

ipmc

Les canaux ioniques dans les arythmies cardiaques

Sophie Demolombe

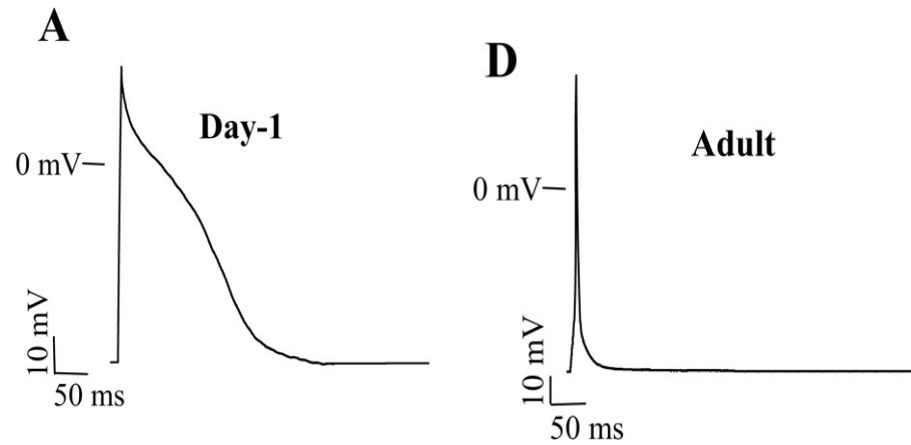
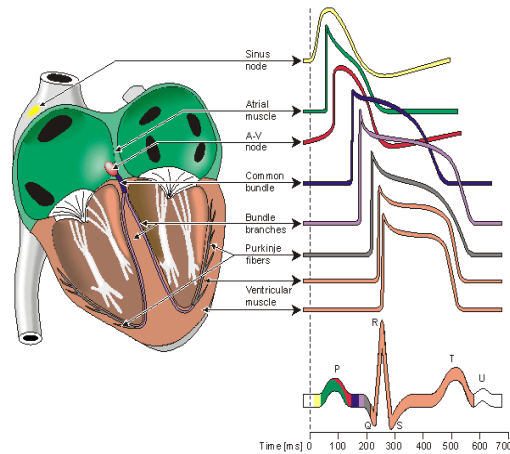
Institut de Pharmacologie Moléculaire et Cellulaire
CNRS UMR7275
Sophia-Antipolis



L'électrogenèse cardiaque

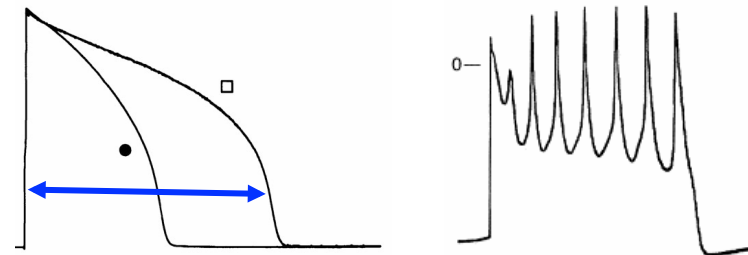
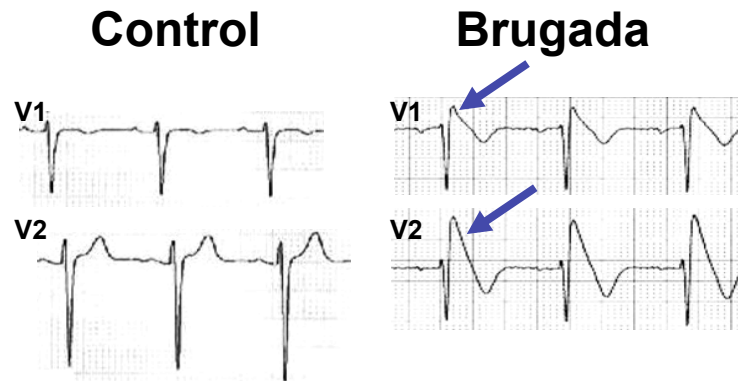
COMPLEXE ET MODULABLE

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



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

✓ **Conditions pathologiques:** mutations, antiarrhythmiques, ...

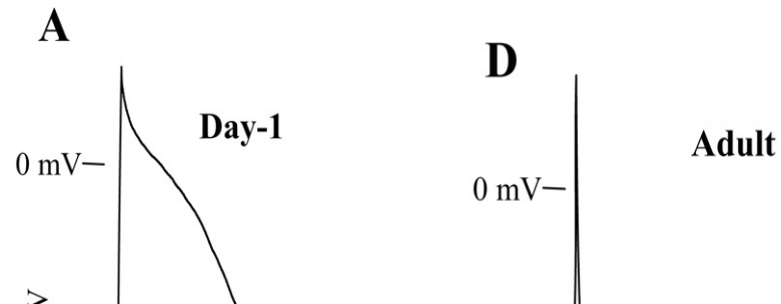
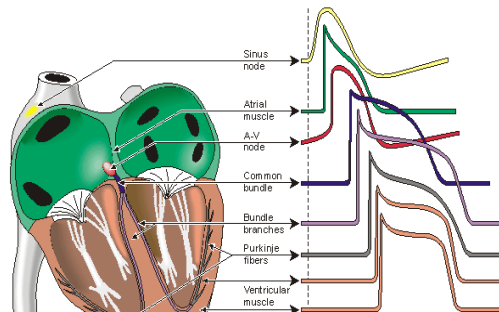


⇒ **Mort subite**

L'électrogenèse cardiaque

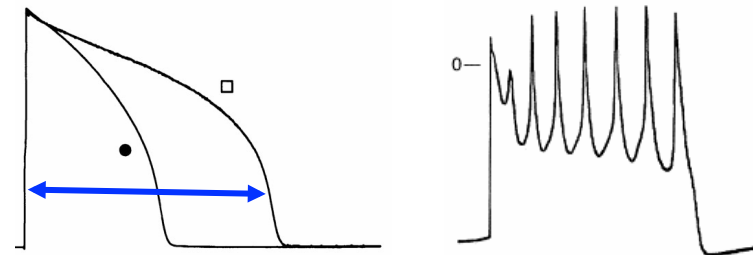
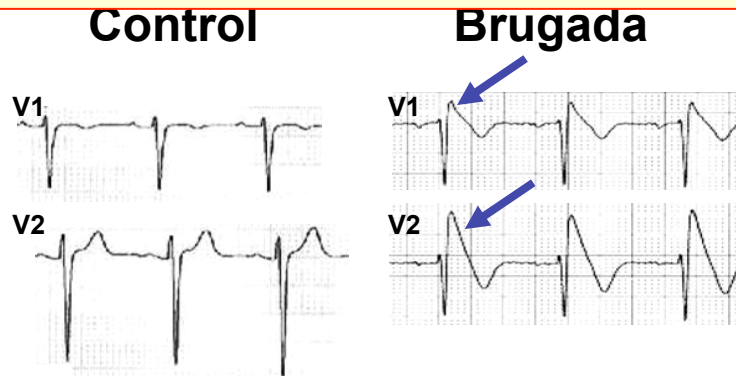
COMPLEXE ET MODULABLE

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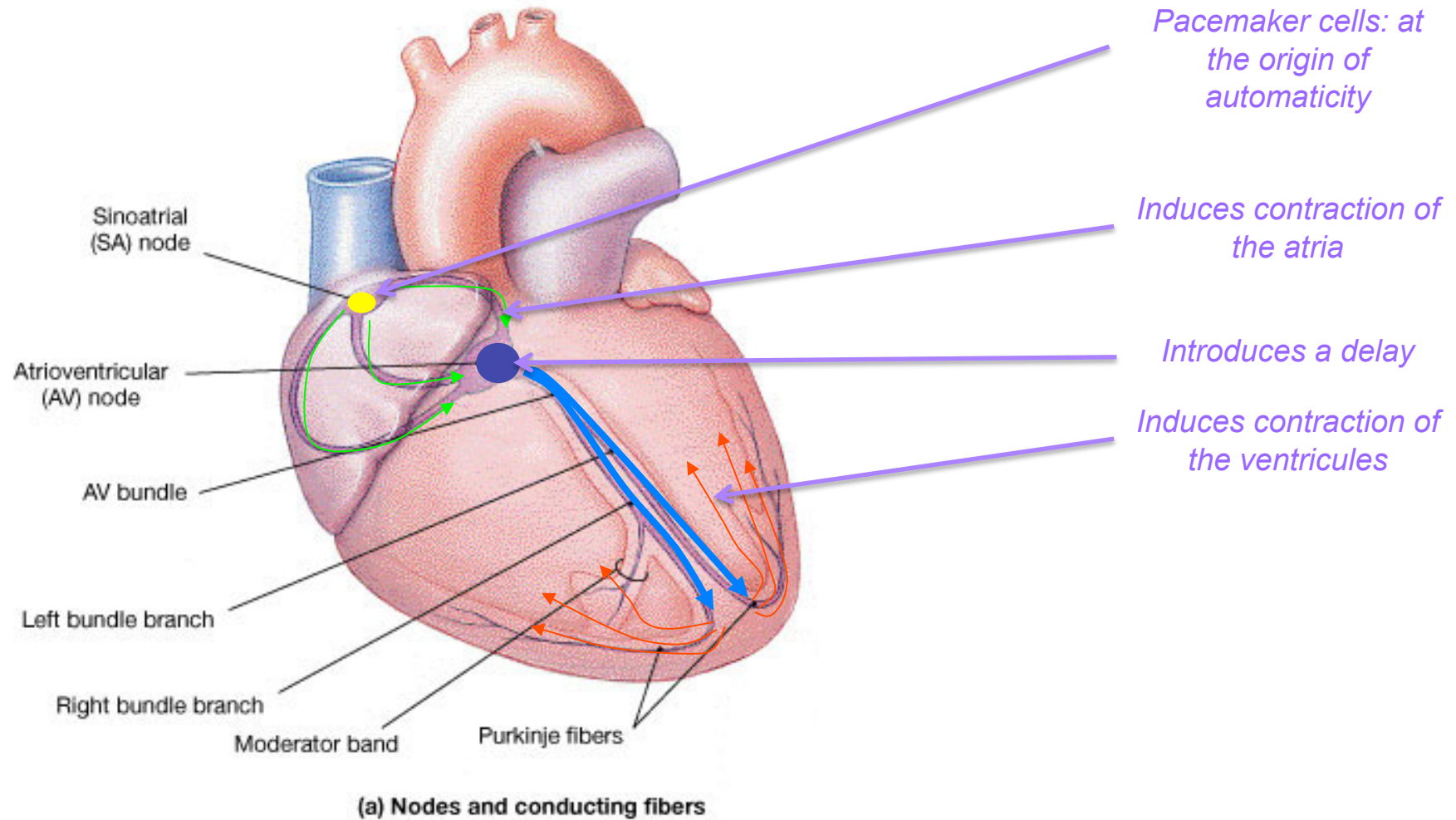
Objectif:
Comprendre les mécanismes fondamentaux qui sous-tendent l'électrogenèse cardiaque et son remodelage

✓ C

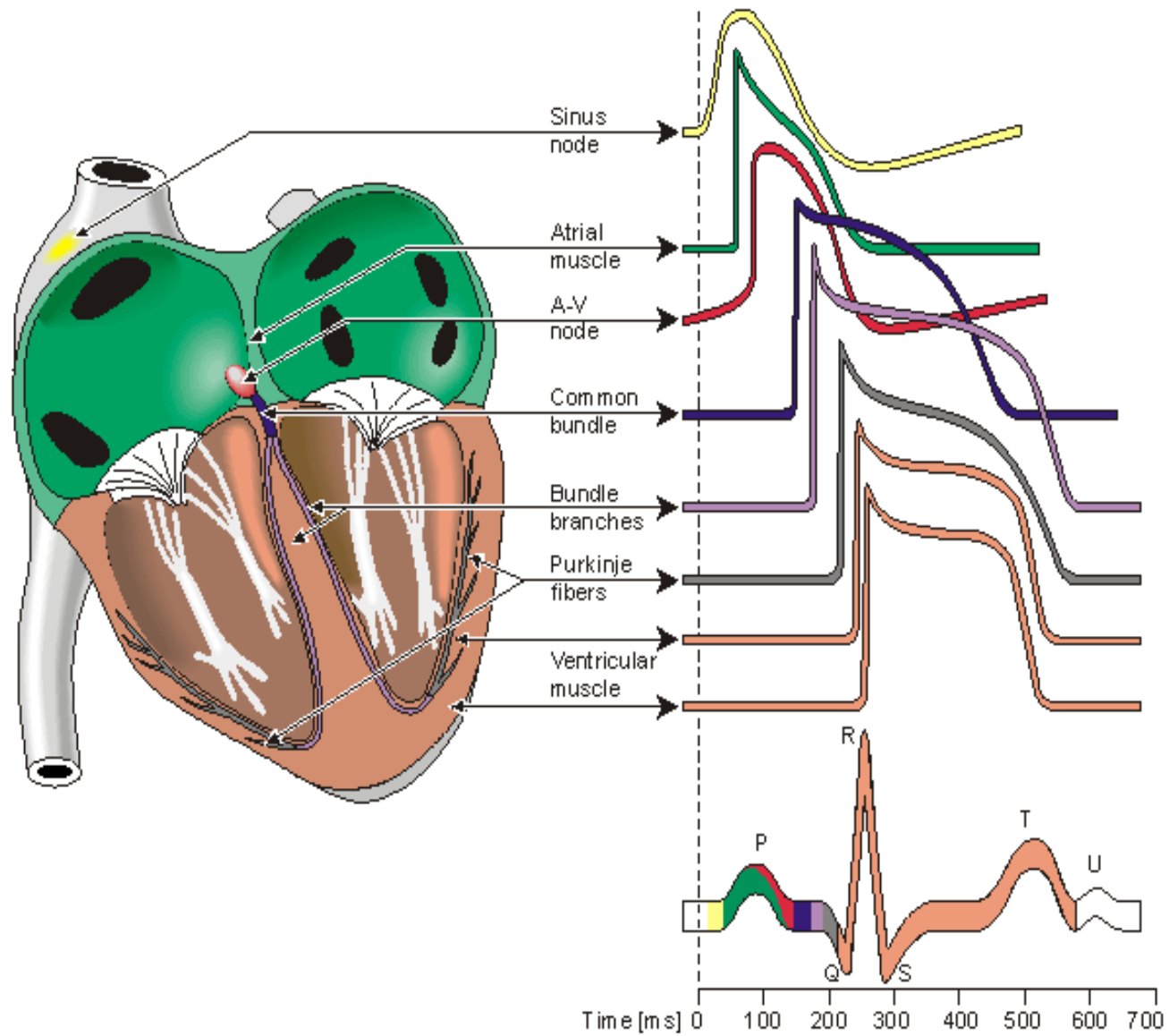


⇒ **Mort subite**

CONDUCTION DU SIGNAL ELECTRIQUE DANS LE COEUR



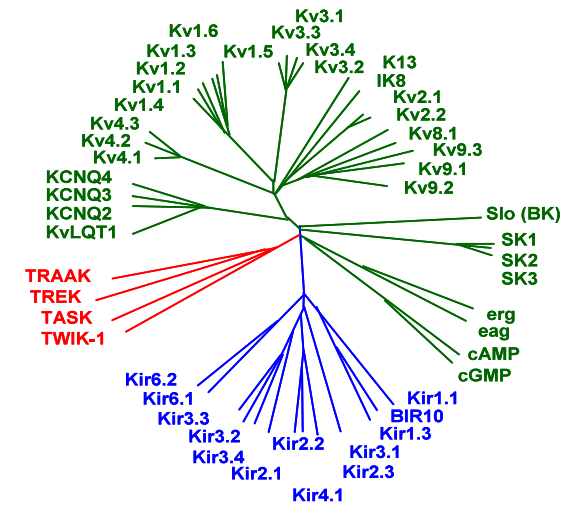
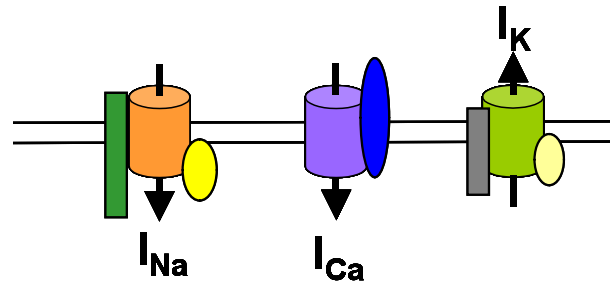
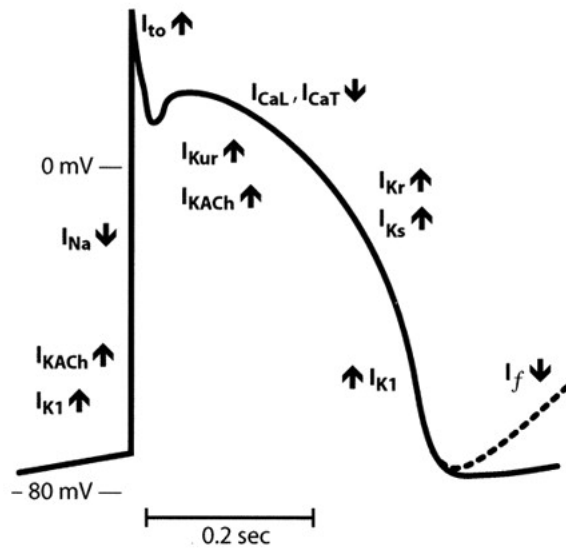
CONDUCTION DU SIGNAL ELECTRIQUE DANS LE COEUR

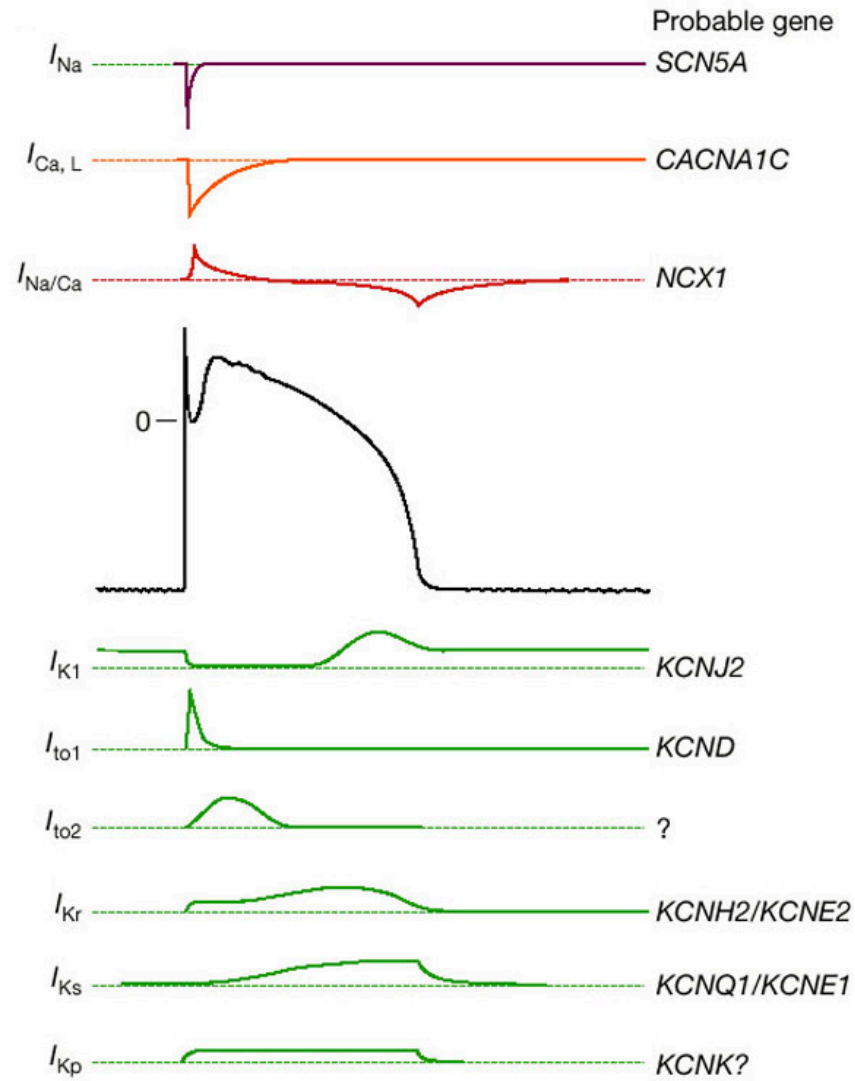


1/ La fonction électrique résulte de l'activité de nombreux courants

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

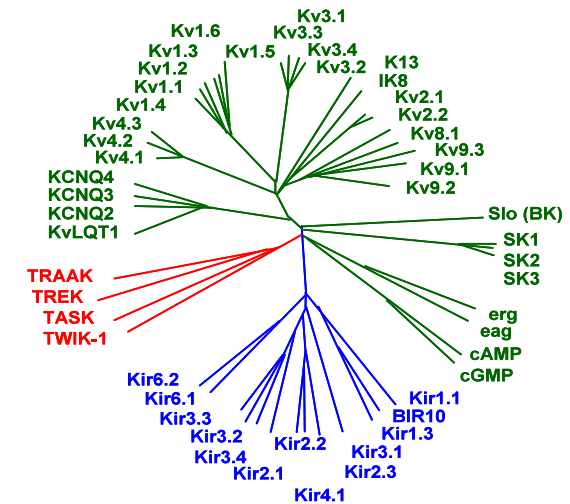
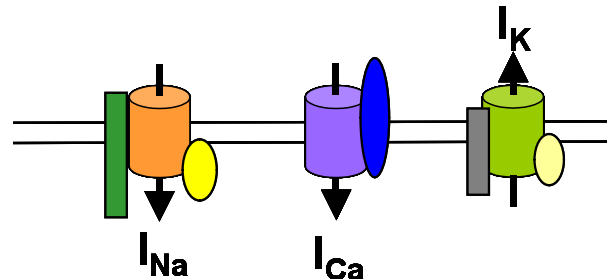
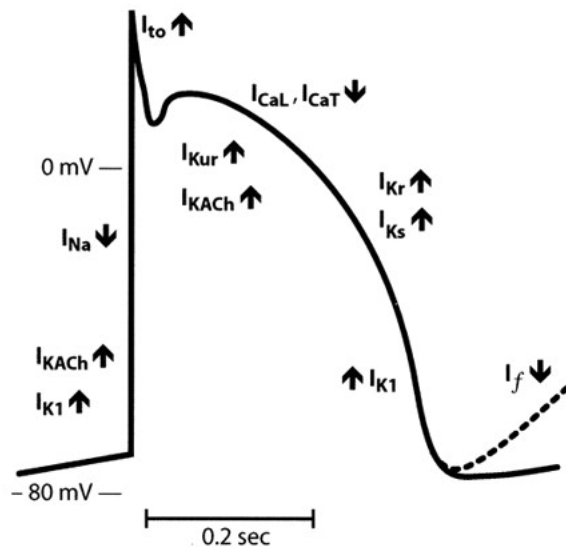




