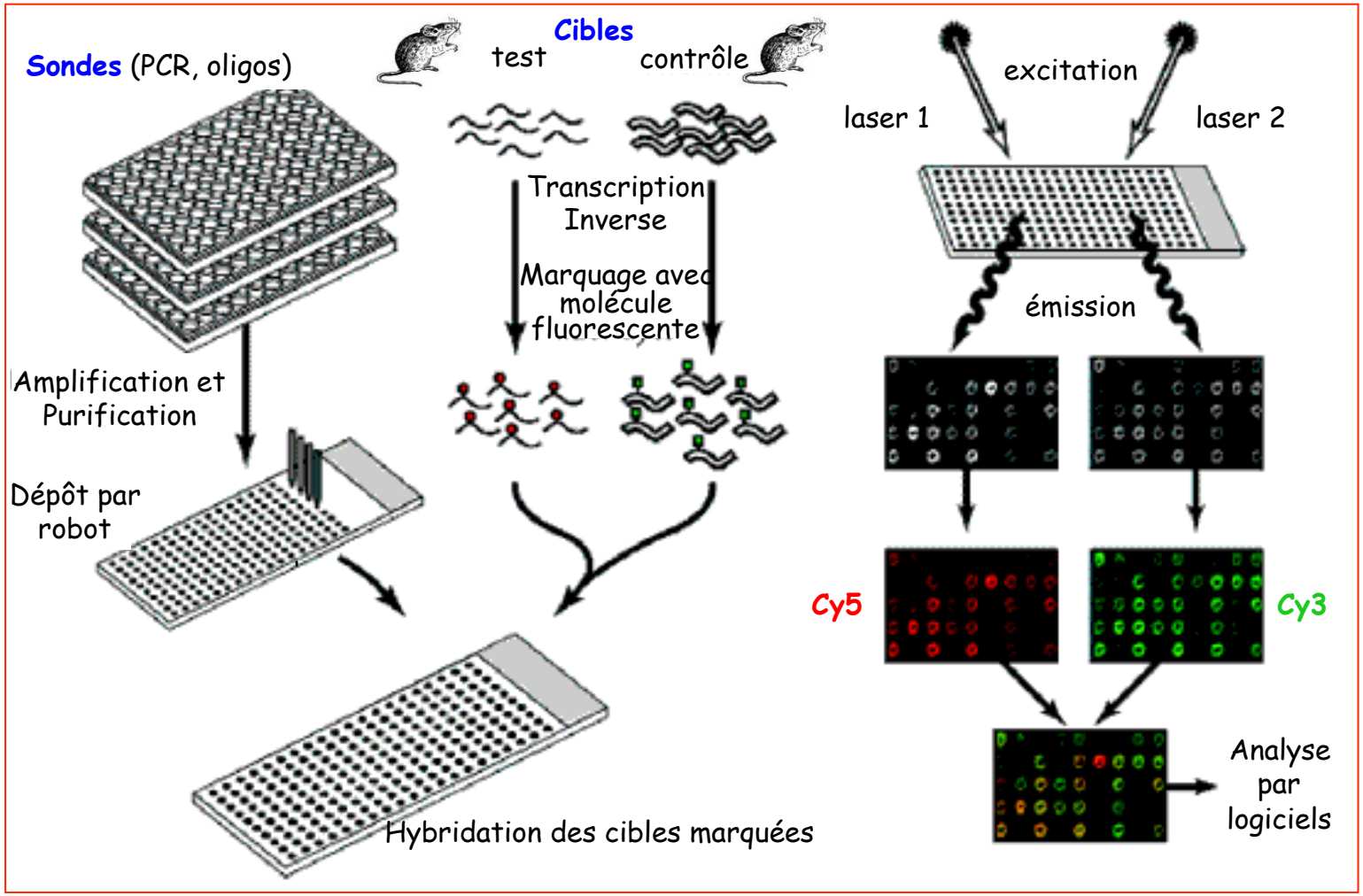


OBJECTIF

Déterminer le rôle et l'organisation de l'expression des canaux ioniques dans le signal électrique physiologie et physiopathologique du cœur

1/ Développer des outils génomiques dédiés aux canaux ioniques:

- les puces à ADN



Duggan DJ et al. *Nature Genetics.* 1999.

Composition des puces dédiées à l'homéostasie ionique



Human

- 137 channel subunits
  - ⇨ (16 Na^+, 22 Ca^{2+}, 13 Cl^-, 75 K^+, 3 f/h, 6 TRPC…)
- 11 connexins
- 20 other genes



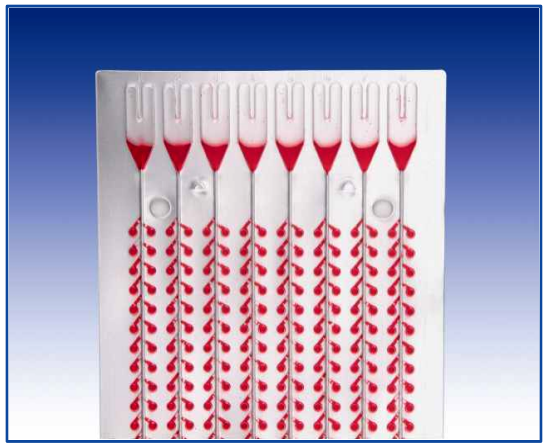
Mouse

- 111 channel subunits
  - ⇨ (10 Na^+, 19 Ca^{2+}, 11 Cl^-, 48 K^+, 4 f/h, 7 TRPC…)
- 11 connexins
- 82 other genes

… + 3000 cardiac genes as controls and references

1/ Développer des outils génomiques dédiés aux canaux ioniques:

- TaqMan Low Density Arrays



Applied Biosystems

- 384 wells format
- 8 entry ports
- **2 ng of cDNA per well**

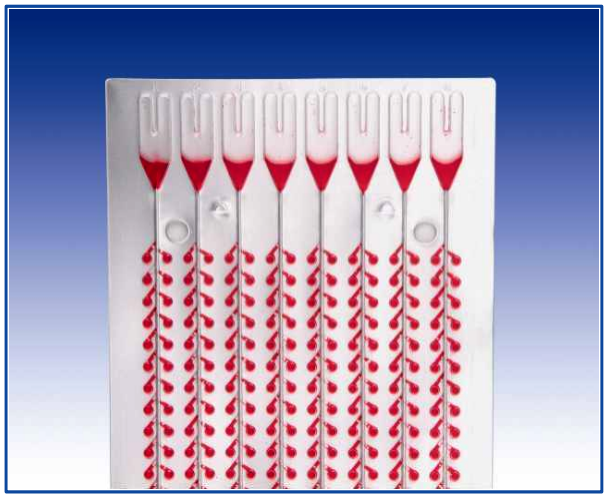
71 ion channel α- and β-subunits
10 Ca^{2+} regulators
1 ion channel expression regulator
6 specific markers of cardiac regions
1 marker of: vascular vessels
neuronal tissue
fibrosis
inflammation
3 reference genes

Stratégie

Loading a TLDA reservoir with 100ng cDNA

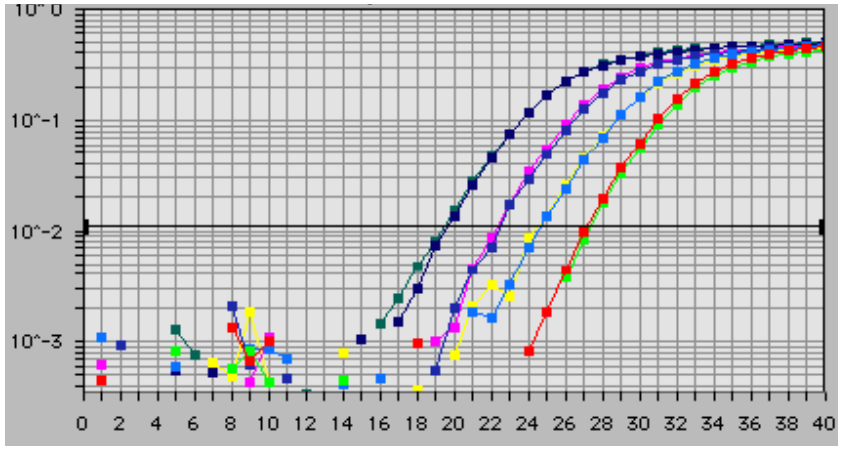


cDNA distribution into each well by centrifugation





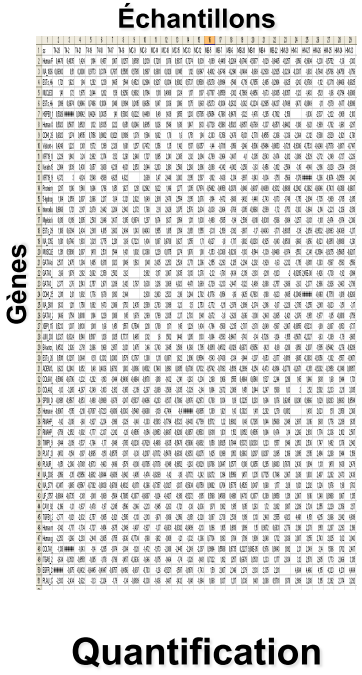
PCR using ABI PRISM® 7900HT Sequence Detection System



Data collection

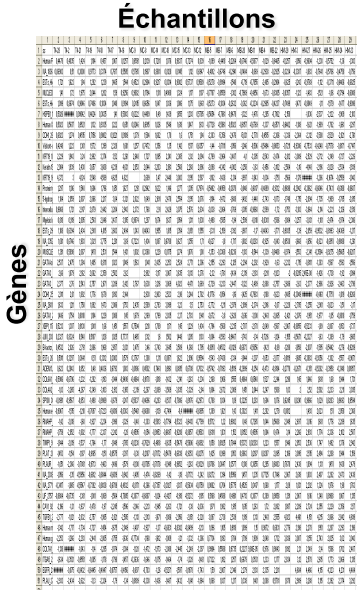
Stratégie

1/ Analyse des données génomiques : Classification hiérarchique

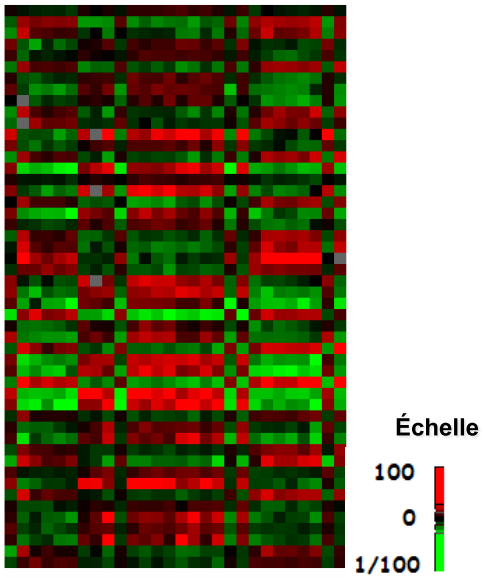


Eisen MB, et al. *PNAS*. 1998

1/ Analyse des données génomiques : Classification hiérarchique



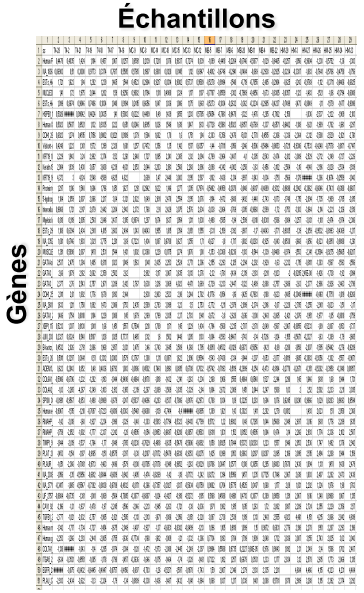
Quantification



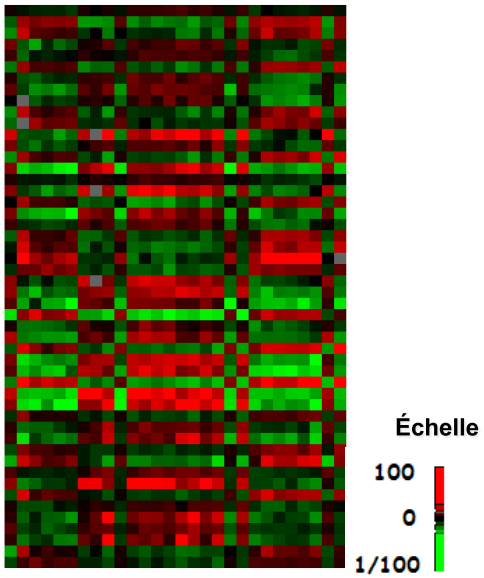
Colorisation
Rouge = sur-exprimé
Vert = sous-exprimé

Eisen MB, et al. *PNAS*. 1998

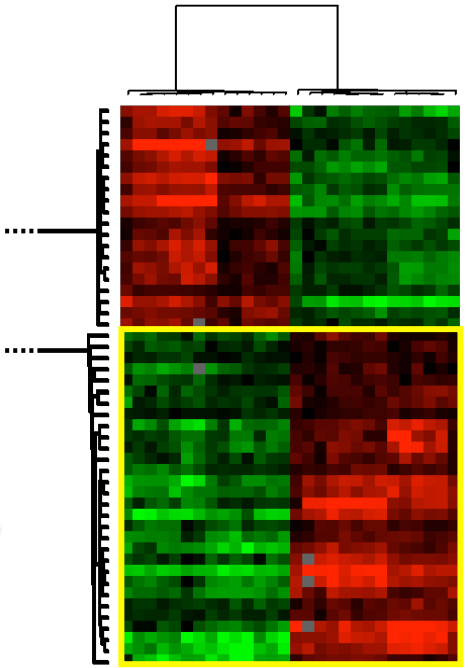
1/ Analyse des données génomiques : Classification hiérarchique



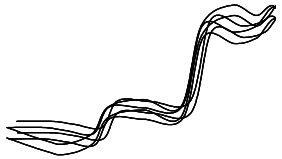
Quantification



Colorisation
Rouge = sur-exprimé
Vert = sous-exprimé



Classification hiérarchique



Gènes co-variants

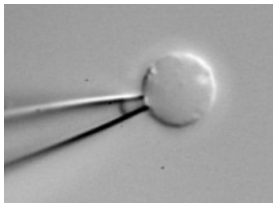
Fonction commune

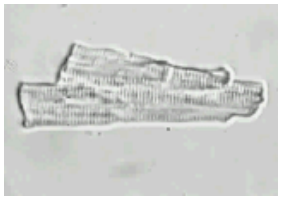
Régulation commune

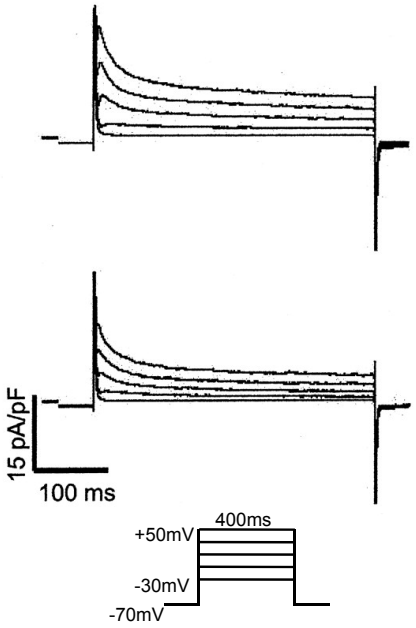
Eisen MB, et al. *PNAS*. 1998

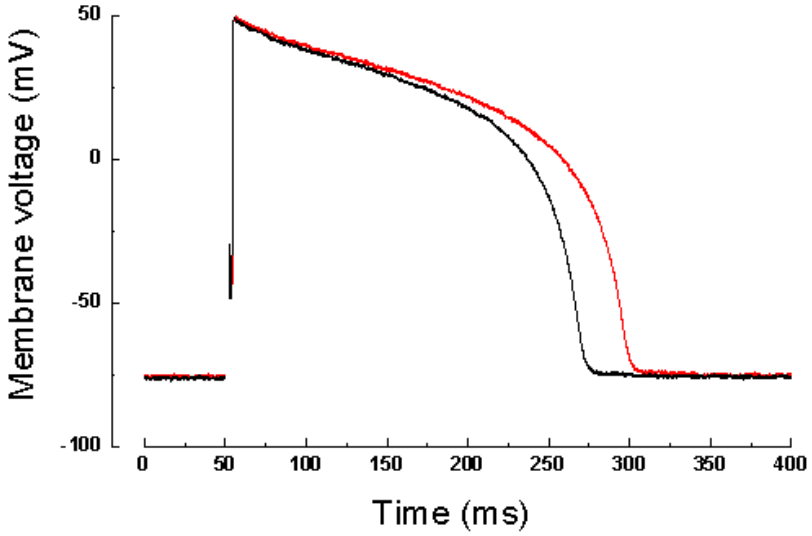
Stratégie

2/ Corréler l'expression transcriptionnelle au phénotype électrophysiologique





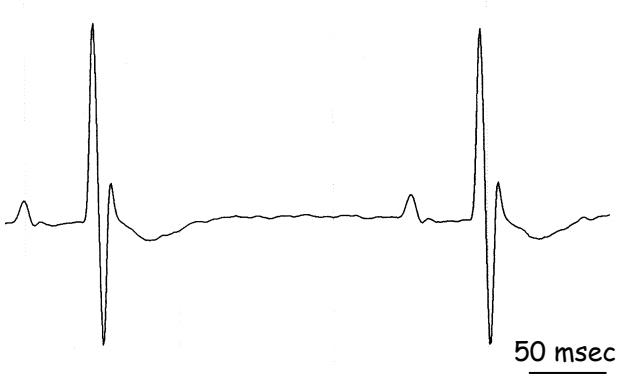




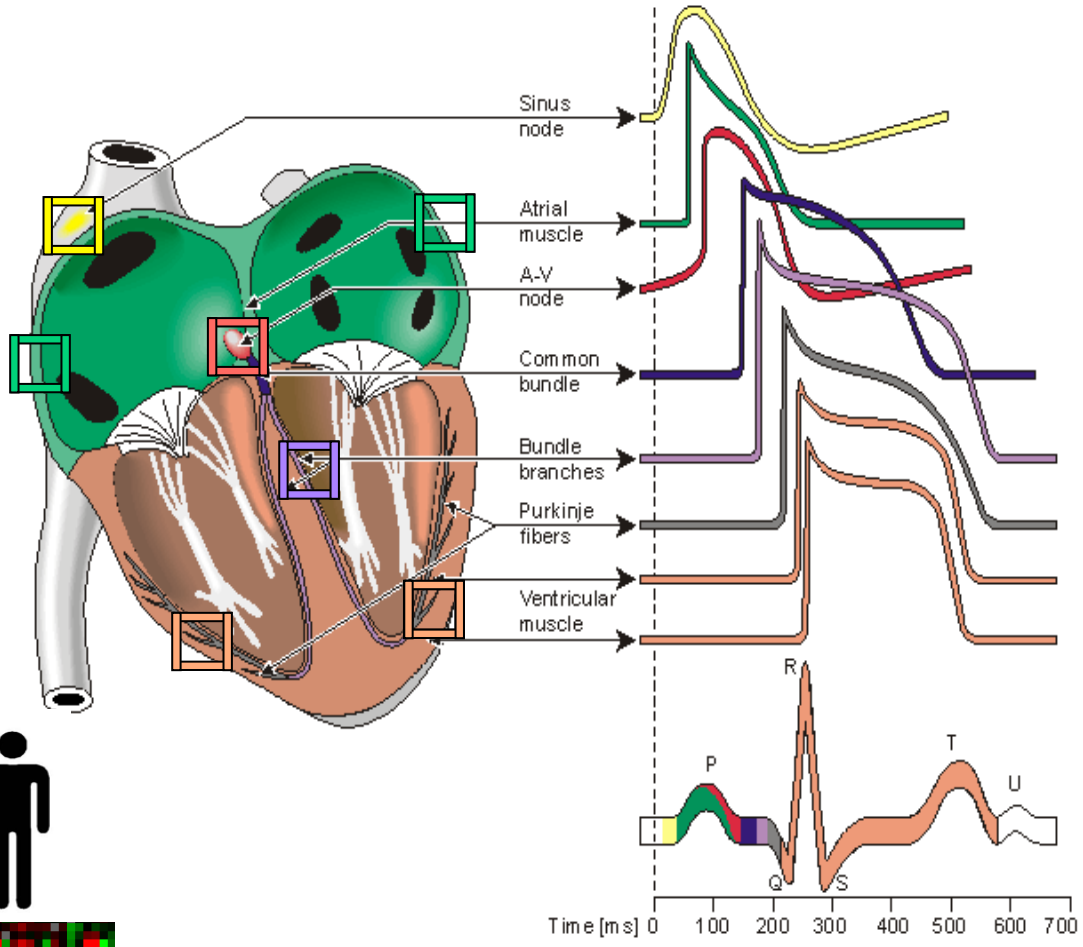




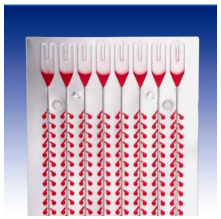


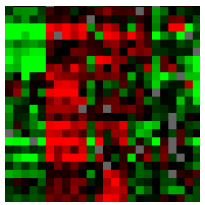


Application 1 : l'hétérogénéité électrique intrinsèque du cœur et la génomique des canaux ioniques

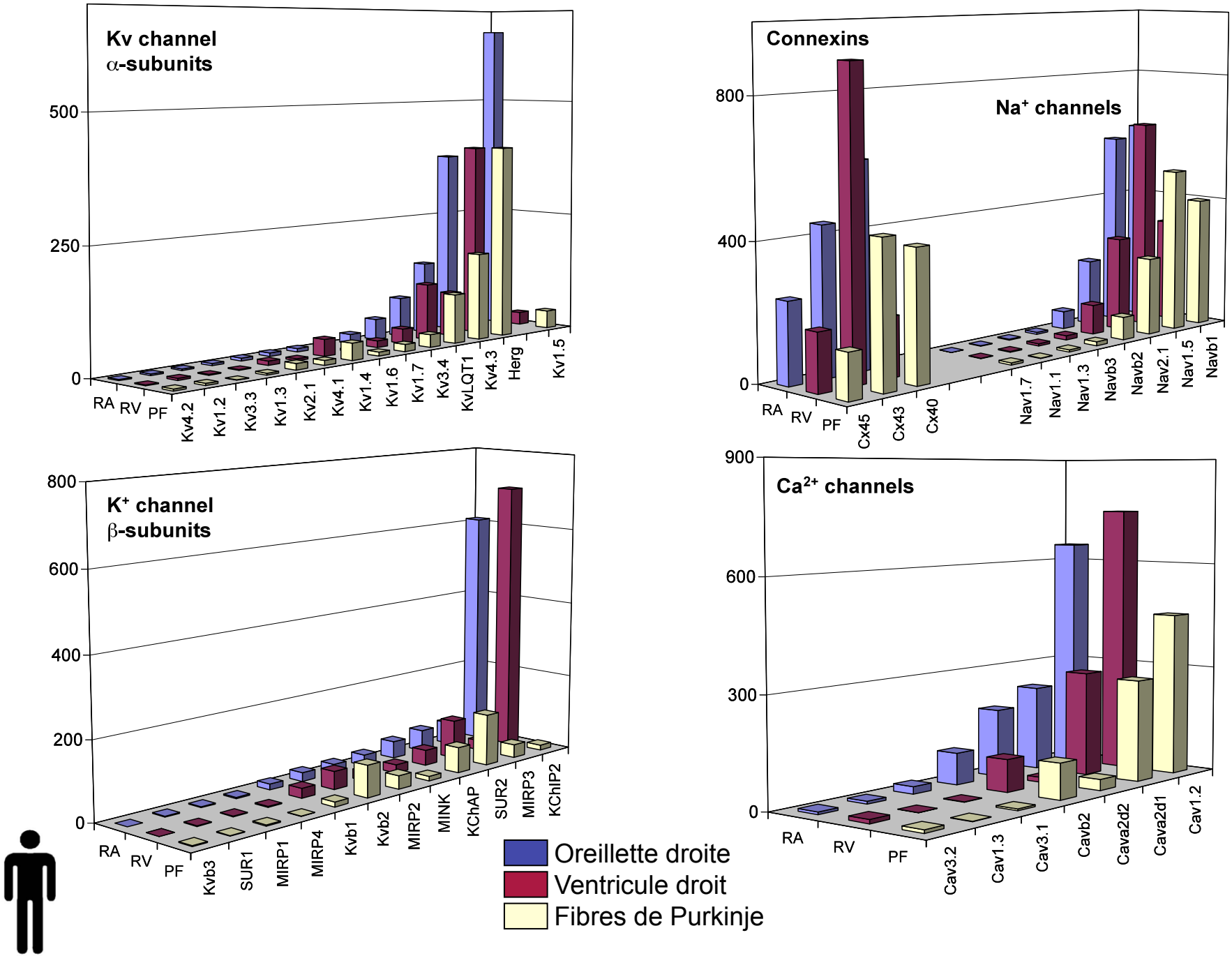


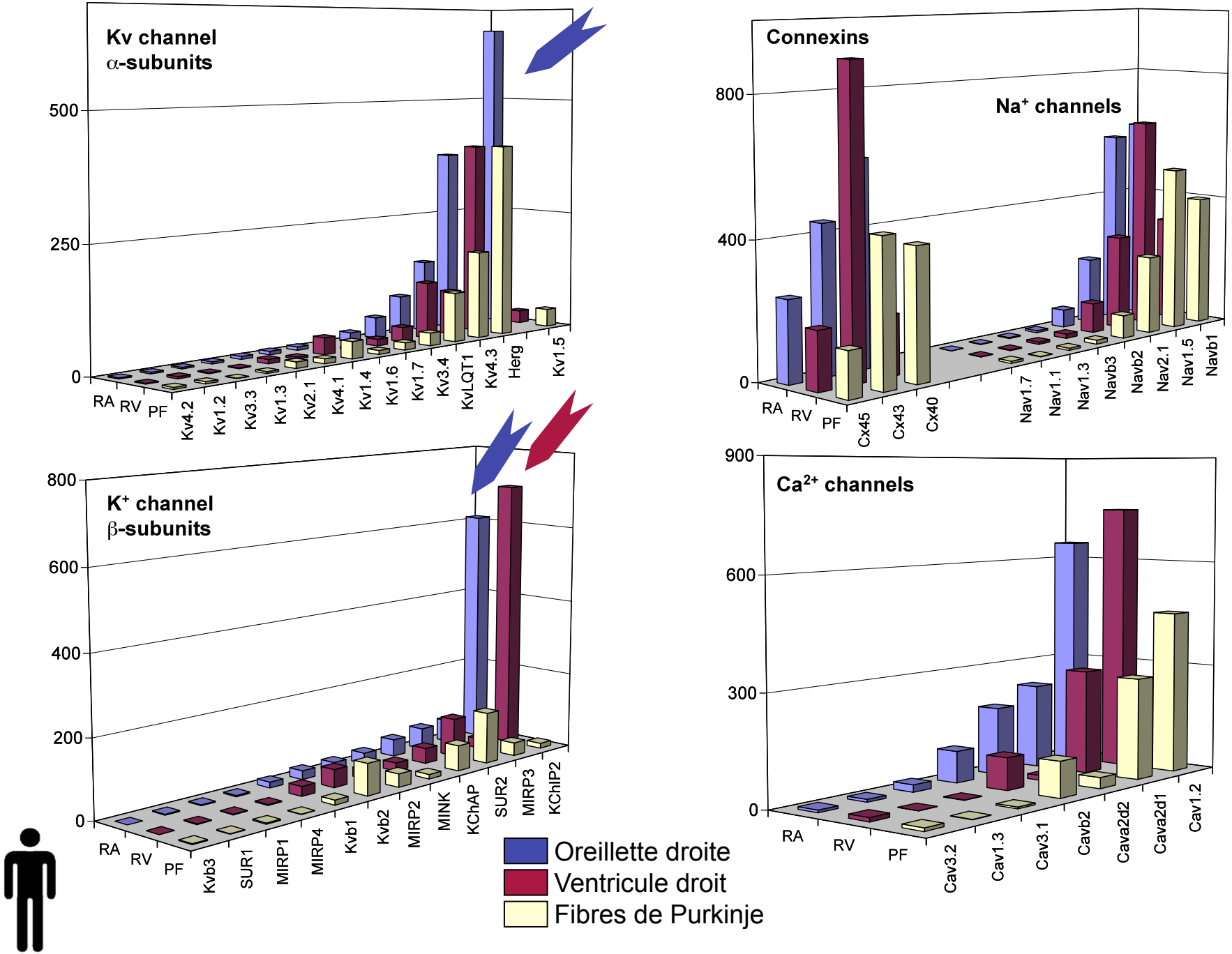


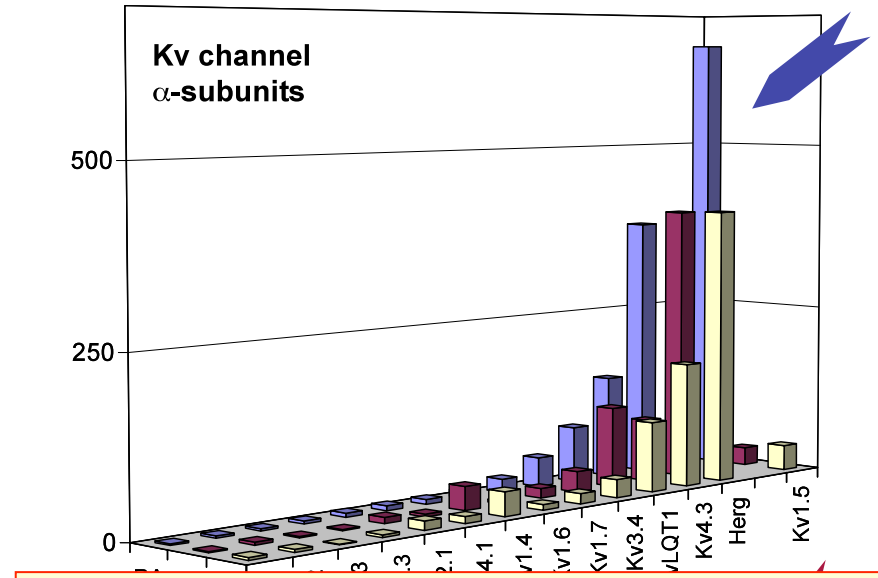


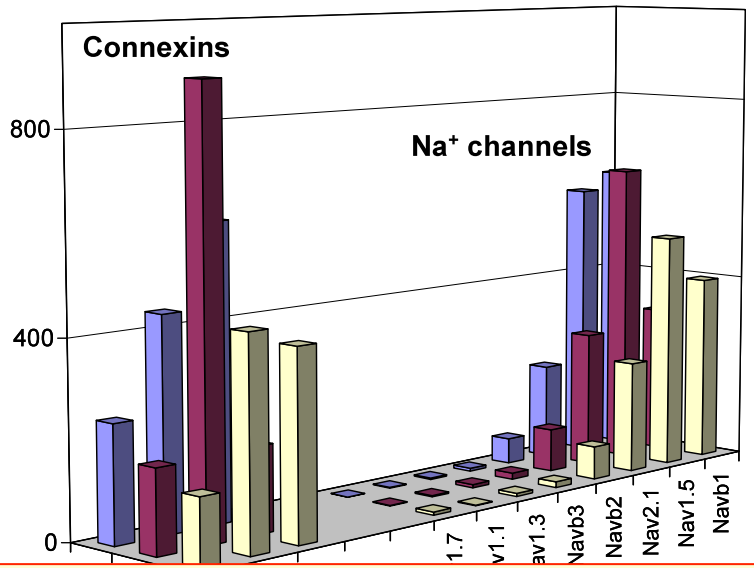


Marionneau C,…, Demolombe S. *J Physiol.* 2005.
Gaborit N,…, Demolombe S. *J. Physiol.* 2007.

