Electrophysiologie Moléculaire - troubles du rythme et de la conduction cardiaques

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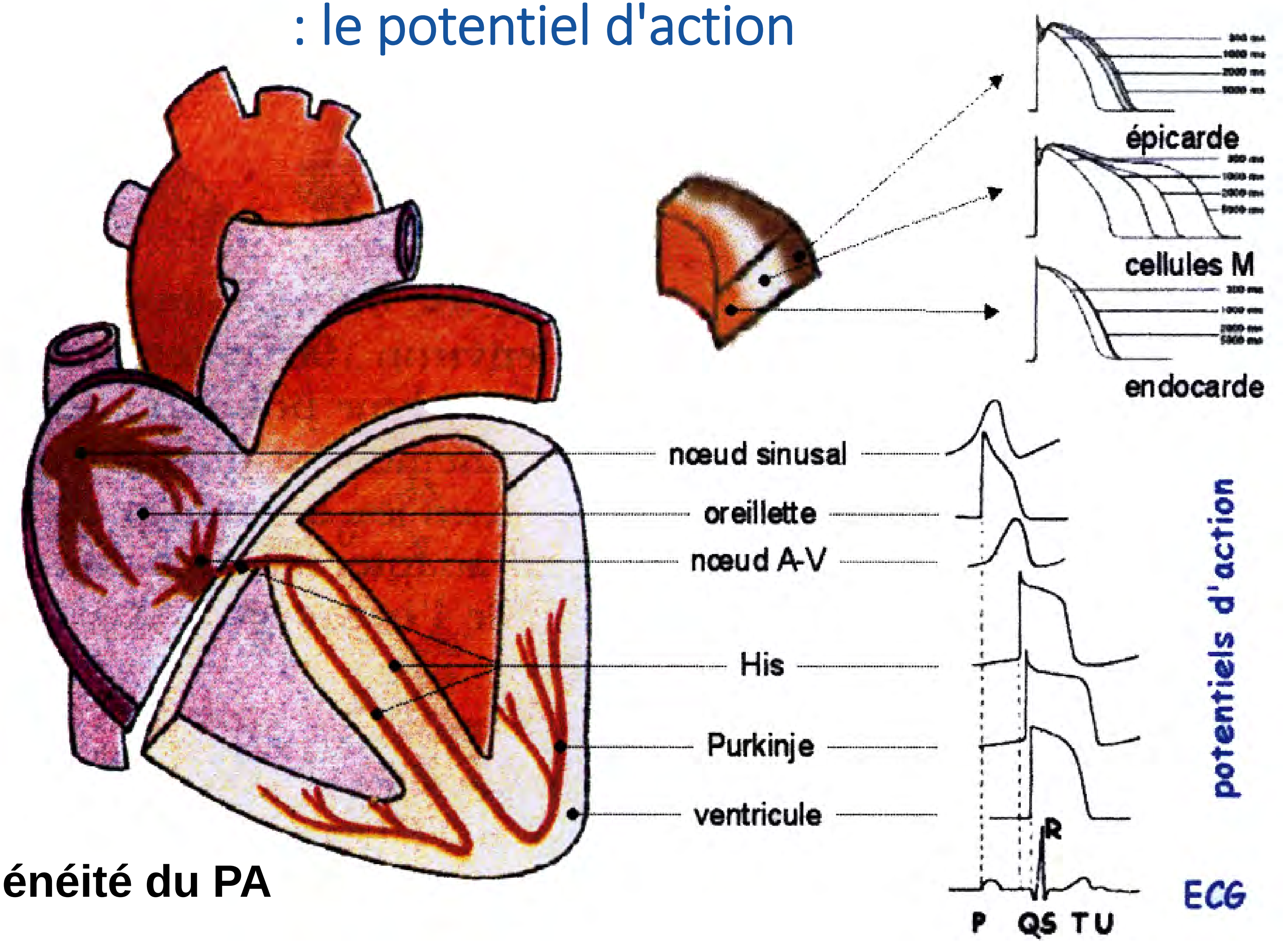
UNIVERSITÉ DE NANTES

CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

MASTER 1 Biologie et Santé - Thorax

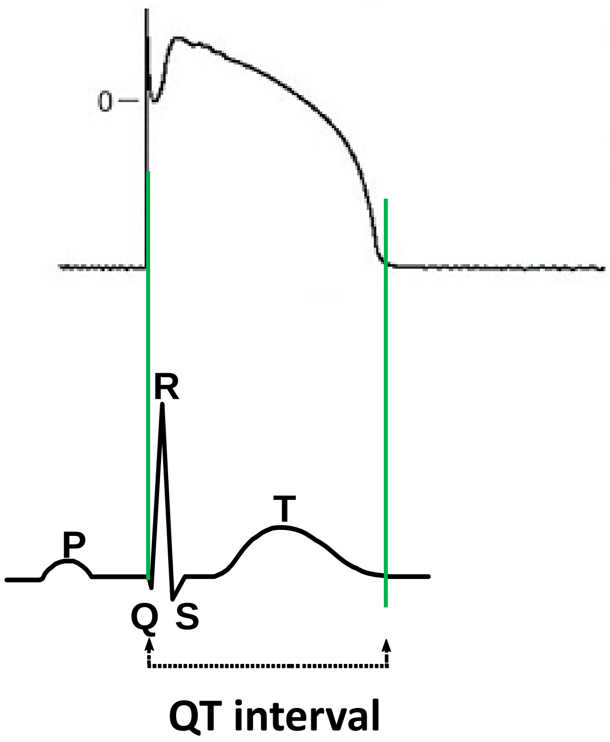
2022-2023

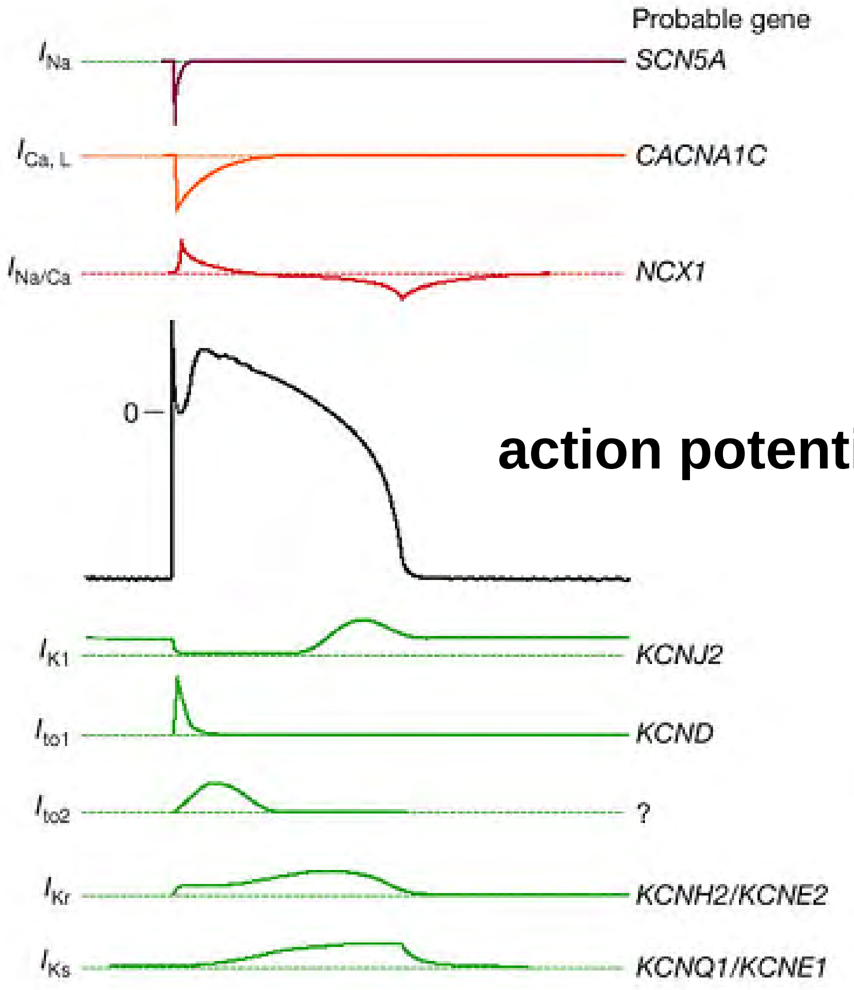
Activité électrique du cardiomyocyte : le potentiel d'action



hétérogénéité du PA

Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action





early depolarization

plateau

action potential (AP)

diastolic potential

early repolarization

late repolarization

from Marbán (2002) Nature 415:213-218 ← **https://pubmed.ncbi.nlm.nih.gov/**

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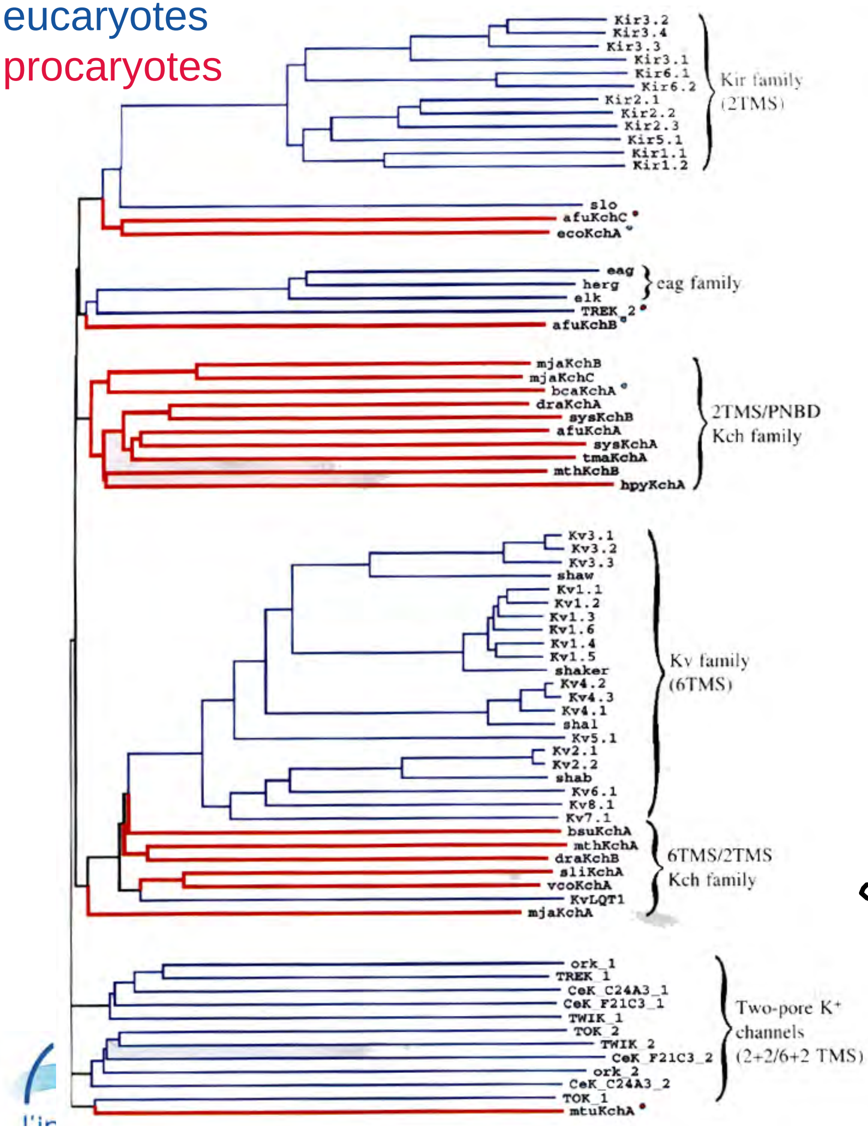
Canaux potassiques

dendrogramme

eucaryotes

procaryotes



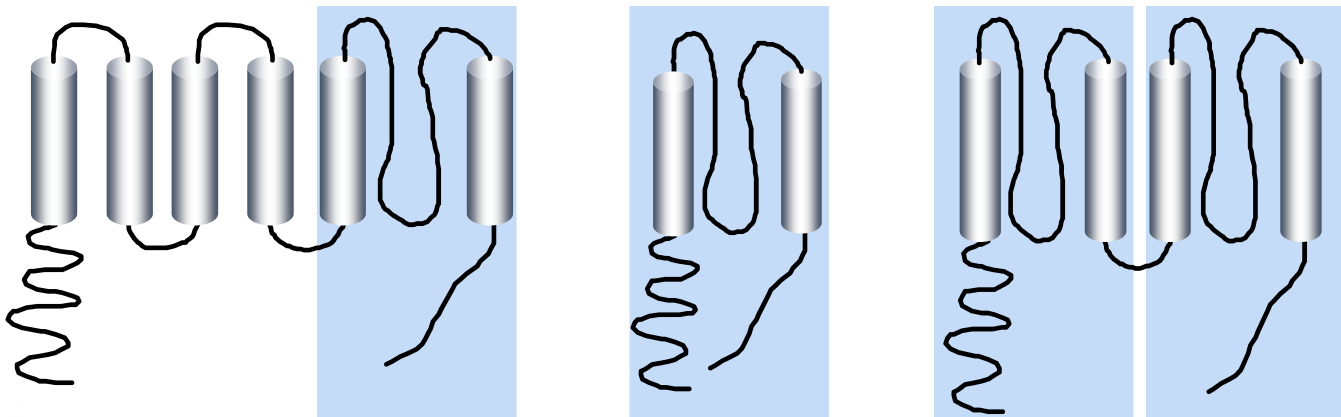
d'après Derst et Karschin J exp Biol (1998) 201:2791

→ 3 classes

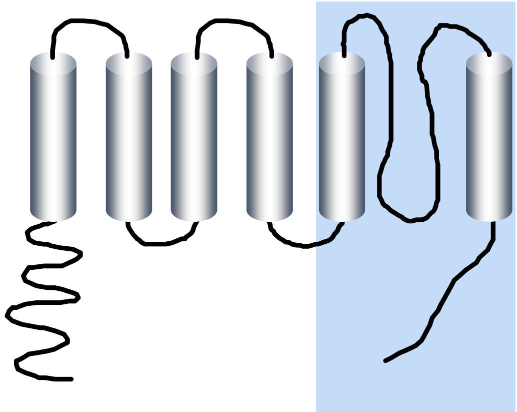
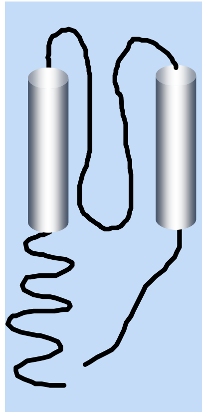
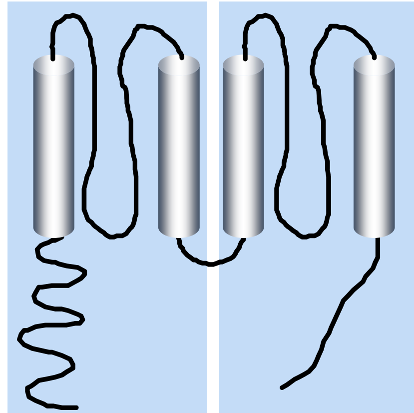
6 segments transmembranaires (TMS) et 1 boucle du pore

2 TMS et 1 boucle du pore

4 TMS et 2 boucles du pore

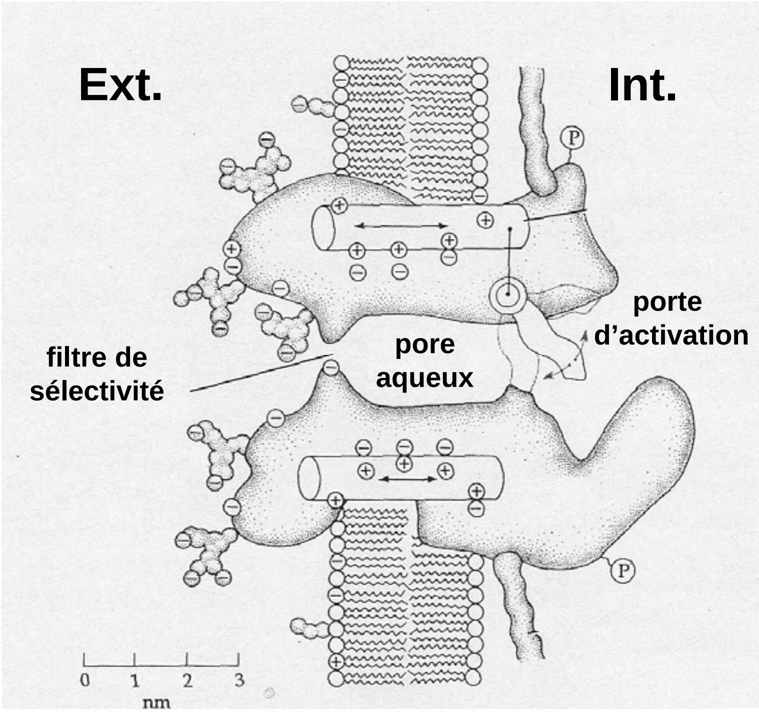


3 classes de canaux K$^+$

	6 segments transmembranaires (TMS) et 1 boucle du pore	2 TMS et 1 boucle du pore	4 TMS et 2 boucles du pore
Trace hydropathie			
Structure	oui	oui	oui
Régulateurs	principalement $V_{membrane}$	principalement ligand: ATP, Prot G	stimuli physiques chimiques
Cibles		$V_{membrane}$; [ion]	

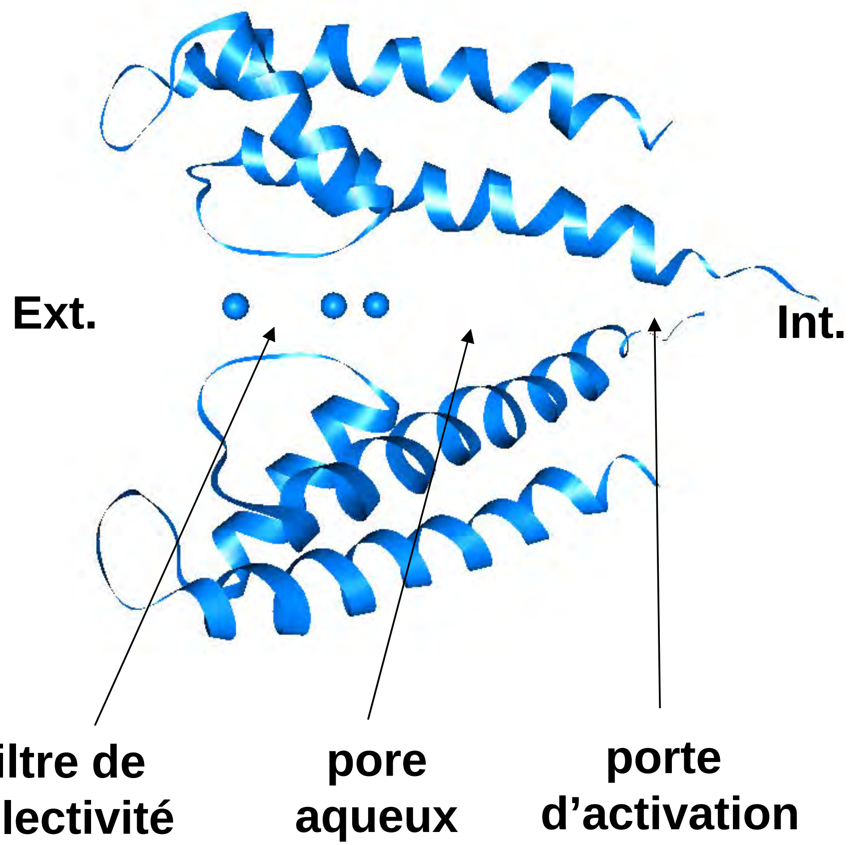
Structure cristallographique d'un canal K^+ à 2 TMS et 1 pore

Hypothèse de travail (1992)



D'après Hille (1992) Sinauer, Sunderland, MA

Structure de KcsA (1998)



D'après Doyle et al. (1998) Science, 280:69

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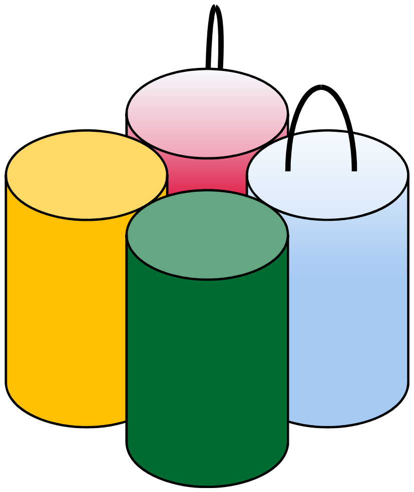
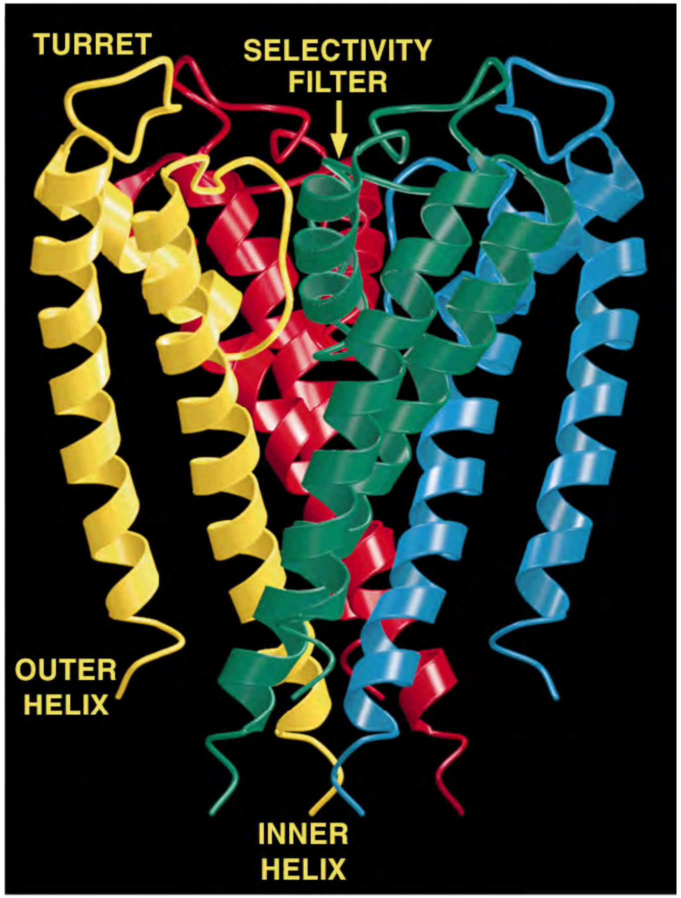
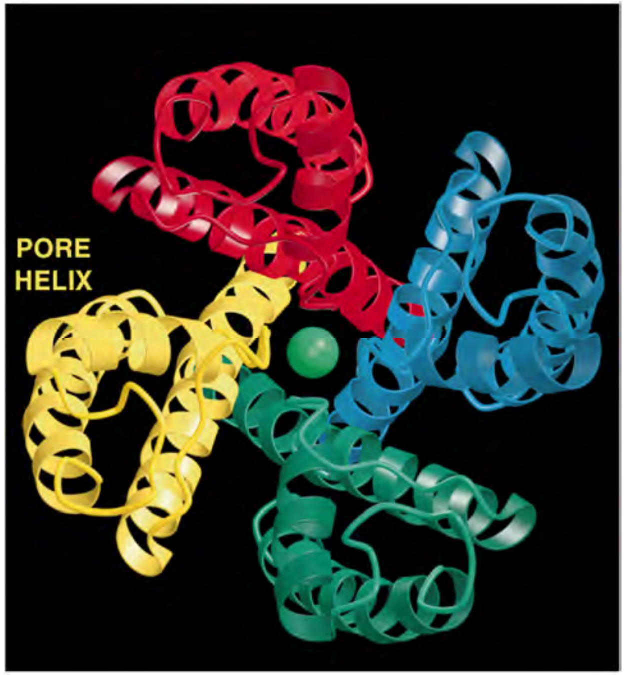
Structure cristallographique d'un canal K^+ à 2 TMS et 1 pore