1/ La fonction électrique résulte de l'activité de nombreux courants

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

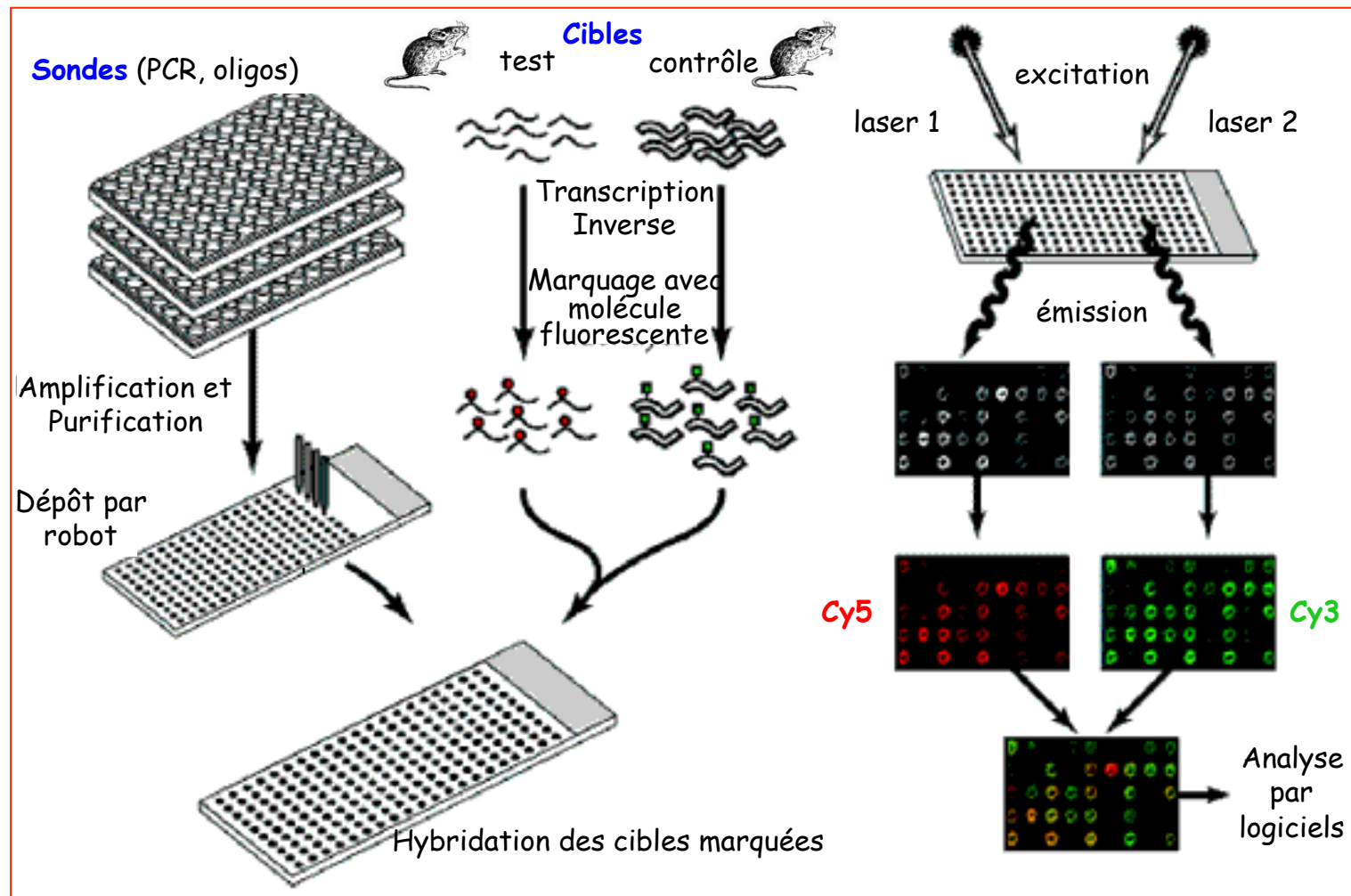
3/ Une famille de canaux est codée par de nombreux gènes



HYPOTHESE

L'expression coordonnée des canaux ioniques gouverne l'hétérogénéité et le remodelage de l'activité électrique cardiaque

1/ Développer des outils génomiques dédiés aux canaux ioniques: - les puces à ADN



Composition des puces dédiées à l'homéostasie ionique

Human

- 137 channel subunits
 - ⇒ (16 Na⁺, 22 Ca²⁺, 13 Cl⁻, 75 K⁺, 3 f/h, 6 TRPC...)
- 11 connexins
- 20 other genes



Mouse

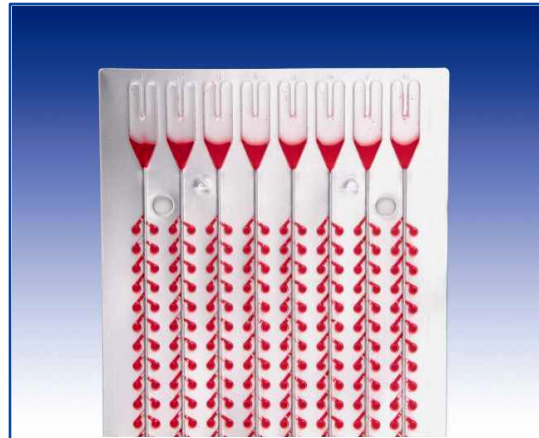
- 111 channel subunits
 - ⇒ (10 Na⁺, 19 Ca²⁺, 11 Cl⁻, 48 K⁺, 4 f/h, 7 TRPC...)
- 11 connexins
- 82 other genes



... + 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**

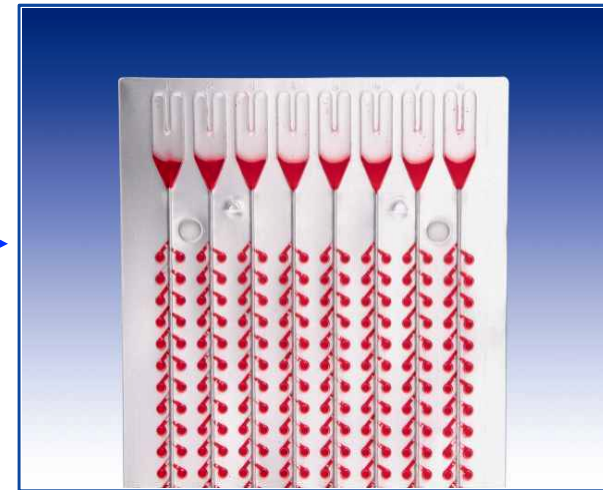
71 ion channel α - and β -subunits
10 Ca^{2+} regulators
1 ion channel expression regulator
6 specific markers of cardiac regions
1 marker of:
 vascular vessels
 neuronal tissue
 fibrosis
 inflammation
3 reference genes

Stratégie

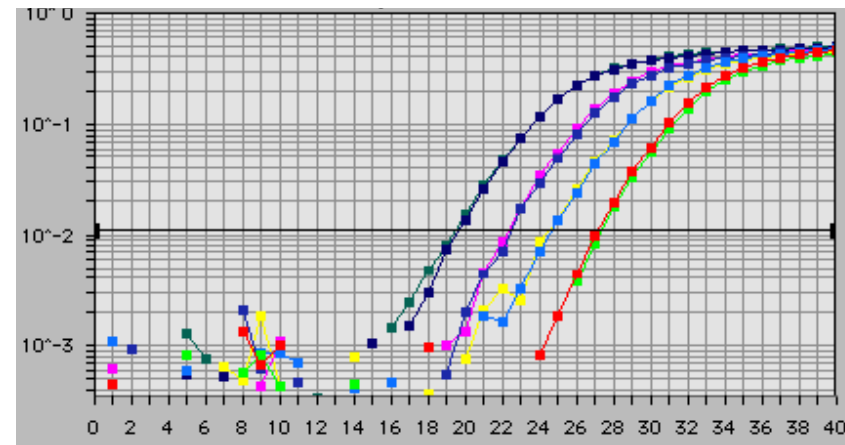
Loading a TLDA reservoir
with 100ng cDNA



cDNA distribution into each
well by centrifugation

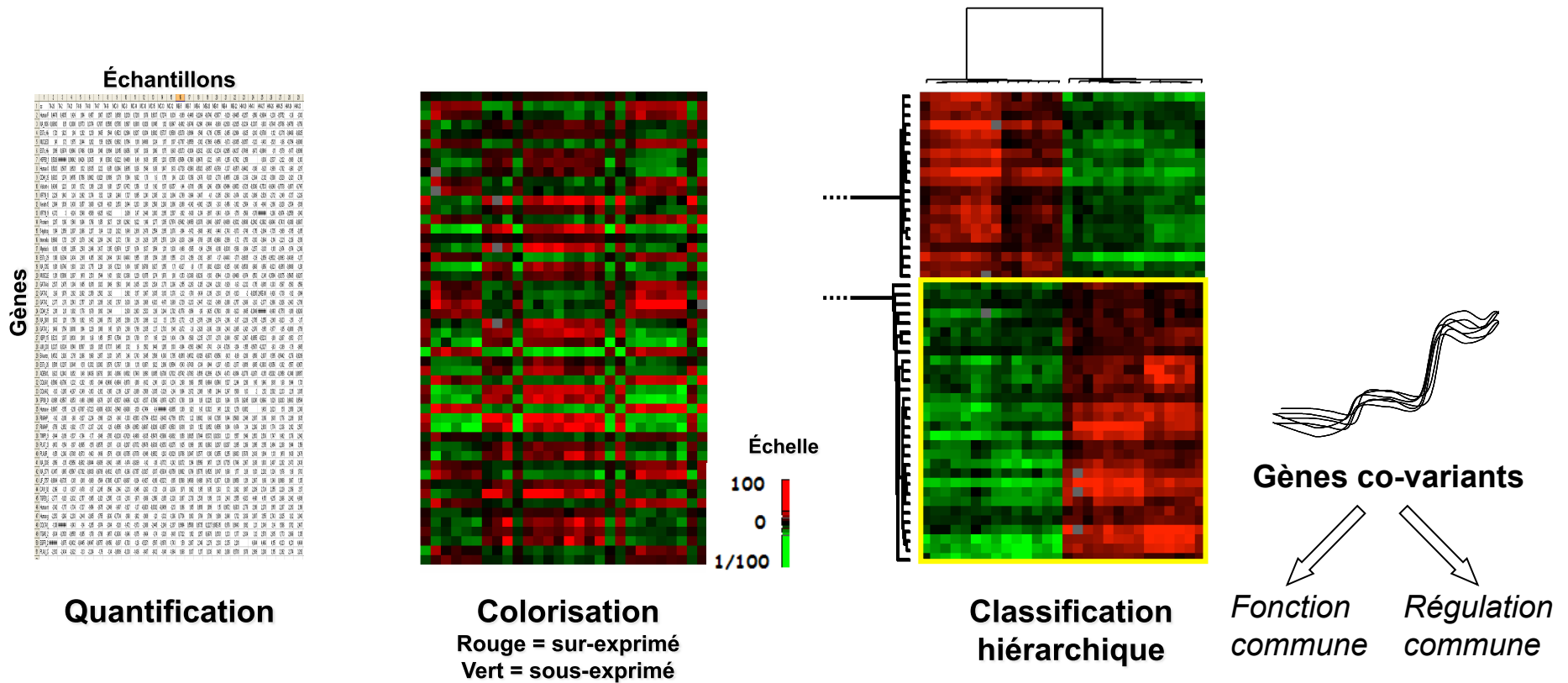


PCR using ABI PRISM[®] 7900HT
Sequence Detection System

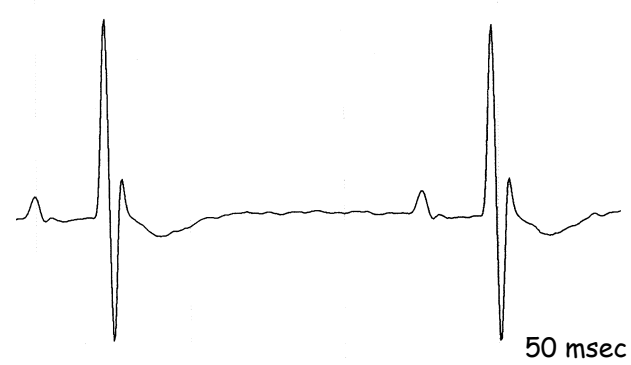
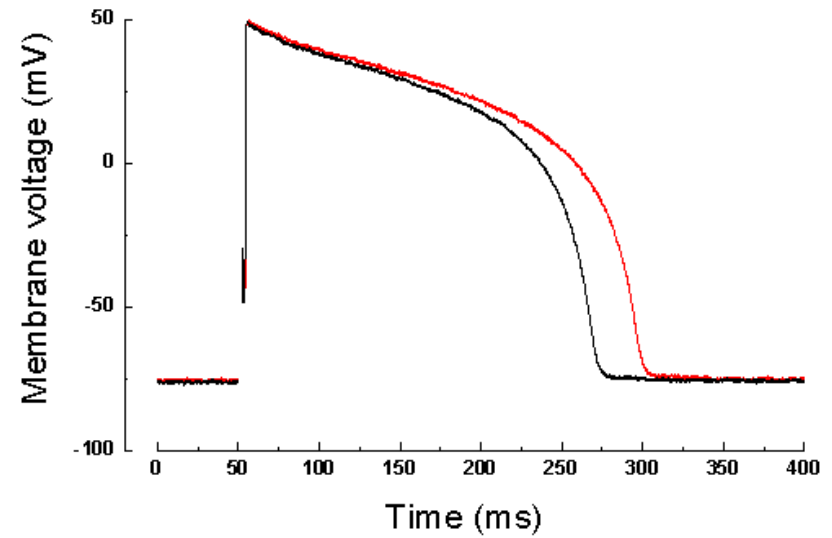
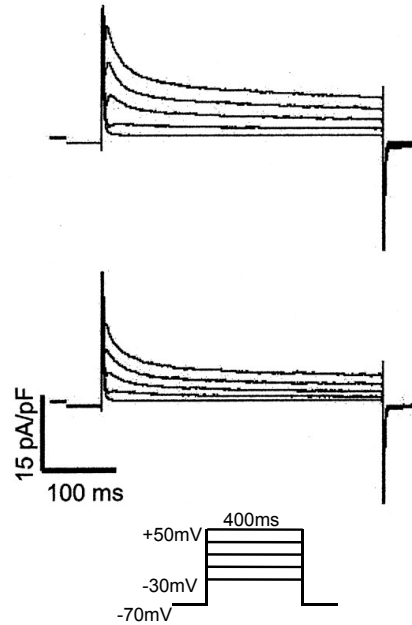
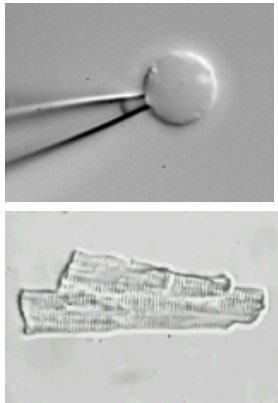


Data collection

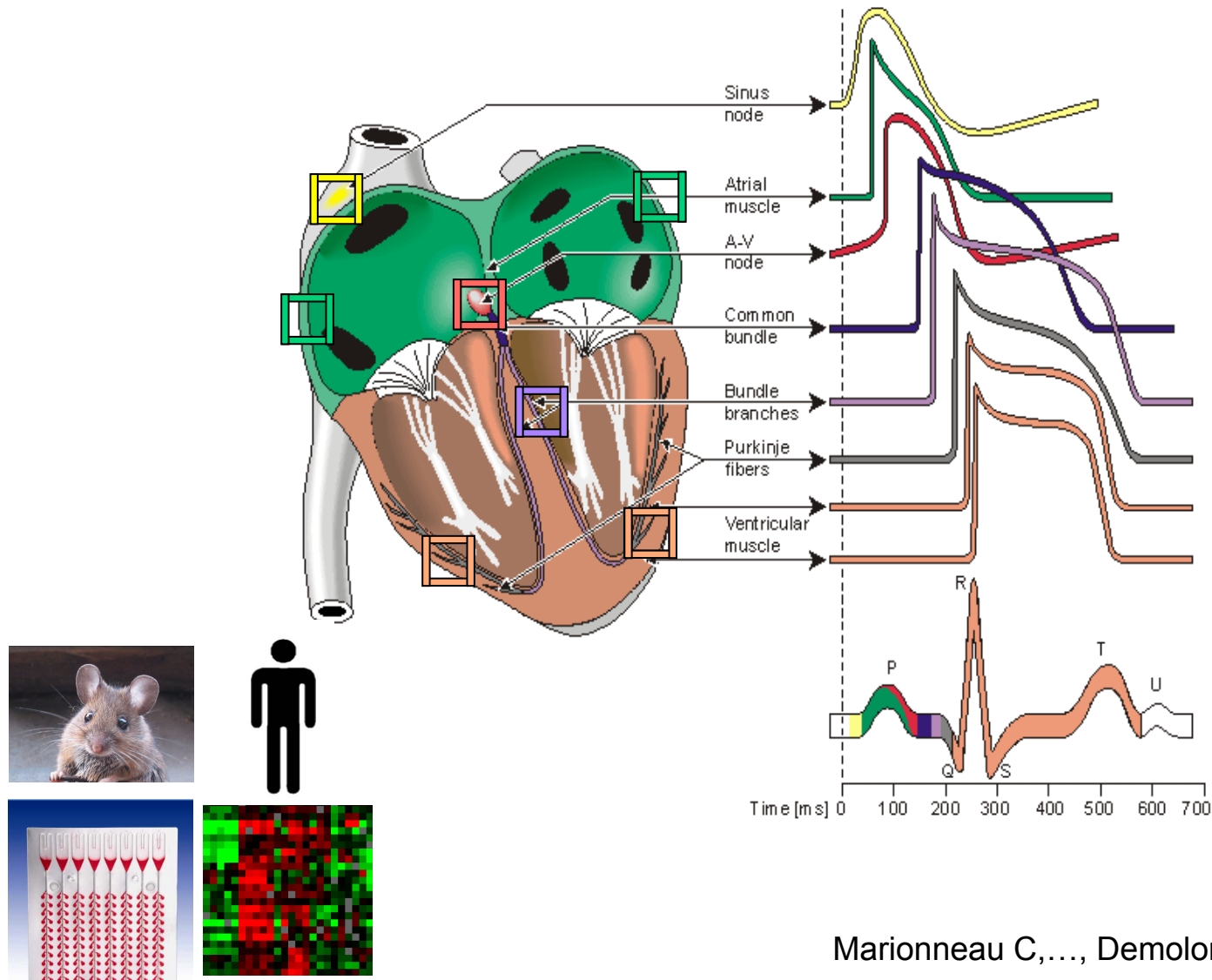
1/ Analyse des données génomiques : Classification hiérarchique



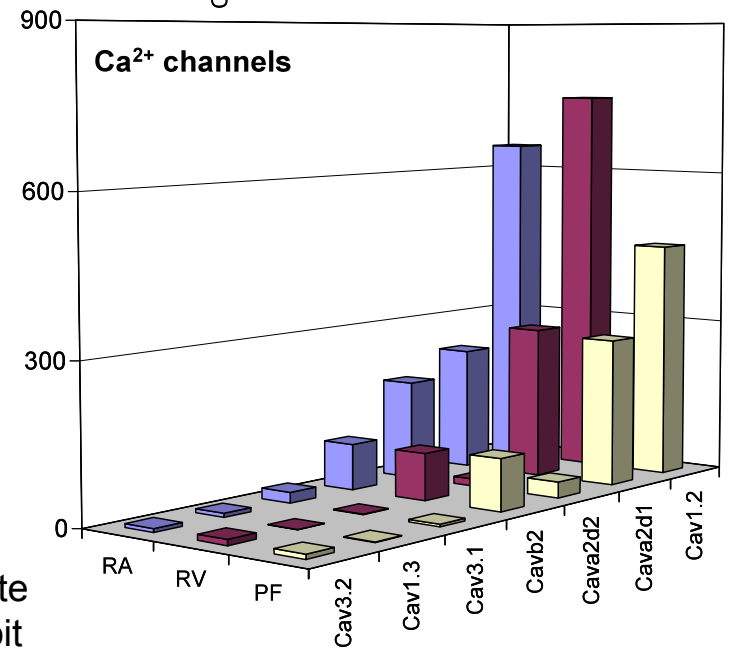
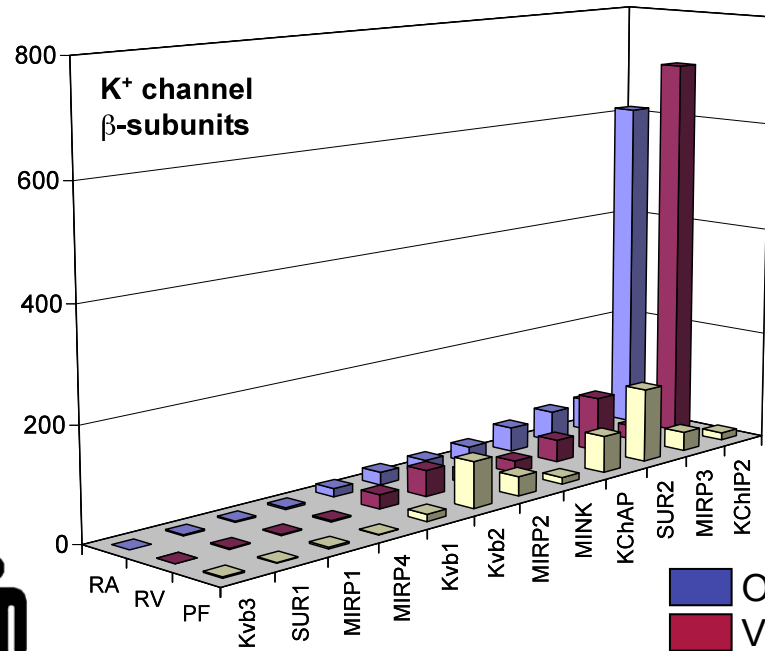
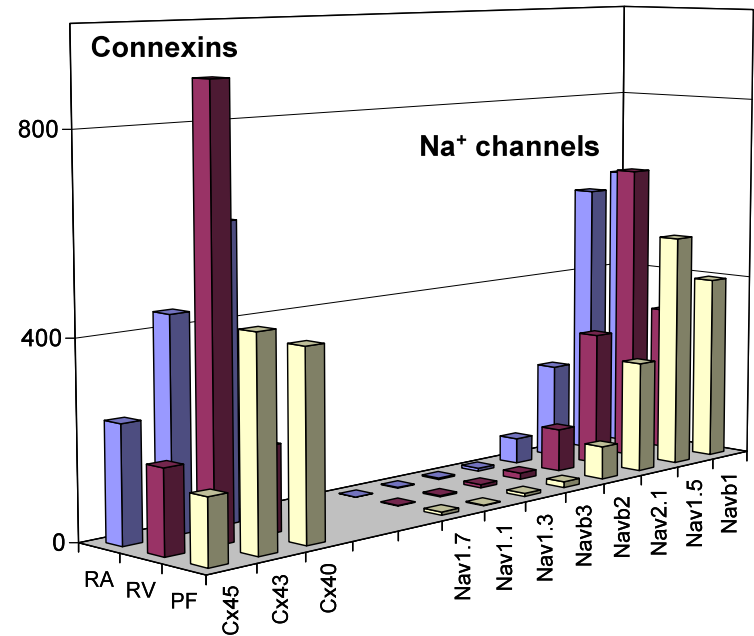
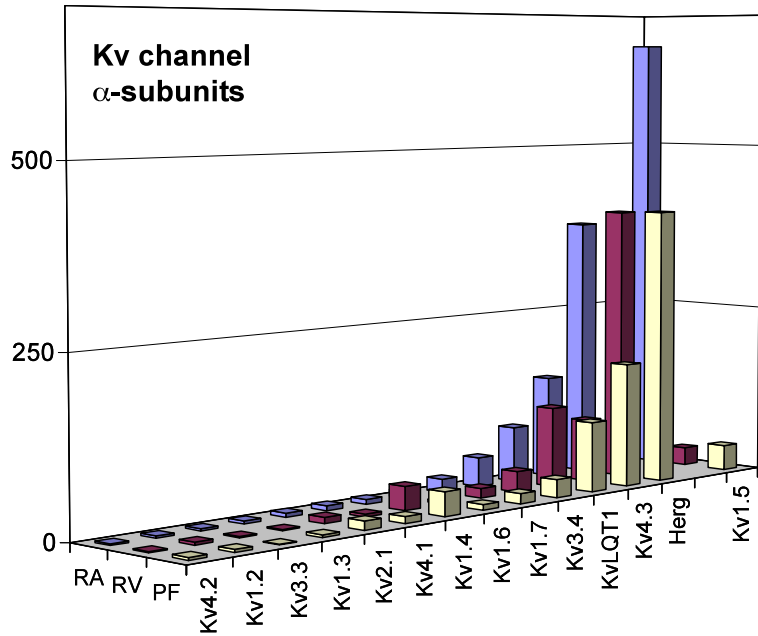
2/ Corréler l'expression transcriptionnelle au phénotype électrophysiologique



