



Electrophysiologie moléculaire - troubles du rythme et de la conduction cardiaques



Gildas Loussouarn, PhD

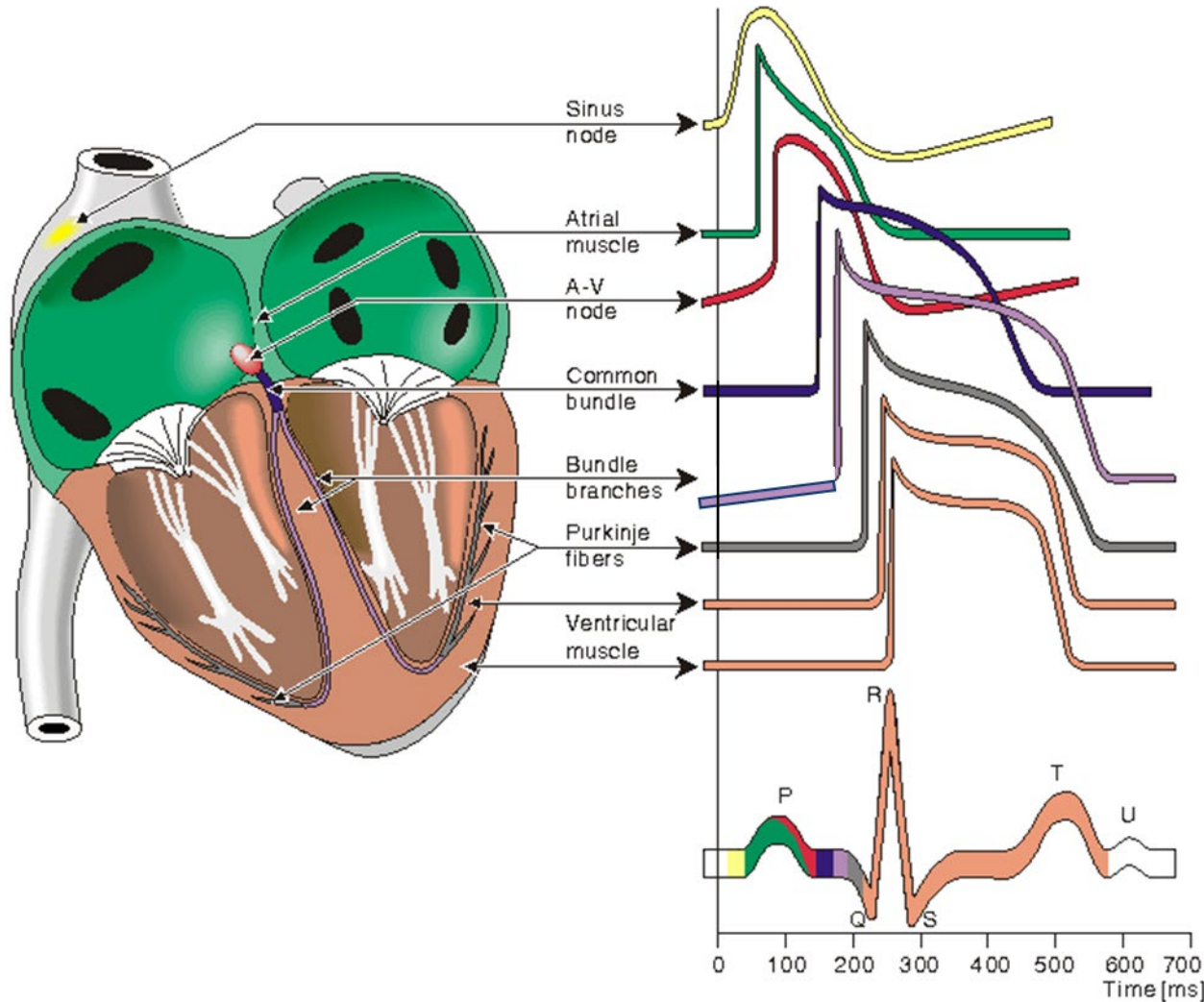
Equipe Canaux ioniques et arythmies
cardiaques

L'unité de recherche de l'institut du thorax

Inserm UMR 1087 / CNRS UMR 6291

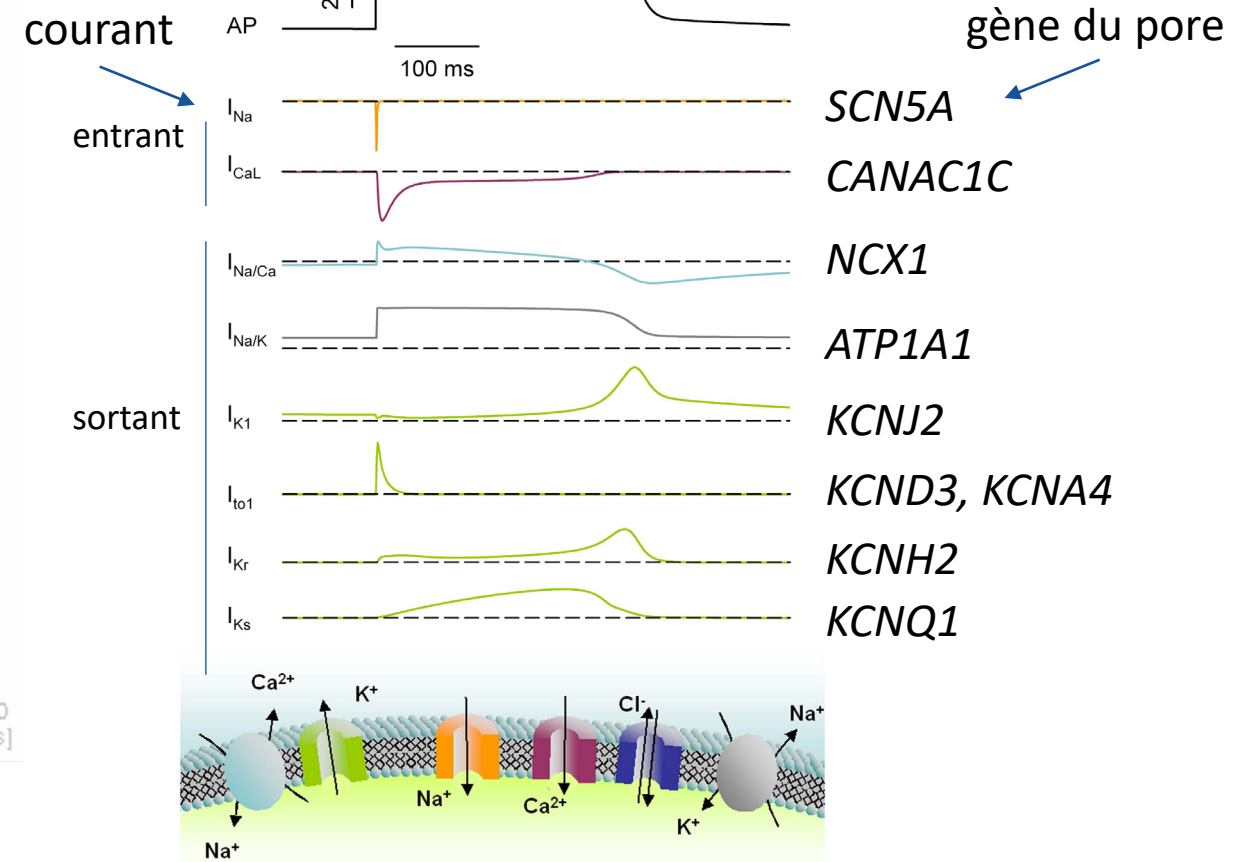
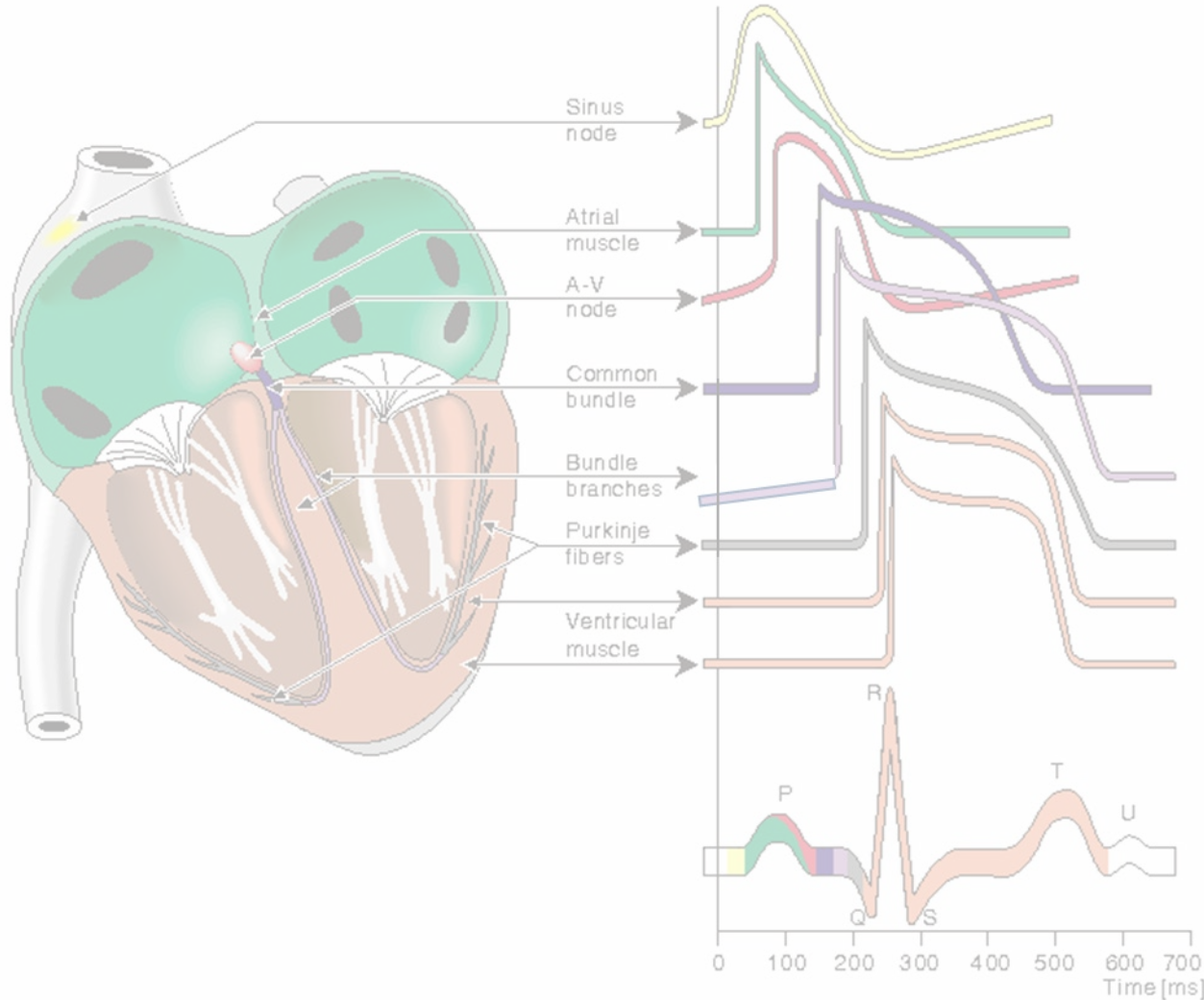
Nantes, France