Tétramères

KcSA



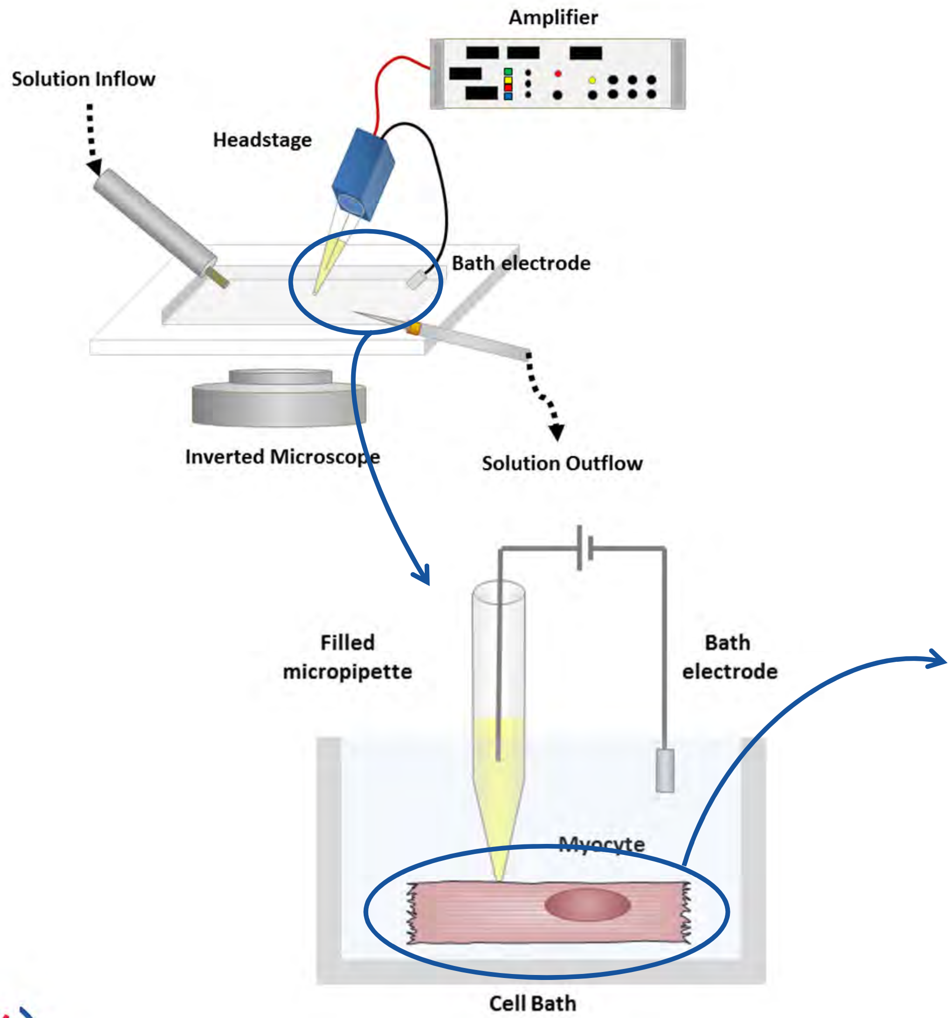


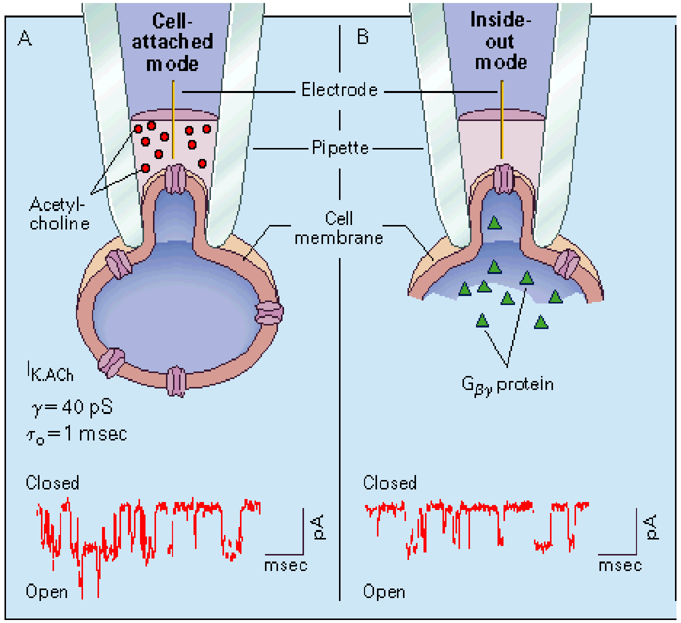
(resolution: 3.2 Å)

Prix Nobel de Chimie 2003 :
Roderick Mac Kinnon

D'après Doyle et al. (1998) Science, 280:69

Enregistrement d'un courant unitaire : le patch-clamp





Prix Nobel de Physiologie 1991 : Erwin Neher & Bert Sakmann

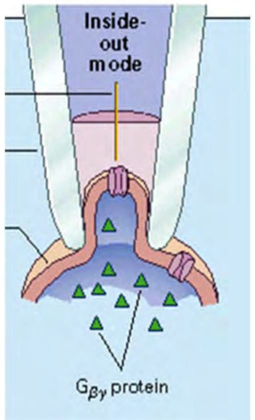
canaux K_{ATP} du pancréas (cellules ß des ilôts de Langerhans)

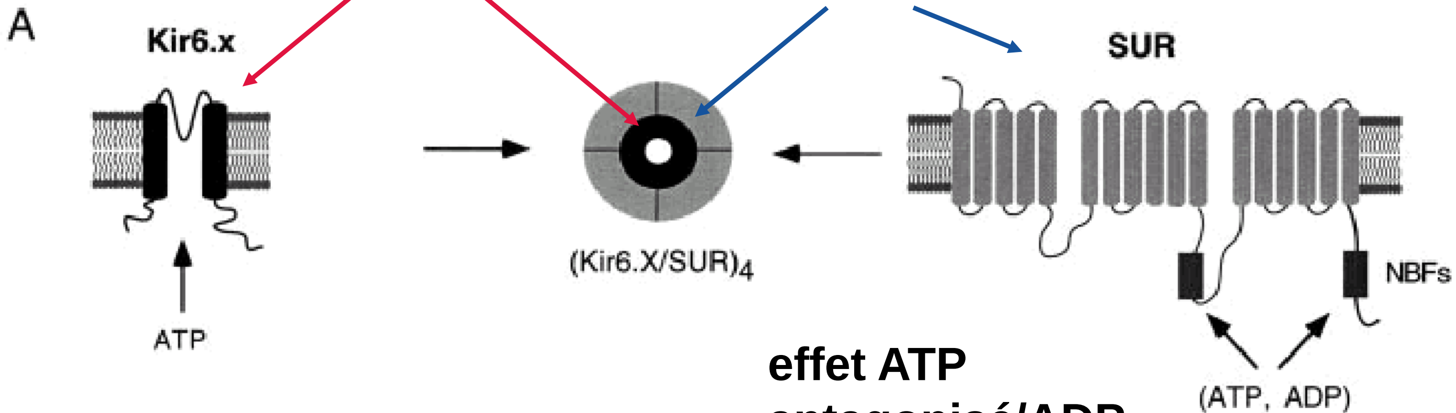
C ⇄ O

fermé ouvert

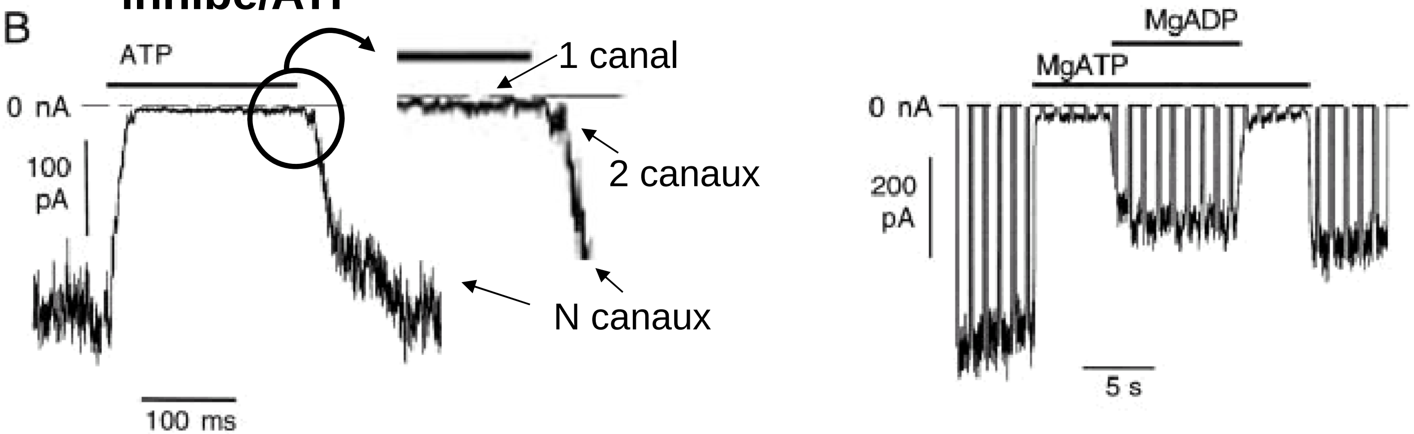
sous-unité α : pore

sous-unité β : auxiliaire

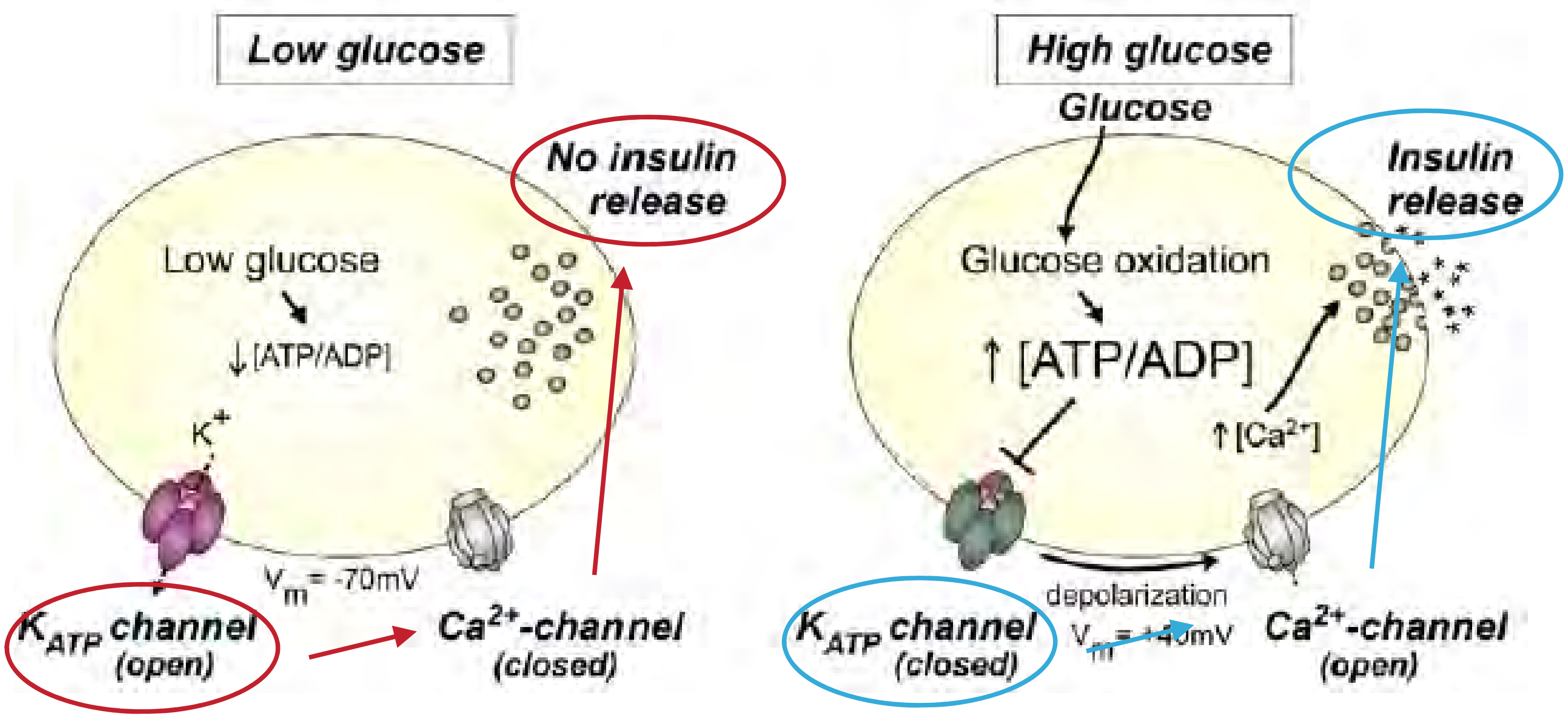




effet ATP antagonisé/ADP



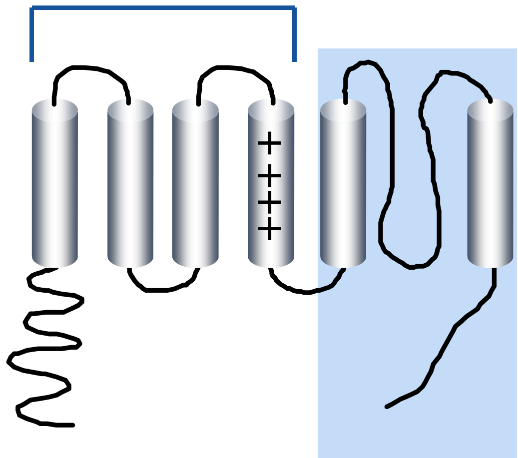
canaux K_{ATP} du pancréas (cellules ß) rôle dans la sécrétion d'insuline

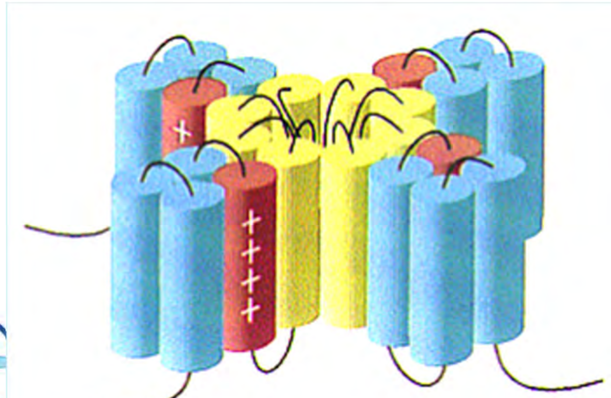


Remedi & Koster (2010) Pflugers Arch - Eur J Physiol 460:307

Structure d'un canal K^+ 6 TMS et 1 pore

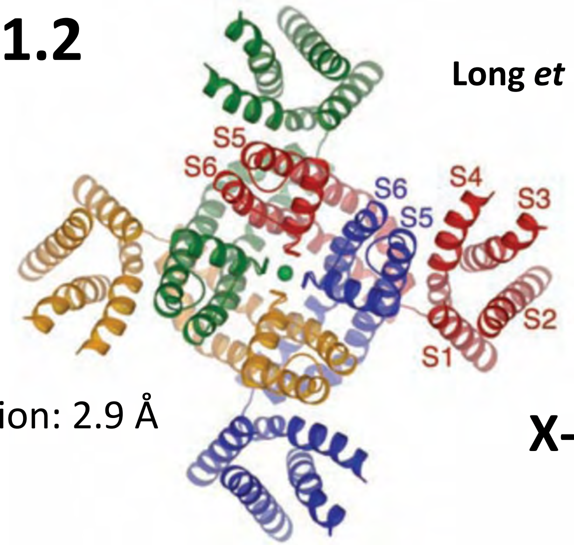
voltage sensor





Tétramères

Kv1.2

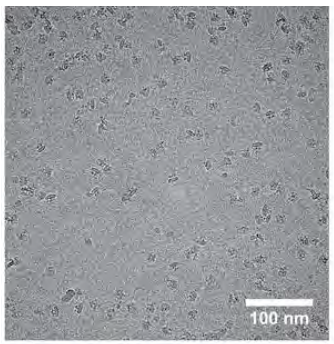


Long *et al.* (2005) Science 309:897

resolution: 2.9 Å

X-ray crystallography

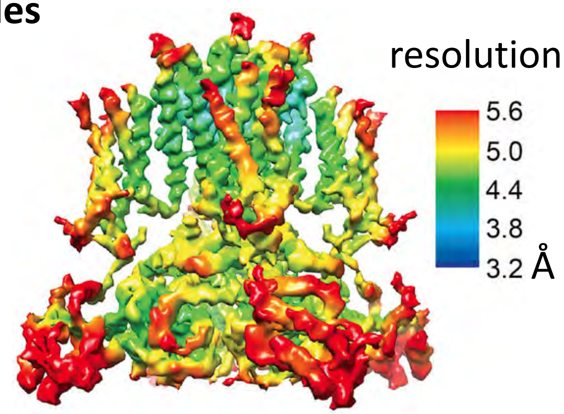
hERG



~144,000 particles

10 nm

resolution:



Cryo-electron microscopy

Wang *et al.* (2017) Cell 169:422

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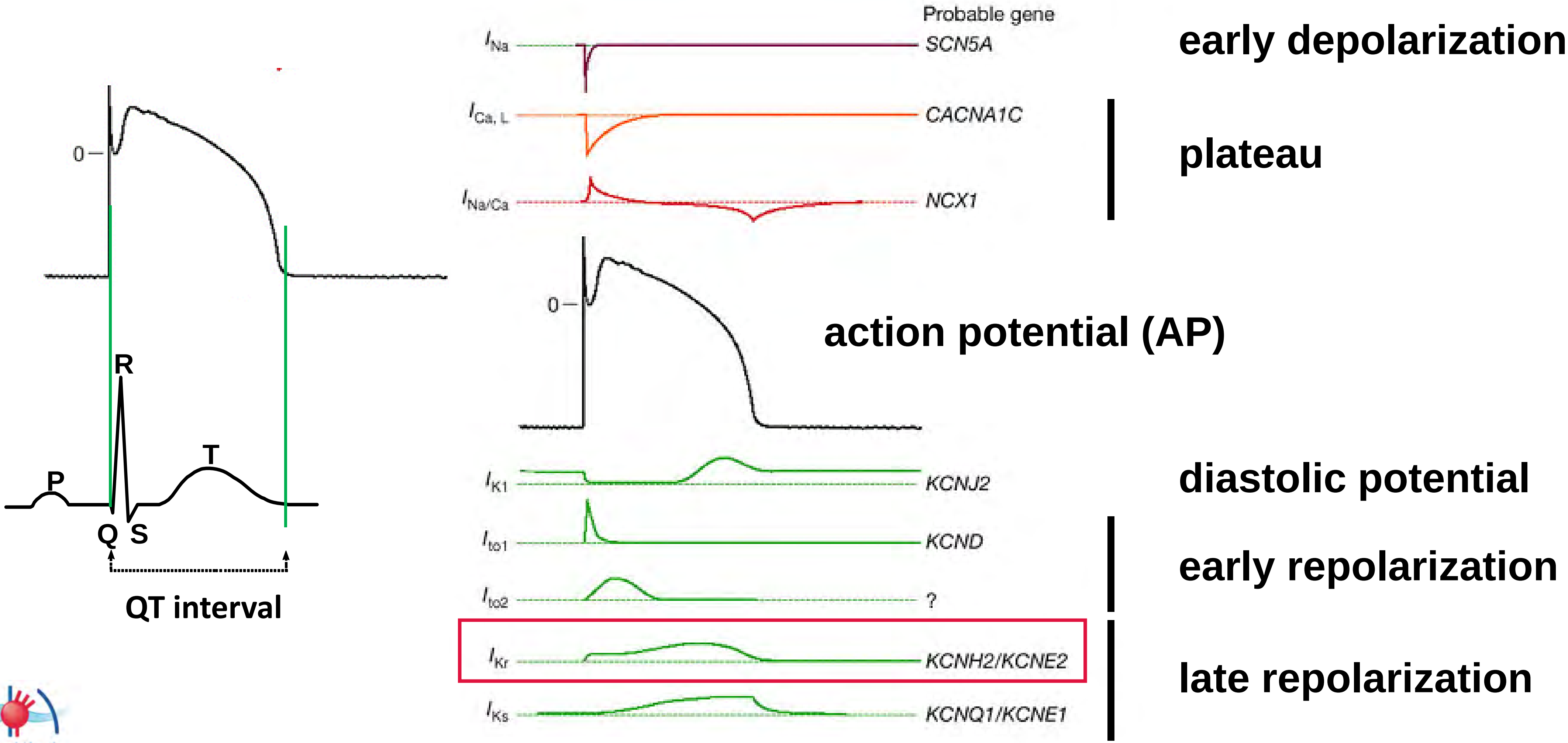
Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



from Marbán (2002) Nature 415:213-218

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**

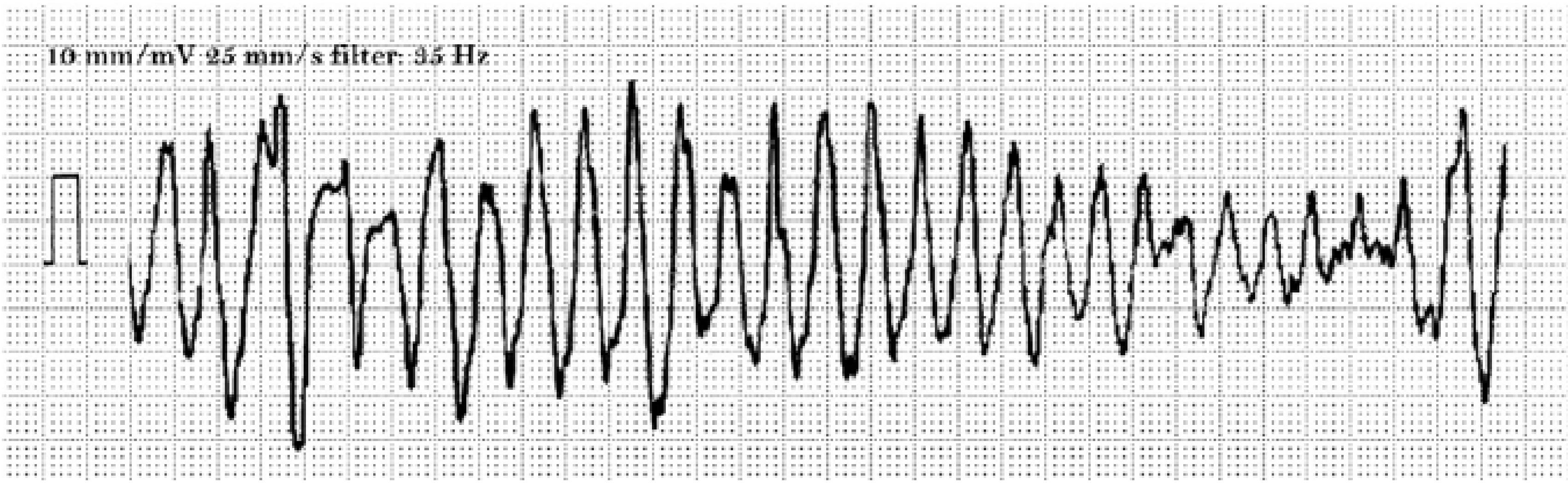


Fig. 1. ECG recording showing an ongoing episode of Torsade de pointes. This polymorphic ventricular arrhythmia is characterized by the progressive rotation of the electrical axis (180 ° in about 10–12 cycles) which shows on the surface ECG as the sinusoidal undulation of the electrical signal.