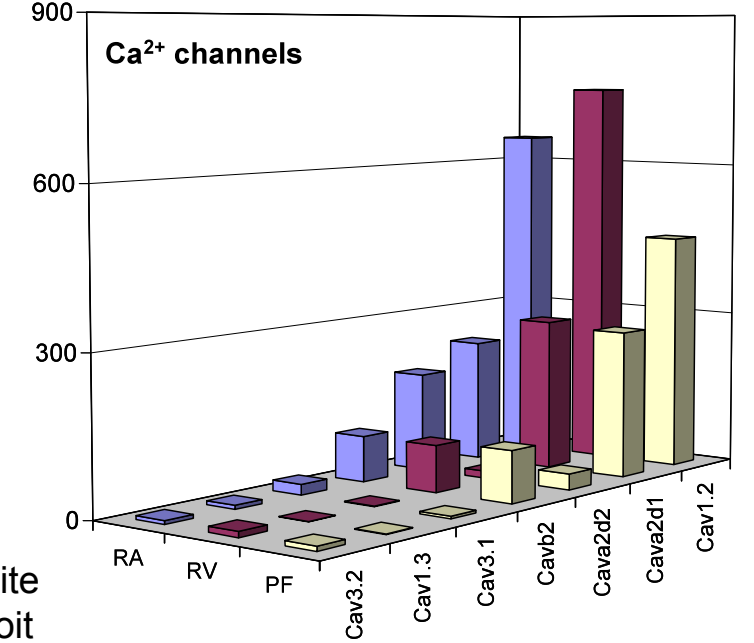
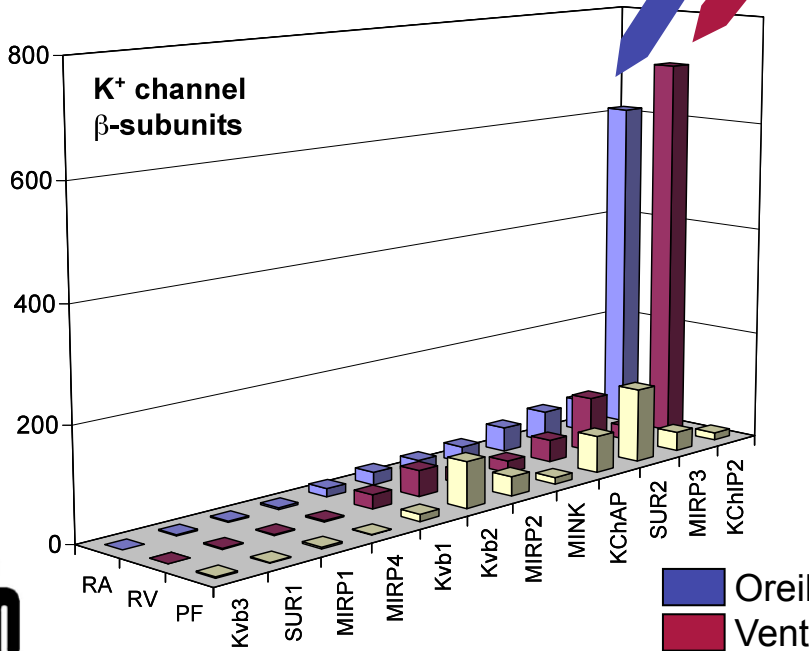
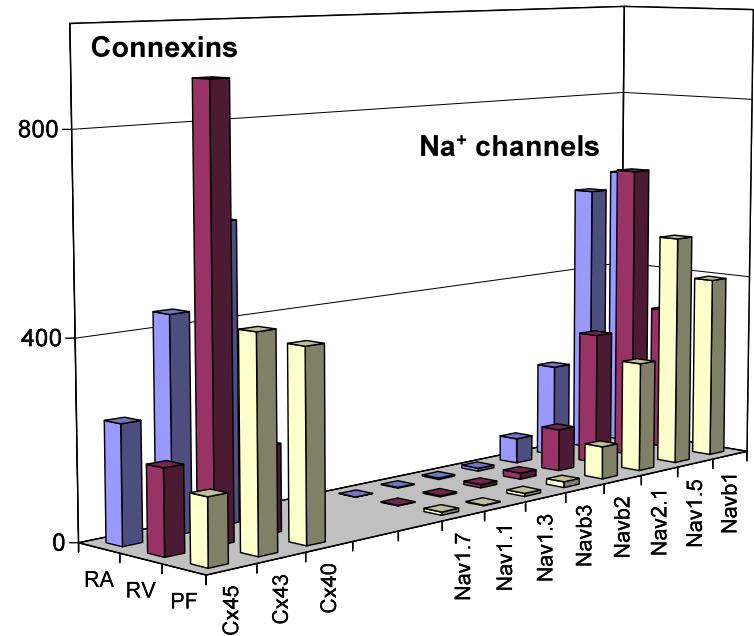
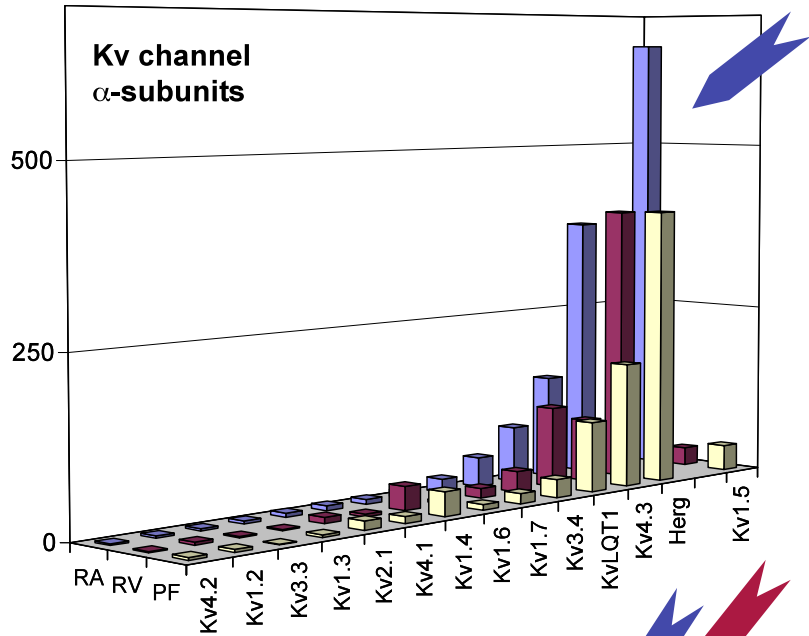
Application 1 : l'hétérogénéité électrique intrinsèque du cœur et la génomique des canaux ioniques



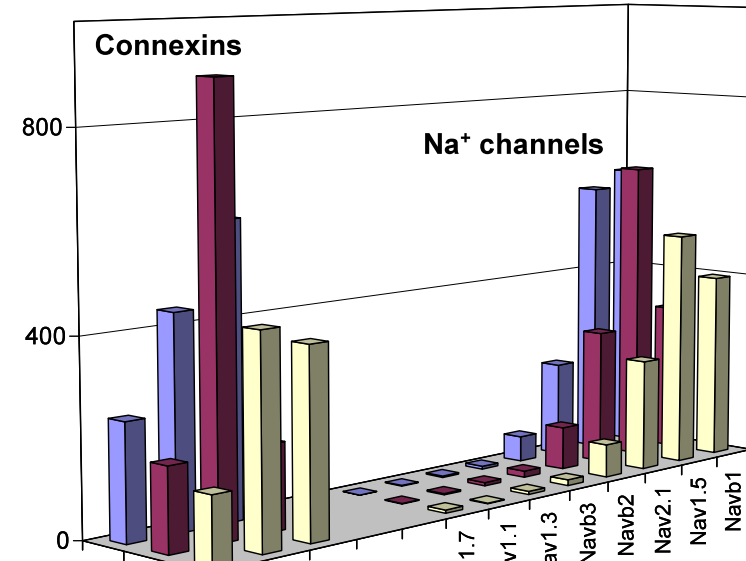
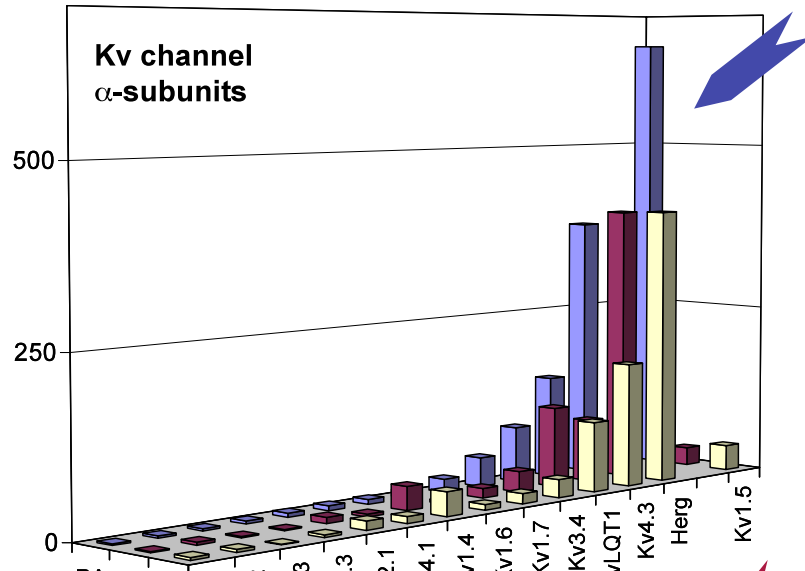
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Gaborit N, ..., Demolombe S. *J. Physiol.* 2007.



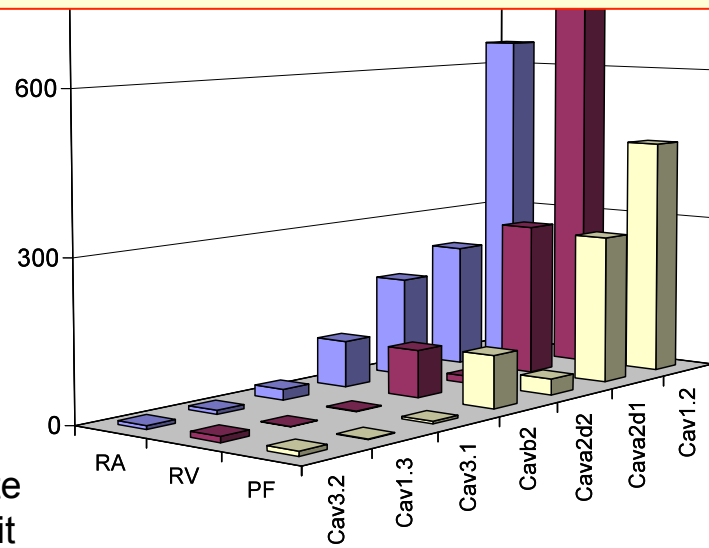
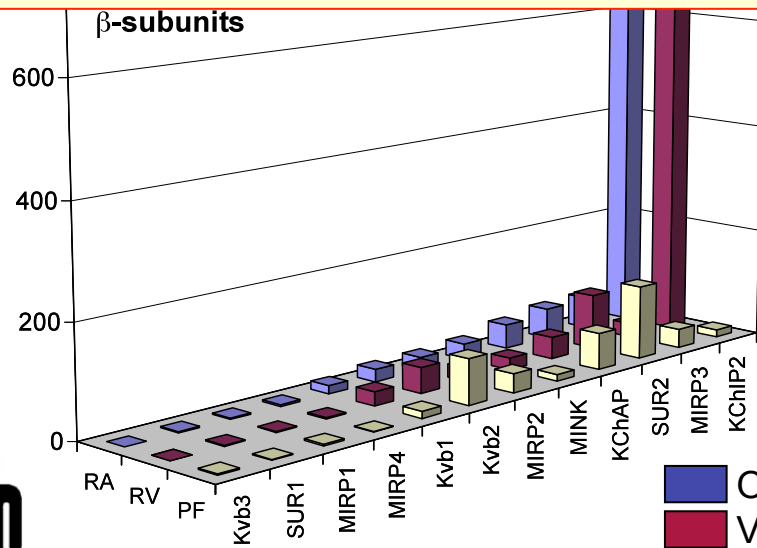
■ Oreillette droite
■ Ventricule droit
■ Fibres de Purkinje



■ Oreillette droite
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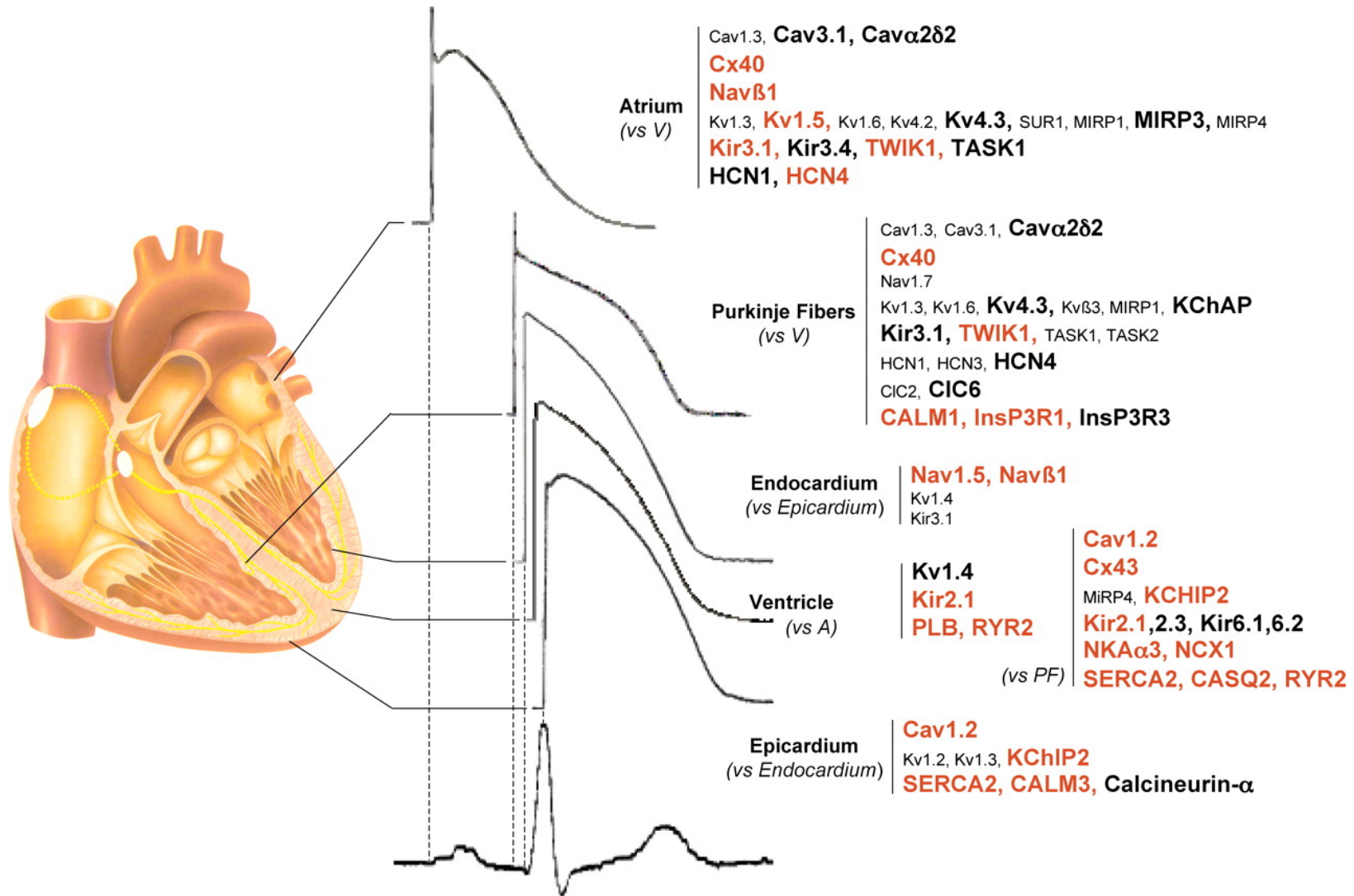


→ Chaque région cardiaque a une signature transcriptionnelle qui assure sa spécificité électrogénique.

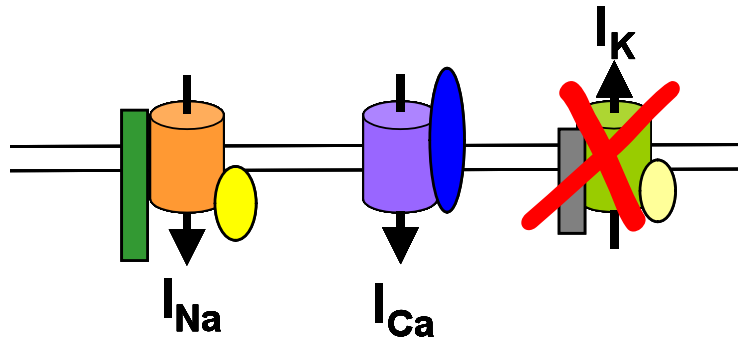


■ Oreillette droite
■ Ventricule droit
■ Fibres de Purkinje

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

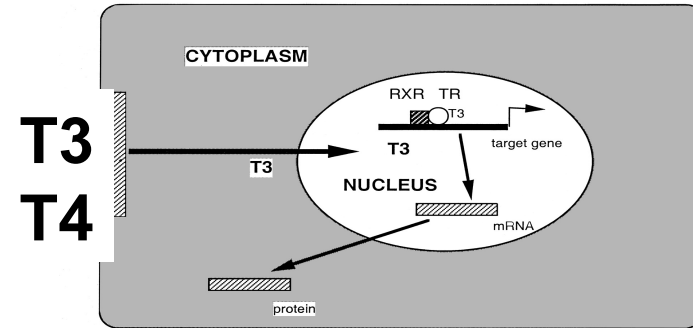
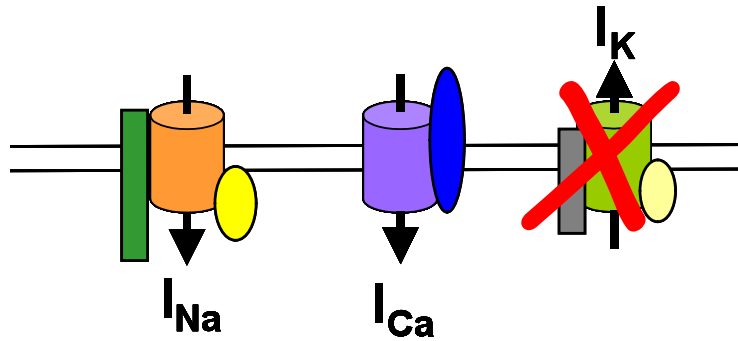


Application 2 : l'antiarrhythmique amiodarone et la génomique des canaux ioniques



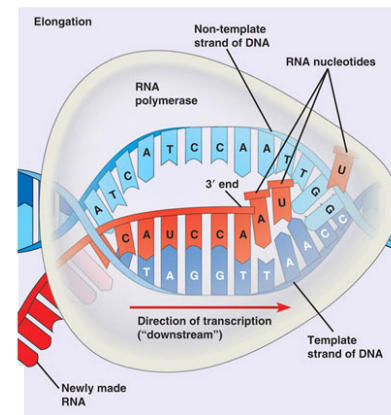
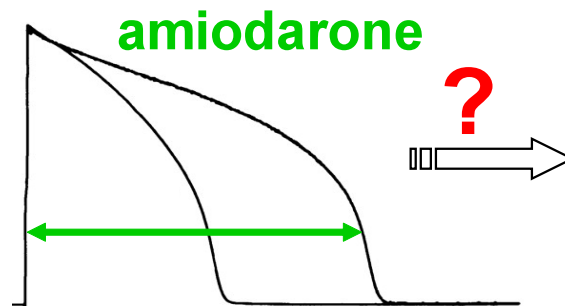
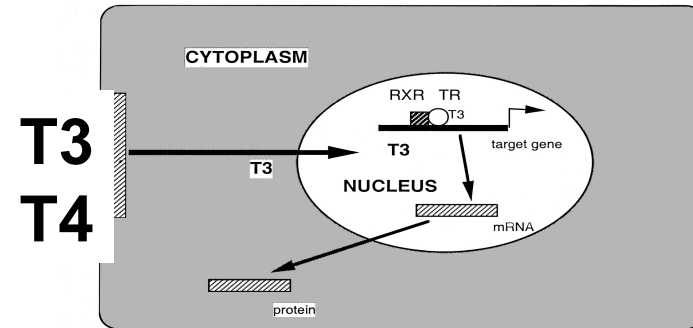
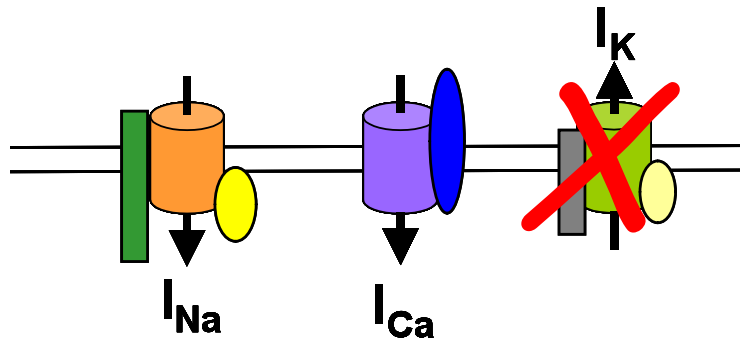
Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,..., Demolombe S. *Circulation.* 2004.

