



# Electrophysiologie moléculaire - troubles du rythme et de la conduction cardiaques



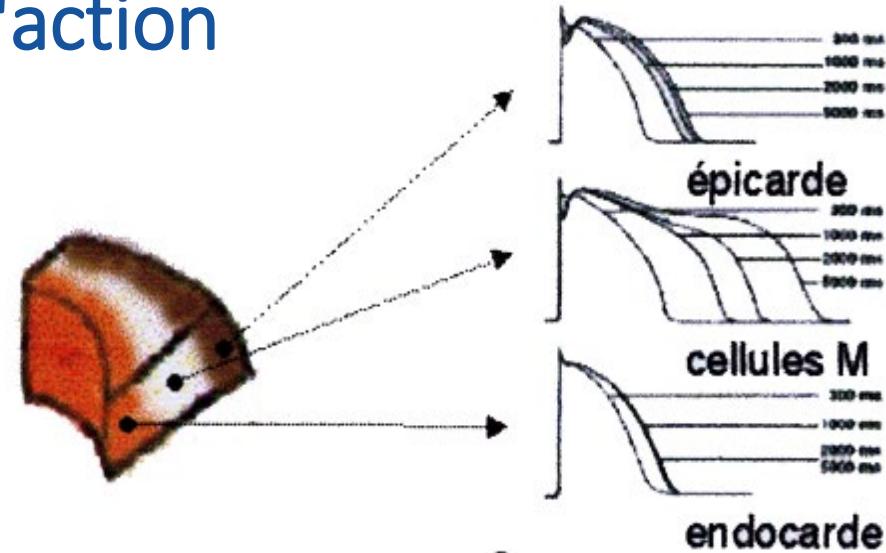
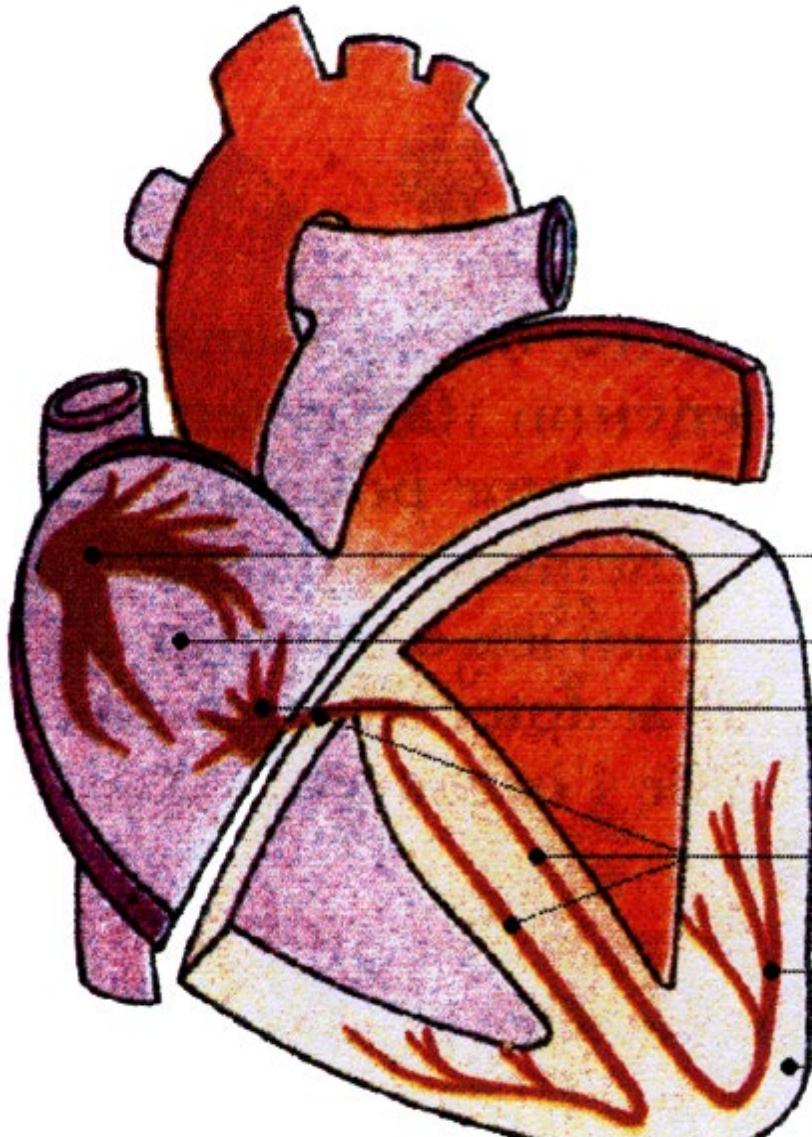
Isabelle BARÓ, PhD

Equipe Canaux ioniques et arythmies  
cardiaques

L'unité de recherche de l'institut du thorax  
Inserm UMR 1087 / CNRS UMR 6291  
Nantes, France

MASTER 1 Biologie et Santé - Thorax

# Activité électrique du cardiomyocyte : le potentiel d'action

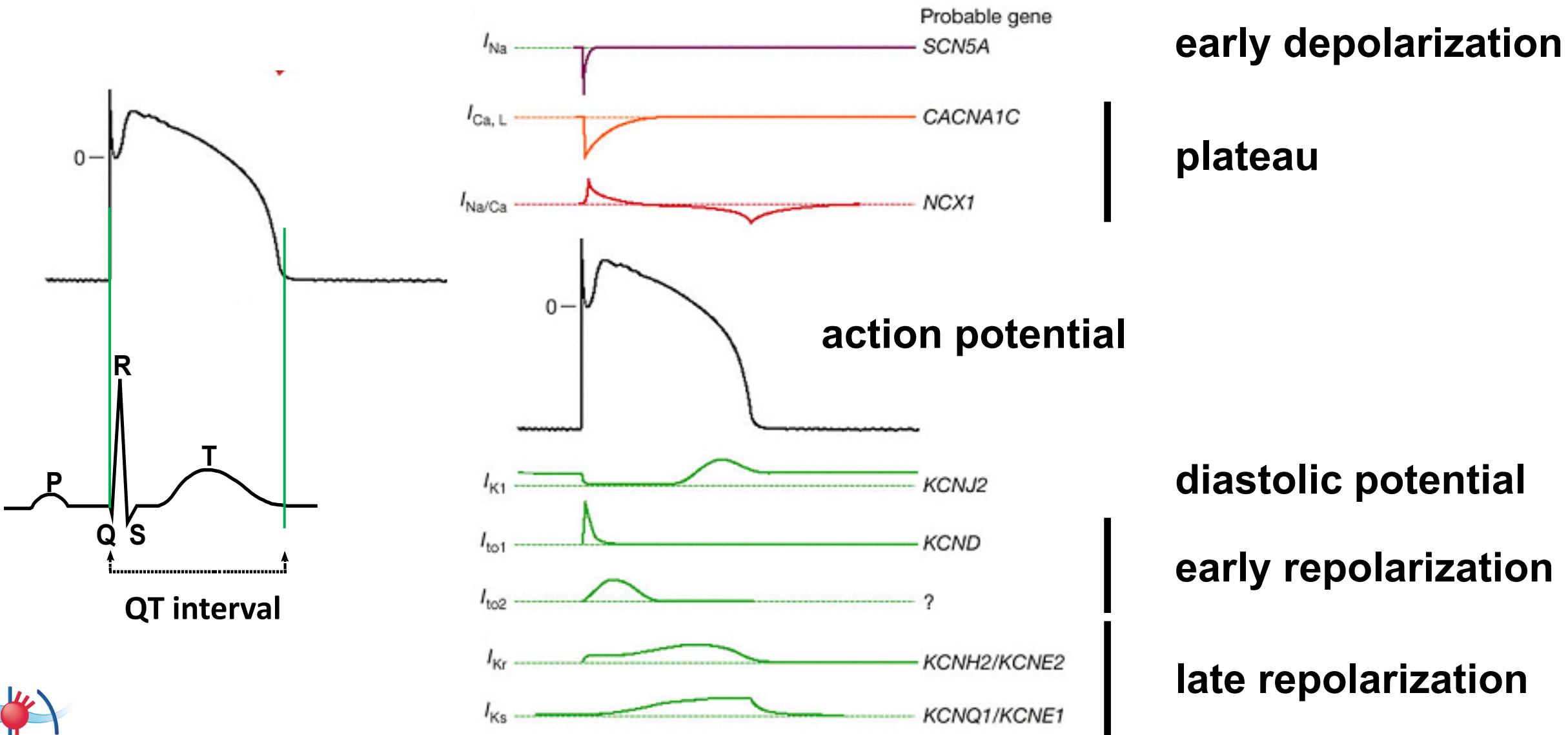


potentiels d'action

ECG

hétérogénéité du potentiel d'action (PA)

# Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



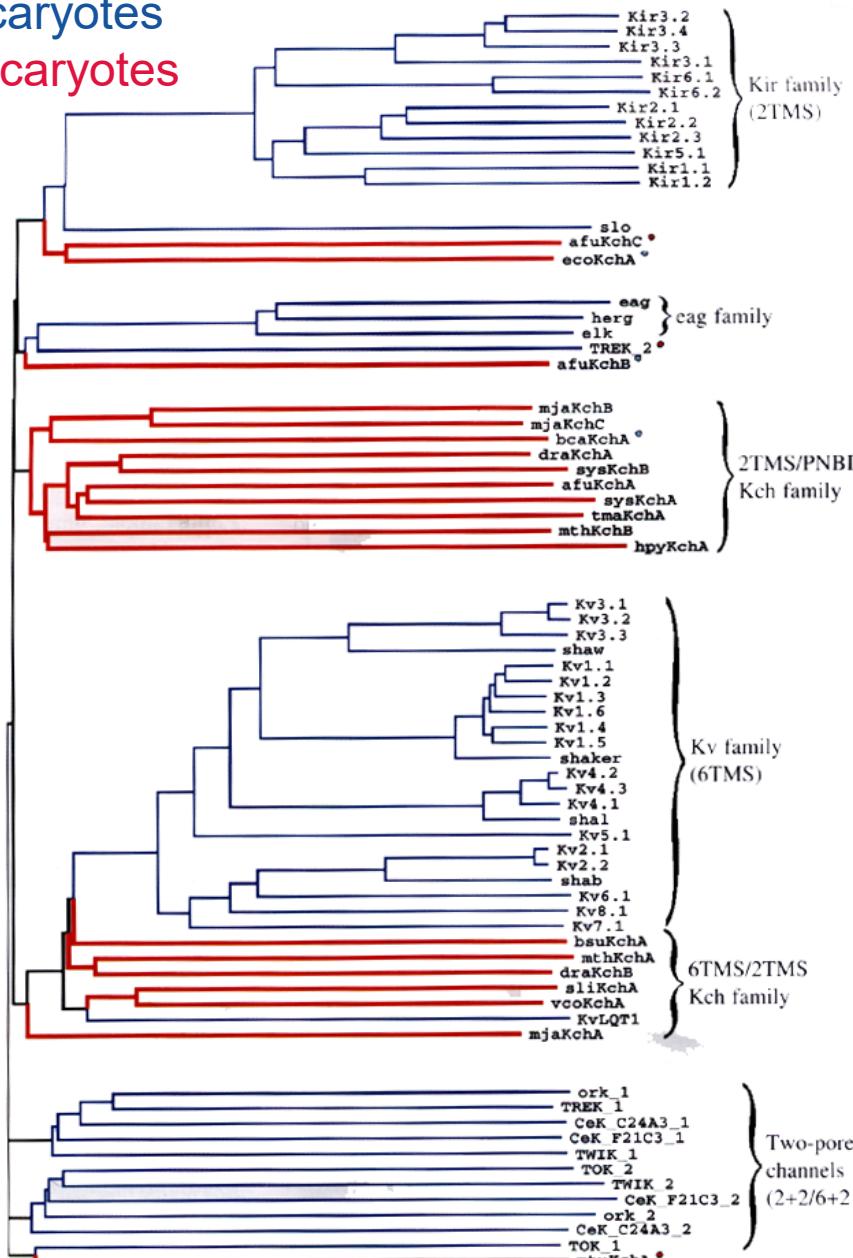
from Marbán E. Nature. 2002 Jan 10;415(6868):213-8

<https://pubmed.ncbi.nlm.nih.gov/>

## dendrogramme

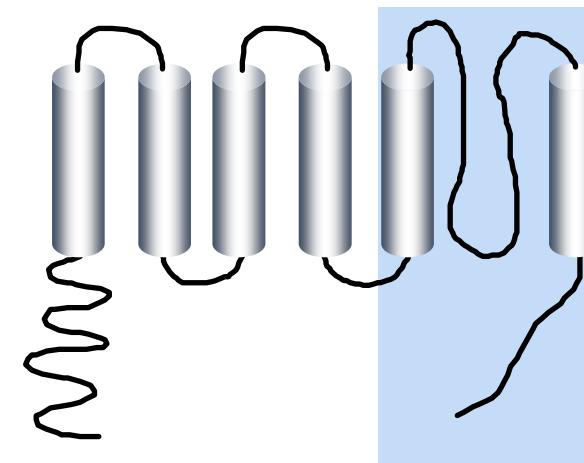
# Canaux potassiques

eucaryotes  
procaryotes

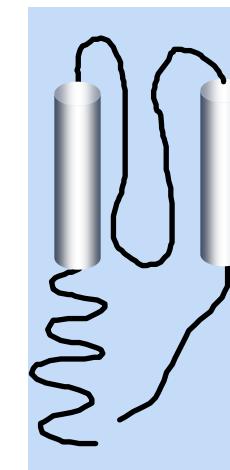


→ 3 classes

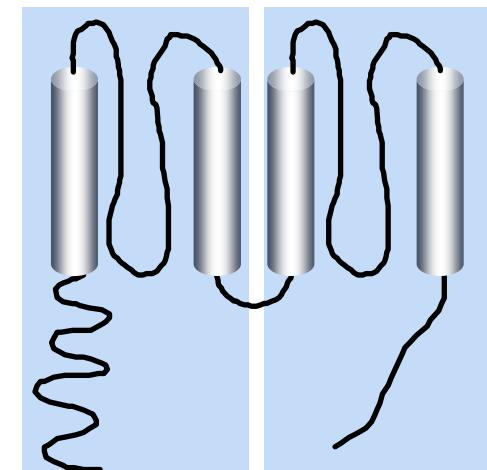
6 segments  
transmembranaires  
(TMS)  
et 1 boucle du  
pore



2 TMS  
et 1 boucle du  
pore

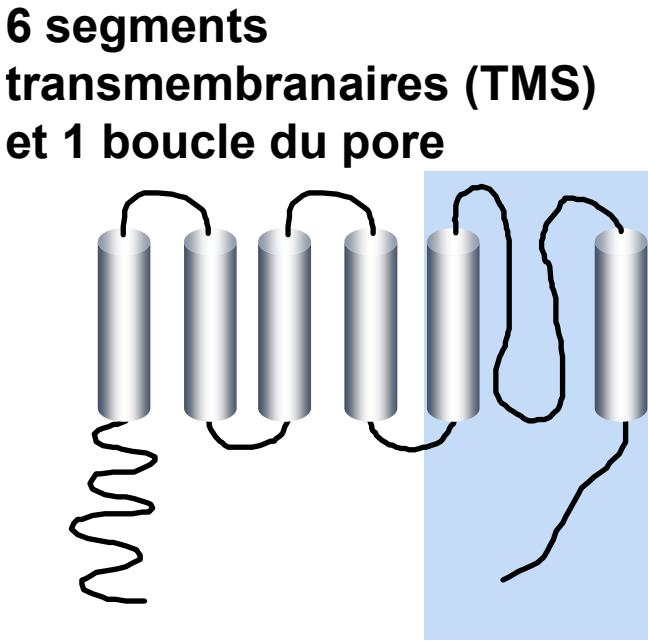


4 TMS  
et 2 boucles du  
pore



# 3 classes de canaux $K^+$

Trace  
hydropathie



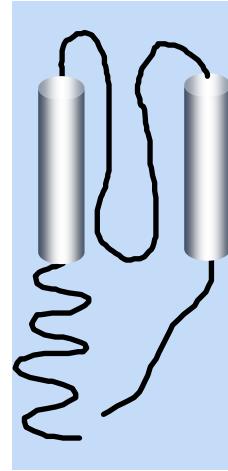
Structure

tétramère

Régulateurs

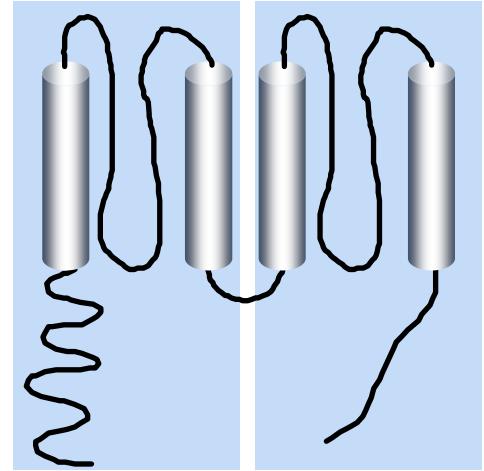
principalement  
 $V_{membrane}$

2 TMS  
et 1 boucle du  
pore



tétramère

4 TMS  
et 2 boucles du  
pore



dimère

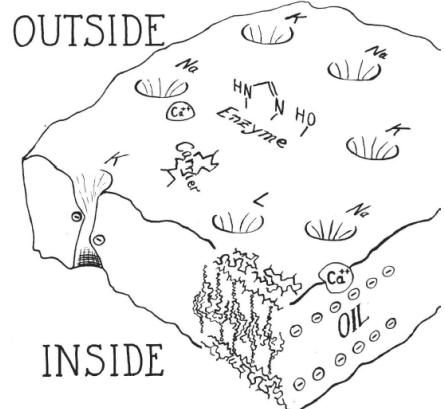
Cibles

$V_{membrane}$ ; [ion]

# De l'hypothèse à la structure cristallographique d'un canal

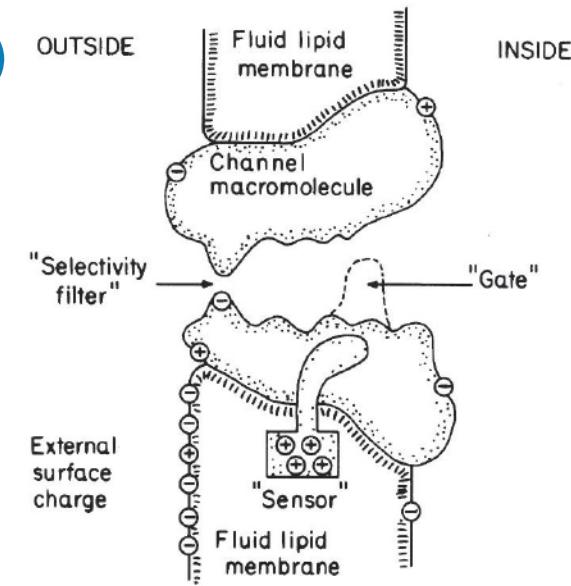
## Hypothèse de travail (1967)

Hille B. 1967. A pharmacological analysis of the ionic channels of nerve. PhD thesis, Rockefeller Univ., New York



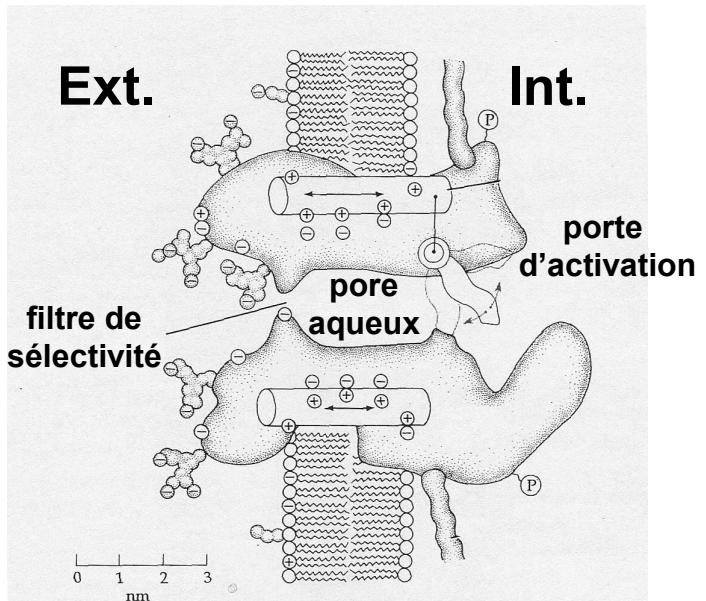
## Hypothèse de travail (1977)

Hille B. 1977 J. Gen. Physiol. 69:497–515



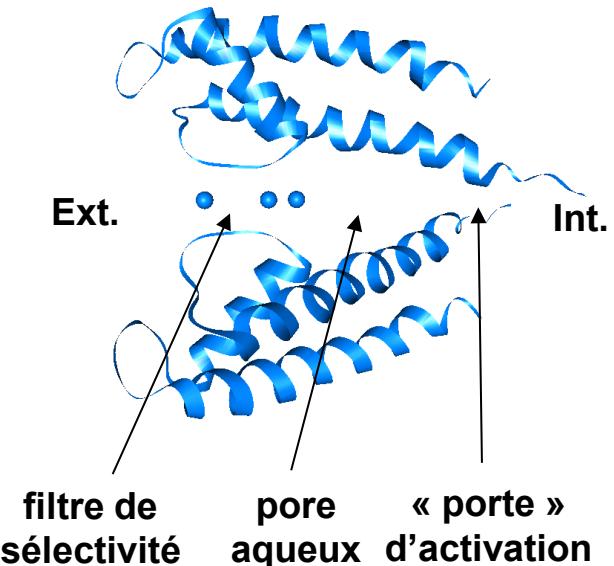
## Hypothèse de travail (1992)

Hille B in Ionic Channels of Excitable Membranes (2<sup>nd</sup> edition). 1992; Sinauer Associated, MA 01375, U.S.A.