Saenen & Vrints (2008) J Mol Cell Cardiol 44:633

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_V7.1 (↓)
LQT2	KCNH2	30–45	7q35–36	K_V11.1 (↓)
LQT3	SCN5A	5–10	3p21–24	Na_V1.5 (↑)
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^{2+} signaling)
LQT15	CALM2	< 1	2p21	Calmodulin 2 (dysfunctional Ca^{2+} signaling)
Jervell and Lange-Nielsen syndrome (JLNS) **mut/mut**				
JLN1	KCNQ1	< 1	11p15.5	K_V7.1 (↓)
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

Wallace *et al.* (2019) Pediatric cardiol 40:1419

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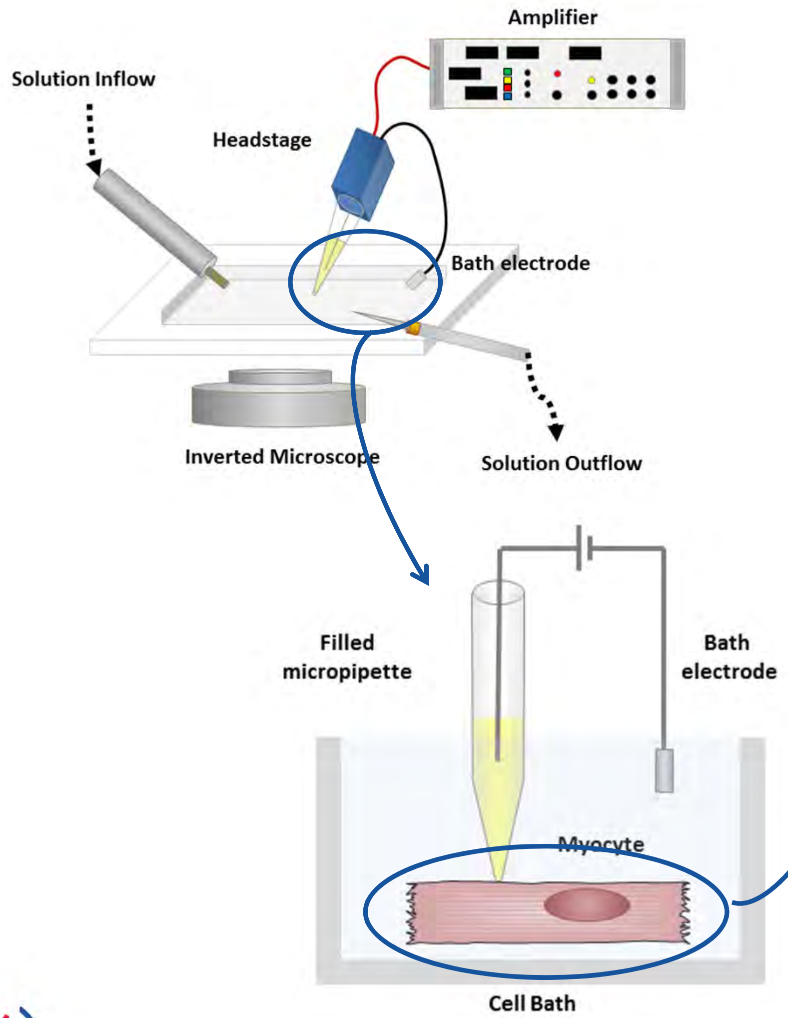
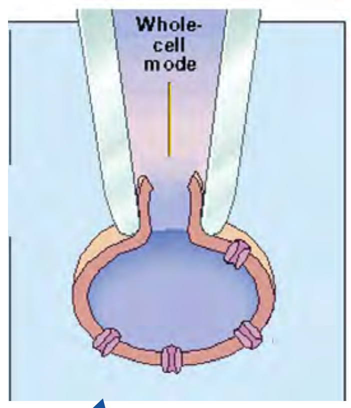
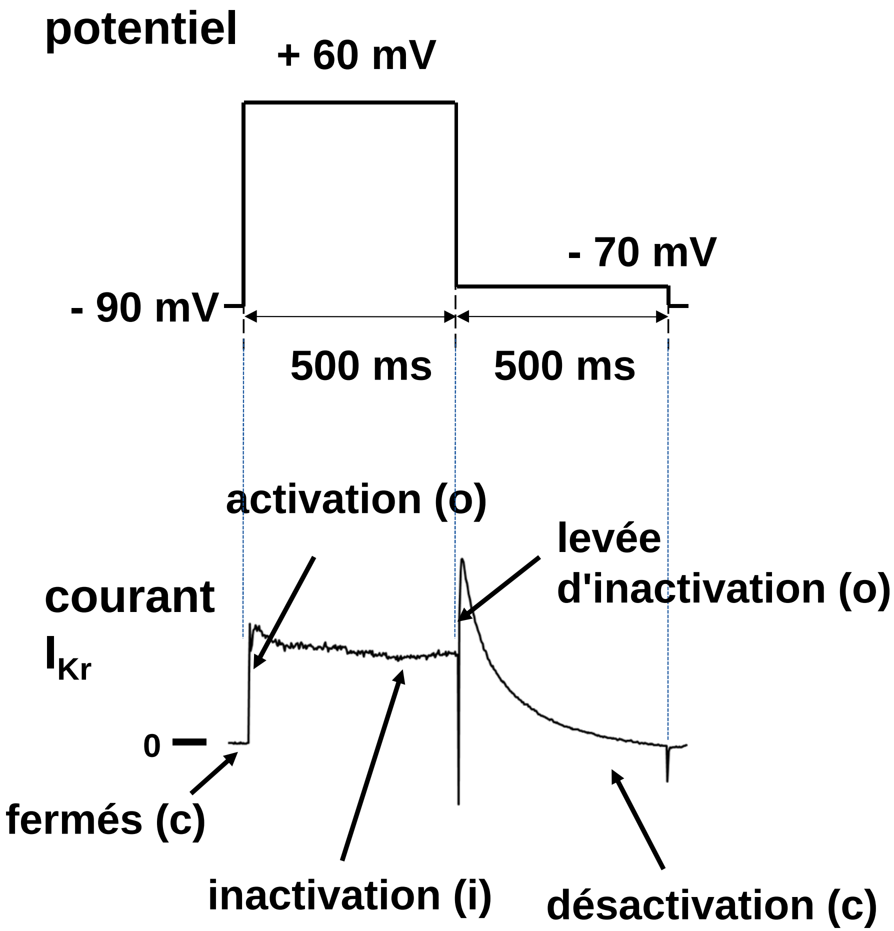
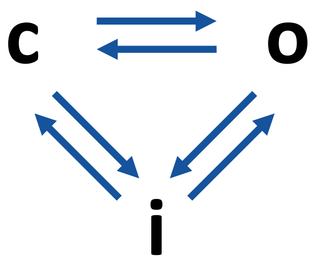
Enregistrement d'un courant trans-membranaire : le patch-clamp









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

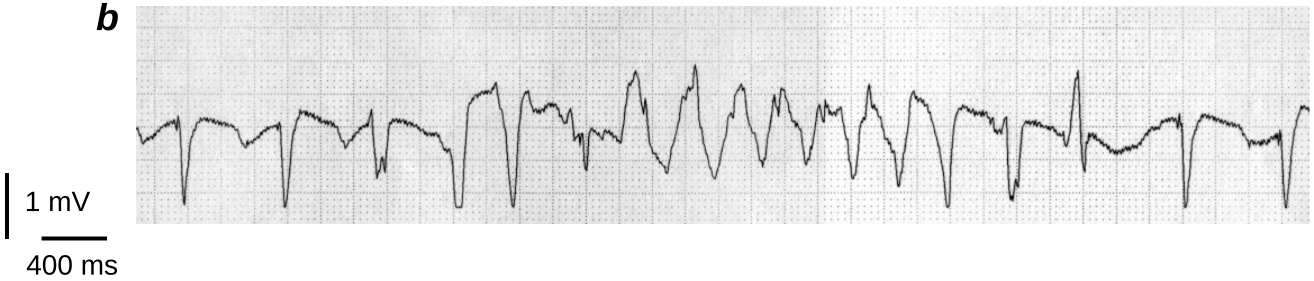
Propositus : fibrillation ventriculaire (torsades de pointes)

a

◂ May-30-97
597 ms
(628 ms)

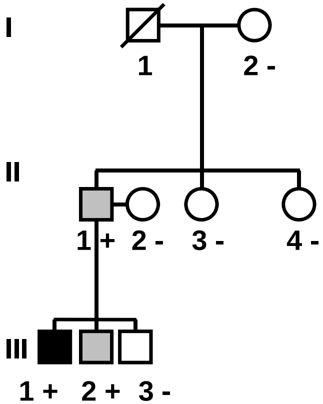
▾ Feb-05-99 – clobutinol

b



Génotypage

porteur hétérozygote :



+ : hERG A561P
- : hERG WT

causal ?

Modèles cellulaires

- **Lignées cellulaires modifiées - canalopathies monogéniques**

expression hétérologue de protéines WT et mutées

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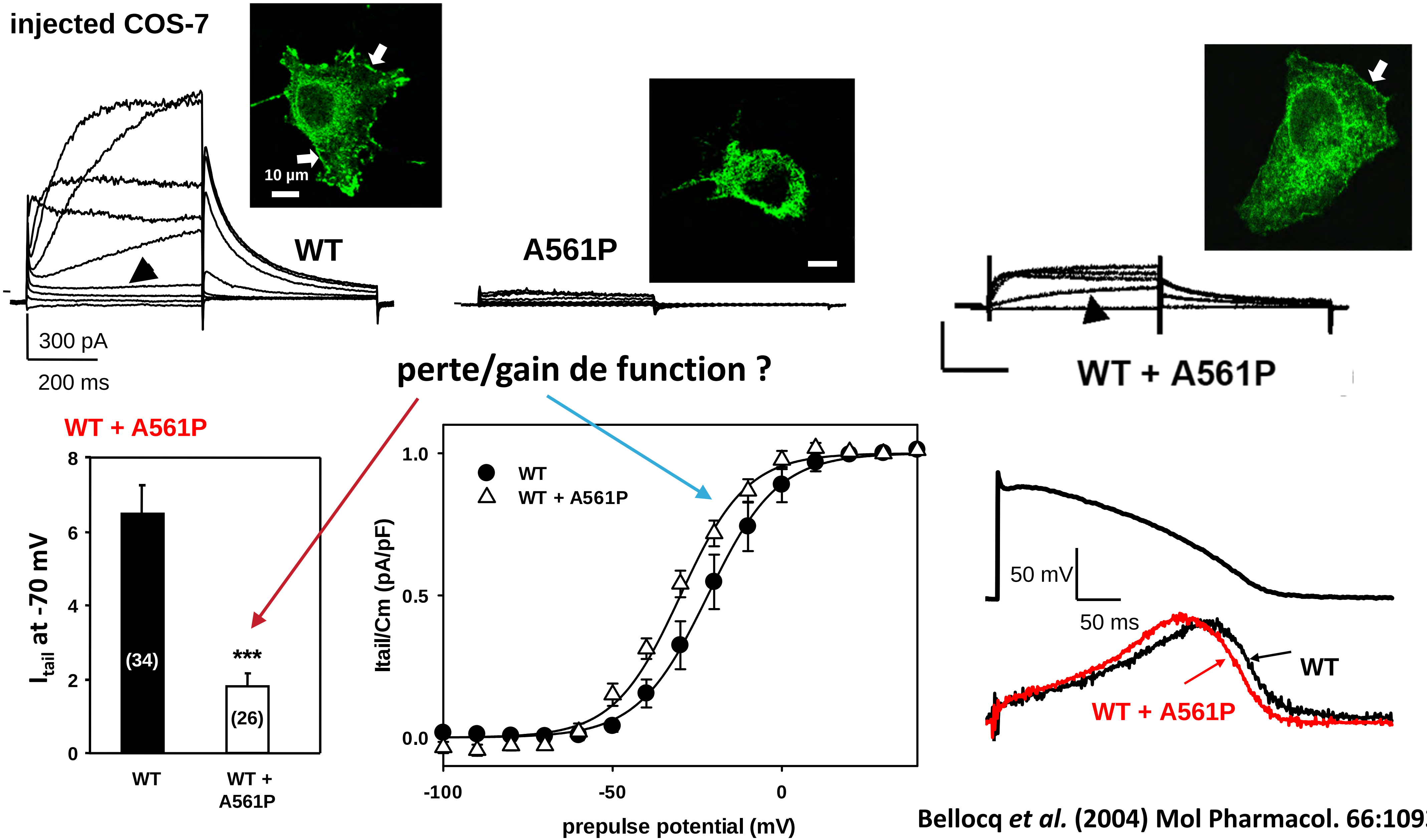
Bellocq *et al.* 2004. Mol Pharmacol. 66:1093



Bellocq *et al.* (2004) Mol Pharmacol. 66:1093

Modèles *in silico*

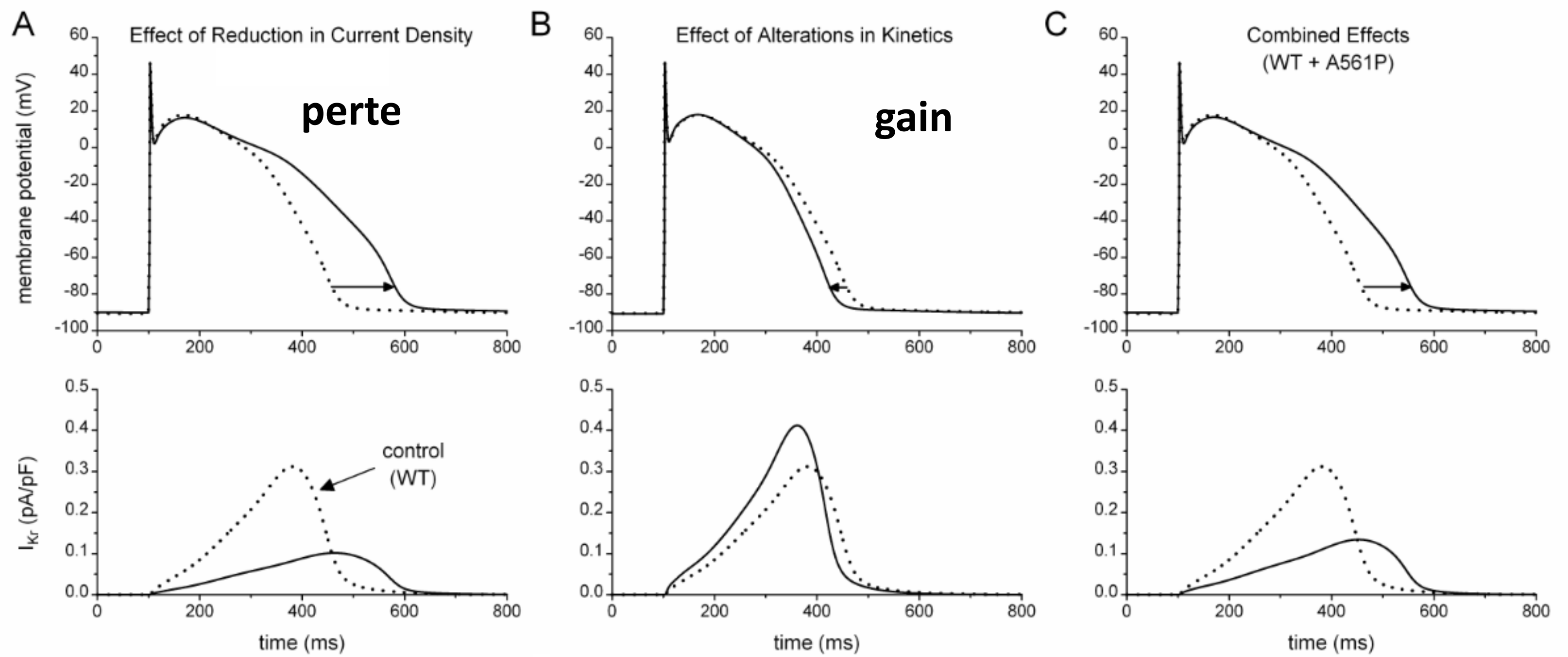
- **Modèle de potentiel d'action ventriculaire humain Priebe and Beuckelmann (1998) - modèle d'Hodgkin et Huxley (Prix Nobel de Physiologie 1963)**

perte/gain de function : allongement ➔ causal

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

$$1-n \underset{\beta_n}{\overset{\alpha_n}{\rightleftarrows}} n$$

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



Rapidly Activating Current: I_{Kr}

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

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

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

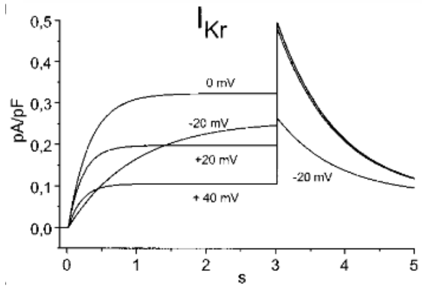
$\alpha_{Xr} = \{0.005 \cdot \exp[5.266 \cdot 10^{-4} \cdot (V+4.067)]\}/\{1+\exp[-0.1262 \cdot (V+4.067)]\}$

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

$$\frac{dXr}{dt} = \alpha_{Xr}(1 - Xr) - \beta_{Xr} Xr = \frac{Xr_\infty - Xr}{\tau_{Xr}}$$ →

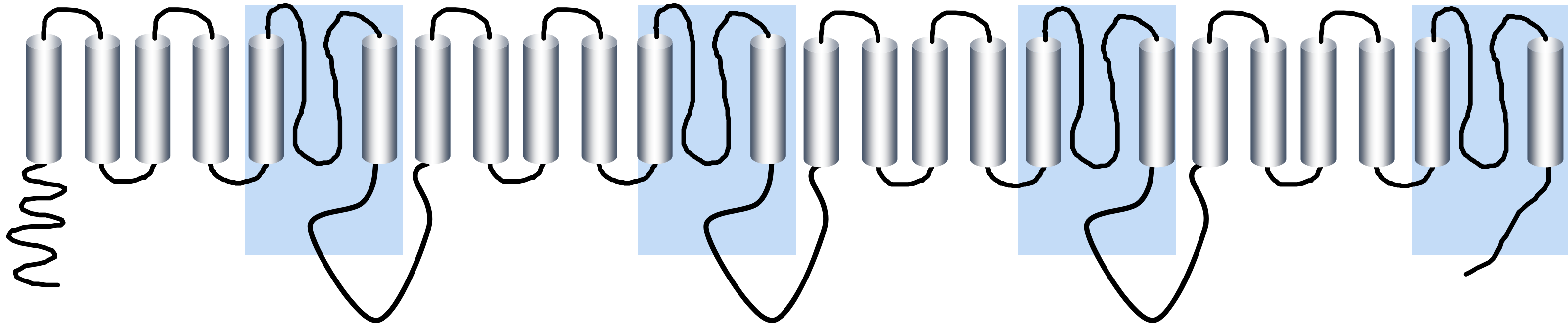
$rik = 1/\{1+\exp[(V+26)/23]\}$

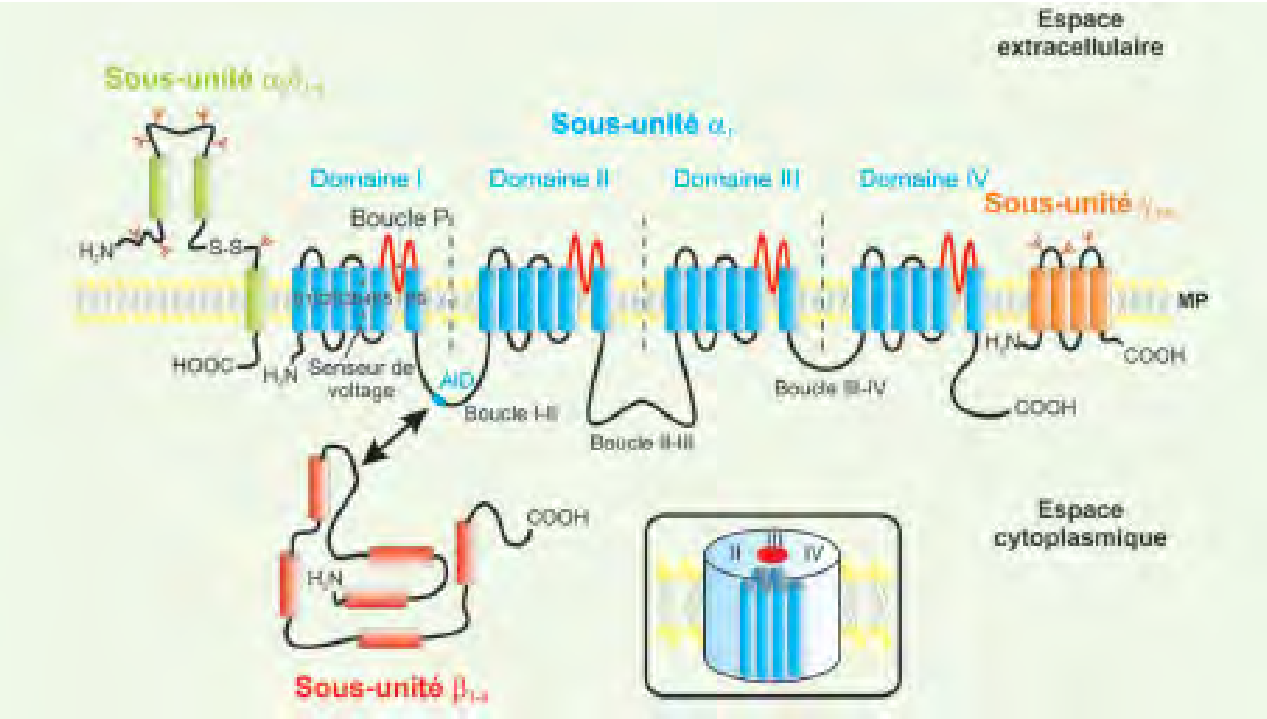
where $g_{Kr,max}$ is g_{max} for I_{Kr}, Xr is the activation gate of I_{Kr}, rik is the inward-rectification factor of I_{Kr}, and E_K is the equilibrium potential for I_K.