Activité électrique du cardiomyocyte : le potentiel d'action



hétérogénéité du potentiel d'action (PA)

Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



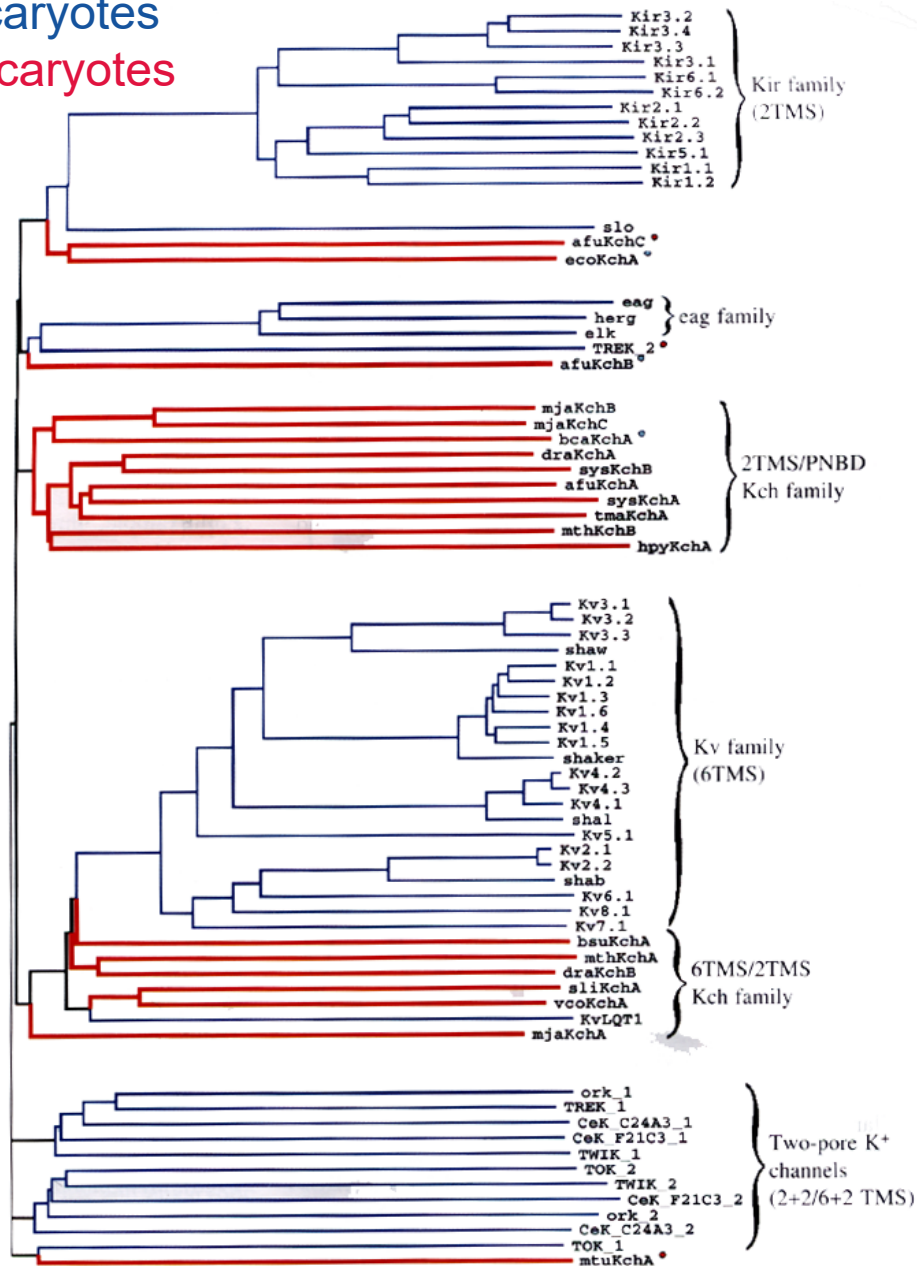
hétérogénéité du potentiel d'action (PA)

dendrogramme

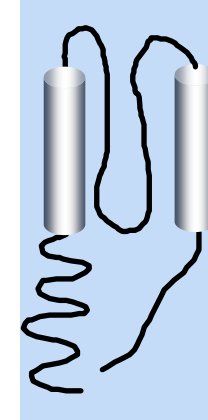
Canaux potassiques

→ 3 classes

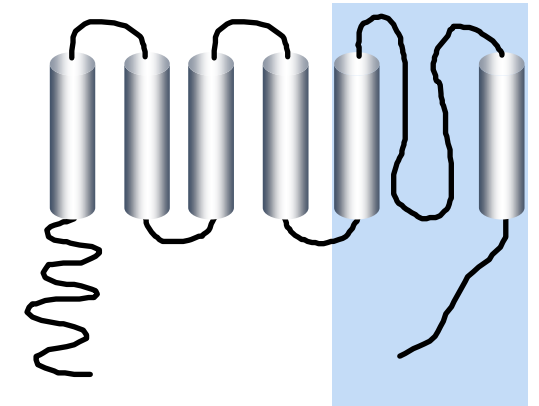
eucaryotes
procaryotes



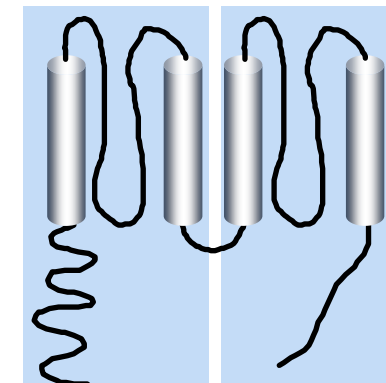
2 TMS
et 1 boucle du
pore



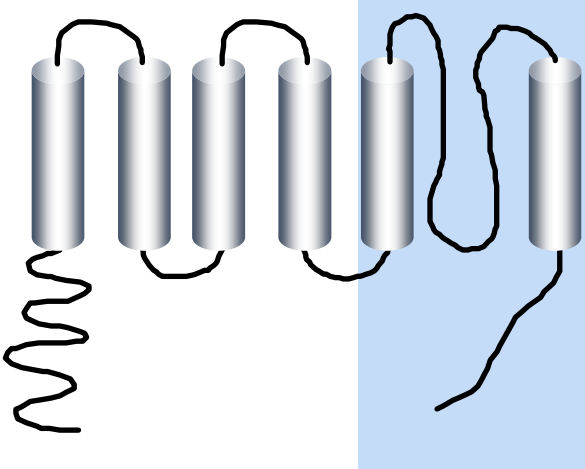
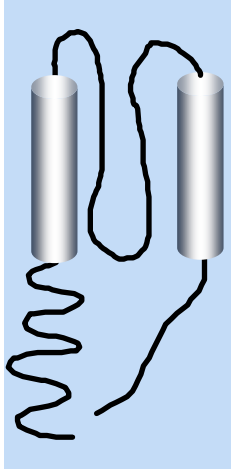
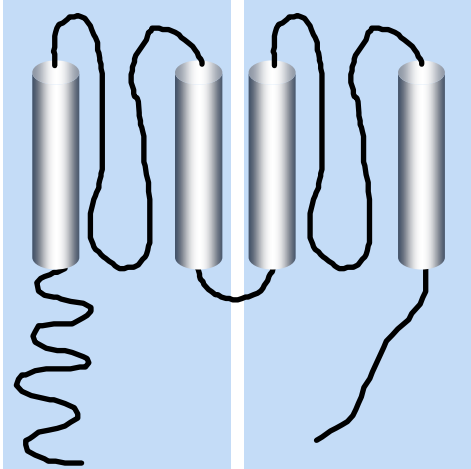
6 segments
transmembranaires
(TMS)
et 1 boucle du pore



4 TMS
et 2 boucles du
pore

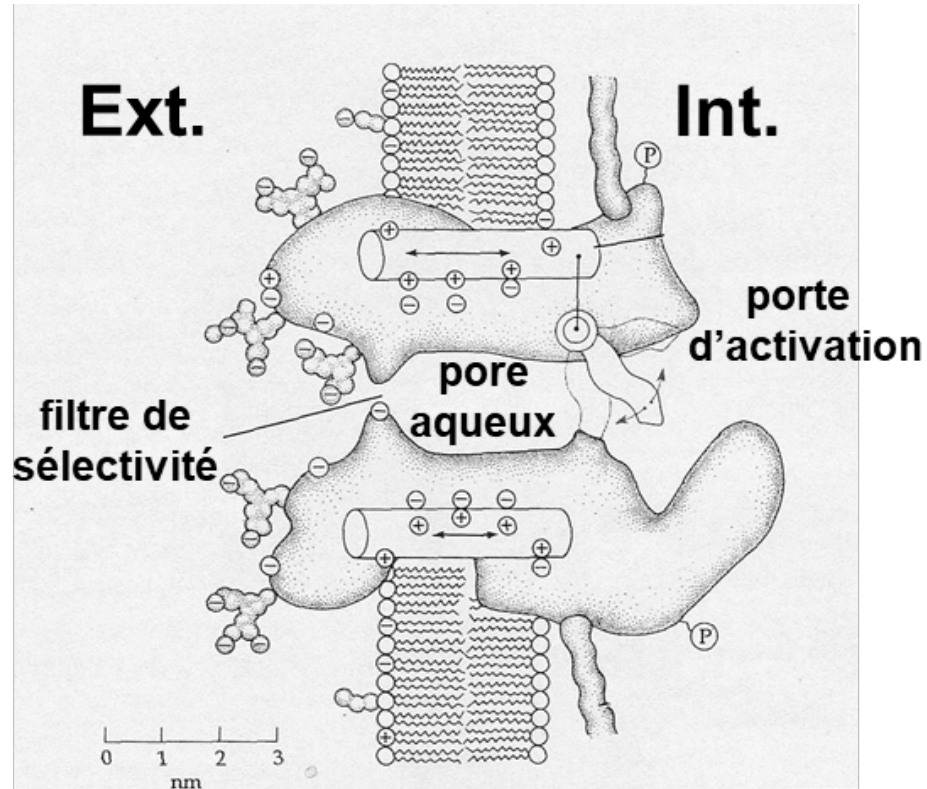


3 classes de canaux potassiques

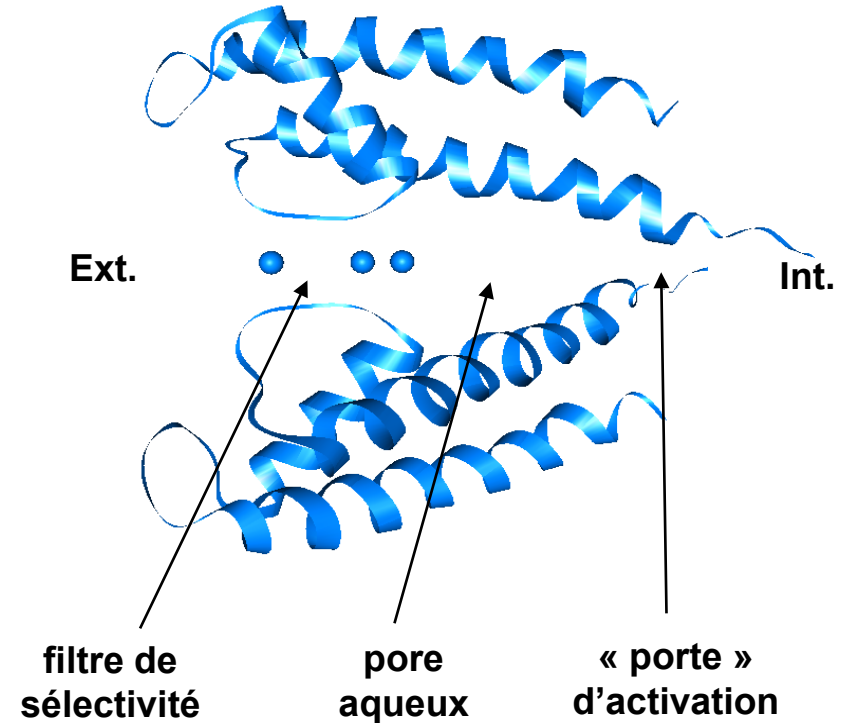
	6 segments transmembranaires (TMS) et 1 boucle du pore	2 TMS et 1 boucle du pore	4 TMS et 2 boucles du pore
Profil hydropathie			
Structure	tétramère	tétramère	dimère
Régulateurs	principalement V_{membrane}	principalement ligand: ATP, Prot G	stimuli physiques chimiques
Cibles	V_{membrane} ; [ion]		