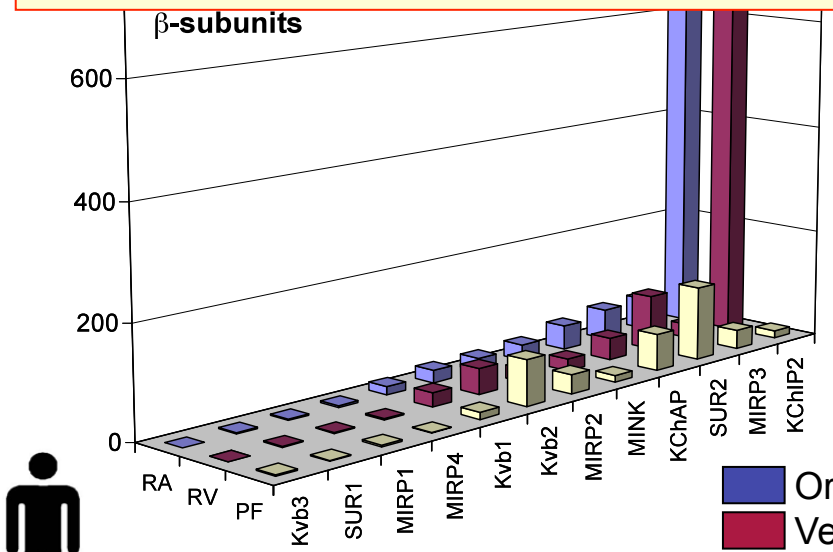

Kv channel
α-subunits
500
250
0
RA RV PF Kv4.2 Kv1.2 Kv3.3 Kv1.3 Kv2.1 Kv4.1 Kv1.4 Kv1.6 Kv1.7 Kv3.4 KvLQT1 Kv4.3 Herg Kv1.5
Connexins
Na+ channels
800
400
0
RA RV PF Cx45 Cx43 Cx40 Nav1.7 Nav1.1 Nav1.3 Navb3 Navb2 Nav2.1 Nav1.5 Navb1
K+ channel
β-subunits
800
600
400
200
0
RA RV PF Kvb3 SUR1 MIRP1 MIRP4 Kvb1 Kvb2 MIRP2 MINK KChAP SUR2 MIRP3 KChIP2
Ca2+ channels
900
600
300
0
RA RV PF Cav3.2 Cav1.3 Cav3.1 Cavb2 Cava2d2 Cava2d1 Cav1.2
Oreillette droite
Ventricule droit
Fibres de Purkinje

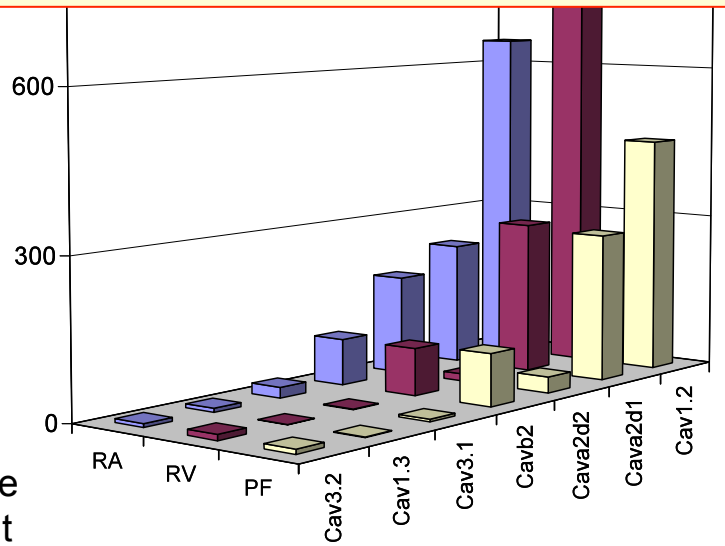

Kv channel
α-subunits
500
250
0
RA RV PF Kv4.2 Kv1.2 Kv3.3 Kv1.3 Kv2.1 Kv4.1 Kv1.4 Kv1.6 Kv1.7 Kv3.4 KvLQT1 Kv4.3 Herg Kv1.5
Connexins
Na+ channels
800
400
0
RA RV PF Cx45 Cx43 Cx40 Nav1.7 Nav1.1 Nav1.3 Navb3 Navb2 Nav2.1 Nav1.5 Navb1
K+ channel
β-subunits
800
600
400
200
0
RA RV PF Kvb3 SUR1 MIRP1 MIRP4 Kvb1 Kvb2 MIRP2 MINK KChAP SUR2 MIRP3 KChIP2
Ca2+ channels
900
600
300
0
RA RV PF Cav3.2 Cav1.3 Cav3.1 Cavb2 Cava2d2 Cava2d1 Cav1.2
Oreillette droite
Ventricule droit
Fibres de Purkinje





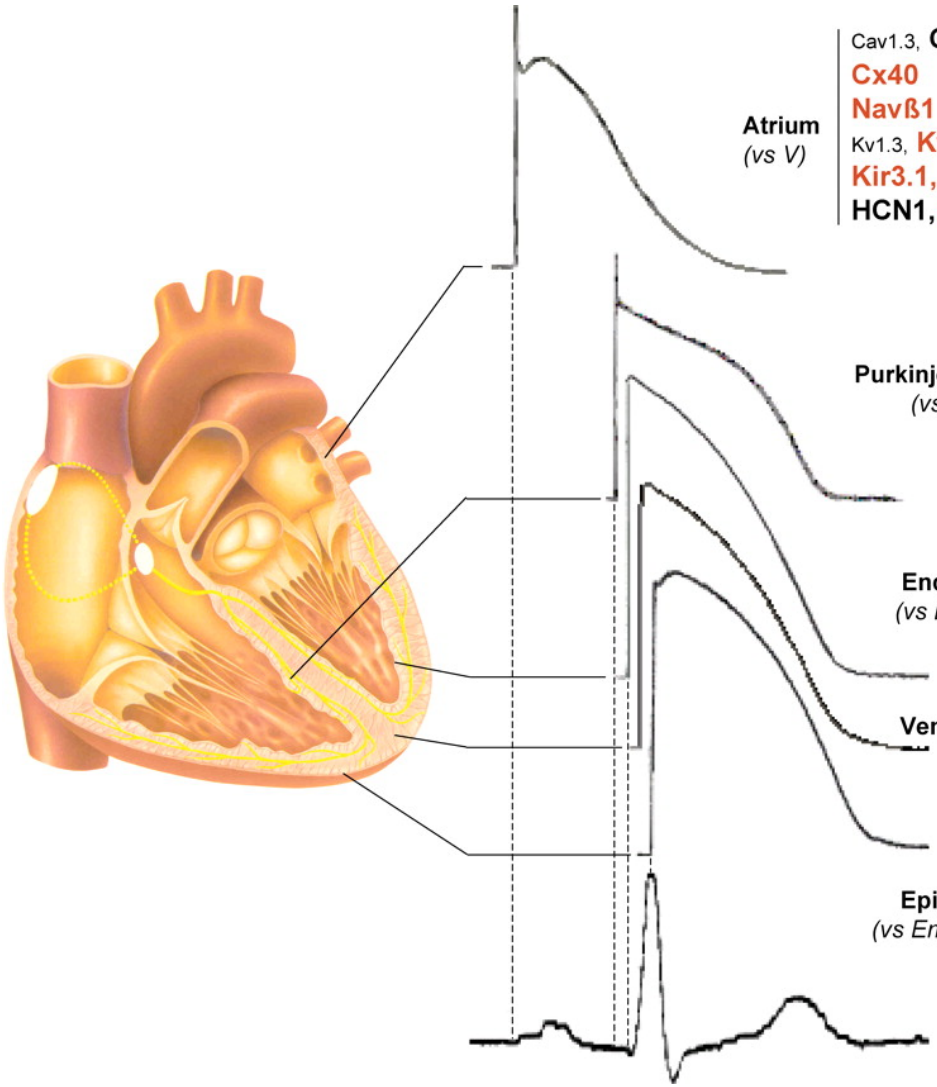
→ **Chaque région cardiaque a une signature transcriptionnelle qui assure sa spécificité électrogénique.**





Oreillette droite
Ventricule droit
Fibres de Purkinje

Portrait moléculaire de l'hétérogénéité électrique dans le cœur humain sain



Atrium *(vs V)*
Cav1.3, **Cav3.1, Cavα2δ2**
Cx40
Navß1
Kv1.3, **Kv1.5,** Kv1.6, Kv4.2, **Kv4.3,** SUR1, MIRP1, **MIRP3,** MIRP4
Kir3.1, Kir3.4, TWIK1, TASK1
HCN1, HCN4

Purkinje Fibers *(vs V)*
Cav1.3, Cav3.1, **Cavα2δ2**
Cx40
Nav1.7
Kv1.3, Kv1.6, **Kv4.3,** Kvß3, MIRP1, **KChAP**
Kir3.1, TWIK1, TASK1, TASK2
HCN1, HCN3, **HCN4**
ClC2, **ClC6**
CALM1, InsP3R1, InsP3R3

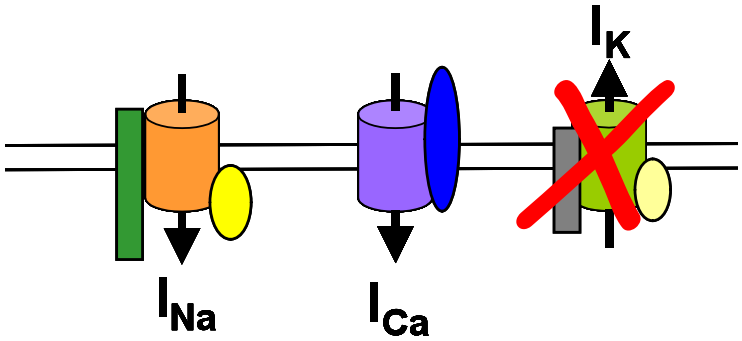
Endocardium *(vs Epicardium)*
Nav1.5, Navß1
Kv1.4
Kir3.1

Ventricle *(vs A)*
Kv1.4
Kir2.1
PLB, RYR2
(vs PF)
Cav1.2
Cx43
MiRP4, **KCHIP2**
Kir2.1,2.3, Kir6.1,6.2
NKAα3, NCX1
SERCA2, CASQ2, RYR2

Epicardium *(vs Endocardium)*
Cav1.2
Kv1.2, Kv1.3, **KChIP2**
SERCA2, CALM3, Calcineurin-α

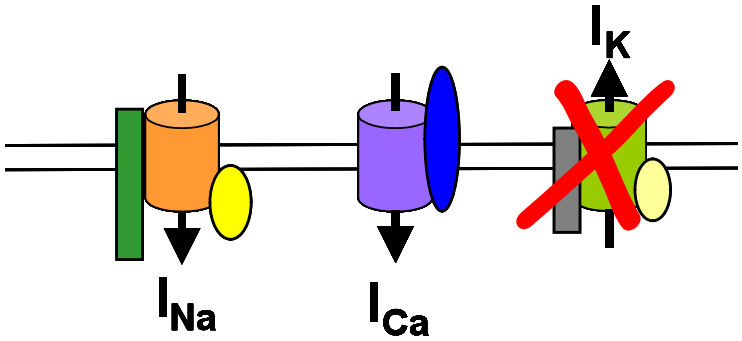
Gaborit N,…, Demolombe S. *J. Physiol.* 2007.

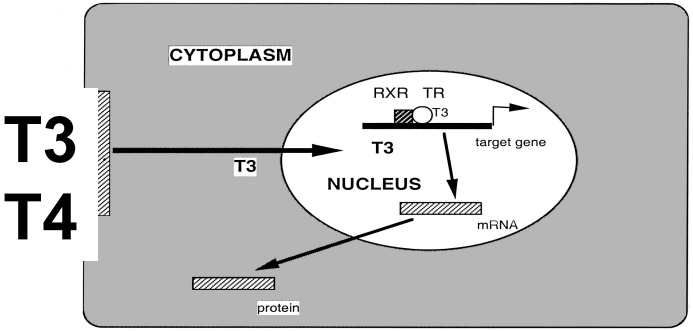
Application 2 : l'antiarrhythmique amiodarone et la génomique des canaux ioniques



Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

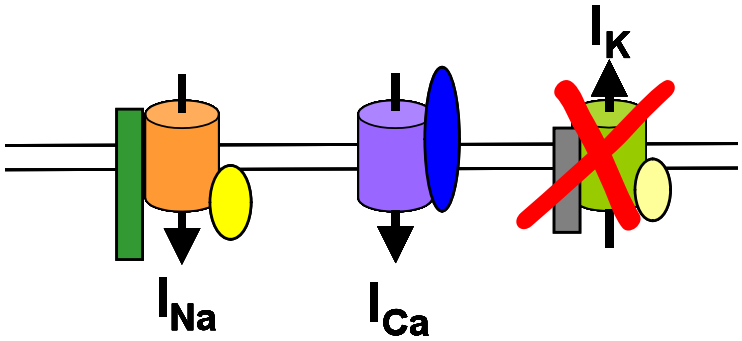
Application 2 : l'antiarrhythmique amiodarone et la génomique des canaux ioniques

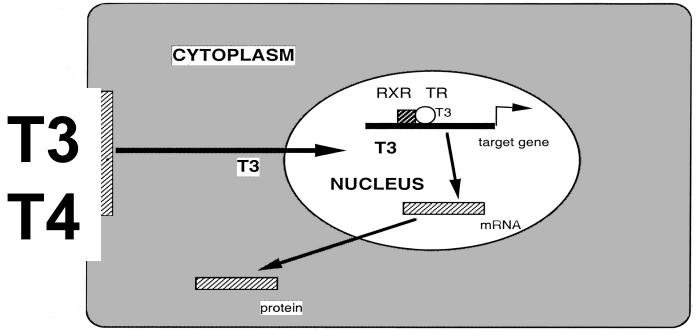


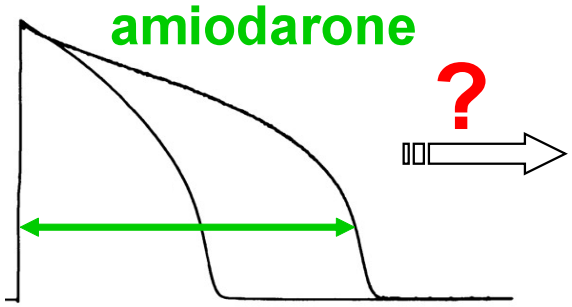


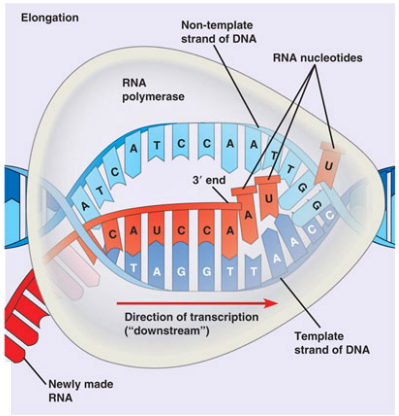
Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

Application 2 : l'antiarrhythmique amiodarone et la génomique des canaux ioniques



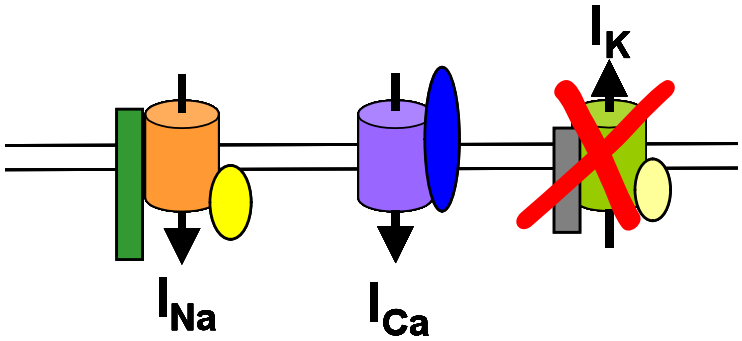


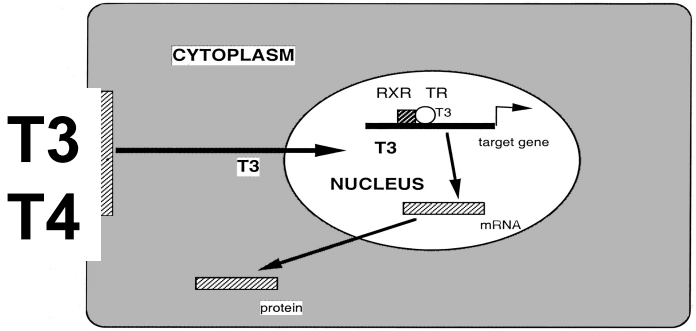


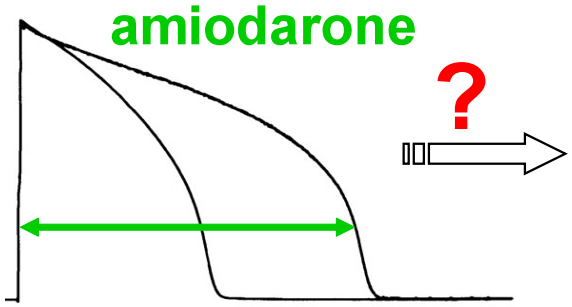


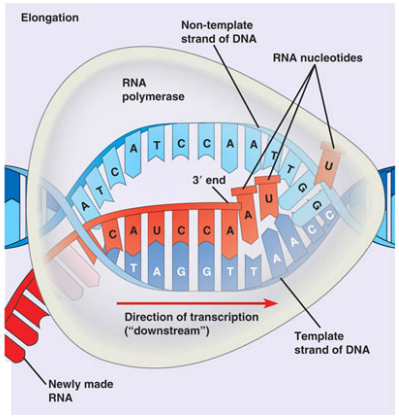
Transcrits des canaux ioniques

Application 2 : l'antiarrhythmique amiodarone et la génomique des canaux ioniques









Transcrits des canaux ioniques



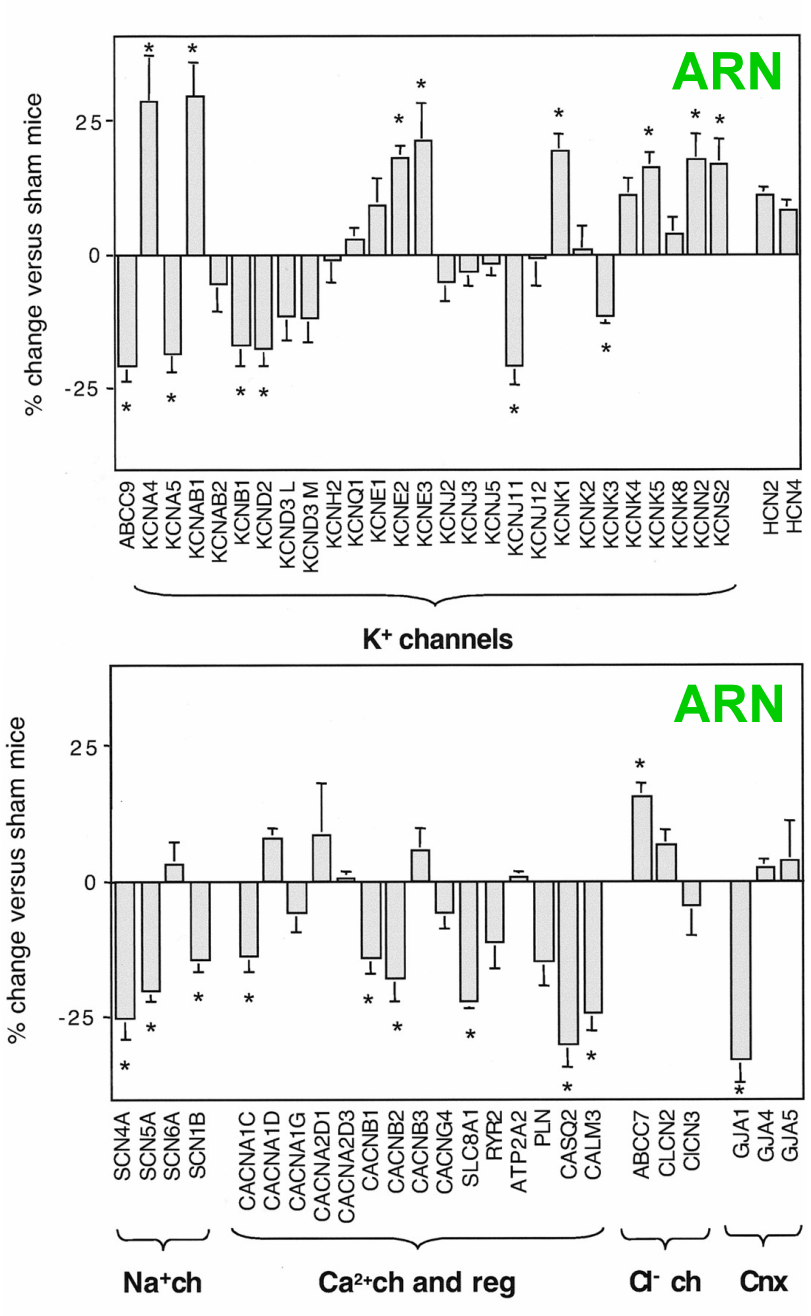
Souris adultes mâles C57BL/6

Amiodarone 30, 90, 180 mg/kg/jour pendant 5 semaines

Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.

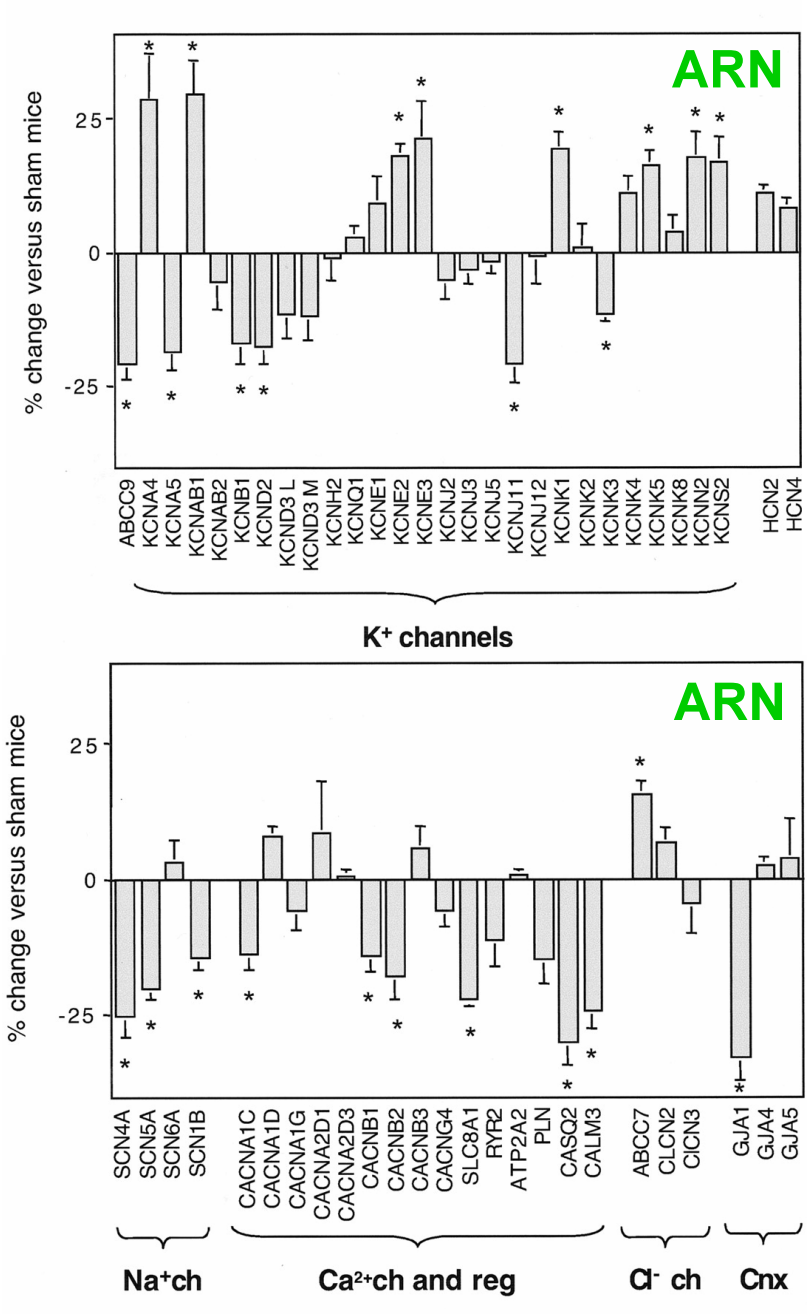
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

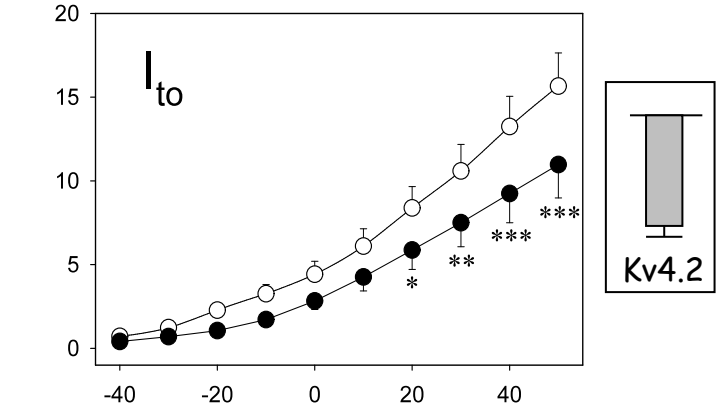
Expression des canaux ioniques et amiodarone