Hodgkin & Huxley (1952) J Physiol 117:500
Priebe & Beuckelmann (1998) Circ Res 82:1206
Bellocq *et al.* (2004) Mol Pharmacol. 66:1093

Canaux Ca^{2+} : 4 domaines homologues

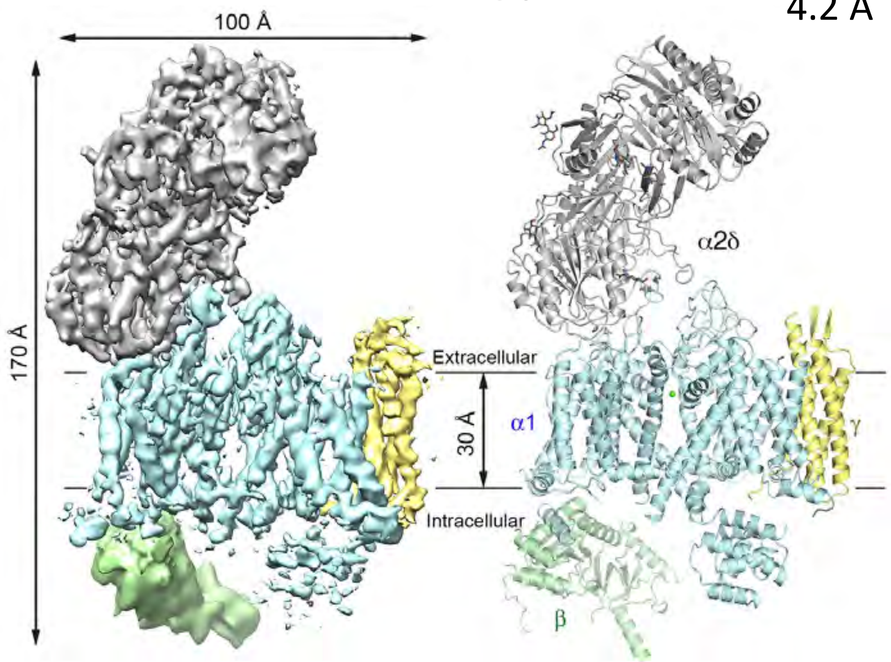




cryo-EM

Cav1.1

4.2 Å



Wu *et al.* (2015) Science 350:aad2395

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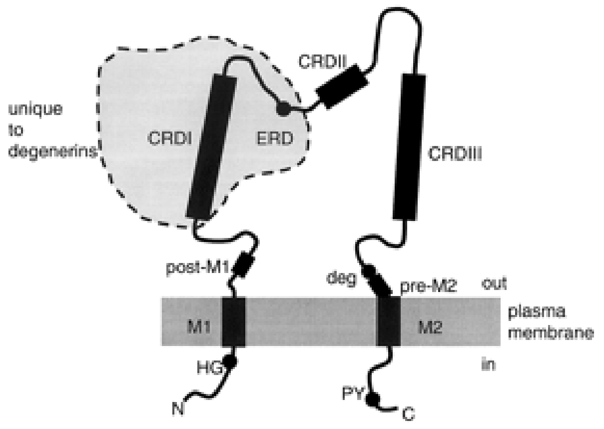
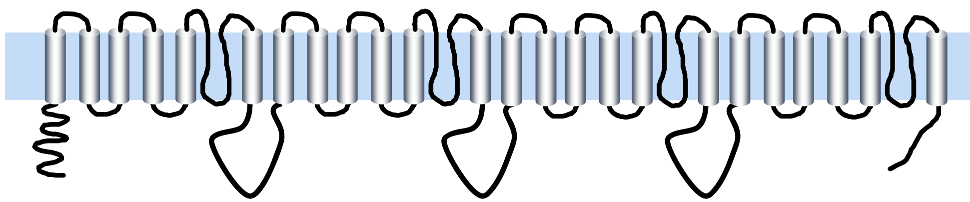
Canaux Na⁺ : 2 classes

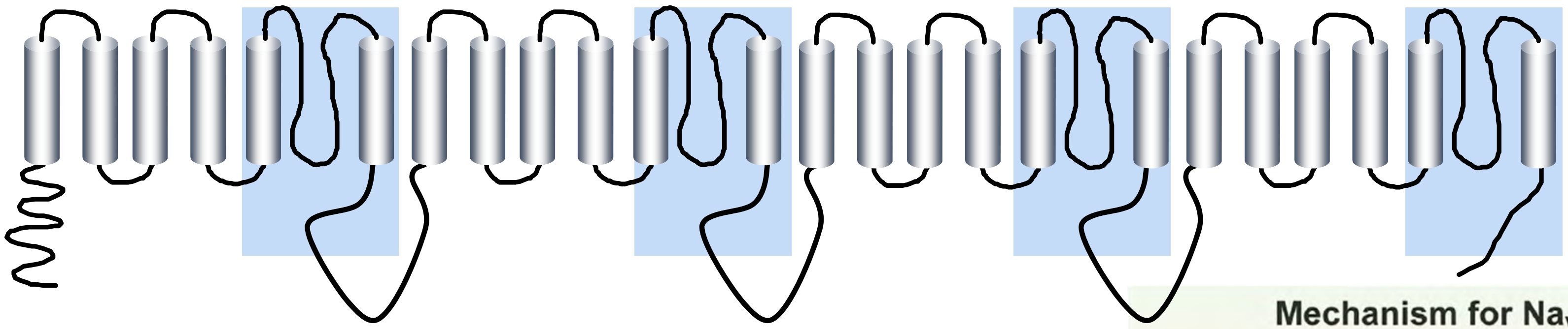


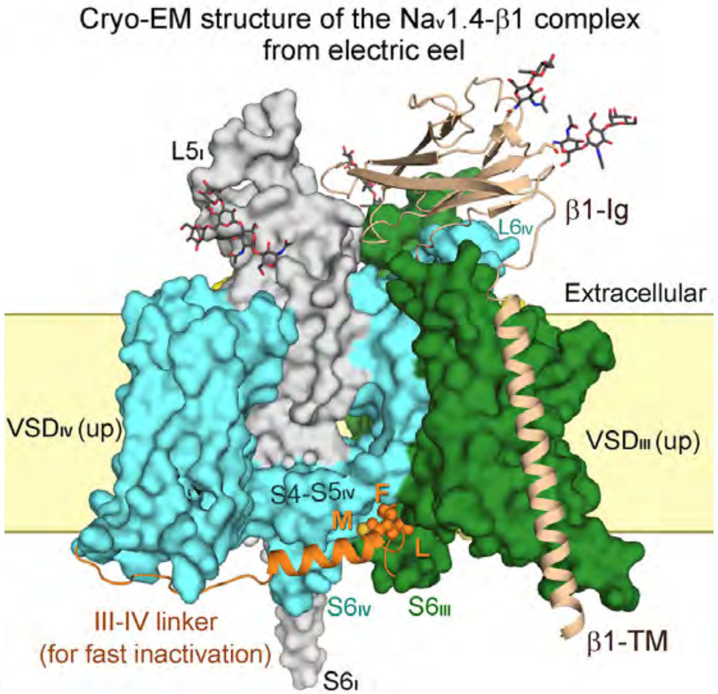


- **ENaC (epith. Na⁺ channel)**

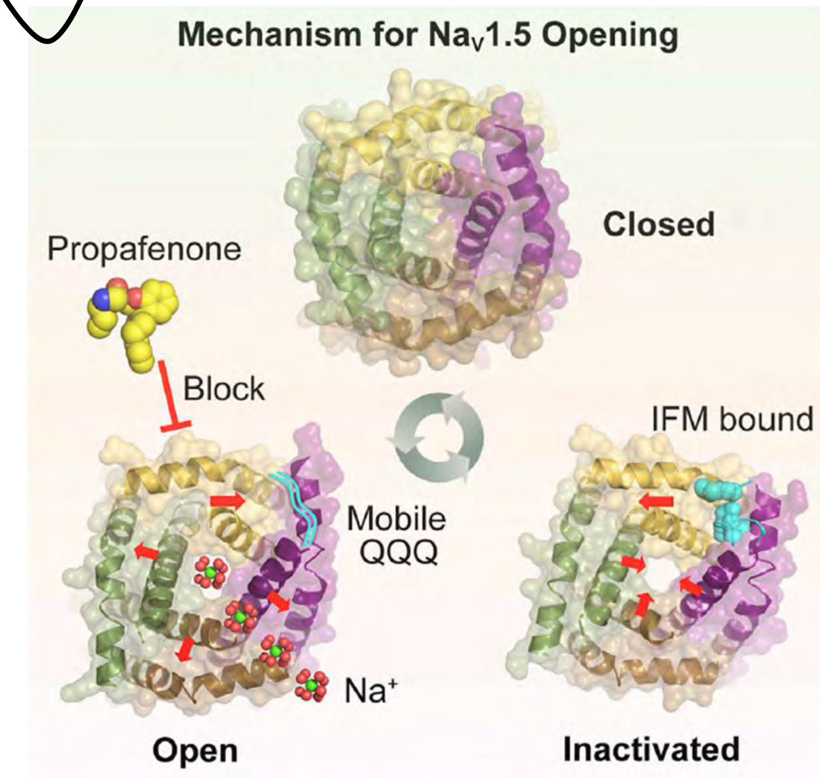
- **Nav: voltage-gated**
- **canal Na⁺ cardiaque : Nav1.5 + Navß1 (*SCN5A* et *SCN1B*)**

Canaux Na$^+$ voltage-gated : structure





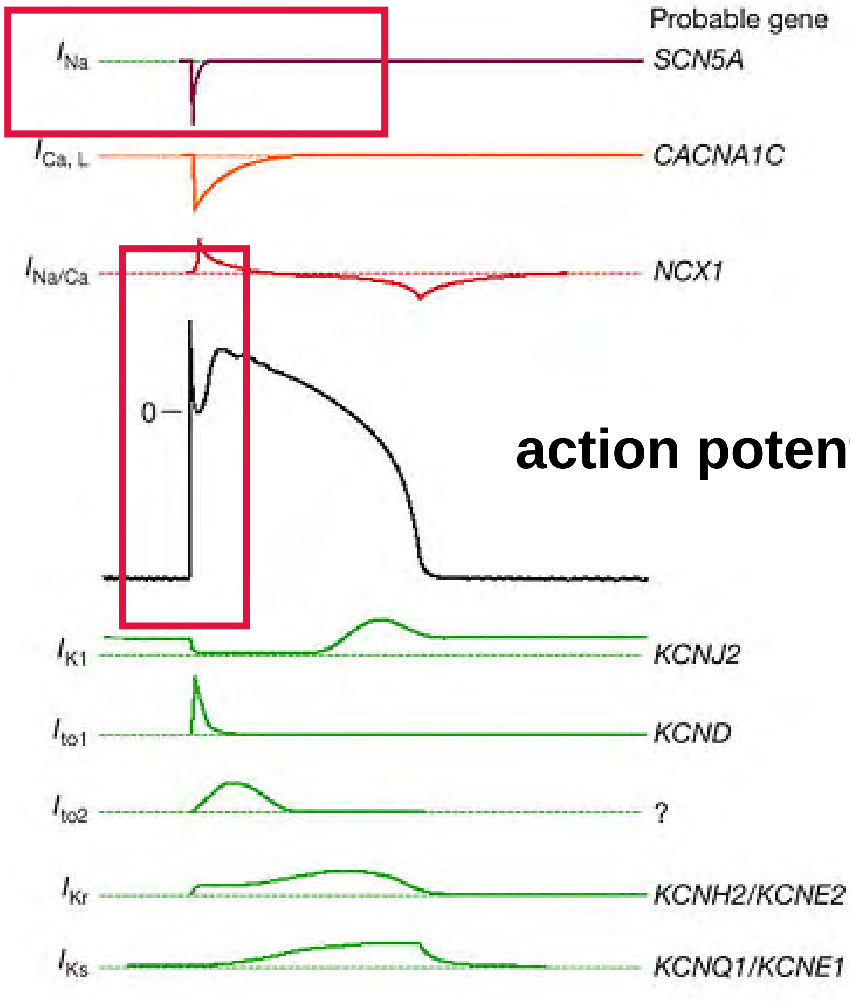
Yan *et al.* (2017) Cell 170:470



Jiang *et al.* (2021) Cell 184:1

Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action

canal Nav1.5



early depolarization

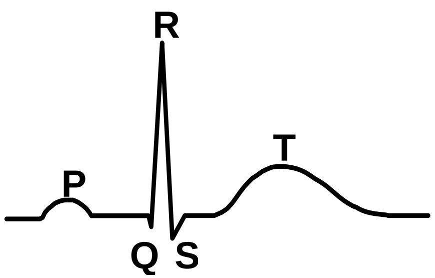
plateau

action potential (AP)

diastolic potential

early repolarization

late repolarization



complexe QRS

from Marbán (2002) Nature 415:213-218

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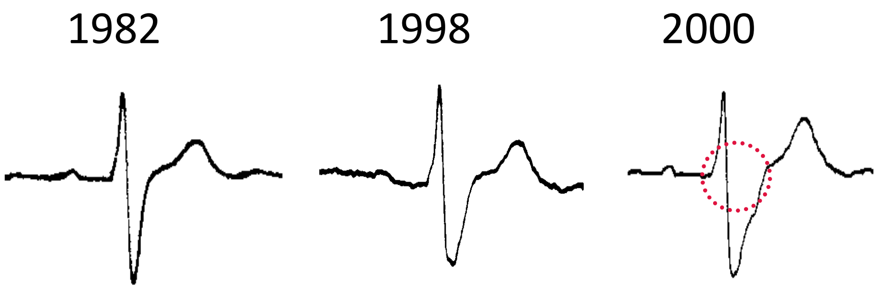
Du modèle cellulaire au modèle murin du syndrome de Lenègre

- **Syndrome de Lenègre : ralentissement de la conduction cardiaque héréditaire**

➔bloc de conduction

- **Modèles cellulaires: lignées cellulaires modifiées - canalopathies monogéniques**

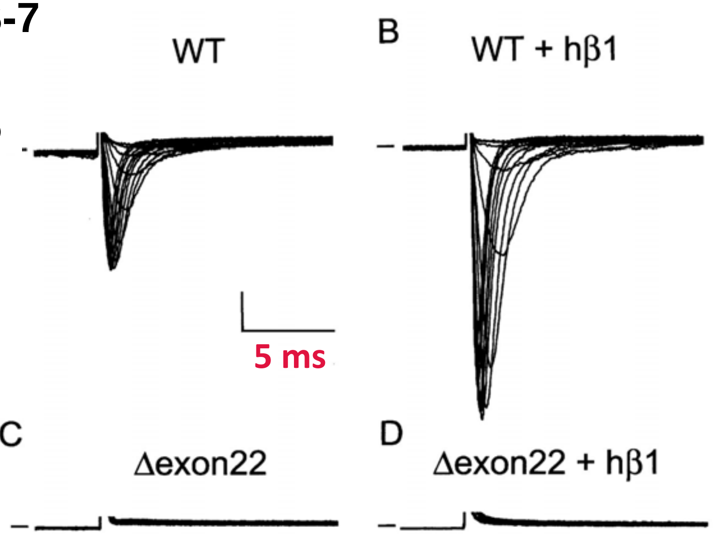
Expression hétérologue de protéines Nav1.5 WT et mutées



QRS prolongation

transfected COS-7

$I_{Nav1.5}$

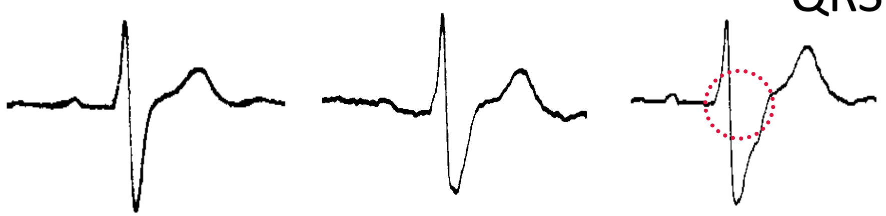


Mutation *SCN5A* : canal Nav1.5 Δexon22 ← causal?

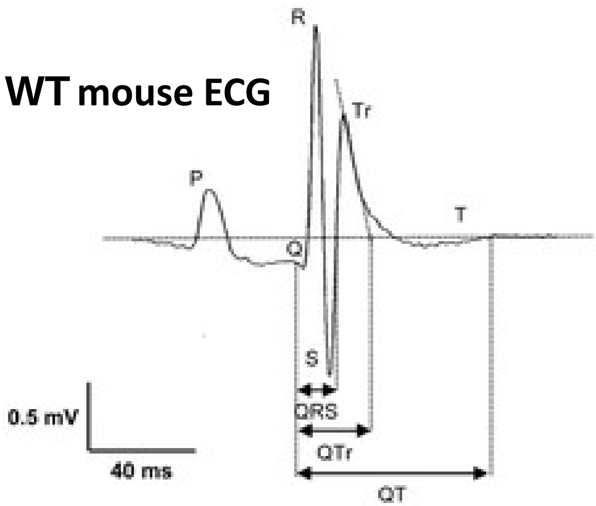
Schott *et al.* (1999) Nat Genet 23:20
Probst *et al.* (2003) J Am Coll Cardiol 41:643

Du modèle cellulaire au modèle murin du syndrome de Lenègre

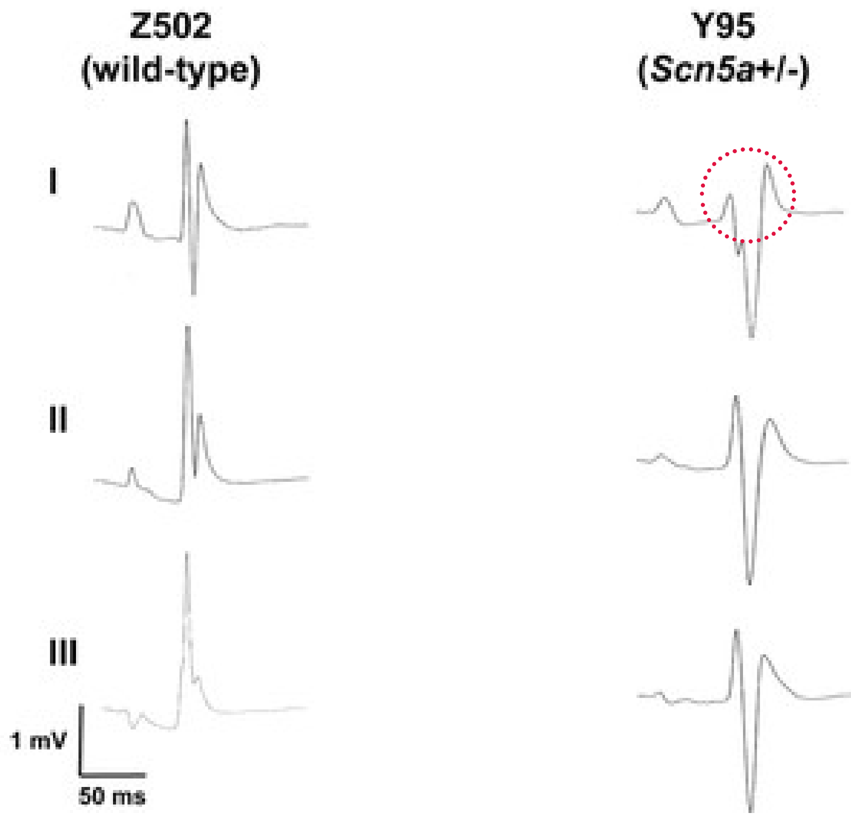
1982 1998 2000 QRS prolongation



***SCN5A* mut : exon 22 absent**



***Scn5a*$^{+/-}$ mouse**



Haplo-insufisance de *SCN5A* → causal

Schott *et al.* (1999) Nat Genet 23:20
Probst *et al.* (2003) J Am Coll Cardiol 41:643
Royer *et al.* (2005) Circ 111:1738

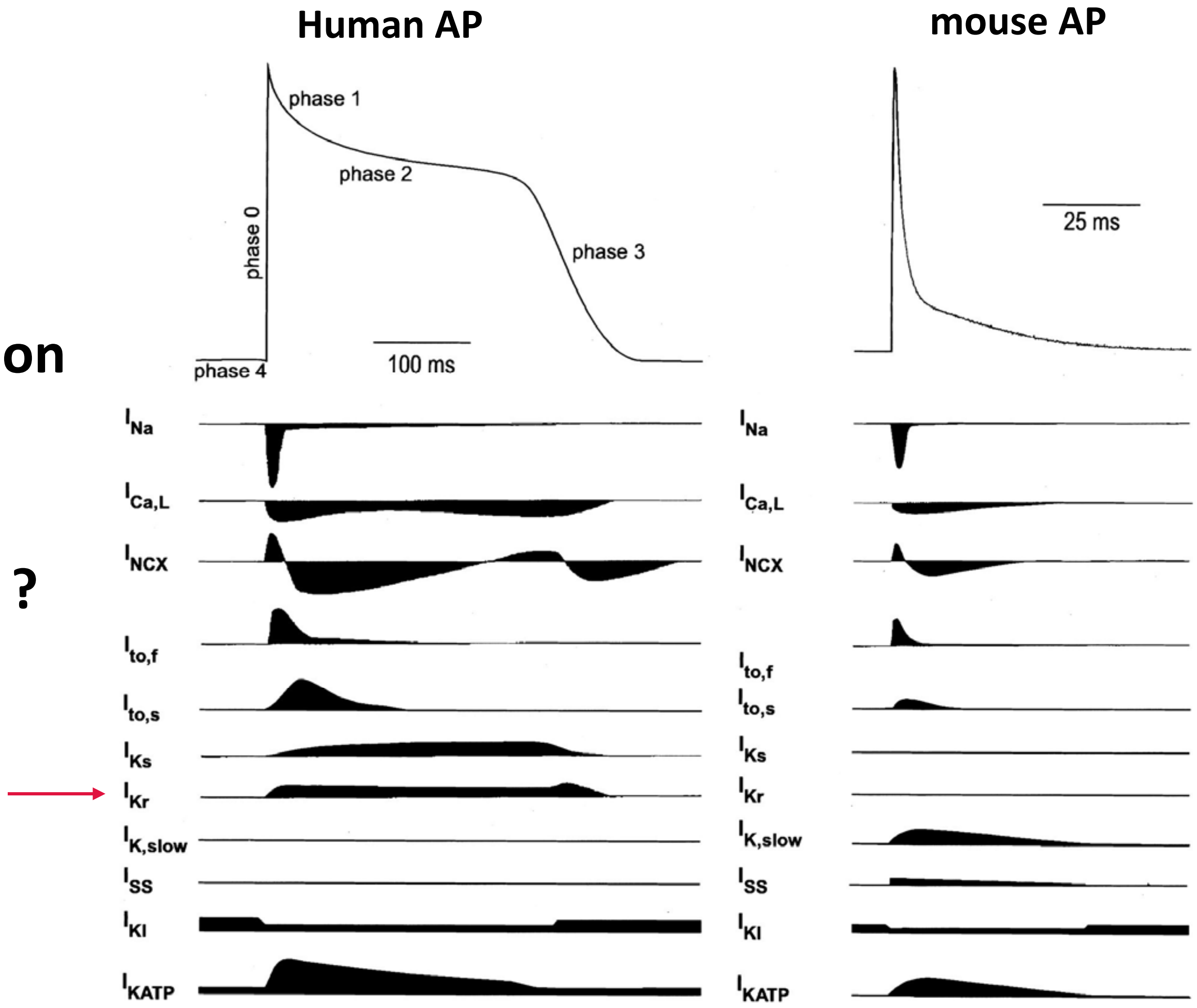
Limites

Modèles unicellulaires

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

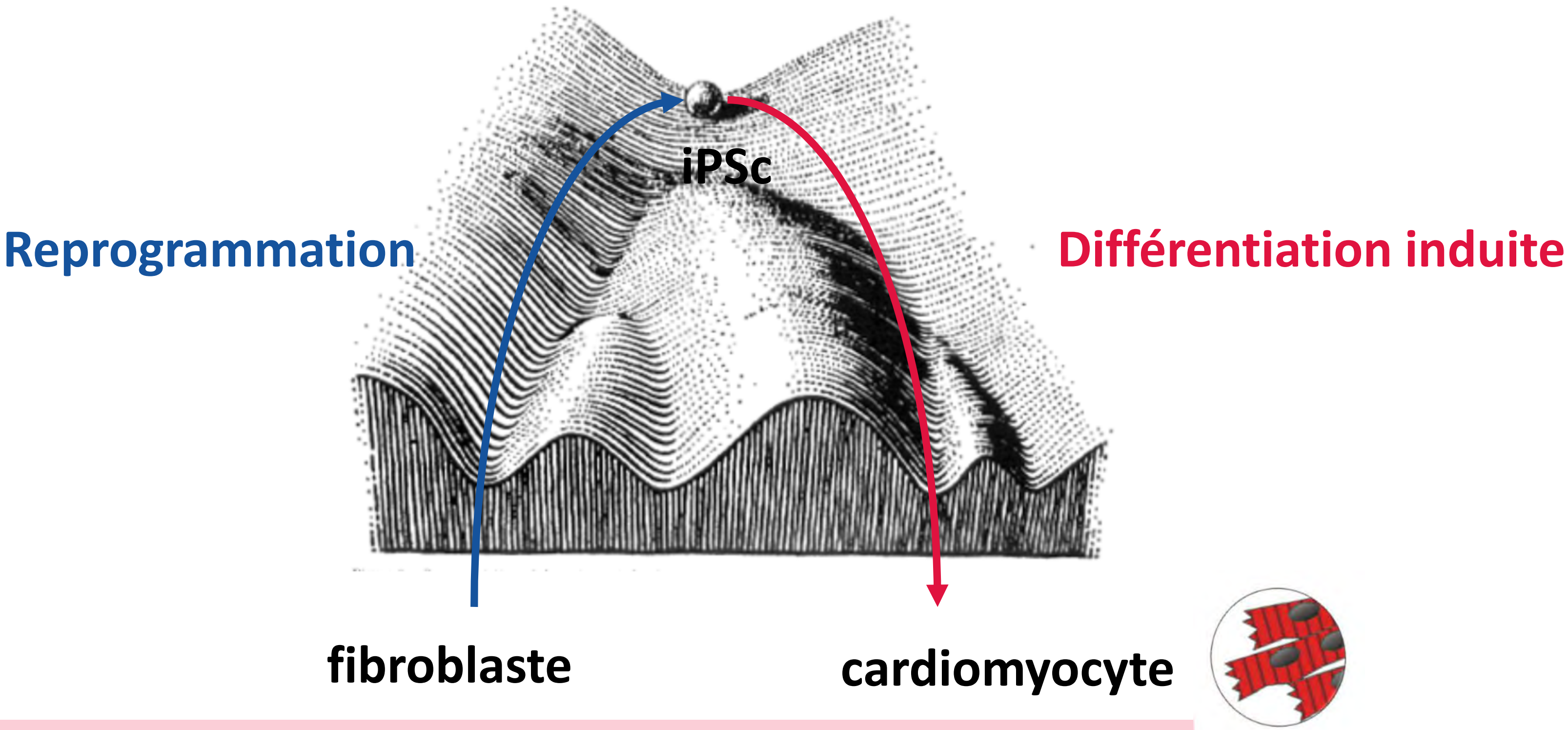
Modèles informatiques

- **environnement fonctionnel ?**



Nerbonne *et al.* (2001) Circ Res 89:944

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."