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

Hypothèse de travail (1992)



Structure de KcsA (1998)

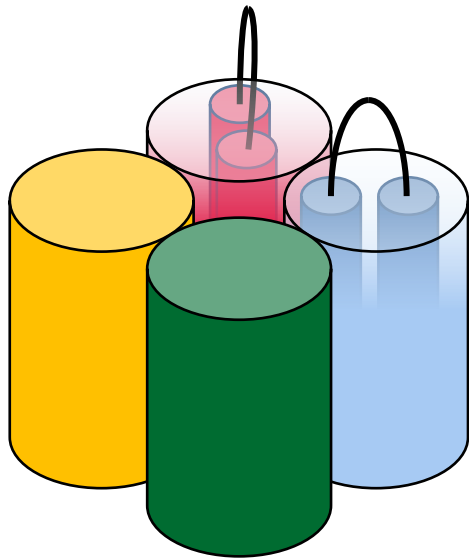


6

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

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

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

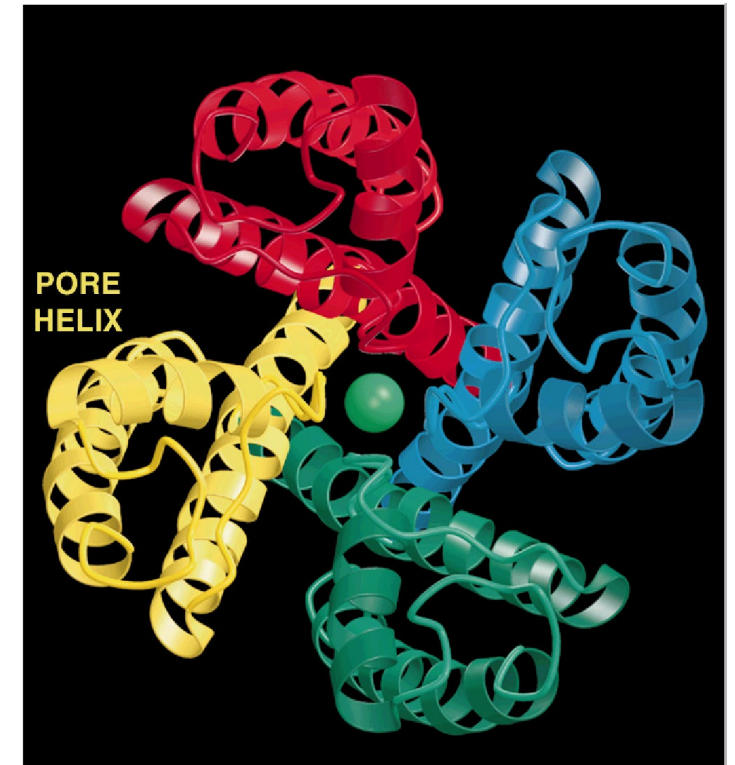


Tétramères

KcsA

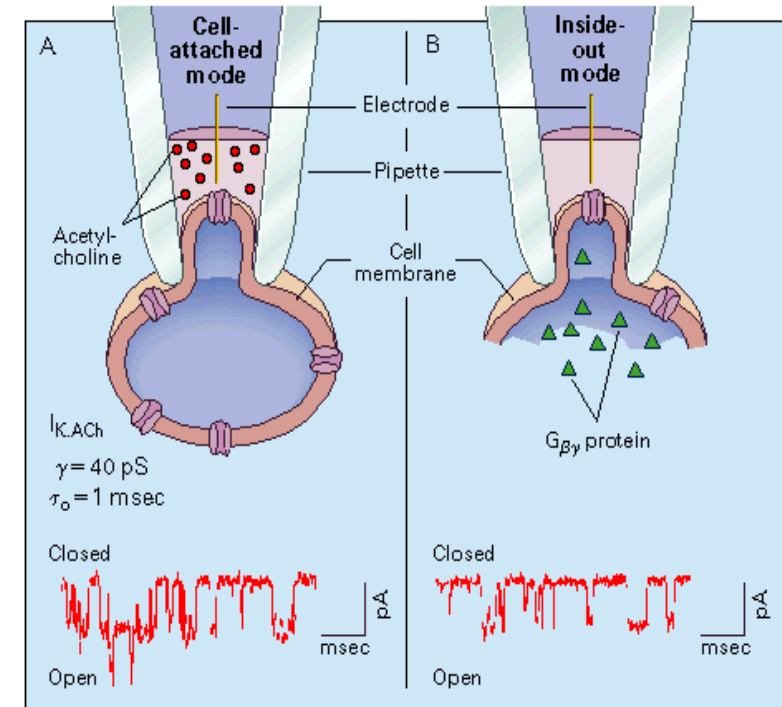
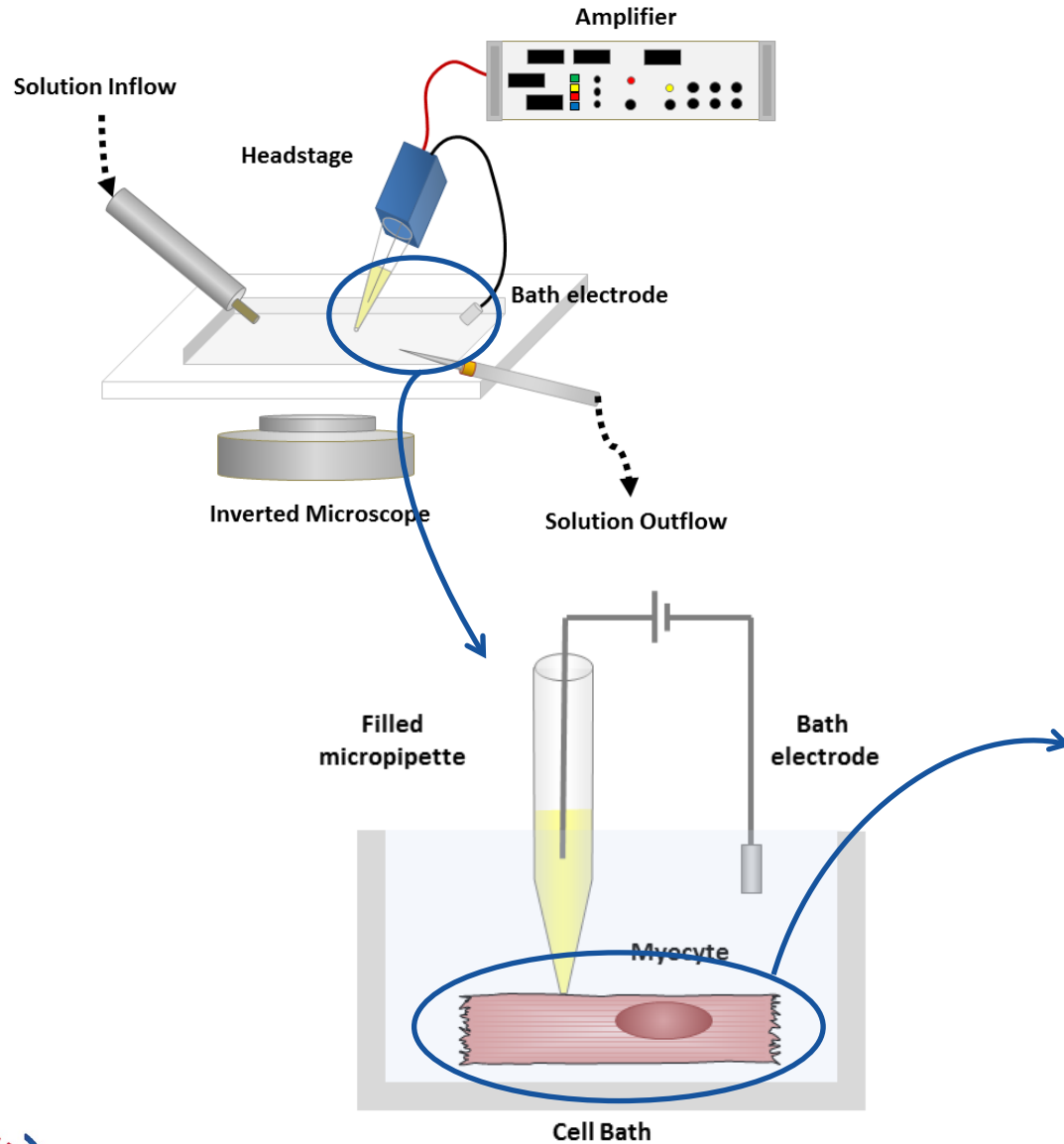


(resolution: 3.2 Å)