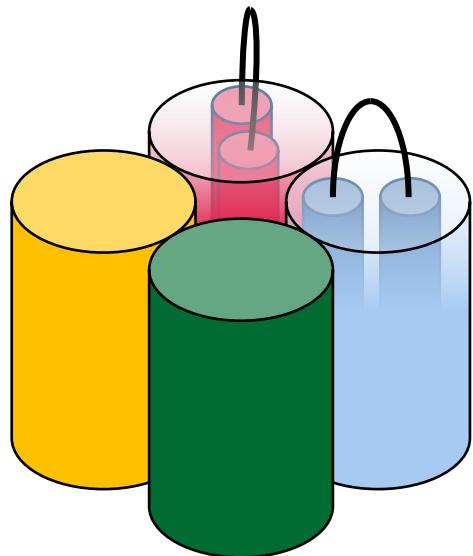


## Structure de KcsA (1998)

from Doyle DA et al. Science. 1998 Apr 3;280(5360):69-77

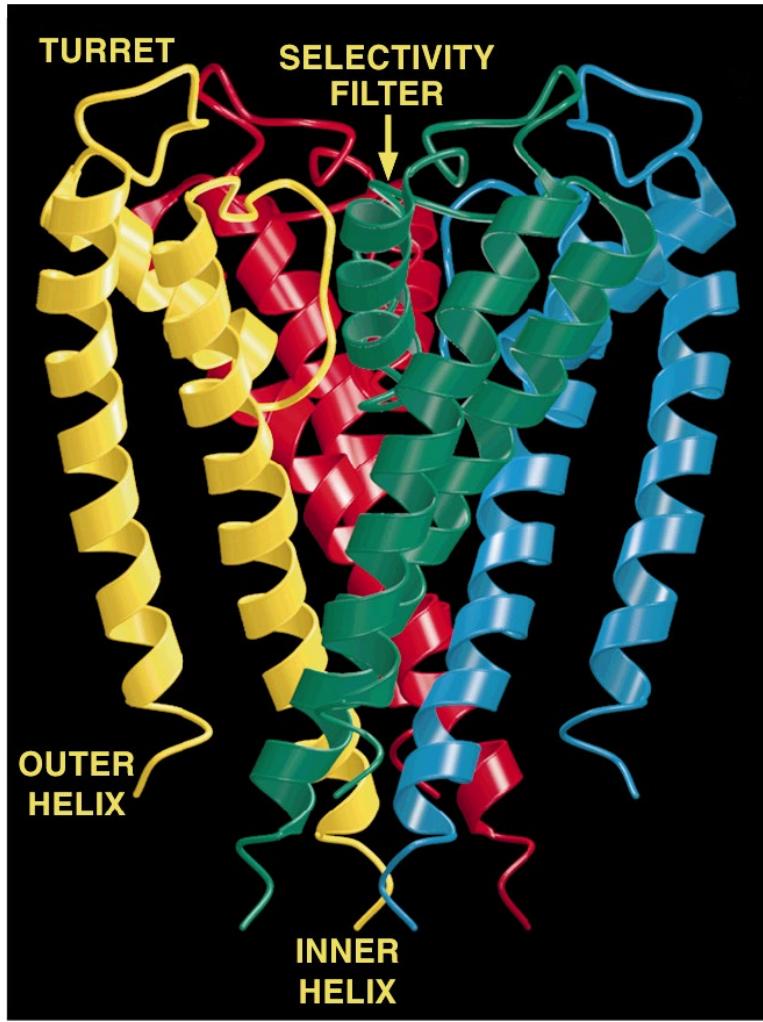


# Structure cristallographique d'un canal K<sup>+</sup> à 2 TMS et 1 pore



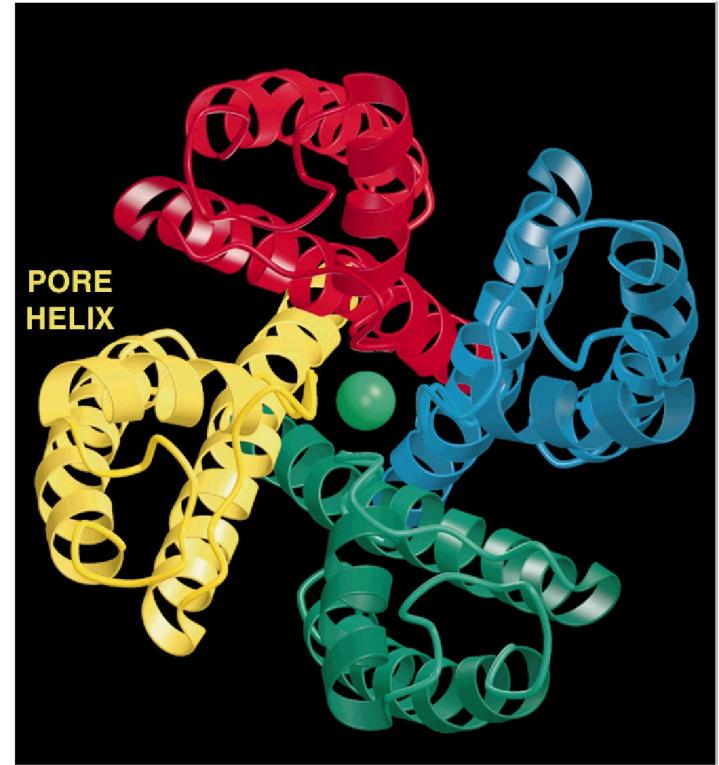
Tétramères

KcSA



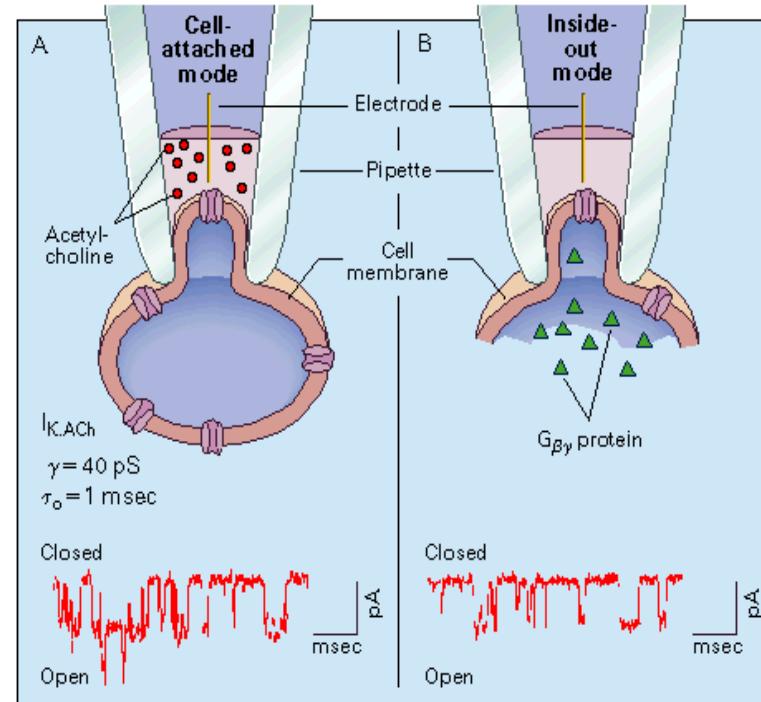
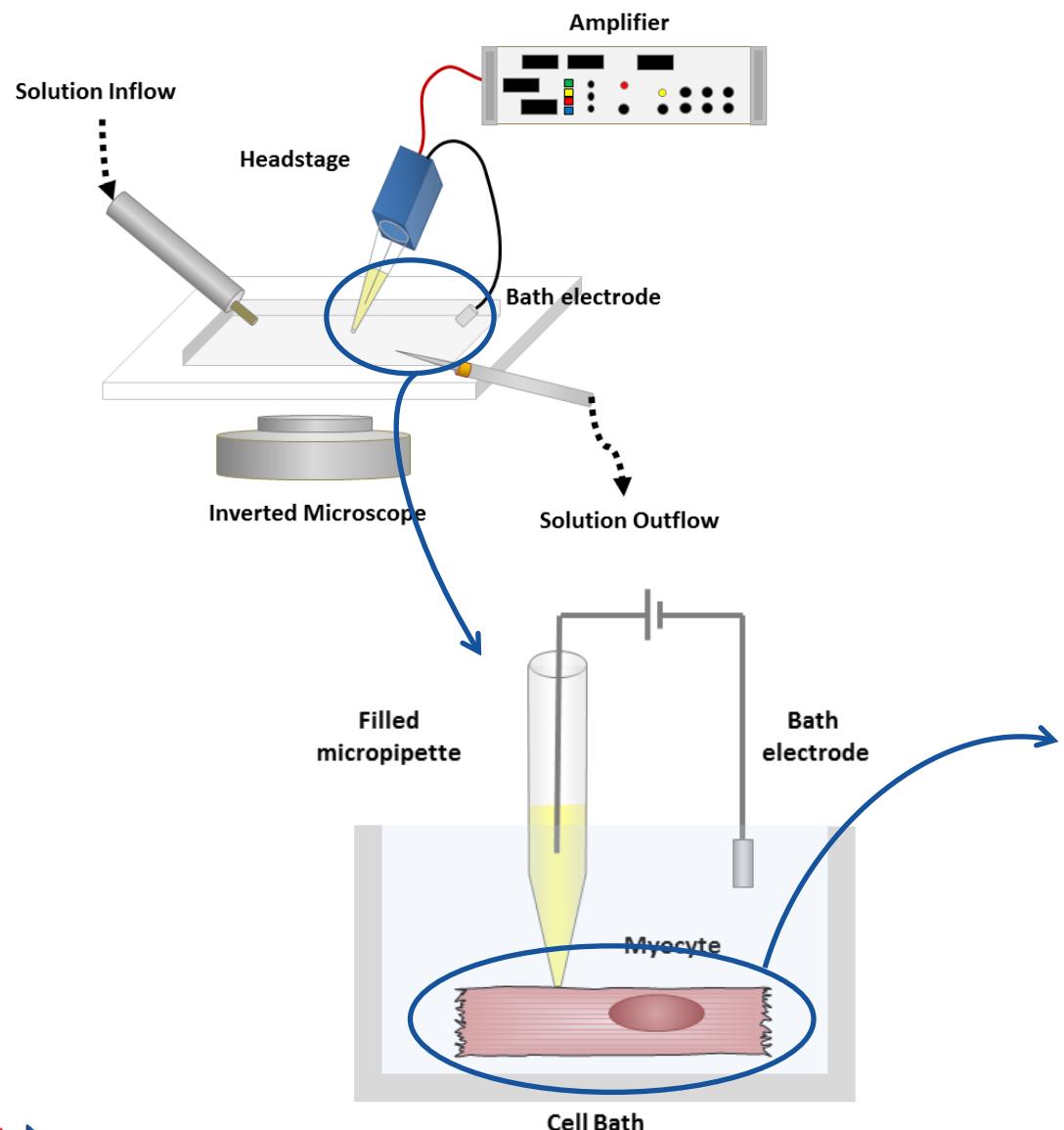
(resolution: 3.2 Å)

D'après Doyle DA et al. Science. 1998 Apr 3;280(5360):69-77

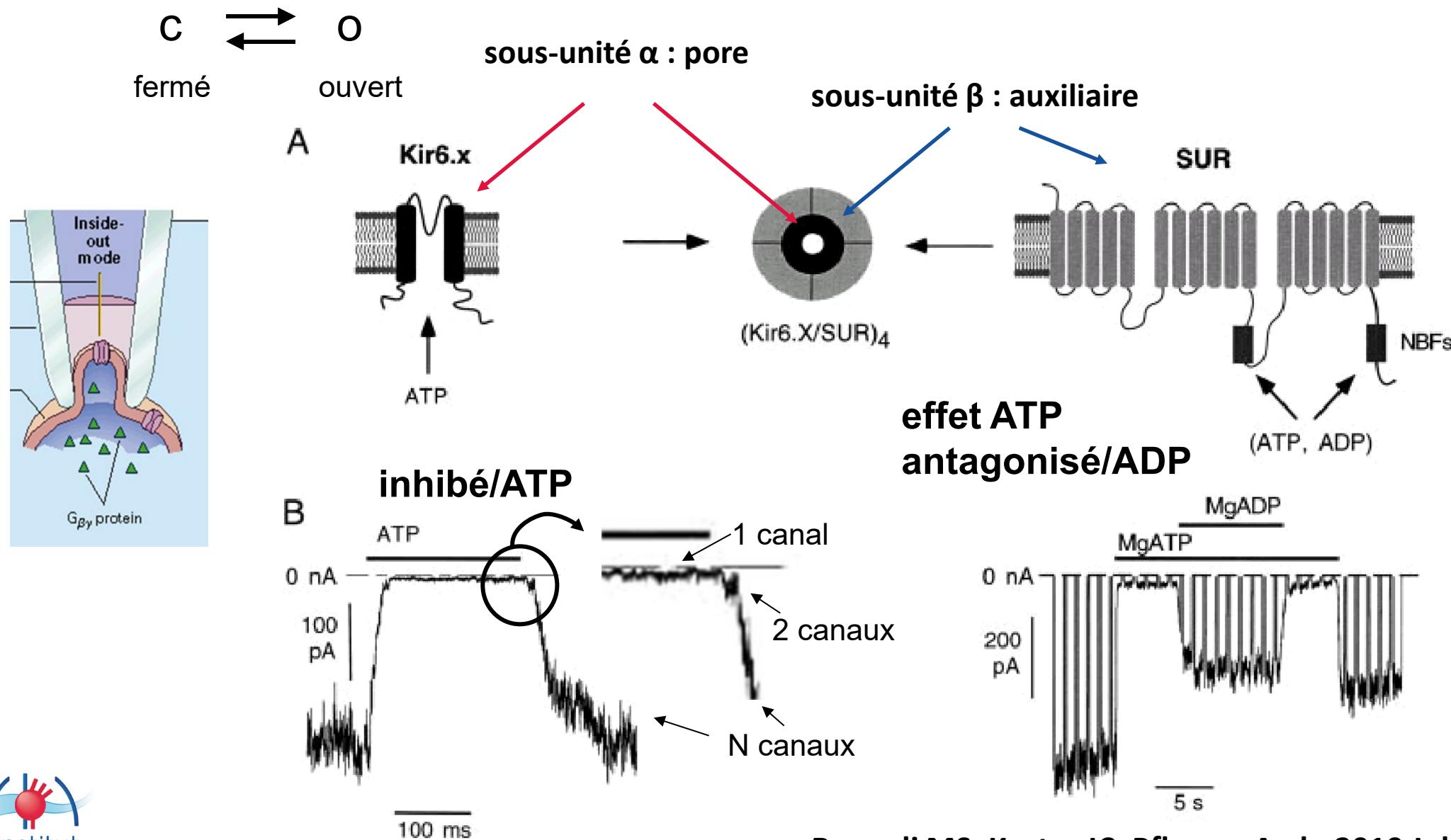


Prix Nobel de Chimie 2003 :  
Roderick Mac Kinnon

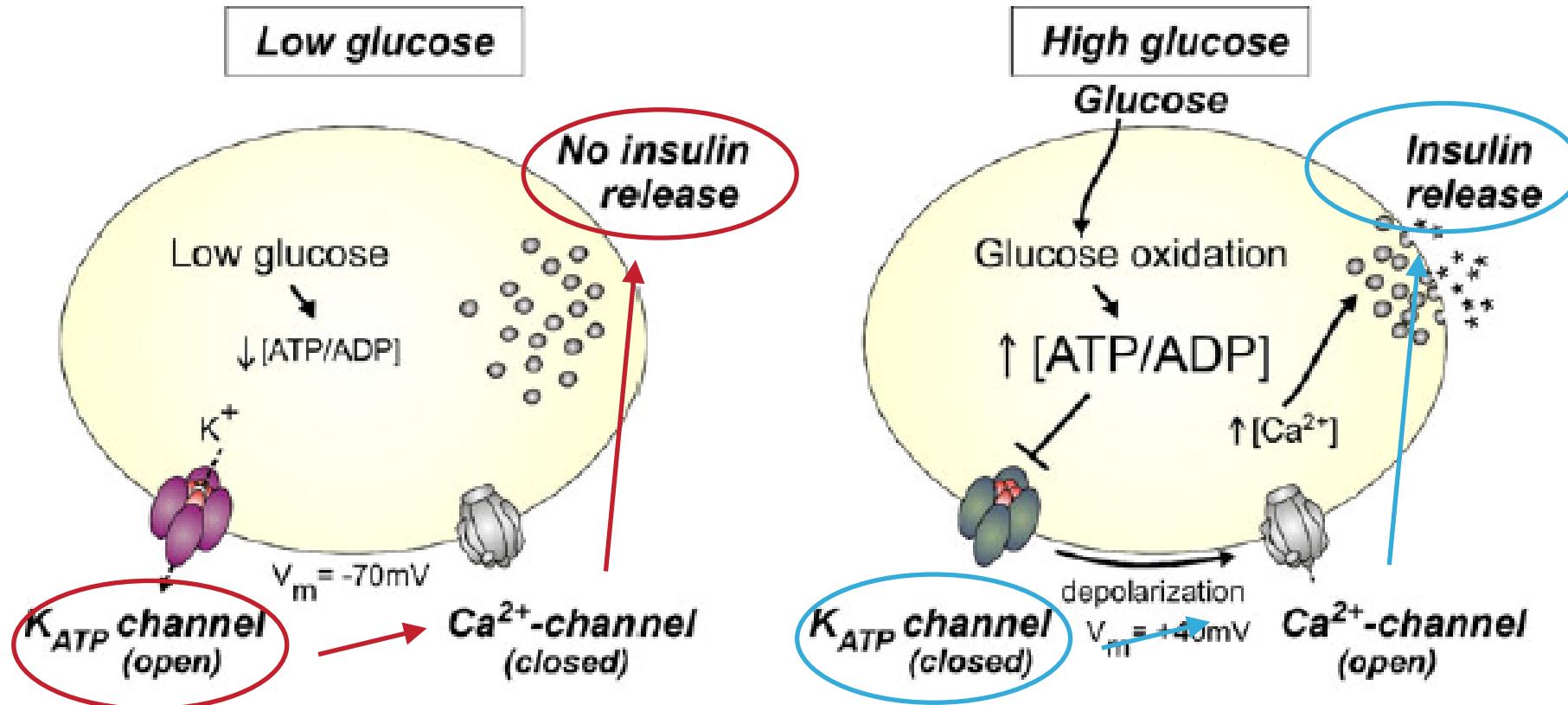
# Enregistrement d'un courant unitaire : le patch-clamp



# canaux $K_{ATP}$ du pancréas (cellules $\beta$ des îlots de Langerhans)

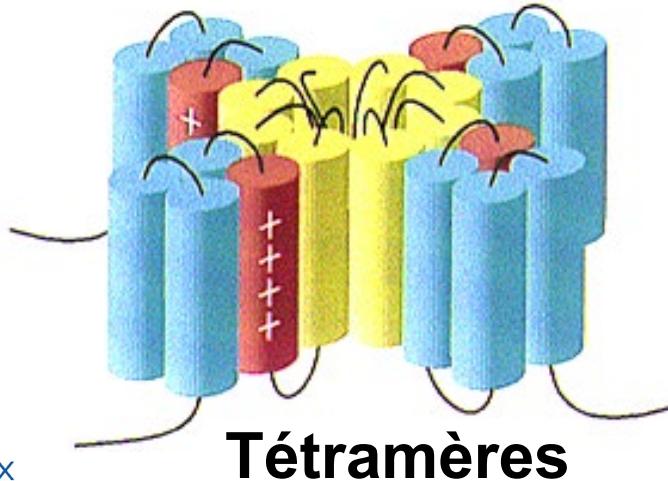
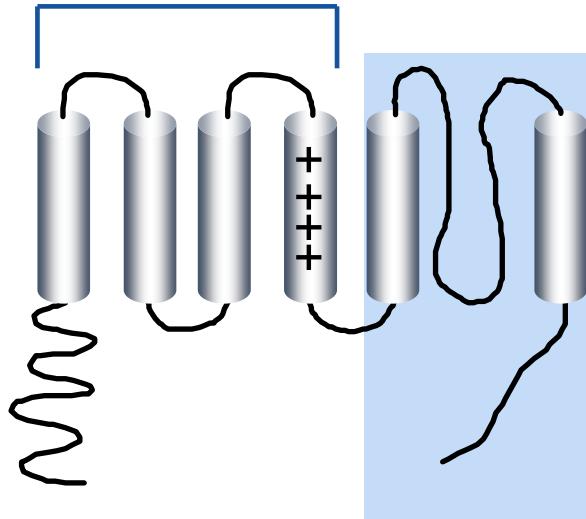


# canaux $K_{ATP}$ du pancréas (cellules $\beta$ ) rôle dans la sécrétion d'insuline

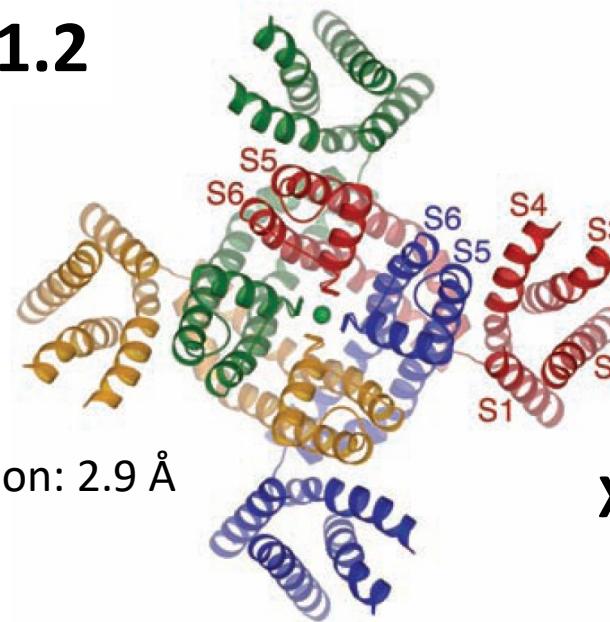


# Structure d'un canal K<sup>+</sup> 6 TMS et 1 pore

voltage sensor



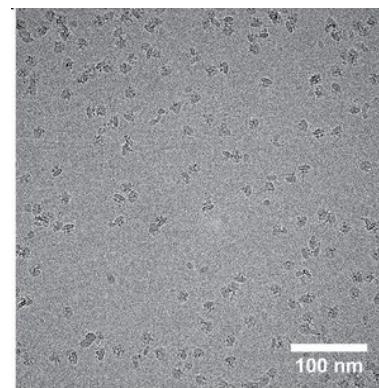
Kv1.2



Long SB et al. Science. 2005 Aug 5;309(5736):897-903.

X-ray crystallography

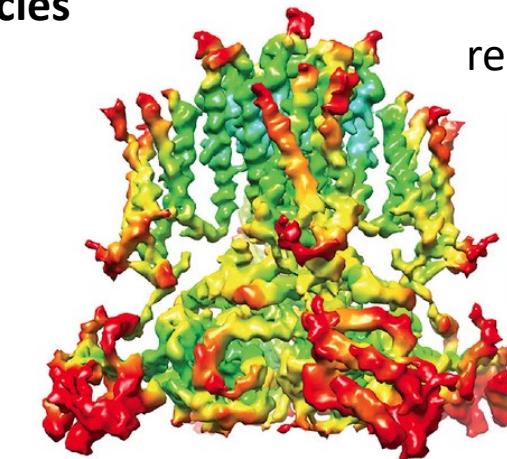
hERG



~144,000 particles



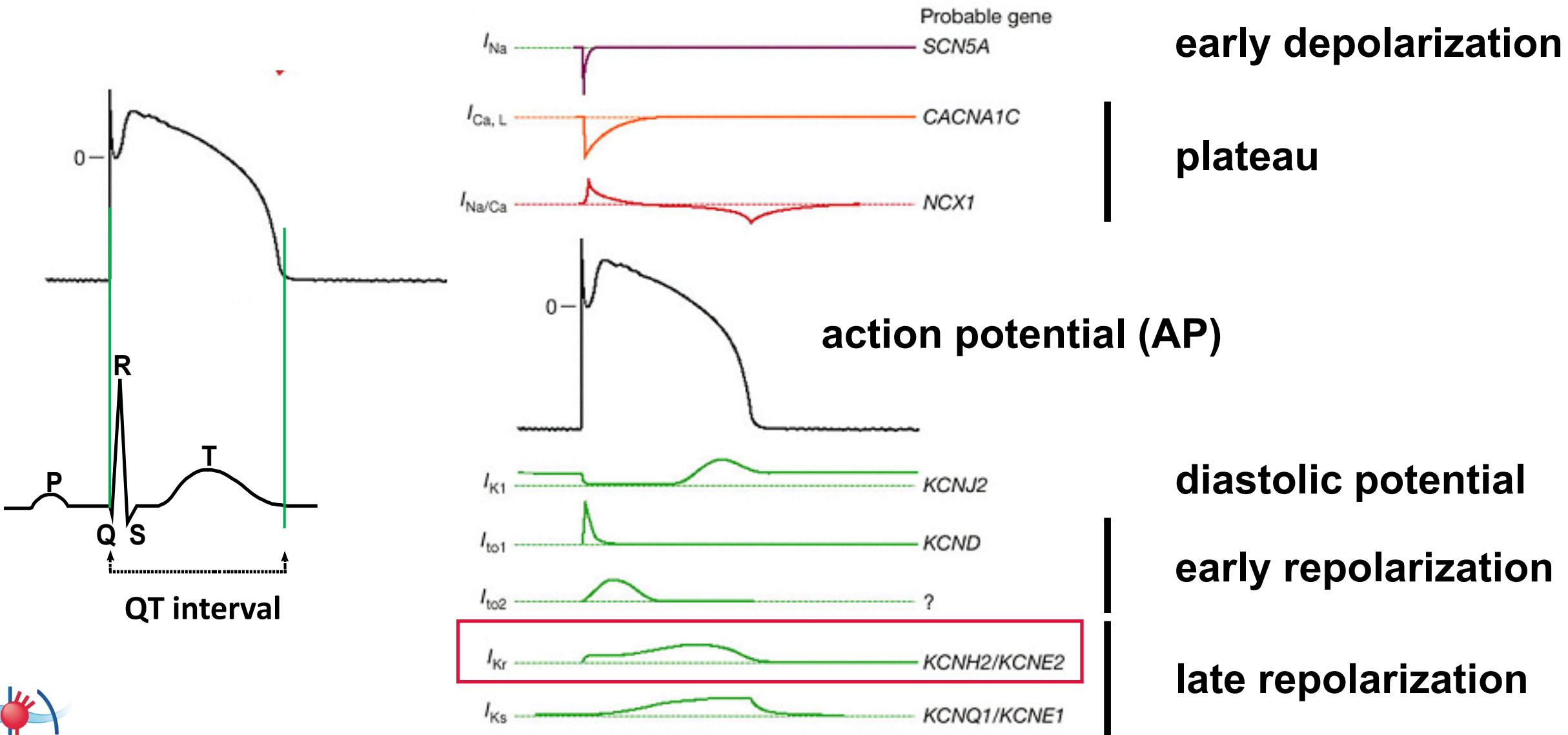
resolution:  
5.6  
5.0  
4.4  
3.8  
3.2 Å



Cryo-electron microscopy

Wang W et al. Cell. 2017 Apr 20;169(3):422-430.e10.

# Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



from Marbán E. Nature. 2002 Jan 10;415(6868):213-8

# Etude d'une mutation de hERG associée au Syndrome du QT long

- **Syndrome du QT long (ECG : intervalle QTc > 450-470 ms), fibrillation ventriculaire (torsades de pointes), mort subite**

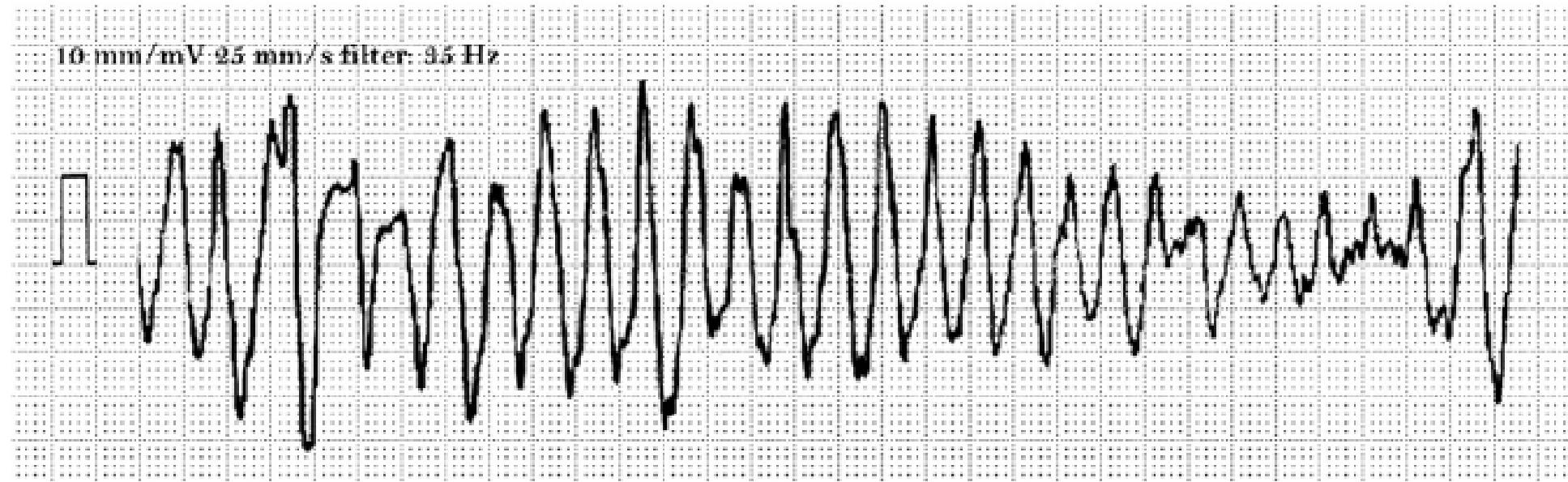


Fig. 1. ECG recording showing an ongoing episode of Torsade de pointes. This polymorphic ventricular arrhythmia is characterized by the progressive rotation of the electrical axis (180 ° in about 10–12 cycles) which shows on the surface ECG as the sinusoidal undulation of the electrical signal.

# Etude d'une mutation de hERG associée au Syndrome du QT long

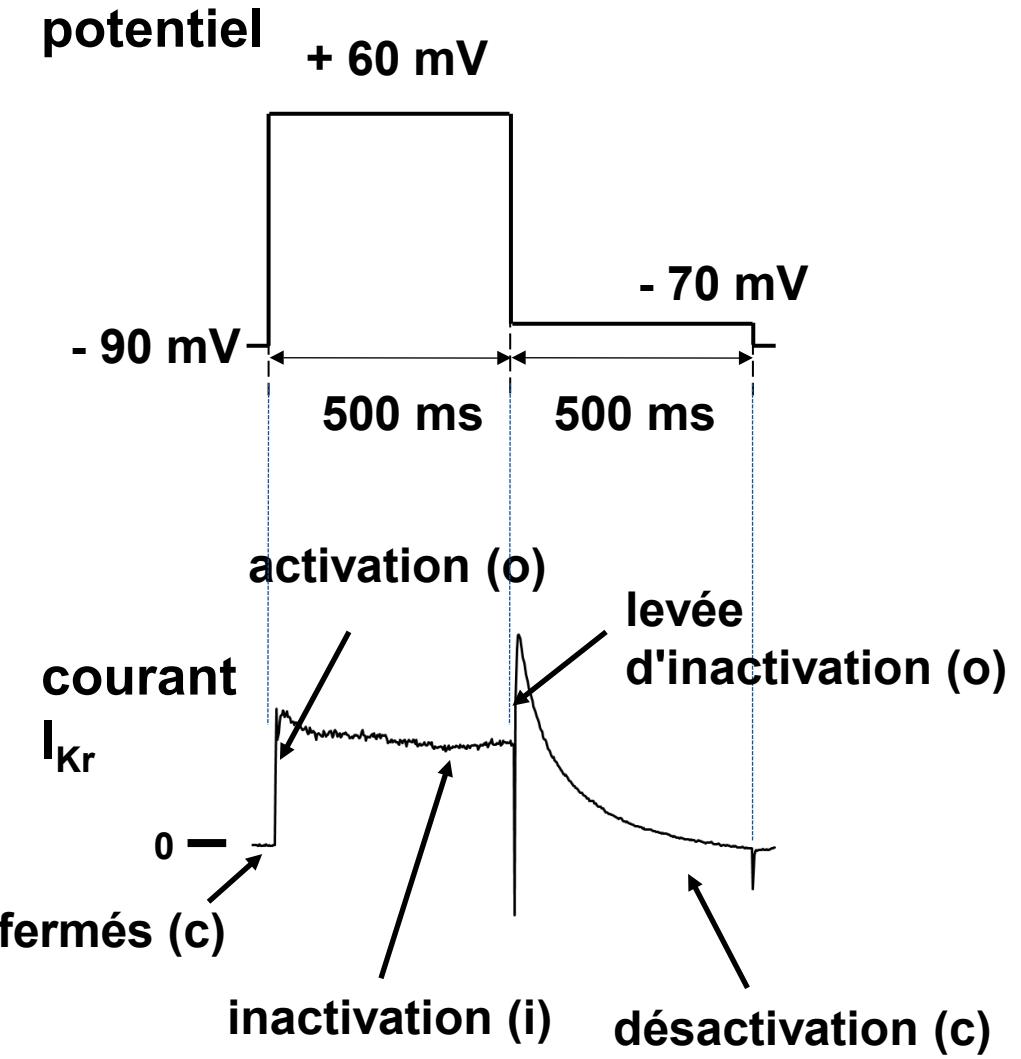
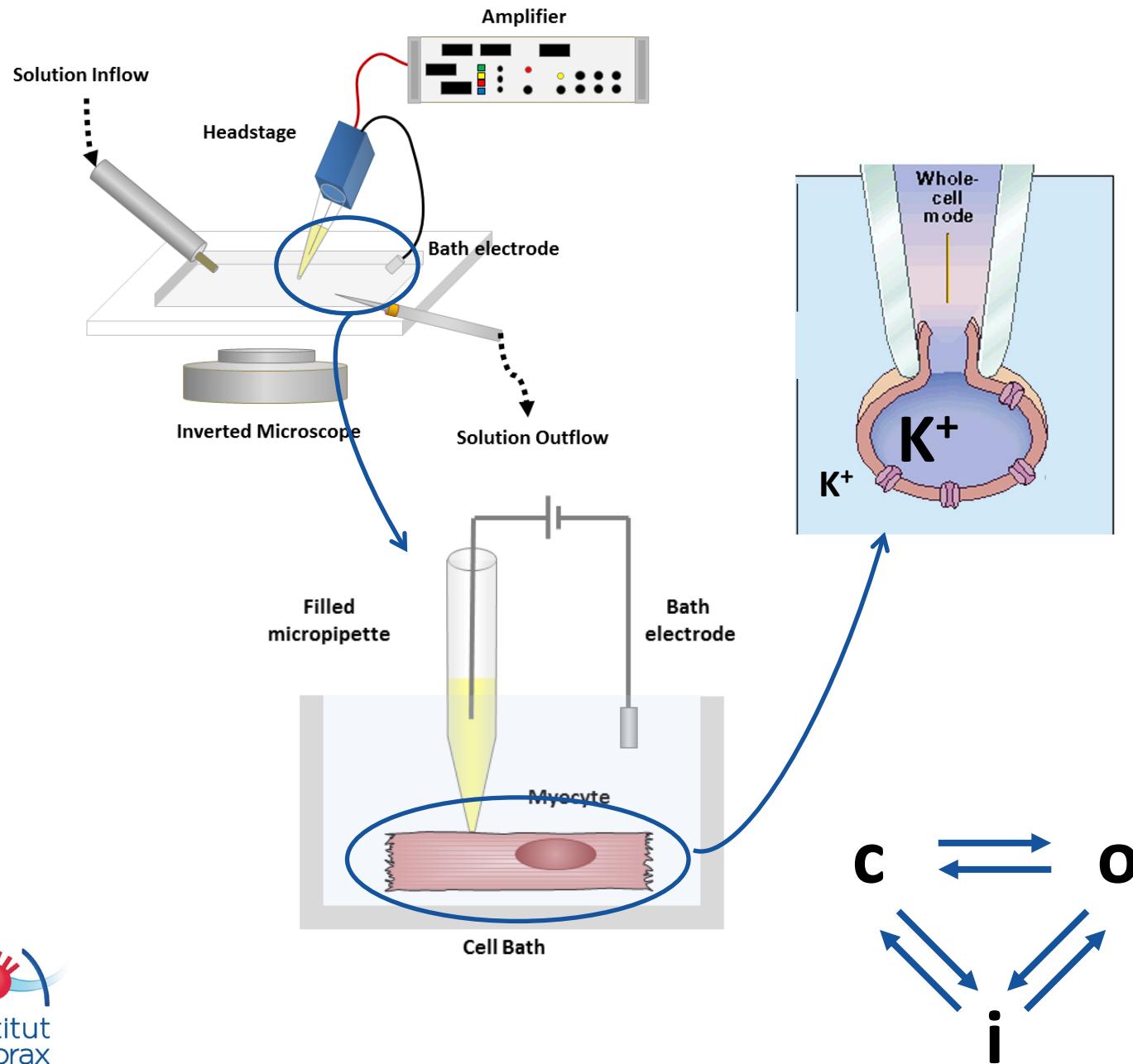
Table 1 Classification of genes responsible for cardiac channelopathies. Adapted from Schwartz et al. [2]

LQTS type	Gene	Mutation frequency among LQTS population (%)	Locus	Protein (functional effect)
Romano-Ward (RWS) mut/WT				
LQT1	KCNQ1	40–55	11p15.5	K <sub>V</sub> 7.1 (↓)
LQT2	KCNH2	30–45	7q35–36	K <sub>V</sub> 11.1 (↓)
LQT3	SCN5A	5–10	3p21–24	Na <sub>V</sub> 1.5 (↑)
LQT4	ANKB	< 1	4q25–27	Ankyrin B (↓)
LQT5	KCNE1	< 1	21q22.1	MinK (↓)
LQT6	KCNE2	< 1	21q22.1	MiRP1 (↓)
LQT7	KCNJ2	< 1	17q23	Kir2.1 (↓)
LQT8	CACNA1C	< 1	12p13.3	L-type calcium channel (↑)
LQT9	CAV3	< 1	3p25	Caveolin 3 (↓)
LQT10	SCN4B	< 1	11q23.3	Sodium channel-β4 (↓)
LQT11	AKAP9	< 1	7q21–22	Yotiao (↓)
LQT12	SNTA1	< 1	20q11.2	Syntrophin α1 (↓)
LQT13	KCNJ5	< 1	11q24	Kir3.4 (↓)
LQT14	CALM1	< 1	14q32.11	Calmodulin 1 (dysfunctional Ca <sup>2+</sup> signaling)
LQT15	CALM2	< 1	2p21	Calmodulin 2 (dysfunctional Ca <sup>2+</sup> signaling)
Jervell and Lange-Nielsen syndrome (JLNS) mut/mut				
JLN1	KCNQ1	< 1	11p15.5	K <sub>V</sub> 7.1 (↓)
JLN2	KCNE1	< 1	21q22.1–22.2	MinK (↓)

Arrows up (↑) or down (↓) showing gain or loss of protein function, respectively

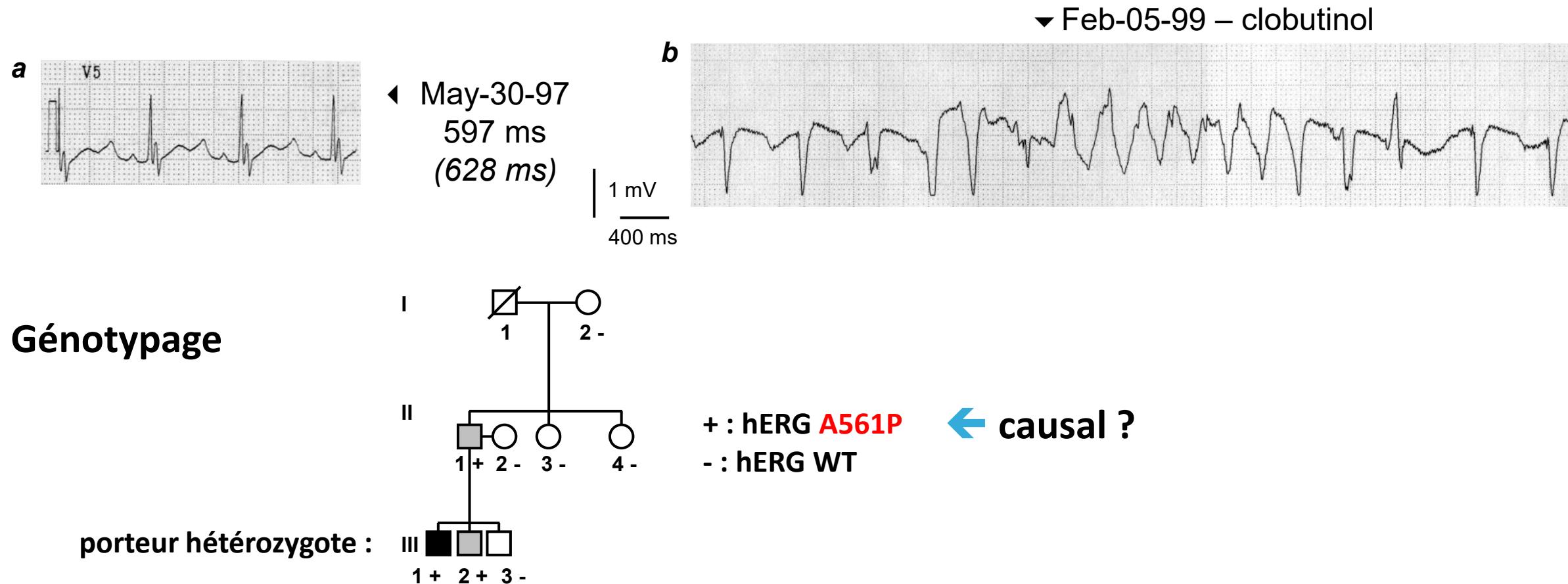
LQT long QT, RWS Romano–Ward syndrome, JLNS Jervell and Lange-Nielsen syndrome

# Enregistrement d'un courant trans-membranaire : le patch-clamp



# Etude d'une mutation de hERG associée au Syndrome du QT long

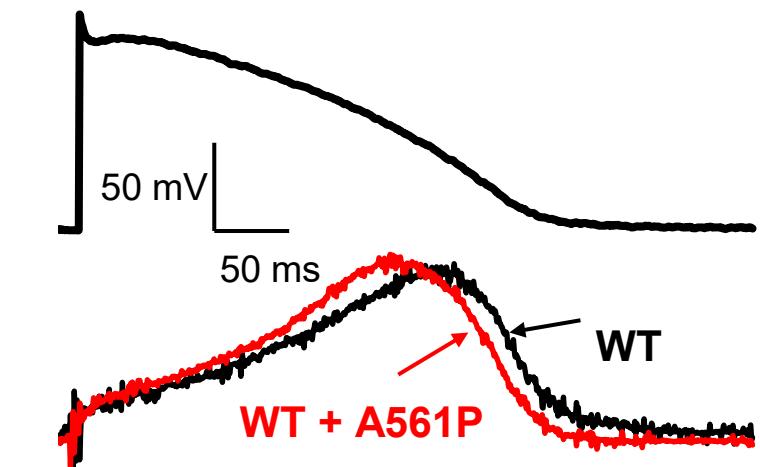
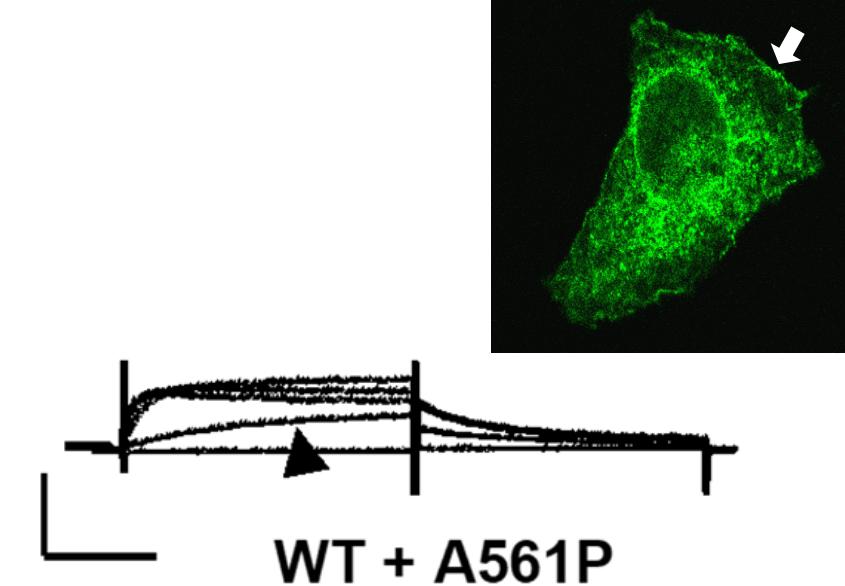
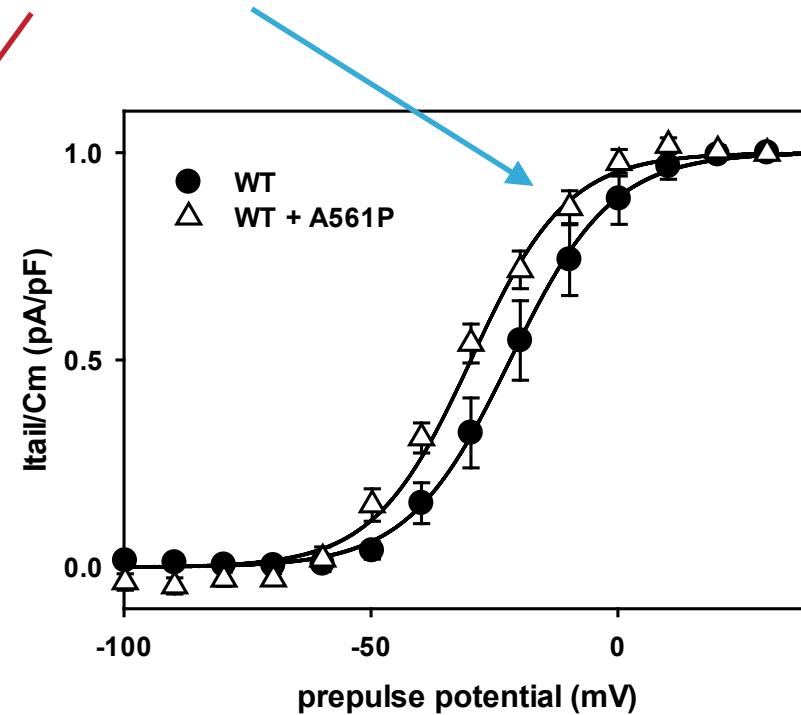
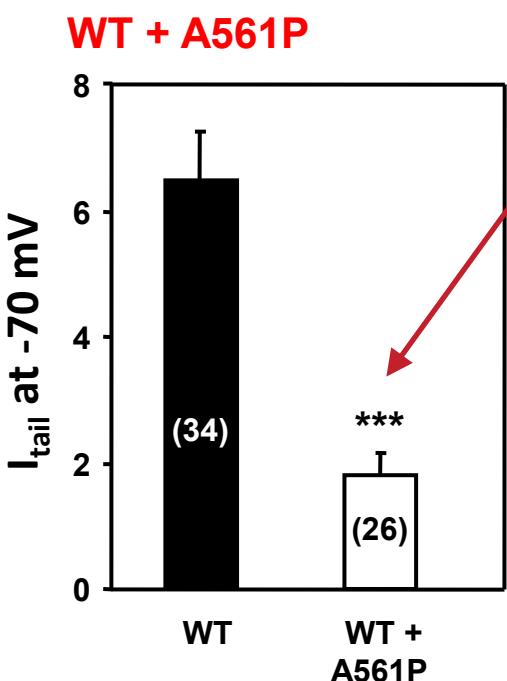
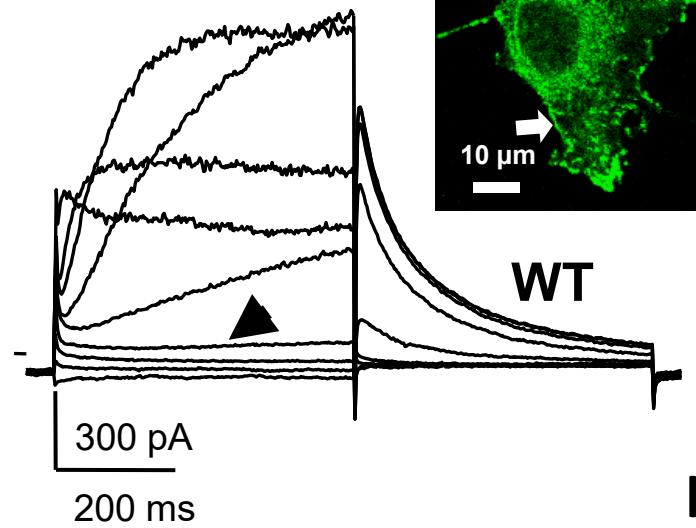
## Propositus : fibrillation ventriculaire (torsades de pointes)