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

Cellules d'origine :

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

Cardiomyocytes issus d'iPSc

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

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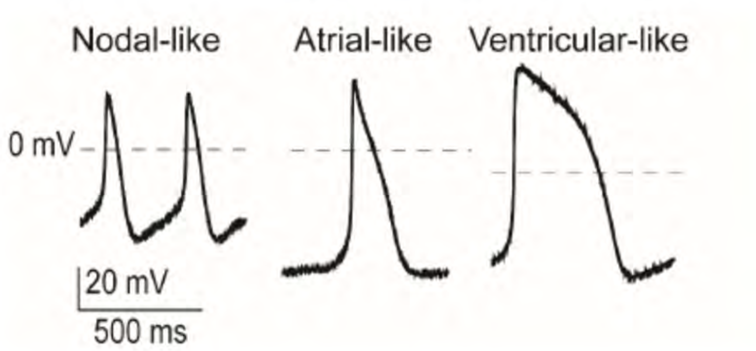
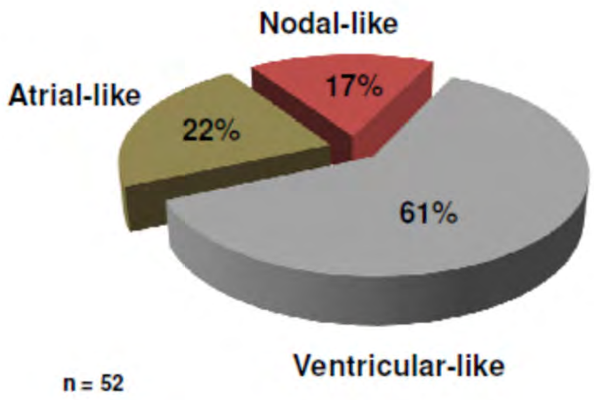
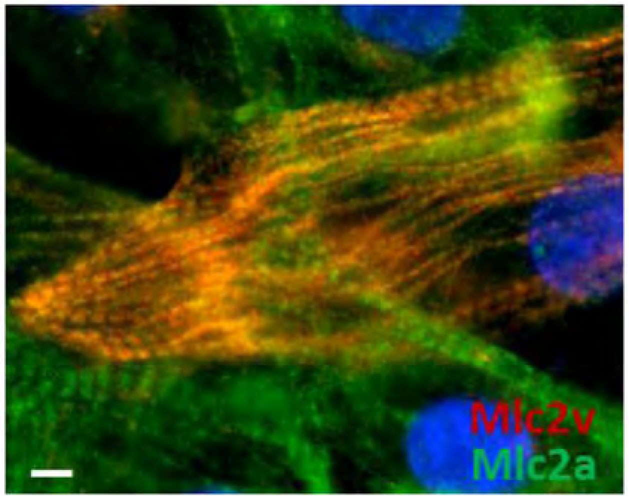
hiPS-cardiomyocytes





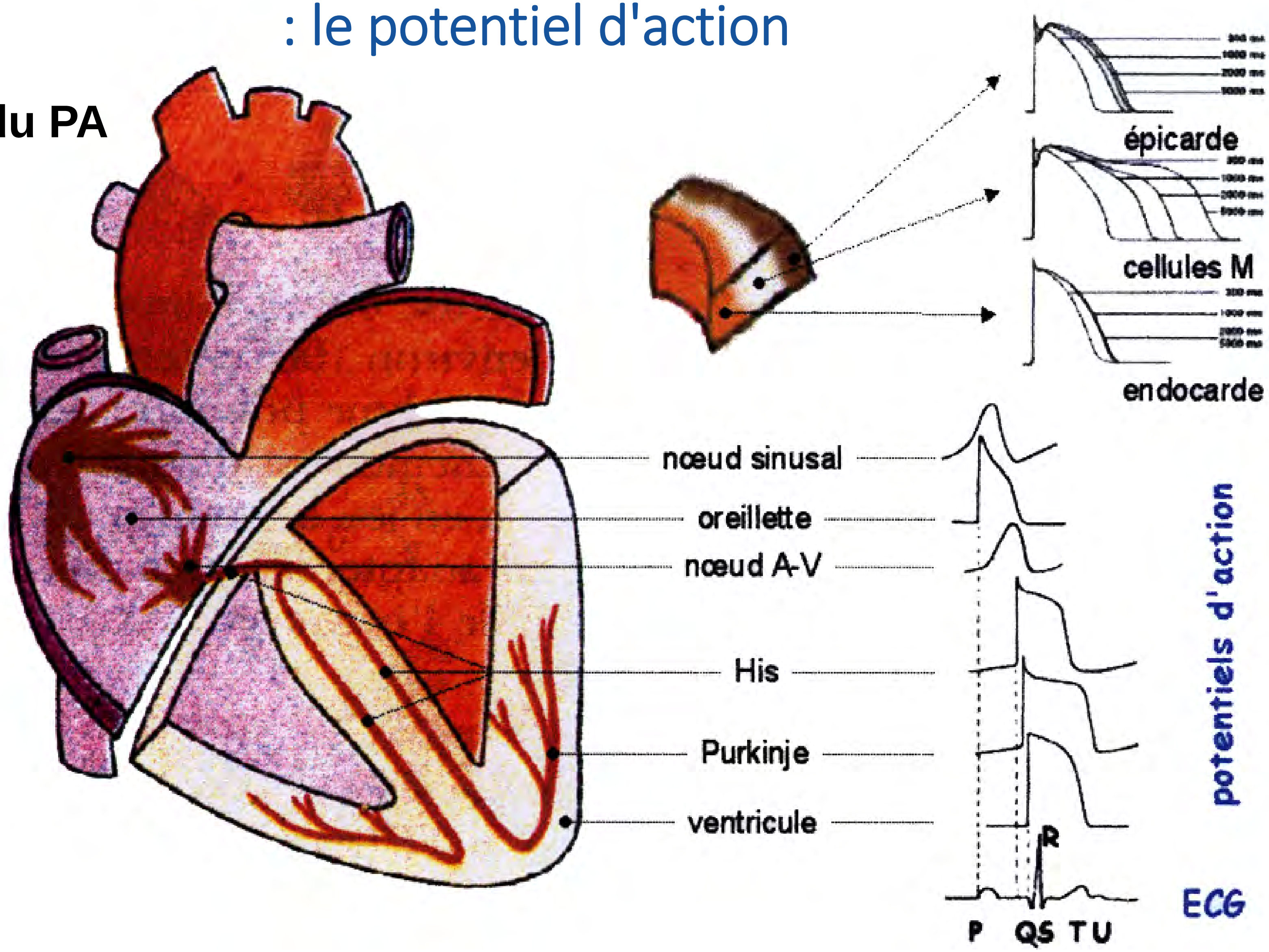


Hétérogénéité phénotypique

Jouni *et al.* (2015) JAHA 4:e002159

Activité électrique du cardiomyocyte : le potentiel d'action

hétérogénéité du PA



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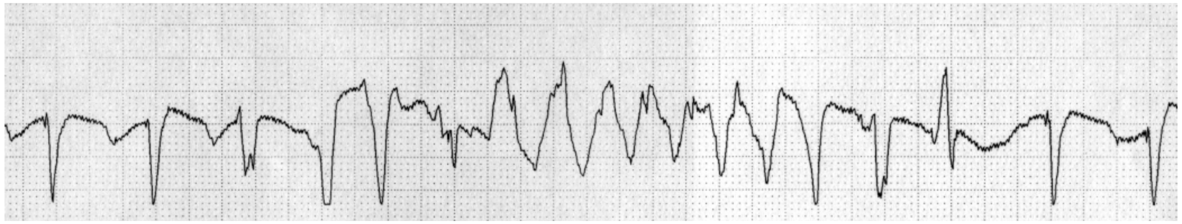
modélisation du syndrome du QT long: A561P HERG

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

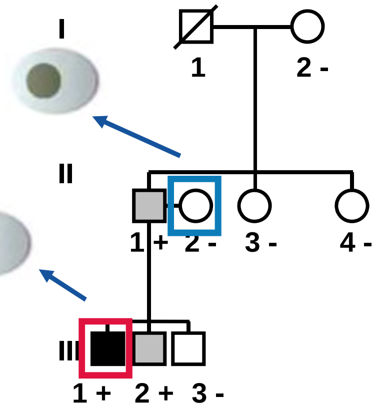
▾ Feb-05-99 – clobutinol



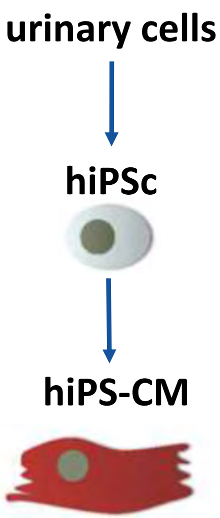
hIPSc Ctrl

hIPSc A561P

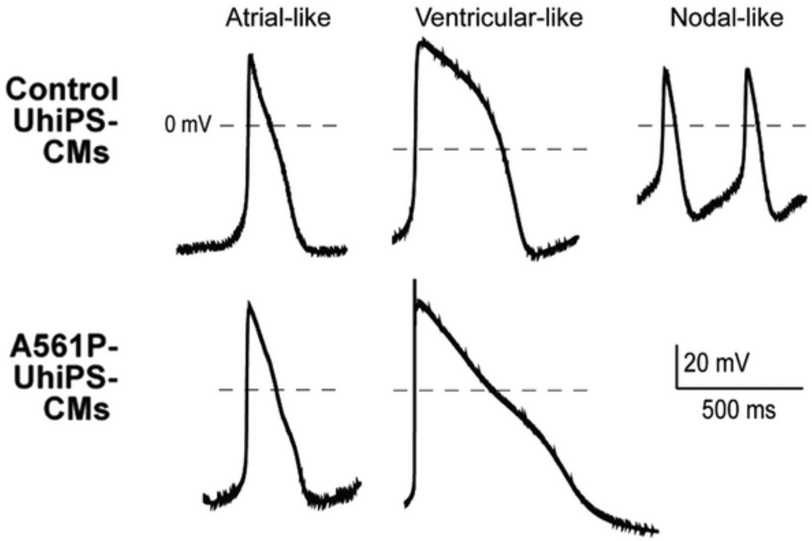
+ : hERG A561P
- : hERG WT



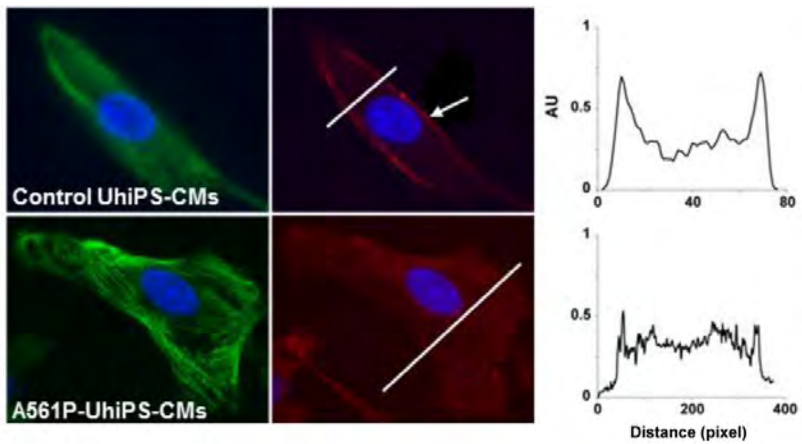
- **re-programmation**



- **current-clamp**



- **immuno-cytofluorimétrie**

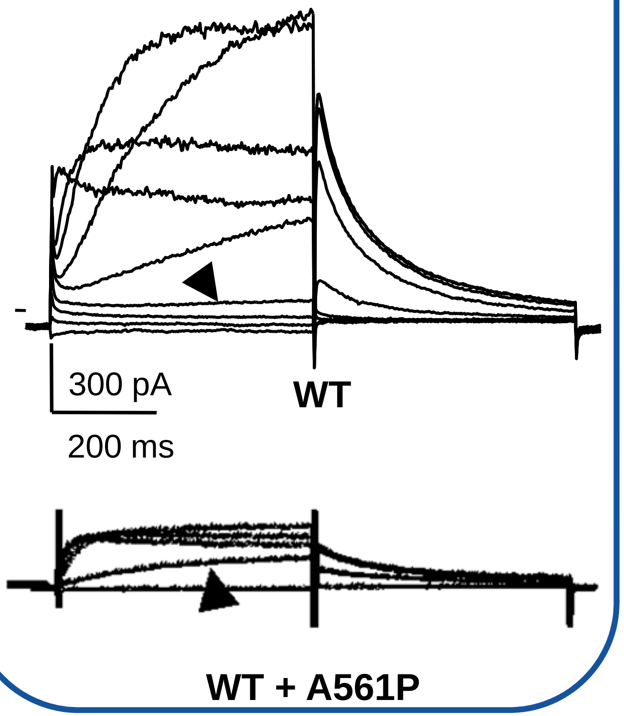


Jouni *et al.* (2015) JAHA 4:e002159

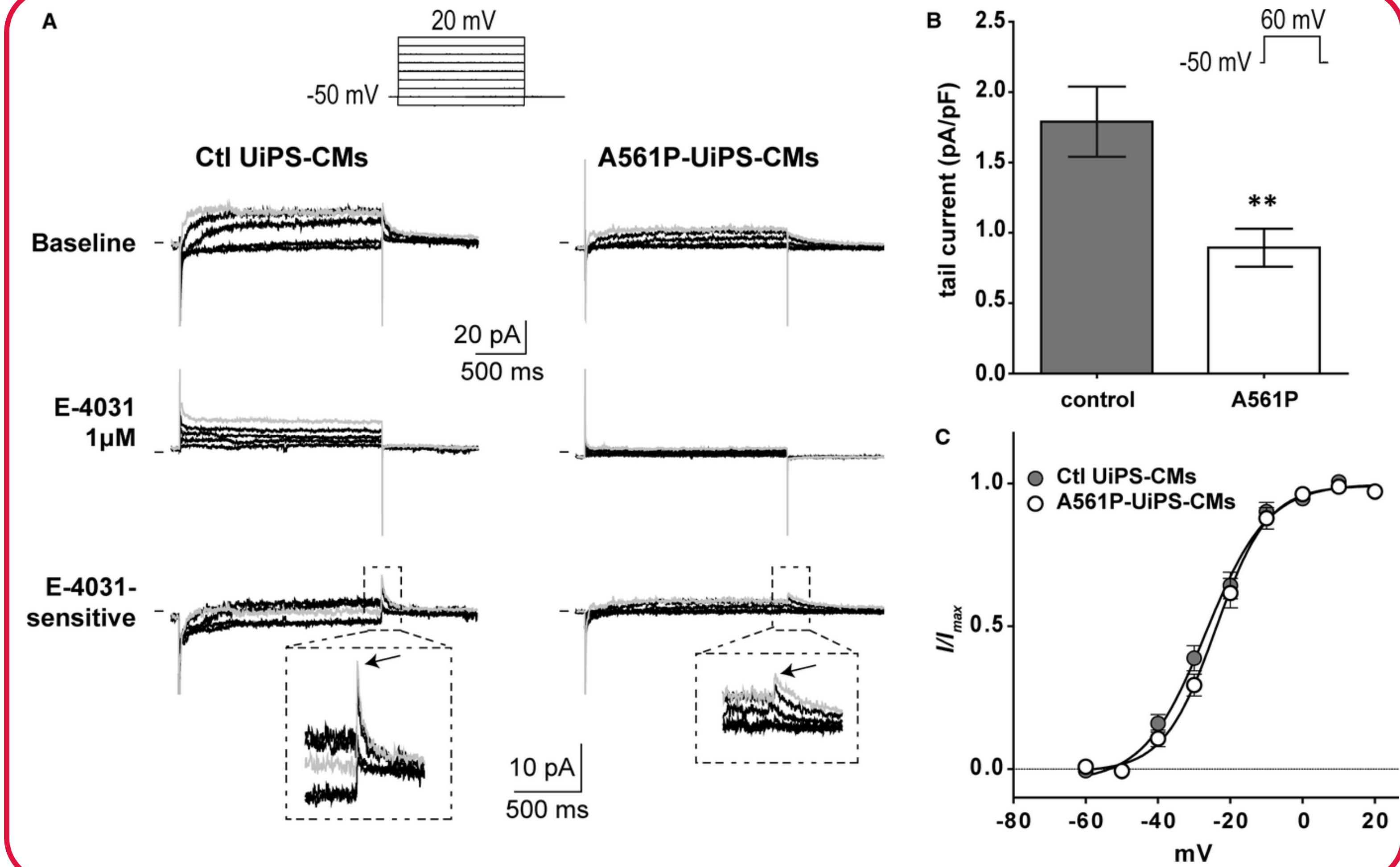
Transfected cells *vs* hiPS-cardiomyocytes

- **voltage-clamp**

transfected COS-7 cells



Bellocq *et al.* 2004. Mol Pharmacol. 66:1093



Jouni *et al.* (2015) JAHA 4:e002159

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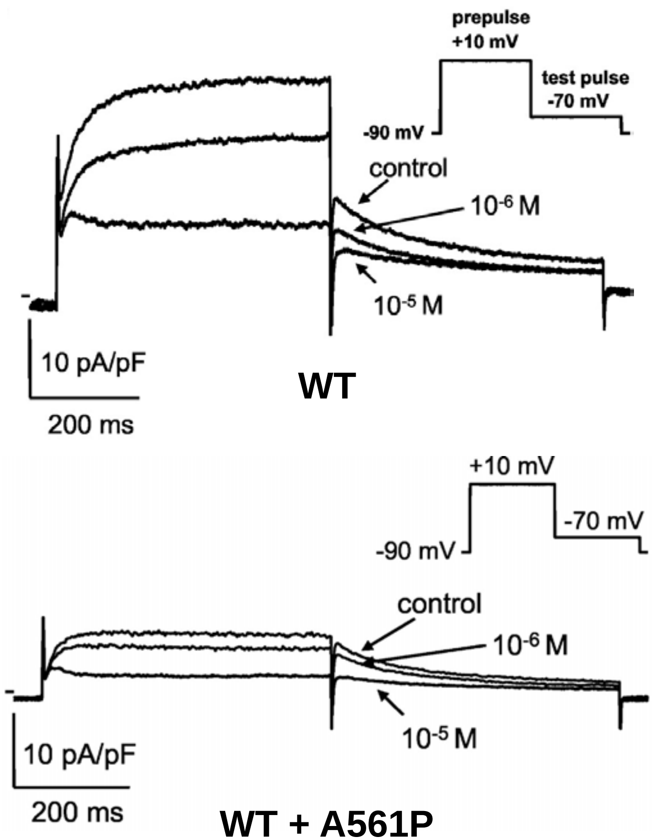
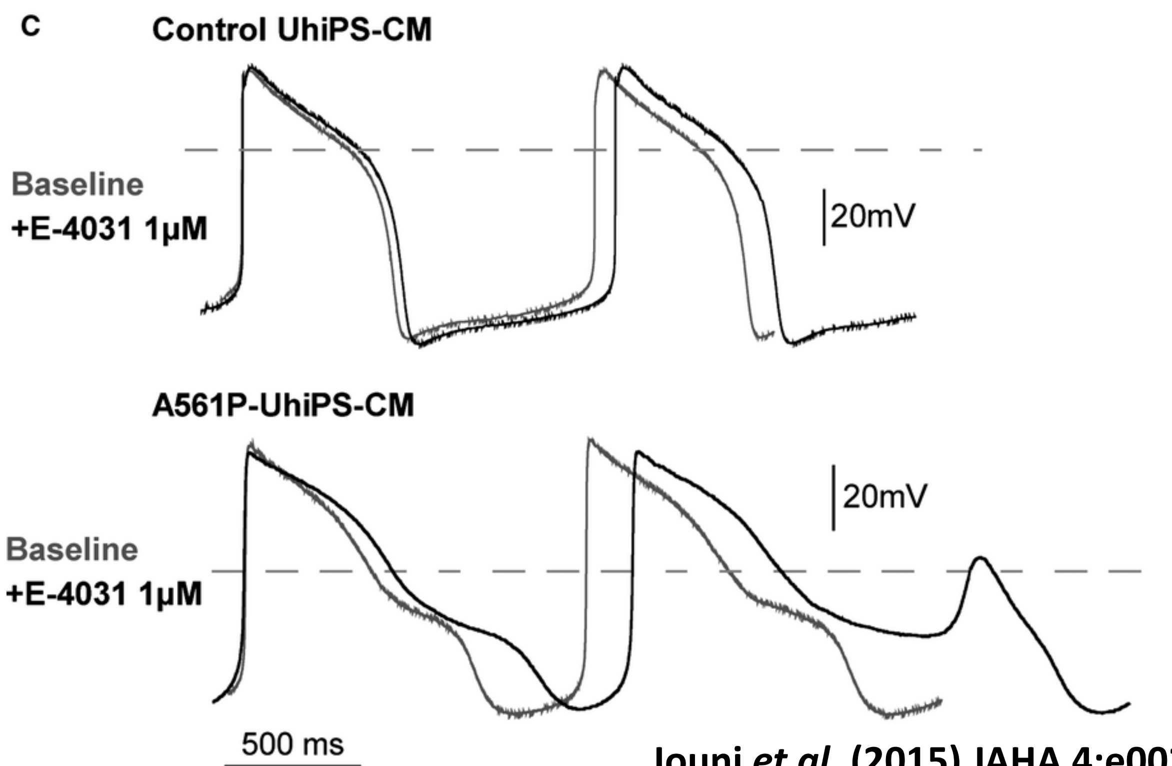
Transfected cells *vs* hiPS-cardiomyocytes

+ clobutinol (I_{Kr} inhib.)



Bellocq *et al.* 2004. Mol Pharmacol. 66:1093

+ E4031 (I_{Kr} inhib.)