**Prix Nobel de Chimie 2003 :
Roderick MacKinnon**

Enregistrement d'un courant unitaire : le patch-clamp



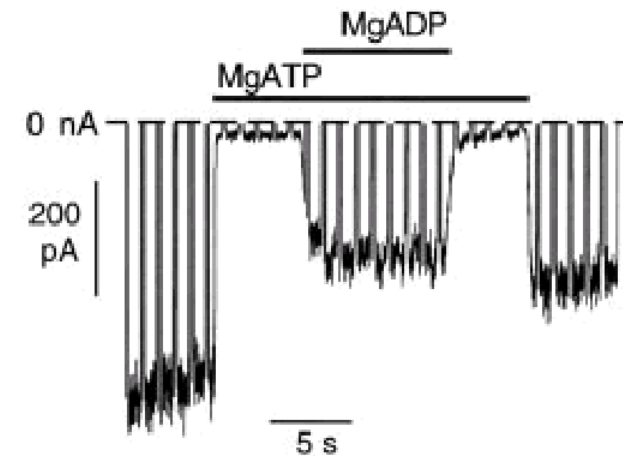
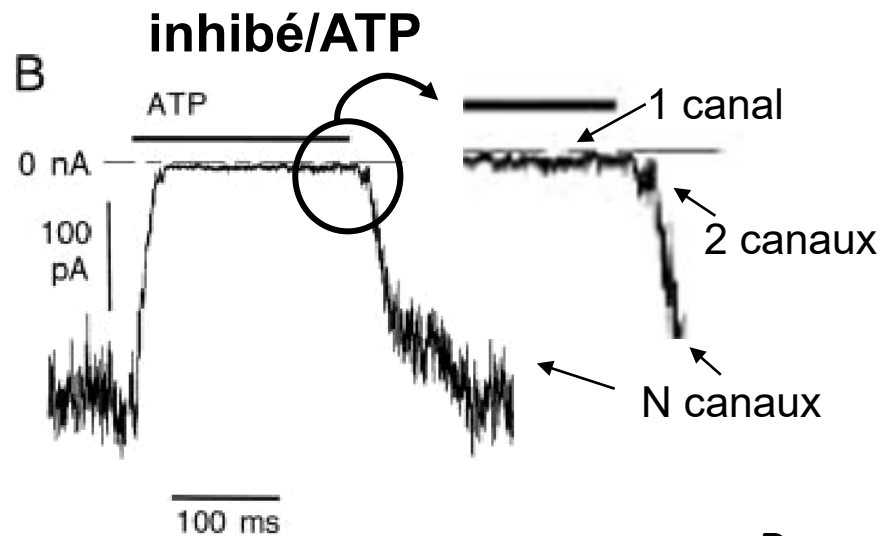
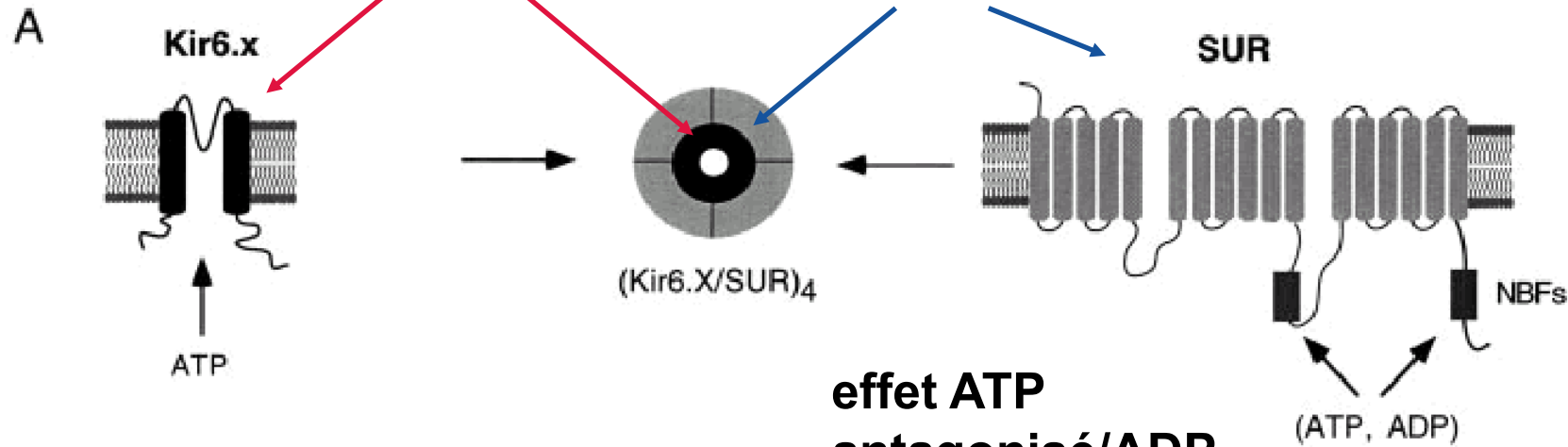
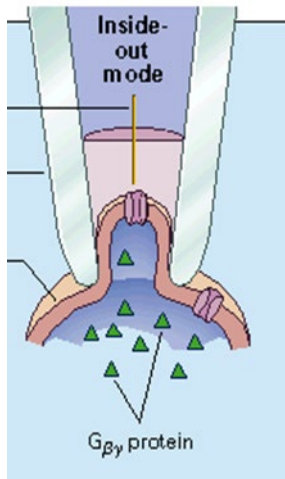
Prix Nobel de Physiologie 1991 : Erwin Neher & Bert Sakmann

canaux K_{ATP} du pancréas (cellules β des îlots de Langerhans)

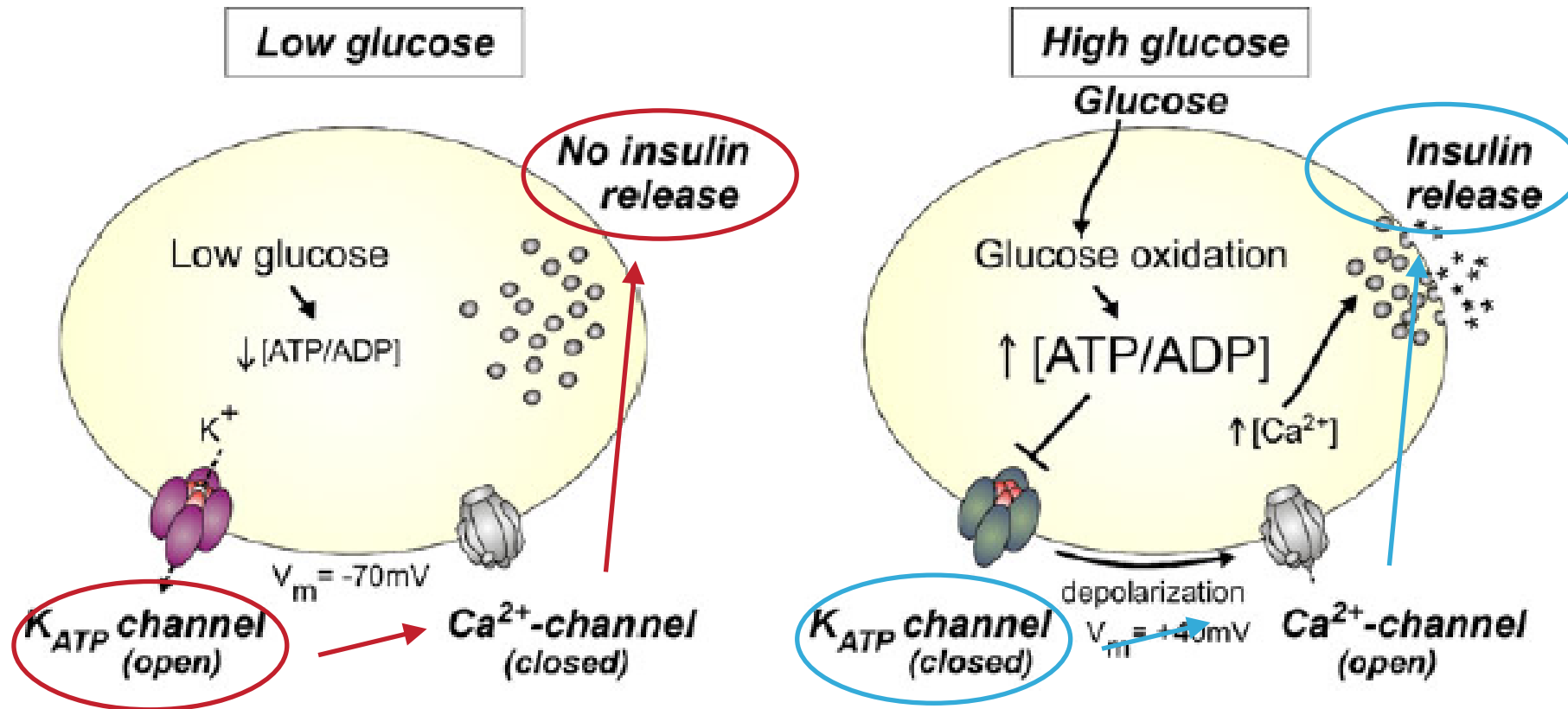
C $\rightleftharpoons$ O
fermé ouvert

sous-unité α : pore

sous-unité β : auxiliaire

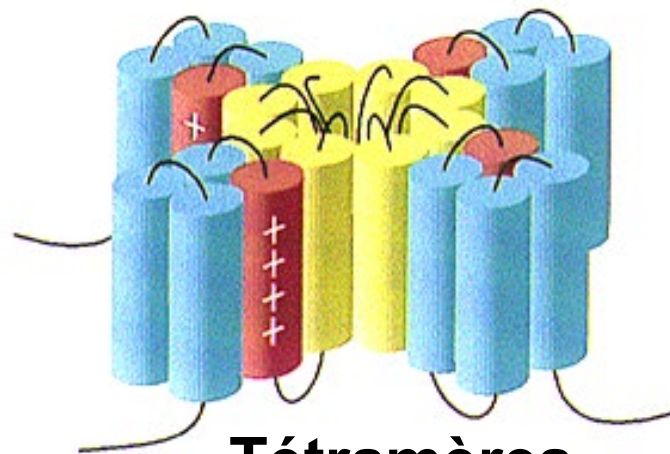
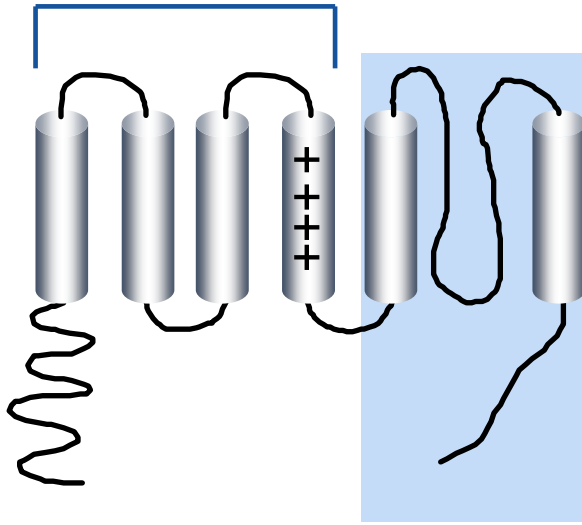


canaux K_{ATP} du pancréas (cellules β) rôle dans la sécrétion d'insuline



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

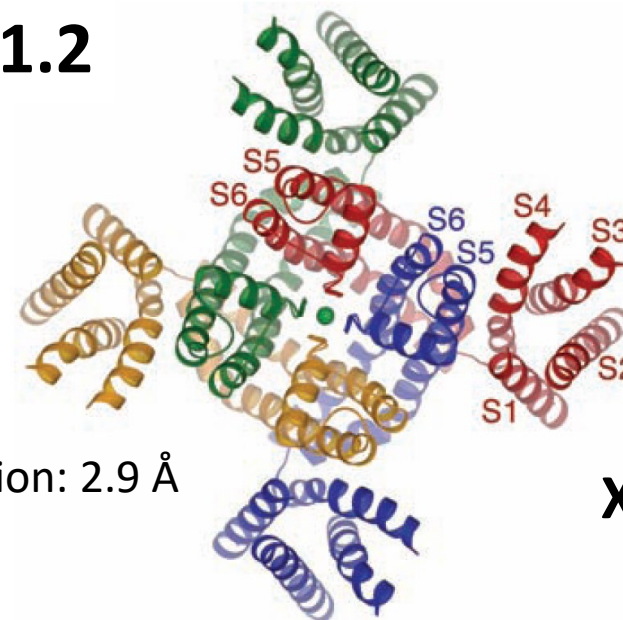
voltage sensor



Tétramères

Kv1.2

resolution: 2.9 Å

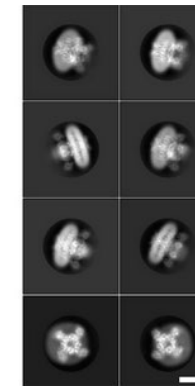
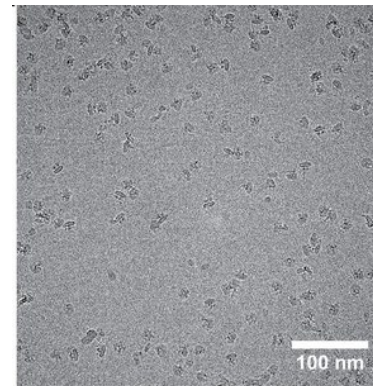


Long SB *et al.* Science. 2005 Aug 5;309(5736):897-903.