## Modèles cellulaires

- Lignées cellulaires modifiées - canalopathies monogéniques  
expression hétérologue de protéines WT et mutées

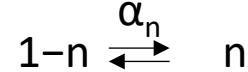
## injected COS-7



# Modèles *in silico*

- Modèle de potentiel d'action ventriculaire humain Priebe and Beuckelmann (1998) -  
modèle d'Hodgkin et Huxley (Prix Nobel de Physiologie 1963)

$$I_K = n^4 \bar{g}_K (V - E_K)$$



$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

Rapidly Activating Current:  $I_{Kr}$

$$I_{Kr} = g_{Kr,max} \cdot Xr \cdot rik \cdot (V - E_K)$$

$$E_K = (RT/F) \cdot \ln([K^+]_o/[K^+]_i)$$

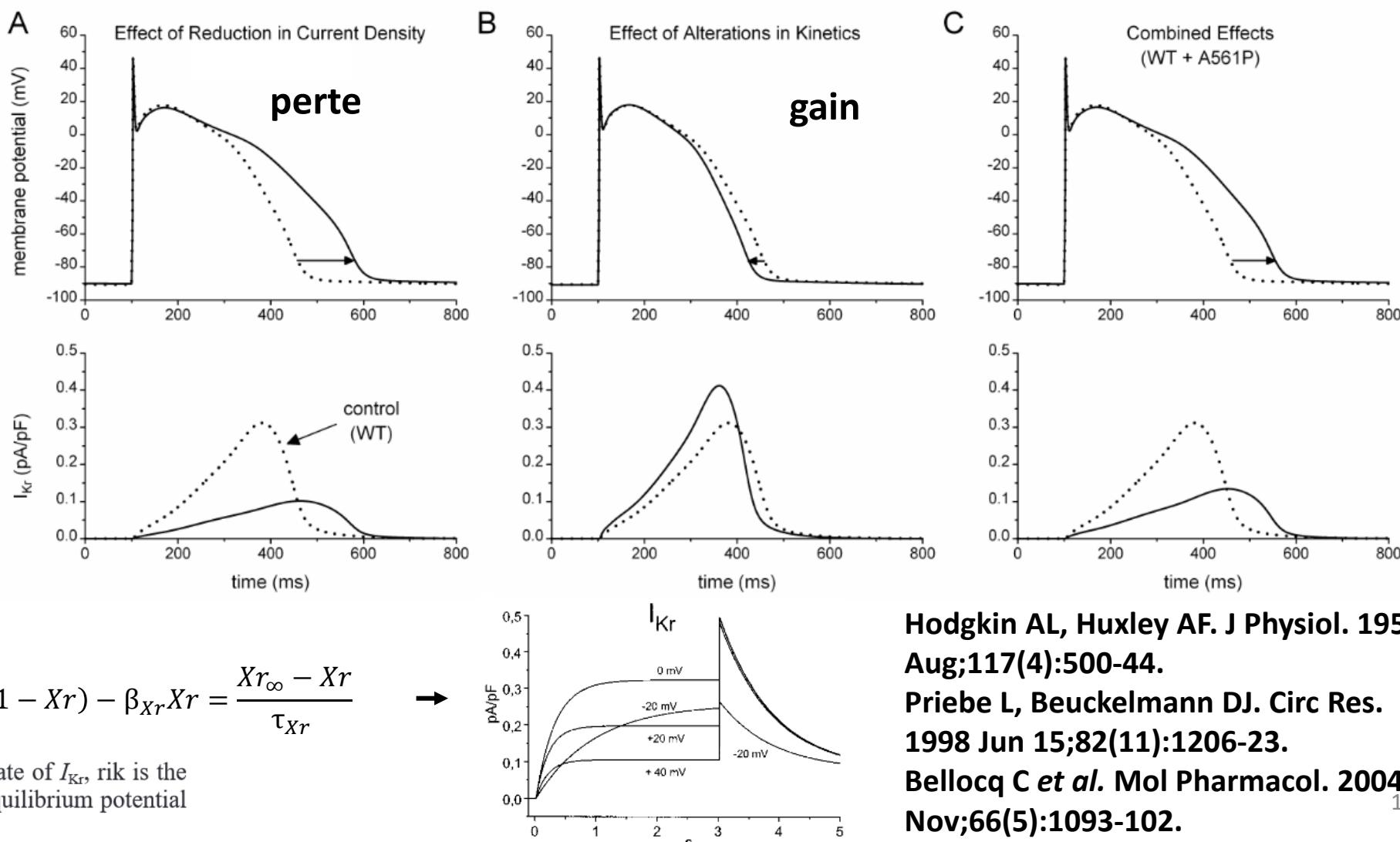
$$g_{Kr,max} = 0.015 \text{ mS}/\mu\text{F}$$

$$\alpha_{Xr} = \{0.005 \cdot \exp[5.266 \cdot 10^{-4} \cdot (V + 4.067)]\} / \{1 + \exp[-0.1262 \cdot (V + 4.067)]\}$$

$$\beta_{Xr} = \{0.016 \cdot \exp[1.6 \cdot 10^{-3} \cdot (V + 65.66)]\} / \{1 + \exp[0.0783 \cdot (V + 65.66)]\}$$

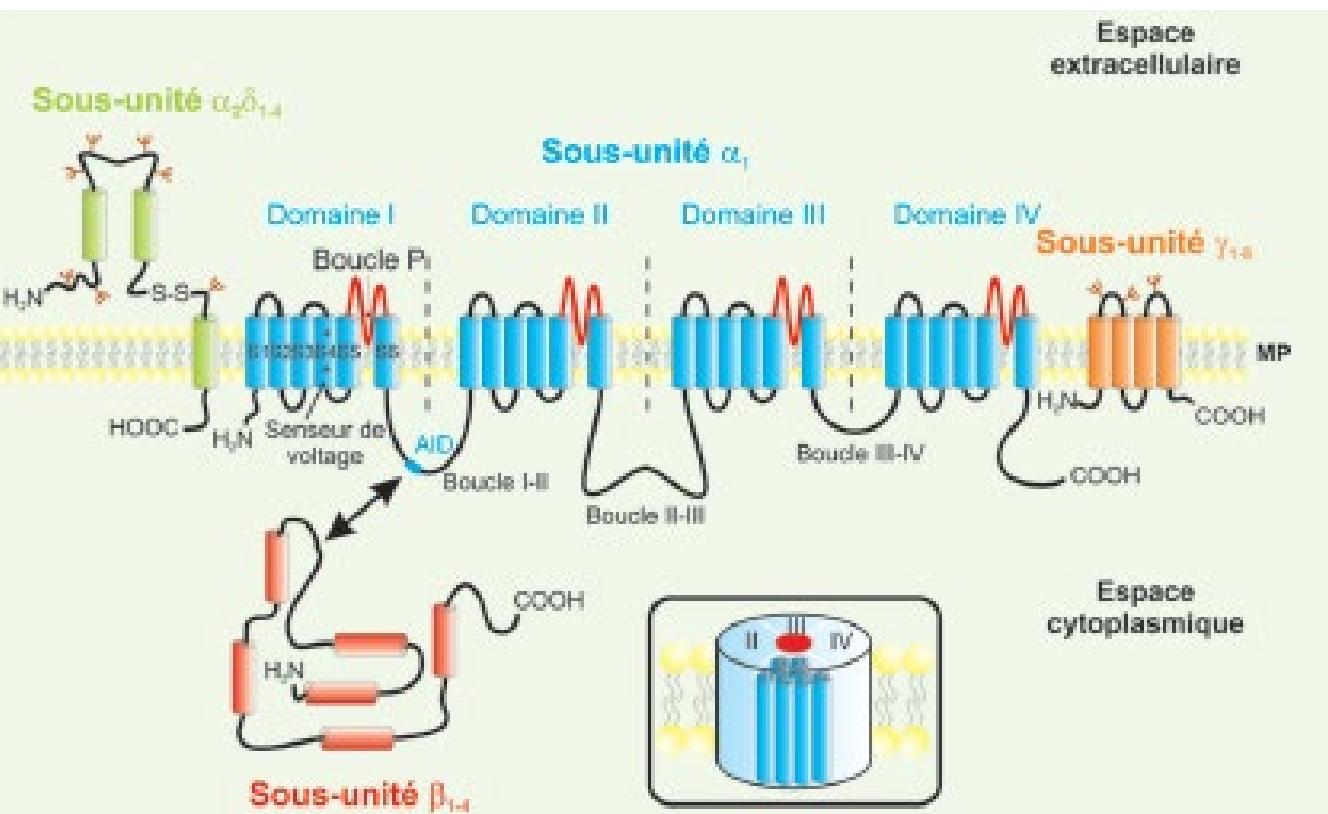
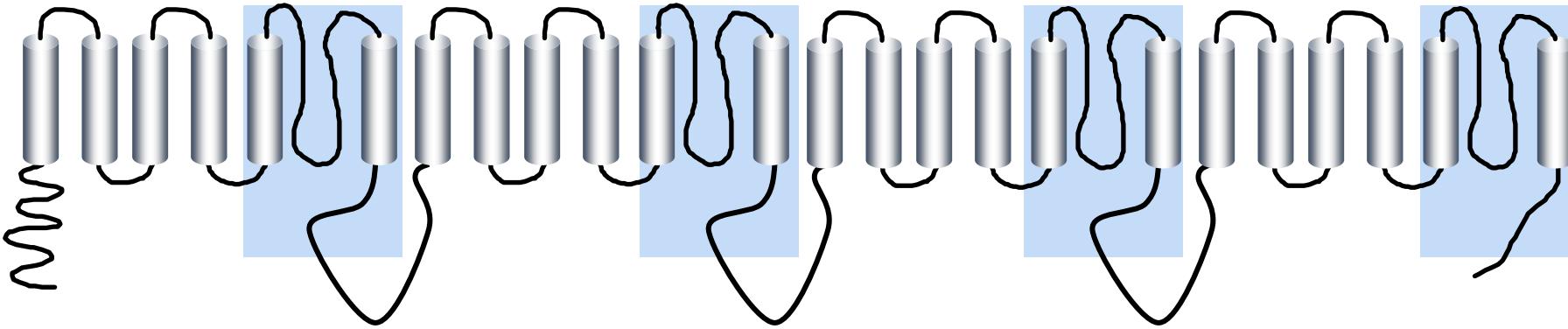
$$rik = 1/\{1 + \exp[(V + 26)/23]\} \quad \frac{dXr}{dt} = \alpha_{Xr}(1 - Xr) - \beta_{Xr}Xr = \frac{Xr_\infty - Xr}{\tau_{Xr}}$$

where  $g_{Kr,max}$  is  $g_{max}$  for  $I_{Kr}$ ,  $Xr$  is the activation gate of  $I_{Kr}$ ,  $rik$  is the inward-rectification factor of  $I_{Kr}$ , and  $E_K$  is the equilibrium potential for  $I_K$ .

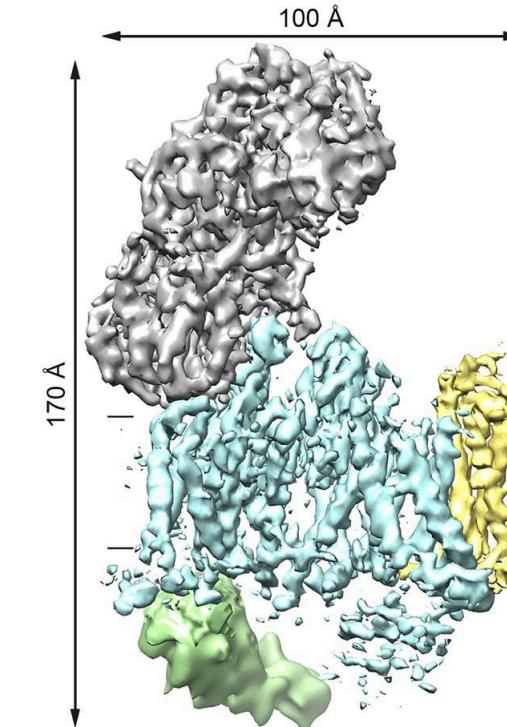


Hodgkin AL, Huxley AF. J Physiol. 1952 Aug;117(4):500-44.  
Priebe L, Beuckelmann DJ. Circ Res. 1998 Jun 15;82(11):1206-23.  
Bellocq C et al. Mol Pharmacol. 2004 Nov;66(5):1093-102.

# Canaux $\text{Ca}^{2+}$ : 4 domaines homologues

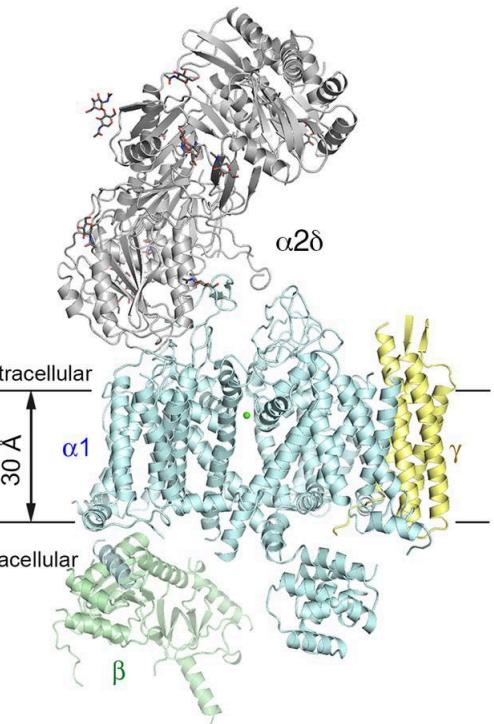


cryo-EM



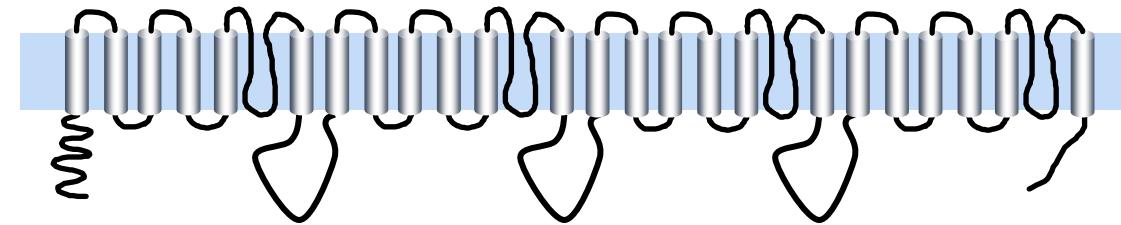
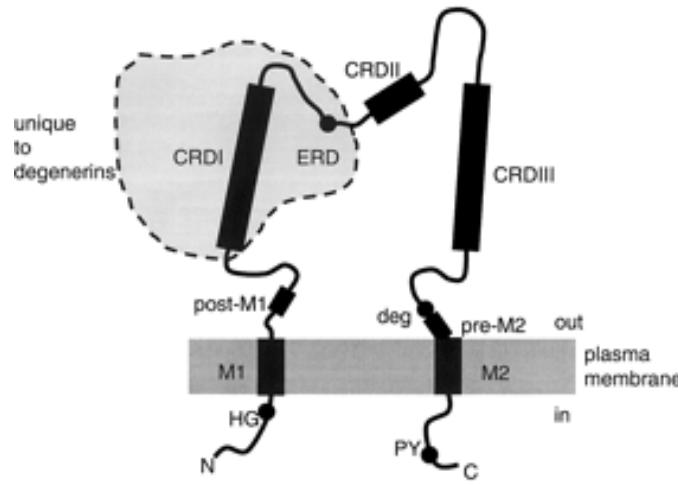
**Cav1.1**

4.2 Å



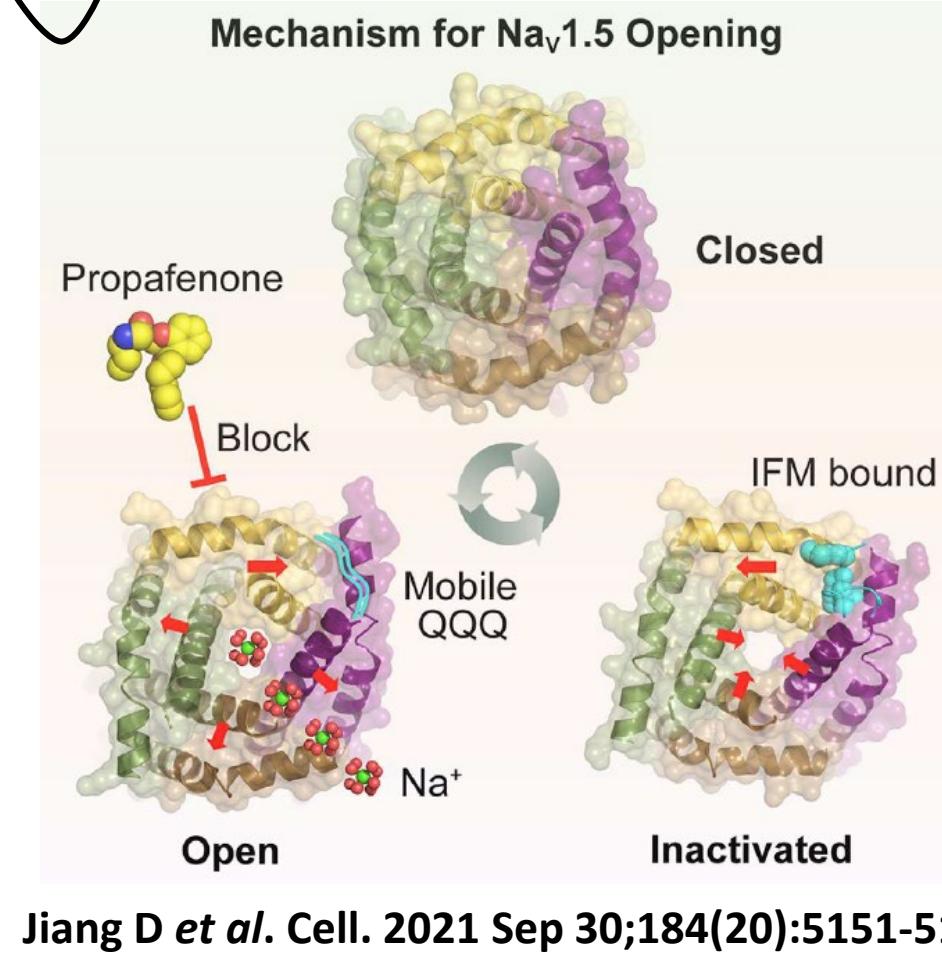
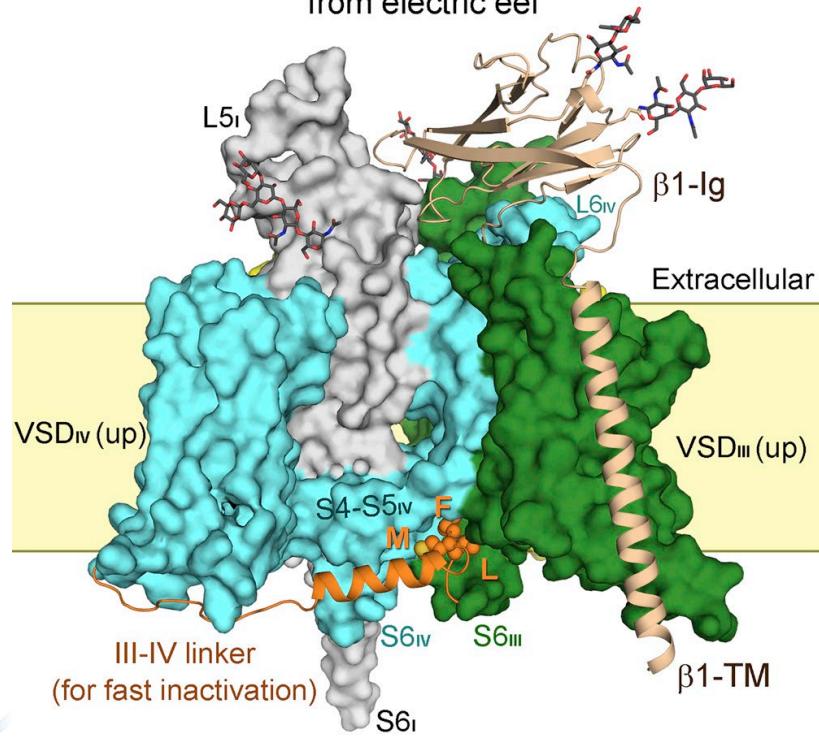
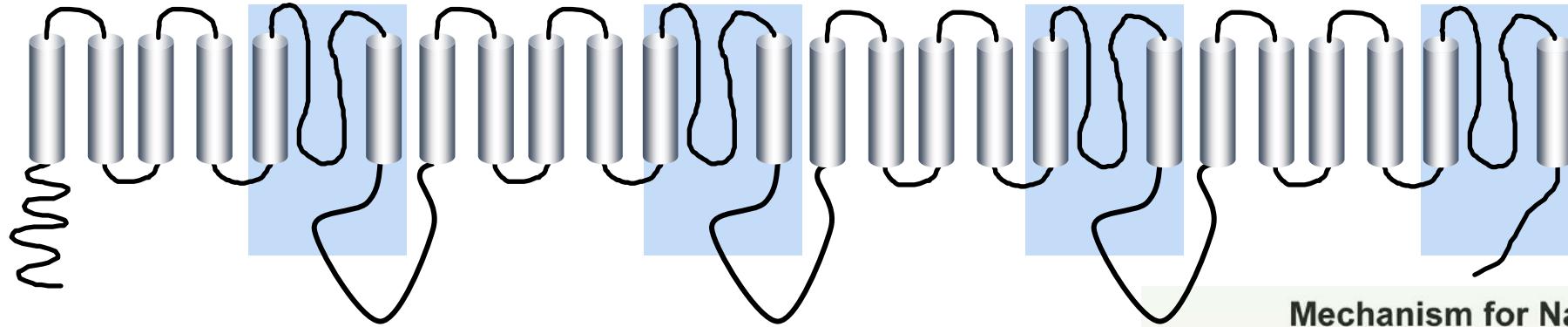
Wu J et al. Science. 2015 Dec 18;350(6267):aad2395<sup>19</sup>

# Canaux $\text{Na}^+$ : 2 classes

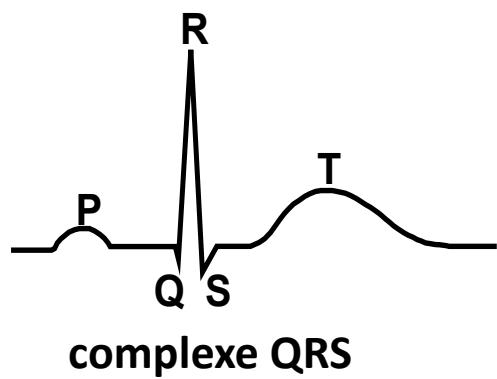


- **ENaC (epith.  $\text{Na}^+$  channel)**
- **Nav: voltage-gated**
- **canal  $\text{Na}^+$  cardiaque : Nav1.5 + Nav $\beta$ 1 (*SCN5A* et *SCN1B*)**