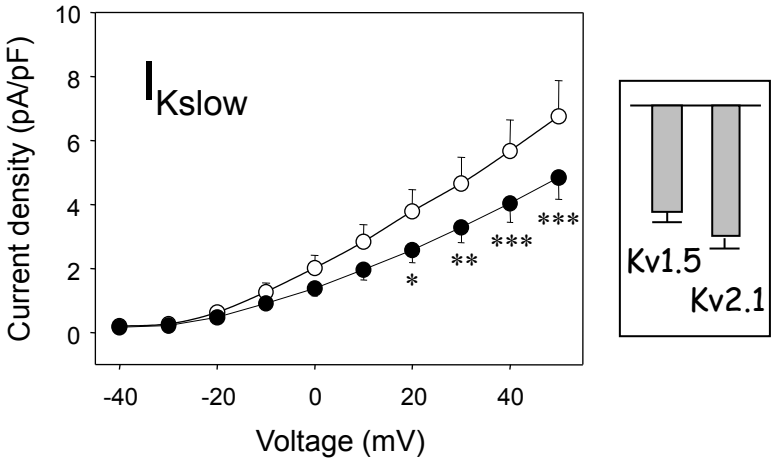


Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

Expression des canaux ioniques et corrélations fonctionnelles

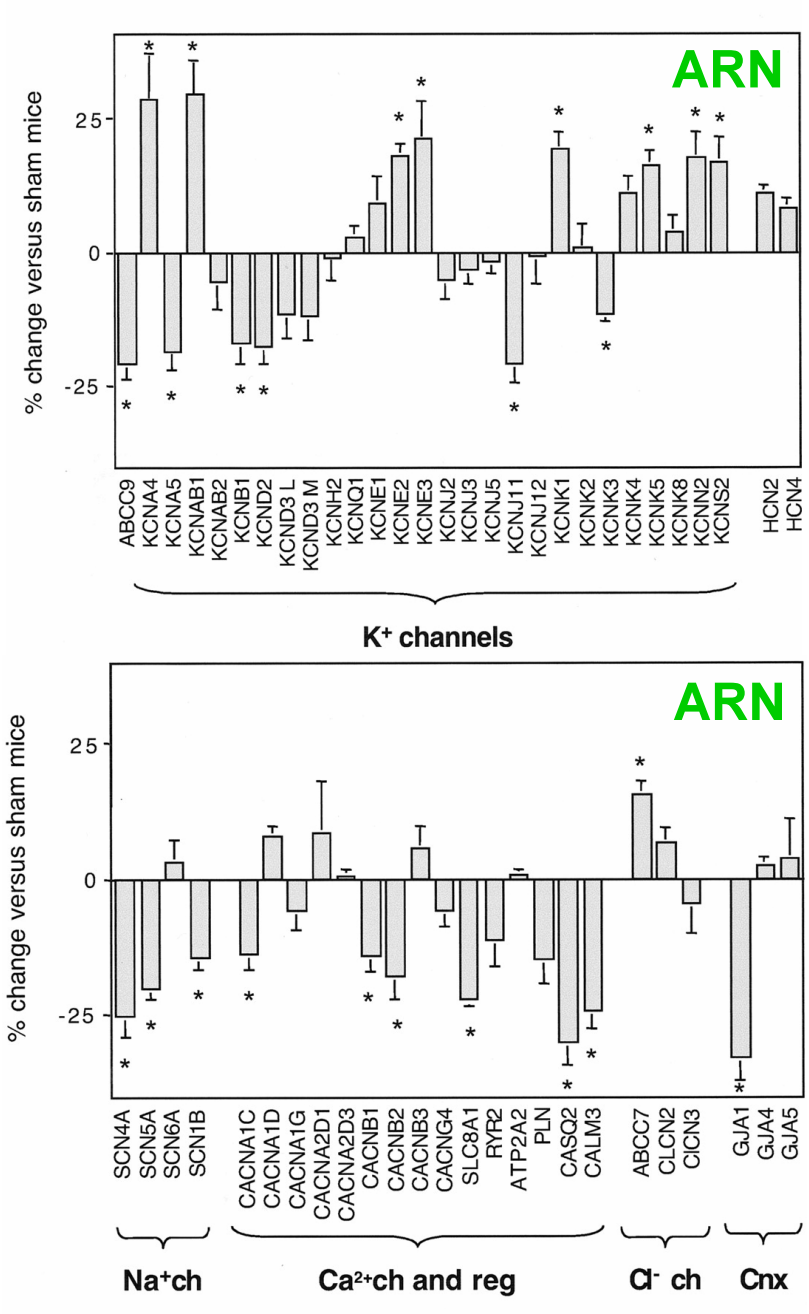


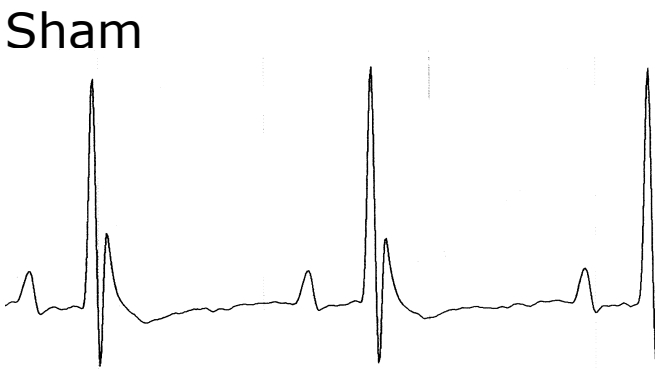


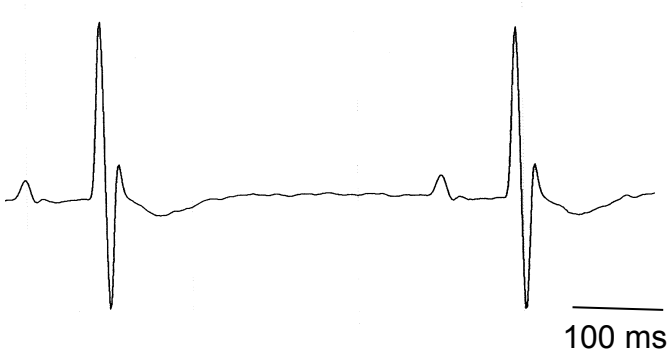


Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

Expression des canaux ioniques et corrélations fonctionnelles

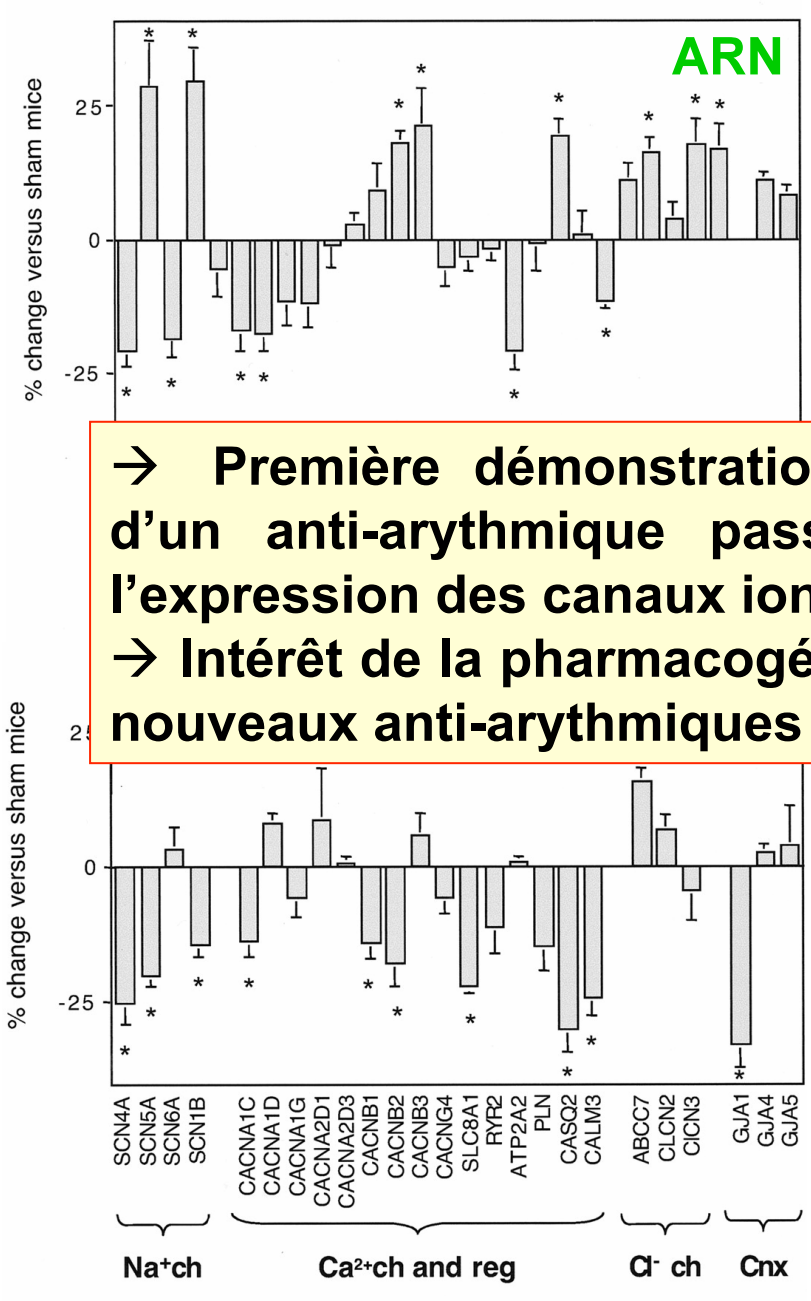


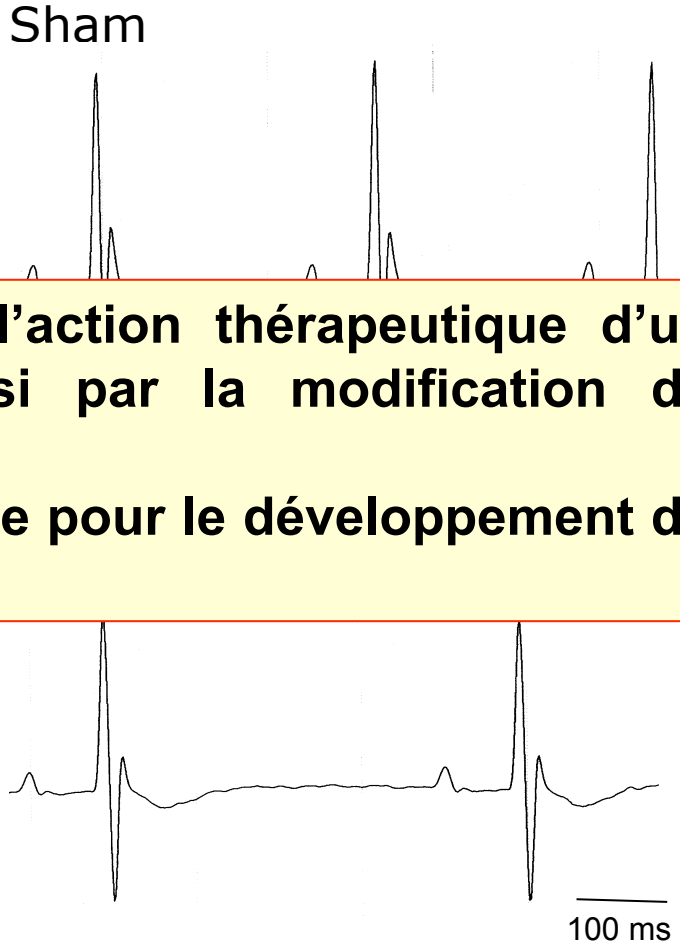




Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

Le remodelage induit par l'amiodarone



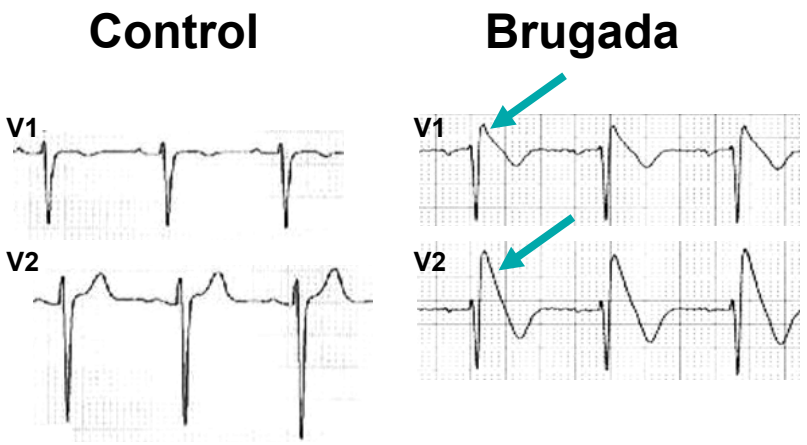


→ Première démonstration que l'action thérapeutique d'un d'un anti-arythmique passe aussi par la modification de l'expression des canaux ioniques

→ Intérêt de la pharmacogénomique pour le développement de nouveaux anti-arythmiques

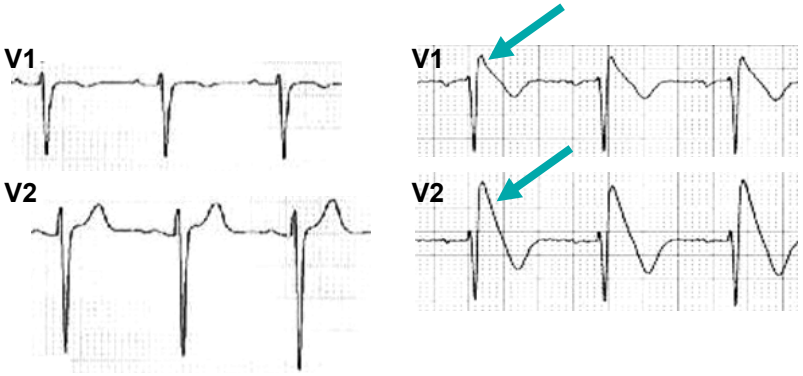
Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,…, Demolombe S. *Circulation*. 2004.

Application 3: Le remodelage ionique et le syndrome de Brugada

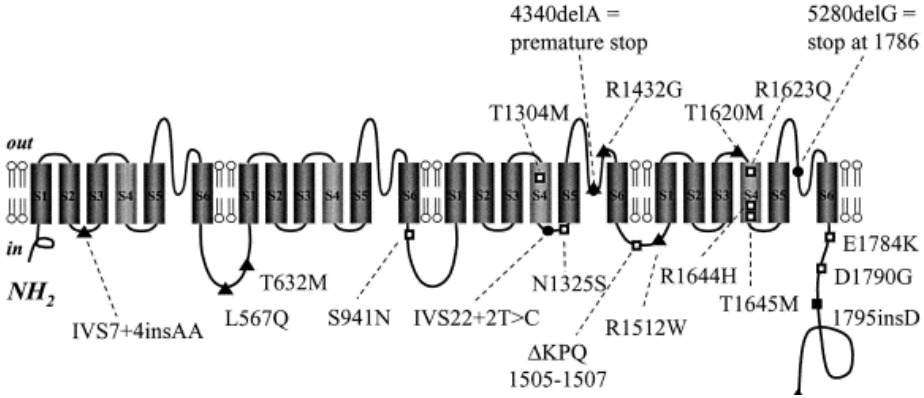


Application 3: Le remodelage ionique et le syndrome de Brugada

Control **Brugada**

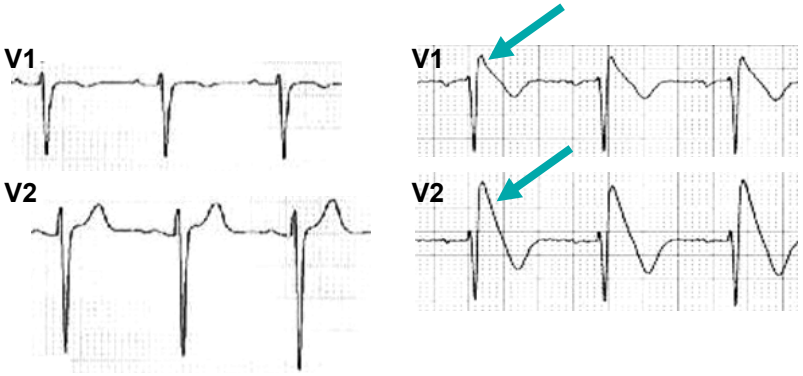


SCN5A (Nav1.5) mutations

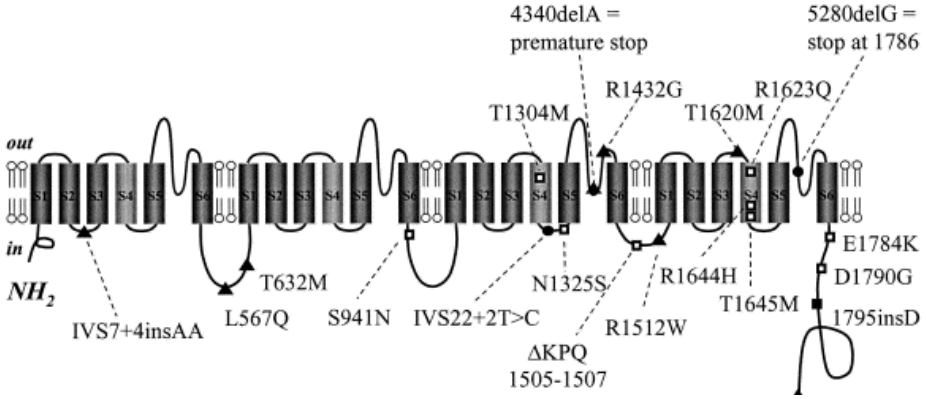


Application 3: Le remodelage ionique et le syndrome de Brugada

Control **Brugada**



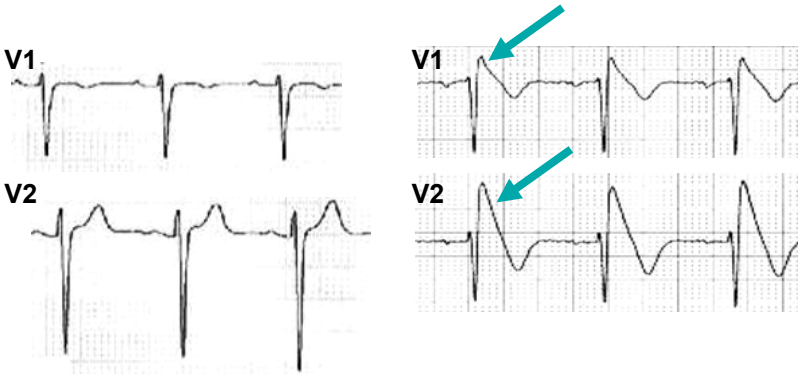
SCN5A (Nav1.5) mutations



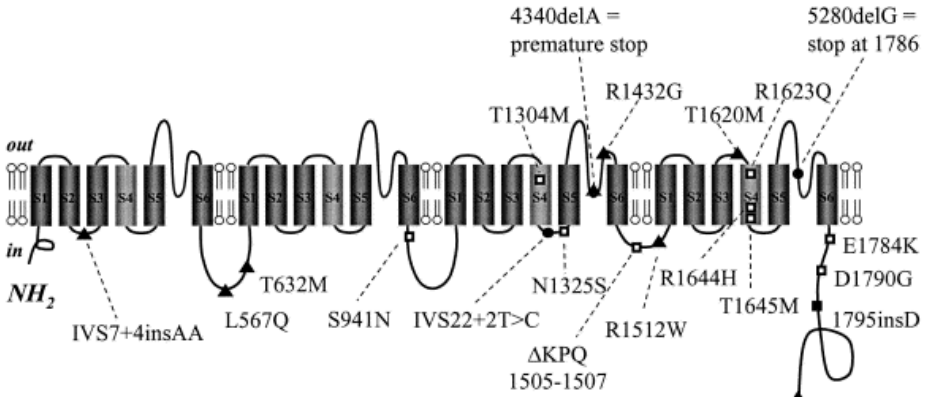
Only 20% of the patients are carrying a mutation in _SCN5A_

Application 3: Le remodelage ionique et le syndrome de Brugada

Control **Brugada**



***SCN5A* (Nav1.5) mutations**



Only 20% of the patients are carrying a mutation in *SCN5A*

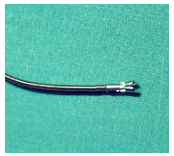
Hypothesis: alternative disease mechanism/determinants:

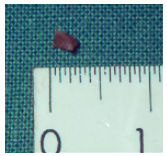
Brugada patients have in common a specific ion channel expression pattern with or without a *SCN5A* mutation.

METHODS

✓ Biological samples

Catheter protocol biopsies



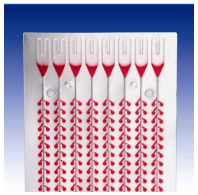


- 11 Brugada patients : 5 harboring *SCN5A* mutation, 6 non-harboring
- 7 Controls: early cardiac transplanted (Ctl-2)
- 10 arrhythmogenic right ventricular cardiomyopathy (ARVC)
- 9 idiopathic right ventricular outflow-tract tachycardia (RVOT)

Scalpel biopsies

- 11 Controls: organ donors (Ctl-1)

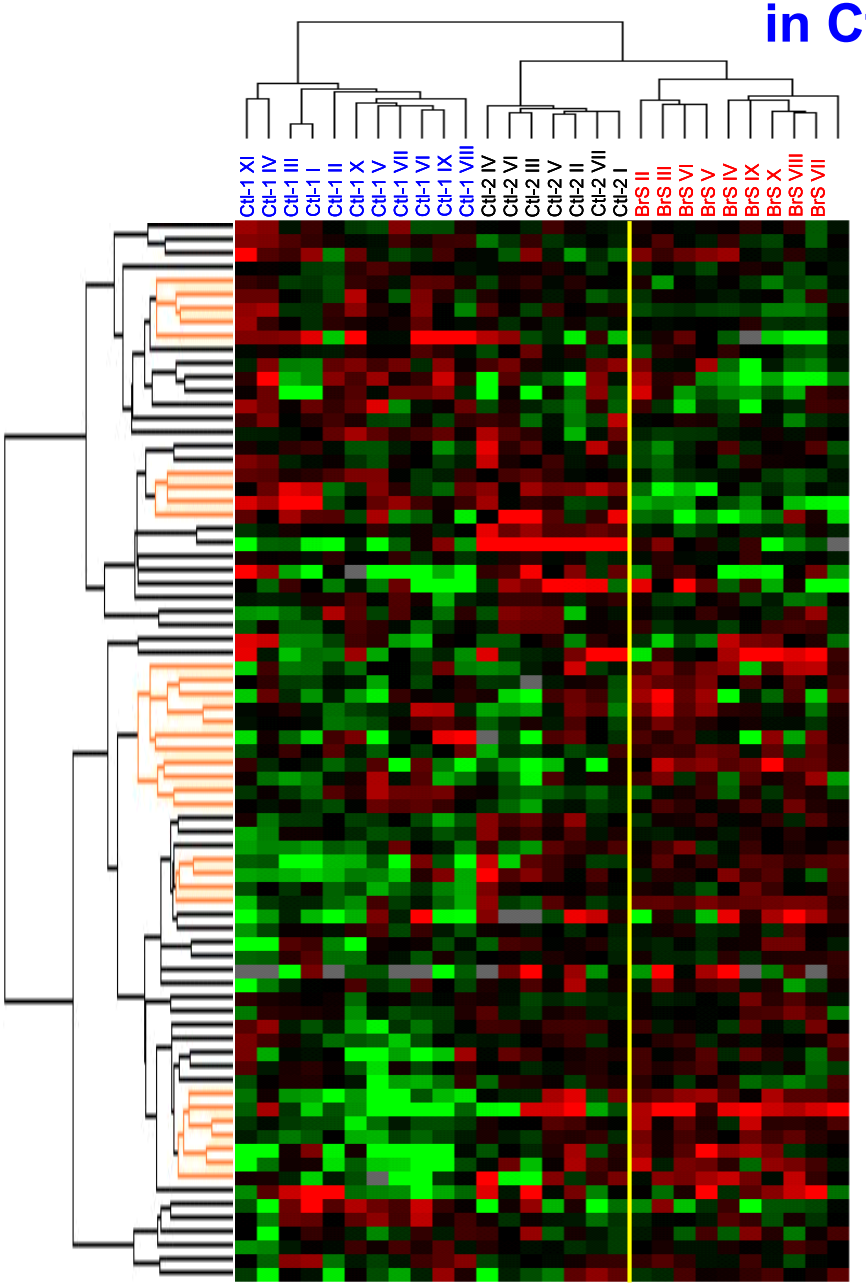
✓ High-throughput real-time PCR

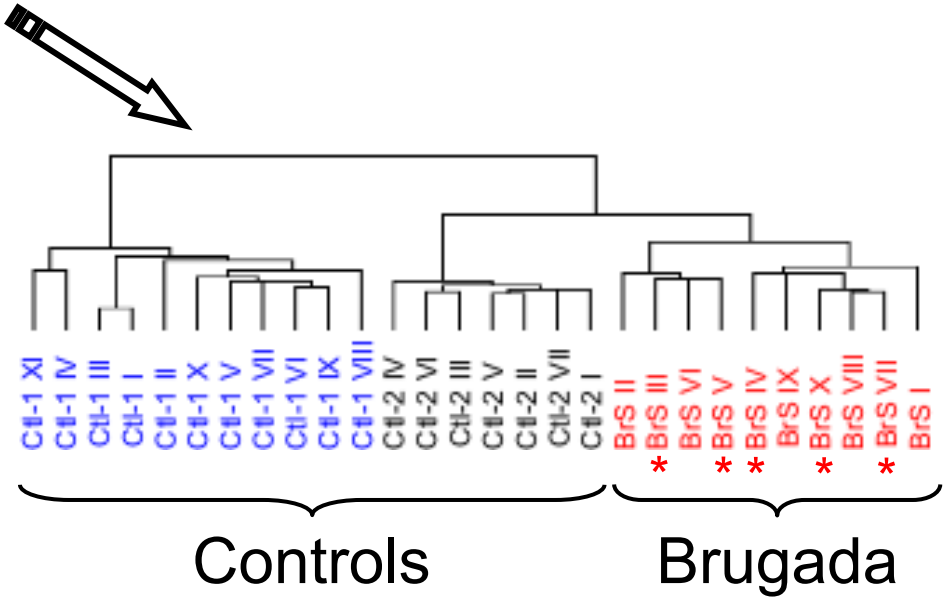


- 67 α- and β-ion channel subunits
- 17 Na/K-ATPase isoforms and proteins involved in Ca^{2+}-homeostasis
- 8 markers (ANF, BNP, β-MHC, CD4, CNN1, COL6A1, IL6, UCHL1)
- 4 reference genes

Gaborit N,…, Demolombe S. *Eur Heart J*. 2009.

Global analysis of ion-channel expression profiles in Ctl-1, Ctl-2 and BrS patients





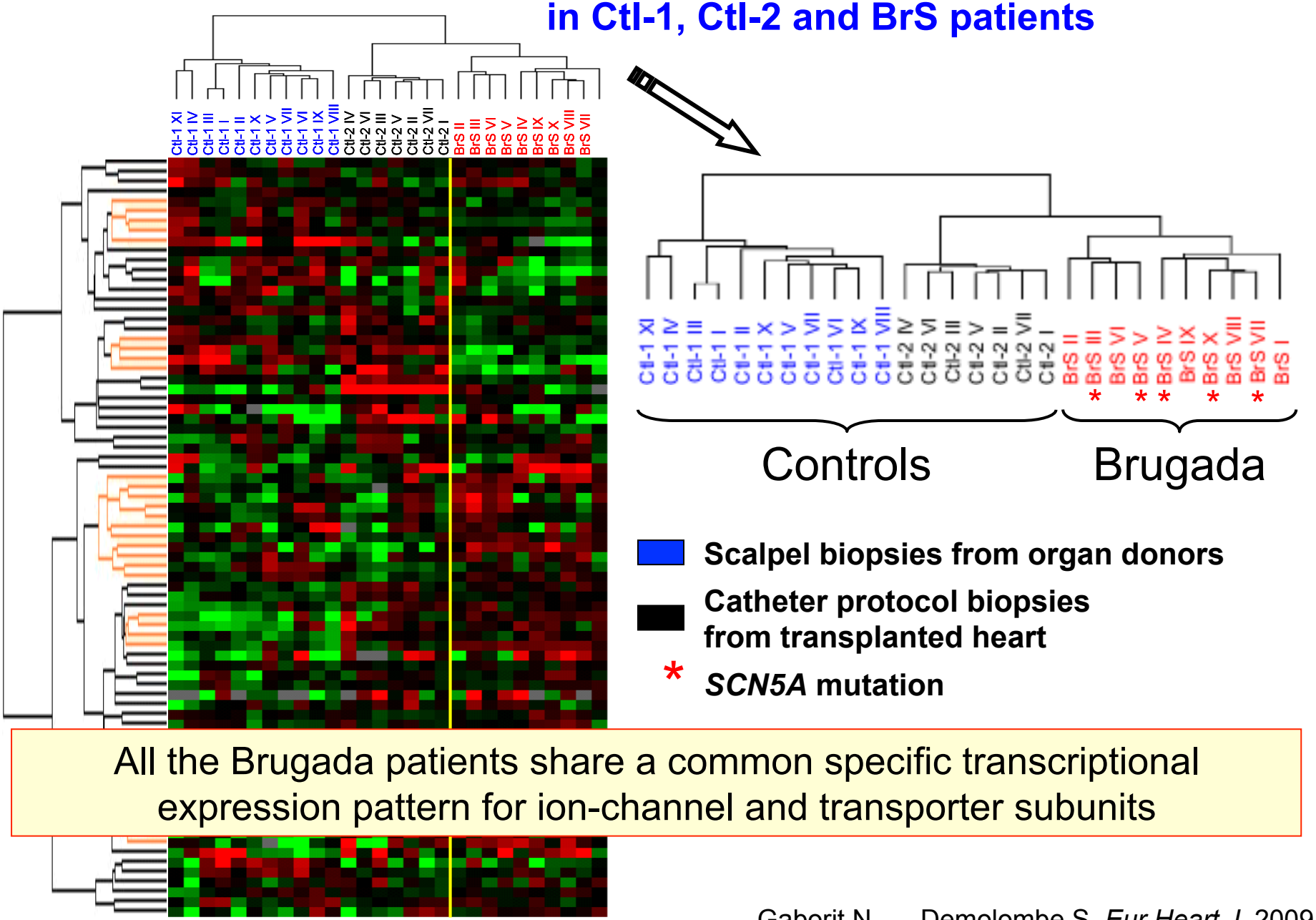
Scalpel biopsies from organ donors

Catheter protocol biopsies from transplanted heart

* ***SCN5A* mutation**

Gaborit N,…, Demolombe S. *Eur Heart J*. 2009.

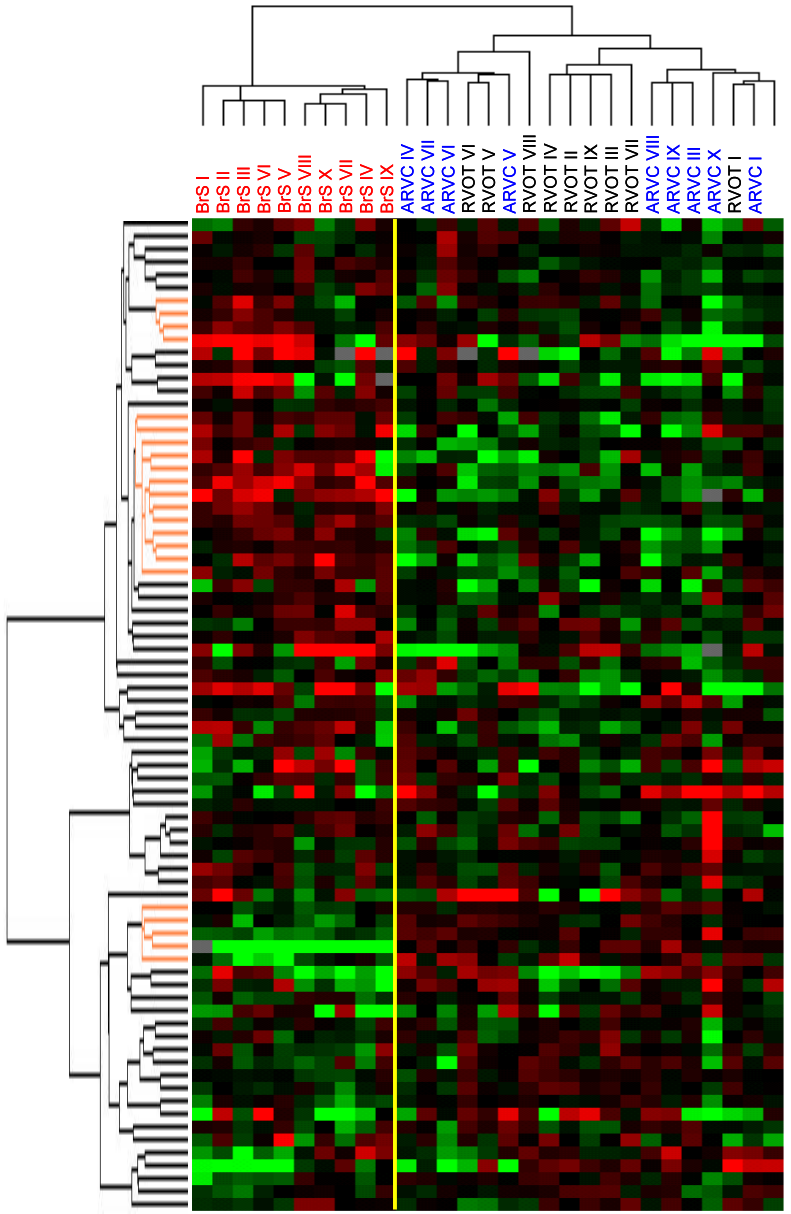
Global analysis of ion-channel expression profiles in Ctl-1, Ctl-2 and BrS patients

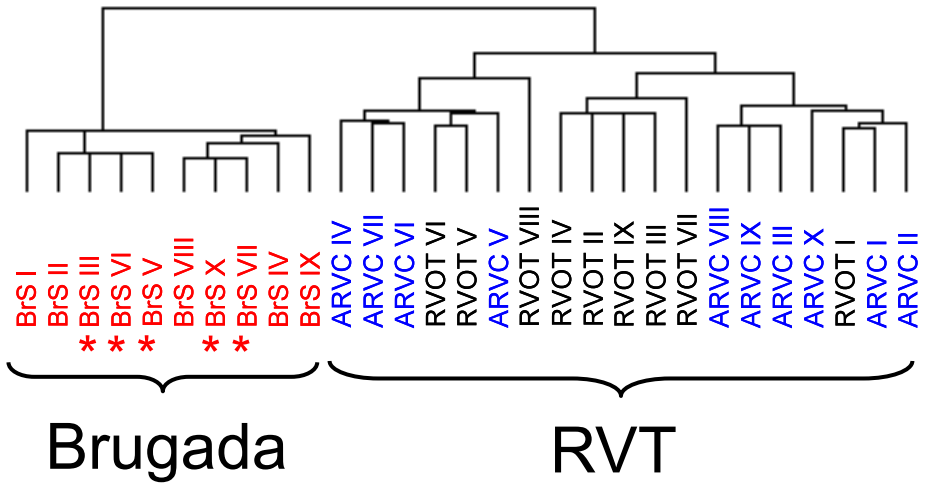


All the Brugada patients share a common specific transcriptional expression pattern for ion-channel and transporter subunits

Gaborit N,…, Demolombe S. *Eur Heart J*. 2009.