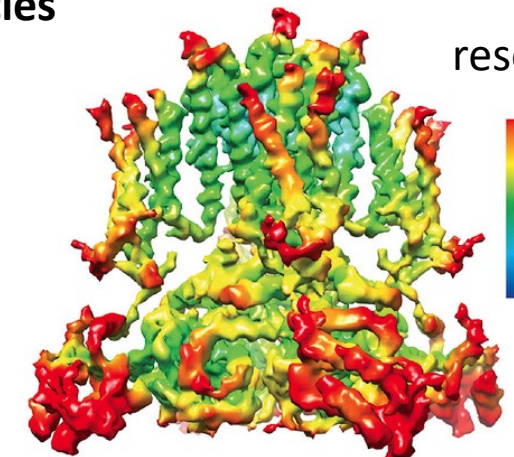
X-ray crystallography

hERG

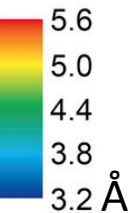
~144,000 particles



10 nm



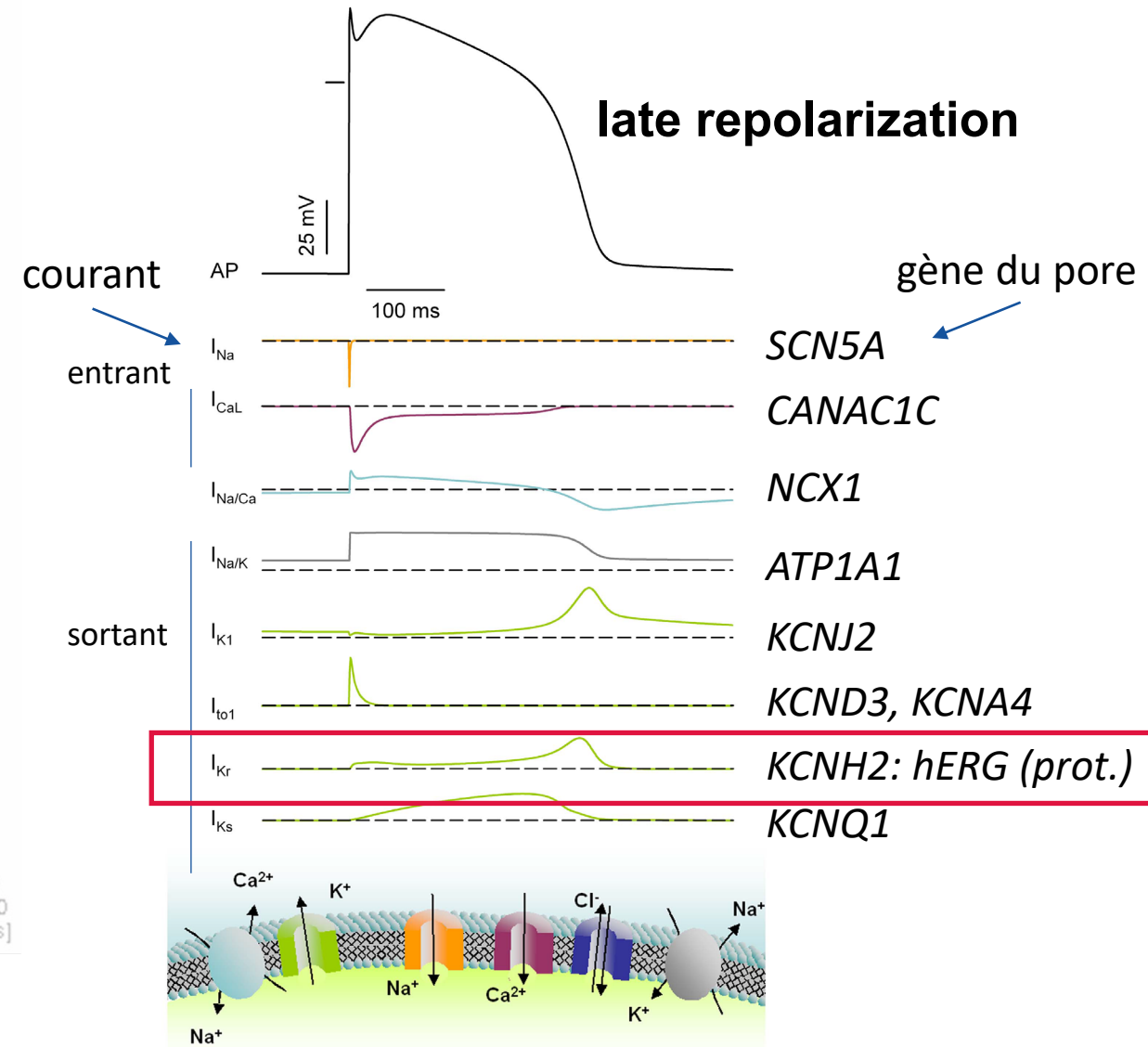
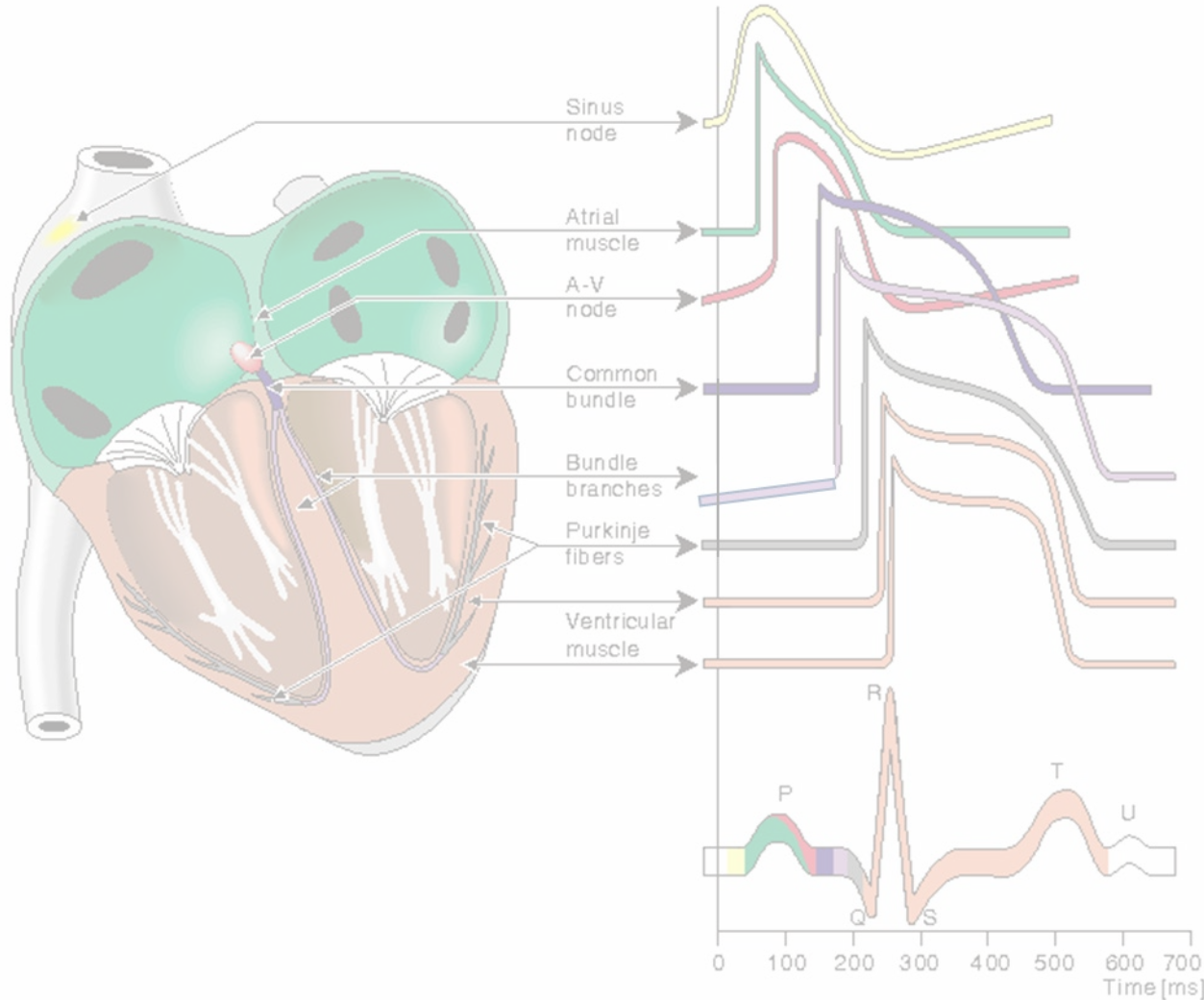
resolution:



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



hétérogénéité du potentiel d'action (PA)

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

- **Syndrome du QT long (ECG : intervalle QTc > 450-460 ms), torsades de pointes, fibrillation ventriculaire, mort subite**

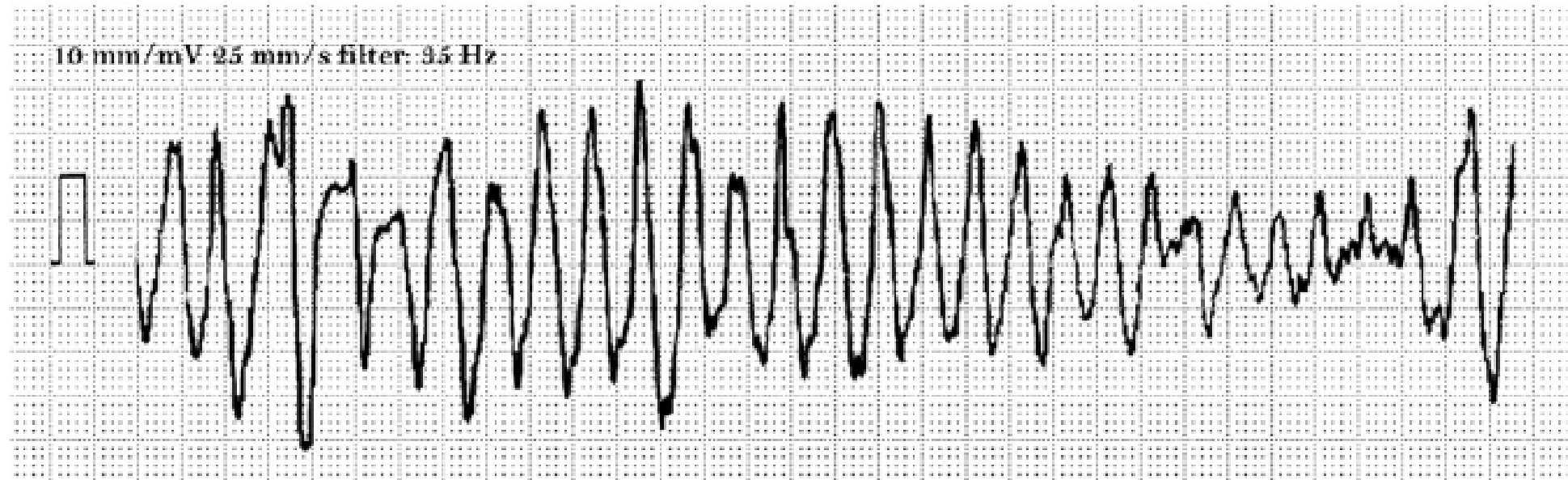


Fig. 1. ECG recording showing an ongoing episode of Torsade de pointes. This polymorphic ventricular arrhythmia is characterized by the progressive rotation of the electrical axis (180 ° in about 10–12 cycles) which shows on the surface ECG as the sinusoidal undulation of the electrical signal.