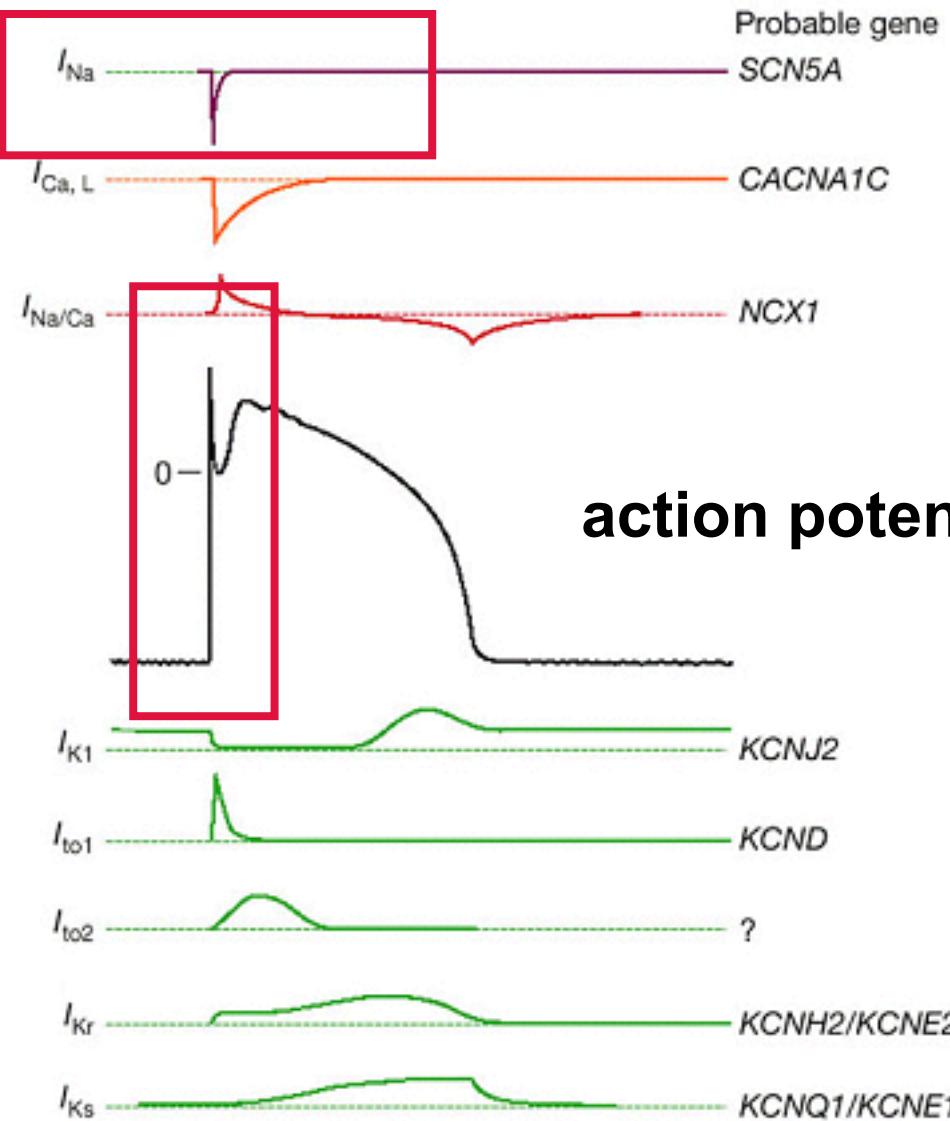
# Canaux Nav : structure



# Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



canal Nav1.5



early depolarization

plateau

action potential (AP)

diastolic potential

early repolarization

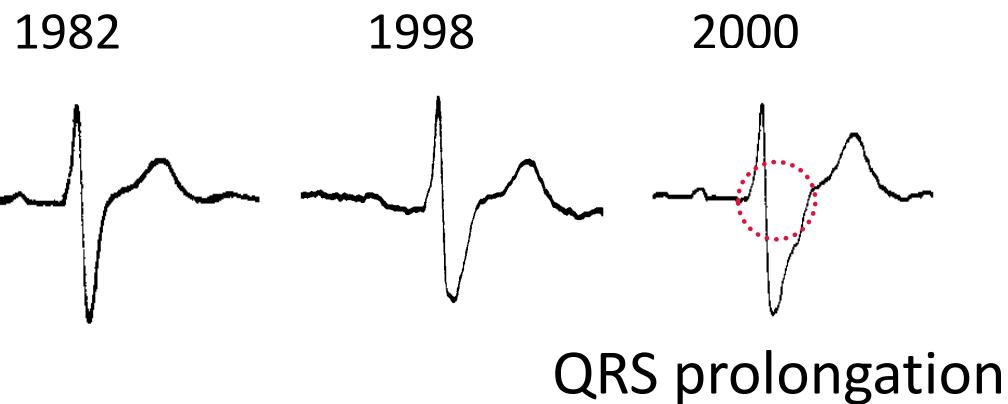
late repolarization

from Marbán E. Nature. 2002 Jan 10;415(6868):213-8

# Du modèle cellulaire au modèle murin du syndrome de Lenègre

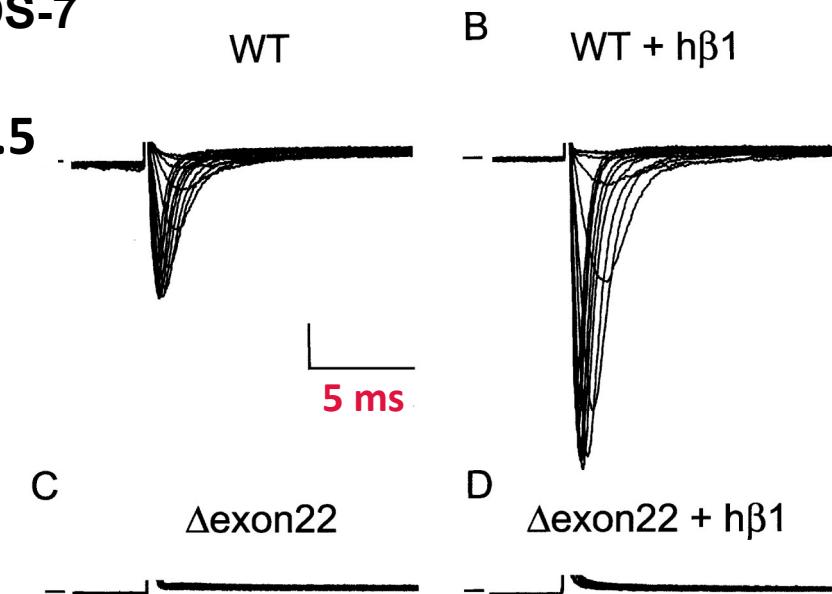
- Syndrome de Lenègre : ralentissement de la conduction cardiaque héréditaire

→ bloc de conduction



Mutation *SCN5A* : canal Nav1.5  $\Delta$ exon22 ← causal?

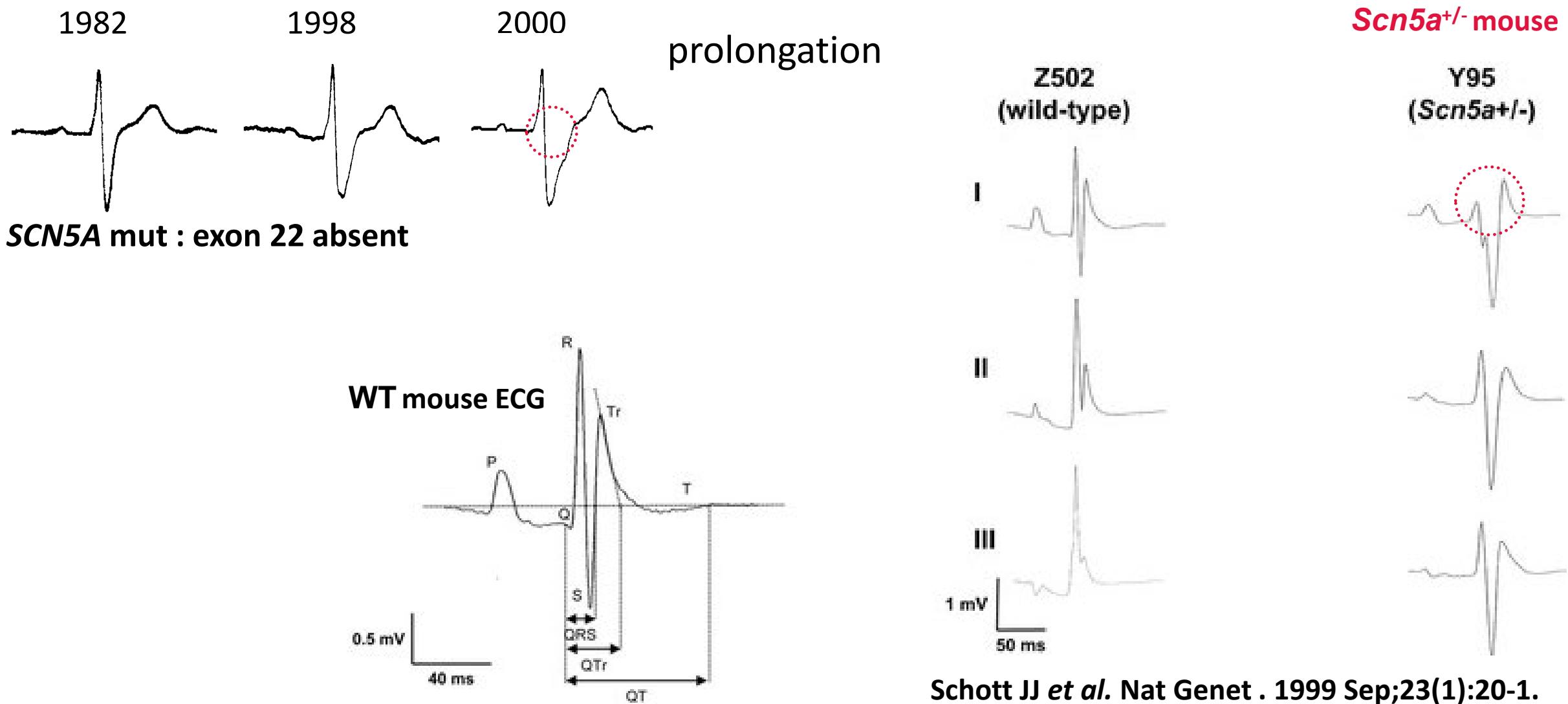
- Modèles cellulaires: lignées cellulaires modifiées - canalopathies monogéniques
- Expression hétérologue de protéines Nav1.5 WT et mutées



Schott JJ et al. Nat Genet. 1999 Sep;23(1):20-1.

Probst V et al. J Am Coll Cardiol. 2003 Feb 19;41(4):643-52.

# Du modèle cellulaire au modèle murin du syndrome de Lenègre



Schott JJ et al. Nat Genet. 1999 Sep;23(1):20-1.

Probst V et al. J Am Coll Cardiol. 2003 Feb 19;41(4):643-52.

Royer et al. Circulation. 2005 Apr 12;111(14):1738-46

Haplo-insuffisance de *SCN5A* → causal

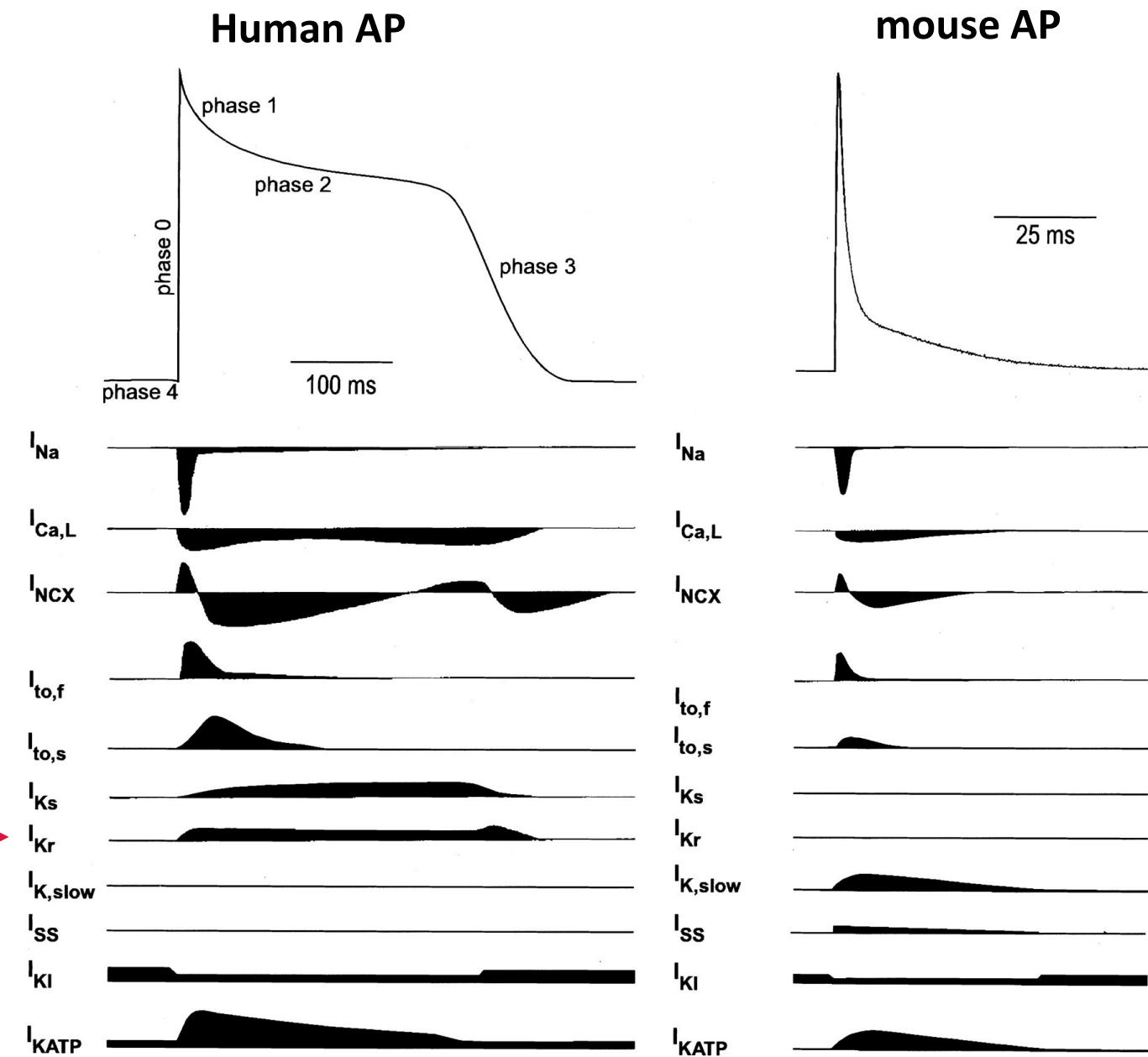
# Limites

## Modèles unicellulaires

- lignées cellulaires : environnement protéique ? (complexe canalaire)
- modèles animaux : expression espèce-spécifique ?

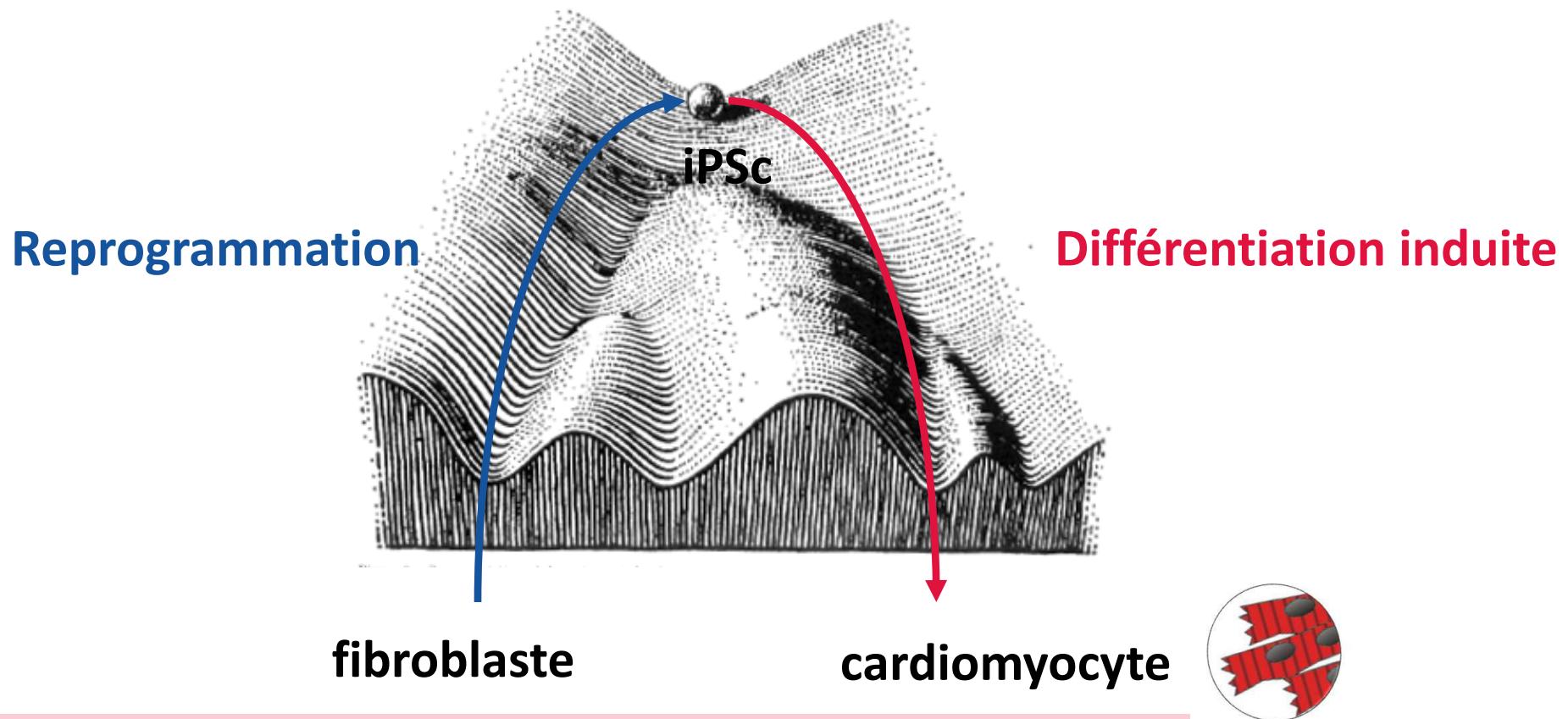
## Modèles informatiques

- environnement fonctionnel ?



Nerbonne JM et al. Circ Res. 2001 Nov 23;89(11):944

# Cardiomyocytes issus de cellules souches pluripotentes induites humaines (hiPS-CM)



**Yamanaka S, Gurdon J: Prix Nobel de médecine 2012**

"...for the discovery that mature cells can be reprogrammed to become pluripotent."

# Cardiomyocytes issus de cellules souches pluripotentes induites (iPS-CM)

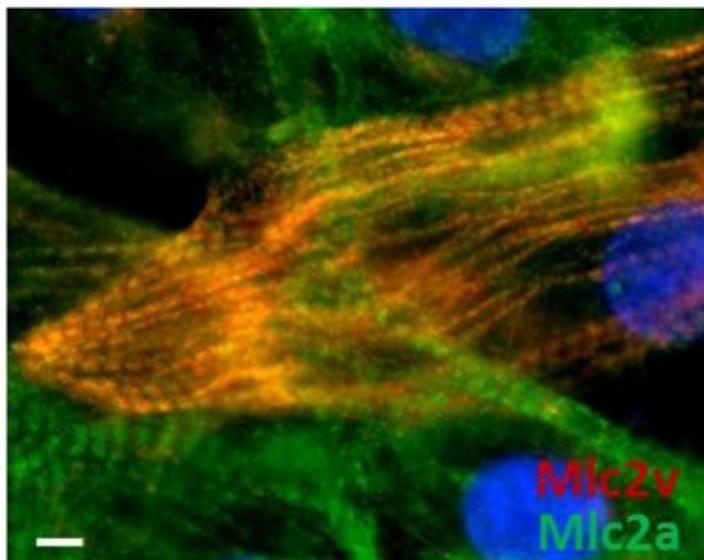
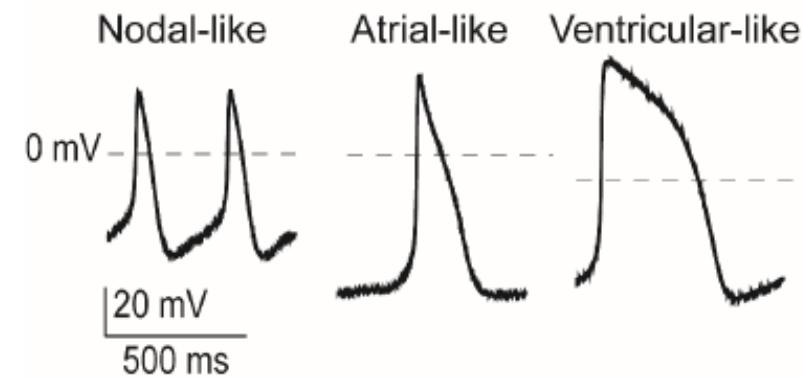
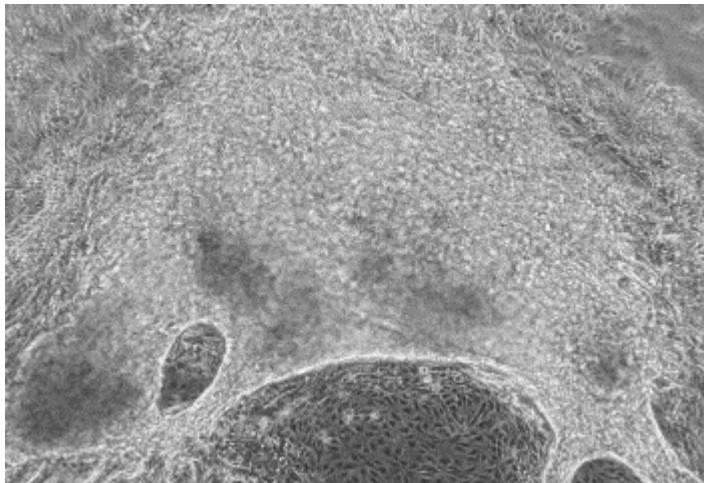
## Cellules d'origine :

- **peau, sang, urine**
- **porte le patrimoine génétique du patient (mutations, variants, SNP...)**

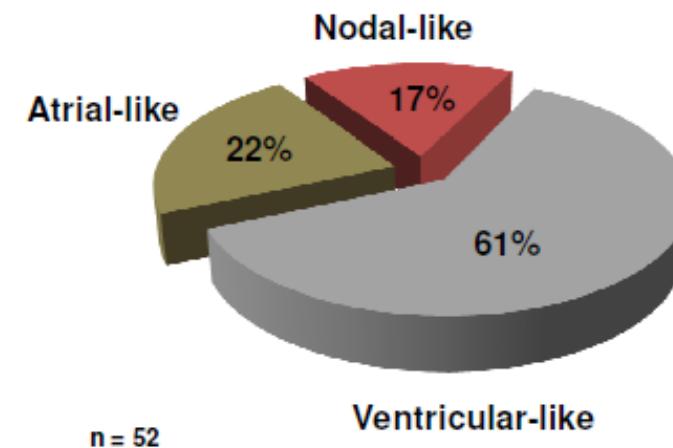
## Cardiomyocytes issus d'iPSC

- **générés au laboratoire dans des conditions acceptables (éthique, temps et budget)**
- **environnement génétique humain**