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



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



Transcrits des canaux ioniques

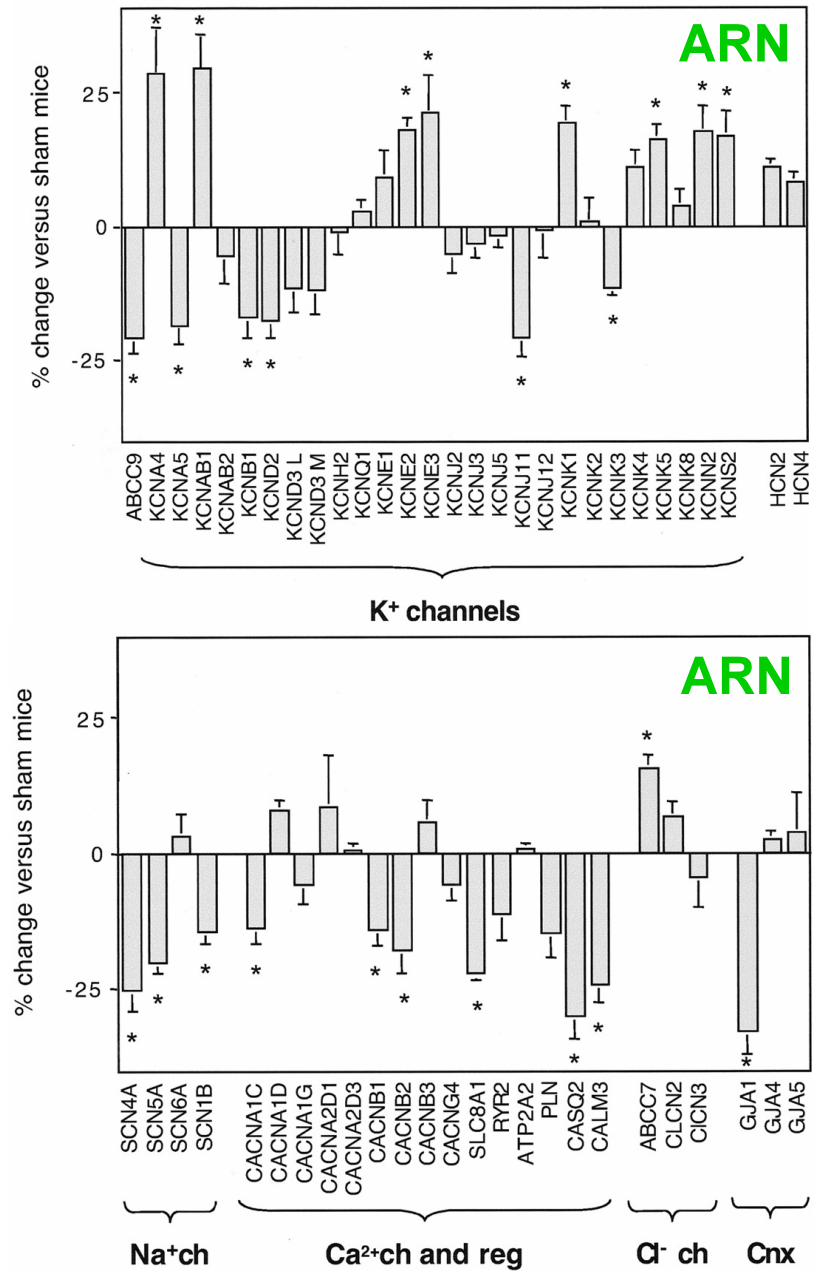


Souris adultes mâles C57BL/6

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

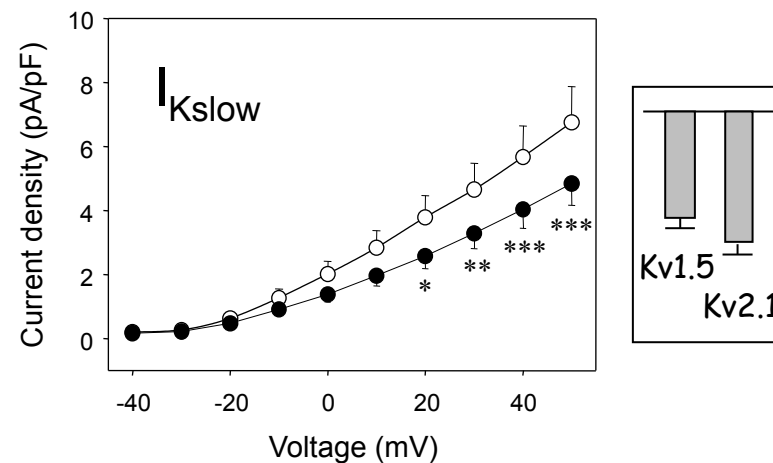
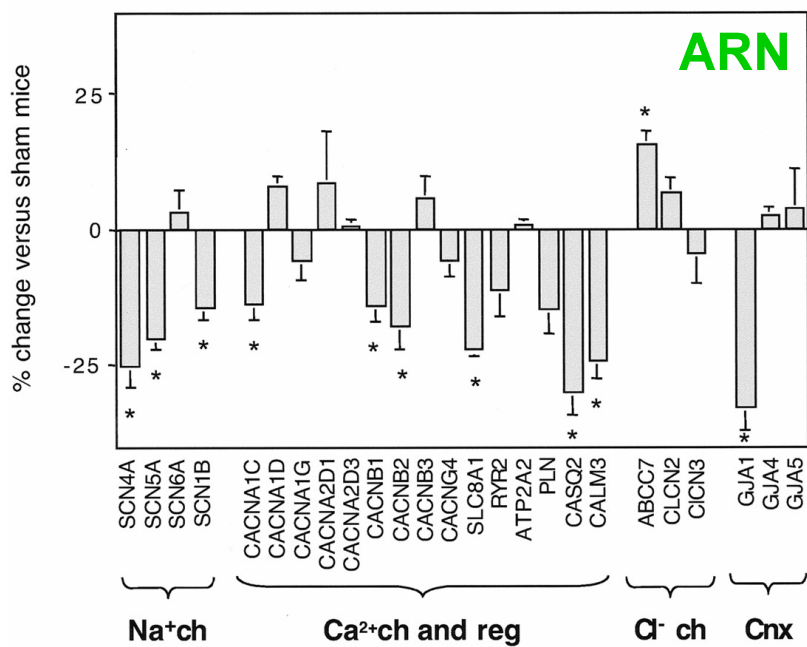
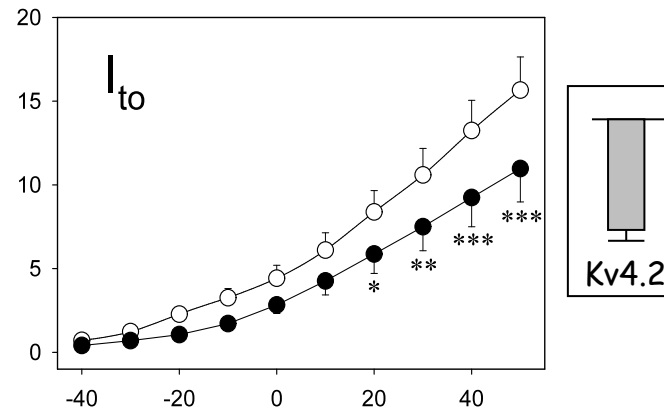
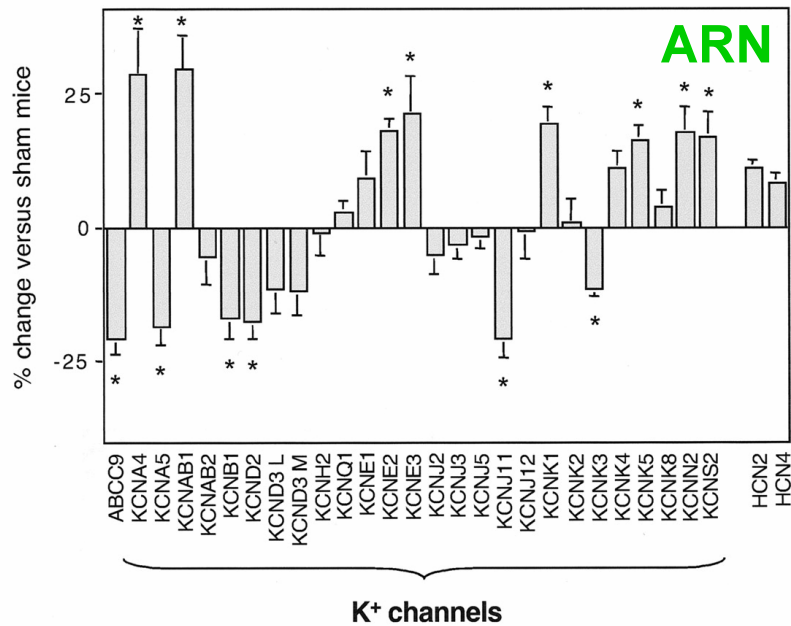
Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
Le Bouter S,..., Demolombe S. *Circulation.* 2004.

Expression des canaux ioniques et amiodarone



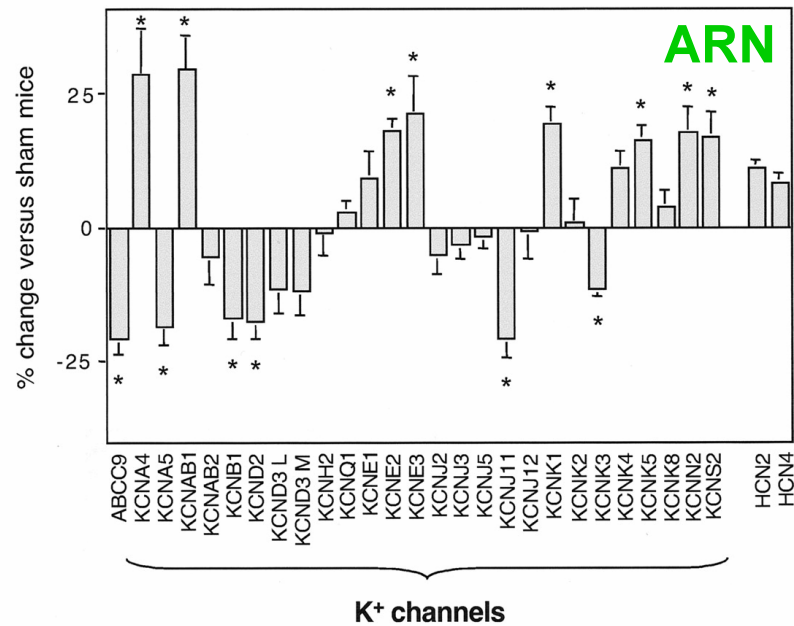
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 Le Bouter S, ..., Demolombe S. *Circulation.* 2004.

Expression des canaux ioniques et corrélations fonctionnelles

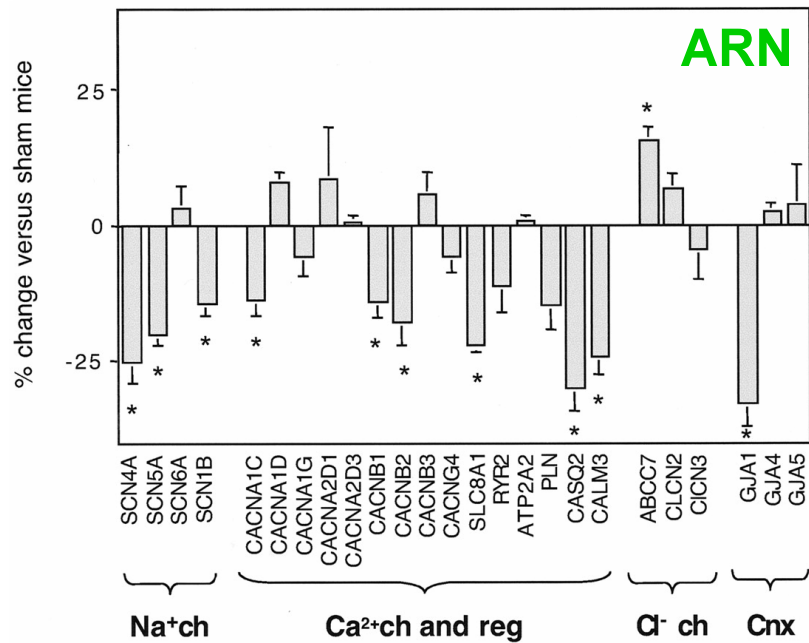
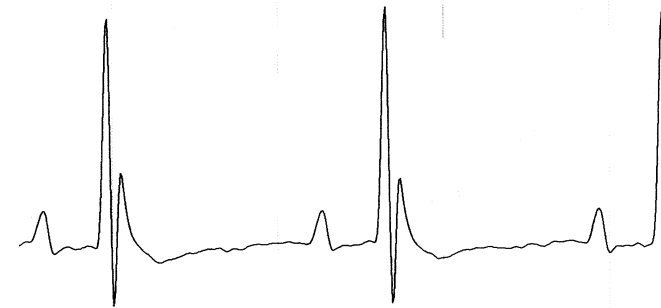


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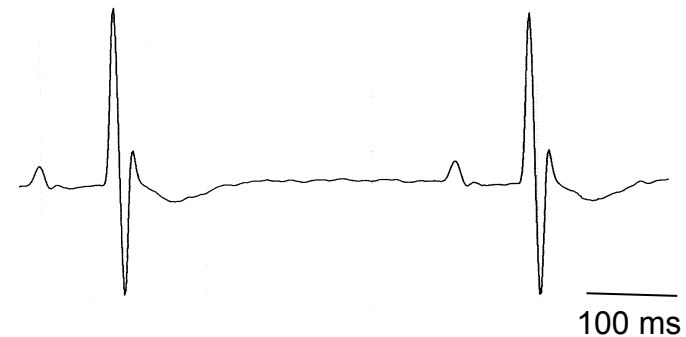
Expression des canaux ioniques et corrélations fonctionnelles



Sham

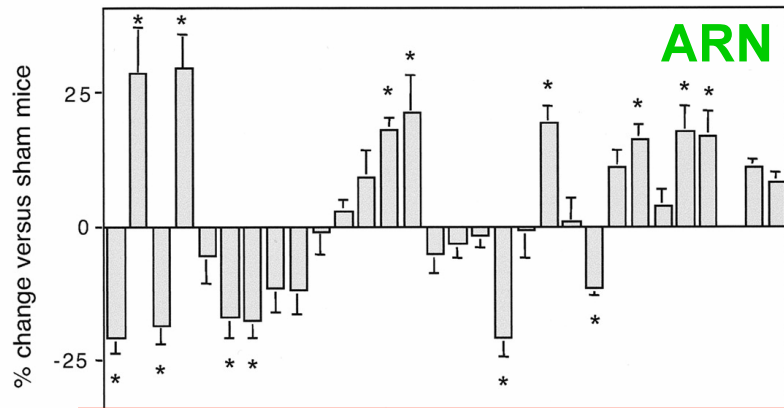


Amiodarone 180

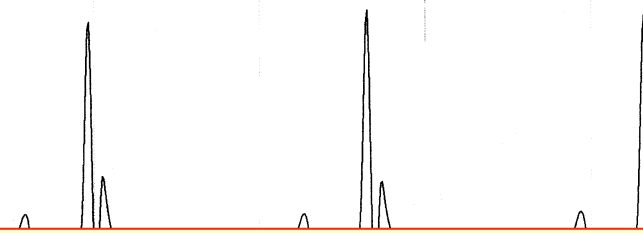


Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
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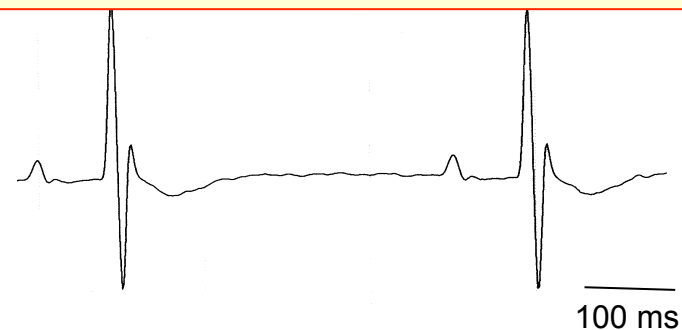
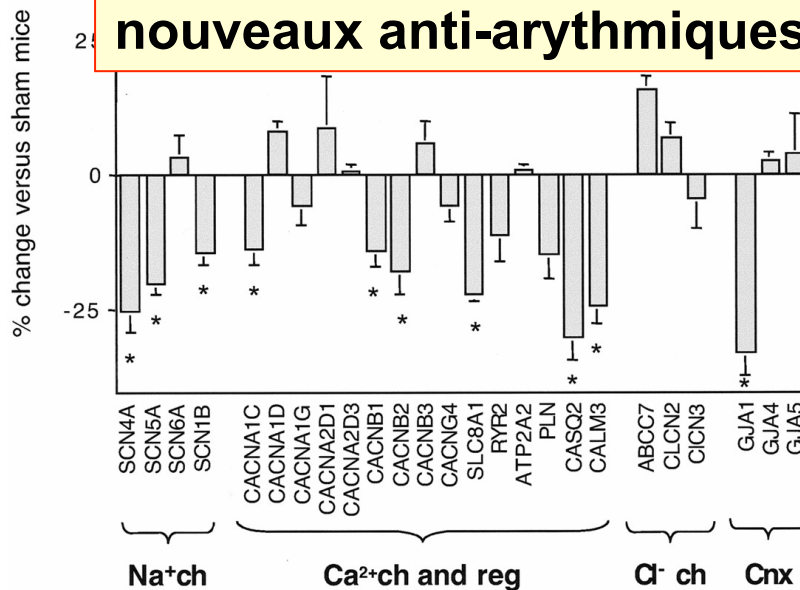
Le remodelage induit par l'amiodarone



Sham

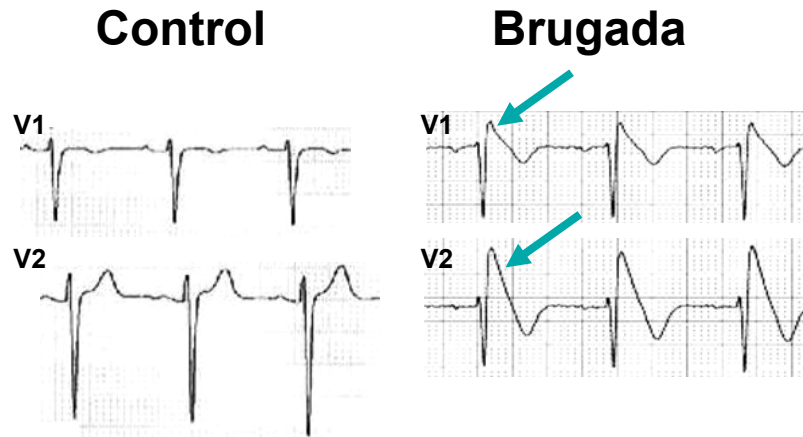


→ Première démonstration que l'action thérapeutique d'un anti-arythmique passe aussi par la modification de l'expression des canaux ioniques
 → Intérêt de la pharmacogénomique pour le développement de nouveaux anti-arythmiques

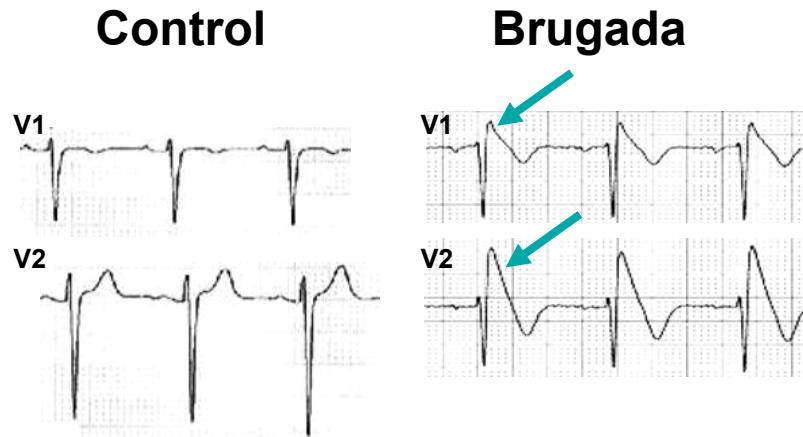


Le Bouter S*, Demolombe S* et coll. *Circ Res.* 2003.
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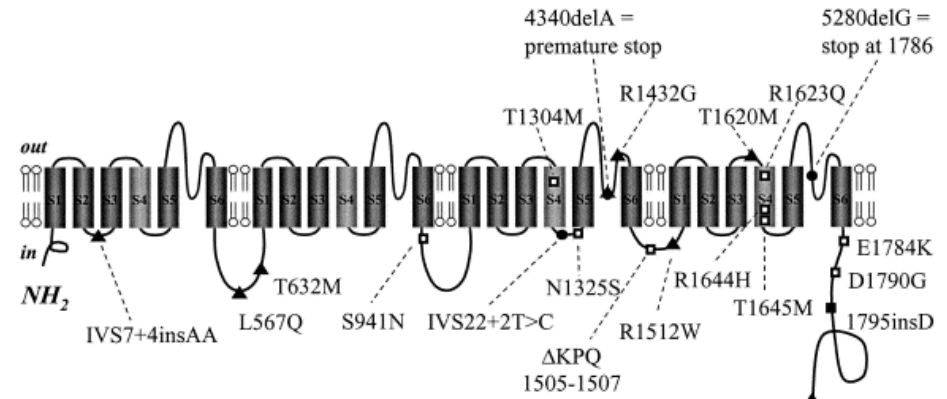
Application 3: Le remodelage ionique et le syndrome de Brugada



Application 3: Le remodelage ionique et le syndrome de Brugada

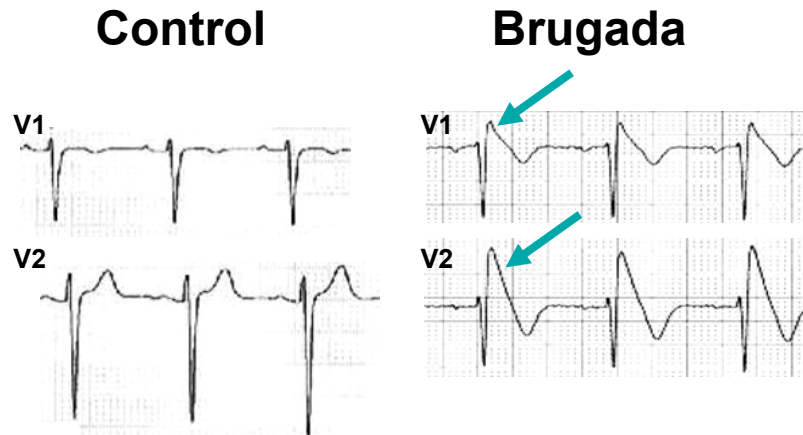


SCN5A (Nav1.5) mutations

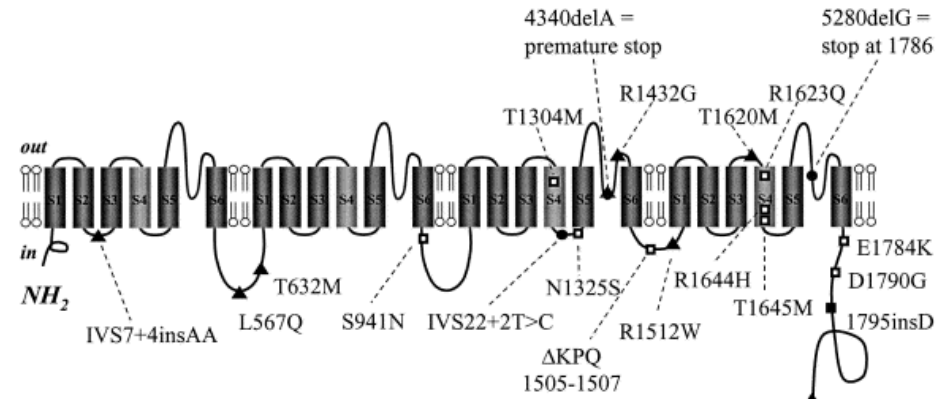


Only 20% of the patients are carrying a mutation in SCN5A

Application 3: Le remodelage ionique et le syndrome de Brugada



SCN5A (Nav1.5) mutations



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

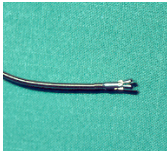
Hypothesis: alternative disease mechanism/determinants:

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

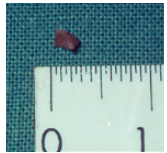
METHODS

✓ Biological samples

Catheter protocol biopsies



- 11 Brugada patients : 5 harboring *SCN5A* mutation, 6 non-harboring
- 7 Controls: early cardiac transplanted (**Ctl-2**)

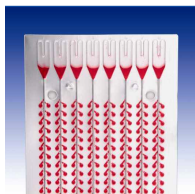


- 10 arrhythmogenic right ventricular cardiomyopathy (**ARVC**)
- 9 idiopathic right ventricular outflow-tract tachycardia (**RVOT**)

Scalpel biopsies

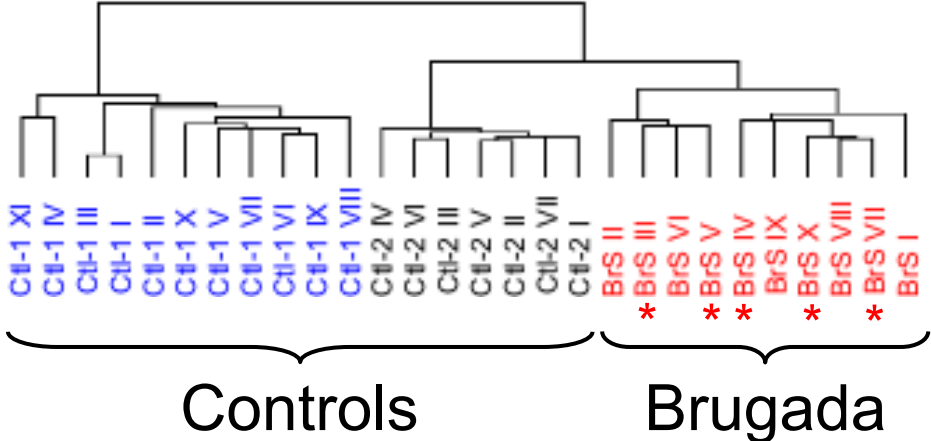
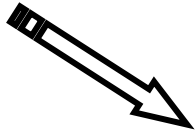
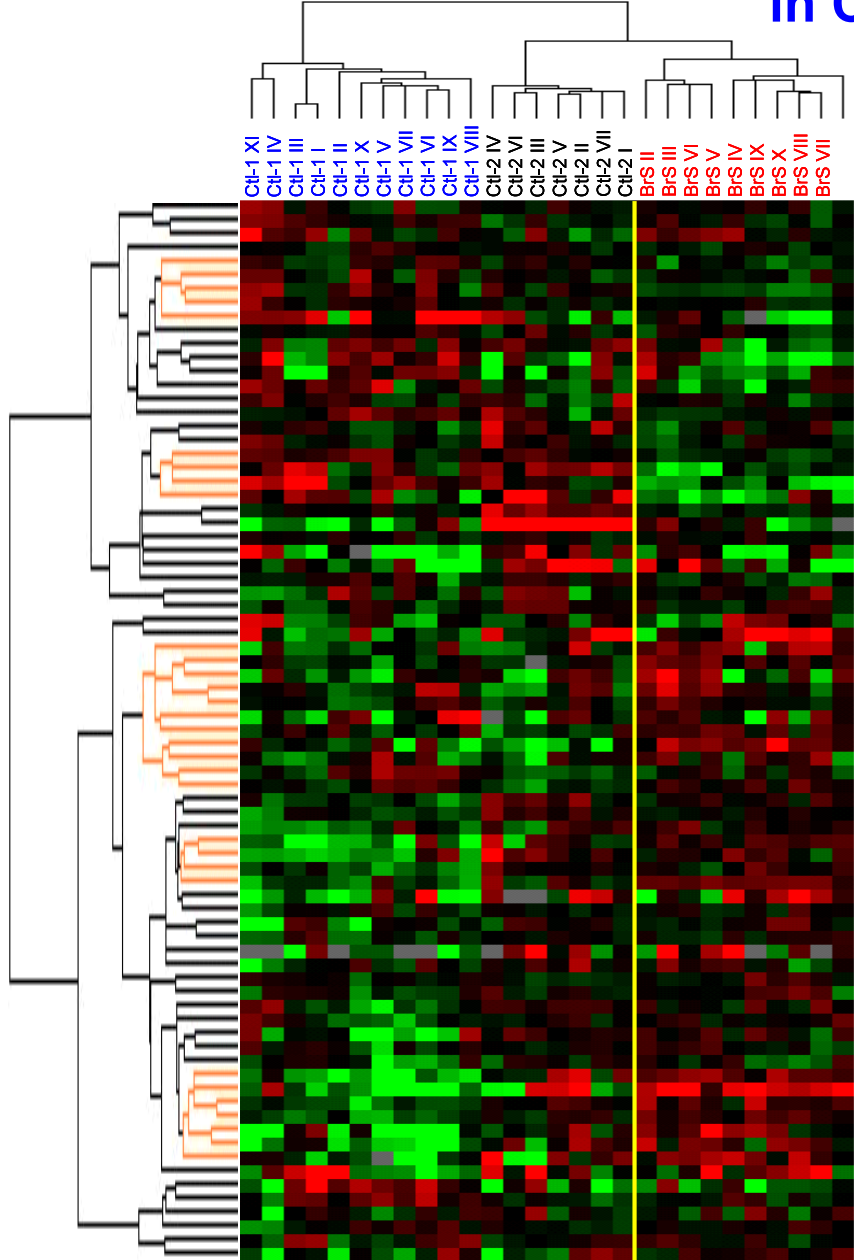
- 11 Controls: organ donors (**Ctl-1**)

✓ High-throughput real-time PCR



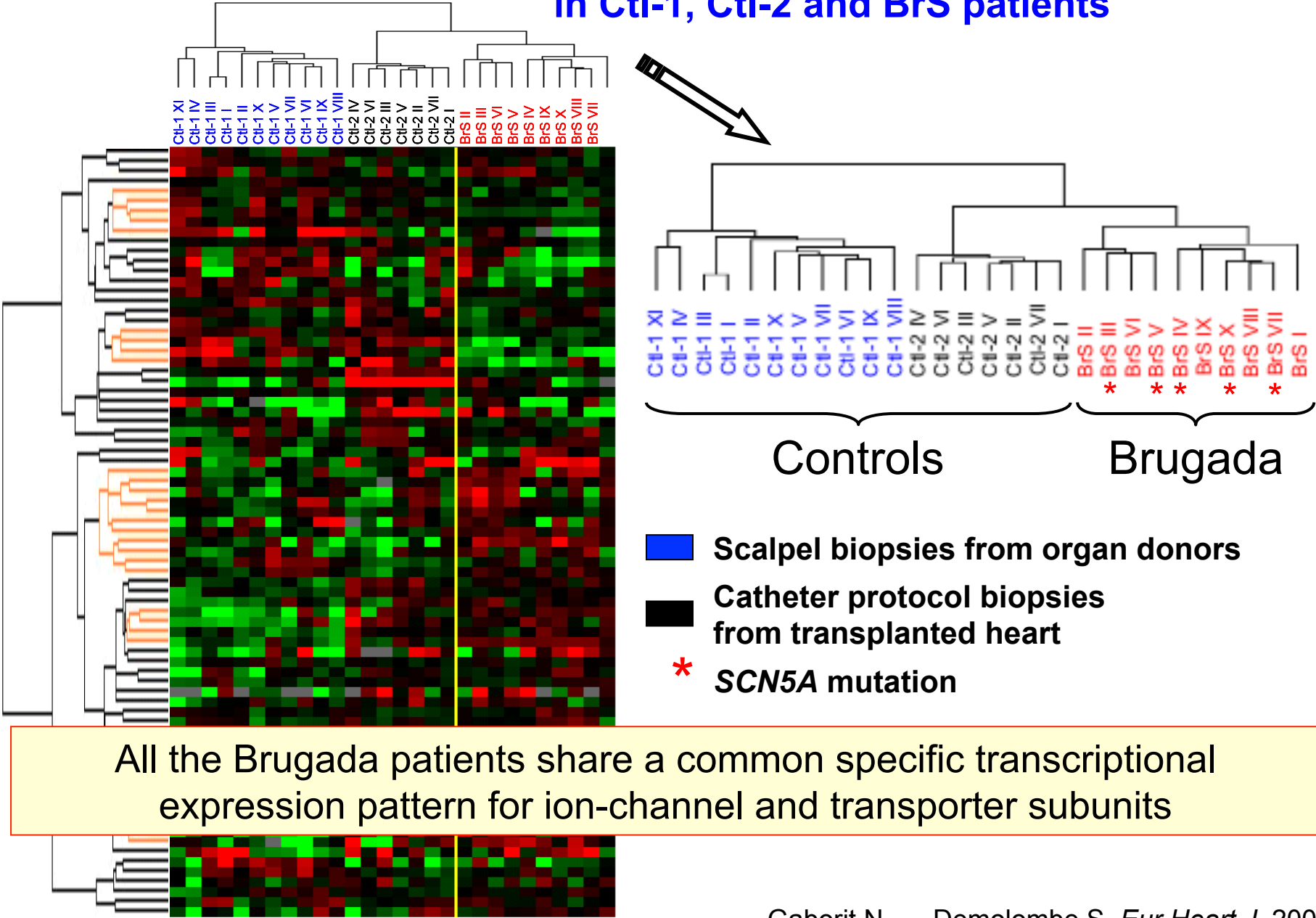
- 67 α - and β -ion channel subunits
- 17 Na/K-ATPase isoforms and proteins involved in Ca^{2+} -homeostasis
- 8 markers (ANF, BNP, β -MHC, CD4, CNN1, COL6A1, IL6, UCHL1)
- 4 reference genes

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

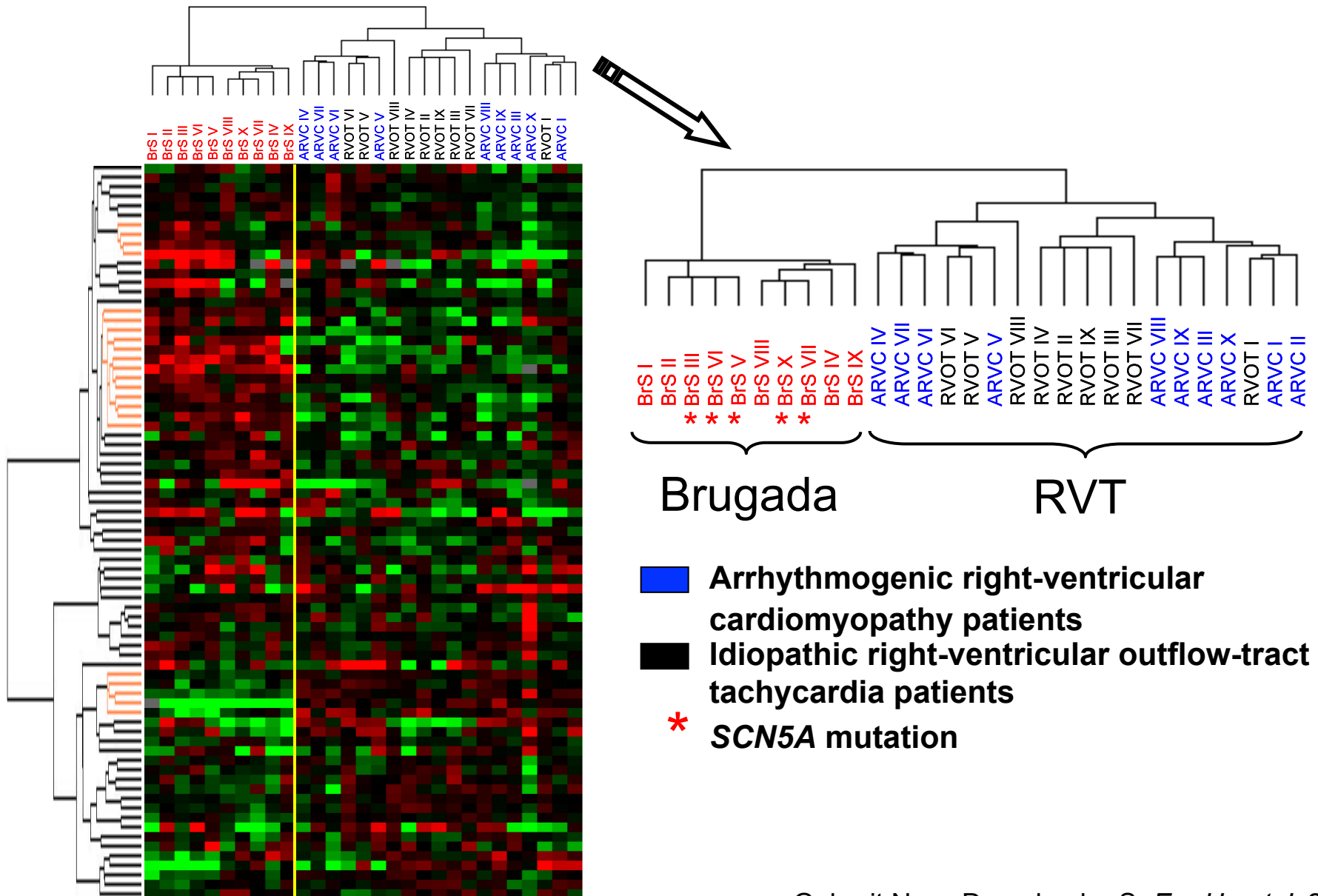


- Scalpel biopsies from organ donors
- Catheter protocol biopsies from transplanted heart
- * SCN5A mutation

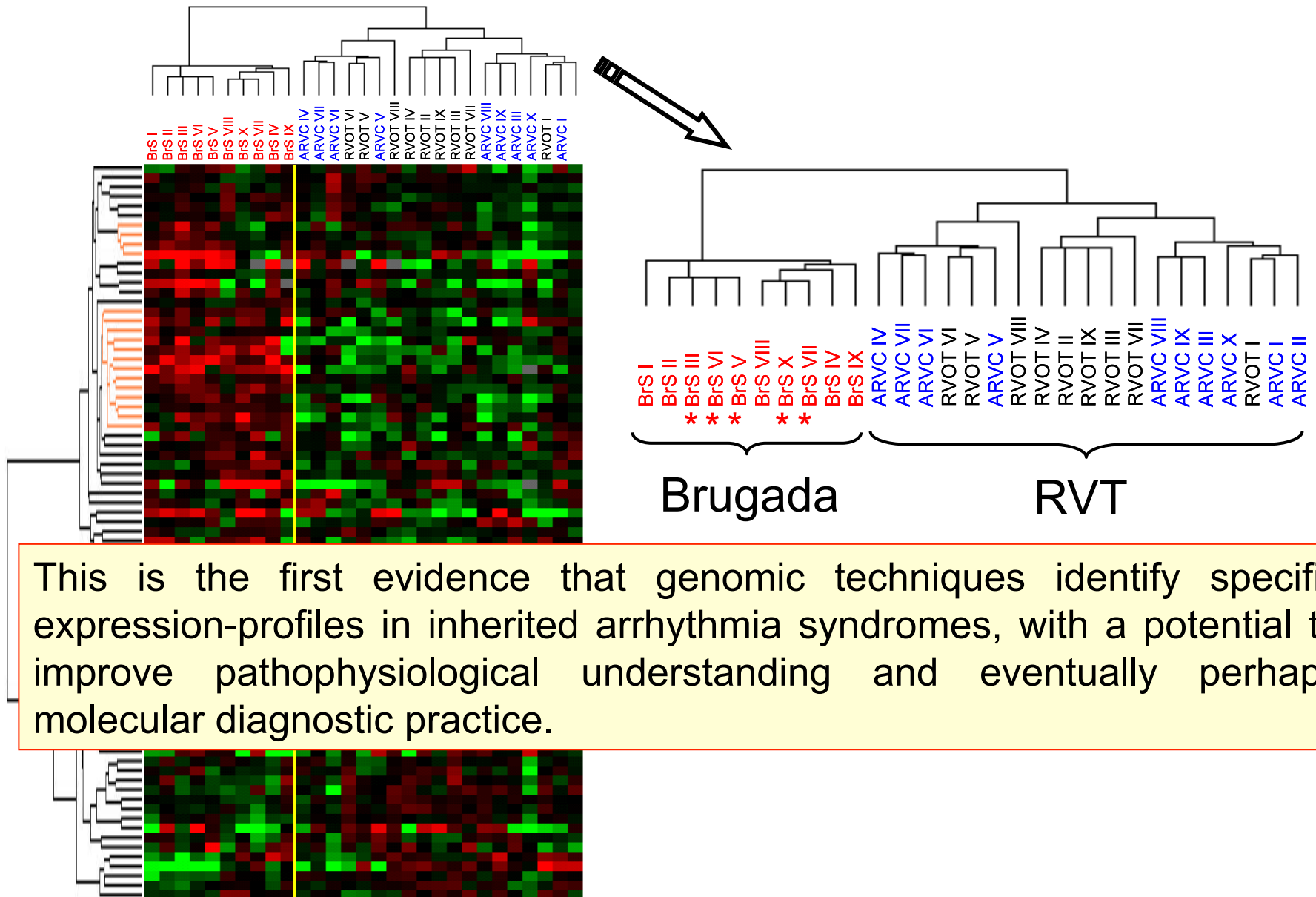
Global analysis of ion-channel expression profiles in Ctl-1, Ctl-2 and BrS patients



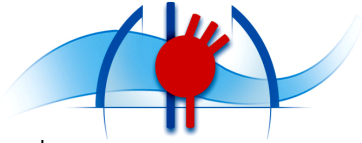
Global analysis of ion-channel expression profiles in ARVC, RVOT and BrS patients



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

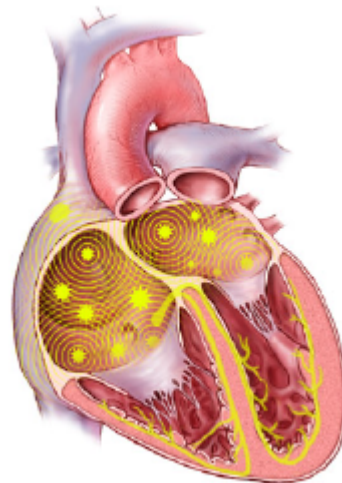


This is the first evidence that genomic techniques identify specific expression-profiles in inherited arrhythmia syndromes, with a potential to improve pathophysiological understanding and eventually perhaps molecular diagnostic practice.



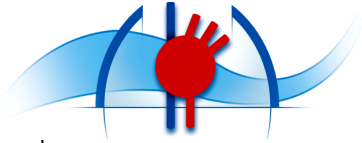
Application 4:

Ion-Channel Transcriptional Signature Associated with Atrial Fibrillation



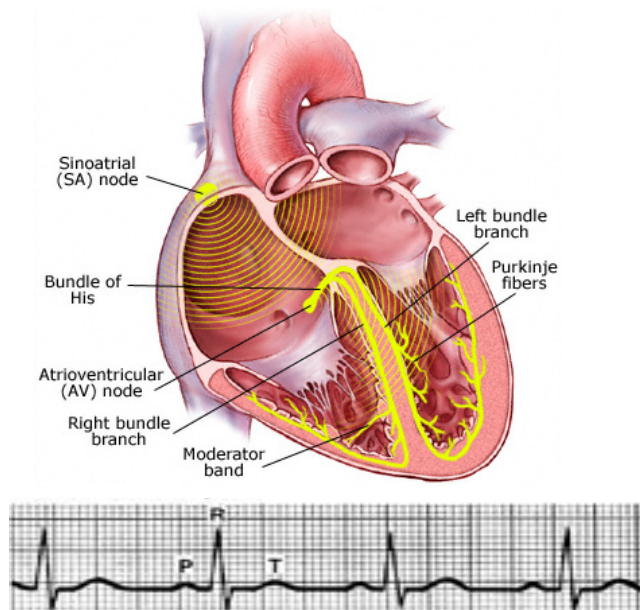
Gaborit et al., Circulation 2005;112:471-81

Lamirault G, Gaborit N, et al., J Mol Cell Cardiol. 2006;40:173-84

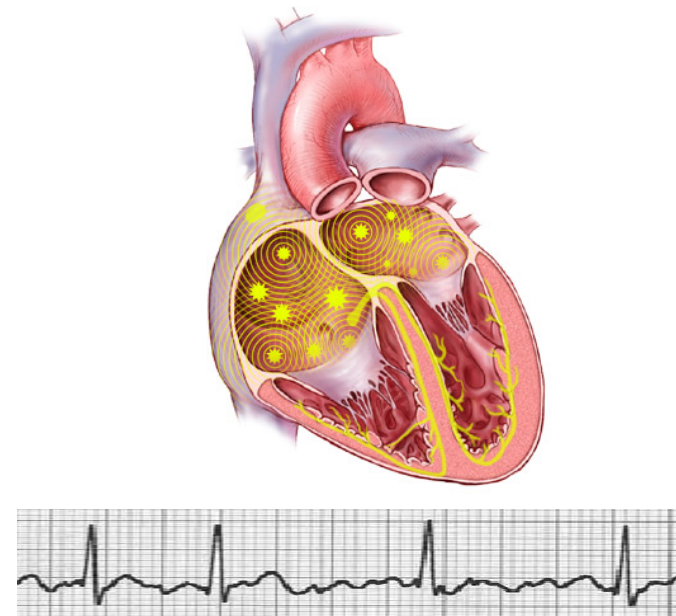


Atrial Fibrillation

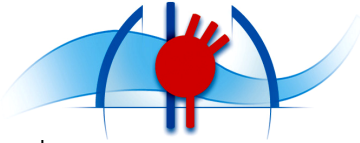
- ✓ *Most frequent cardiac arrhythmia (2 000 000 in USA)*
- ✓ *Occurrence of AF increases with age*
- ✓ *Rapid and irregular activation of the atrium (400-600/min)*



Sinus rythm

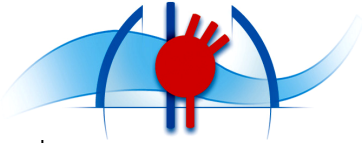


Atrial fibrillation



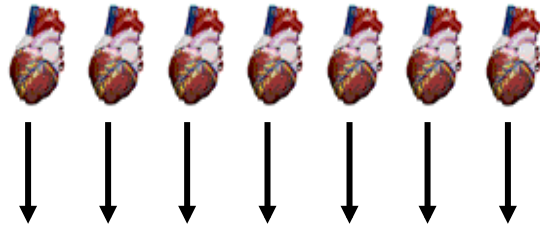
Atrial Fibrillation

- *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*

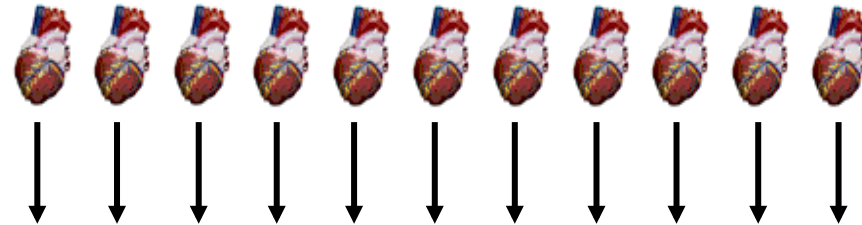


Study Design

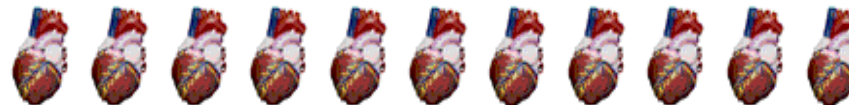
**Valvular heart disease +
sinus rhythm
(VHD)**