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

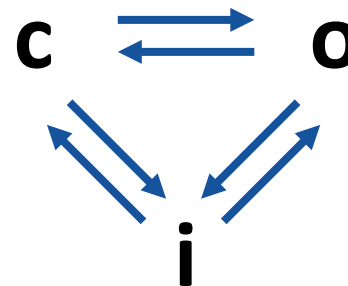
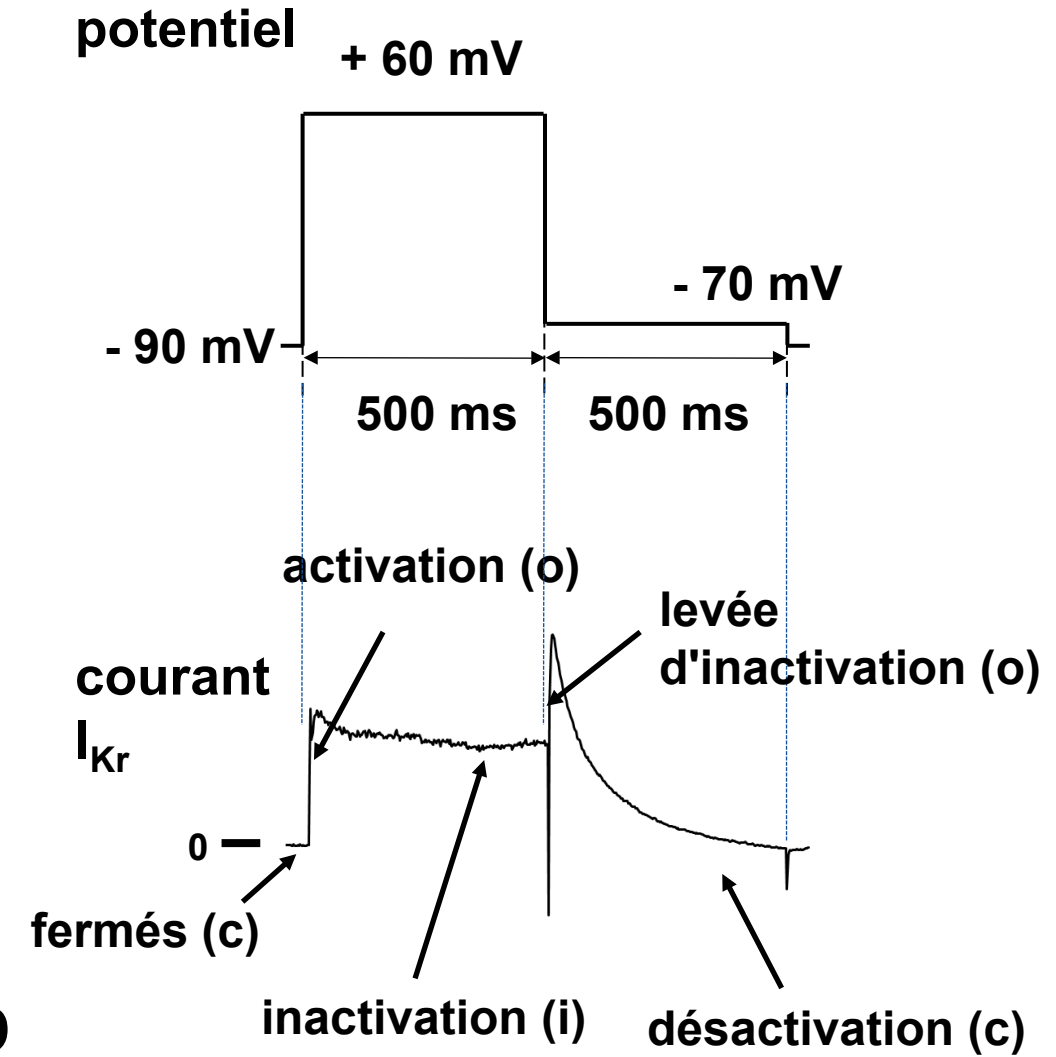
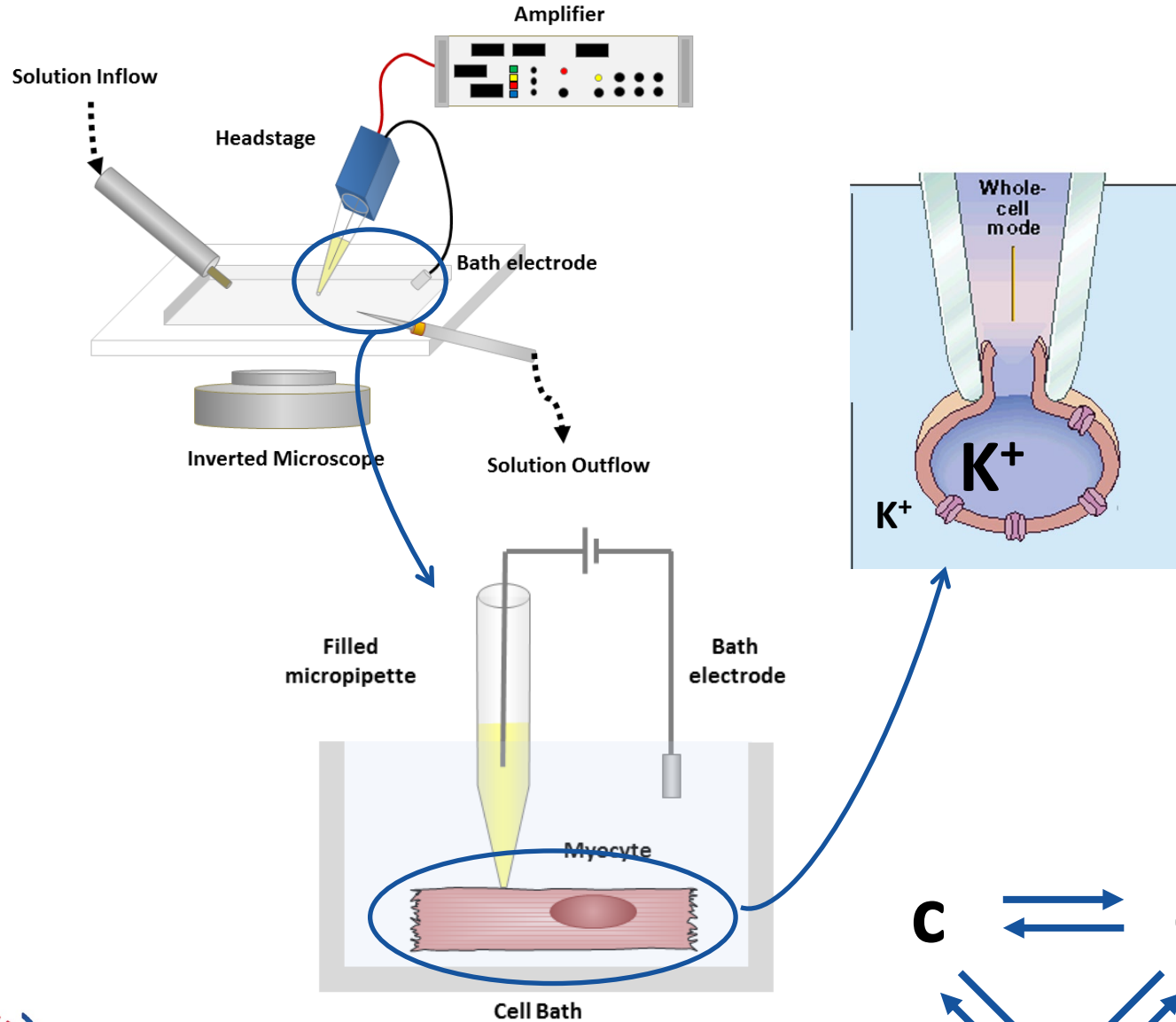
Table 1 Classification of genes responsible for cardiac channelopathies. Adapted from Schwartz et al. [2]

LQTS type	Gene	Mutation frequency among LQTS population (%)	Locus	Protein (functional effect)
Romano–Ward (RWS) <i>mut/WT</i>				
LQT1	KCNQ1	40–55	11p15.5	K _V 7.1 (↓)
LQT2	KCNH2	30–45	7q35–36	K _V 11.1 (↓)
LQT3	SCN5A	5–10	3p21–24	Na _V 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 ²⁺ signaling)
LQT15	CALM2	< 1	2p21	Calmodulin 2 (dysfunctional Ca ²⁺ signaling)
Jervell and Lange-Nielsen syndrome (JLNS) <i>mut/mut</i>				
JLN1	KCNQ1	< 1	11p15.5	K _V 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

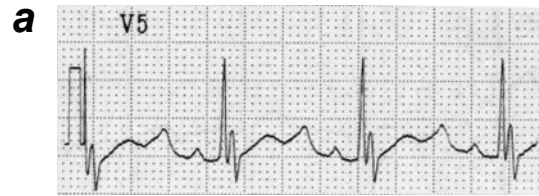
Enregistrement d'un courant trans-membranaire : le patch-clamp



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

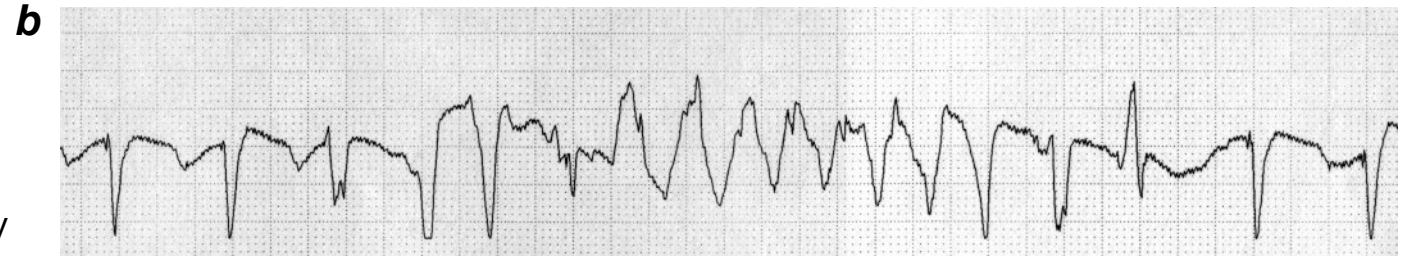
Propositus : fibrillation ventriculaire (torsades de pointes)