# hiPS-cardiomyocytes



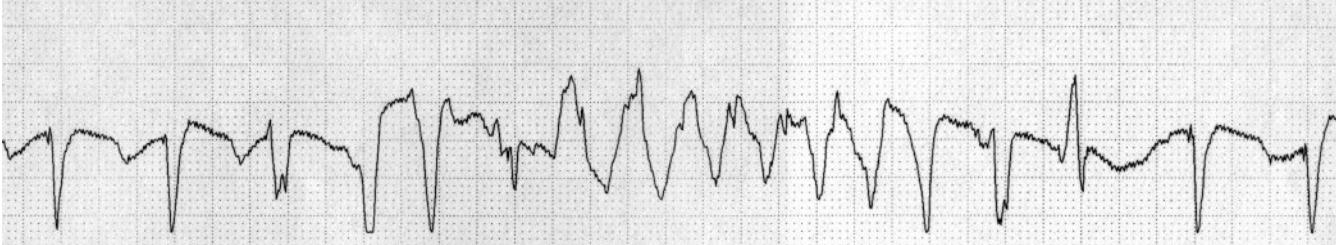
## Hétérogénéité phénotypique



# Modélisation du syndrome du QT long: A561P HERG

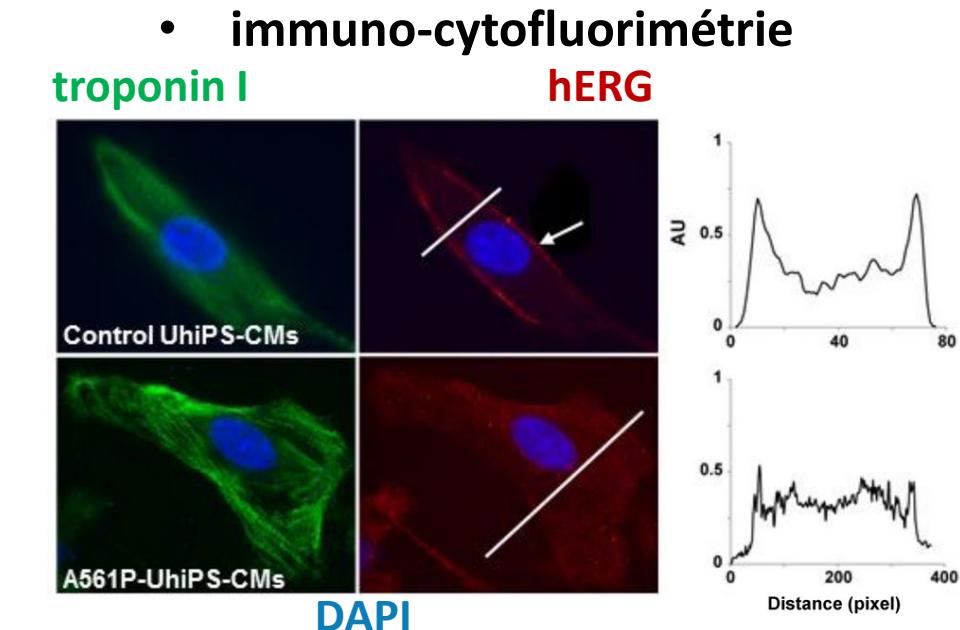
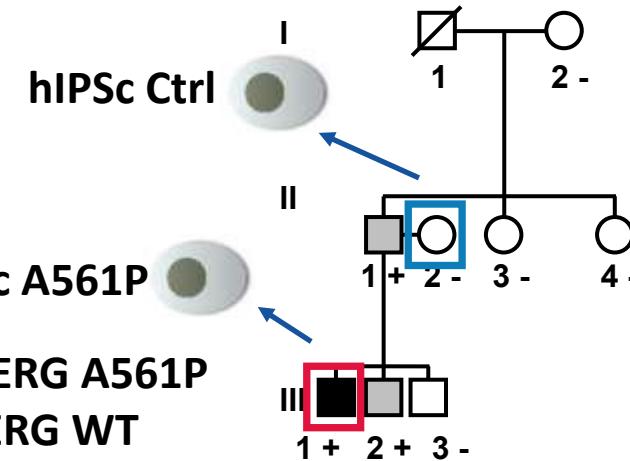
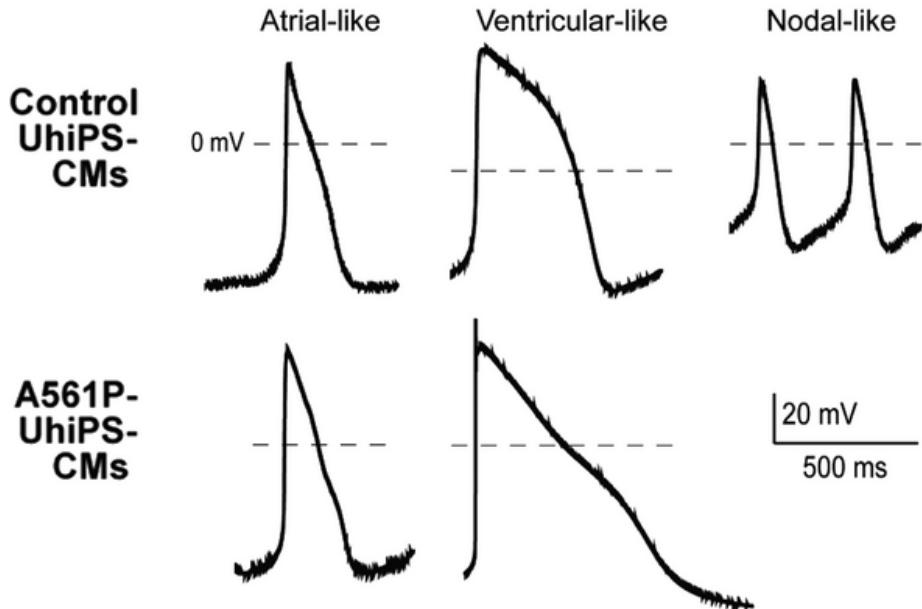
→ hERG A561P modifie-t-il le potentiel d'action des cardiomyocytes du malade ?

▼ Feb-05-99 – clobutinol



- re-programmation
- current-clamp

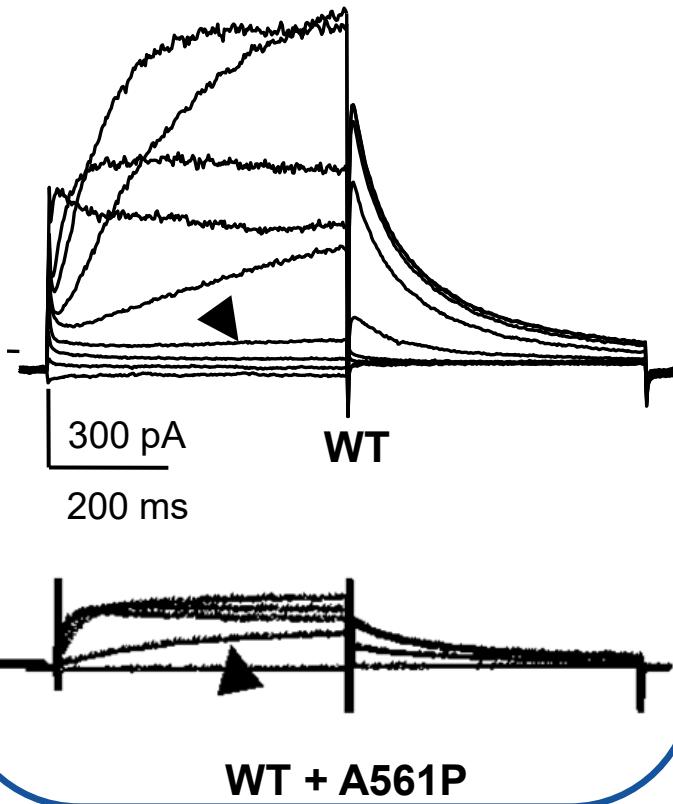
urinary cells



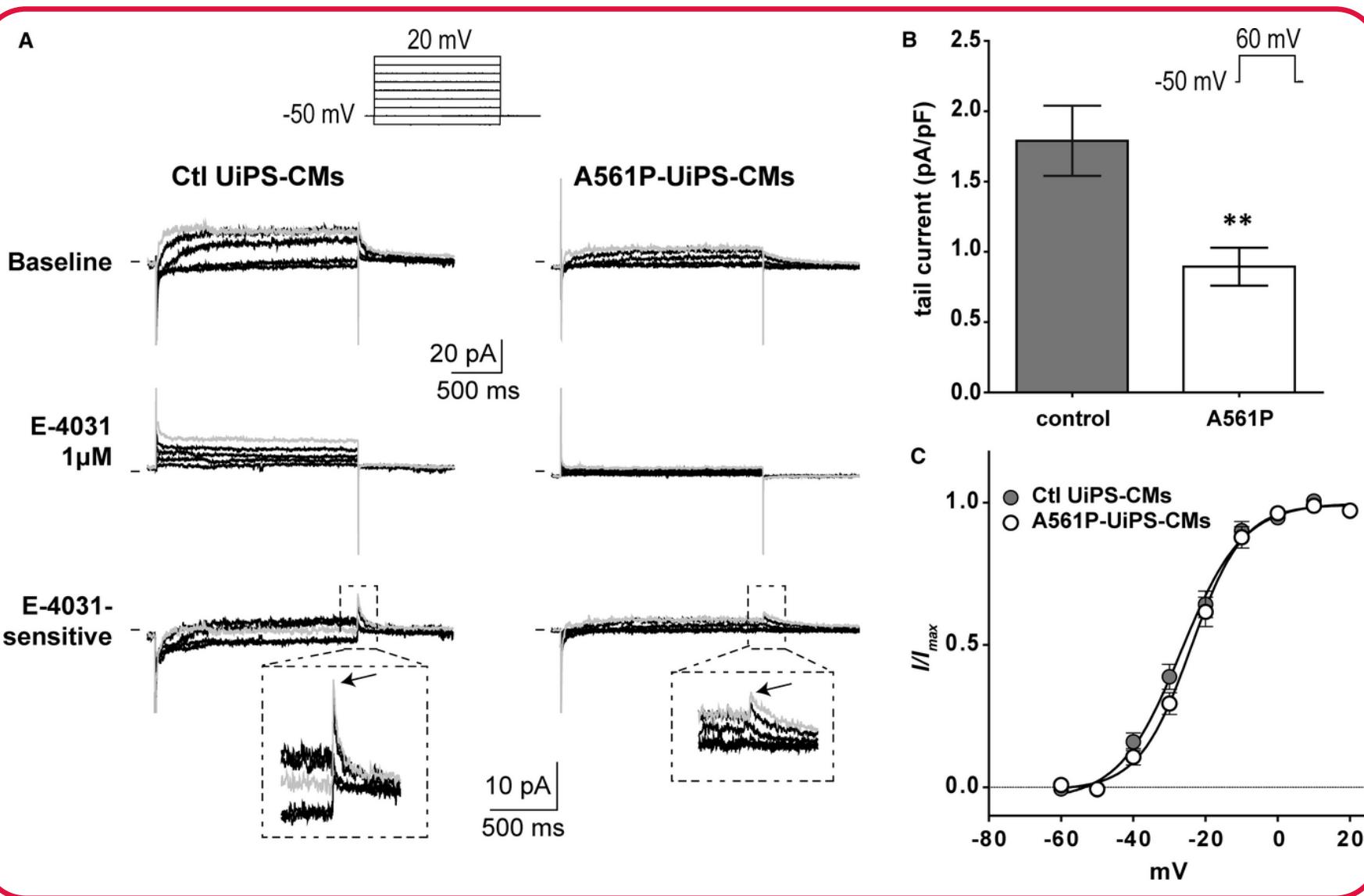
# Transfected cells vs hiPS-cardiomyocytes

- voltage-clamp

## transfected COS-7 cells



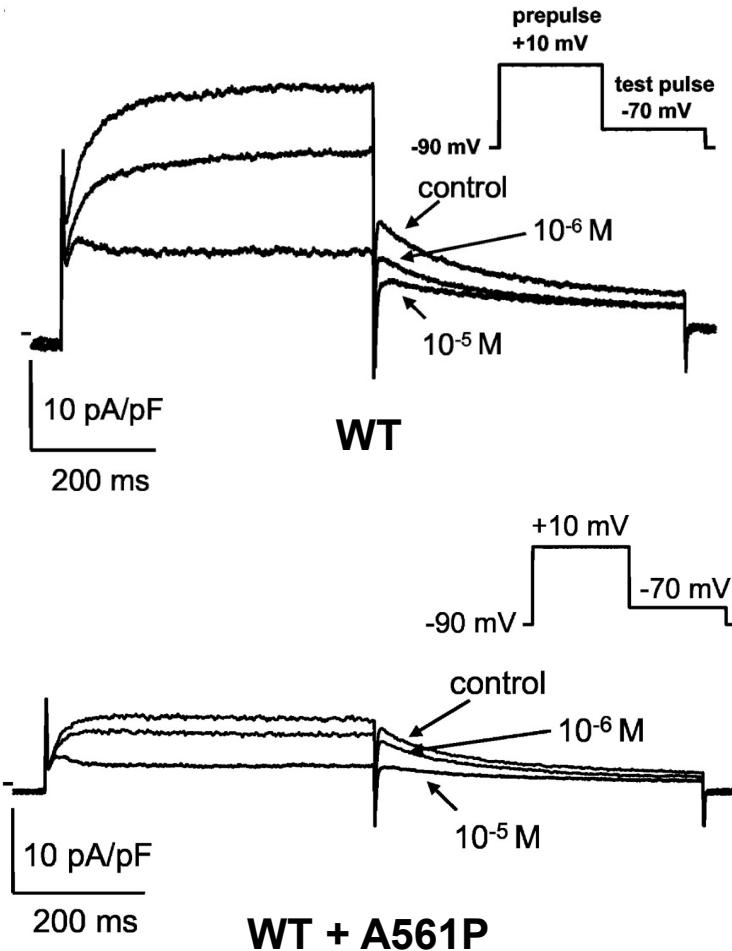
Bellocq C et al. Mol Pharmacol. 2004 Nov;66(5):1093-102



Jouni M et al. J Am Heart Assoc. 2015 Sep 1;4(9):e002159.

# Transfected cells vs hiPS-cardiomyocytes

+ clobutinol ( $I_{Kr}$  inhib.)

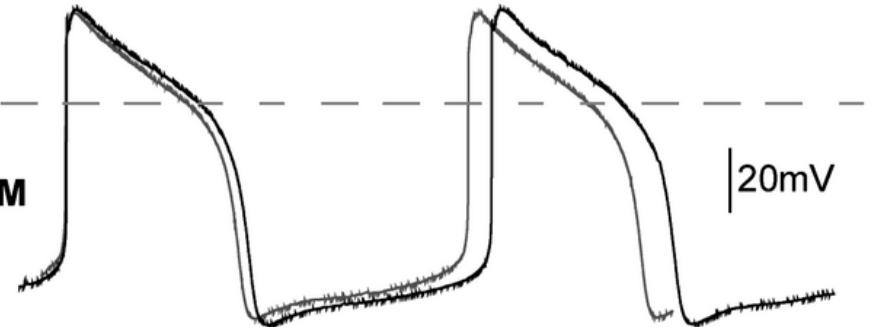


Bellocq C et al. Mol Pharmacol. 2004  
Nov;66(5):1093-102

+ E4031 ( $I_{Kr}$  inhib.)

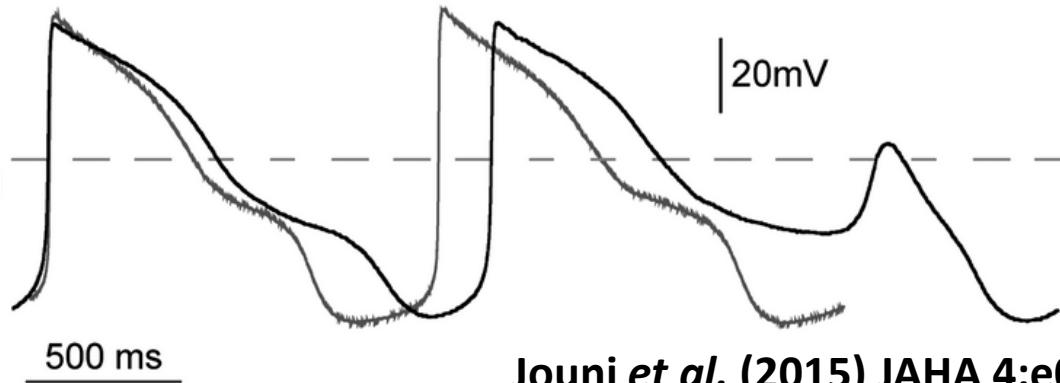
C Control UhiPS-CM

Baseline  
+E-4031 1 $\mu$ M



A561P-UhiPS-CM

Baseline  
+E-4031 1 $\mu$ M

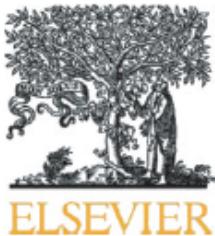


Jouni et al. (2015) JAHA 4:e002159

- ✓ Défaut de transport
- ✓ Réduction d' $I_{Kr}$
- Prolongation durée du PA
- Evènements arythmiques

Perte de fonction et allongement confirmés → causal

# Analyse d'article



Contents lists available at ScienceDirect

# Journal of Molecular and Cellular Cardiology

journal homepage: [www.elsevier.com/locate/yjmcc](http://www.elsevier.com/locate/yjmcc)



Original Article

## HIV-Tat induces a decrease in $I_{Kr}$ and $I_{Ks}$ via reduction in phosphatidylinositol-(4,5)-bisphosphate availability



Zeineb Es-Salah-Lamoureaux <sup>a,1</sup>, Mariam Jouni <sup>a,1</sup>, Olfat A. Malak <sup>a</sup>, Nadjet Belbachir <sup>a</sup>, Zeina Reda Al Sayed <sup>a</sup>, Marine Gandon-Renard <sup>a</sup>, Guillaume Lamirault <sup>b</sup>, Chantal Gauthier <sup>a</sup>, Isabelle Baró <sup>a</sup>, Flavien Charpentier <sup>b</sup>, Kazem Zibara <sup>c</sup>, Patricia Lemarchand <sup>b</sup>, Bruno Beaumelle <sup>d</sup>, Nathalie Gaborit <sup>a,\*1</sup>, Gildas Loussouarn <sup>a,1</sup>

<sup>a</sup> *l'institut du thorax, Inserm, CNRS, Université de Nantes, Nantes, France*

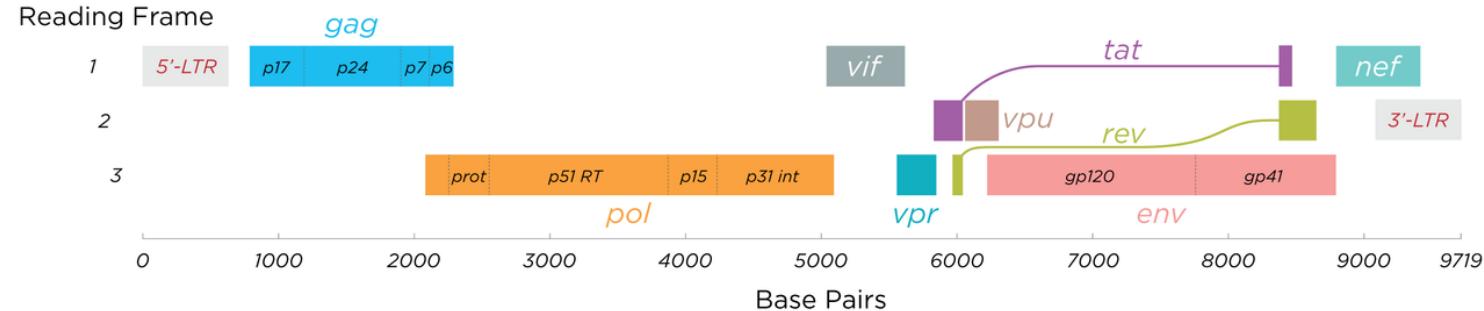
<sup>b</sup> *l'institut du thorax, Inserm, CNRS, Université de Nantes, CHU Nantes, Nantes, France*

<sup>c</sup> *ER045, PRASE, Laboratory of stem cells, Lebanese university, Beirut, Lebanon*

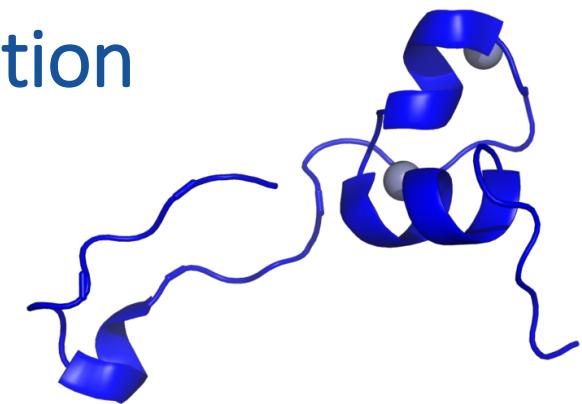
<sup>d</sup> *Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé, CNRS, Université de Montpellier, Montpellier, France*

- **Les patients HIV+ présentent un intervalle QT allongé et un risque de mort subite élevé par rapport à la population générale. Origine ?**
- **Des études précédentes suggèrent un effet direct du virus (plus que des anti-viraux)**

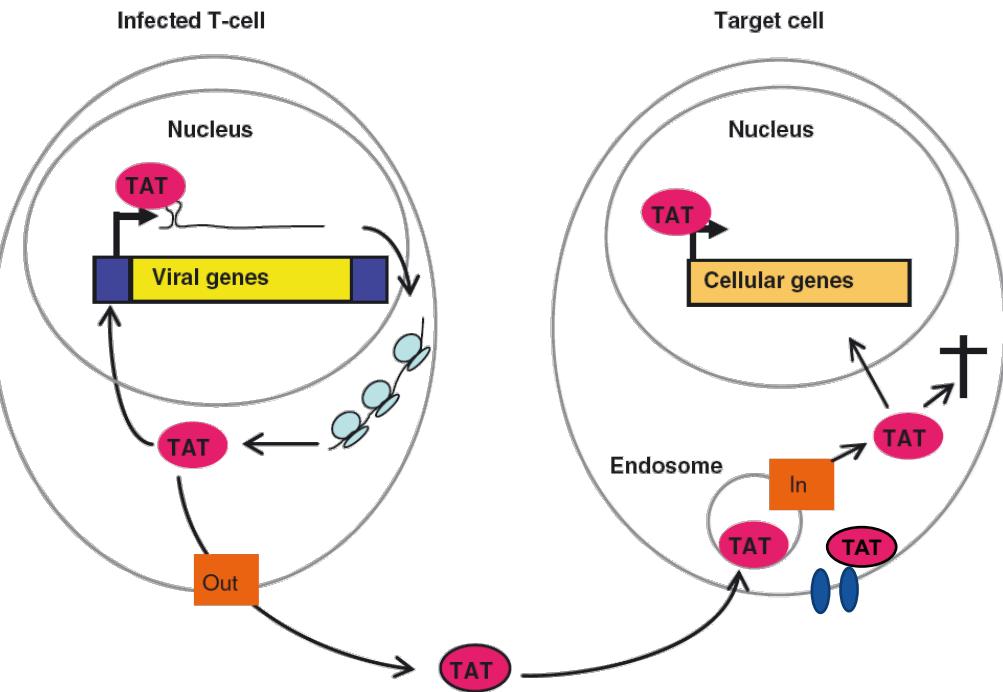
# Tat = Trans-Activator of Transcription



<https://commons.wikimedia.org/w/index.php?curid=33943759>



<https://commons.wikimedia.org/w/index.php?curid=79119847>



d'après Debaisieux et al. *Traffic*. 2012 Mar;13(3):355-63.