Jouni *et al.* (2015) JAHA 4:e002159

- ✓ **Défaut de transport**
- ✓ **Réduction d'I_{Kr}**

- ➢ **Prolongation durée du PA**
- ➢ **Evènements arythmiques**

Perte de function et allongement confirmés ➔ causal

Analyse d'article

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Journal of Molecular and Cellular Cardiology 99 (2016) 1–13

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Original Article

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

CrossMark

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

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[b] l'institut du thorax, Inserm, CNRS, Université de Nantes, CHU Nantes, Nantes, France
[c] ER045, PRASE, Laboratory of stem cells, Lebanese university, Beirut, Lebanon
[d] 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 les anti-viraux)**

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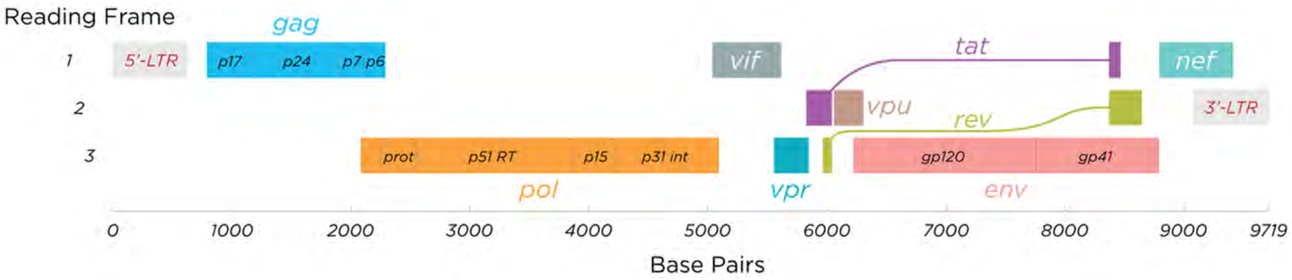
Tat = Trans-Activator of Transcription



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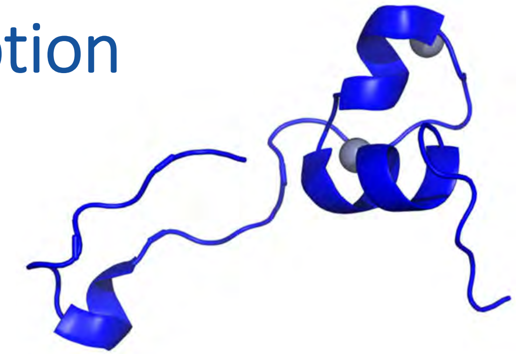

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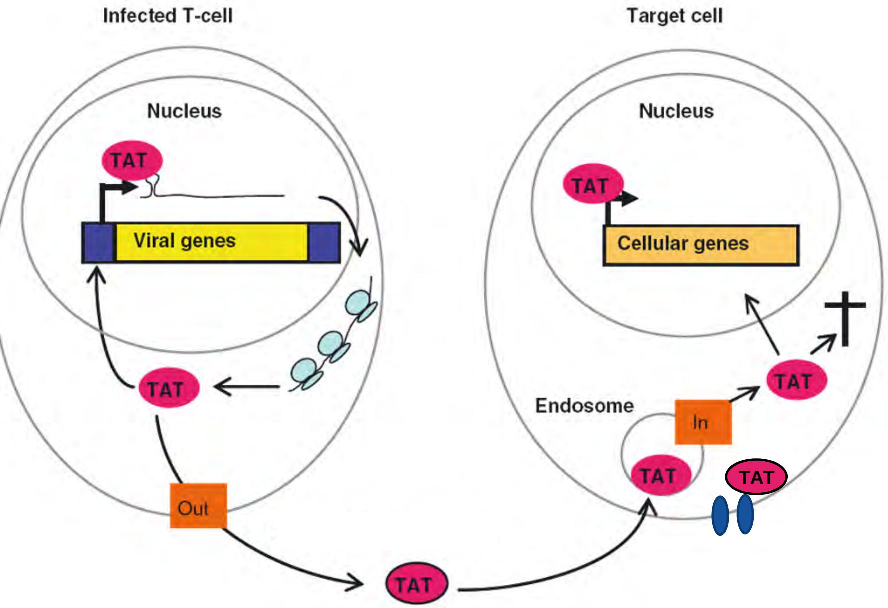

d'après Debaisieux et al., (2012)Traffic 13:355

- **Protéine Tat du HIV-1 : présente dans le sérum des patients, peut pénétrer les cellules et interagir avec le PIP$_2$ (phosphoinositide).**
- **KCNQ1 et hERG, canaux repolarisants, nécessitent du PIP$_2$ pour être fonctionnels**

➔ **Les canaux KCNQ1 ou hERG sont-ils impliqués dans les effets cardiaques de l'infection HIV par l'intermédiaire de Tat?**

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

cellules COS-7

- **Courant I_{hERG}**

Figure 1

W11Y-Tat : pas d'interaction avec le PIP_2

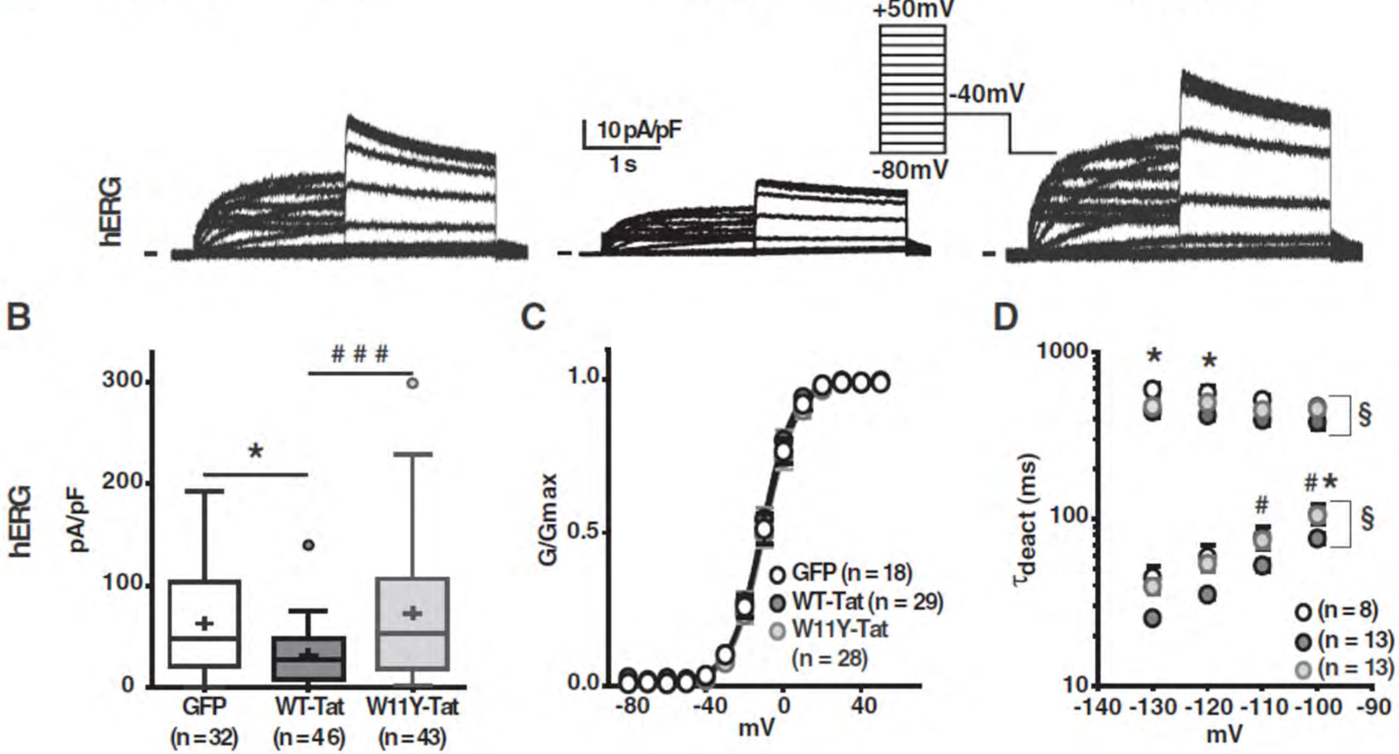
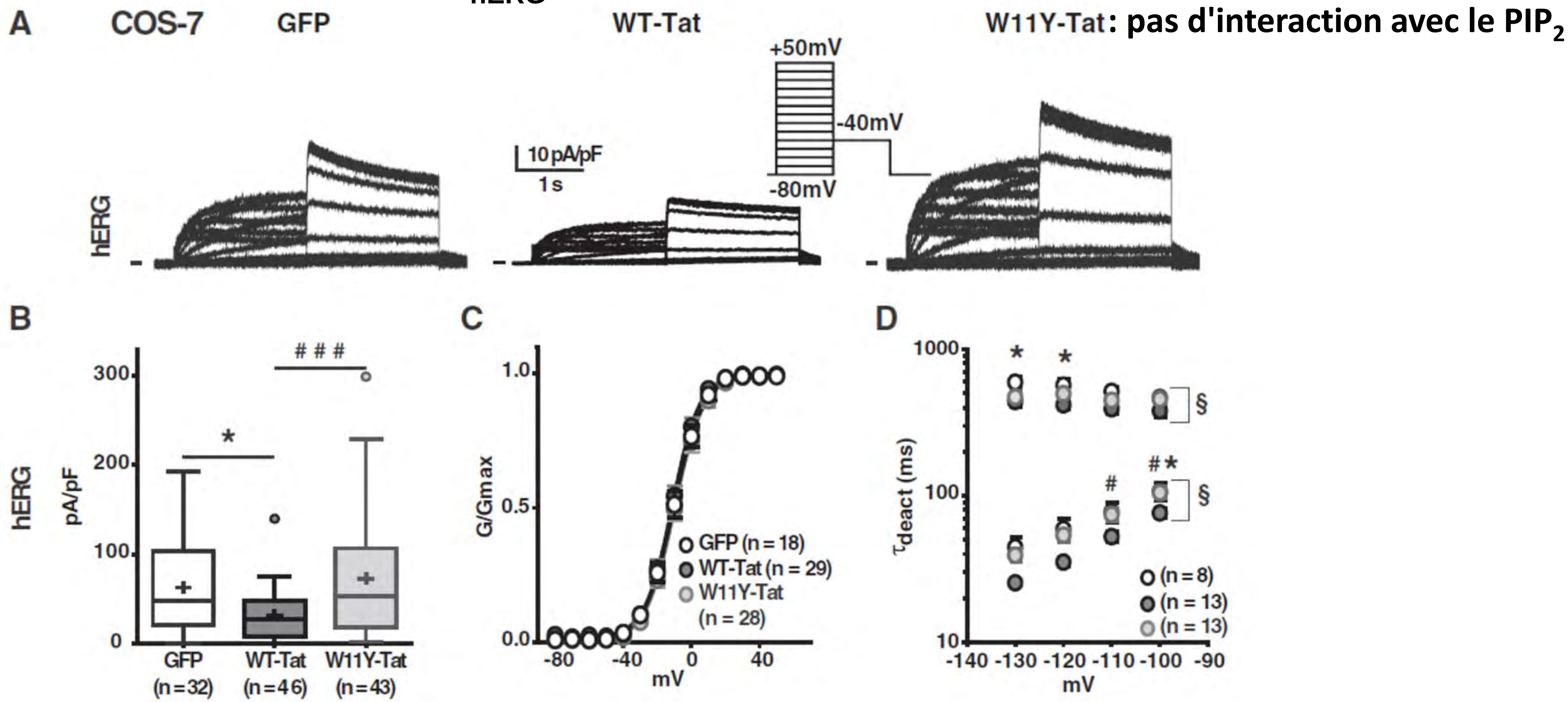


Figure 1

cellules COS-7

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

- Courant I_{hERG}



- **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**

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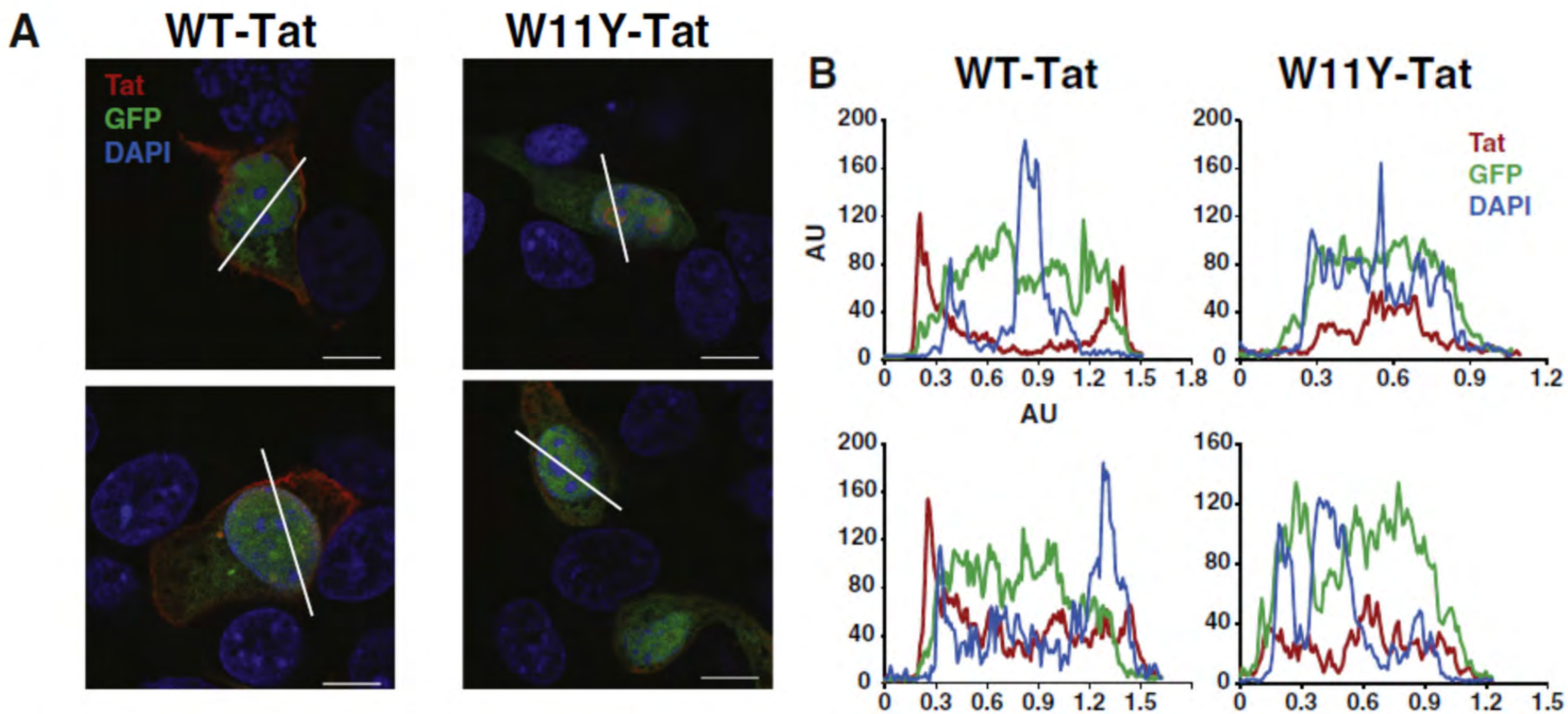
cellules COS-7

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



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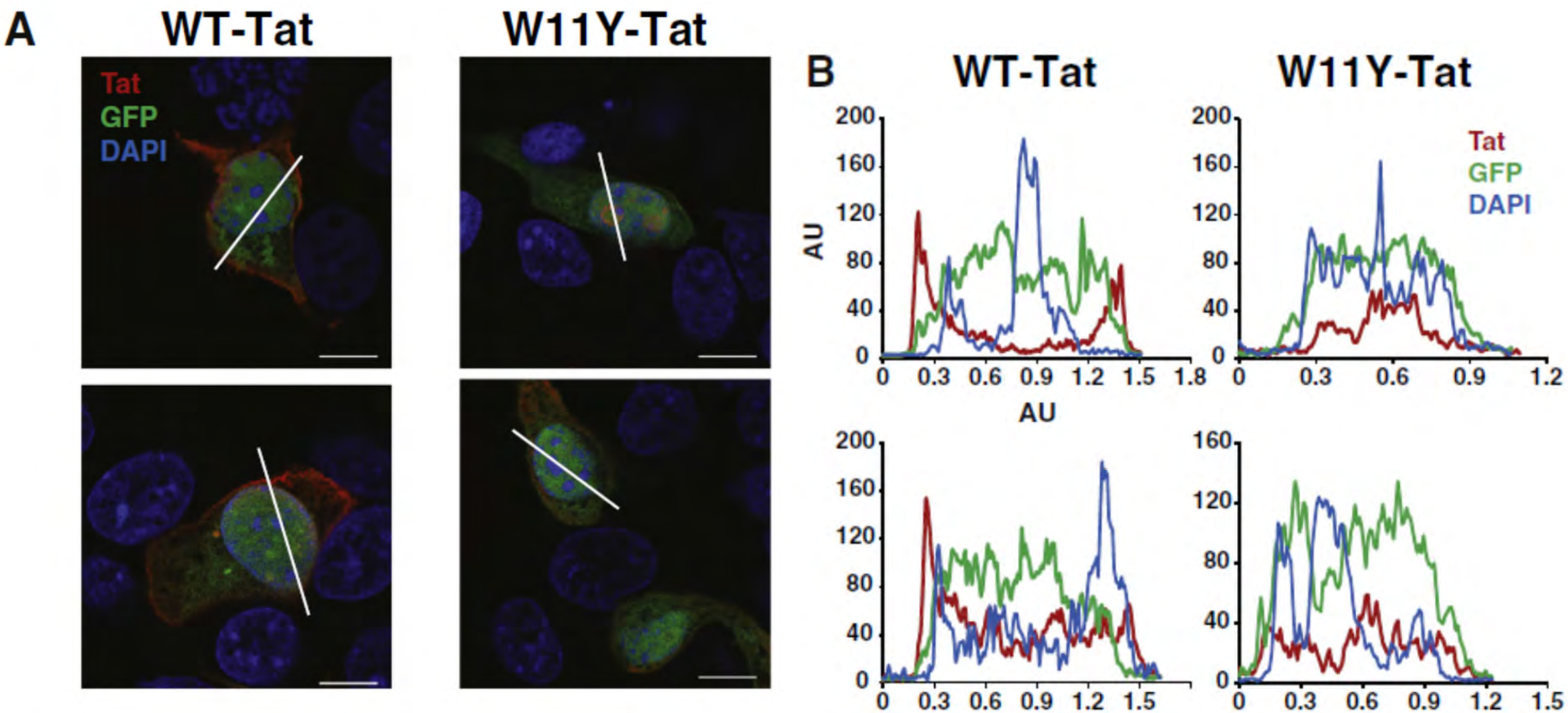
cellules
COS-7

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



- **WT-Tat : membranaire, comme le canal**
- **W11Y-Tat : non membranaire, car elle n'interagit pas avec le PIP_2**

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cellules COS-7

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

- **Courant $I_{KCNE1\text{-}KCNQ1}$**

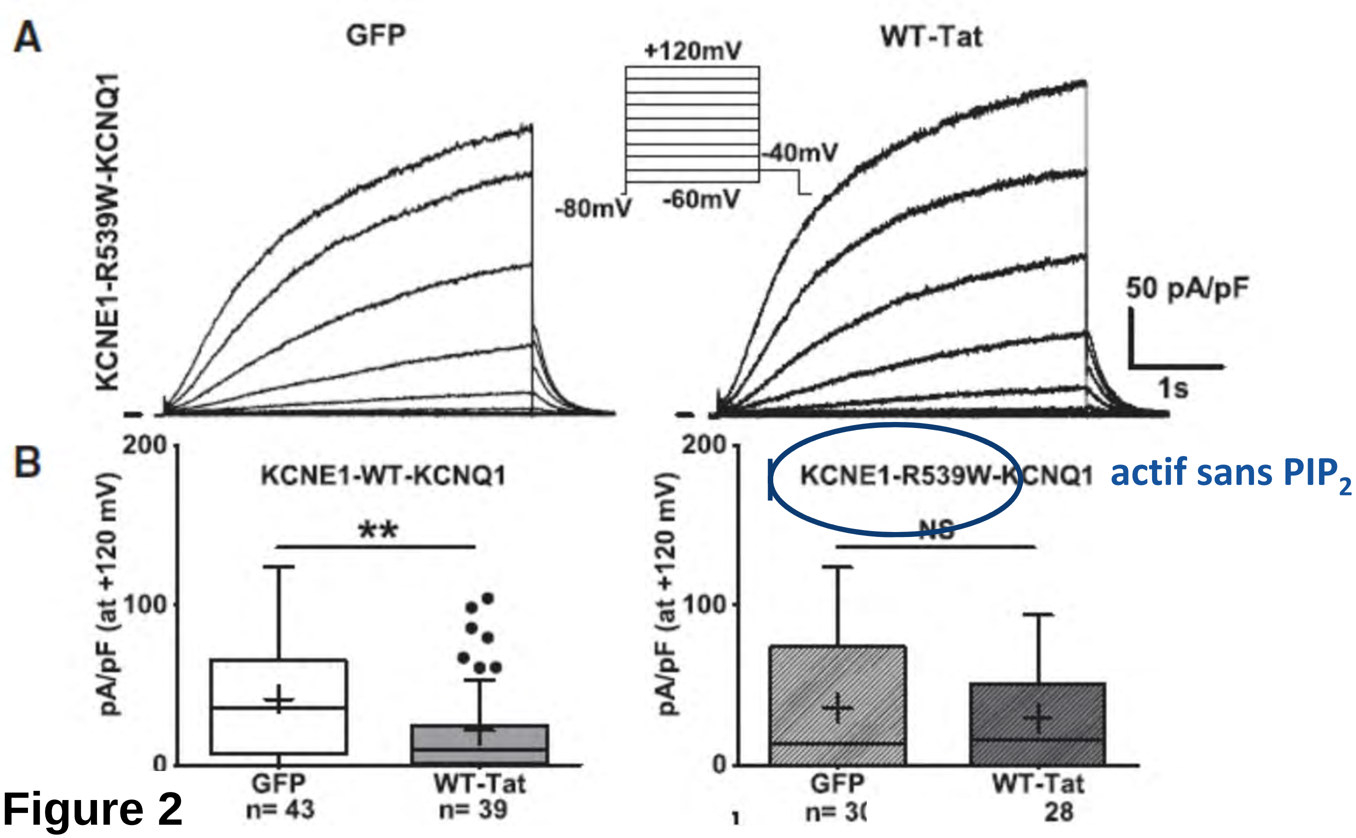

Figure 2

cellules COS-7

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

- **Courant $I_{KCNE1\text{-}KCNQ1}$**

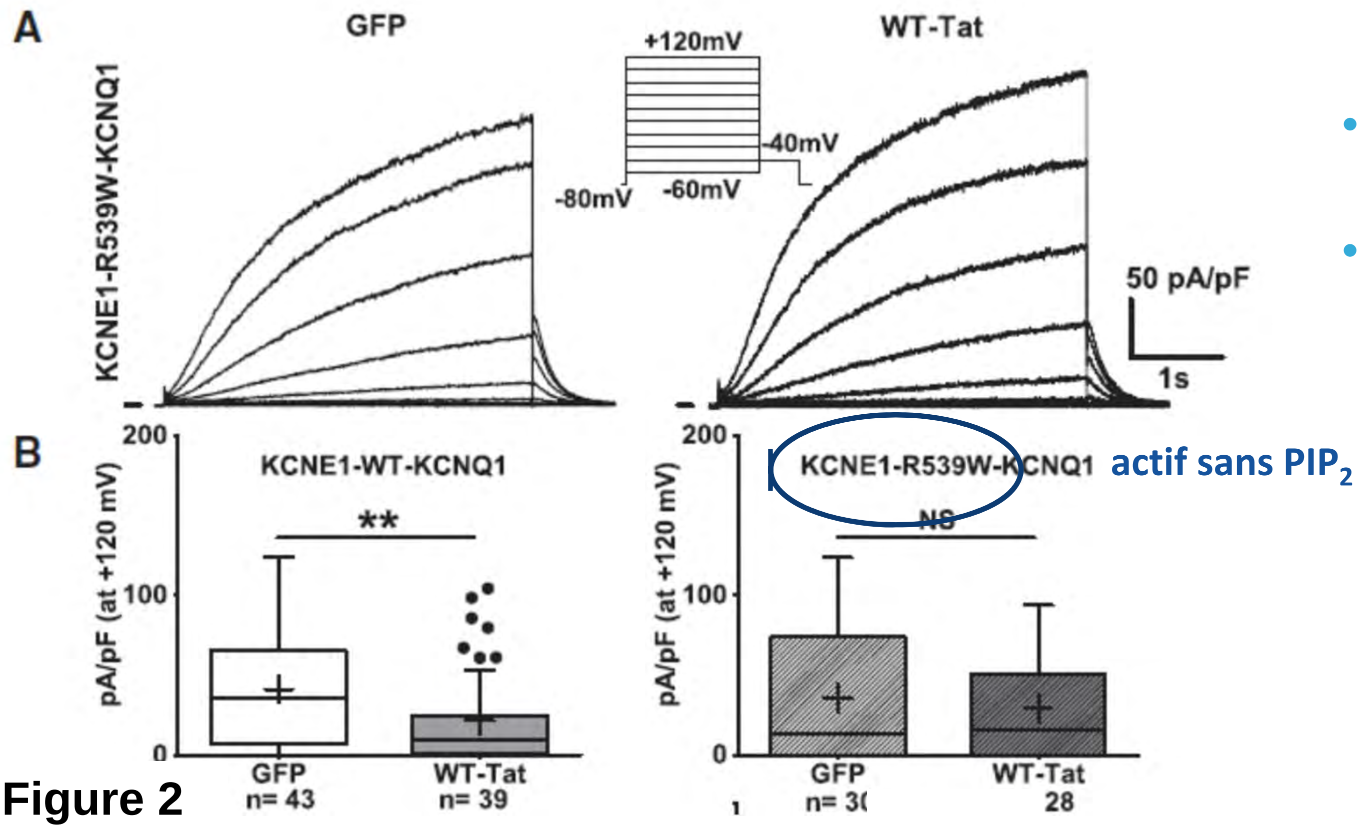

Figure 2

- **3B: canal WT sensible au PIP_2 : sensible à la Tat**
- **3A & B canal insensible au PIP_2 : insensible à la Tat**