Global analysis of ion-channel expression profiles in ARVC, RVOT and BrS patients





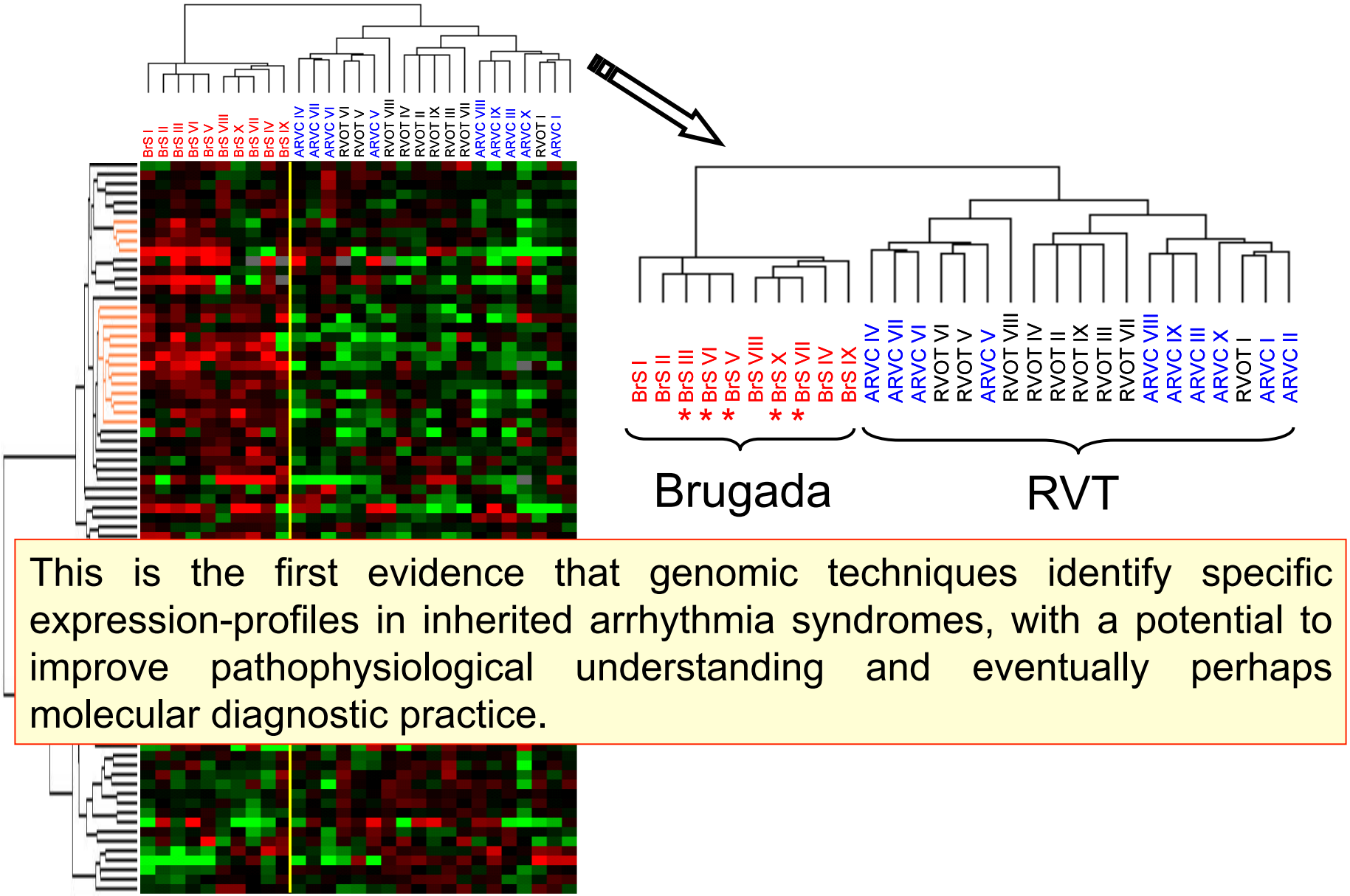
Arrhythmogenic right-ventricular cardiomyopathy patients

Idiopathic right-ventricular outflow-tract tachycardia patients

* ***SCN5A* mutation**

Gaborit N,…, Demolombe S. *Eur Heart J*. 2009.

Global analysis of ion-channel expression profiles in ARVC, RVOT and BrS patients

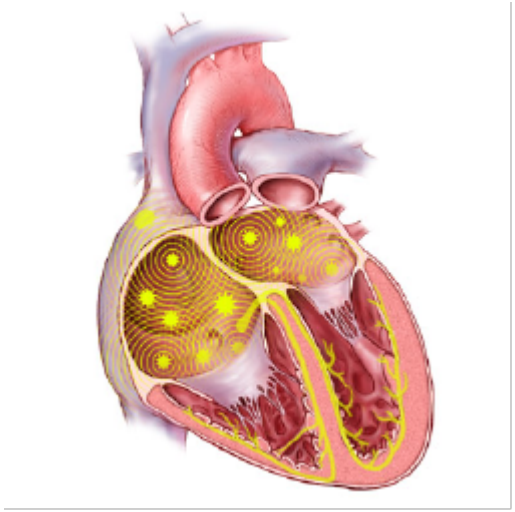


This is the first evidence that genomic techniques identify specific expression-profiles in inherited arrhythmia syndromes, with a potential to improve pathophysiological understanding and eventually perhaps molecular diagnostic practice.

l'institut du thorax

Application 4:

Ion-Channel Transcriptional Signature Associated with Atrial Fibrillation



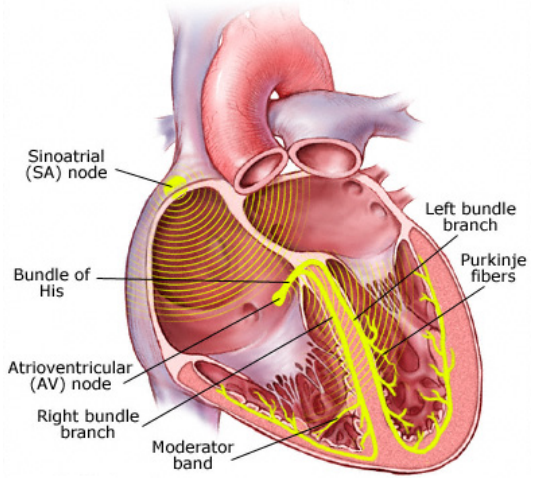
Gaborit et al., Circulation 2005;112:471-81
Lamirault G, Gaborit N, et al., J Mol Cell Cardiol. 2006;40:173-84

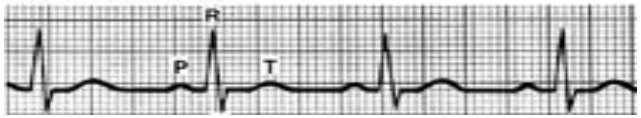
Inserm

l'institut du thorax

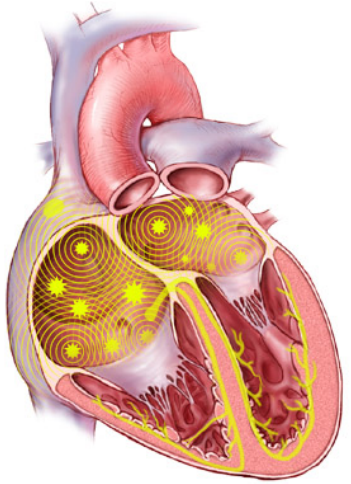
Atrial Fibrillation

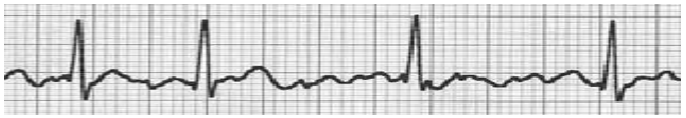
- ✓ *Most frequent cardiac arrhythmia (2 000 000 in USA)*
- ✓ *Occurrence of AF increases with age*
- ✓ *Rapid and irregular activation of the atrium (400-600/min)*





Sinus rythm





Atrial fibrillation

l'institut du thorax

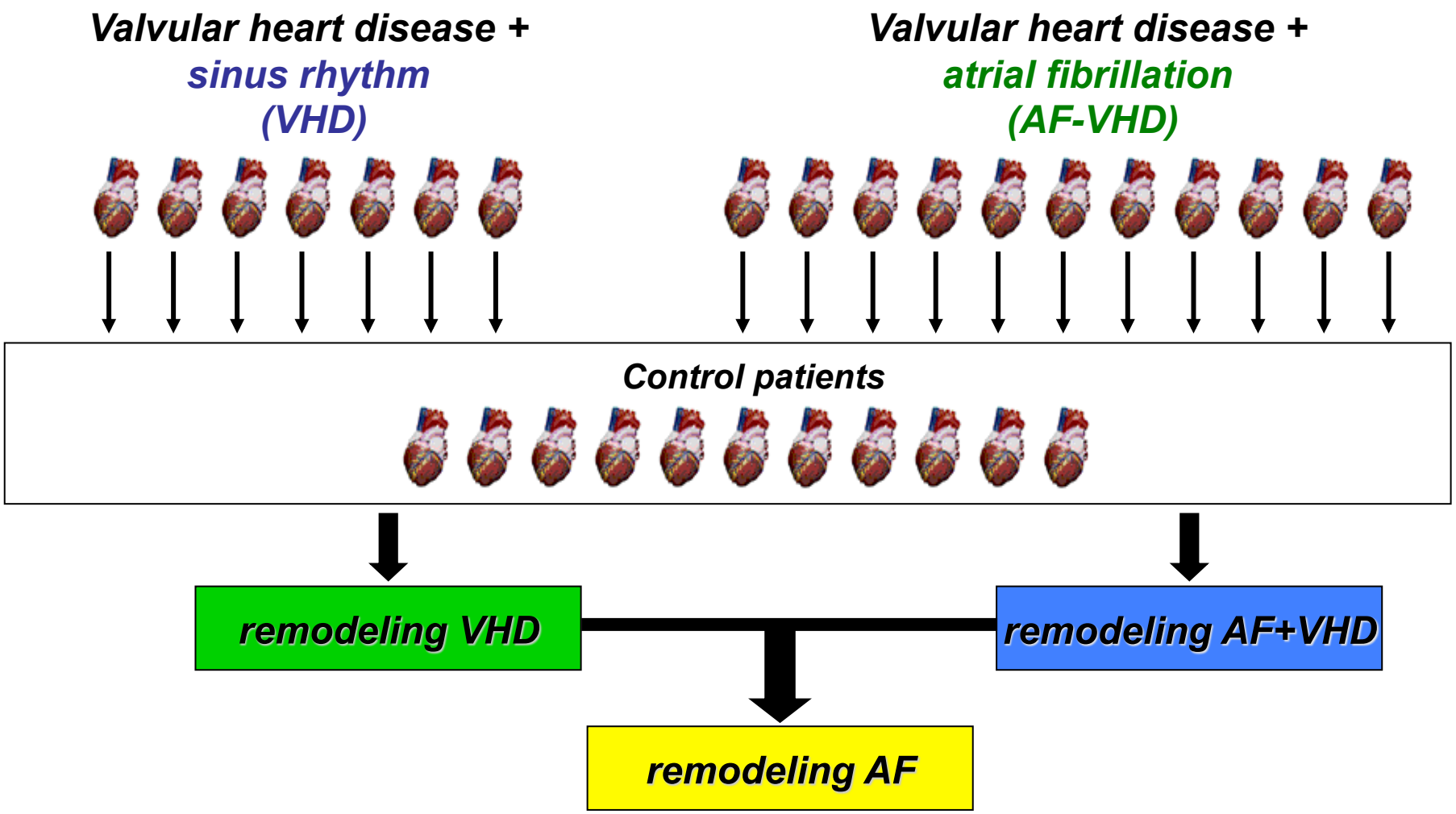
Atrial Fibrillation

- *Electrical remodeling plays an important role in the pathophysiology of AF*
- *Previous studies limited to small number of candidate subunits*
- *Valvular heart disease = frequent clinical cause of AF*
- *The independent contributions of AF and underlying heart disease not adequately addressed*

Inserm

l'institut du thorax

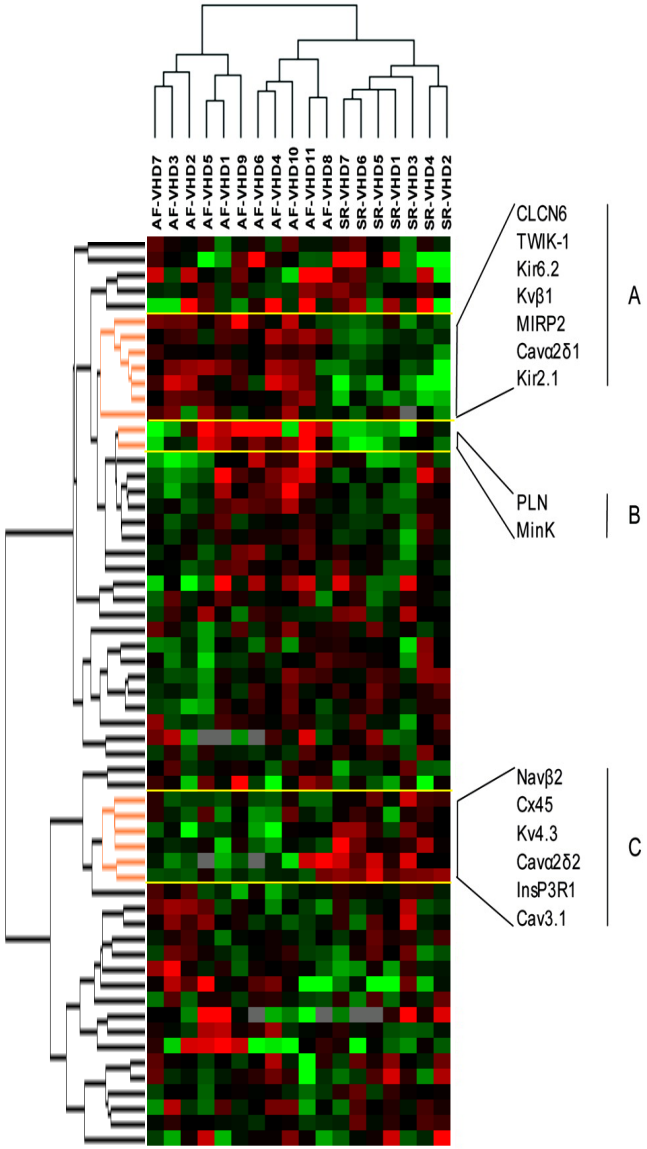
Study Design



Inserm

l'institut du thorax

Hierarchical Clustering

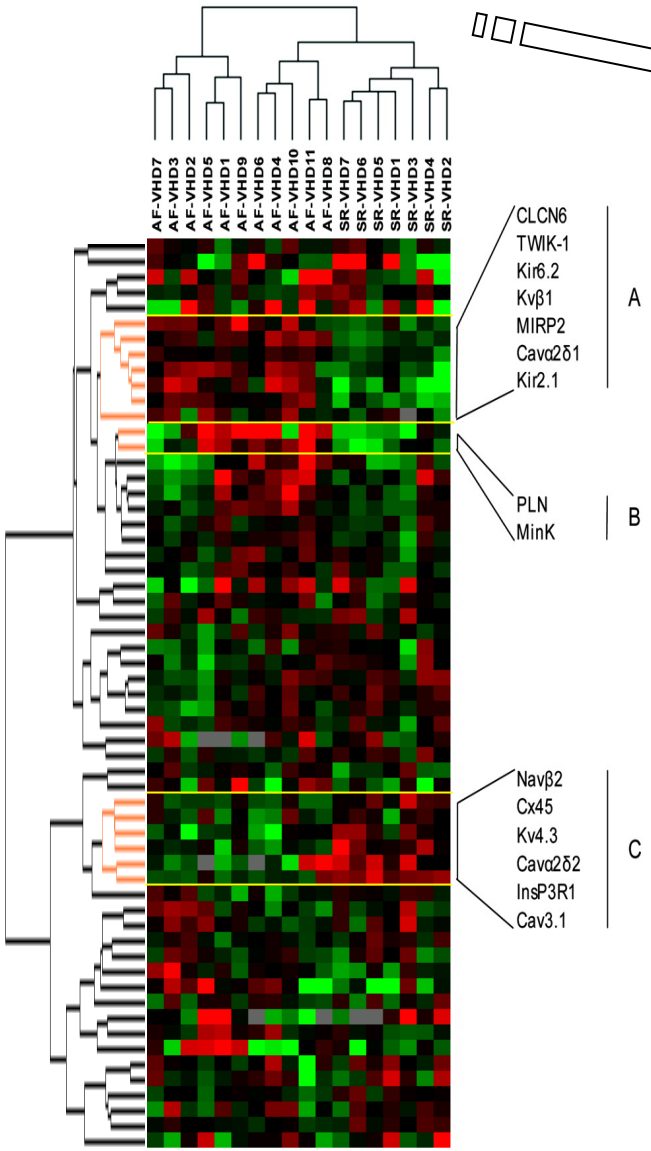


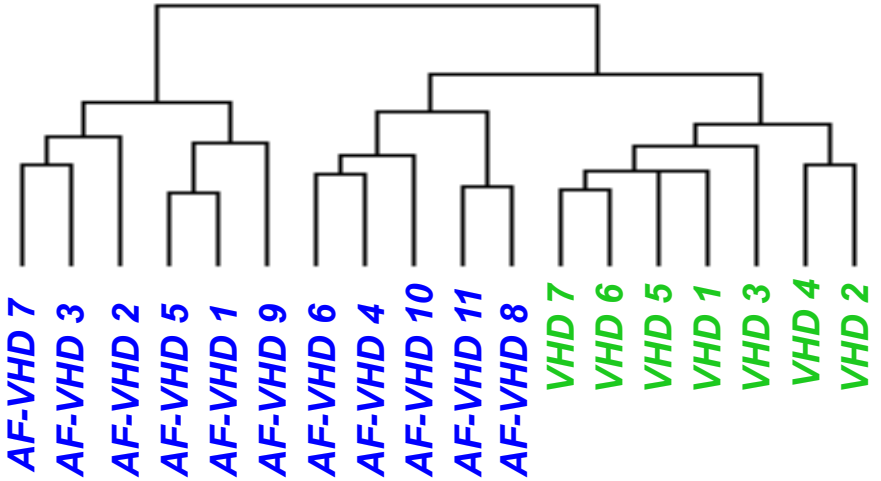
Gaborit et al., Circulation 2005;112:471-81

Inserm

l'institut du thorax

Hierarchical Clustering



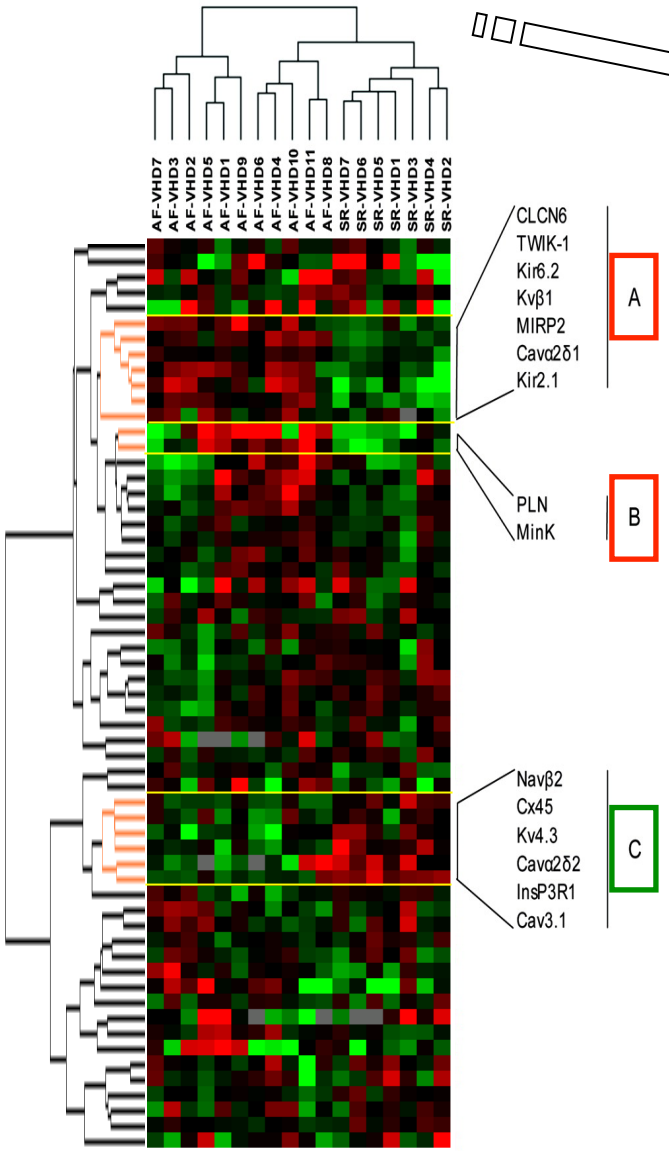


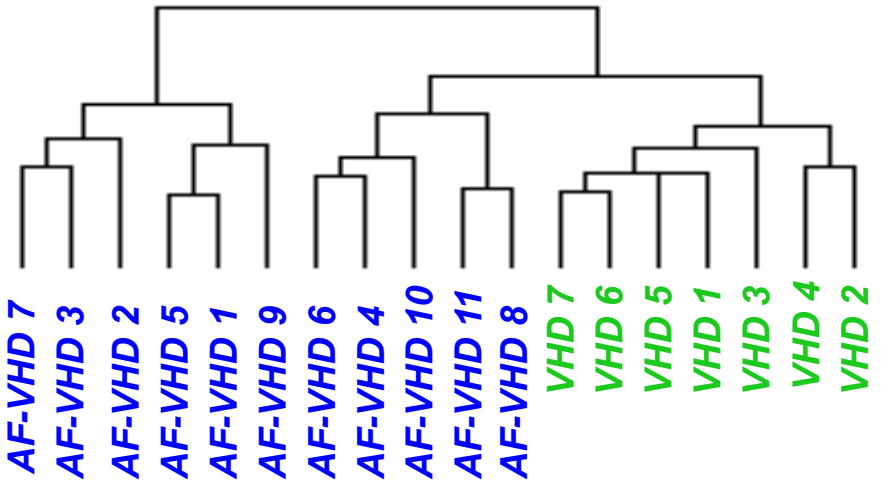
Gaborit et al., Circulation 2005;112:471-81

Inserm

l'institut du thorax

Hierarchical Clustering





- *Ion channel gene expression profile discriminates patients with AF-VHD from patients with VHD*

A and B : *ion channels up-regulated in AF-VHD*

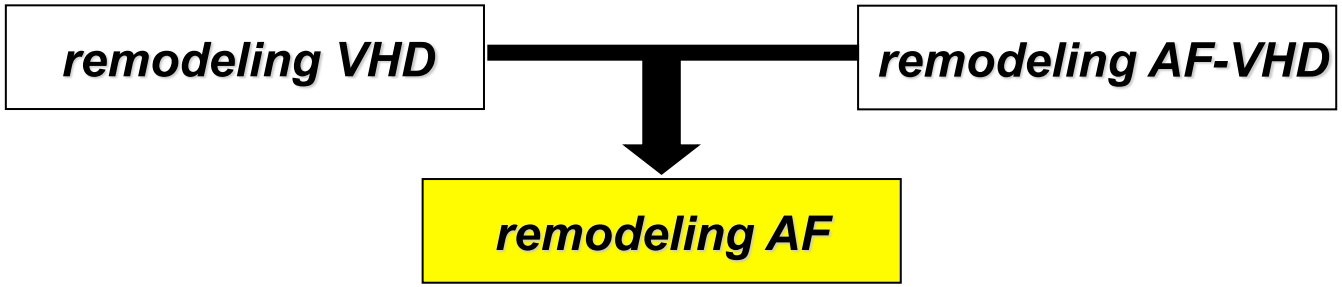
C : *ion channels down-regulated in AF-VHD*

AF-specific ion channel expression profile

Gaborit et al., Circulation 2005;112:471-81

Inserm

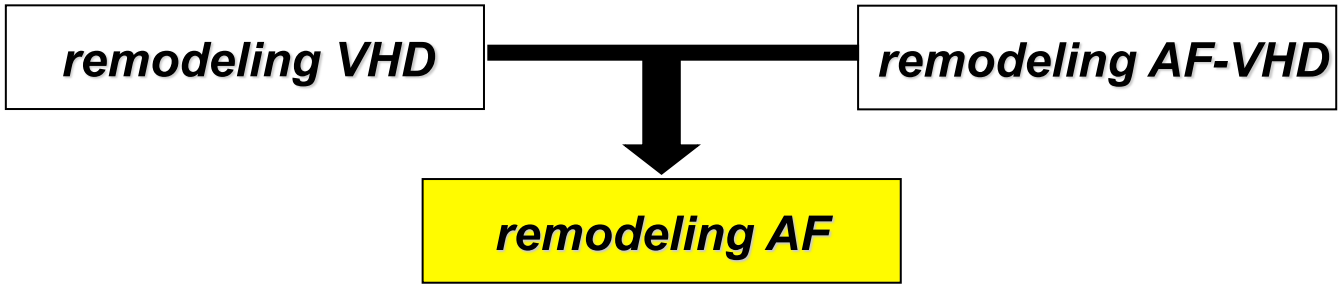
l'institut du thorax



Name	identifier	description	AF-specific variations
Cav3.1	NM_018896	calcium channel, voltage-dependent, alpha 1G subunit	↘
Cav$\alpha 2\delta 1$	NM_000722	calcium channel, voltage-dependent, alpha 2/delta subunit 1	↗
Cav$\alpha 2\delta 2$	NM_006030	calcium channel, voltage-dependent, alpha 2/delta subunit 2	↘
ITPR1	NM_002222	inositol 1,4,5-triphosphate receptor, type 1	↘
PLN	NM_002667	phospholamban	↗
Nav$\beta 2$	NM_004588	sodium channel, voltage-gated, type II, beta polypeptide	↘
CIC6	NM_021737	chloride channel 6	↗
Kv$\beta 1$	NM_003471	potassium voltage-gated channel, shaker-related subfamily, beta member 1	↗
MinK	NM_000219	potassium voltage-gated channel, Isk-related family, member 1	↗
MIRP2	NM_005472	potassium voltage-gated channel, Isk-related family, member 3	↗
Kv4.3	NM_004980	potassium voltage-gated channel, Shal-related subfamily, member 3	↘
TWIK1	NM_002245	potassium channel, subfamily K, member 1	↗

Inserm

l'institut du thorax

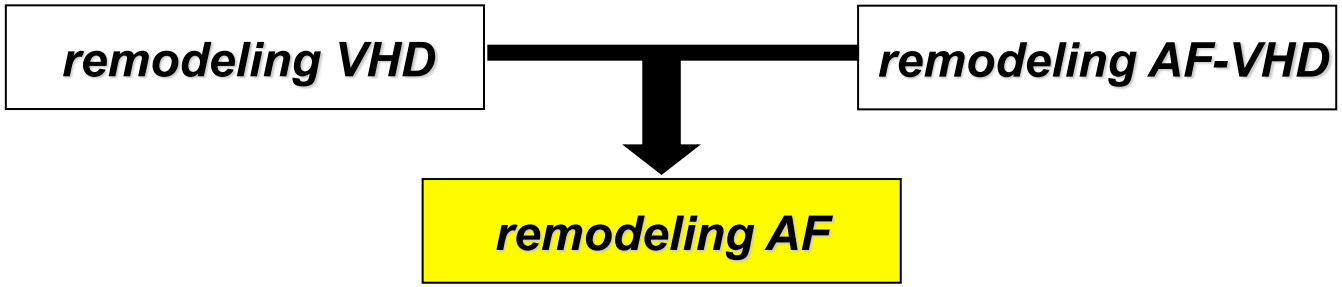


Name	identifier	description	AF-specific variations
Cav3.1	NM_018896	calcium channel, voltage-dependent, alpha 1G subunit	↘
Cav$\alpha 2\delta 1$	NM_000722	calcium channel, voltage-dependent, alpha 2/delta subunit 1	↗
Cav$\alpha 2\delta 2$	NM_006030	calcium channel, voltage-dependent, alpha 2/delta subunit 2	↘
ITPR1	NM_002222	inositol 1,4,5-triphosphate receptor, type 1	↘
PLN	NM_002667	phospholamban	↗
Nav$\beta 2$	NM_004588	sodium channel, voltage-gated, type II, beta polypeptide	↘
CIC6	NM_021737	chloride channel 6	↗
Kv$\beta 1$	NM_003471	potassium voltage-gated channel, shaker-related subfamily, beta member 1	↗
MinK	NM_000219	potassium voltage-gated channel, Isk-related family, member 1	↗
MIRP2	NM_005472	potassium voltage-gated channel, Isk-related family, member 3	↗
Kv4.3	NM_004980	potassium voltage-gated channel, Shal-related subfamily, member 3	↘
TWIK1	NM_002245	potassium channel, subfamily K, member 1	↗