- Protéine Tat du HIV-1 : présente dans le sérum des patients, peut pénétrer les cellules et interagir avec le PIP<sub>2</sub> (phosphoinositide).
  - KCNQ1 et hERG, canaux repolarisants, nécessitent du PIP<sub>2</sub> pour être fonctionnels
- Les canaux KCNQ1 ou hERG sont-ils impliqués dans les effets cardiaques de l'infection HIV par l'intermédiaire de Tat?



cellules  
COS-7

Condition : sur-expression du canal hERG et de la protéine Tat (WT et mutée) après transfection (plasmides)

- Courant  $I_{hERG}$

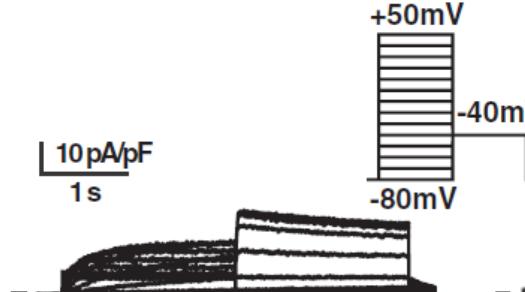
A COS-7

GFP

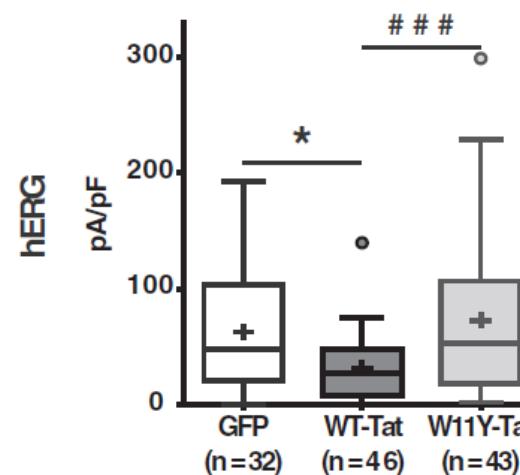
WT-Tat

W11Y-Tat: pas d'interaction avec le  $PIP_2$

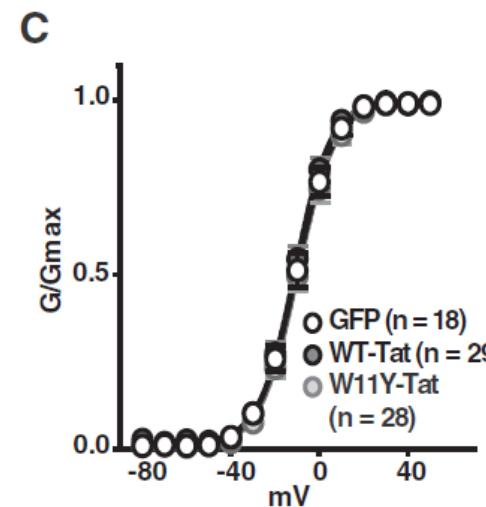
WT-Tat



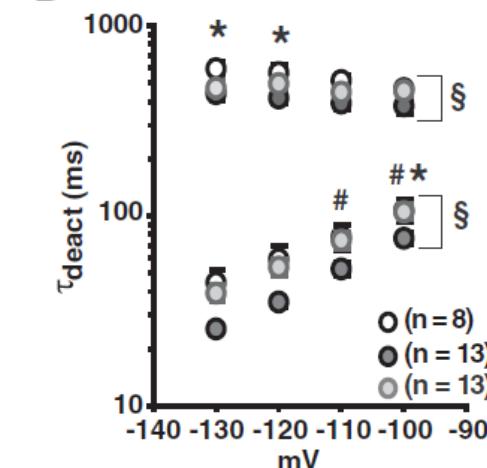
B



C



D



- WT-Tat : 1A & B : diminution du courant hERG (et KCNQ1)
- WT-Tat : 1D : accélération de la désactivation du courant hERG (et KCNQ1)
- W11Y-Tat, qui n'interagit pas avec le  $PIP_2$  : pas d'effet

Figure 1

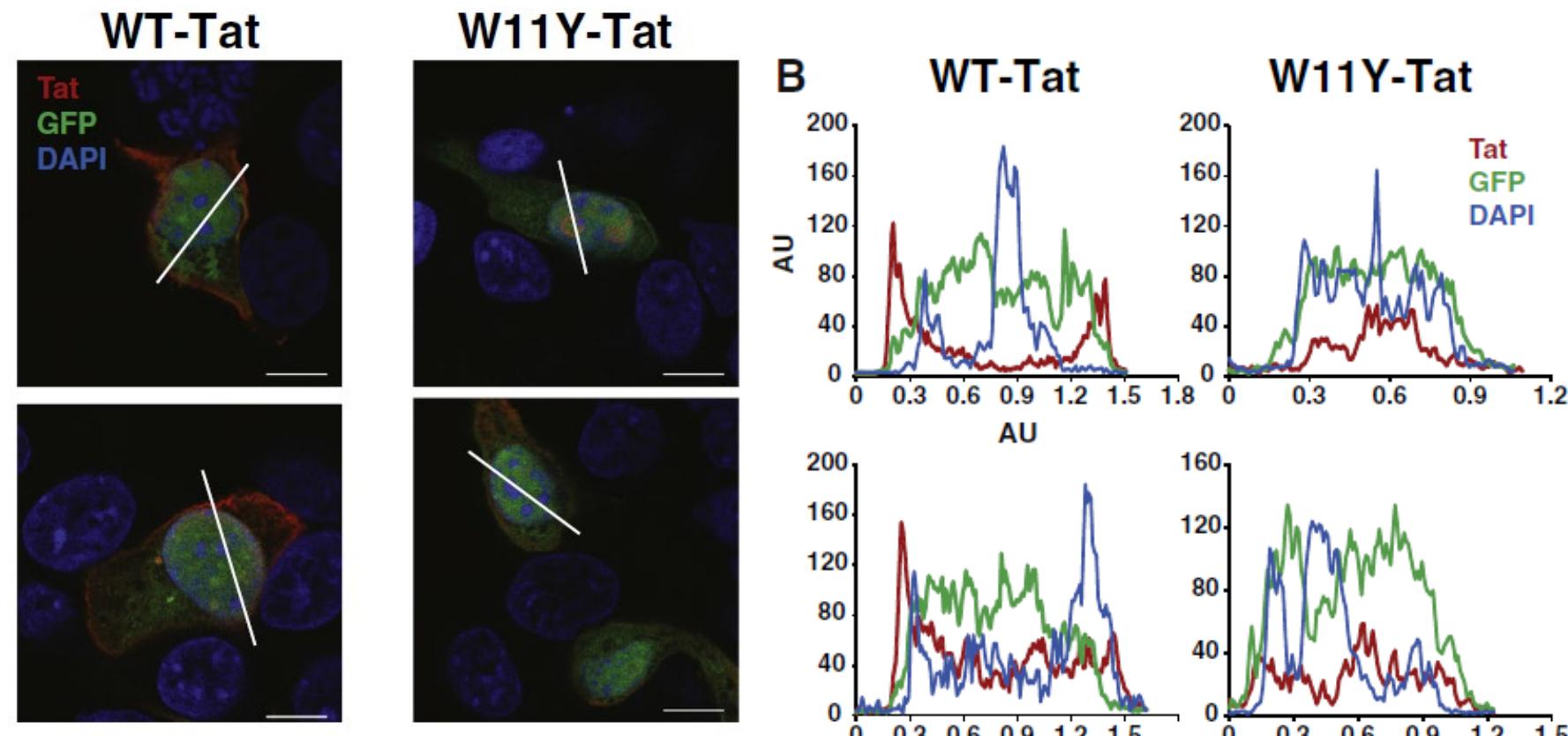


cellules  
COS-7

Condition : sur-expression du canal hERG et de la protéine Tat (WT et mutée) après transfection (plasmides)

- Localisation de la protéine Tat (immunomarquage et microscopie confocale)

Figure 3 A



- WT-Tat : membranaire, comme le canal
- W11Y-Tat : non membranaire, car elle n'interagit pas avec le PIP<sub>2</sub>

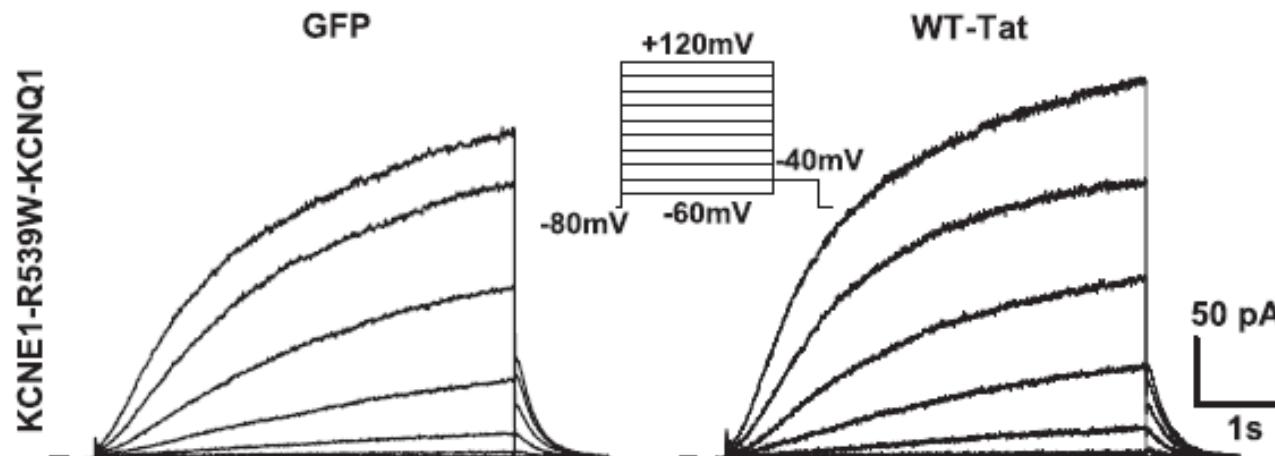


cellules  
COS-7

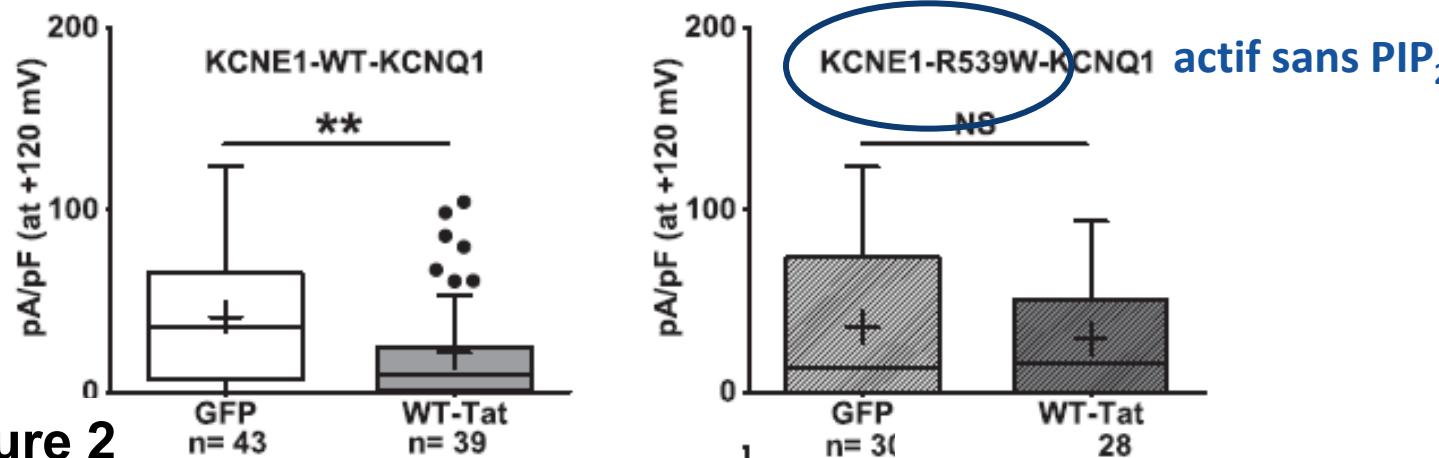
Condition : sur-expression du canal KCNE1-KCNQ1 (WT et muté) et de la protéine WT-Tat après transfection (plasmides)

- Courant  $I_{KCNE1-KCNQ1}$

A



B



- 3B: canal WT sensible au PIP<sub>2</sub> : sensible à la Tat
- 3A & B canal insensible au PIP<sub>2</sub> : insensible à la Tat

Figure 2



cellules  
COS-7

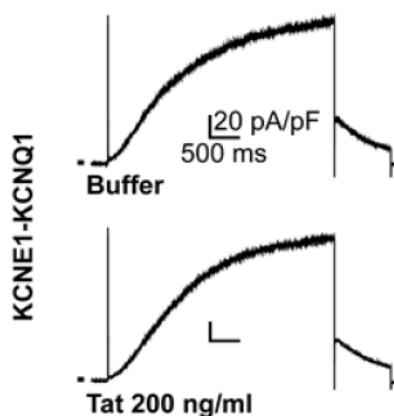
Condition : sur-expression du canal KCNE1-WT- KCNQ1 après transfection (plasmides), application extracellulaire de la protéine Tat

- Courant  $I_{KCNE1-KCNQ1}$

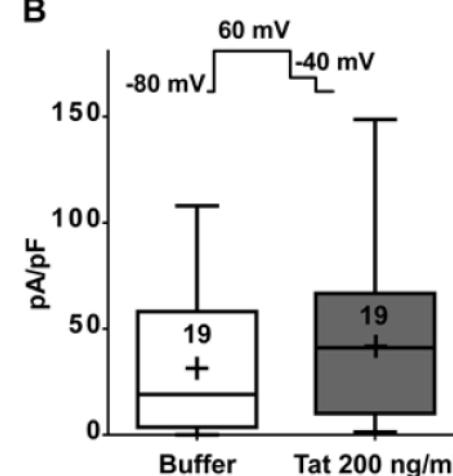
Suppl.  
Figure 4

A

COS-7

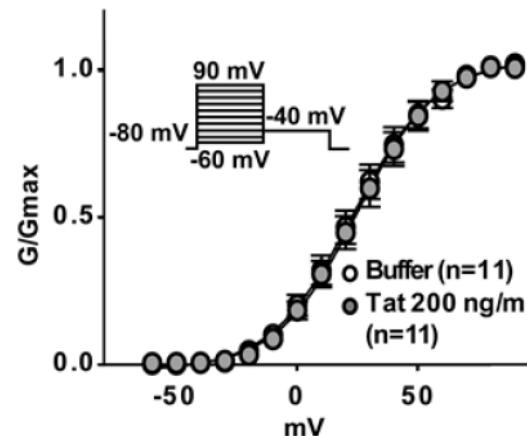


B

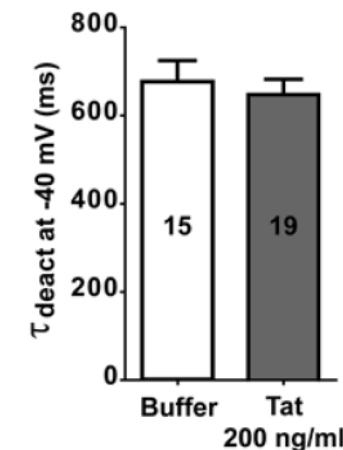


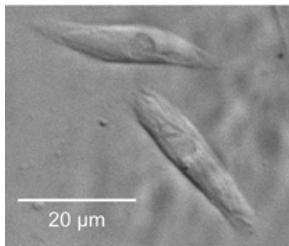
- application de la protéine Tat : pas d'effet

C

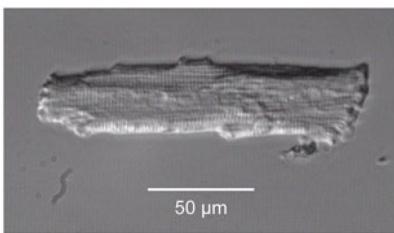


D





cardiomyocytes  
issus de cellules hiPS



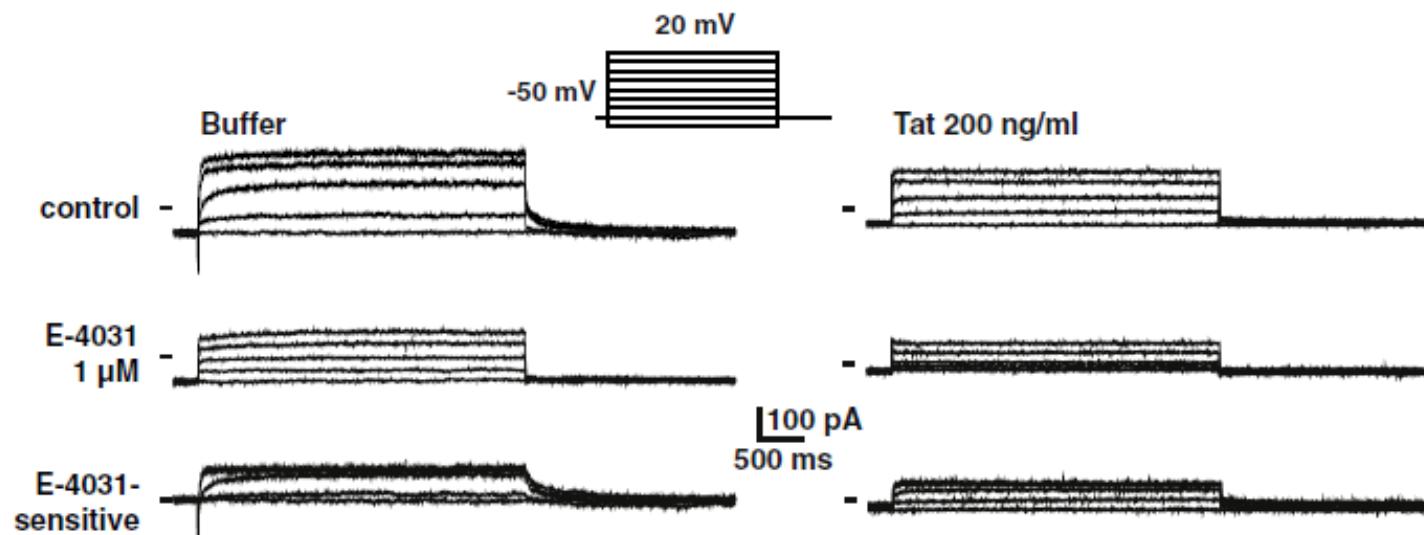
cardiomyocytes humains  
fraîchement isolés

- application de la protéine Tat : effet sur le courant E-4031-sensible,  $I_{Kr}$

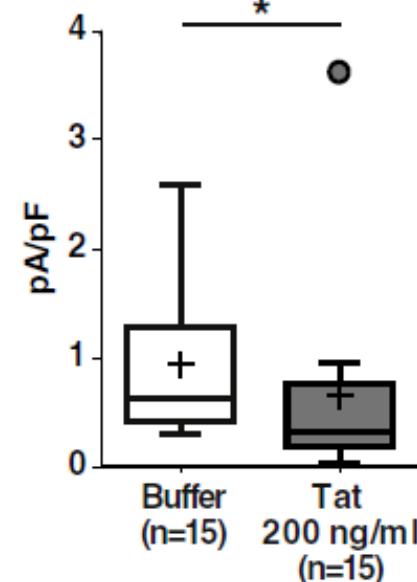
## Condition : application extracellulaire de la protéine Tat

- Courant  $I_{Kr}$  (hERG, inhibé par E-4031)

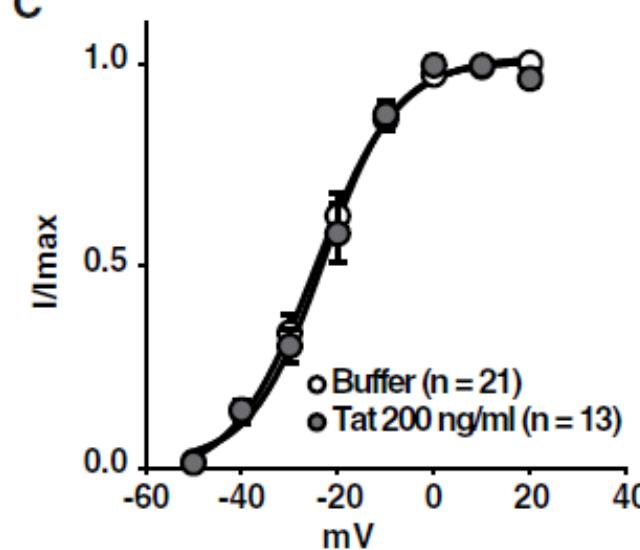
### A hiPS-CMs



### B



### C



### D

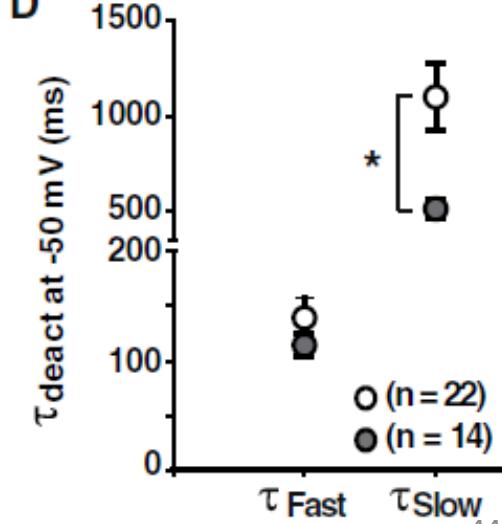
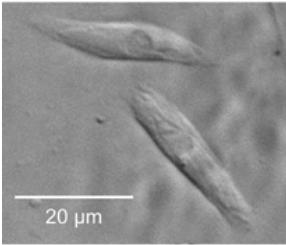


Figure 4

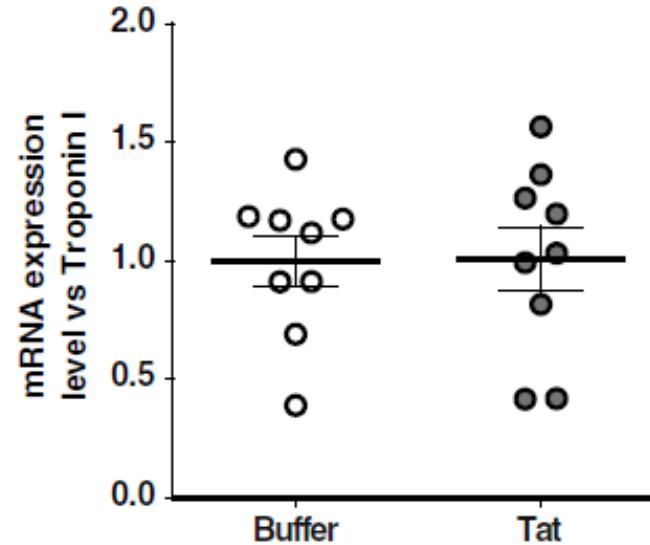


cardiomyocytes  
issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- expression de hERG ARNm et protéine