**Valvular heart disease +
atrial fibrillation
(AF-VHD)**



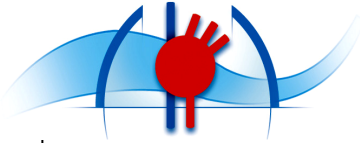
Control patients



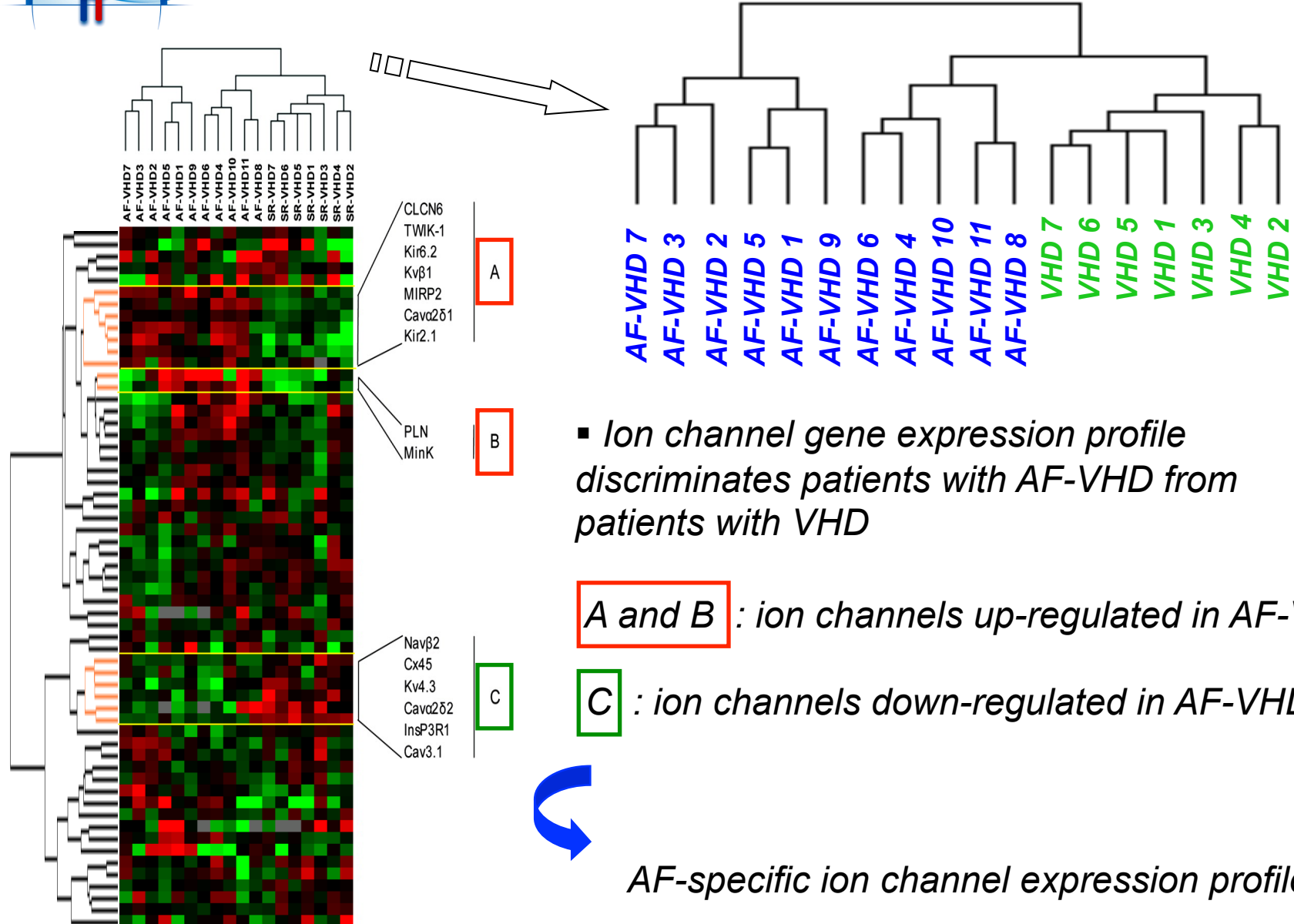
remodeling VHD

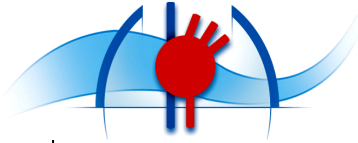
remodeling AF+VHD

remodeling AF



Hierarchical Clustering





remodeling VHD

remodeling AF-VHD

remodeling AF

Name	identifier	description	AF-specific variations
Cav3.1	NM_018896	calcium channel, voltage-dependent, alpha 1G subunit	↓
Cav α 2 δ 1	NM_000722	calcium channel, voltage-dependent, alpha 2/delta subunit 1	↑
Cav α 2 δ 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 β 2	NM_004588	sodium channel, voltage-gated, type II, beta polypeptide	↓
CIC6	NM_021737	chloride channel 6	↑
Kv β 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**

➔ **Potential therapeutic targets?**



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



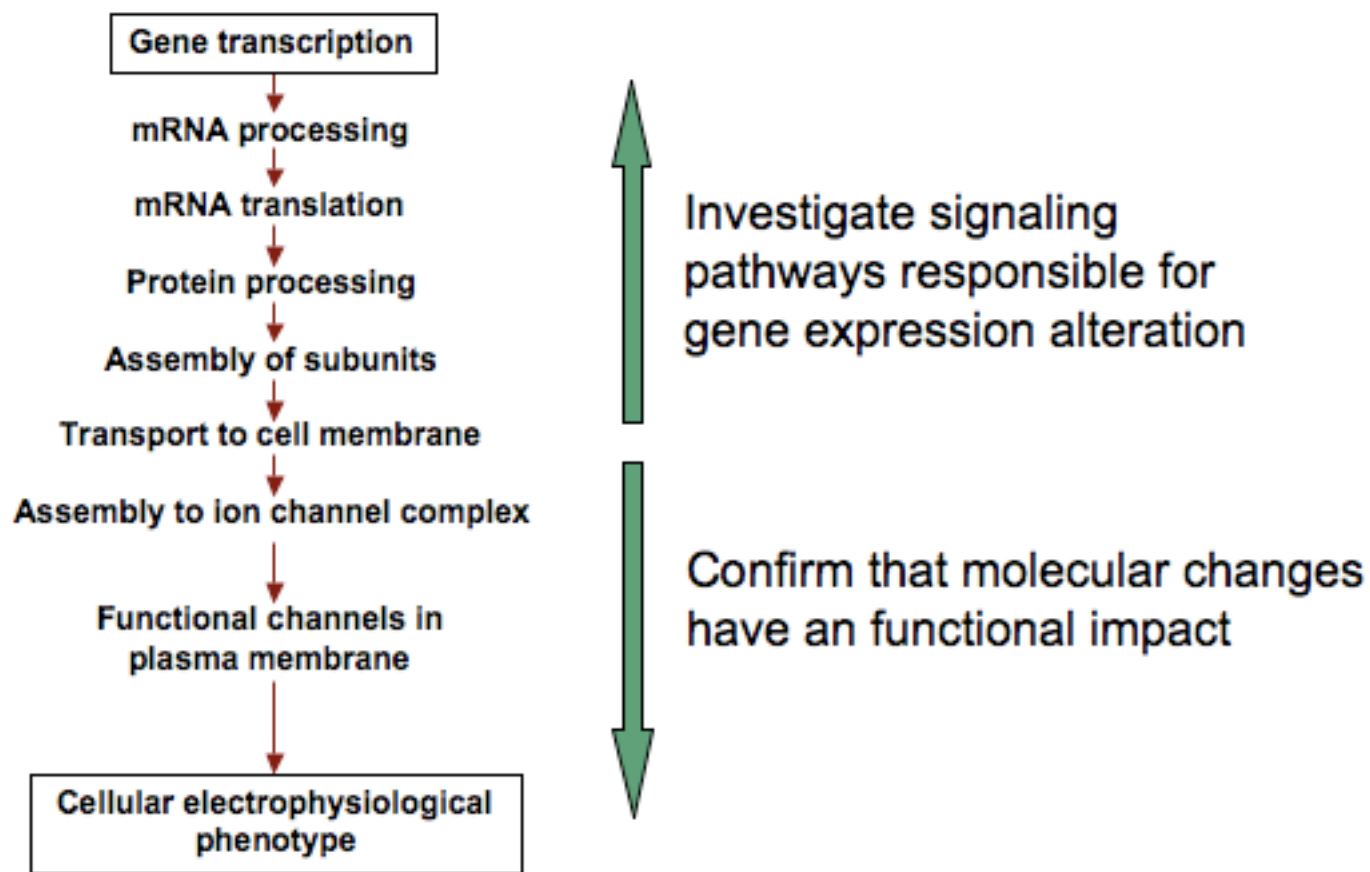
Possibilities and Promises of Transcriptomics

- Provide a tool for target discovery through drug profiling
- Provide a molecular correlate of disease progression
- Provide a disease classification
- Provide a tool for personalized medicine



From transcriptomic to drug target discovery: Challenges

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

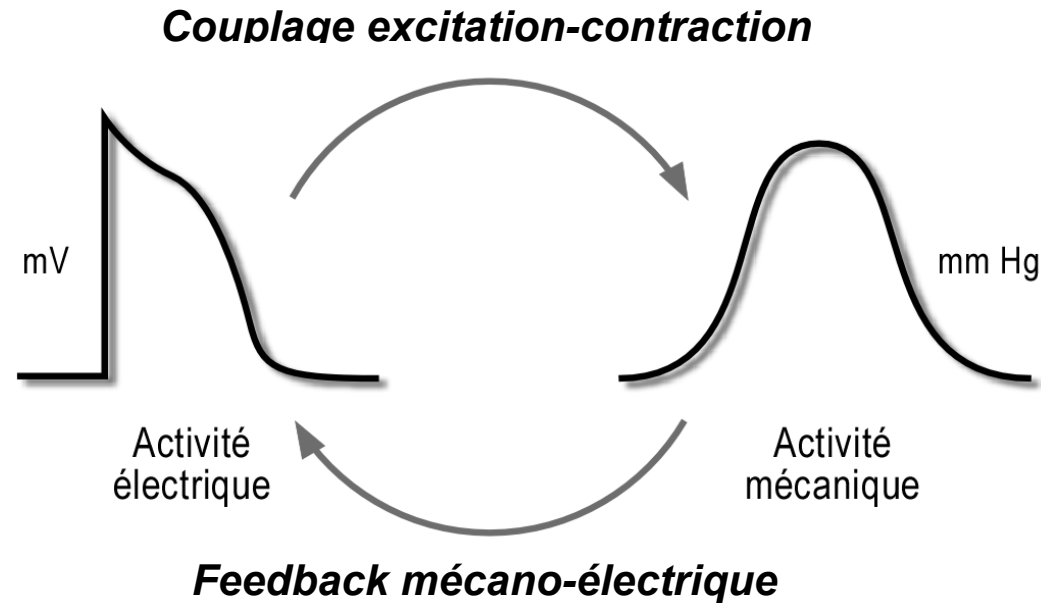


- In arrhythmia: Reduction of symptoms without side effects

PROJET :

Stress mécanique et remodelage électrique cardiaque

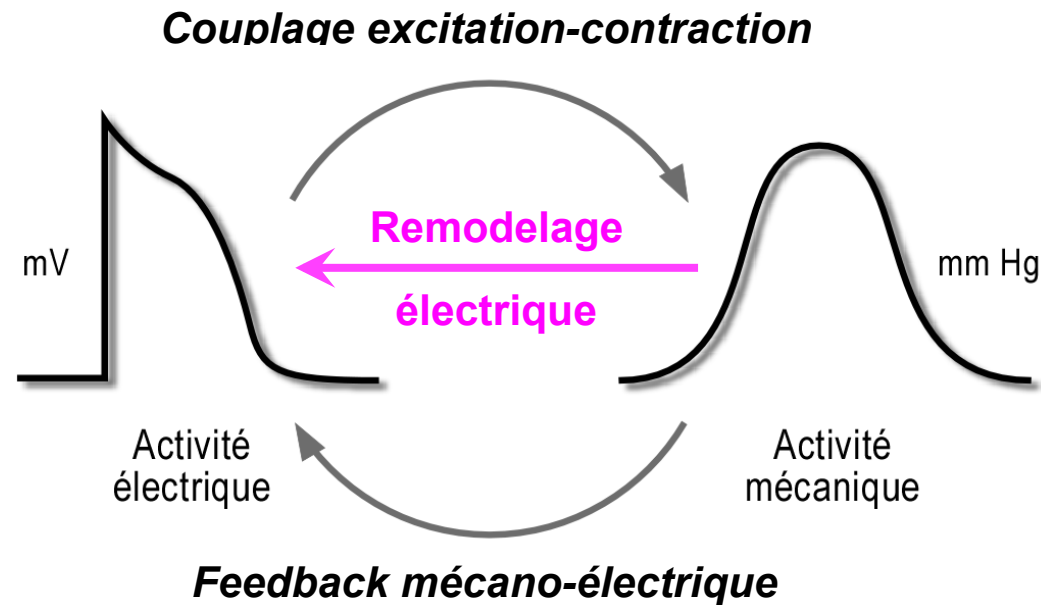
De nombreuses situations arythmogènes, dont la fibrillation auriculaire et l'insuffisance cardiaque, sont associées à une altération de la contractilité musculaire et à une hétérogénéité mécanique.



PROJET :

Stress mécanique et remodelage électrique cardiaque

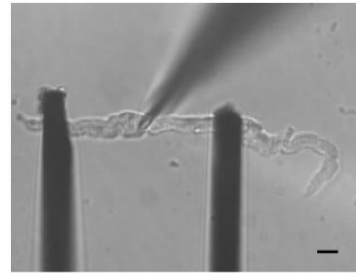
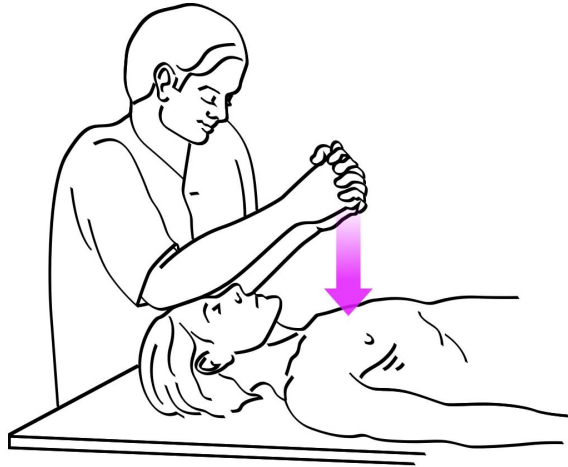
De nombreuses situations arythmogènes, dont la fibrillation auriculaire et l'insuffisance cardiaque, sont associées à une altération de la contractilité musculaire et à une hétérogénéité mécanique.



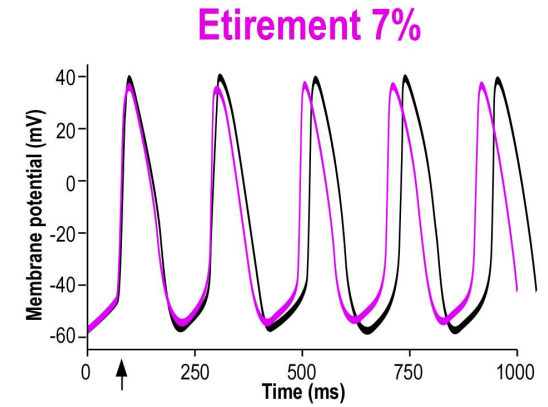
OBJECTIF:

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

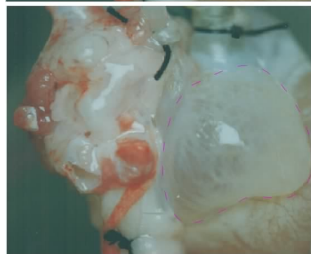
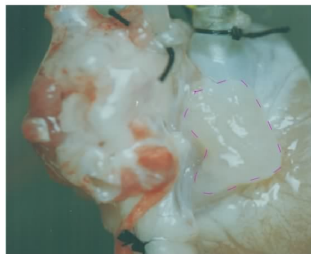
Le couplage mécano-électrique cardiaque



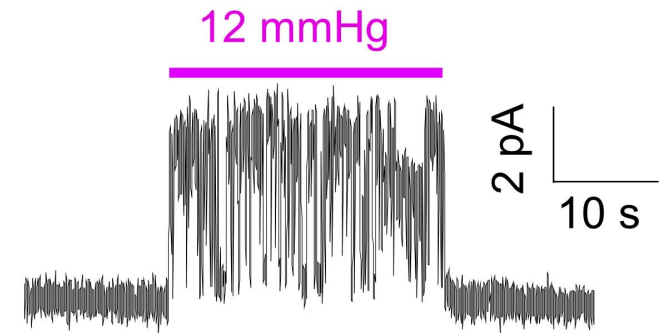
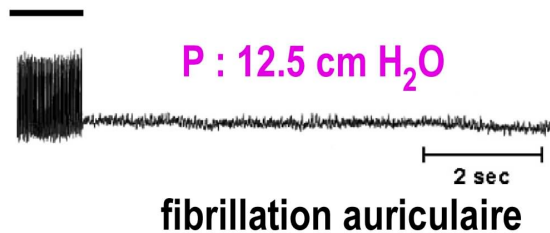
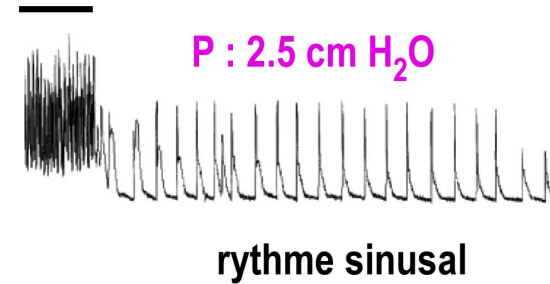
Cooper et al., 2000