→ ***Specific ion channel signature of human AF***

Inserm

l'institut du thorax



Name	identifier	description	AF-specific variations
Cav3.1	NM_018896	calcium channel, voltage-dependent, alpha 1G subunit	↘
Cav$\alpha 2\delta 1$	NM_000722	calcium channel, voltage-dependent, alpha 2/delta subunit 1	↗
Cav$\alpha 2\delta 2$	NM_006030	calcium channel, voltage-dependent, alpha 2/delta subunit 2	↘
ITPR1	NM_002222	inositol 1,4,5-triphosphate receptor, type 1	↘
PLN	NM_002667	phospholamban	↗
Nav$\beta 2$	NM_004588	sodium channel, voltage-gated, type II, beta polypeptide	↘
CIC6	NM_021737	chloride channel 6	↗
Kv$\beta 1$	NM_003471	potassium voltage-gated channel, shaker-related subfamily, beta member 1	↗
MinK	NM_000219	potassium voltage-gated channel, Isk-related family, member 1	↗
MIRP2	NM_005472	potassium voltage-gated channel, Isk-related family, member 3	↗
Kv4.3	NM_004980	potassium voltage-gated channel, Shal-related subfamily, member 3	↘
TWIK1	NM_002245	potassium channel, subfamily K, member 1	↗

→ ***Potential therapeutic targets?***

Inserm

l'institut du thorax

Conclusions

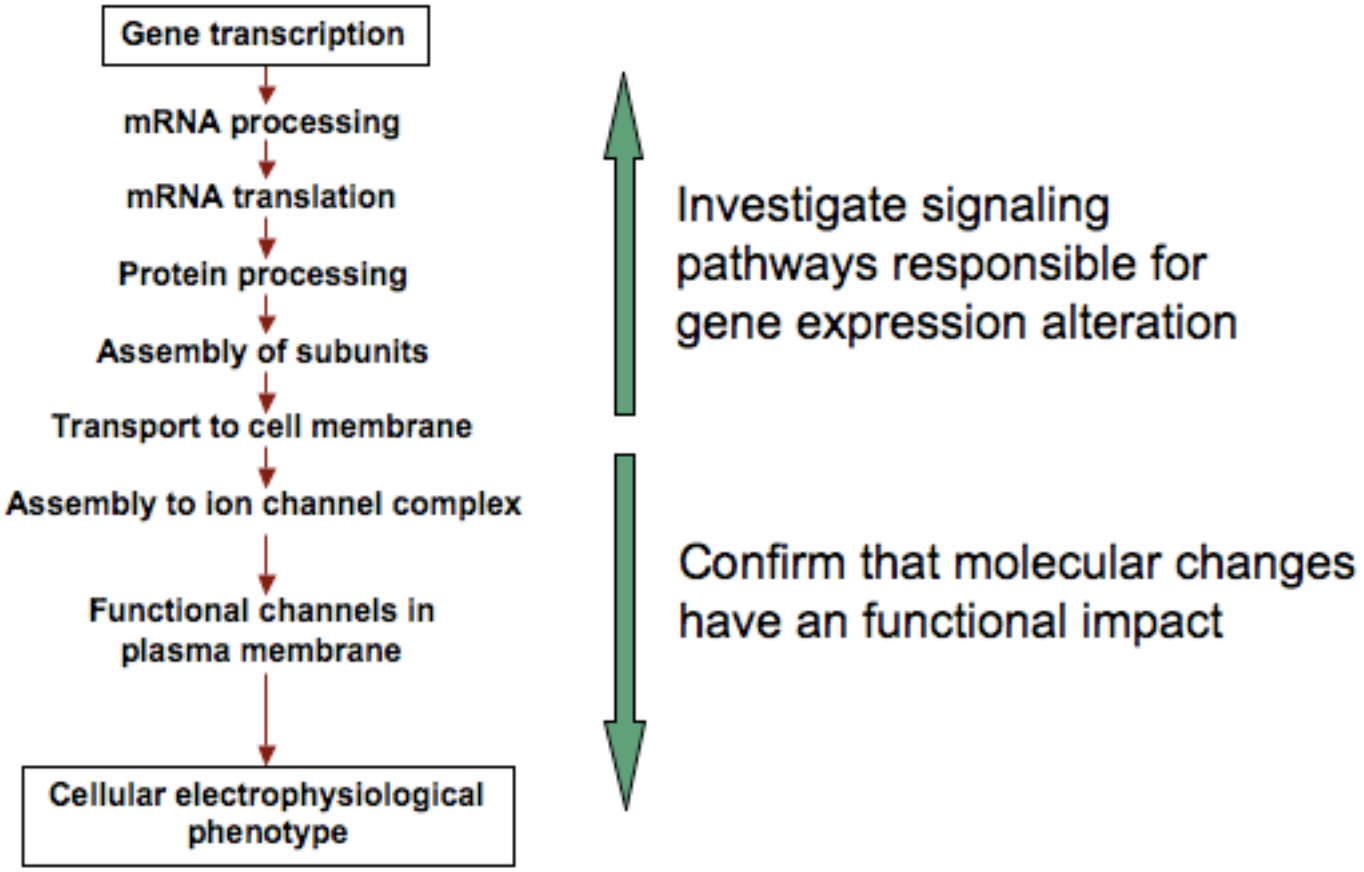
Transcriptomics → **specific ion-channel expression-profiles in arrhythmia syndromes**

- **Uncover new mechanisms of disease**
- **Provide a tool for drug discovery**
- **Provide a new diagnostic tool**

l'institut du thorax

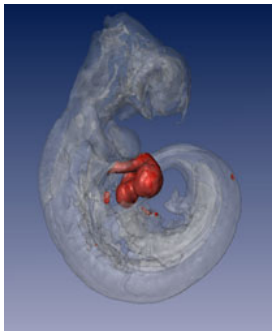
From transcriptomic to drug target discovery: Challenges

- Difficult accessibility to cardiac tissues
- Comprehensive integration and validation of the transcript changes:



- In arrhythmia: Reduction of symptoms without side effects

Specific ion channels profile:
what are the transcription factors involved ?



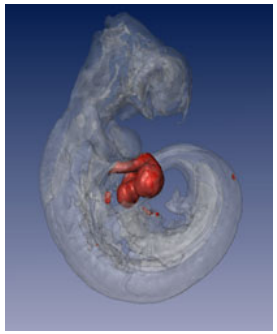
?



Bruneau BG. Circ Res. 2002.

l'institut du thorax

Specific ion channels profile:
what are the transcription factors involved ?



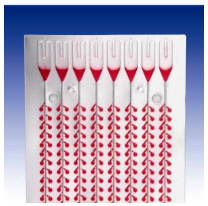
?



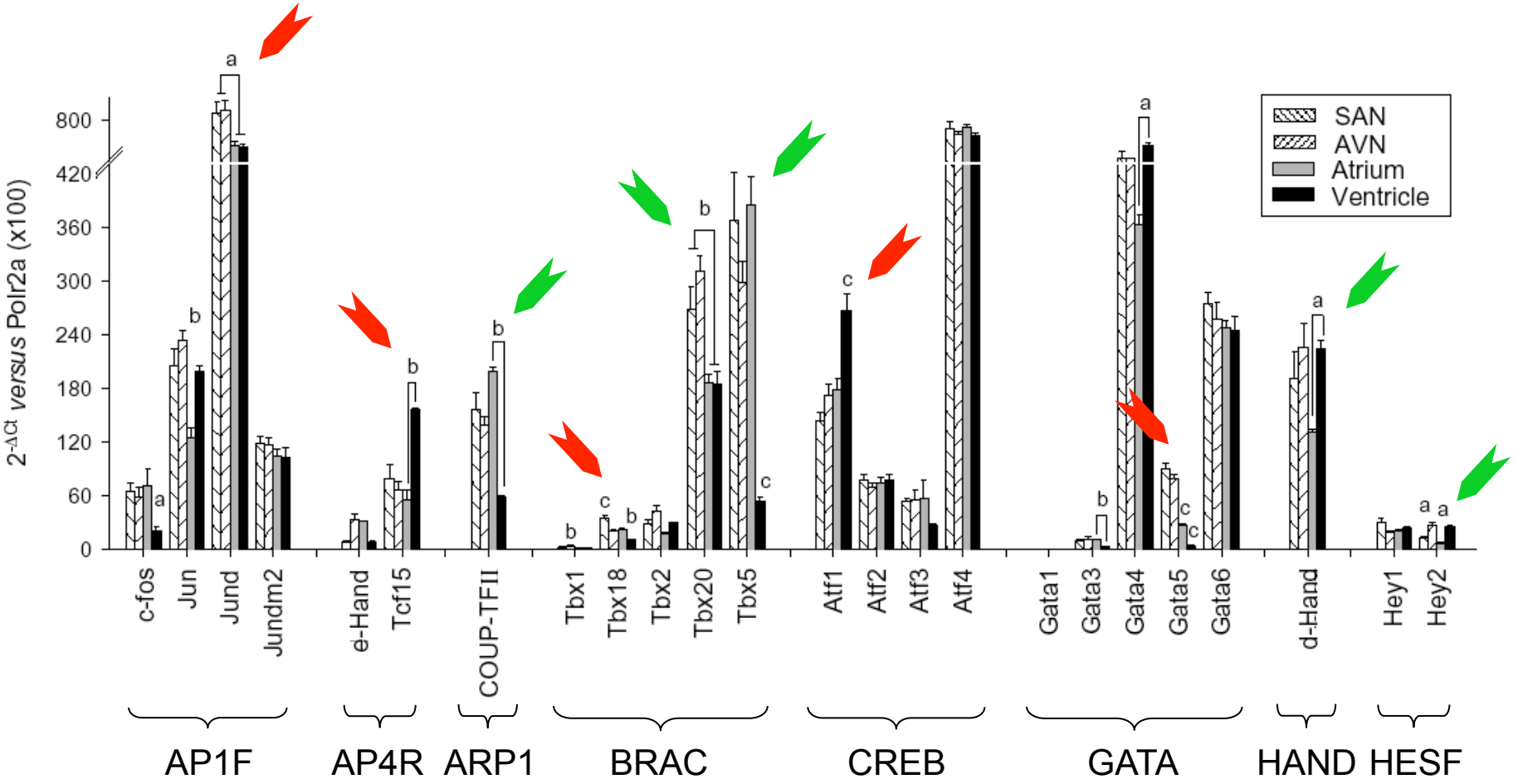
Bruneau BG. Circ Res. 2002.

Tissues: 6 pools of sinoatrial node
6 pools of atrio-ventricular node
6 pools of atria
6 pools of ventricles
(1 pool = 5 adult C57BL/6 male mice, 10 week old)

High-throughput TaqMan: 163 transcription factors
19 ion channel subunits
12 markers



Profile d'expression des facteurs de transcription spécifique à chaque région cardiaque



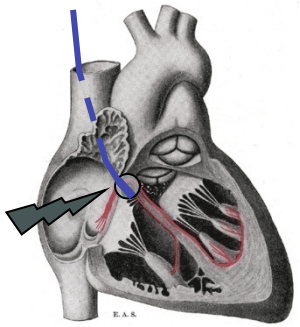
a: p<0.05; b: p<0.01; c: p<0.001.

Marionneau C,…,Demolombe S. *Circulation Suppl.* 2005.

Modèle murin d'hypertrophie



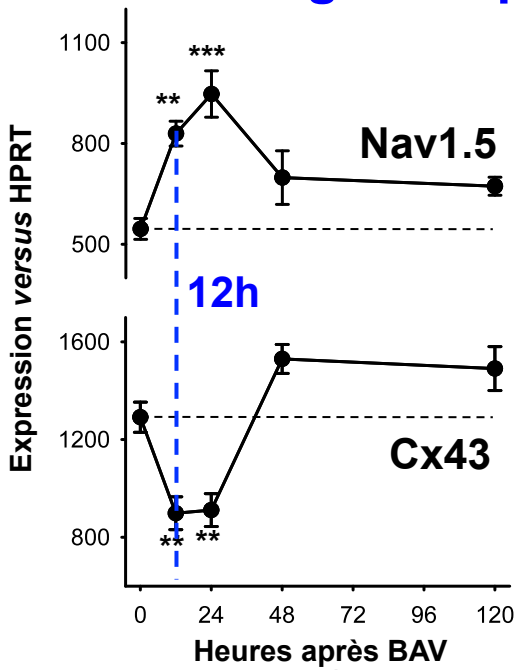
BAV



Troubles du rythme **dès 12 h** après BAV



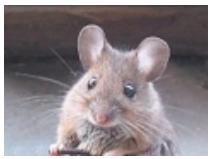
Remodelage ionique



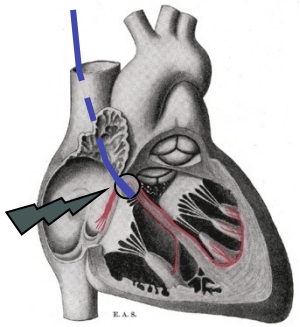
Bignolais O,…, Demolombe S. *Am J Physiol.* (en révision)

l'institut du thorax

Modèle murin d'hypertrophie



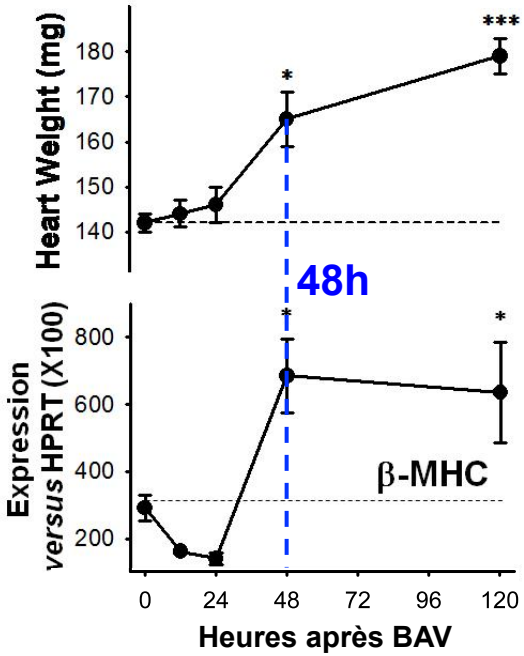
BAV



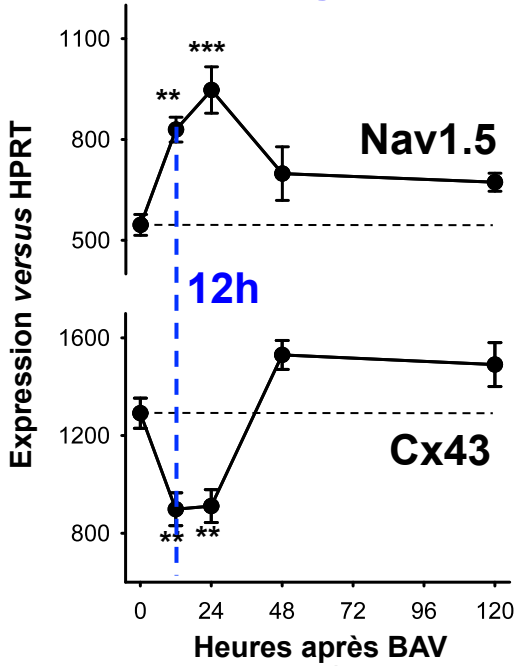
Troubles du rythme **dès 12 h** après BAV



Hypertrophie



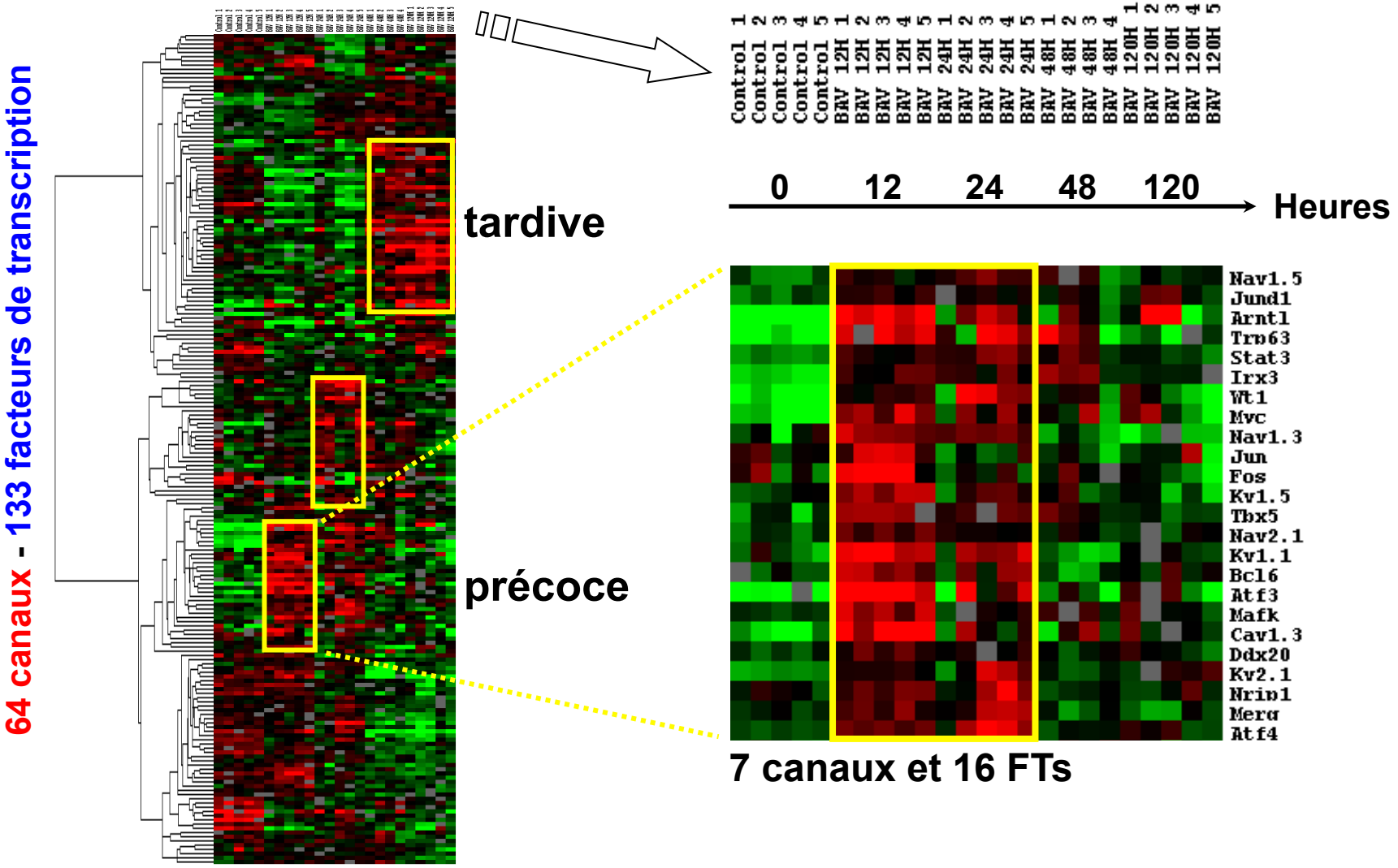
Remodelage ionique



Bignolais O,…, Demolombe S. *Am J Physiol.* (en révision)

CNRS CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE · Inserm · CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES · UNIVERSITE DE NANTES

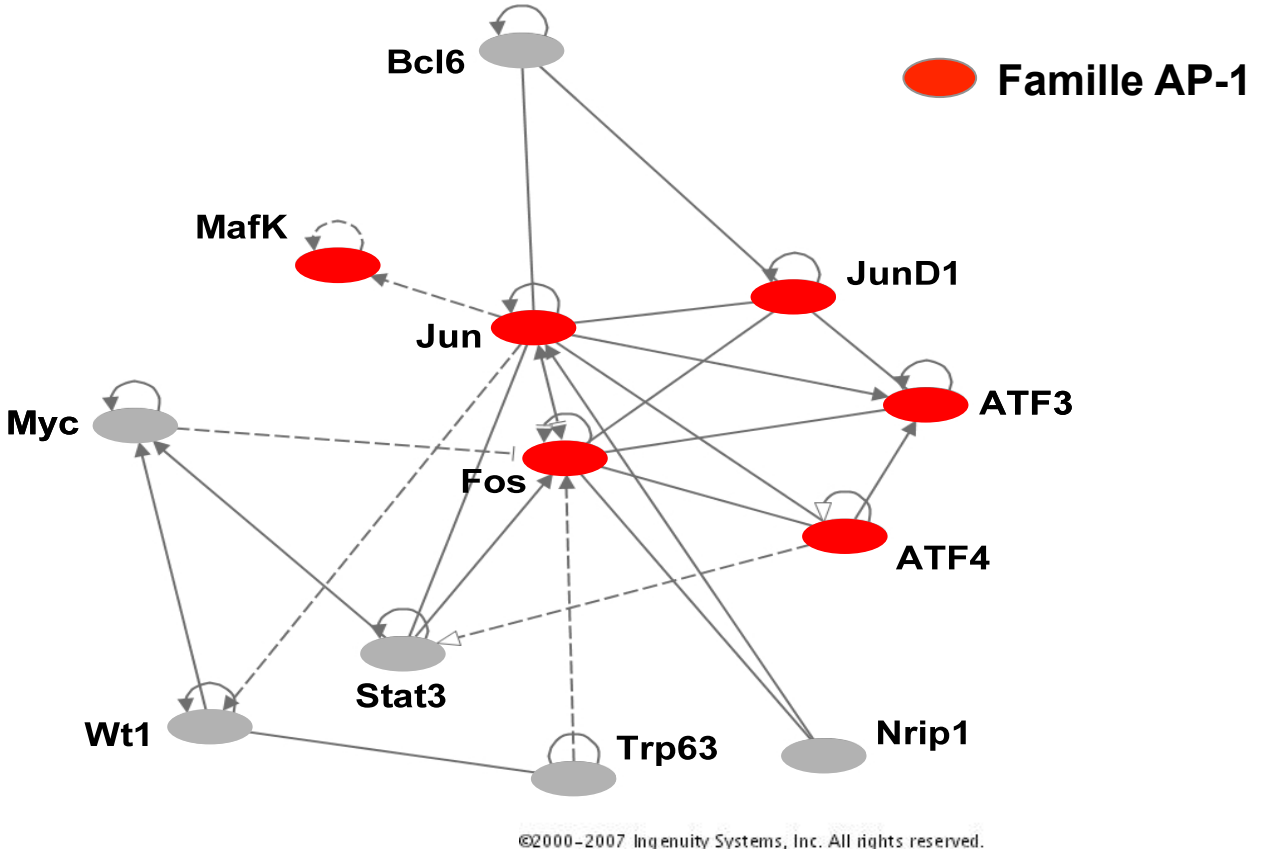
Co-variations des canaux ioniques et des facteurs de transcription dans le modèle BAV



Bignolais O,…, Demolombe S. *Am J Physiol.* (en révision)

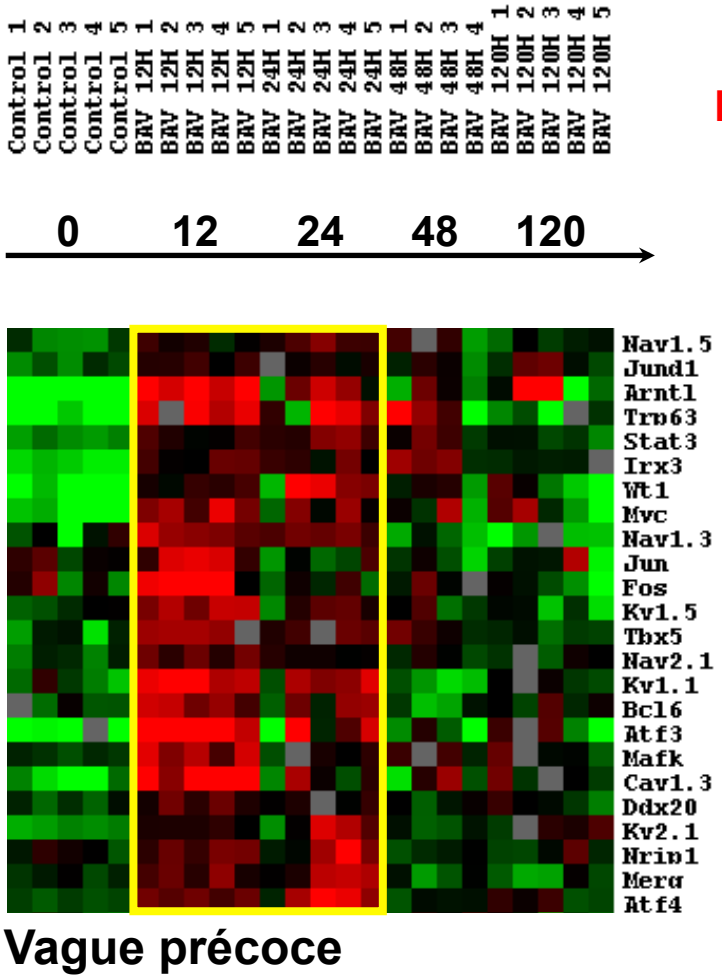
l'institut du thorax

Réseaux d'interaction moléculaire

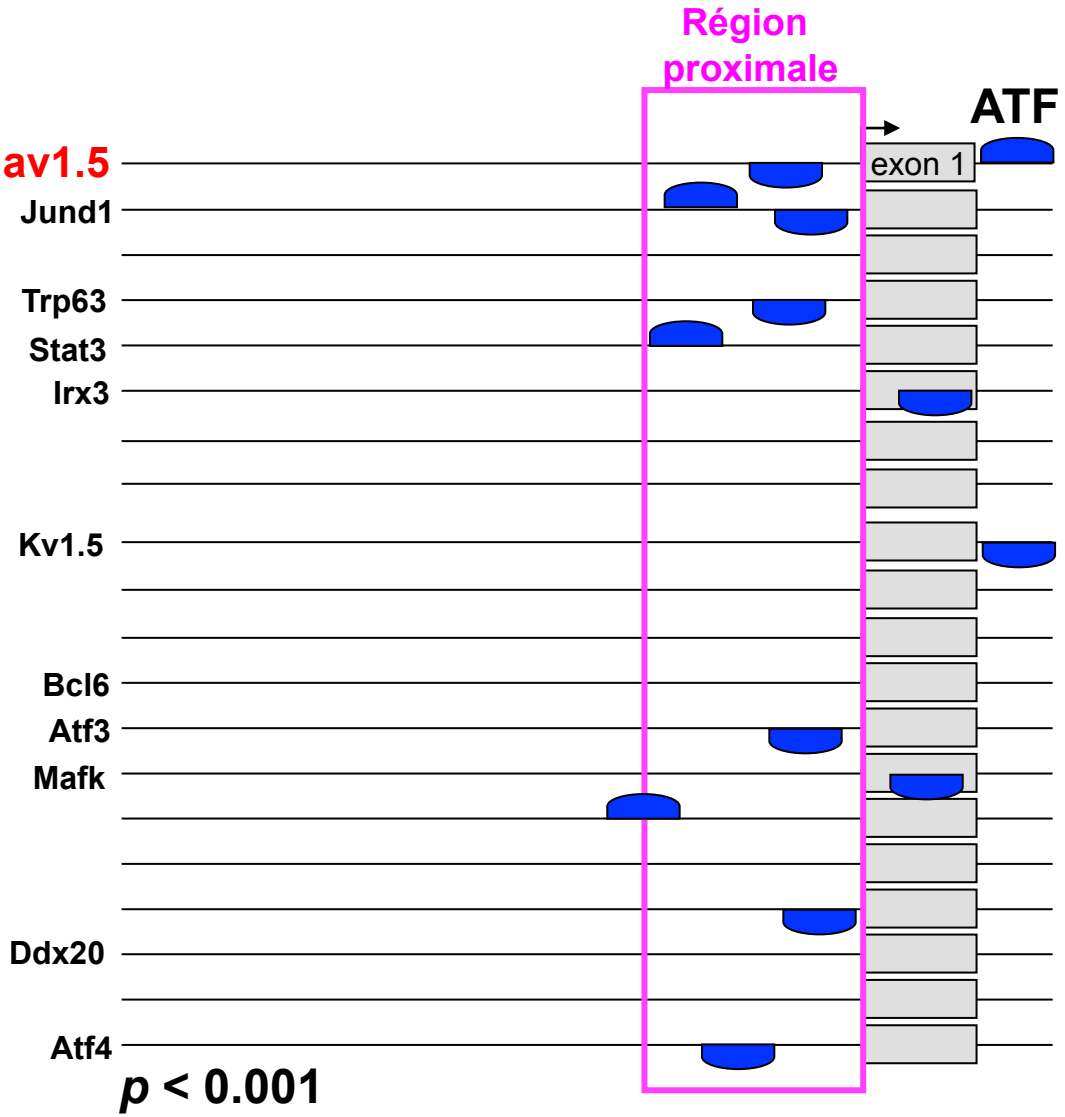


Parmi les 16 FTs de la "vague" précoce, 12 sont connus pour interagir entre eux et 6 appartiennent à la famille des activating protein-1 (AP-1)

Prédiction bioinformatique

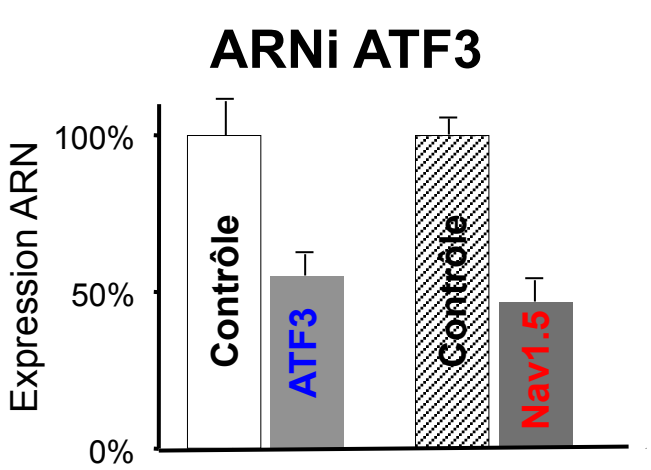


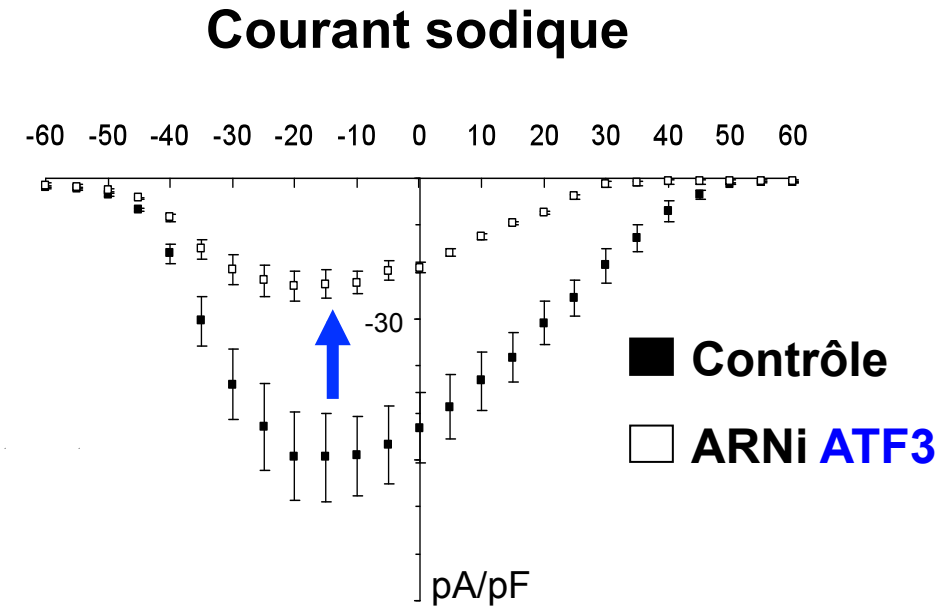
Vague précoce



Validation biologique et conséquences fonctionnelles

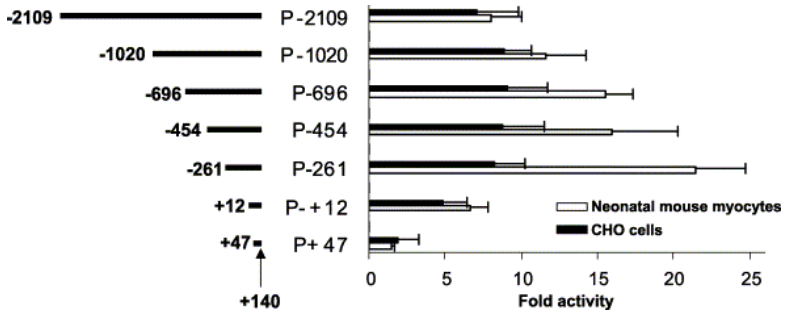




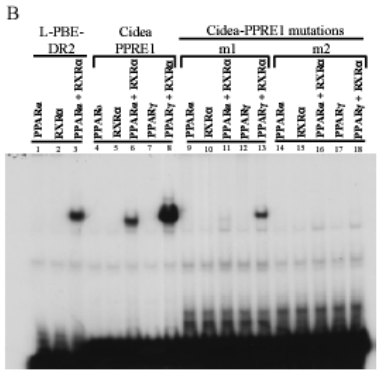


Rôle des ATFs dans l'excitabilité cellulaire ?