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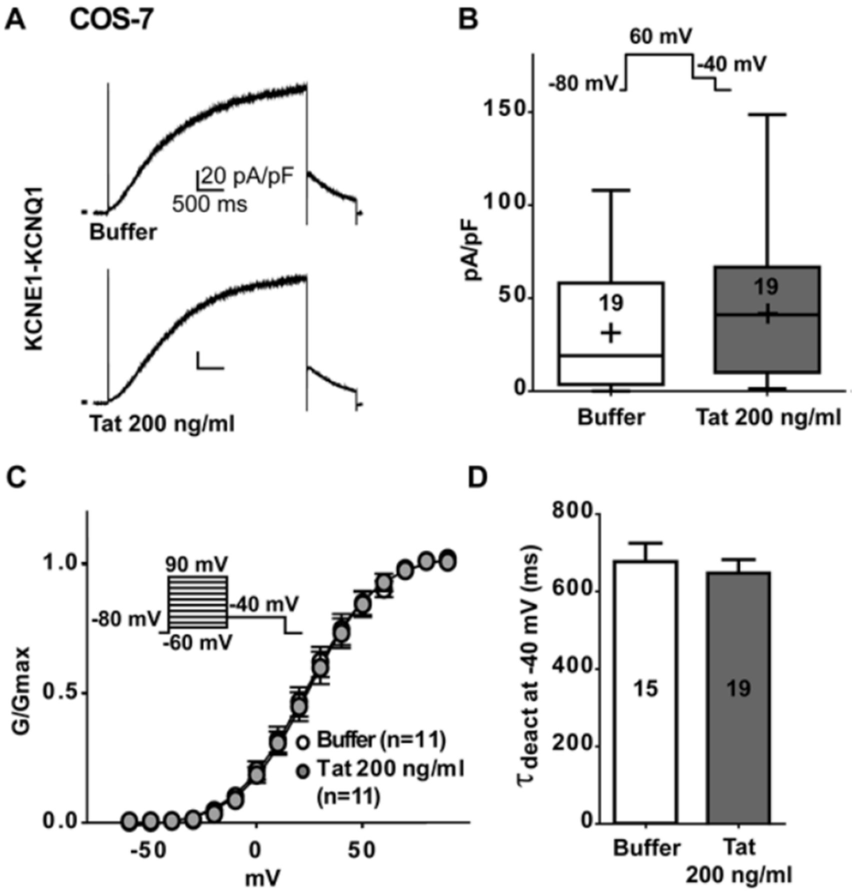
cellules COS-7

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

- **Courant $I_{KCNE1\text{-}KCNQ1}$**

Suppl. Figure 4



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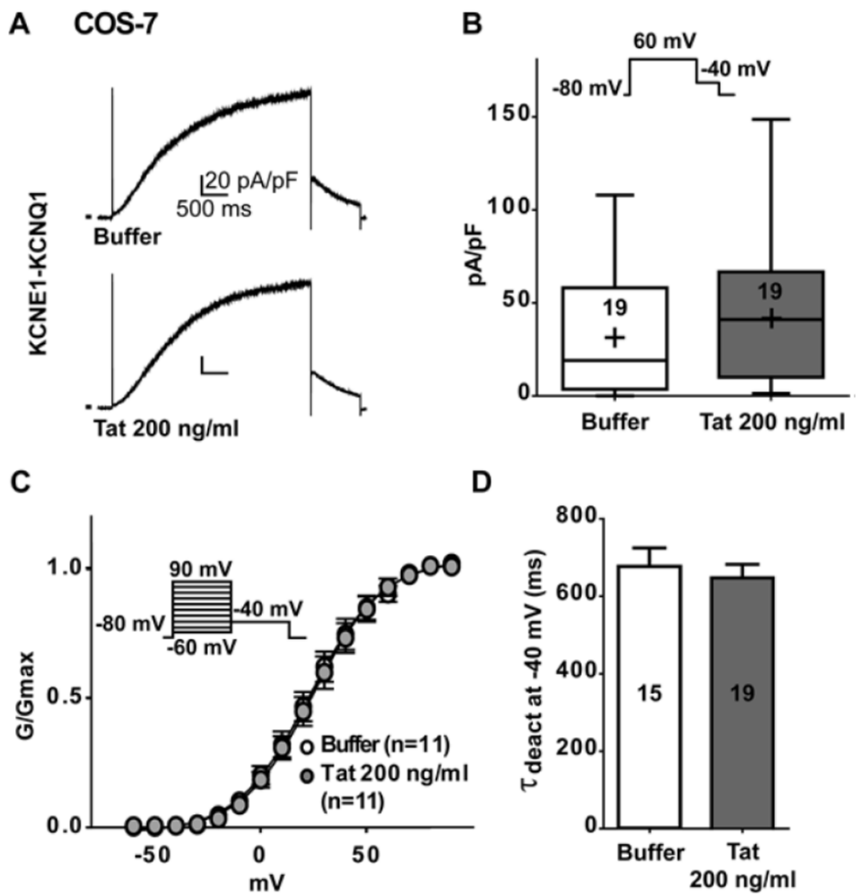
cellules
COS-7

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

- **Courant $I_{KCNE1\text{-}KCNQ1}$**

Suppl. Figure 4



- **application de la protéine Tat : pas d'effet**

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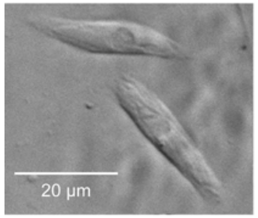
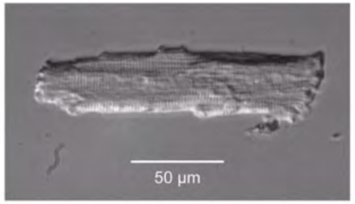


cardiomyocytes
issus de cellules hiPS



cardiomyocytes humains
fraichement isolés

Condition : application extracellulaire de la protéine Tat

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

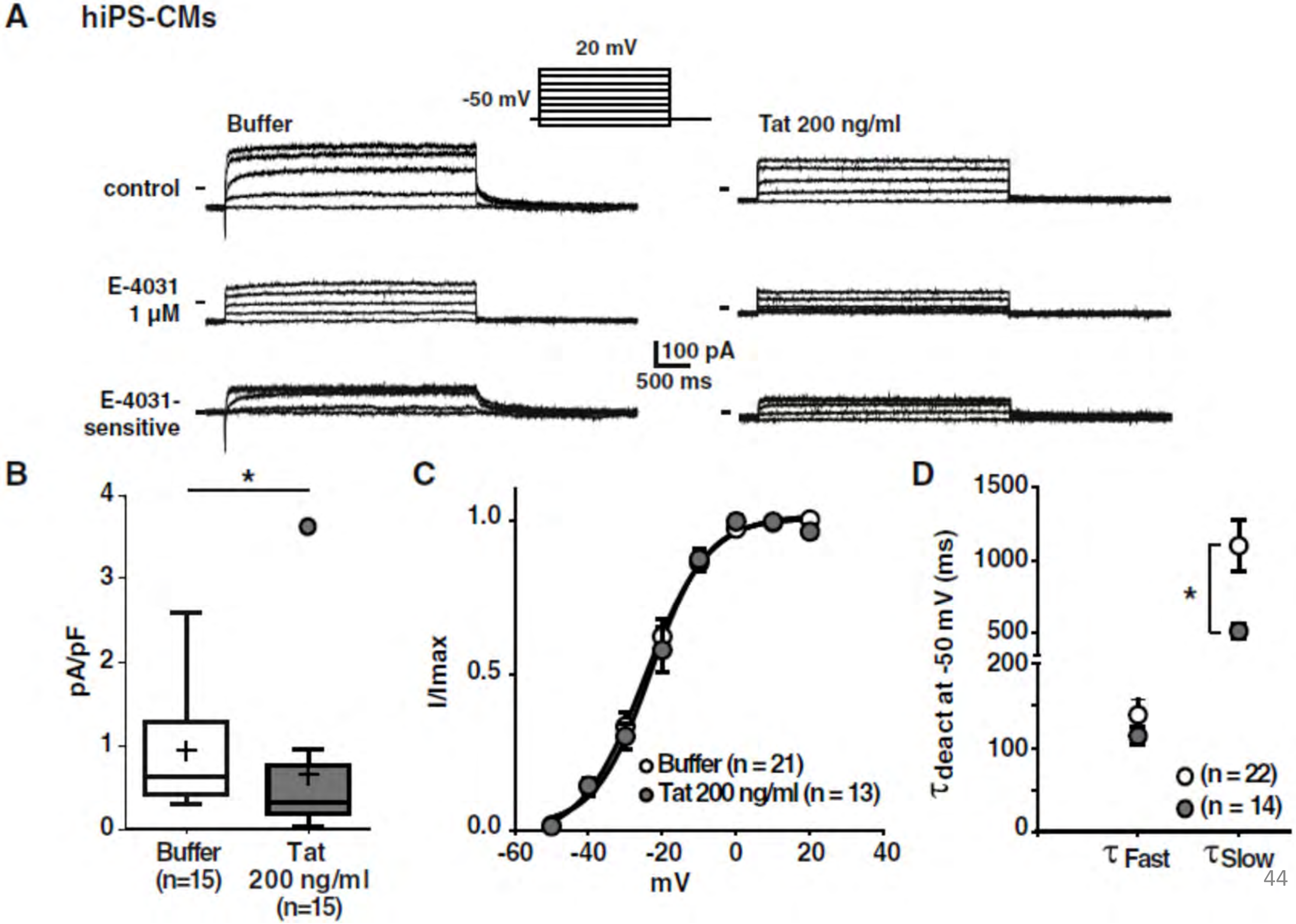


Figure 4

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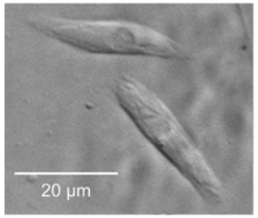
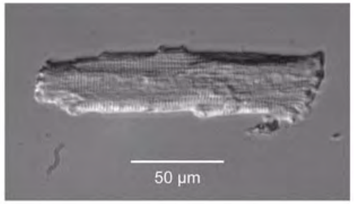


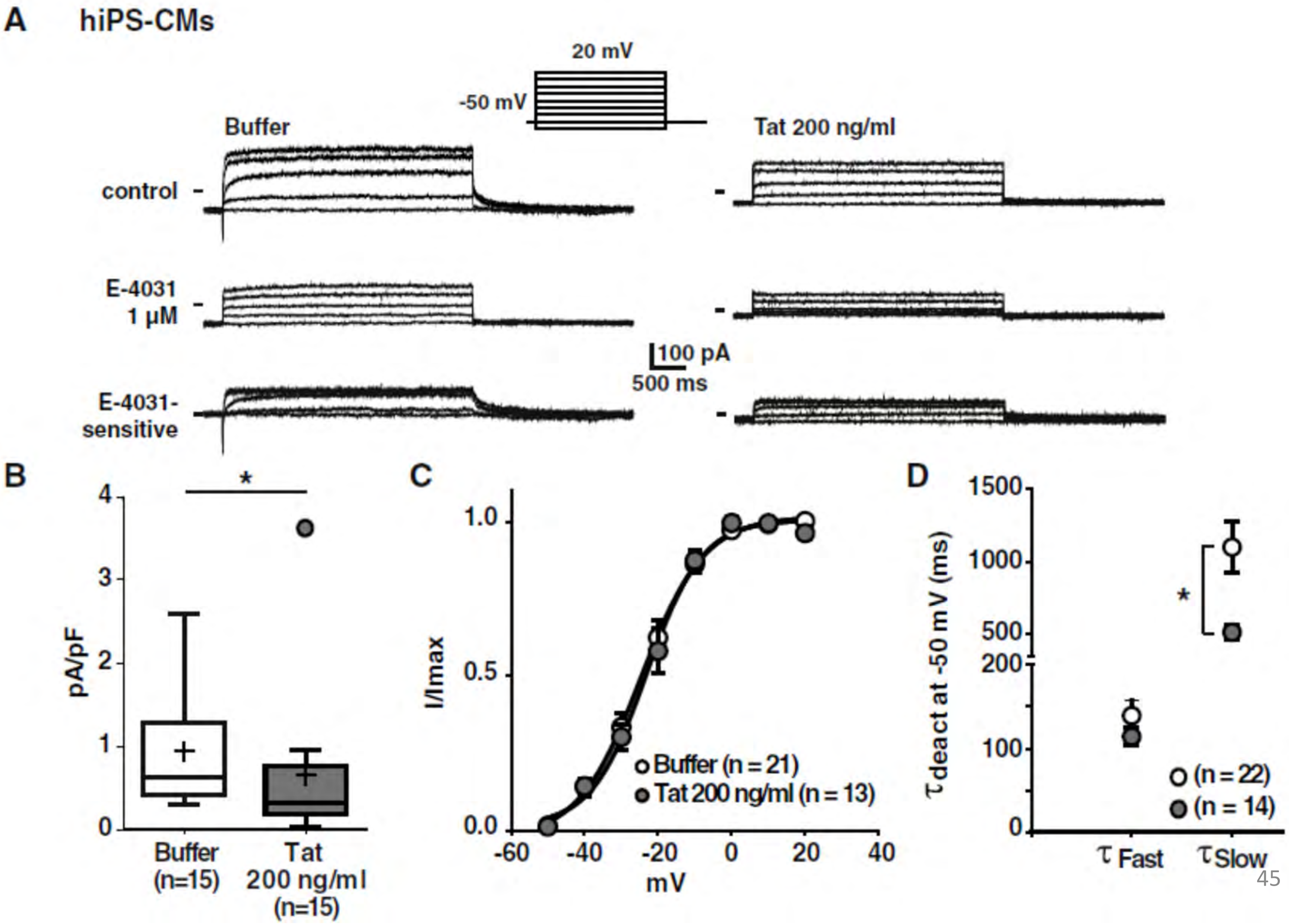
cardiomyocytes issus de cellules hiPS



cardiomyocytes humains fraichement isolés

Condition : application extracellulaire de la protéine Tat

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



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

Figure 4

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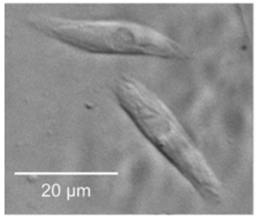


cardiomyocytes issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- **expression de hERG ARNm et protéine**

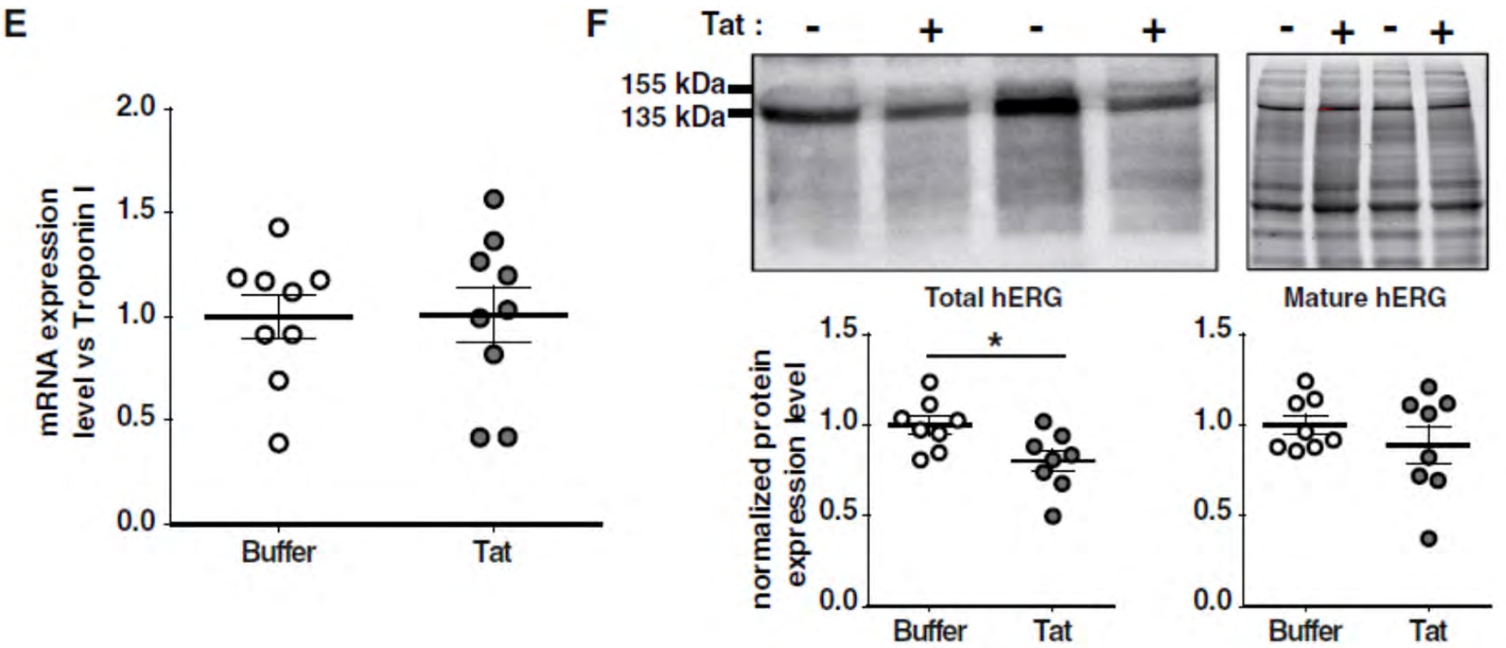
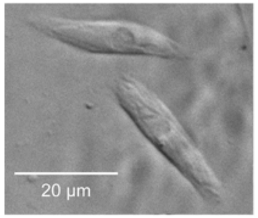


Figure 4



cardiomyocytes
issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- **expression de hERG ARNm et protéine**

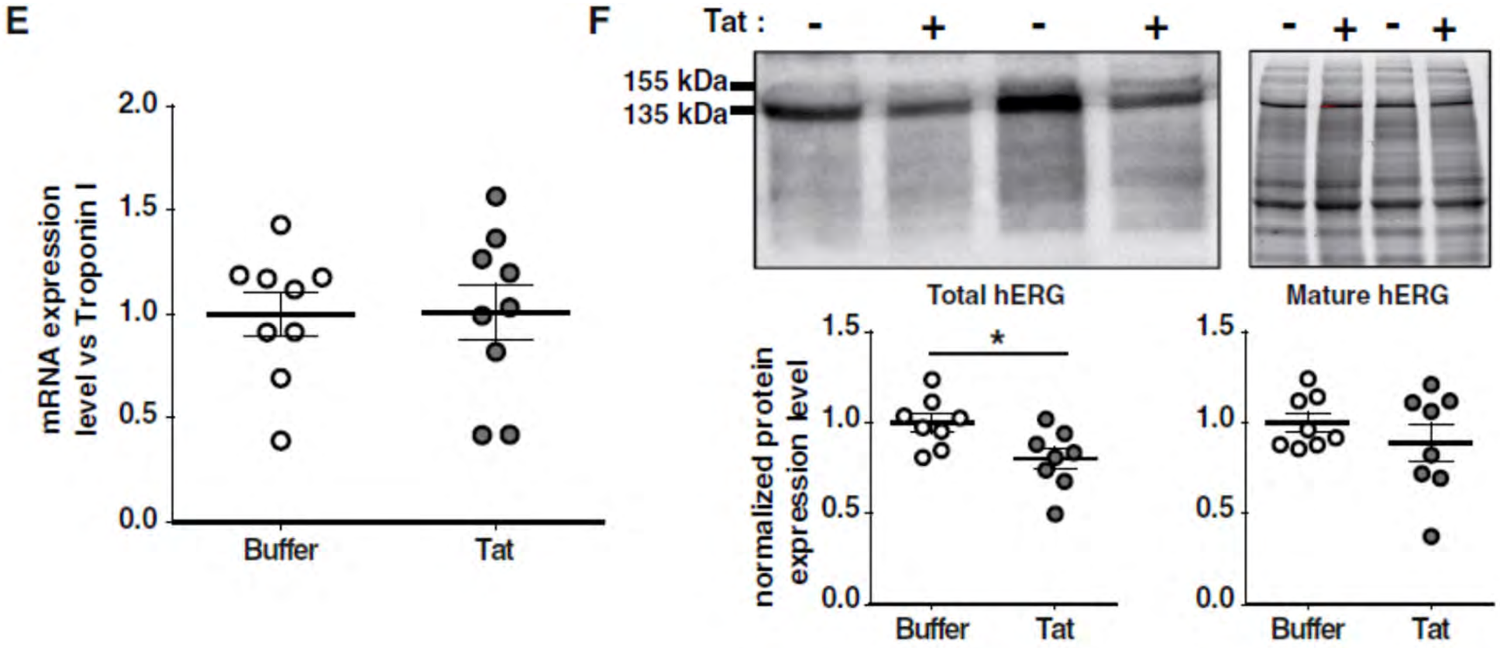


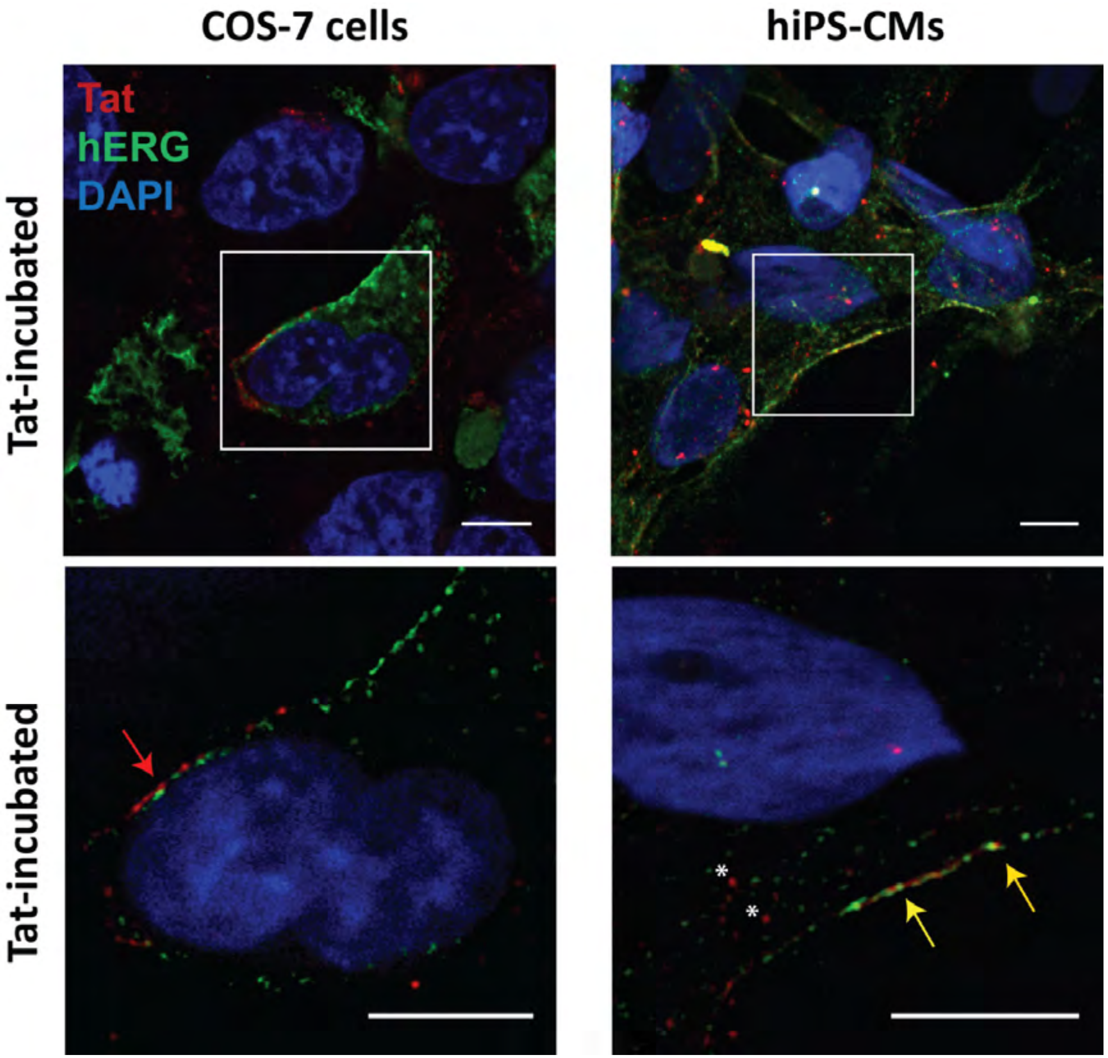
Figure 4

- **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

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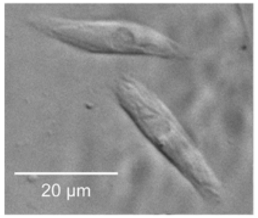

cardiomyocytes
issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- **potentiel d'action**