Haute fréquence



Bode et al., 2001



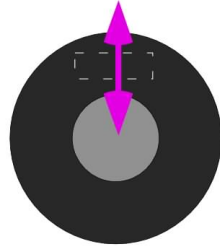
Terrenoire et al., 2001

Stress mécanique et hypertrophie ventriculaire

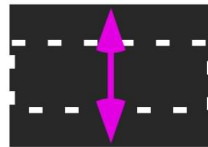
sain



Hypertrophie ventriculaire



surcharge de pression

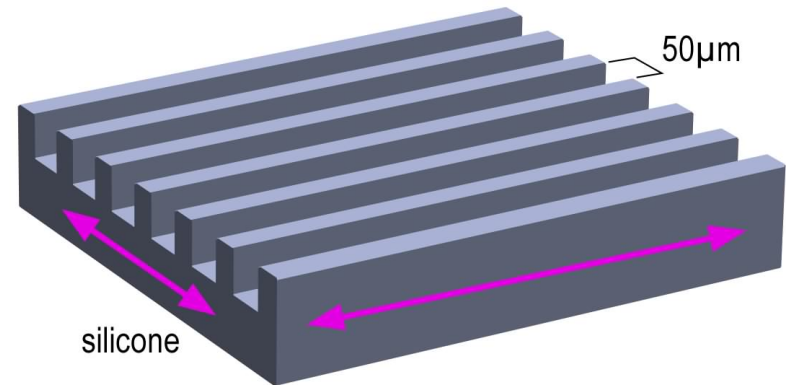


Epaississement

myocyte



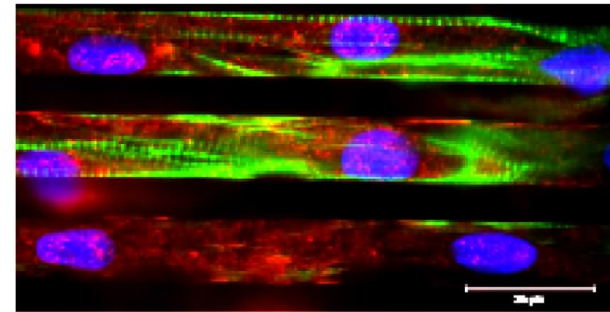
Modèle *in vitro* d'étirement biaxial



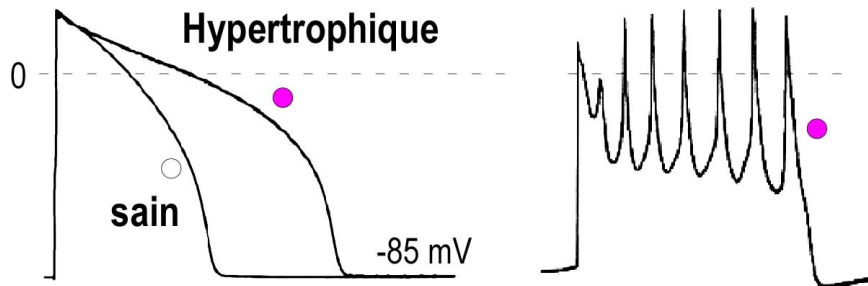
Étirement 10%

génomique, IF, biochimie, physiologie

transversale



noyau, actin et FAKpY397



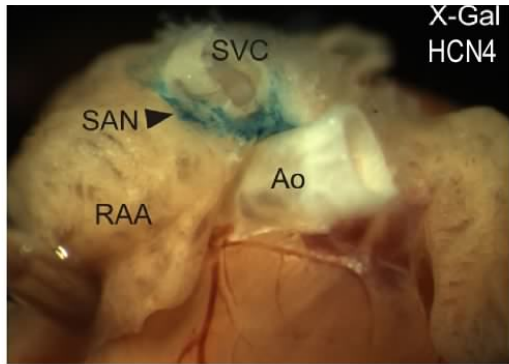
400 ms

Tachycardie ventriculaire
Mort subite

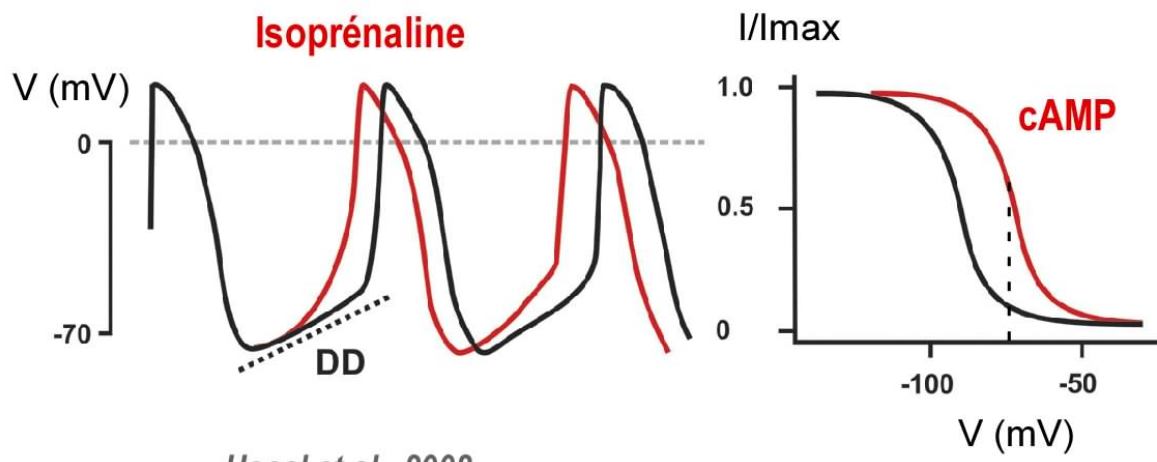
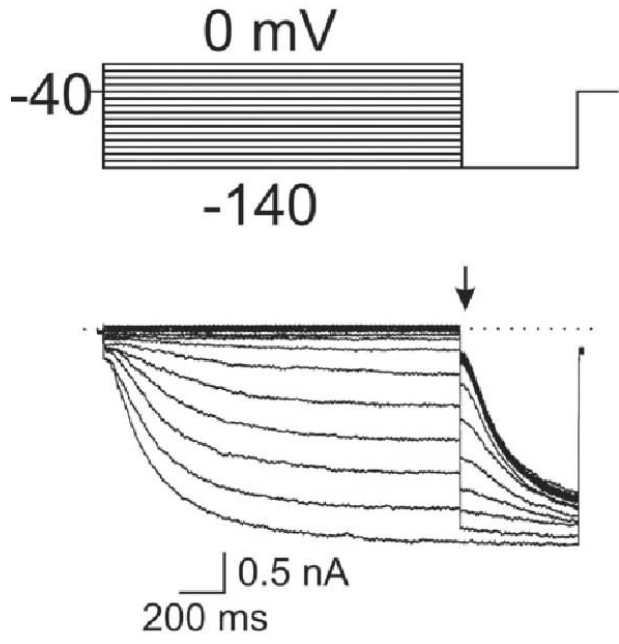
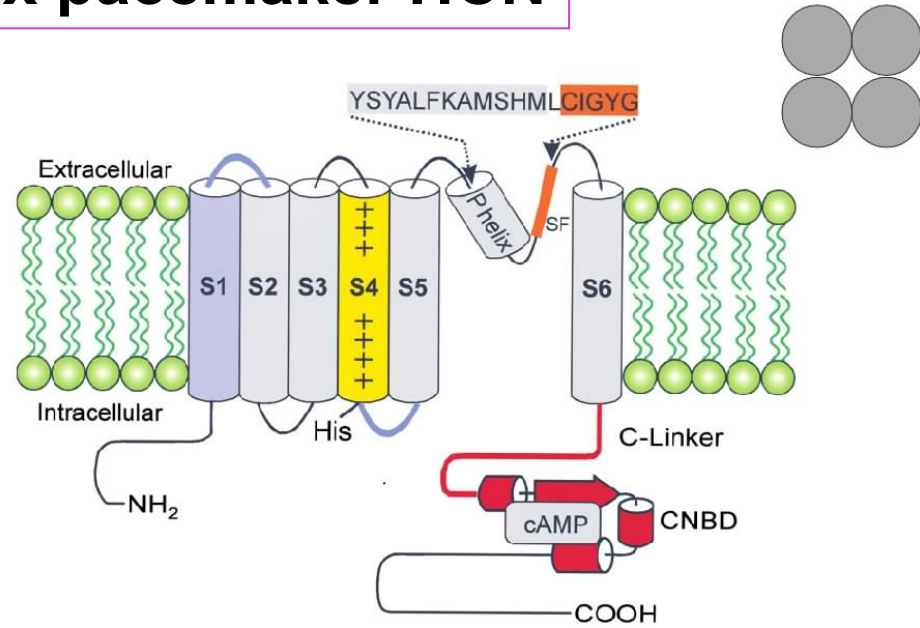
Hill, 2003

Senyo et al., 2007

Les canaux pacemaker HCN

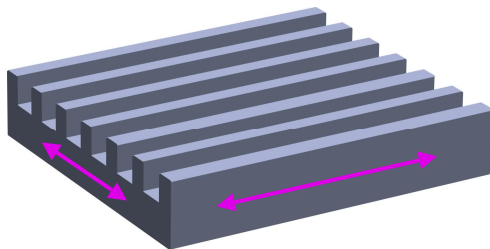
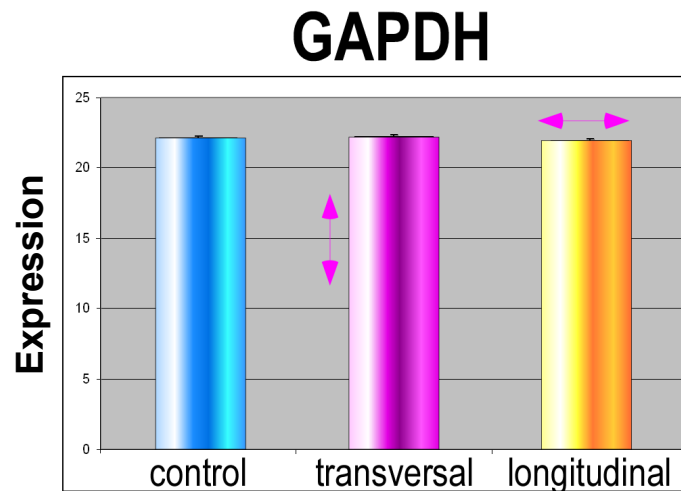
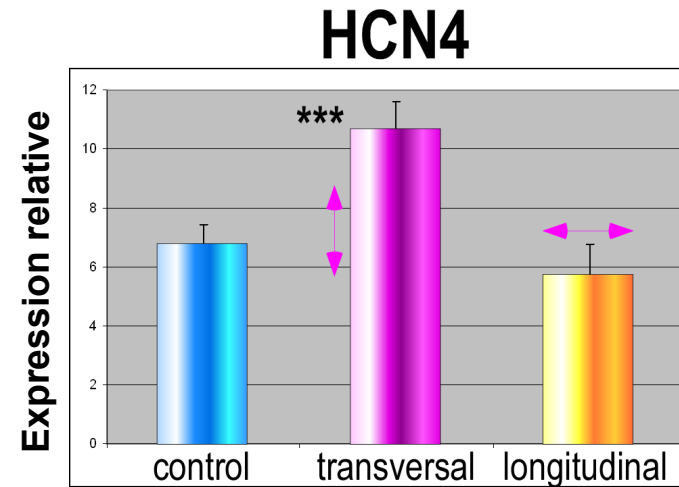
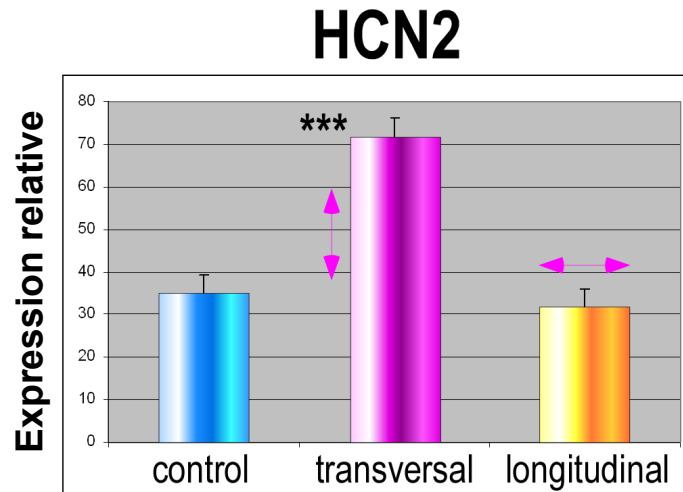


Biel et al., 2009

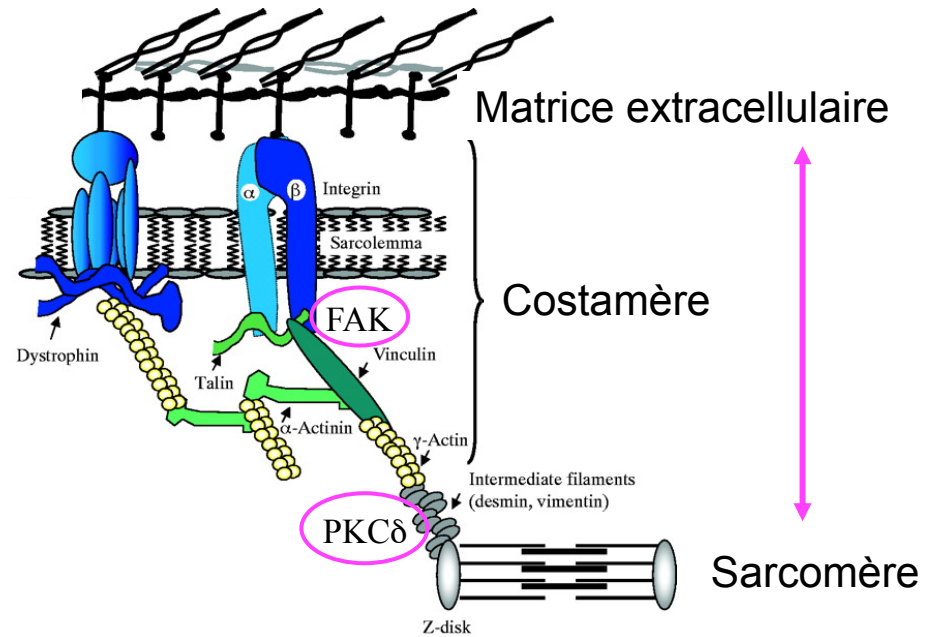
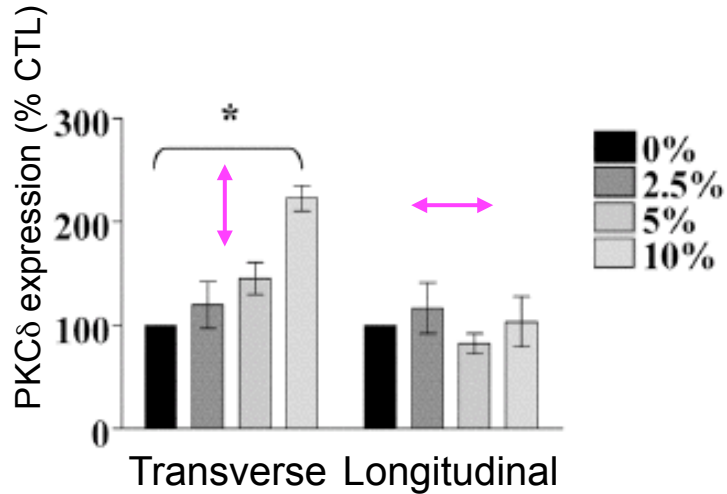
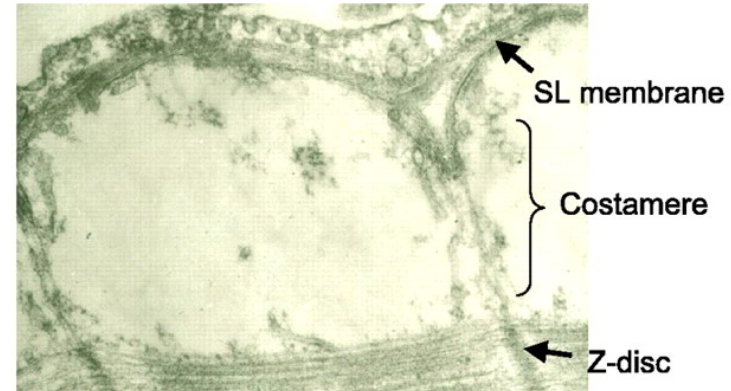
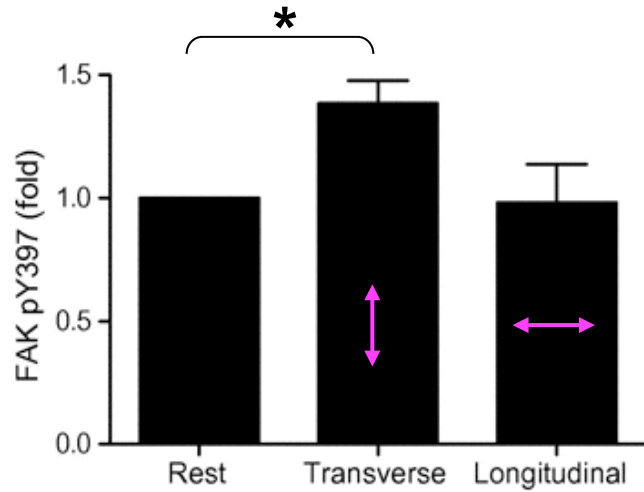


Hoesl et al., 2008

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



Le costamère et la mécanotransduction cardiaque



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

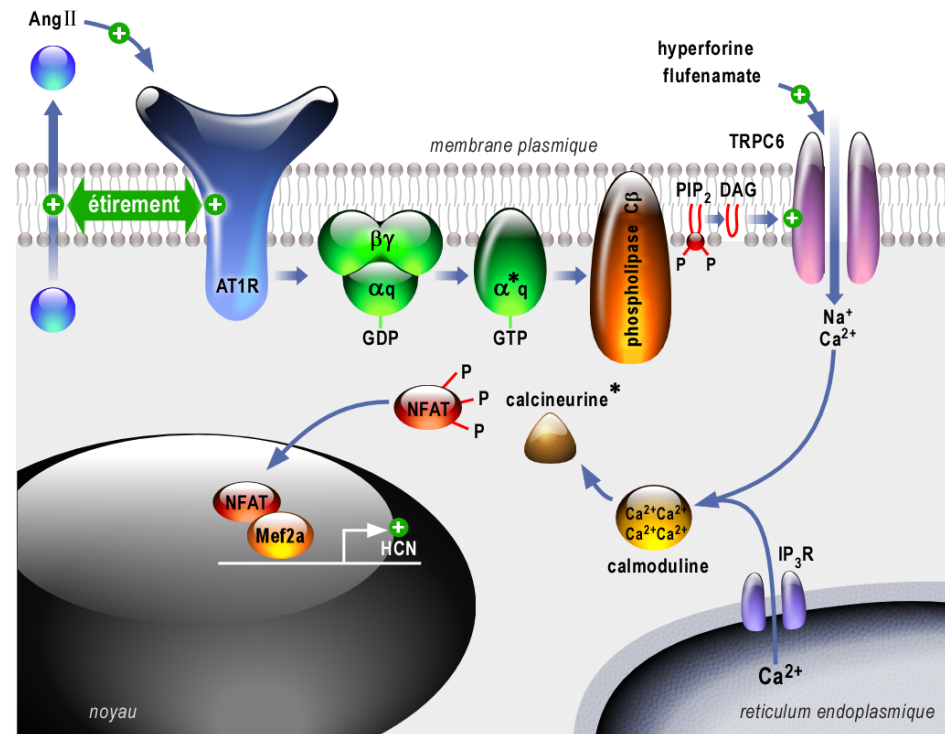
Objectif 1: SIGNALISATION

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

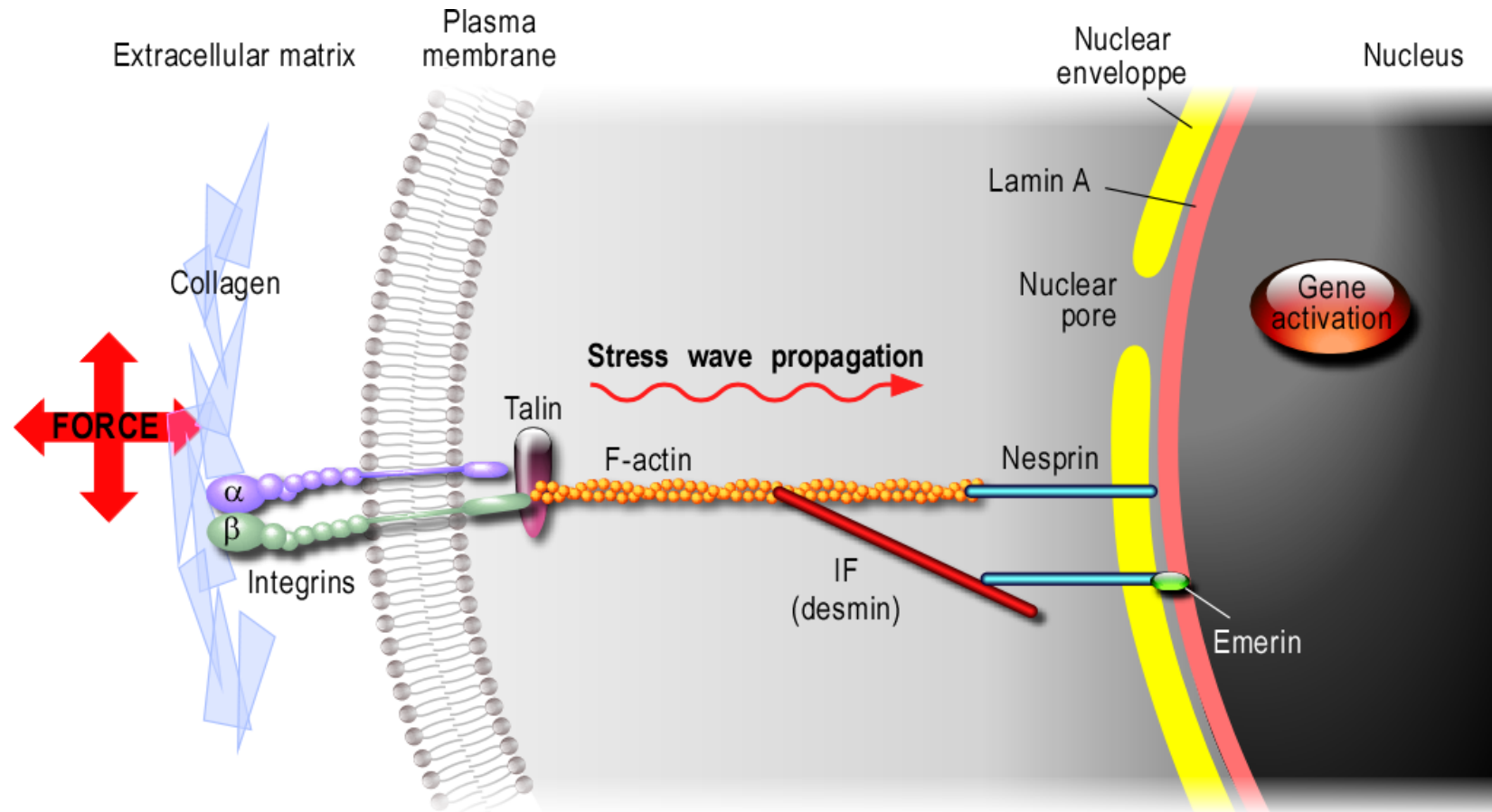
- effets d'une inhibition de son récepteur
- effets de la sous-expression de *miR-1* et *miR-133*
- adressage à la membrane des endosomes contenant les canaux HCN recyclés

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



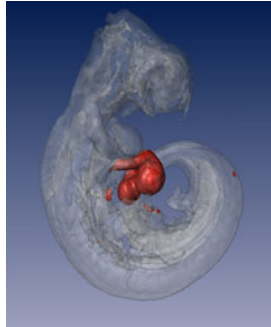
Objectif 2: TRANSMISSION DIRECTE DE LA FORCE AU NOYAU



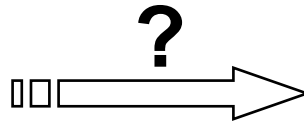
Conséquence de l'invalidation de la lamine A (souris $Lmna^{H222P/H222P}$, collaboration avec Gisèle Bonne, INSERM U974, Paris)

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

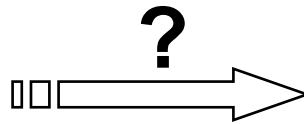
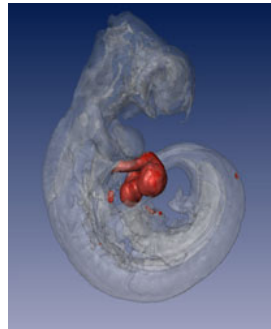
Specific ion channels profile: what are the transcription factors involved ?



Bruneau BG. Circ Res. 2002.



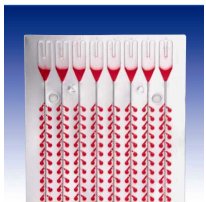
Specific ion channels profile: what are the transcription factors involved ?



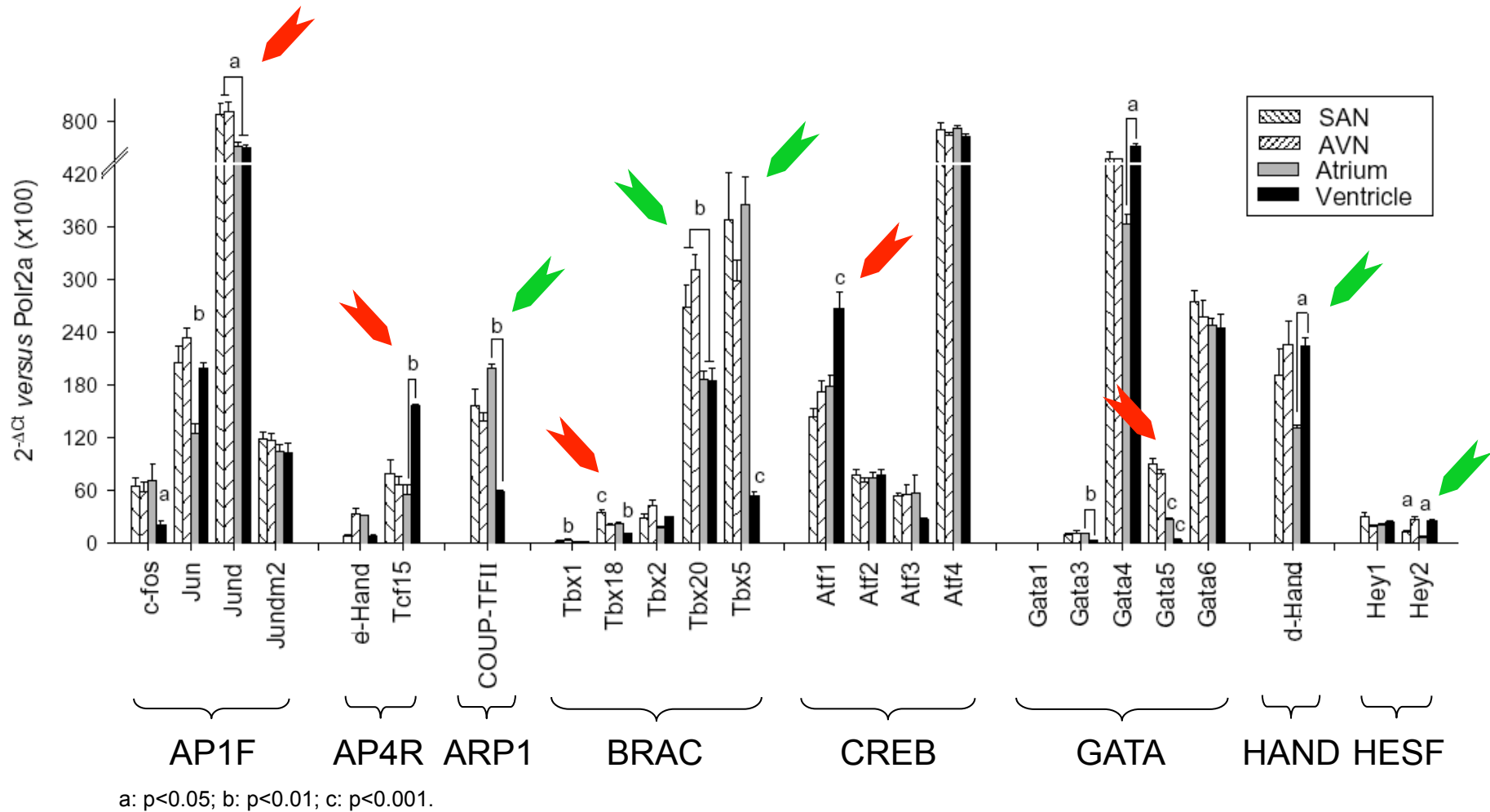
Bruneau BG. Circ Res. 2002.