Analyser la fonction du promoteur du canal sodique

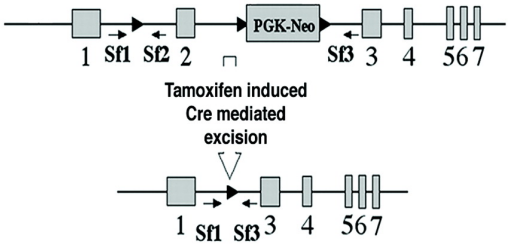


Montrer la fixation directe des ATF sur le promoteur du canal sodique



Evaluer les conséquences fonctionnelles *<u>in vivo</u>*

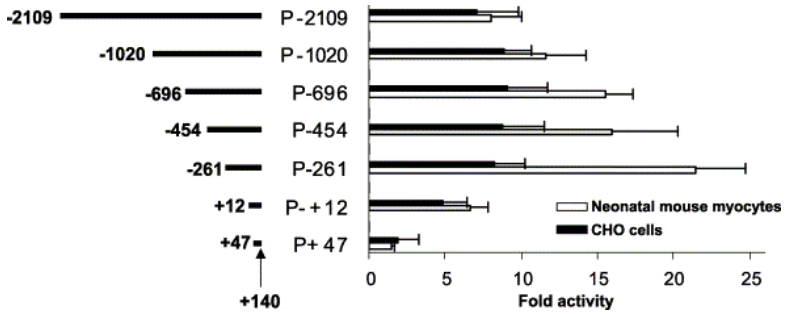




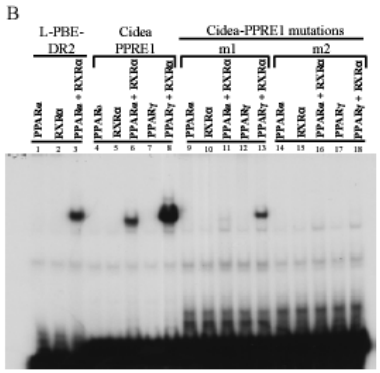


Rôle des ATFs dans l'excitabilité cellulaire ?

Analyser la fonction du promoteur du canal sodique

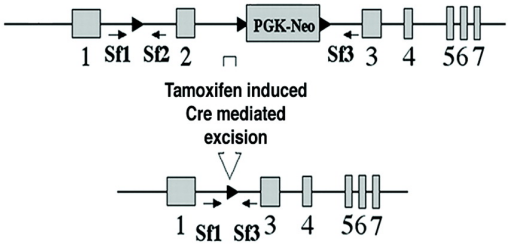


Montrer la fixation directe des ATF sur le promoteur du canal sodique



Evaluer les conséquences fonctionnelles *<u>in vivo</u>*



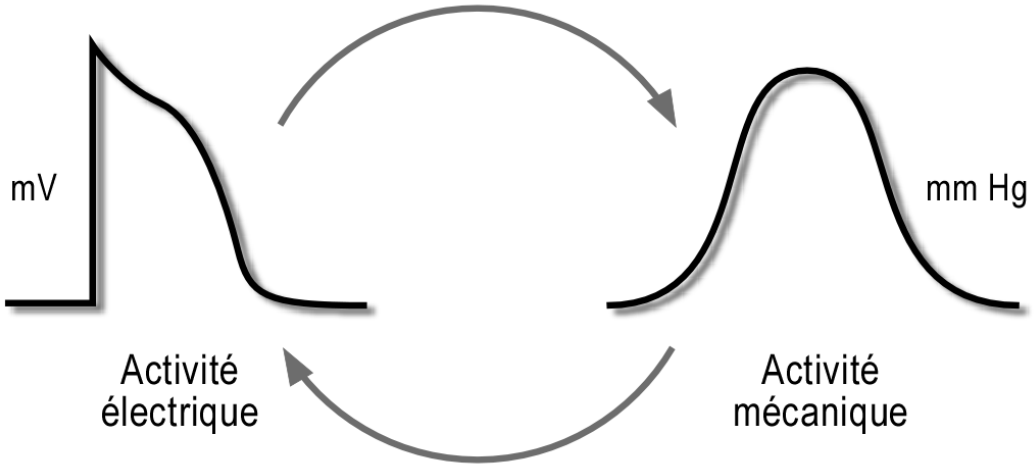




PROJET :
Stress mécanique et remodelage électrique cardiaque

De nombreuses situations arythmogènes, dont la fibrillation auriculaire et l'insuffisance cardiaque, sont associées à une altération de la contractilité musculaire et à une hétérogénéité mécanique.

Couplage excitation-contraction

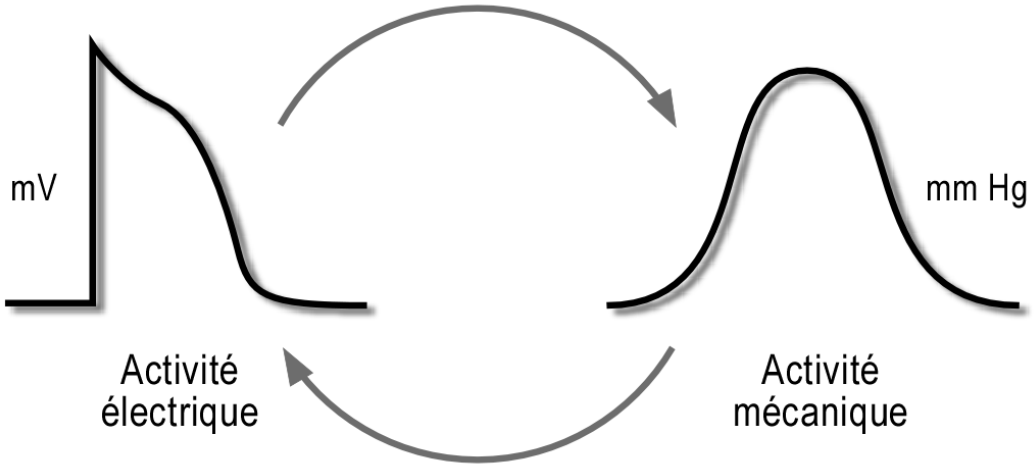


Feedback mécano-électrique

PROJET :
Stress mécanique et remodelage électrique cardiaque

De nombreuses situations arythmogènes, dont la fibrillation auriculaire et l'insuffisance cardiaque, sont associées à une altération de la contractilité musculaire et à une hétérogénéité mécanique.

Couplage excitation-contraction

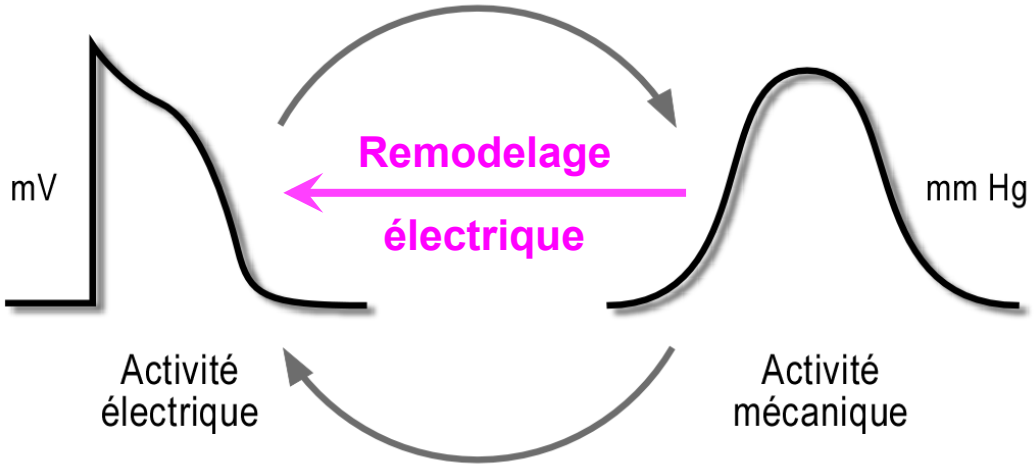


Feedback mécano-électrique

PROJET :
Stress mécanique et remodelage électrique cardiaque

De nombreuses situations arythmogènes, dont la fibrillation auriculaire et l'insuffisance cardiaque, sont associées à une altération de la contractilité musculaire et à une hétérogénéité mécanique.

Couplage excitation-contraction

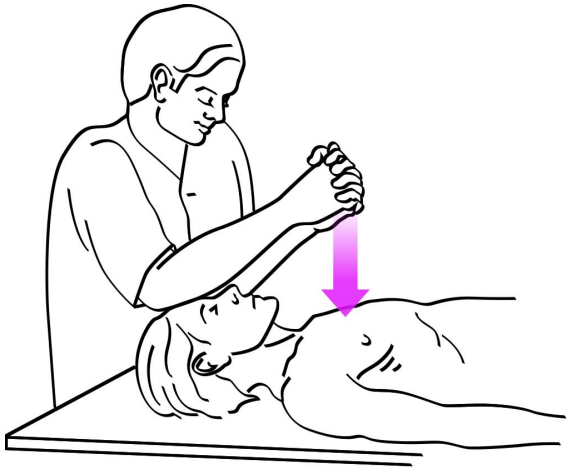


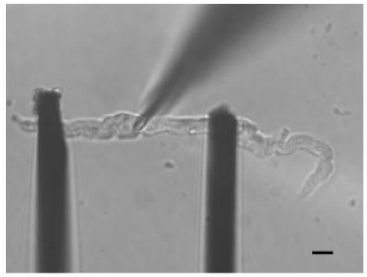
Feedback mécano-électrique

OBJECTIF:

Comprendre comment les stimuli mécaniques à long terme modulent l'expression des canaux ioniques et contribuent au déclenchement d'arythmies.

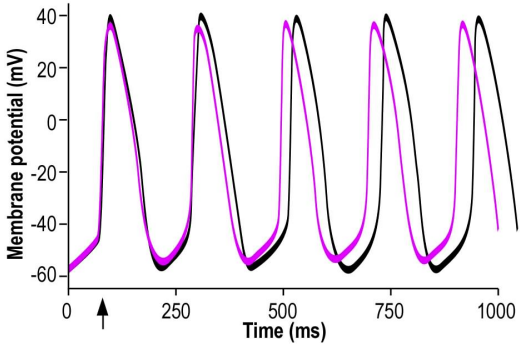
Le couplage mécano-électrique cardiaque





Cooper et al., 2000

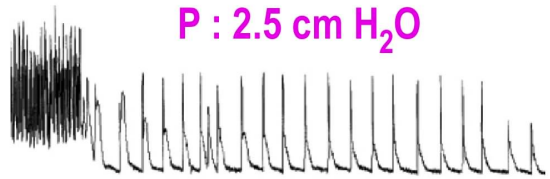
Etirement 7%



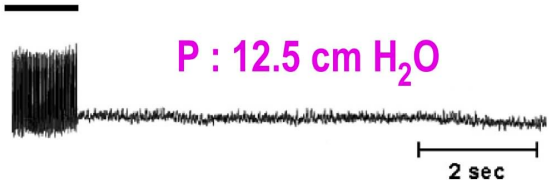
Haute fréquence



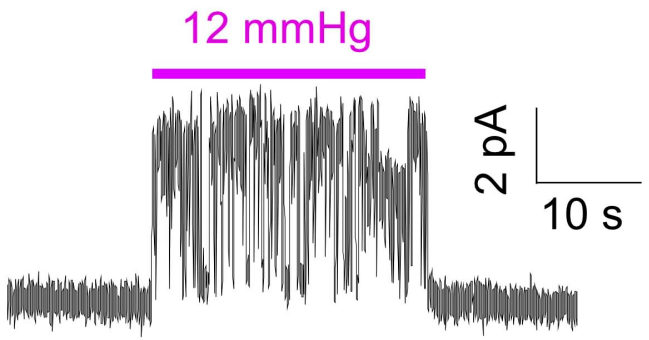
Bode et al., 2001



rythme sinusal



fibrillation auriculaire



Terrenoire et al., 2001

Stress mécanique et hypertrophie ventriculaire

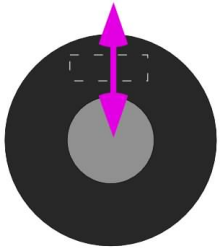
sain



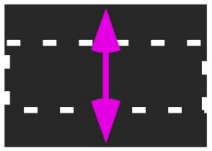
myocyte



Hypertrophie ventriculaire

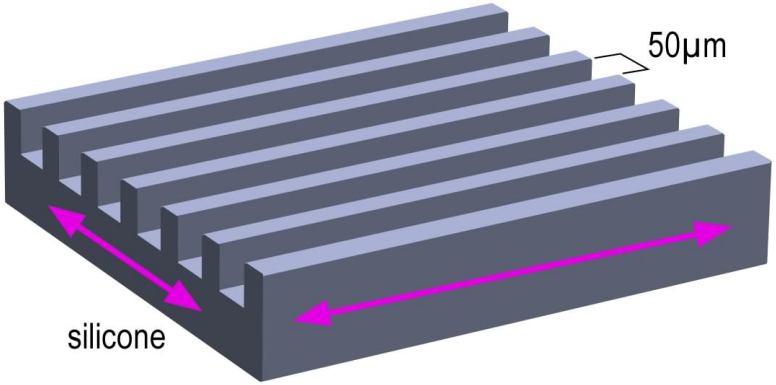


surcharge de pression



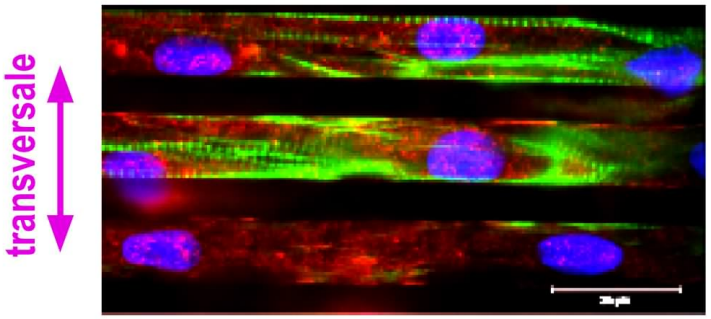
Epaississement

Modèle *in vitro* d'étirement biaxial



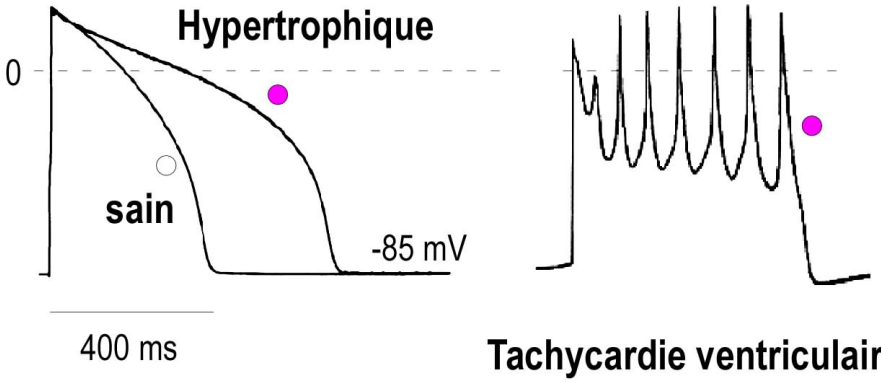
Étirement 10%

génomique, IF, biochimie, physiologie



noyau, actin et FAKpY397

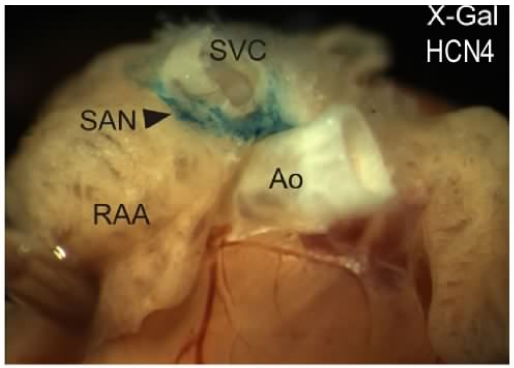
Senyo et al., 2007



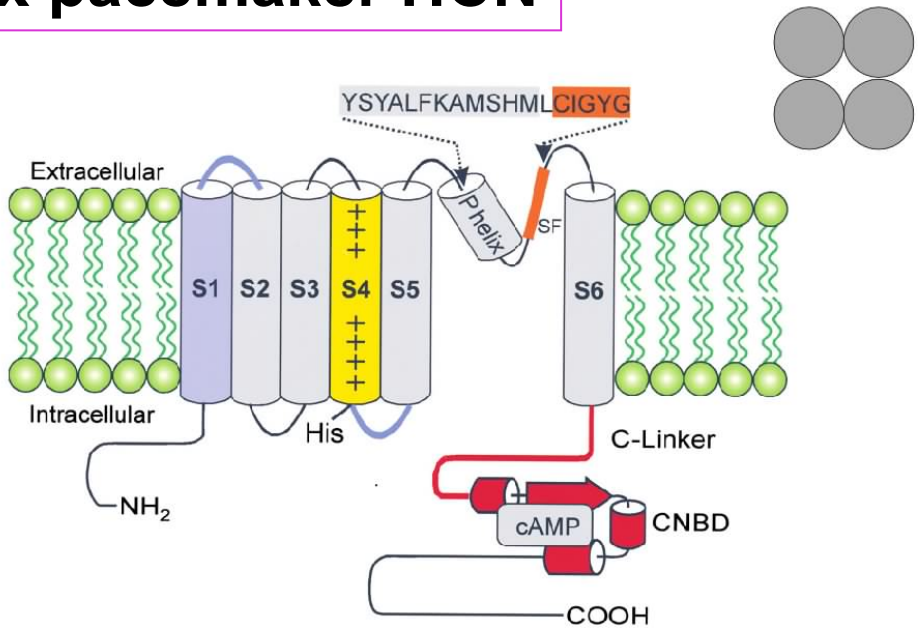
Hill, 2003

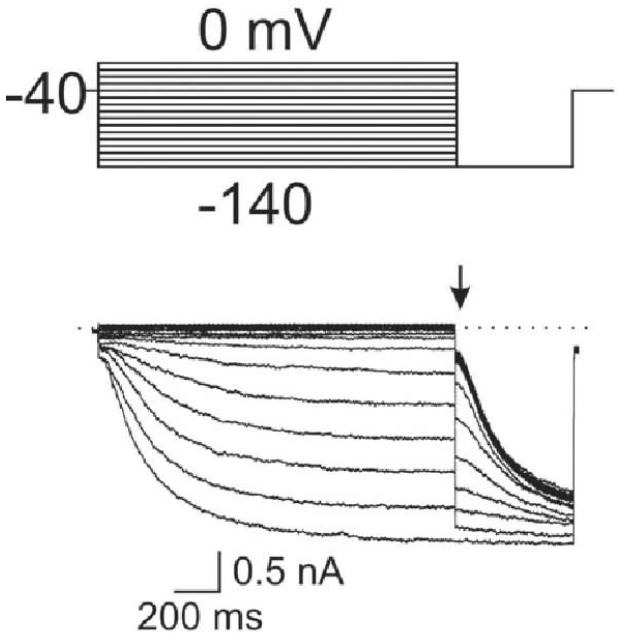
Tachycardie ventriculaire
Mort subite

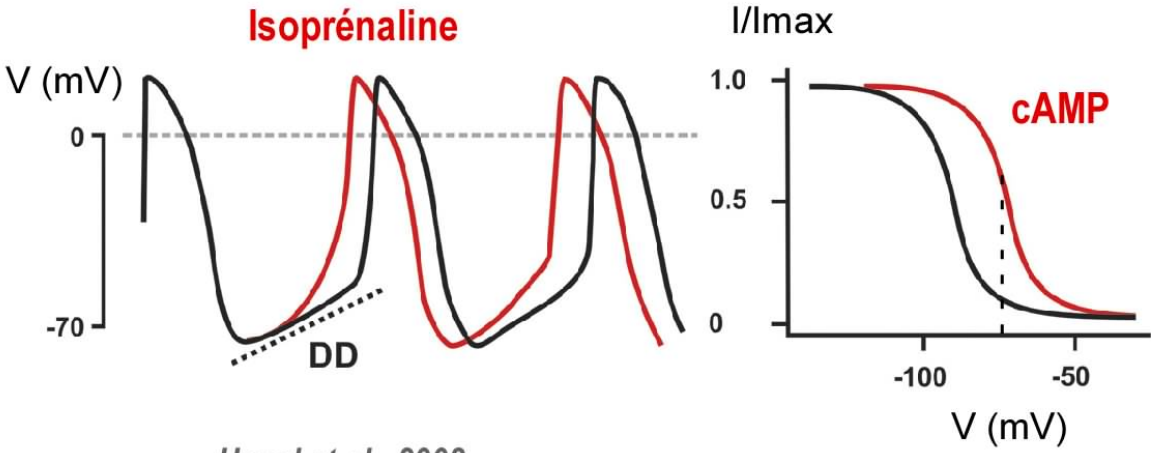
Les canaux pacemaker HCN



Biel et al., 2009





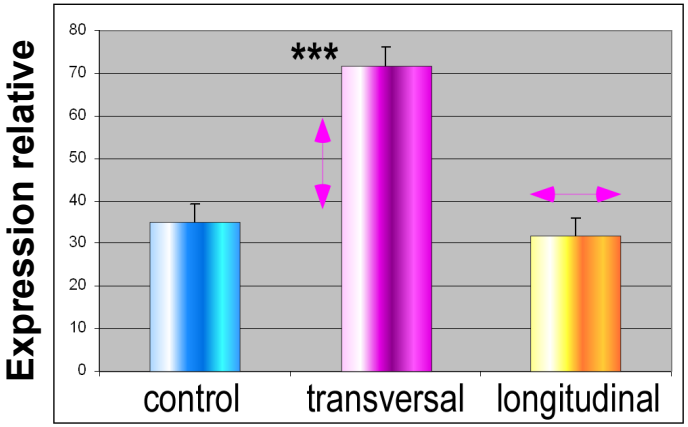


Hoesl et al., 2008

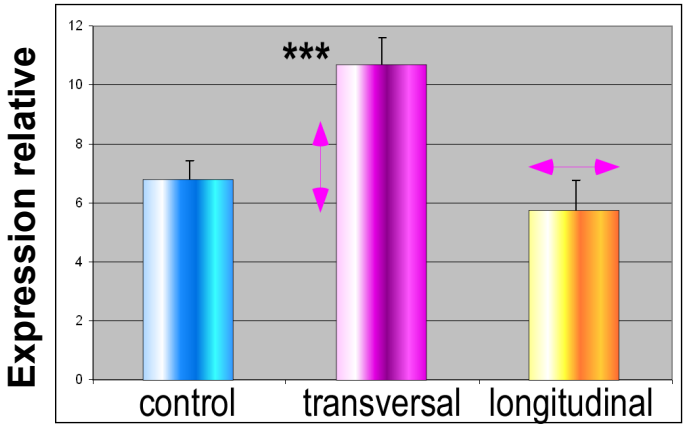
Résultats préliminaires :