E



F

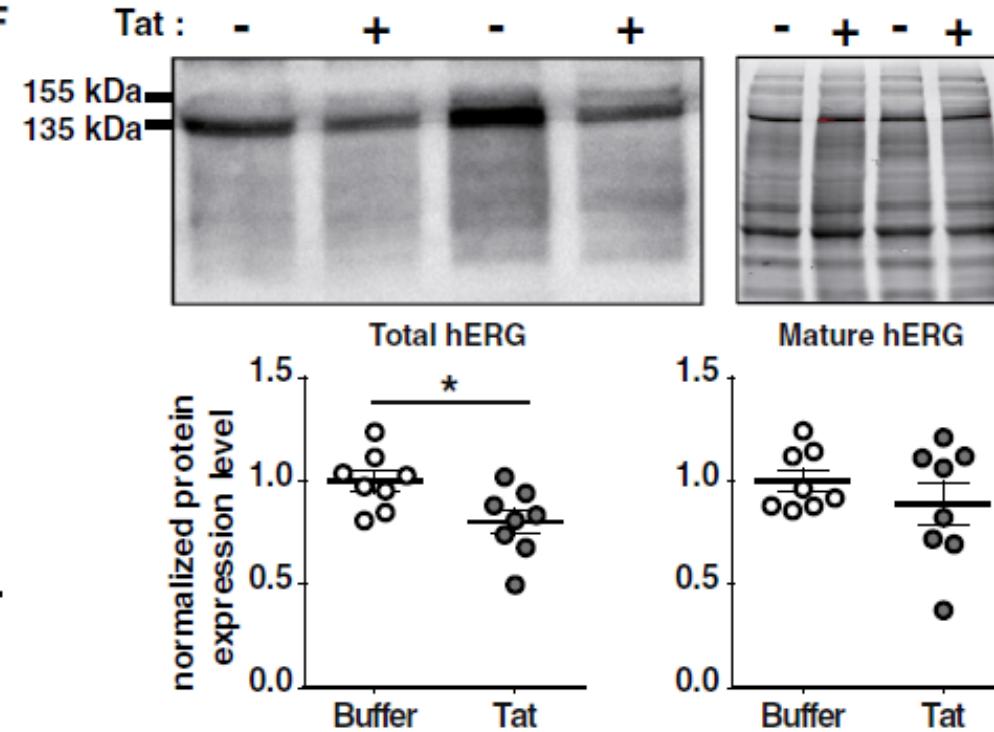


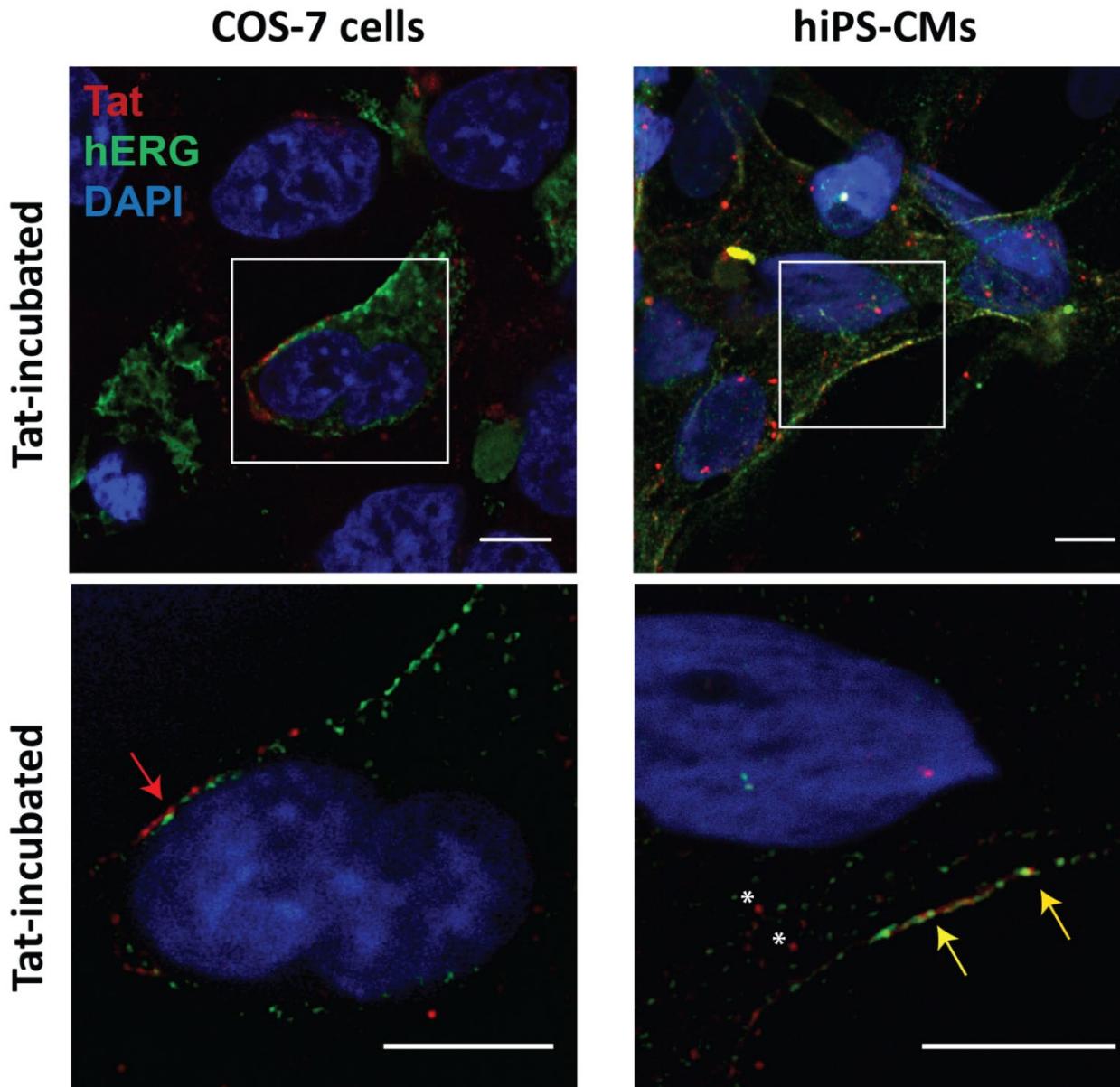
Figure 4

- protéine Tat : pas d'effet sur la densité de canaux à la membrane plasmique
- diminution du courant  $I_{Kr}$  par régulation de la fonction

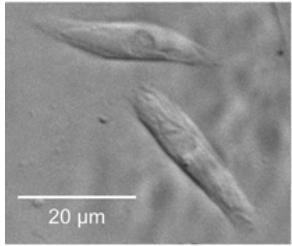
## Condition : application extracellulaire de la protéine Tat

- Localisation de la protéine Tat (immunomarquage et microscopie confocale)

Figure 5



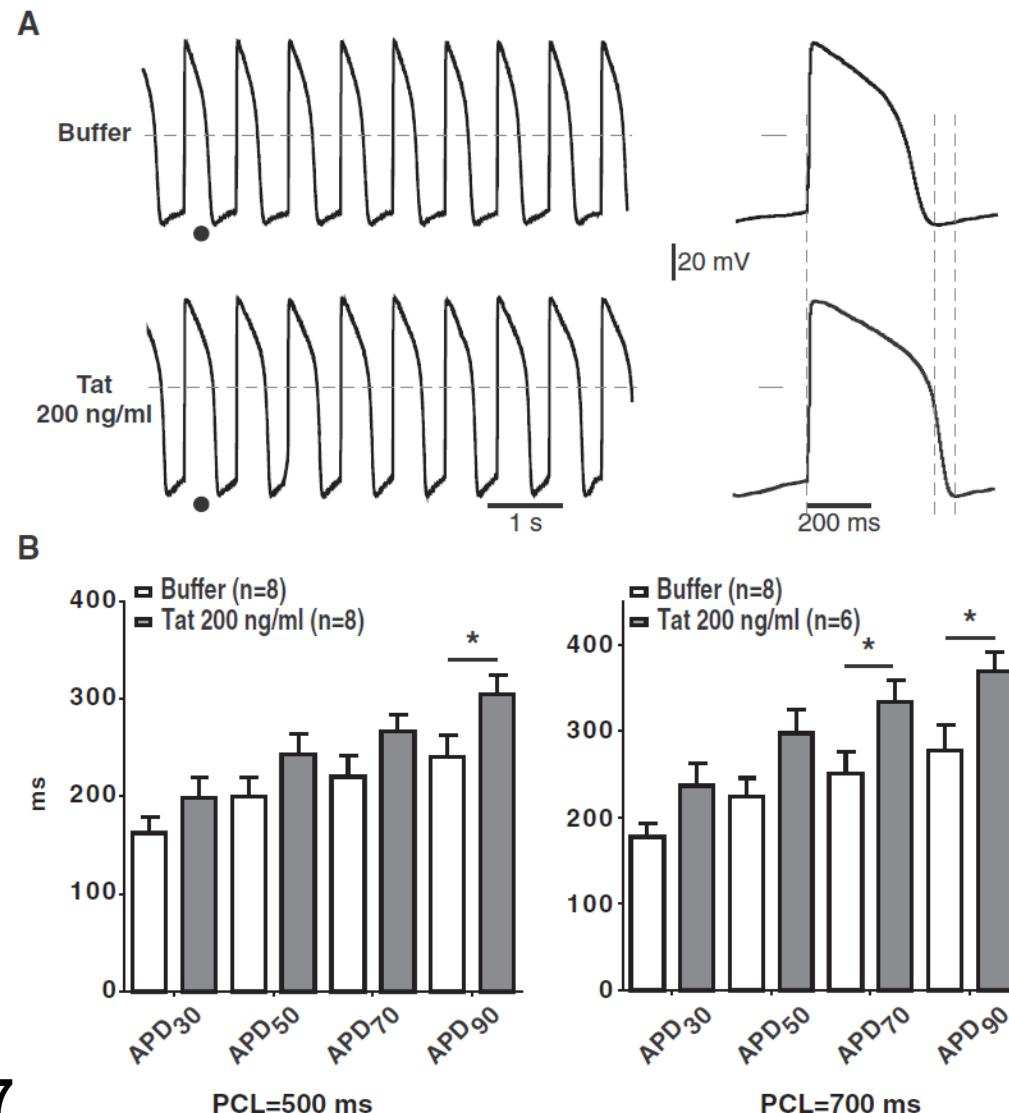
- application de la protéine Tat : endocytose uniquement dans les cardiomyocytes



cardiomyocytes  
issus de cellules hiPSC

Condition : application extracellulaire de la protéine Tat

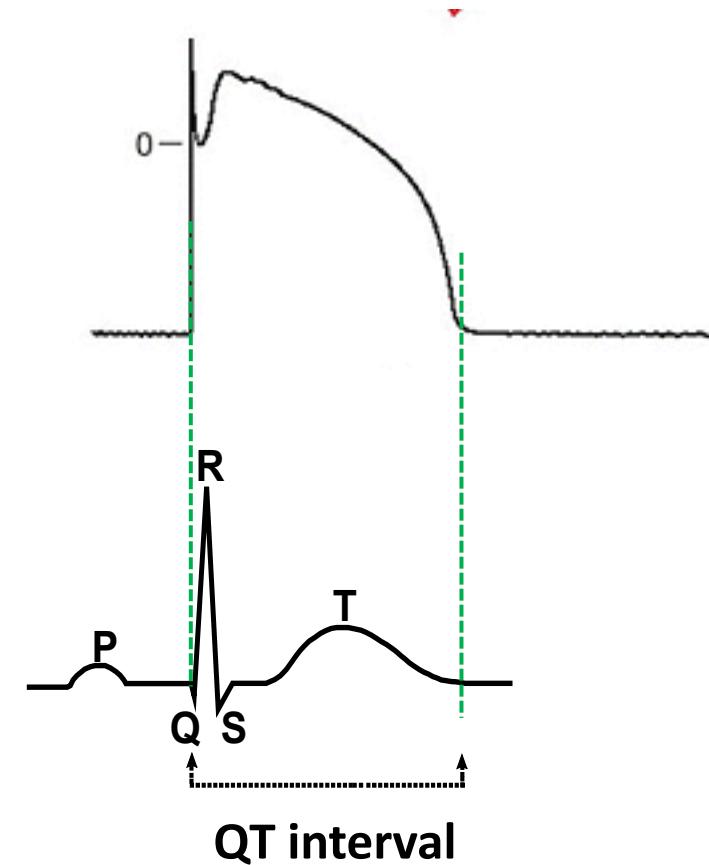
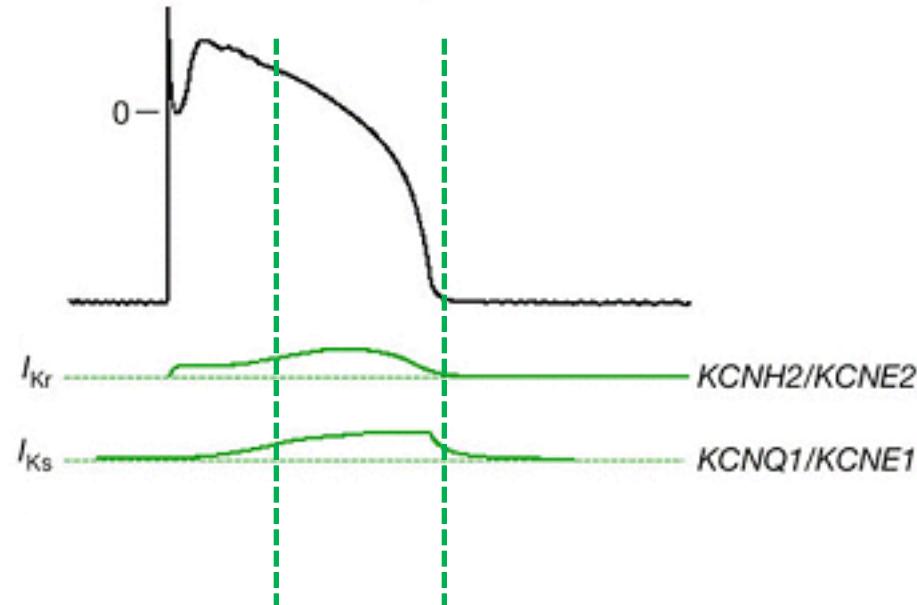
- potentiel d'action



- allongement de la durée du potentiel d'action induit par la Tat (phases tardives)

# Conclusions

"Altogether, these data obtained on human K<sup>+</sup> channels both in heterologous expression systems and in human cardiomyocytes suggest that Tat sequesters PIP<sub>2</sub>, leading to a reduction of  $I_{Kr}$  and  $I_{Ks}$ , and provide a molecular mechanism for QT prolongation in HIV-infected patients."

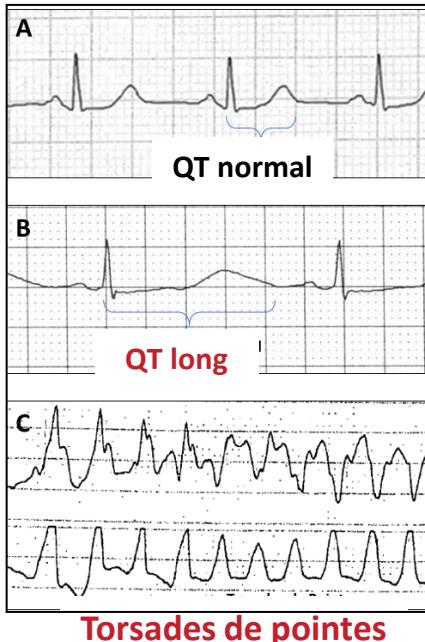


# Syndrome du QT long congénital : variants de *KCNH2*

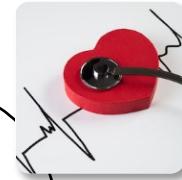
## Contexte du projet

Mutations perte de fonction du gène codant le canal hERG (*KCNH2*)

ECG



Syncopes et mort subite



2800 variants de *KCNH2* (1159 a.a.)  
dont 2057 faux sens



Classe 1



Classe 3  
VSI



Faux sens

1%

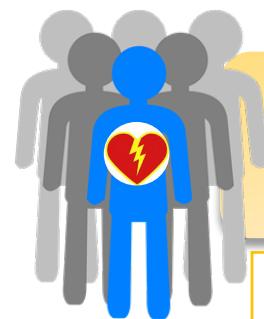
3%

83%

6%

7%

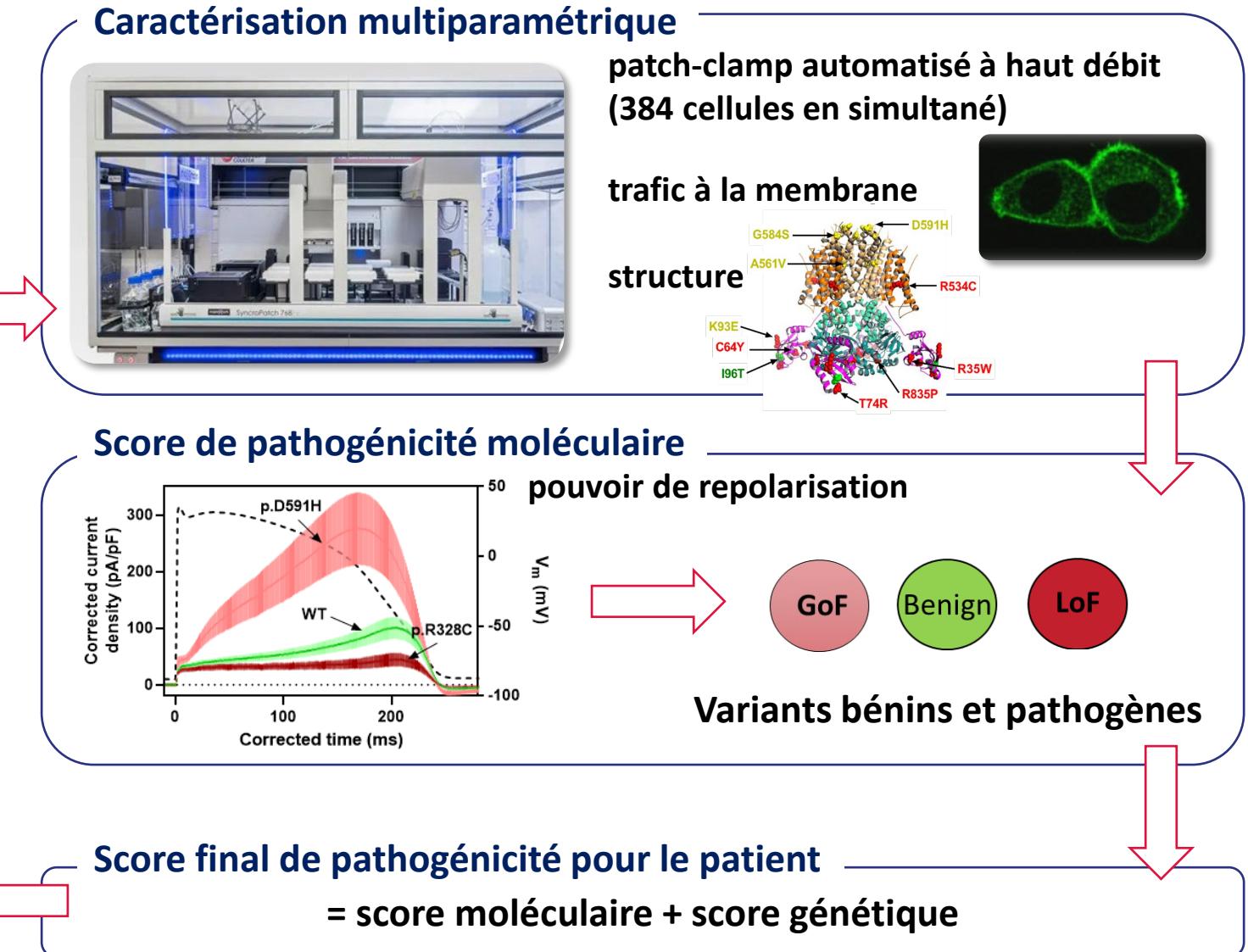
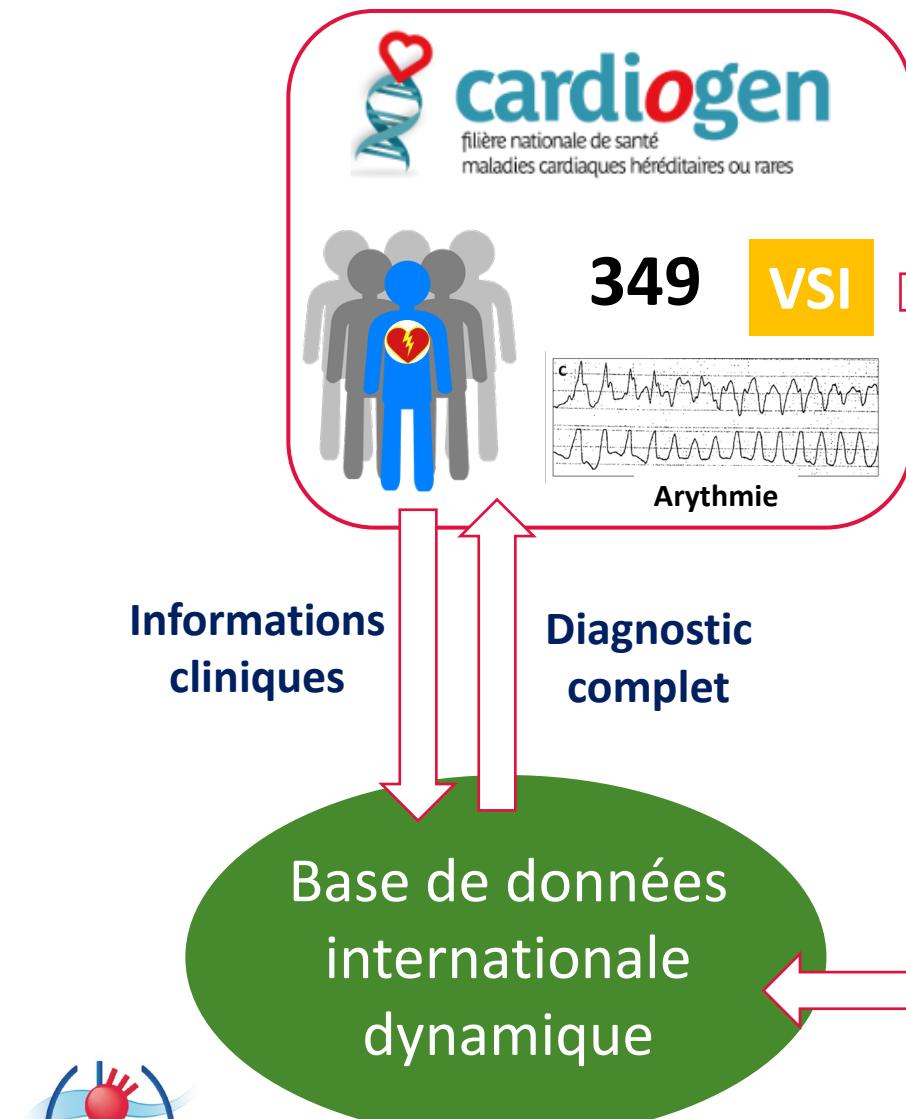
VSI: Variants de Signification Inconnue



Besoin clinique: caractérisation fonctionnelle à haut débit de variants VSI

Stratification optimale du risque rythmique des patients

# Caractérisation de l'ensemble des variants du gène *KCNH2*



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*l'institut du thorax*

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Barbara Ribeiro

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Gildas Loussouarn

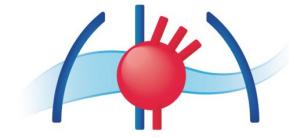
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Richard Redon



l'institut  
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iPSC core facility

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Nantes, France



Daniel Buren et Patrick Bouchain, Les Anneaux, Quai des Antilles, Nantes, création pérenne Estuaire 2007 © Martin Argyroglo/LVAN