▼ Feb-05-99 – clobutinol

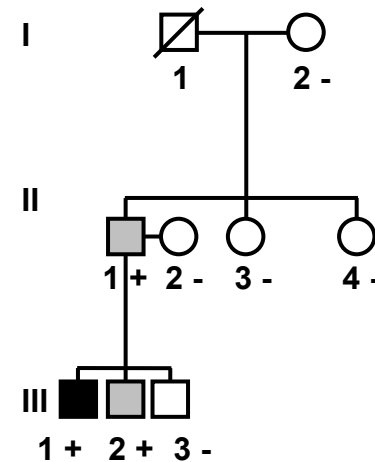


◀ May-30-97
597 ms
(628 ms)

1 mV
400 ms



Génotypage



+ : hERG **A561P**
- : hERG WT

← causal ?

porteur hétérozygote :

Modèles cellulaires

- Lignées cellulaires modifiées - canalopathies monogéniques

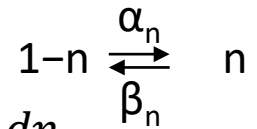
expression hétérologue de protéines WT et mutées

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 fonction :
allongement → causal

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



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

Rapidly Activating Current: I_{Kr}

$$I_{Kr} = g_{Kr, \max} \cdot X_r \cdot r_{ik} \cdot (V - E_K)$$

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

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

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

$$\{1 + \exp[-0.1262 \cdot (V + 4.067)]\}$$

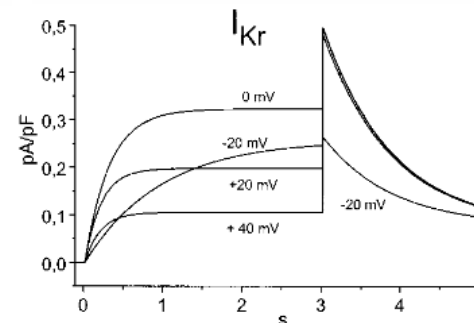
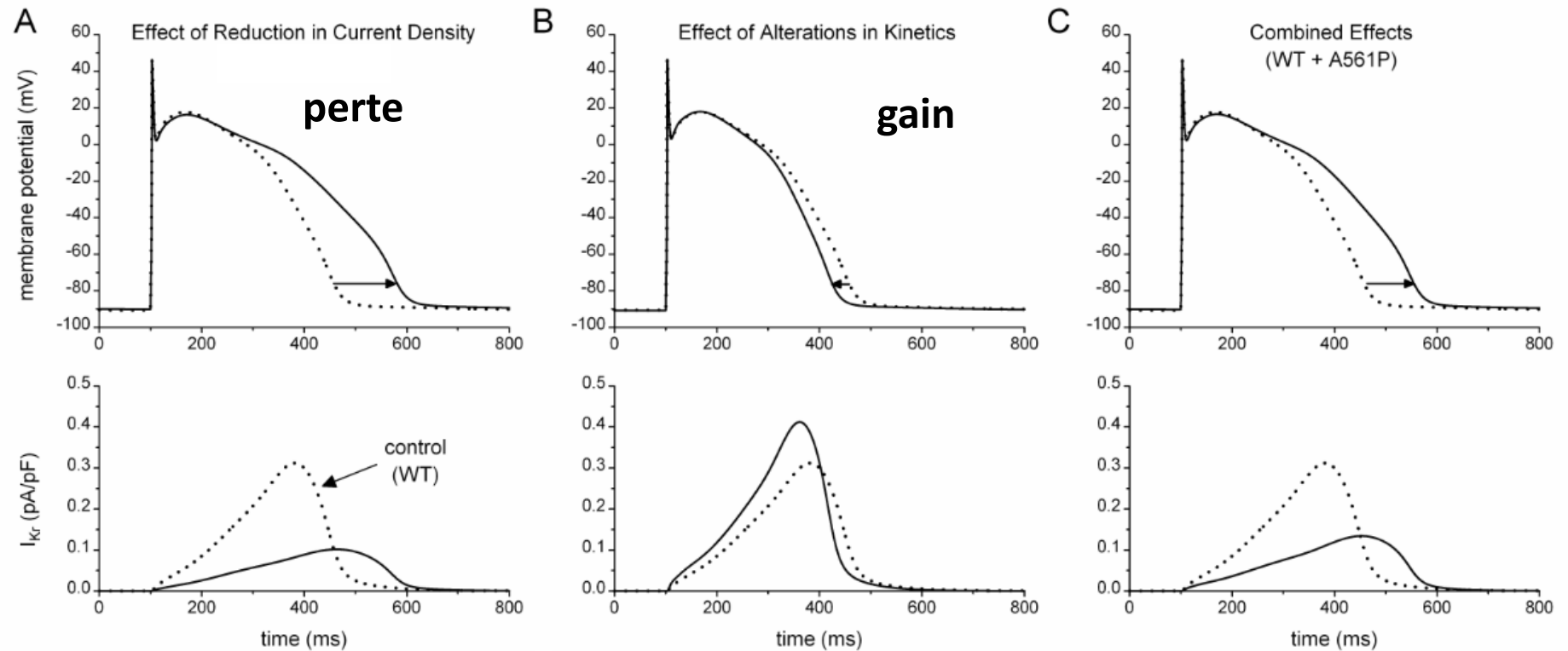
$$\beta_{X_r} = \{0.016 \cdot \exp[1.6 \cdot 10^{-3} \cdot (V + 65.66)]\} /$$

$$\{1 + \exp[0.0783 \cdot (V + 65.66)]\}$$

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

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

$$\frac{dX_r}{dt} = \alpha_{X_r}(1 - X_r) - \beta_{X_r}X_r = \frac{X_{r\infty} - X_r}{\tau_{X_r}}$$

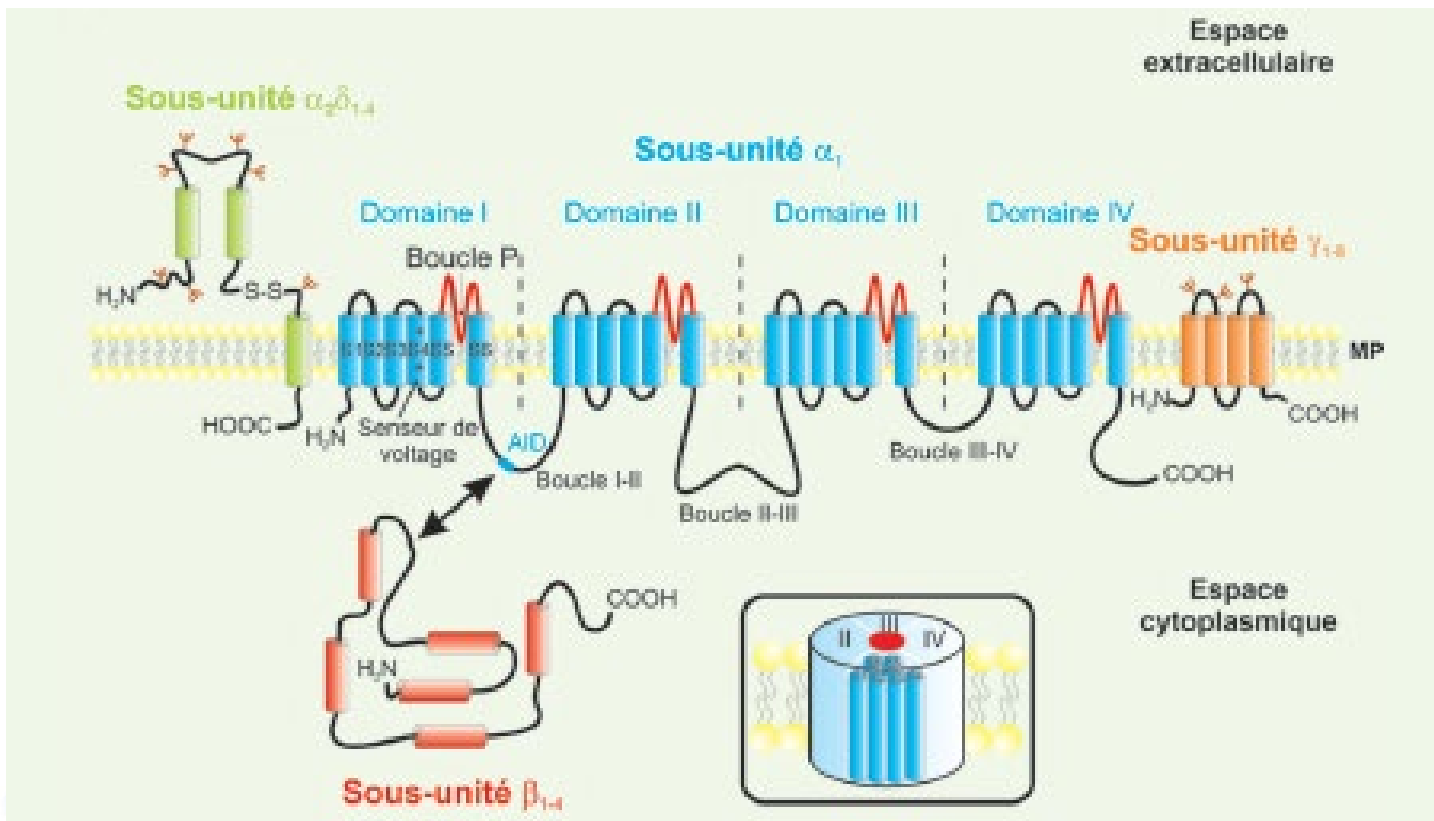
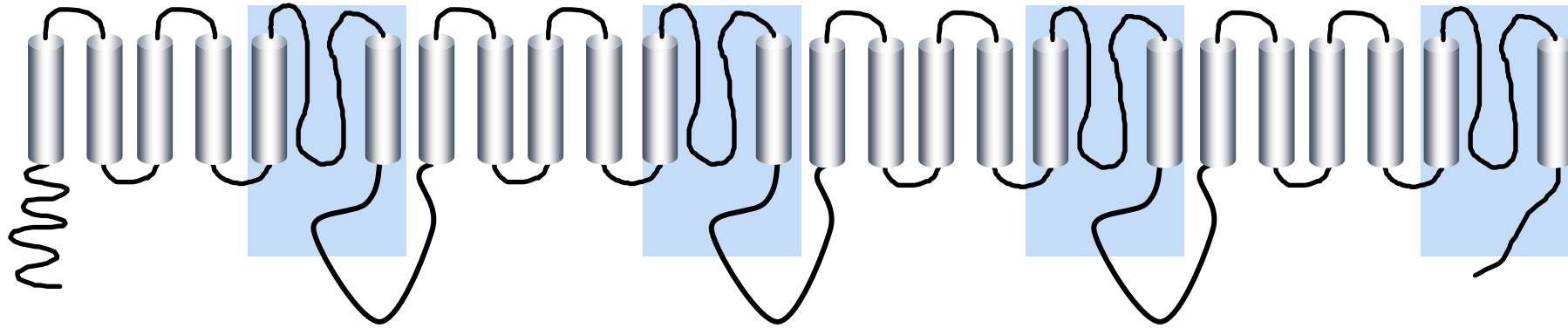


Hodgkin AL, Huxley AF. J Physiol. 1952 Aug;117(4):500-44.