La force transversale augmente l'expression des sous-unités HCN

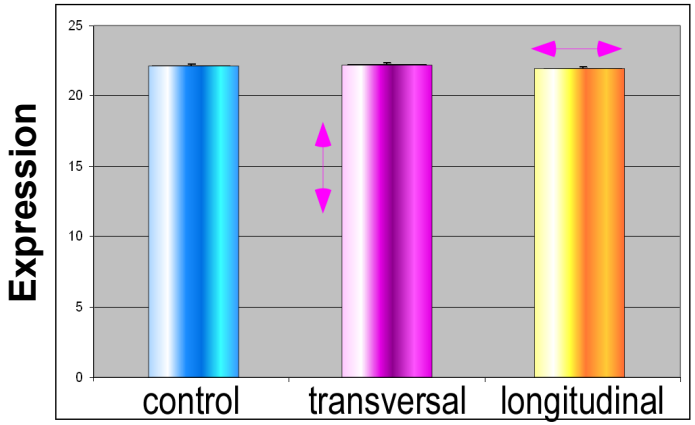
HCN2

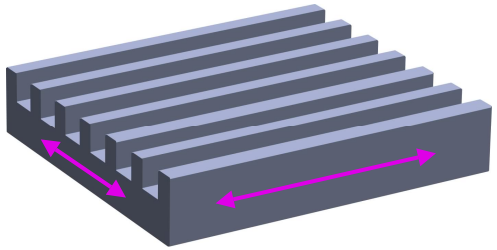


HCN4

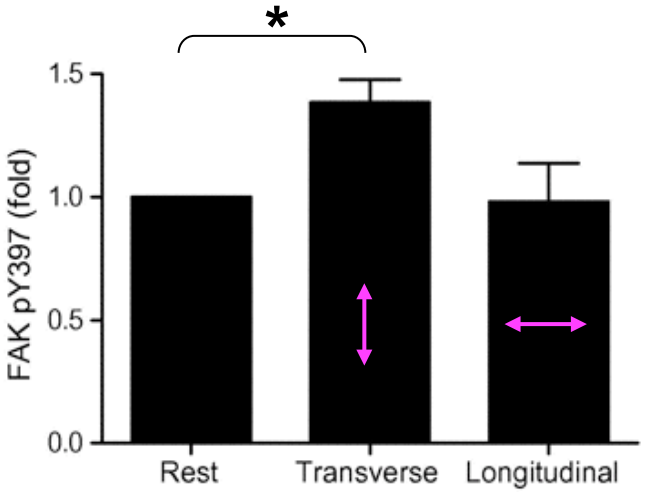


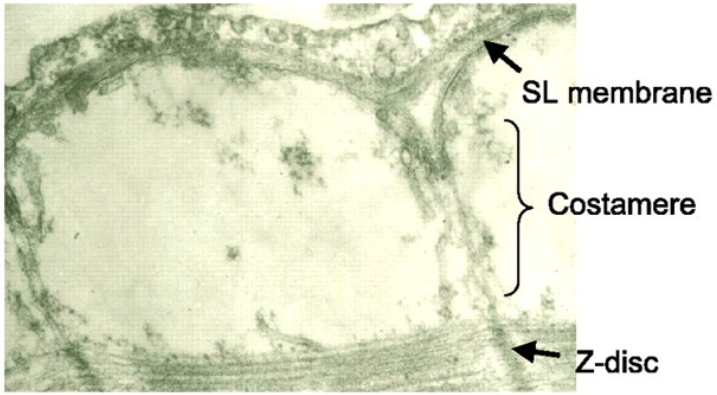
GAPDH

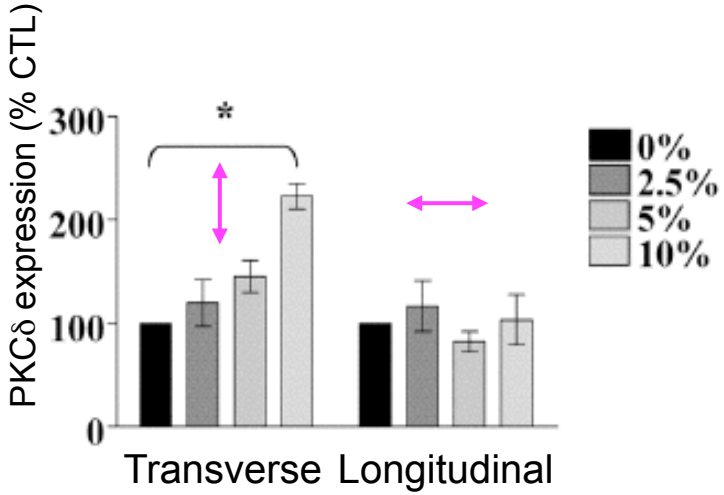


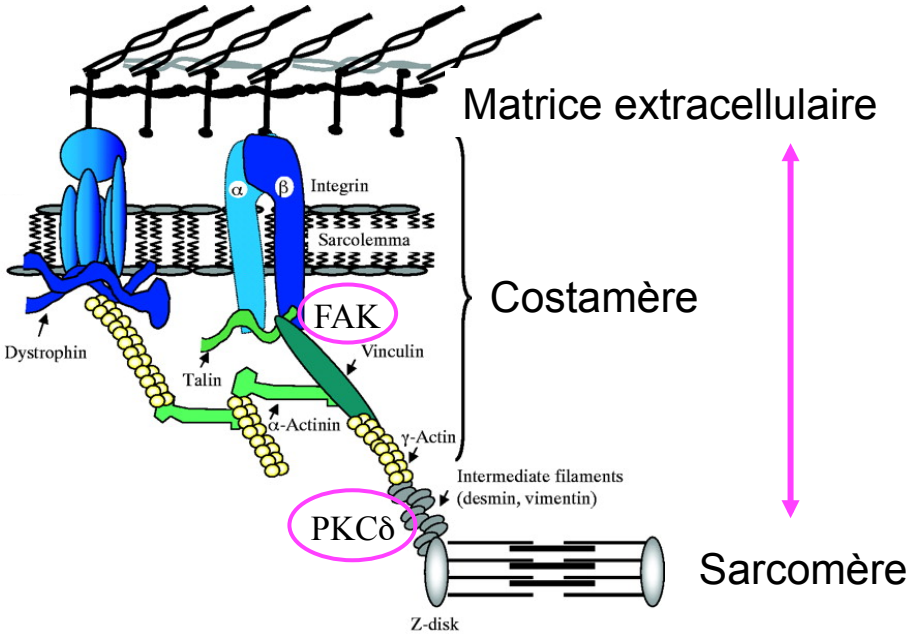


Le costamère et la mécanotransduction cardiaque



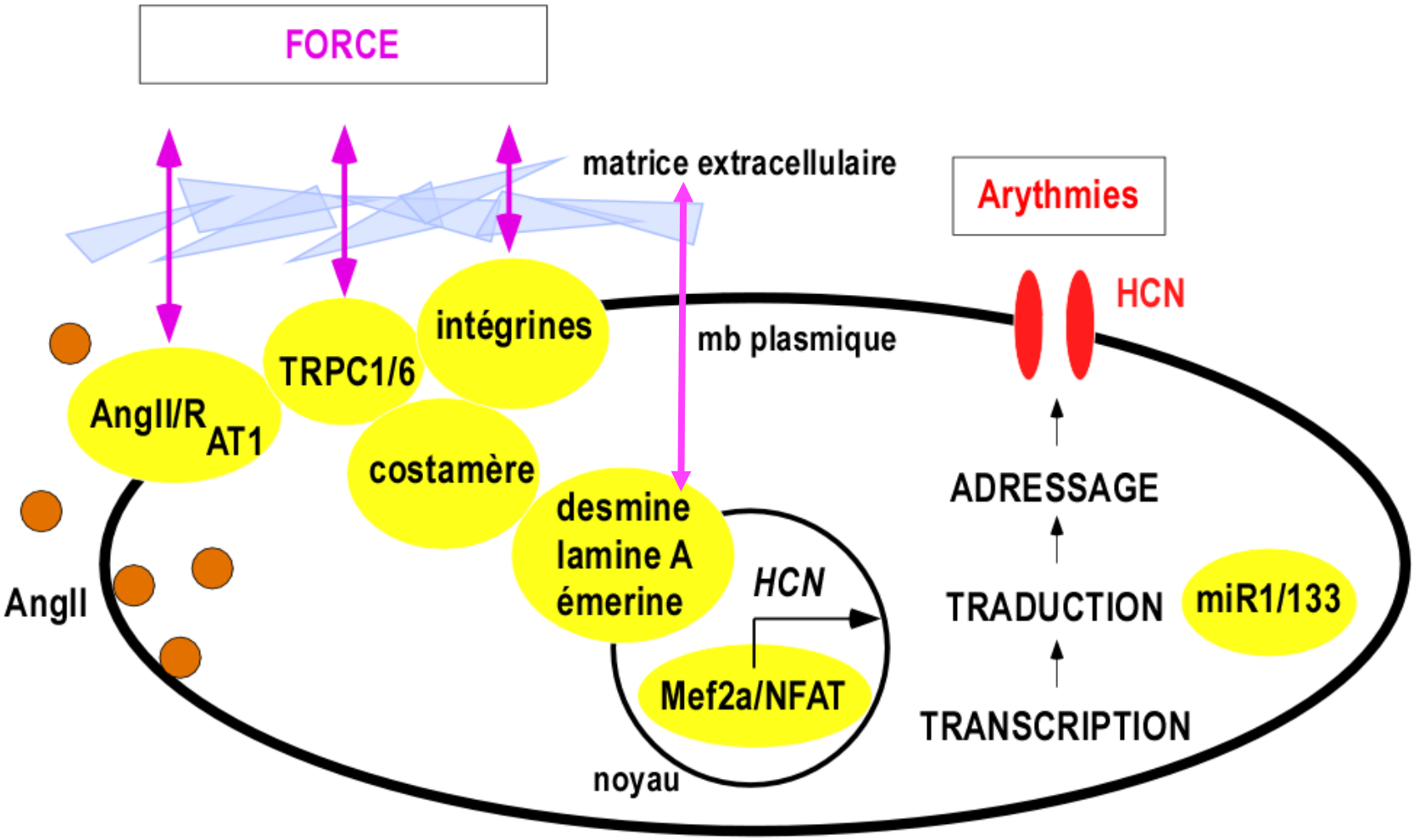






Senyo et al., 2007
Bullard et al., 2007

Mécanotransduction cardiaque et régulation de l'expression des canaux HCN



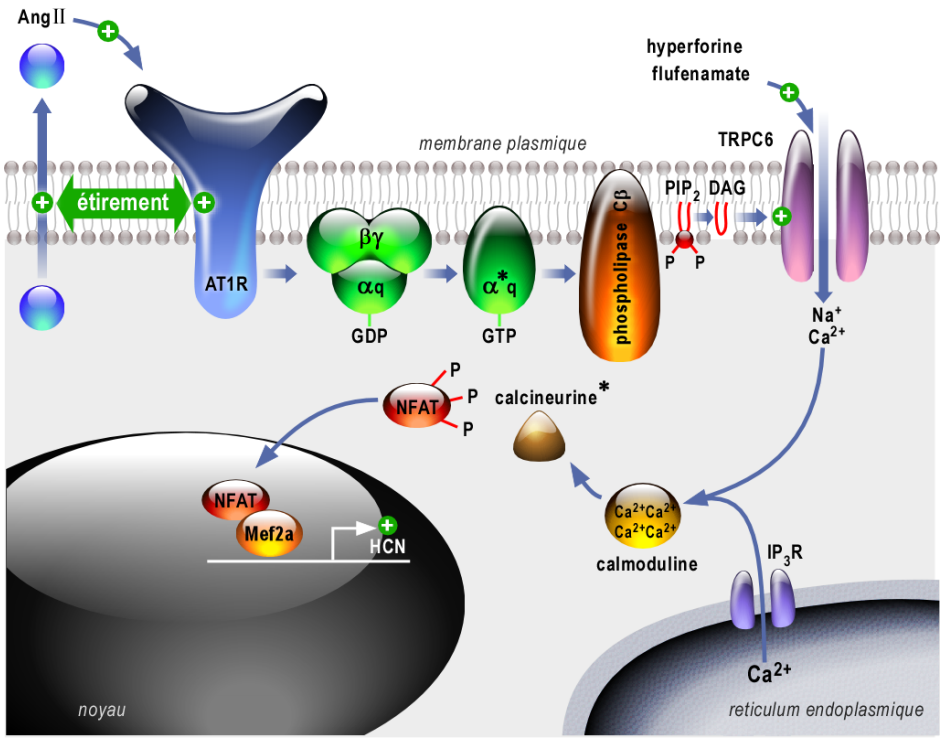
Objectif 1: SIGNALISATION

Contribution de la sécrétion autocrine d'angiotensine II lors d'un stress mécanique

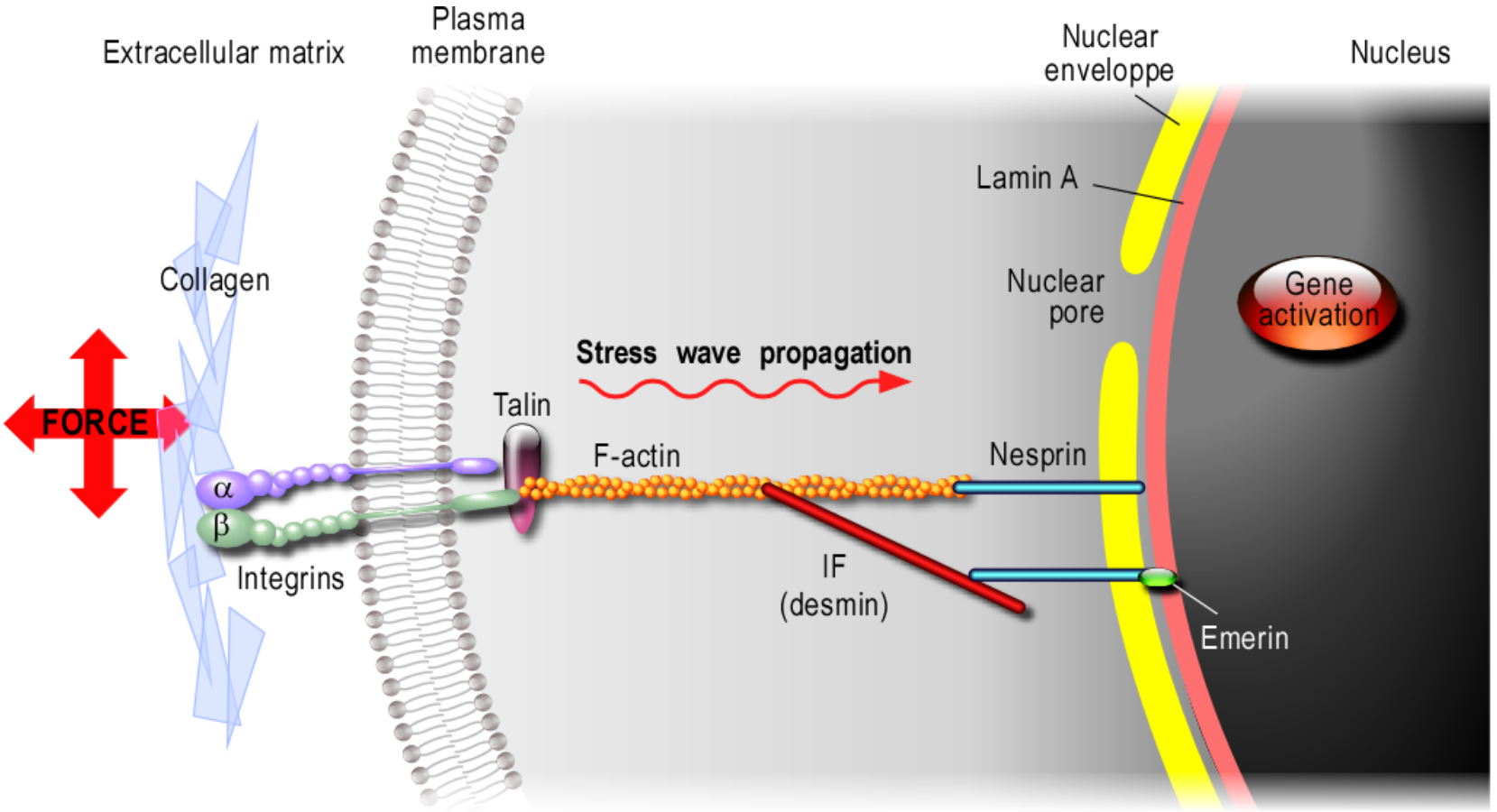
- effets d'une inhibition de son récepteur
- effets de la sous-expression de *miR-1* et *miR-133*
- adressage à la membrane des endosomes contenant les canaux HCN recyclés

Contribution de l'activation directe des récepteurs à l'Ang II par l'étirement membranaire

- conséquences de l'activation des canaux TRPC1 et TRPC6
- rôle de la voie NFAT/Mef2a



Objectif 2:TRANSMISSION DIRECTE DE LA FORCE AU NOYAU



Conséquence de l'invalidation de la lamine A (souris *Lmna*$^{H222P/H222P}$, collaboration avec Gisèle Bonne, INSERM U974, Paris)

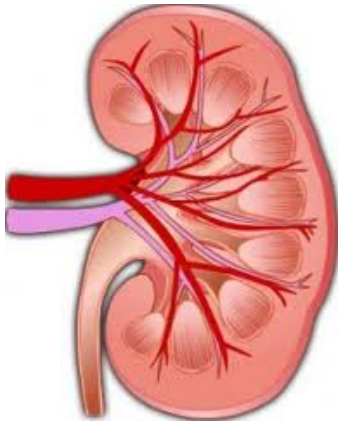
Conséquence de l'invalidation de la desmine (souris Desm$^{-/-}$, collaboration avec Zhenlin Li, CNRS UMR7079, Paris)

Les canaux Piezo dans la mécano-transduction podocytaire

Marine Berguignat

Laboratoire de Physiopathologie réno-vas*culaire*
Dr Eric Honoré

Encadrant: Dr Sophie Demolombe



cnrs dépasser les frontières

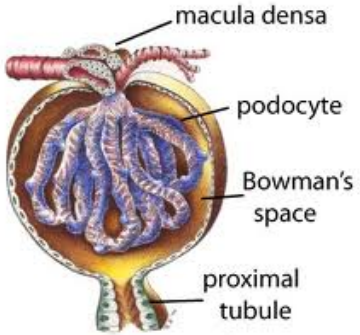
ipmc

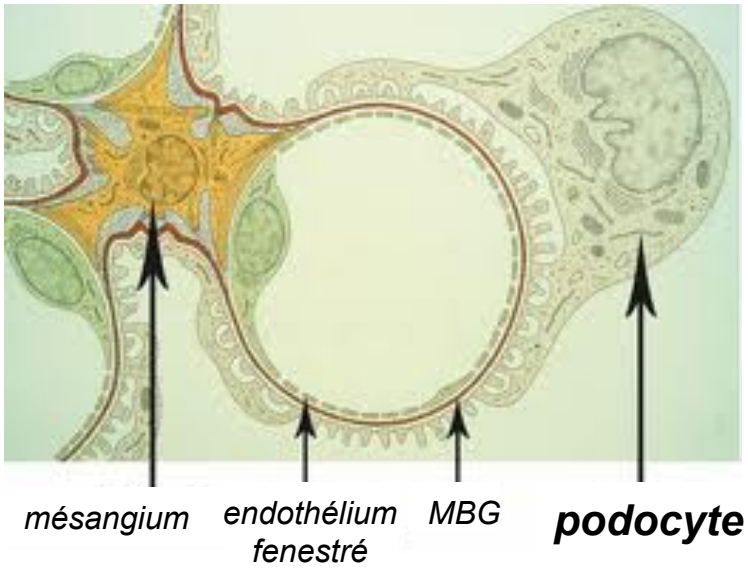
FONDATION RECHERCHE MÉDICALE

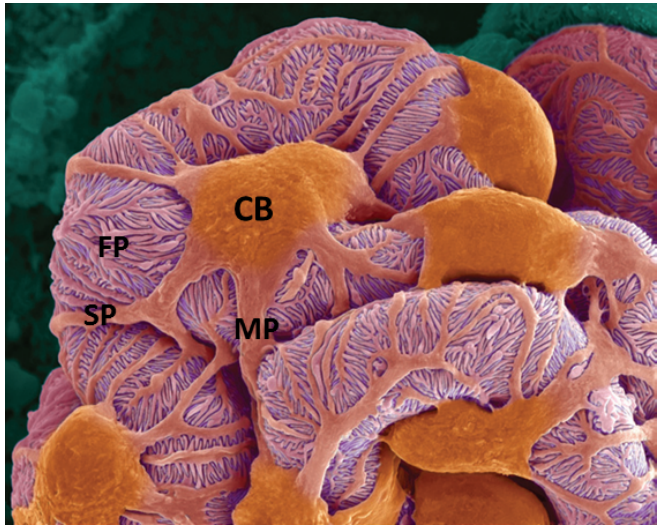
Université Nice SOPHIA ANTIPOLIS

Centre Hospitalier Universitaire de Nice

La filtration glomérulaire







Hypertension artérielle

Diabète

↑↑ PRESSION INTRAGLOMÉRULAIRE

Lésions podocytaires

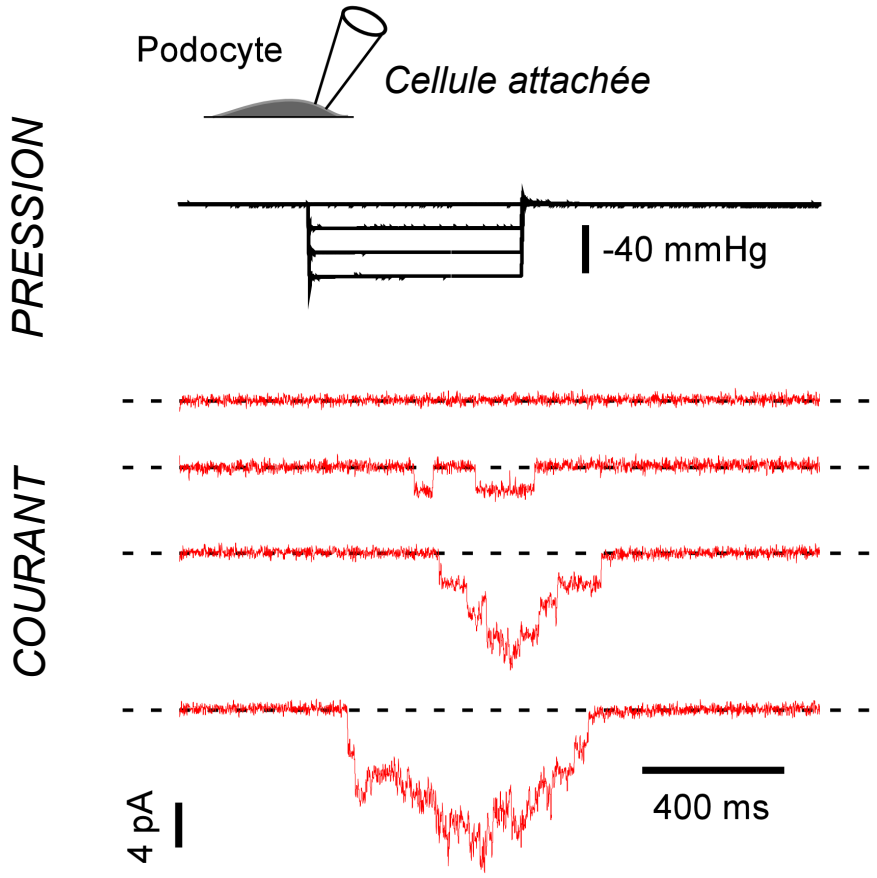
Lésions endothéliales

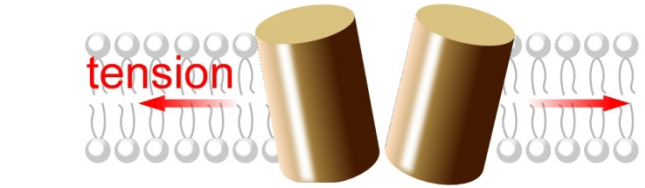
Lésions mésangiales

glomérulosclérose

Mécano-sensibilité du podocyte

Canaux ioniques mécano-sensibles dans les podocytes

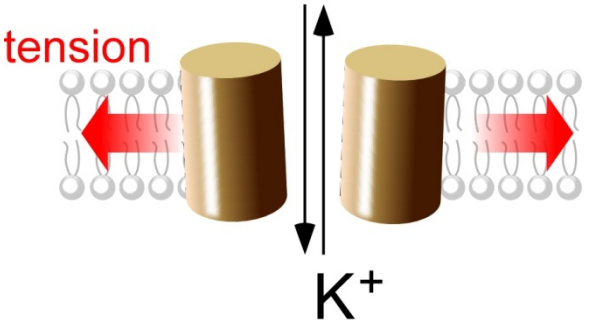




bicouche lipidique Piezo

canal mécano-sensible non sélectif

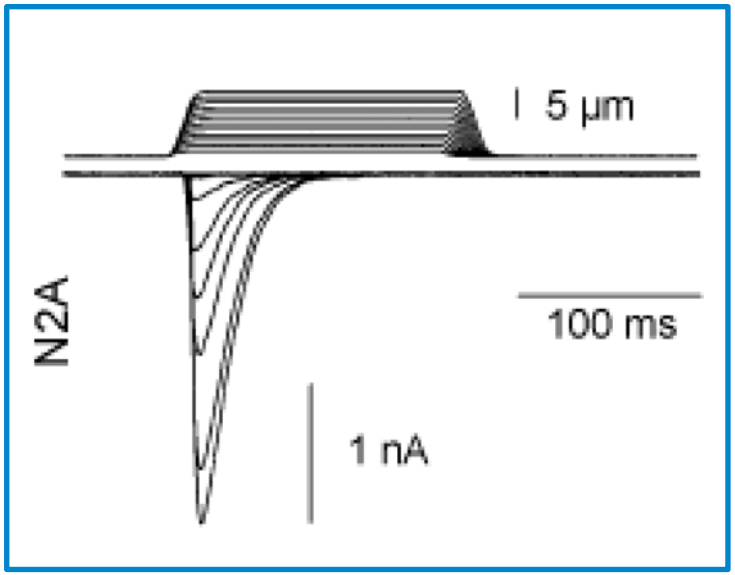
Na^+ Ca^{2+}



dépolarisant

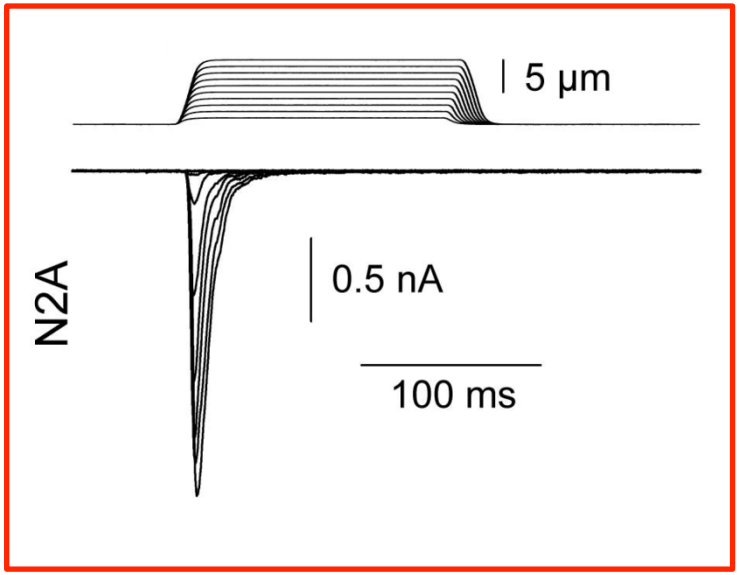
Rémi Peyronnet

Piezo1 et Piezo2: mécano-senseurs du podocyte?

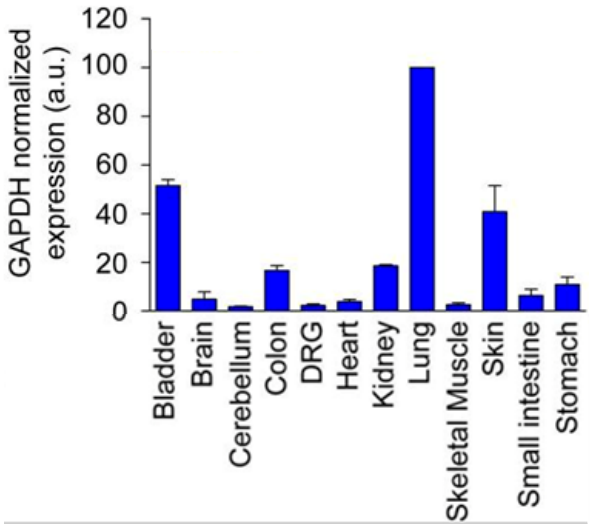




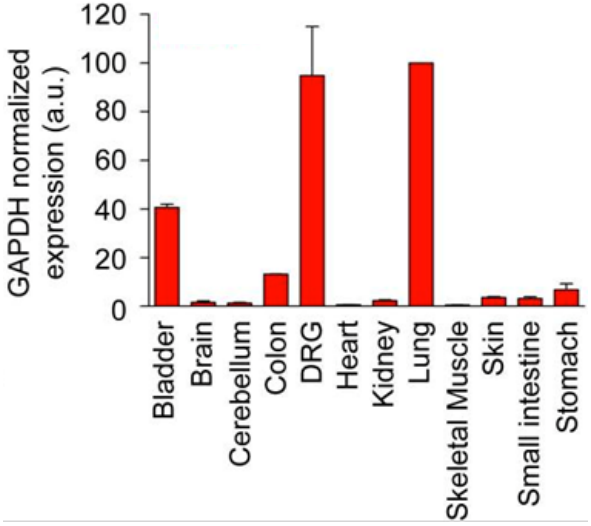
cellule entière



Piezo1



Piezo2



Coste et al., Science (2010)

Materials and Methods

Podocyte cell lines:

- *Derived from mouse: WT-M Cl2; WT-M C3; SVI*
- *Derived from human: WS-C4; AB; ATC*

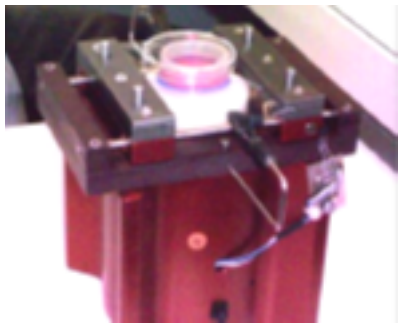
Quantification of transcription by real-time