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

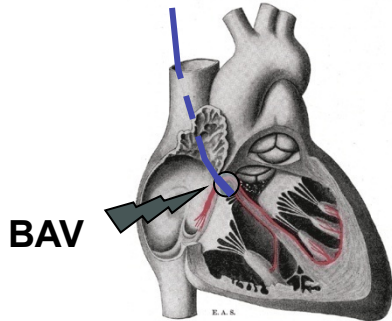


Profil d'expression des facteurs de transcription spécifique à chaque région cardiaque





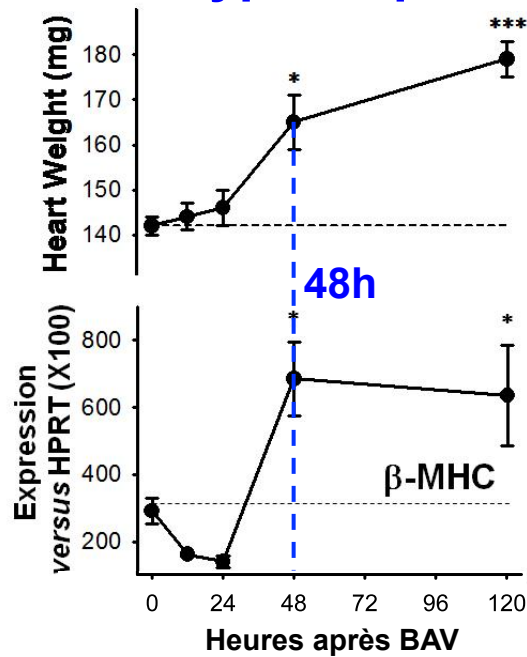
Modèle murin d'hypertrophie



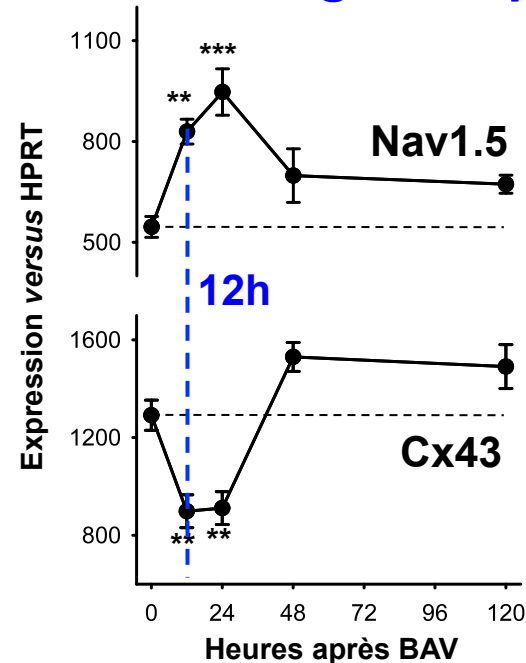
Troubles du rythme dès 12 h après BAV



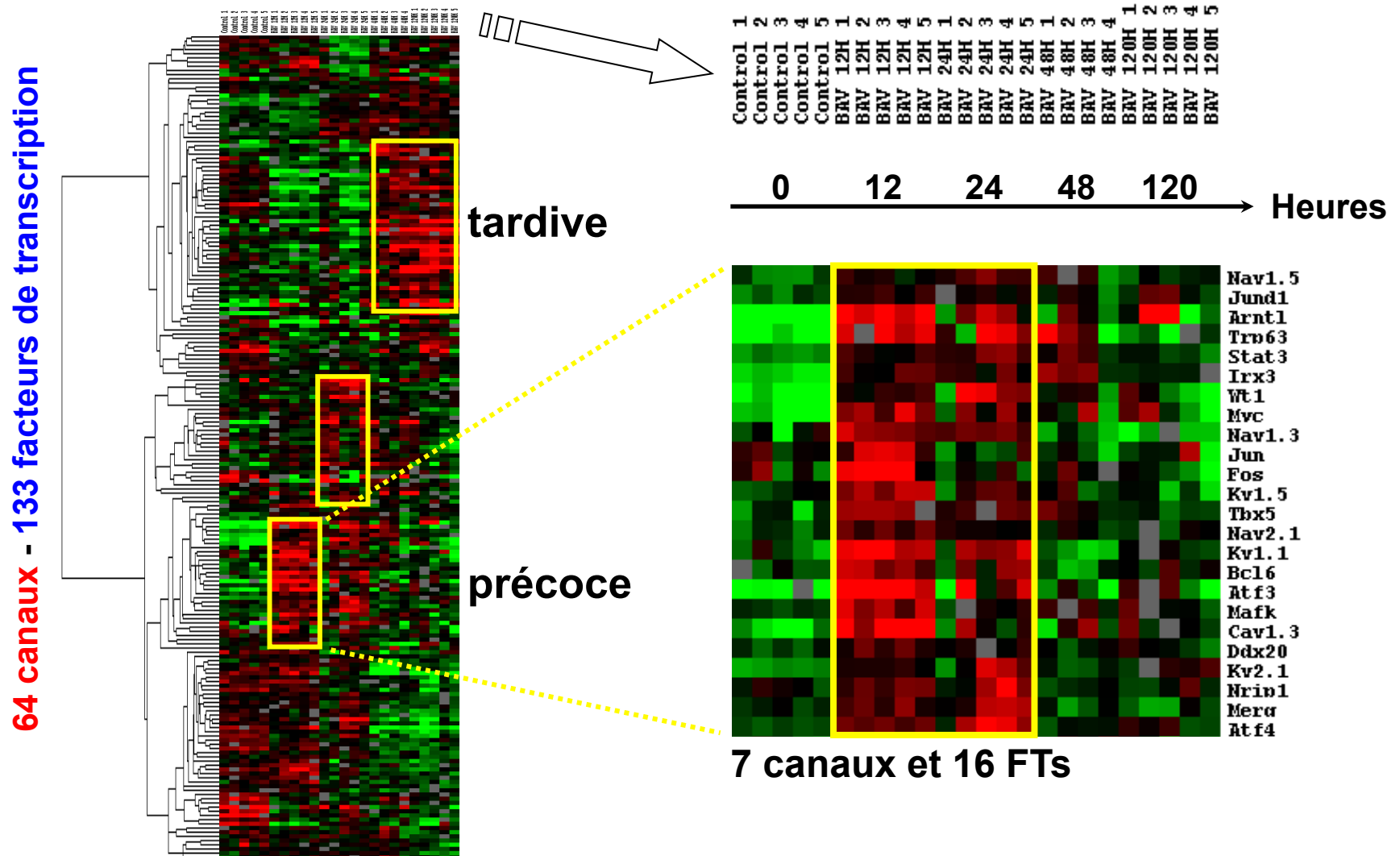
Hypertrophie

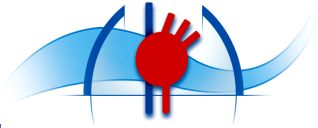


Remodelage ionique

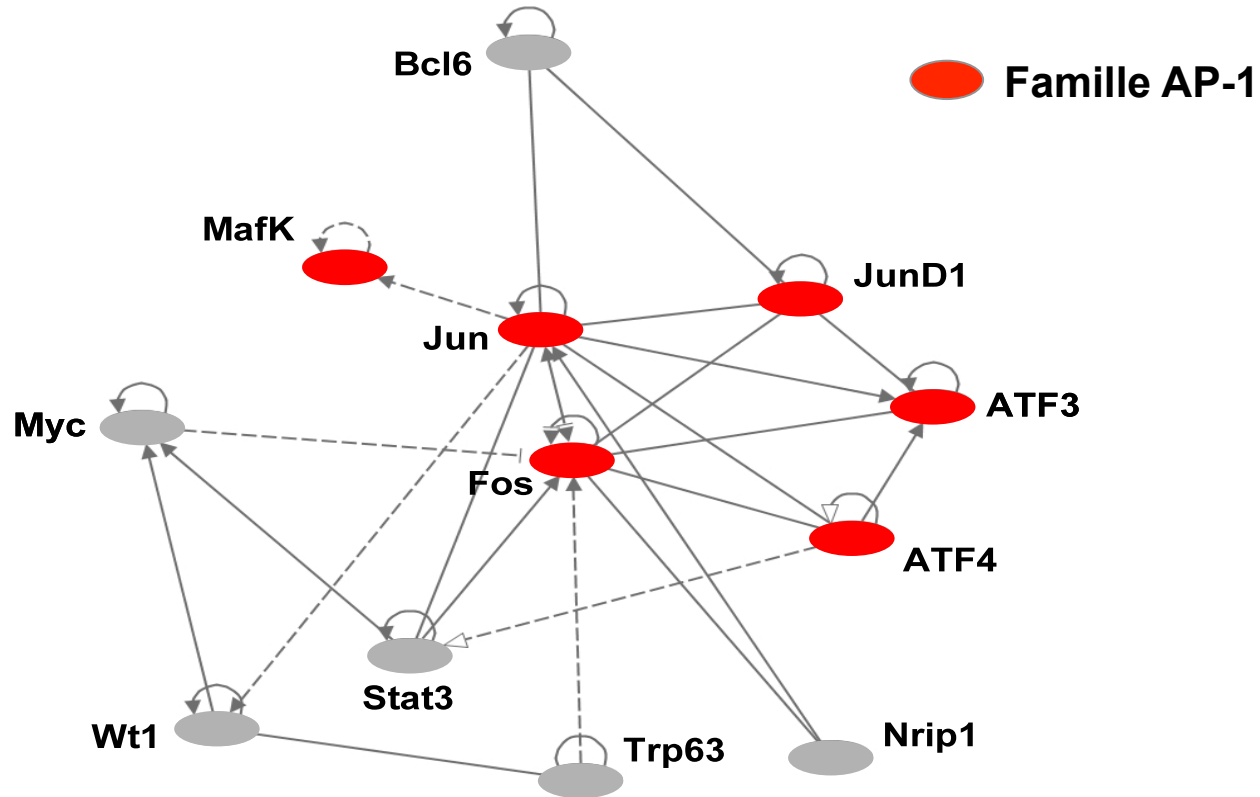


Co-variations des canaux ioniques et des facteurs de transcription dans le modèle BAV





Réseaux d'interaction moléculaire



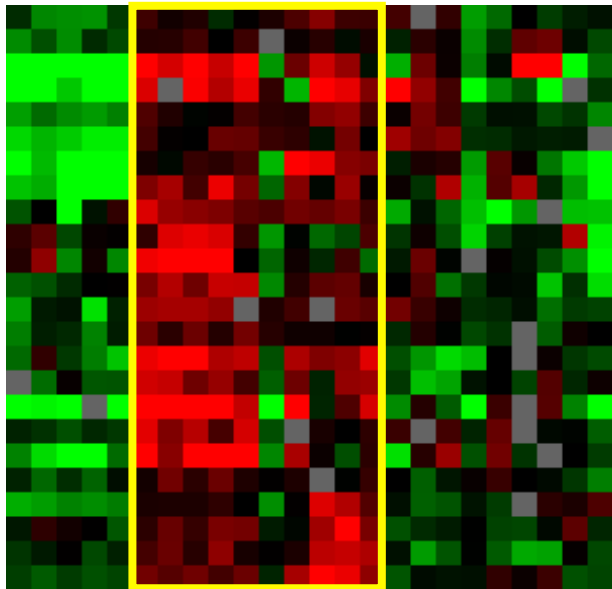
©2000-2007 Inqenuity Systems, Inc. All rights reserved.

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

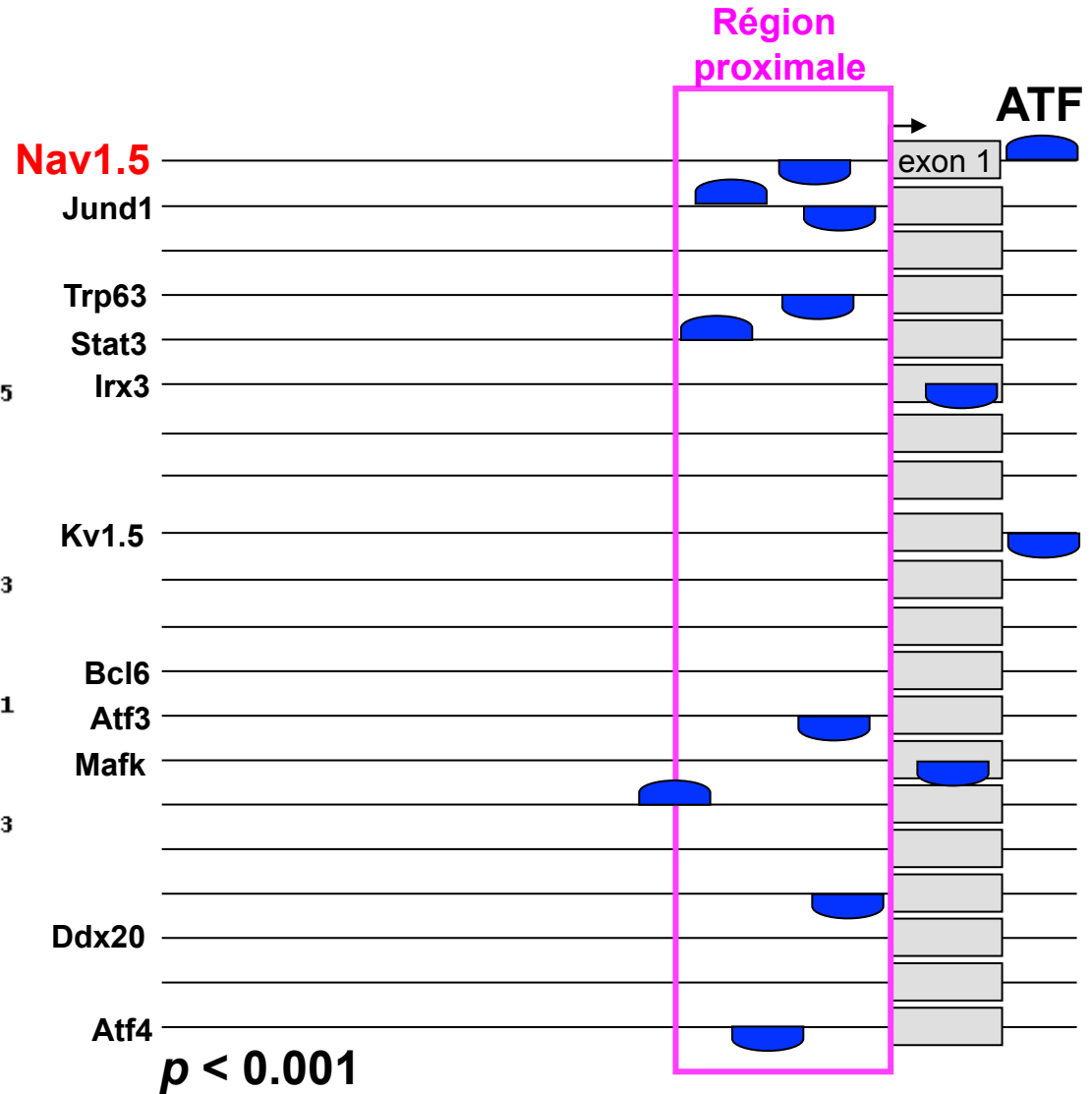
Control 1
Control 2
Control 3
Control 4
Control 5
BAV 12H 1
BAV 12H 2
BAV 12H 3
BAV 12H 4
BAV 12H 5
BAV 24H 1
BAV 24H 2
BAV 24H 3
BAV 24H 4
BAV 24H 5
BAV 48H 1
BAV 48H 2
BAV 48H 3
BAV 48H 4
BAV 120H 1
BAV 120H 2
BAV 120H 3
BAV 120H 4
BAV 120H 5

0 12 24 48 120 →

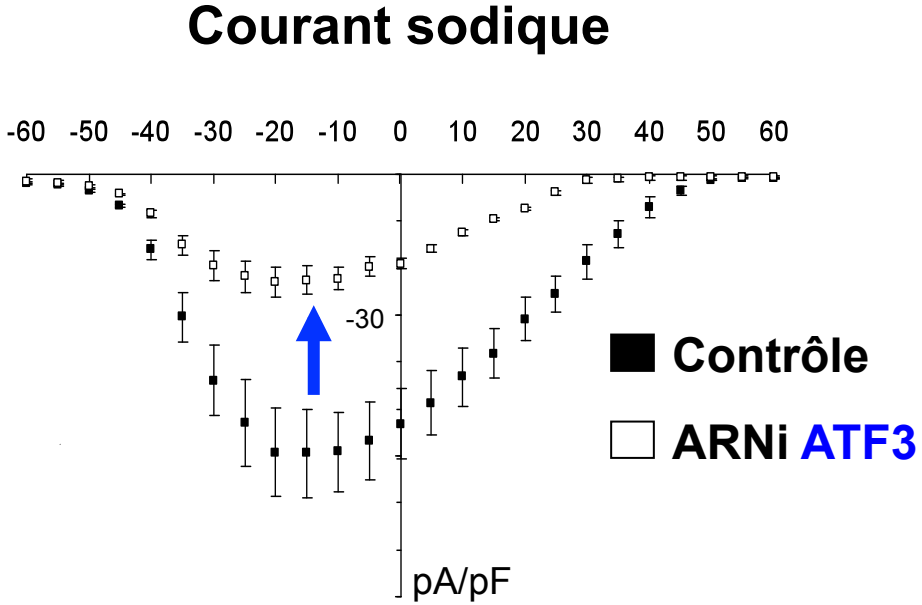
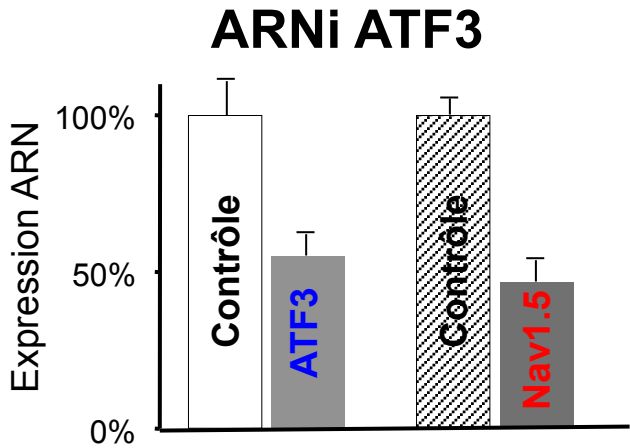


Vague précoce

Nav1.5
Jund1
Arnt1
Trp63
Stat3
Irx3
Wt1
Myc
Nav1.3
Jun
Fos
Kv1.5
Tbx5
Nav2.1
Kv1.1
Bcl6
Atf3
Mafk
Cav1.3
Ddx20
Kv2.1
Nr1h1
Merg
Atf4

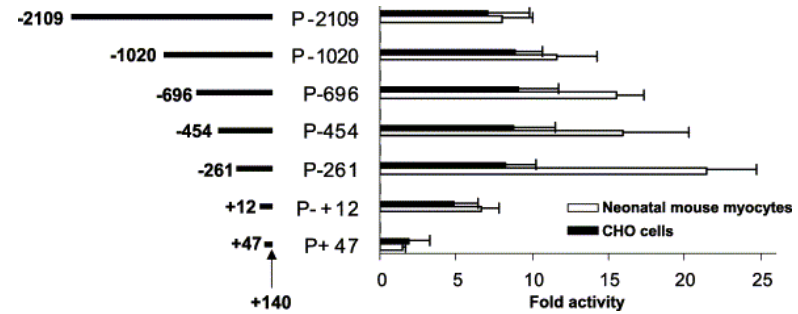


Validation biologique et conséquences fonctionnelles

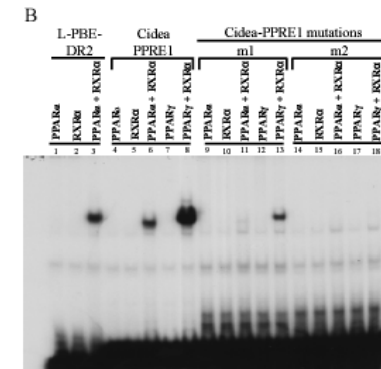


Rôle des ATFs dans l'excitabilité cellulaire ?

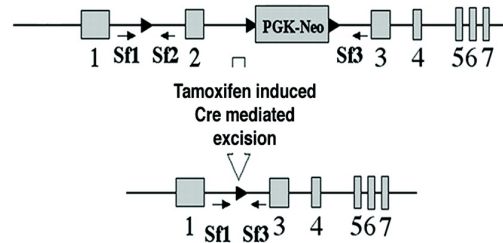
Analyser la fonction du promoteur du canal sodique



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

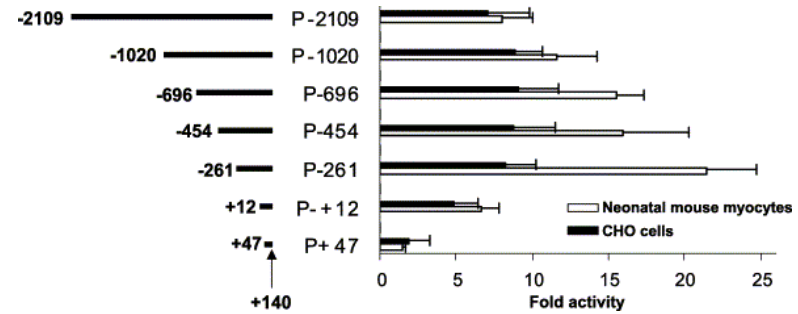


Evaluer les conséquences fonctionnelles *in vivo*

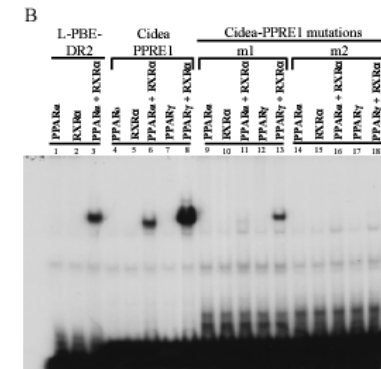


Rôle des ATFs dans l'excitabilité cellulaire ?

Analyser la fonction du promoteur du canal sodique



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



Evaluer les conséquences fonctionnelles *in vivo*