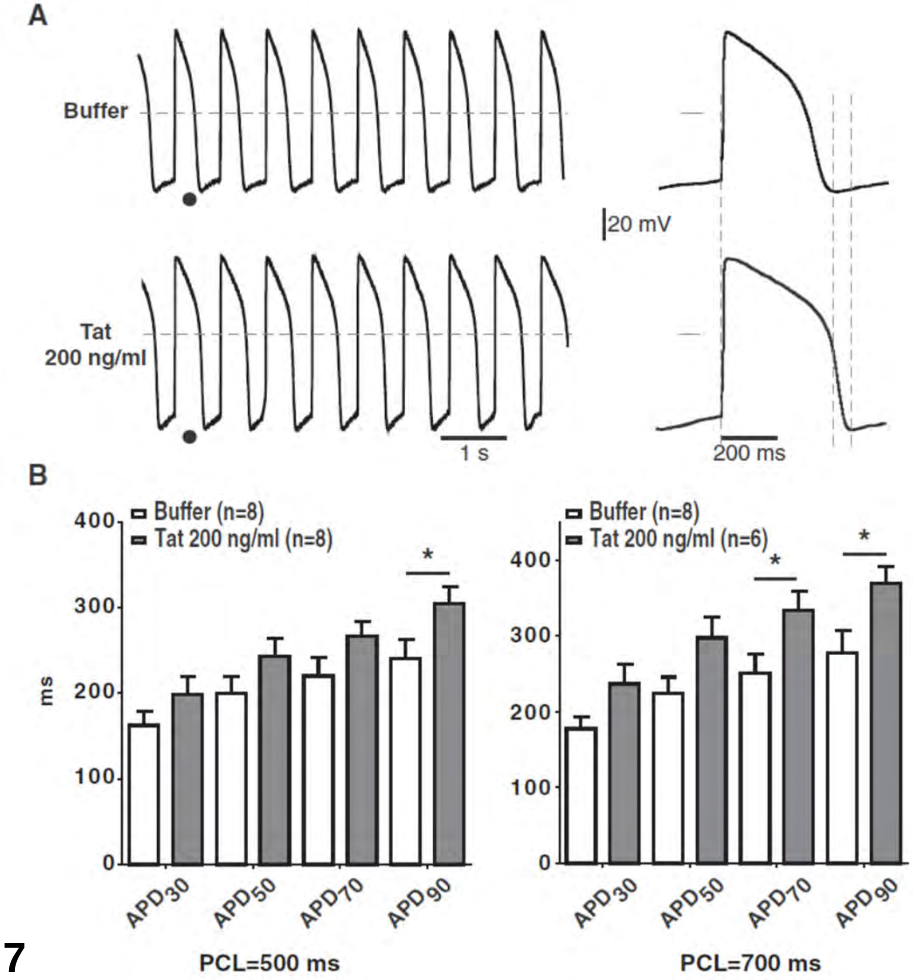

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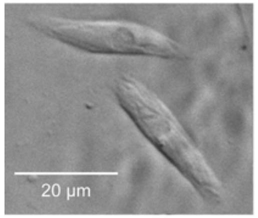
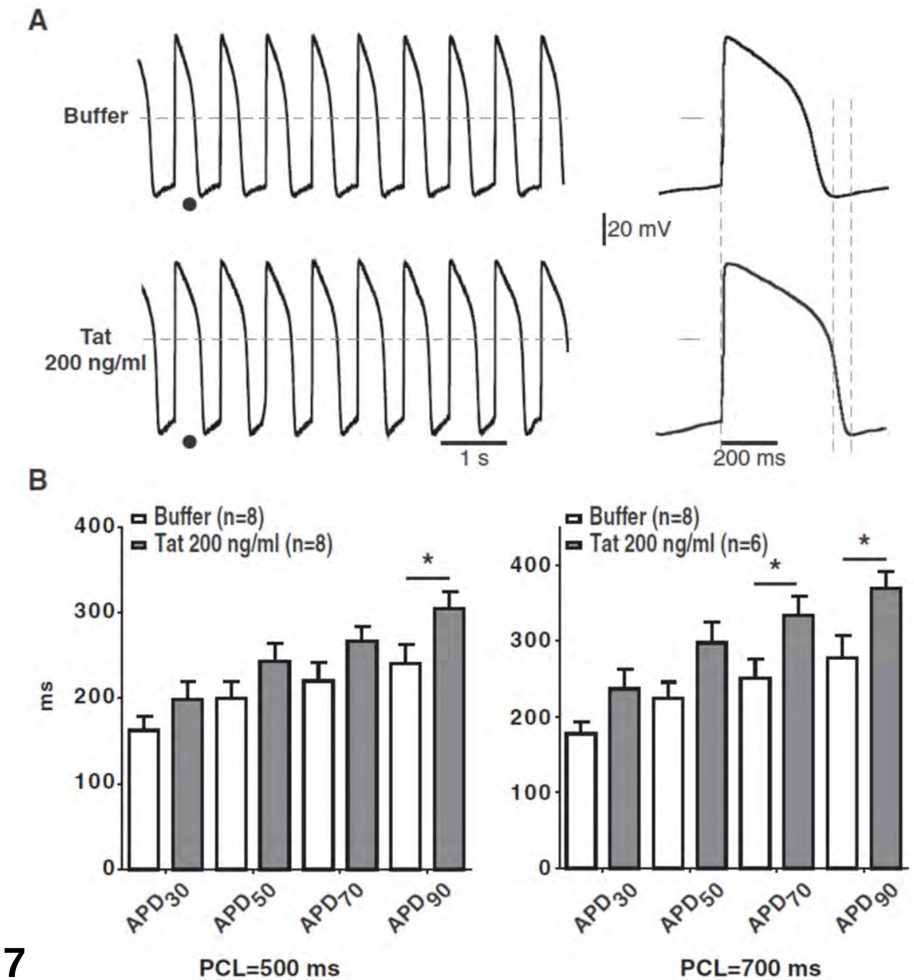
Figure 7



cardiomyocytes
issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- **potentiel d'action**



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

Figure 7

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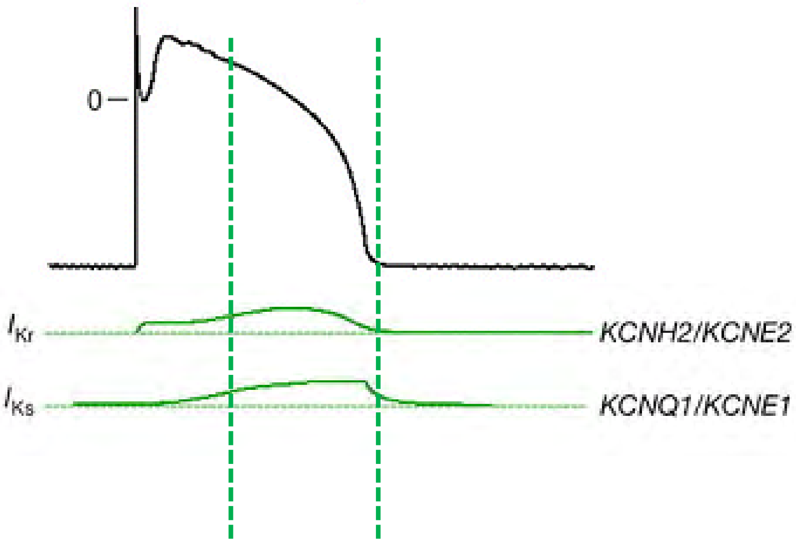
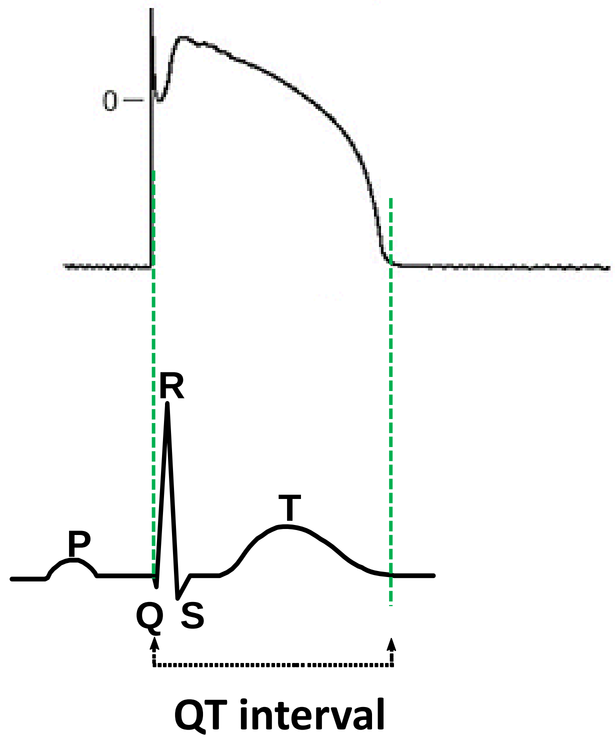
Conclusions

"Altogether, these data obtained on human K^+ channels both in heterologous expression systems and in human cardiomyocytes suggest that Tat sequesters PIP_2, leading to a reduction of I_{Kr} and I_{Ks}, and provide a molecular mechanism for QT prolongation in HIV-infected patients."





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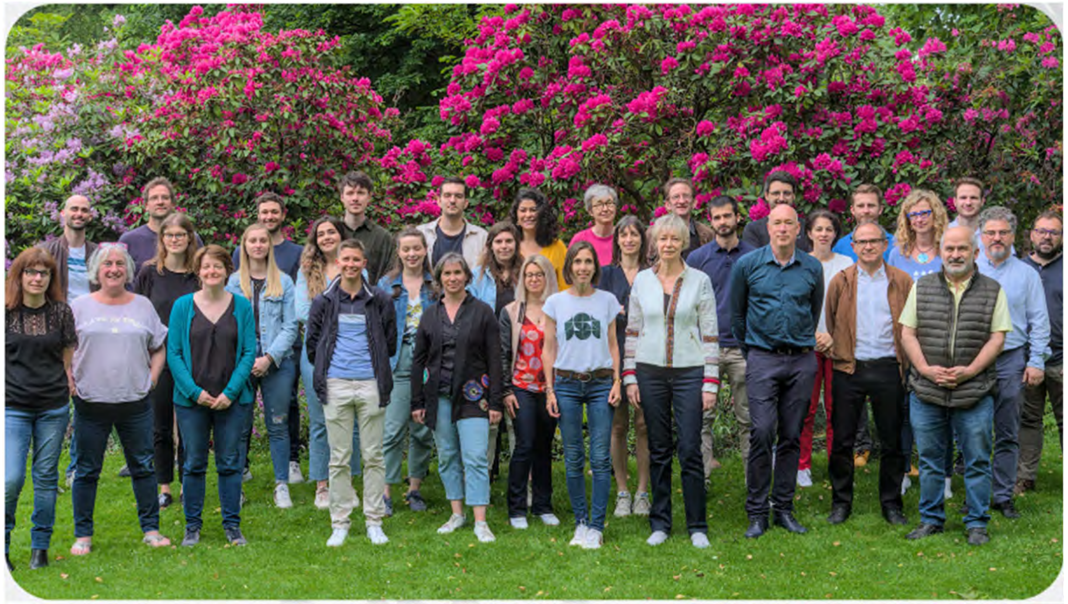

Fundings

umr1087.univ-nantes.fr

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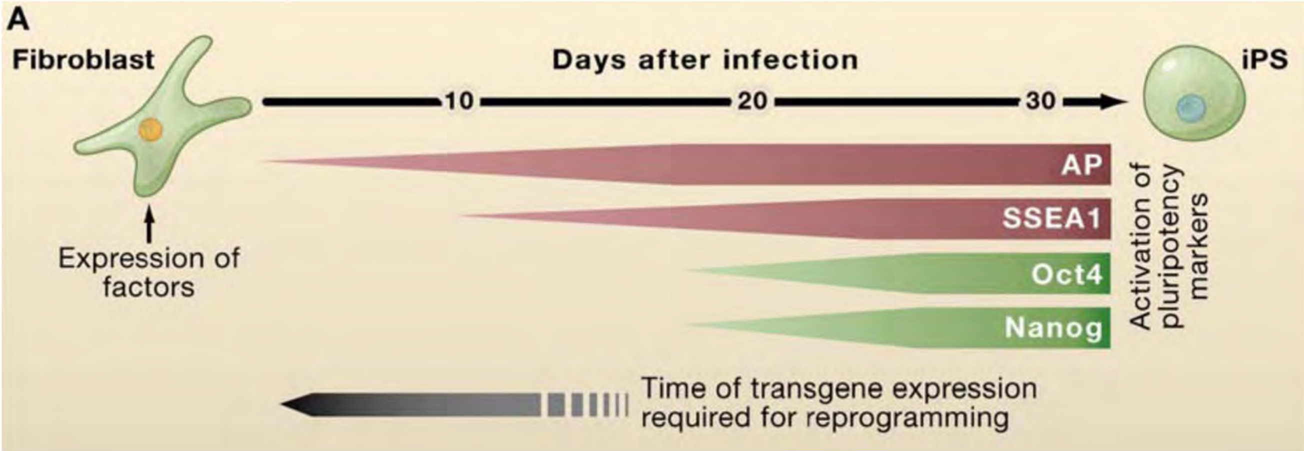
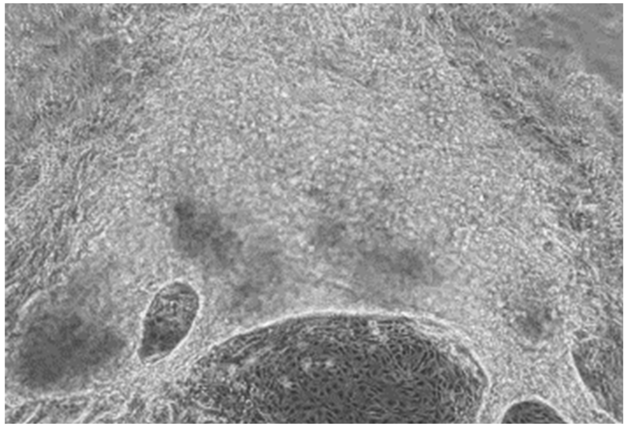
L'unité de recherche de l'institut du thorax
Inserm UMR 1087 / CNRS UMR 6291
Nantes, France

De l’urine au cardiomyocyte



Reprogrammation

Différentiation



Différenciation cardiomyocytaire: caractérisation

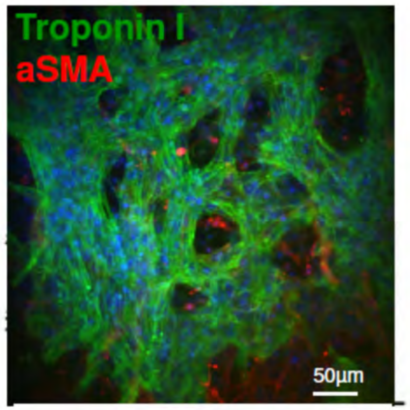
D0 | **D2** | **D5** | **> D18**

cell type: **hiPSc** | **mesoderm** | **cardiac precursor** | **hiPS-CM**

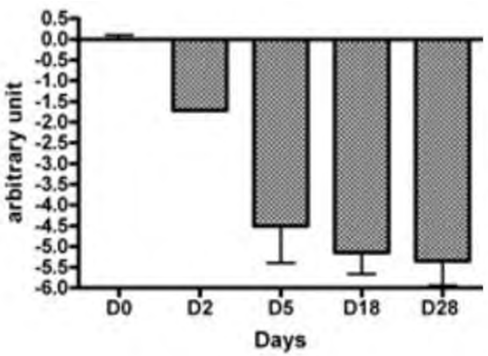
matrigel overlay

+ Activin A
+ FGF2
+ BMP4

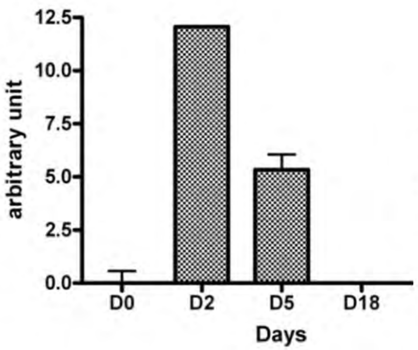
beats



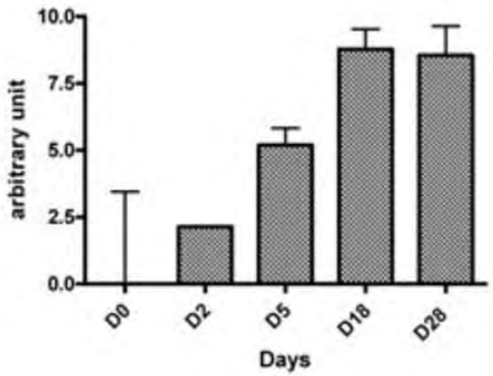
NANOG

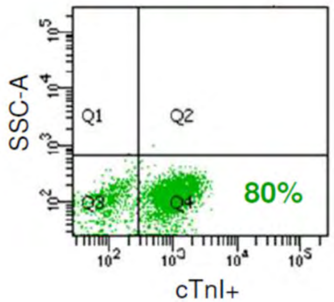


BRACHYURY



NKX2-5





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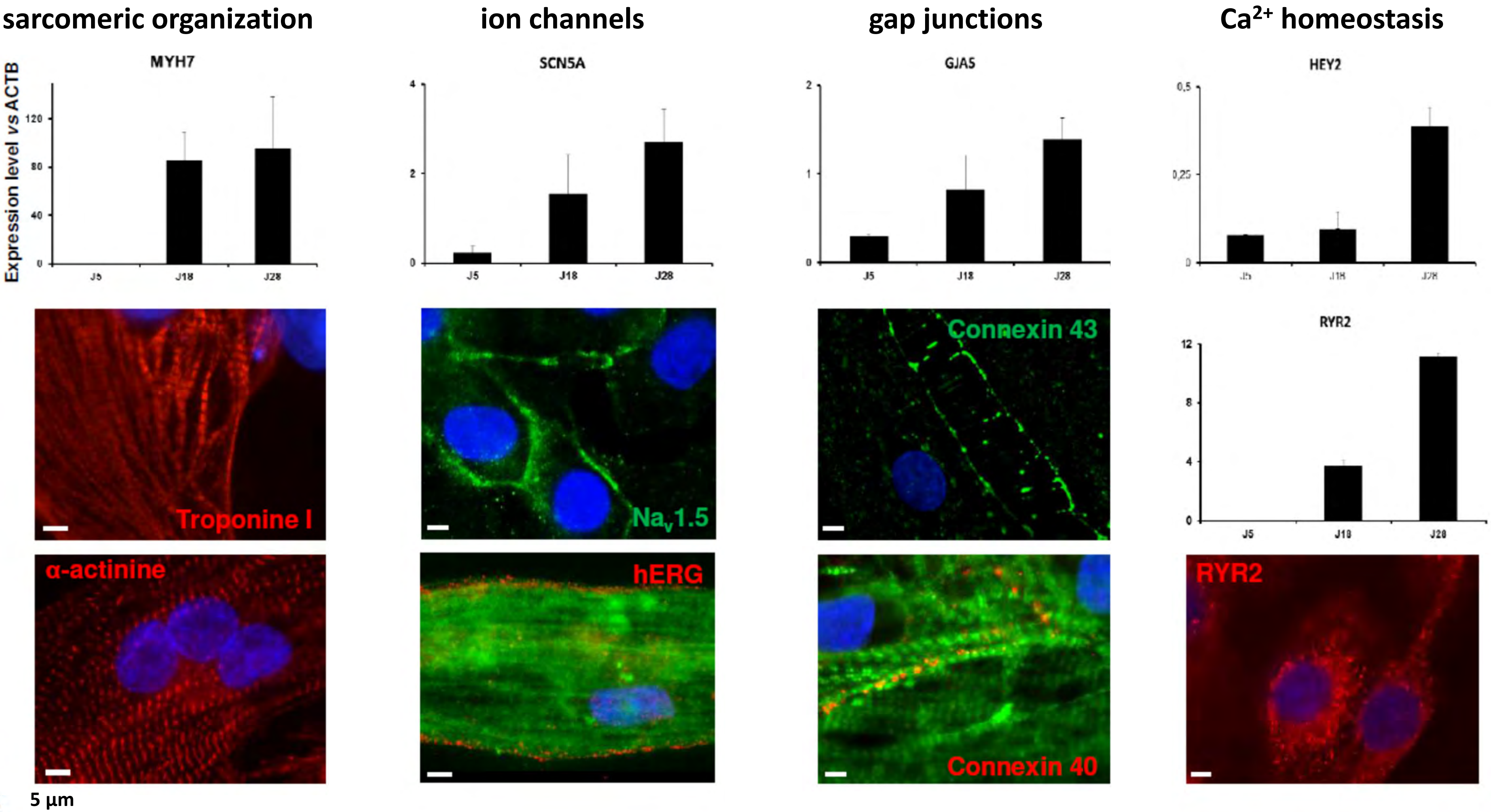
hiPS-cardiomyocytes : caractérisation



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