Patch clamp

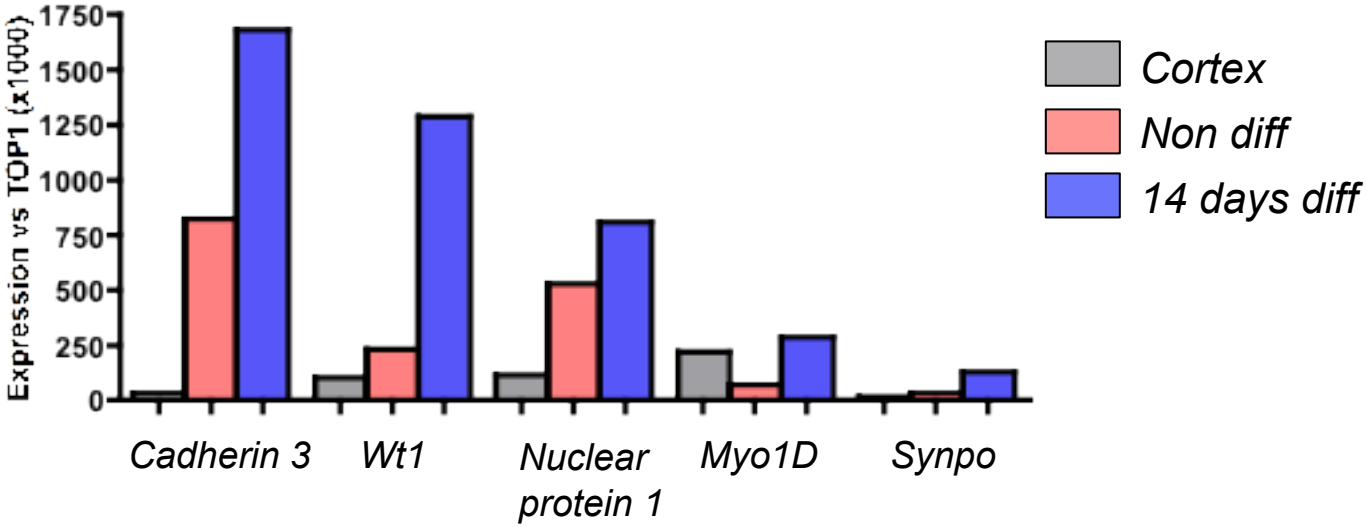
Plasmid and siRNA transfection

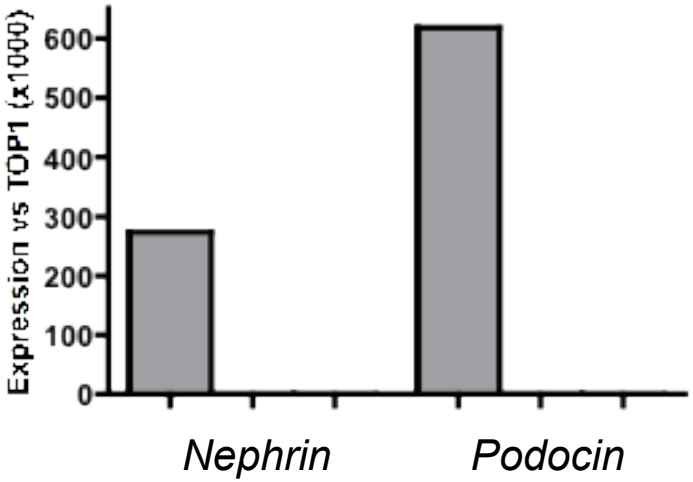
Stretching of podocytes





Expression profile of podocyte markers in mouse *cell line*

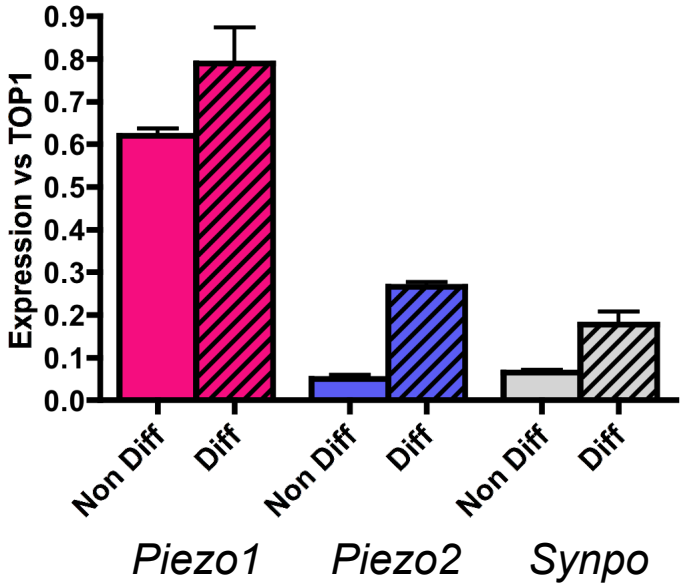




Expression profile of Piezo in podocyte *cell lines*

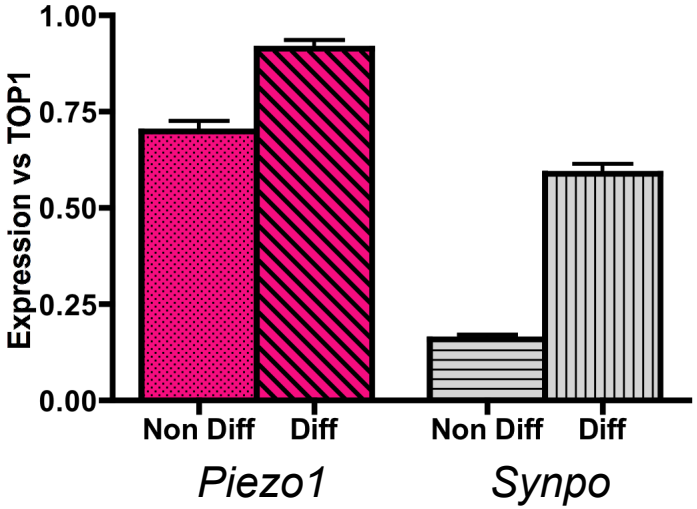


WTM-C3

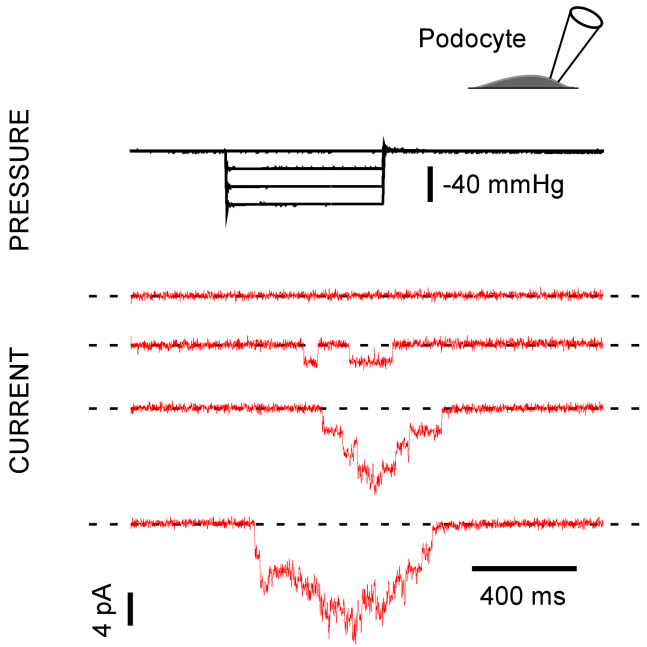


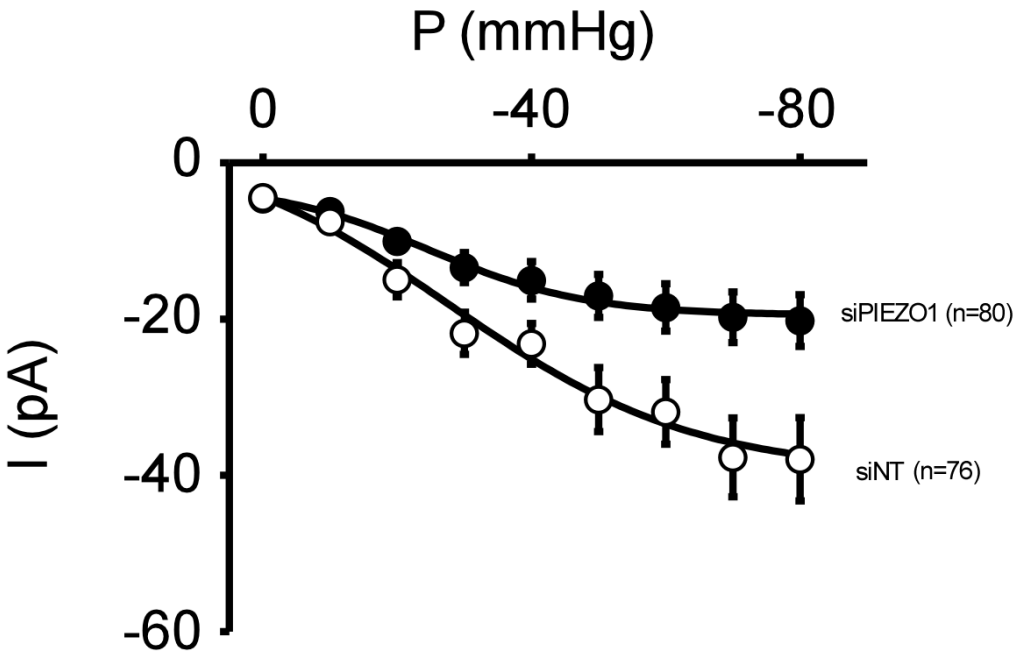


ATC



SACs in a mouse podocyte cell line

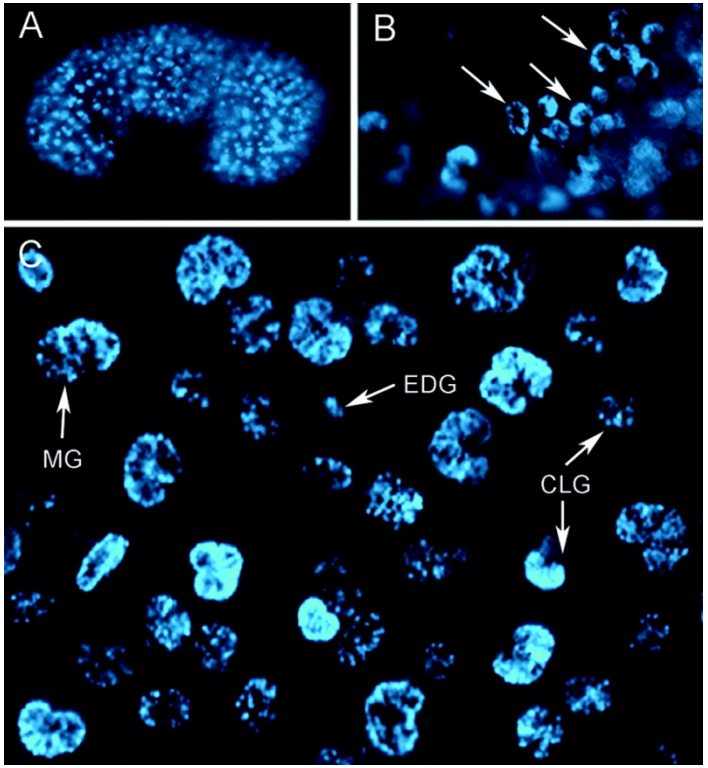




siPiezo1 (H1) effect on endogenous SACs in ***mouse podocyte cell line*** ***7 days of differentiation***

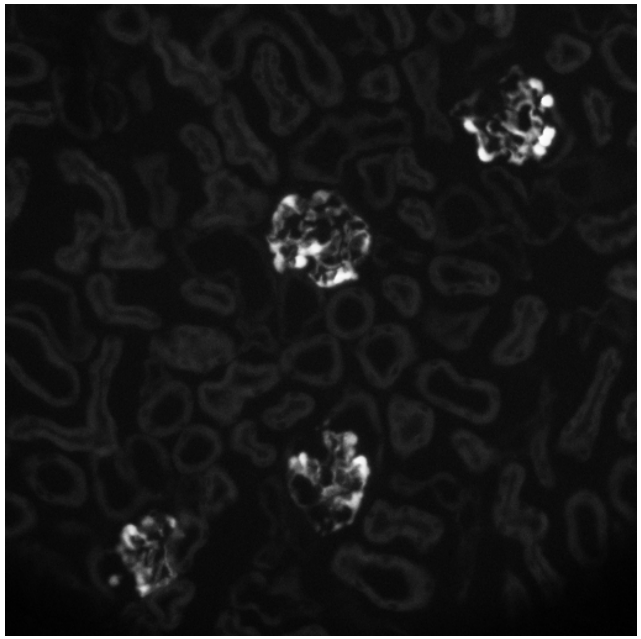
(Lipo 2000 57% efficiency)

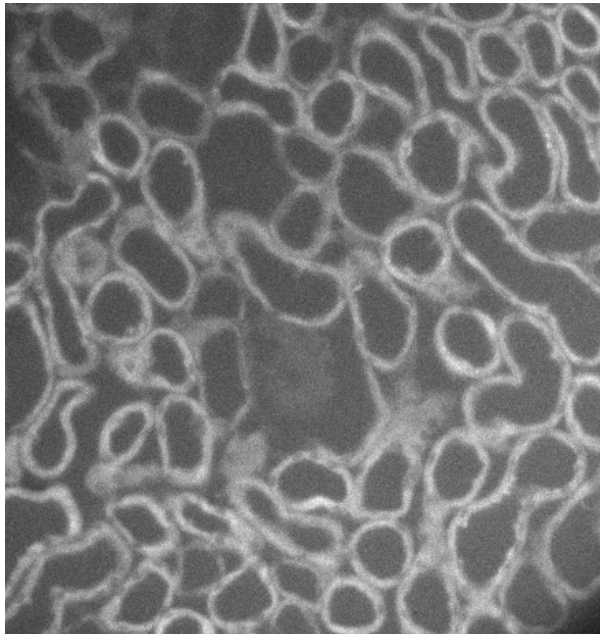
Nephrin-CFP mouse model

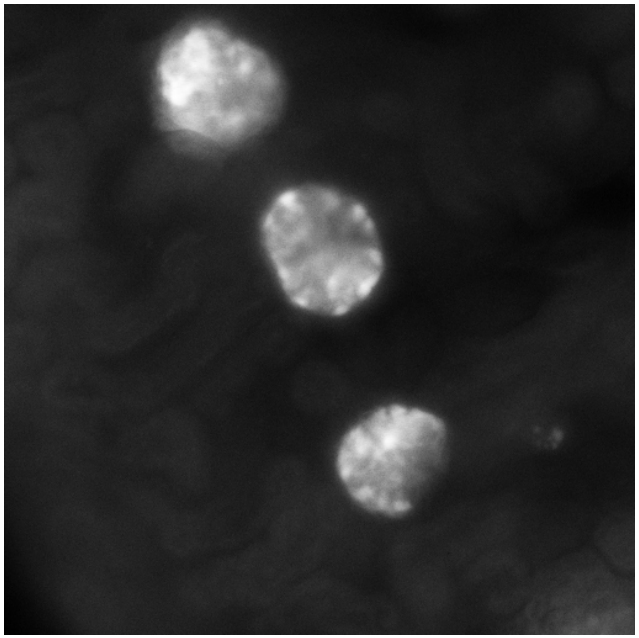


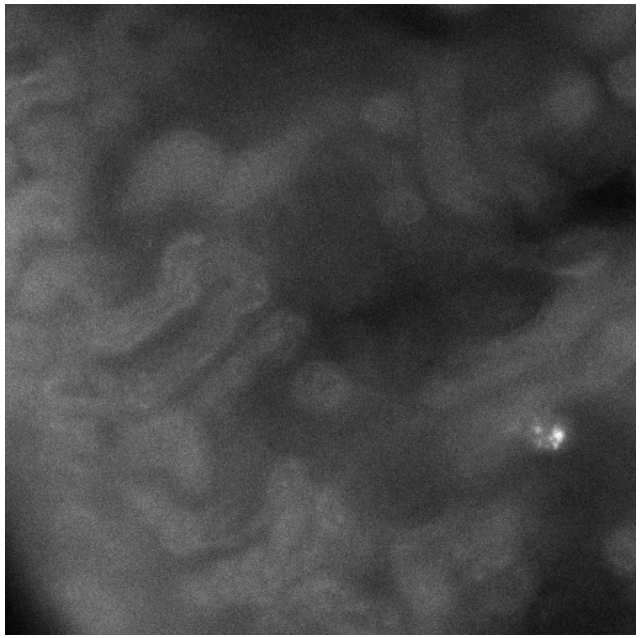
Cui S et al. JASN 2005;16:3247-3255



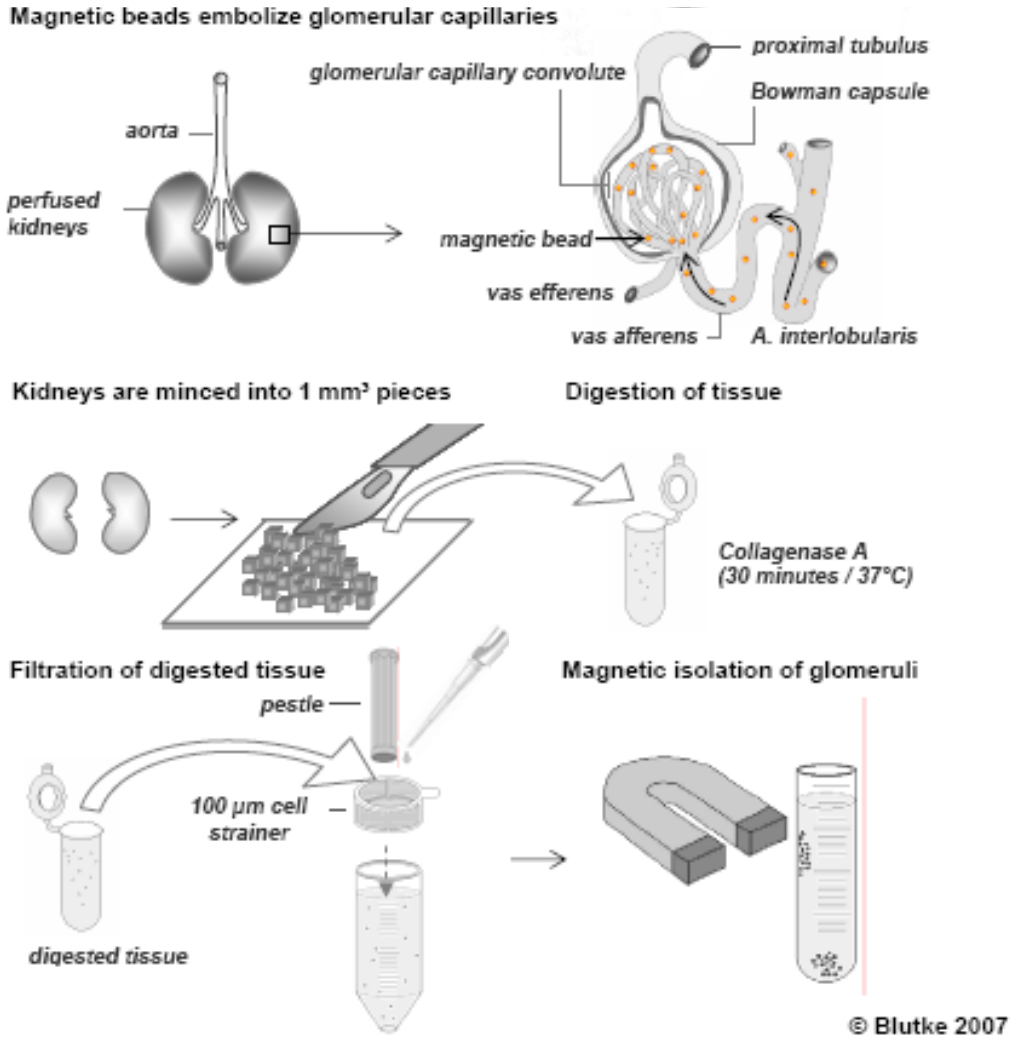


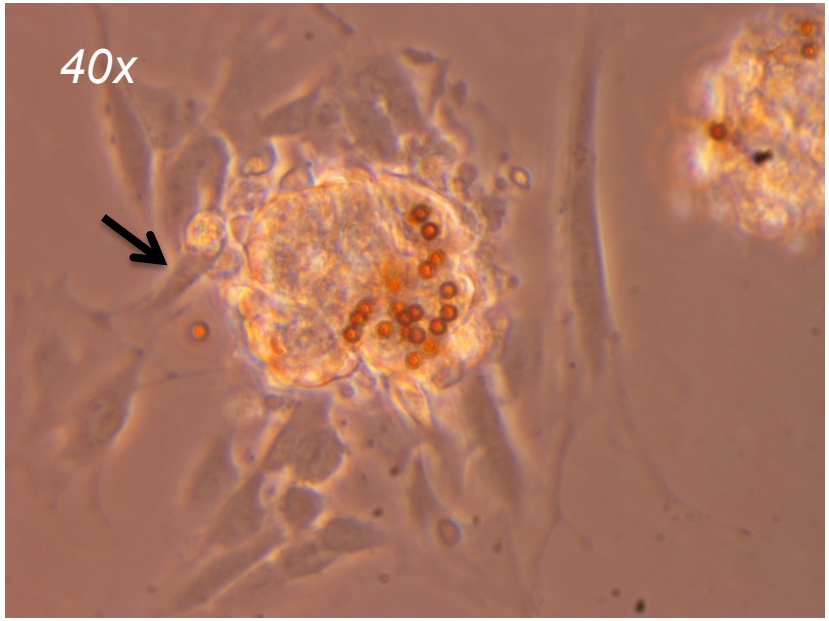


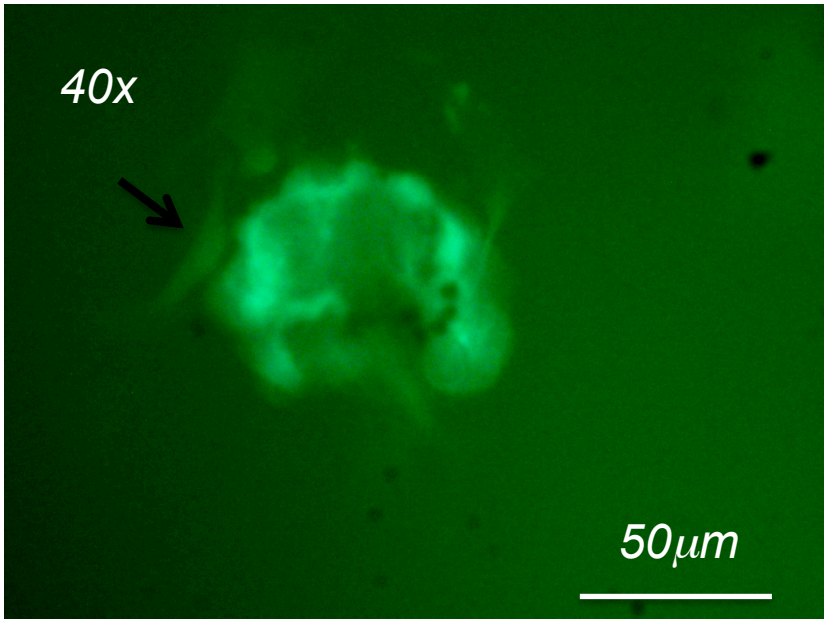


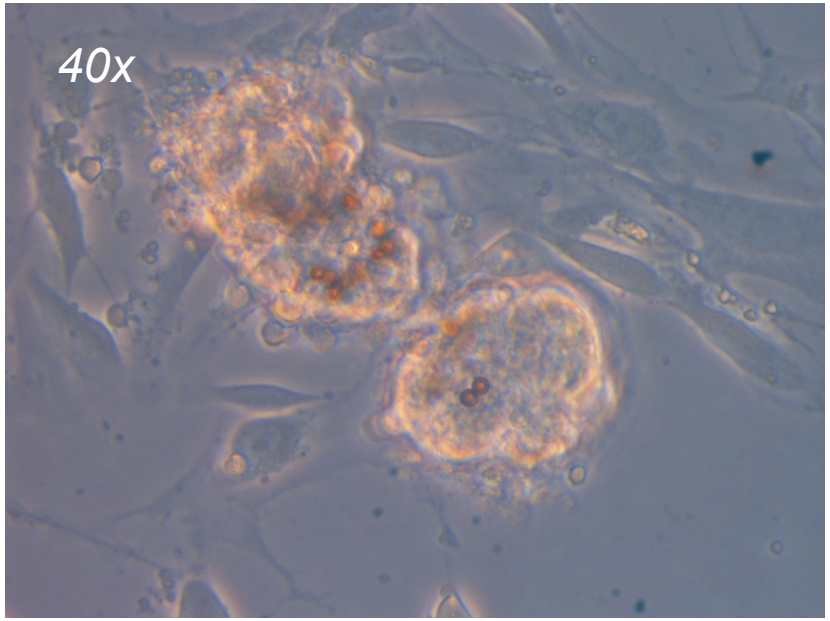


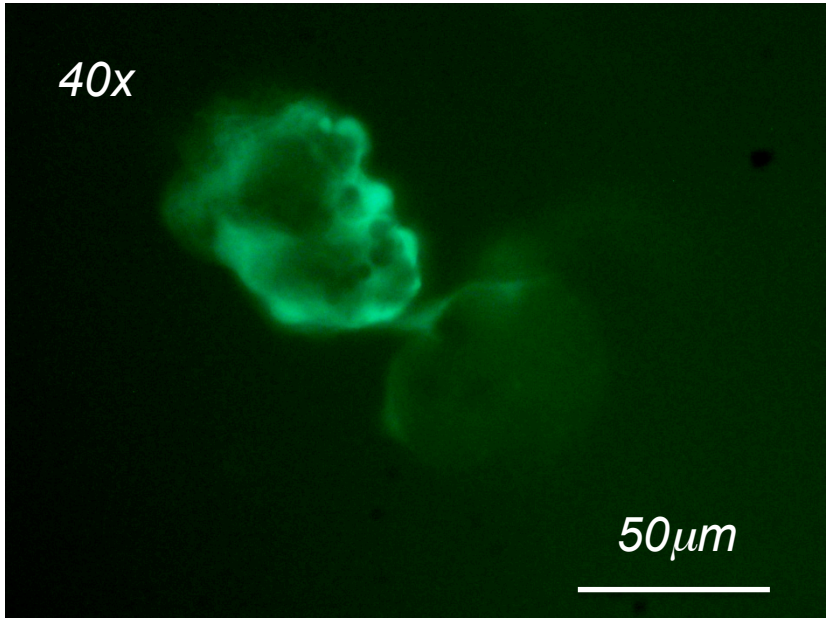
Glomeruli isolation



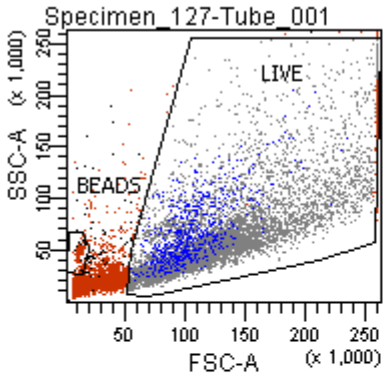

40x


40x
50μm


40x

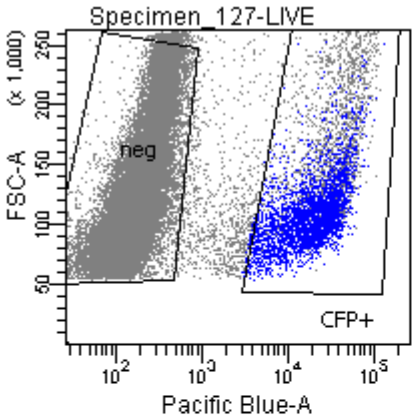

40x
50μm

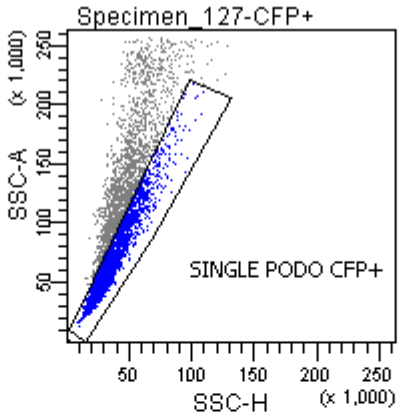
Sorting of CFP + cells

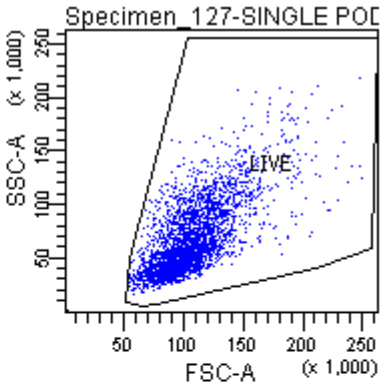


Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	59,421	####	100.0
PI-	56,896	95.8	95.8
LIVE	39,363	66.2	66.2
CFP+	4,706	12.0	7.9
SINGLE PODO CFP+	2,960	62.9	5.0
neg	33,091	84.1	55.7
BEADS	3,527	5.9	5.9

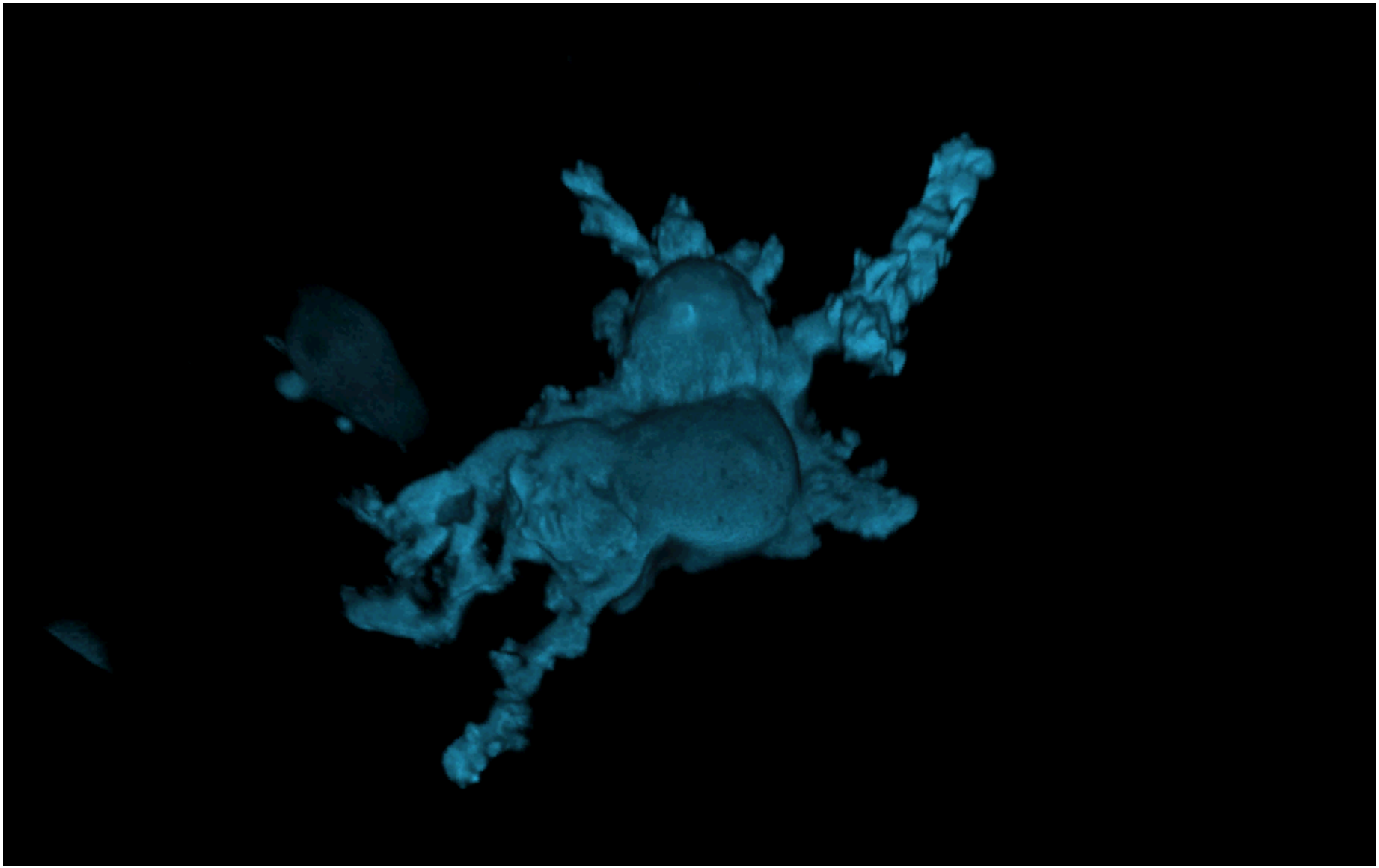






Specimen Name: Specimen_127

Population	Parent N...	%Parent	%Total	Pacific Bl... Geo Mean
CFP+	LIVE	12.0	7.9	24,418



Expression profile of CFP positive cells