Priebe L, Beuckelmann DJ. Circ Res. 1998 Jun 15;82(11):1206-23.

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

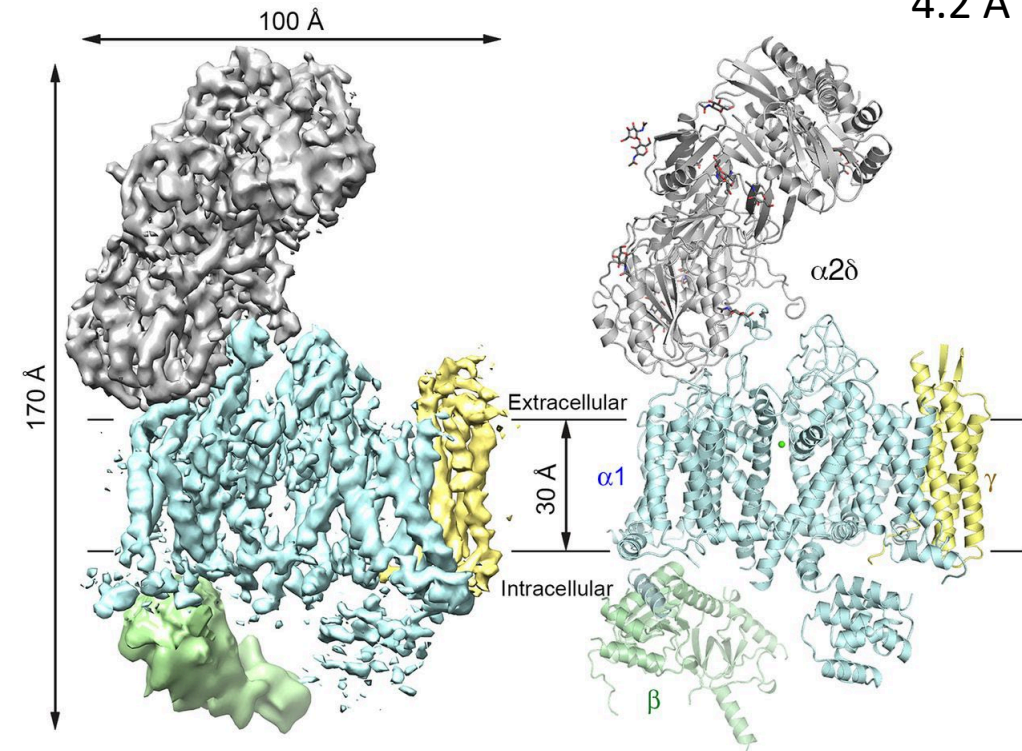
Canaux Ca^{2+} : 4 domaines homologues



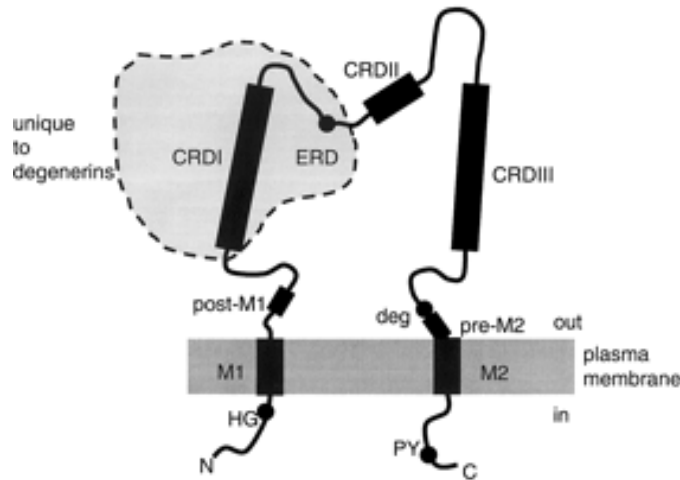
cryo-EM

Cav1.1

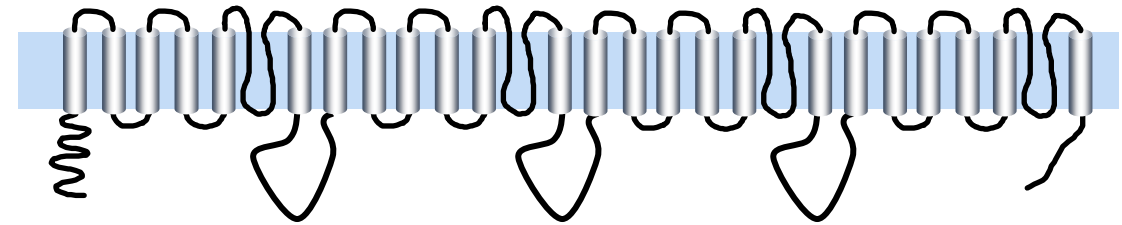
4.2 Å



Canaux Na^+ : 2 classes

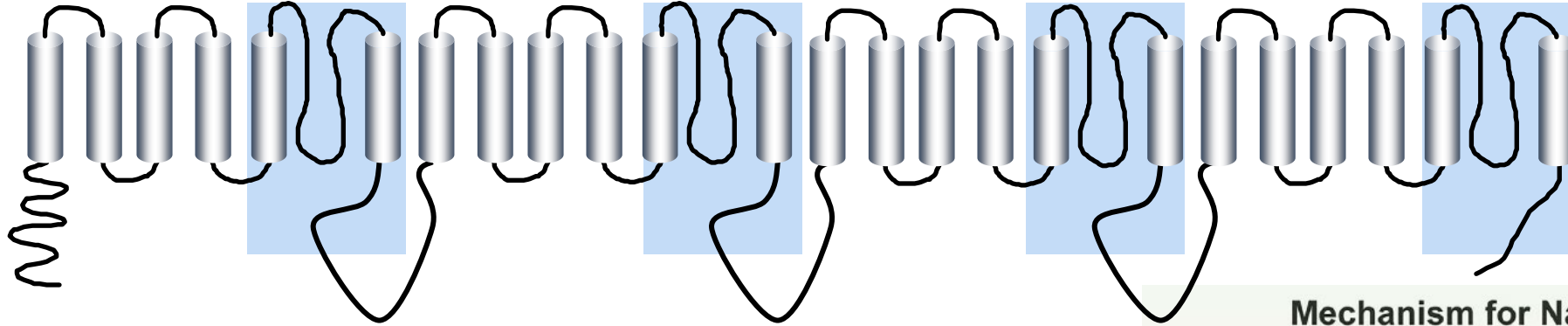


- **ENaC (epith. Na^+ channel)**

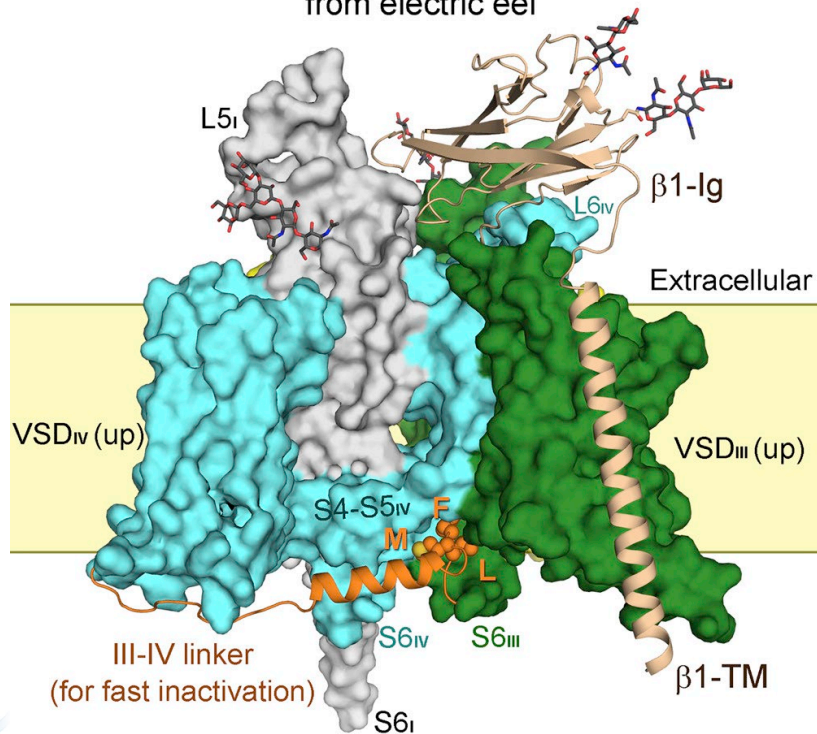


- **Nav: voltage-gated**
- **canal Na^+ cardiaque : Nav1.5 + Nav β 1 (*SCN5A* et *SCN1B*)**

Canaux Nav : structure

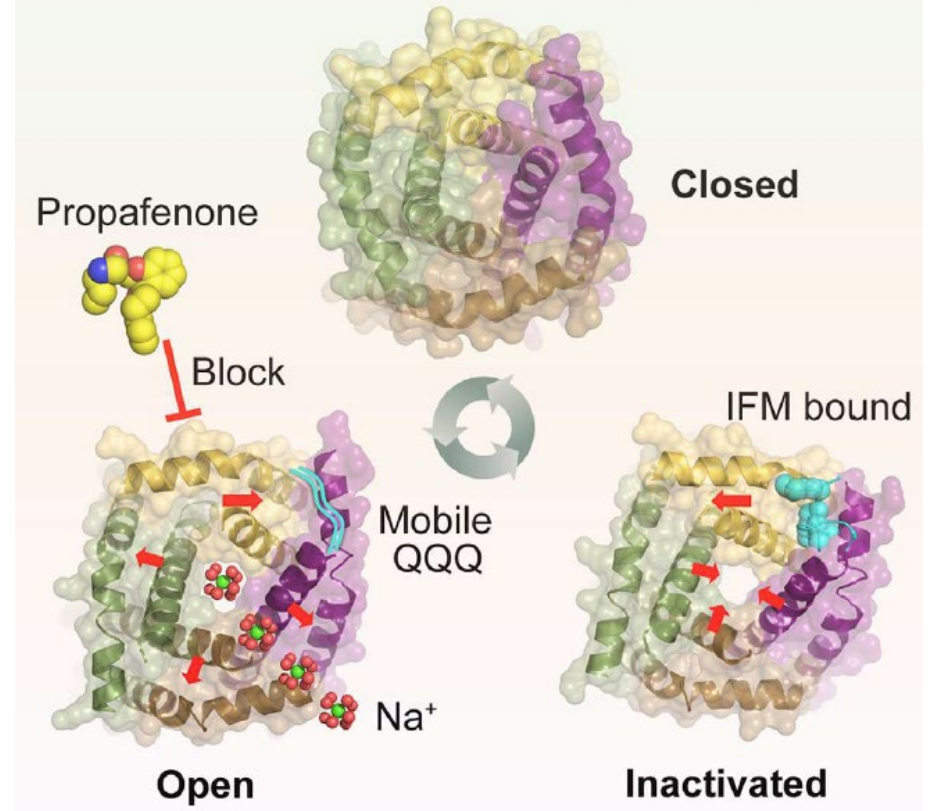


Cryo-EM structure of the Nav1.4- β 1 complex from electric eel



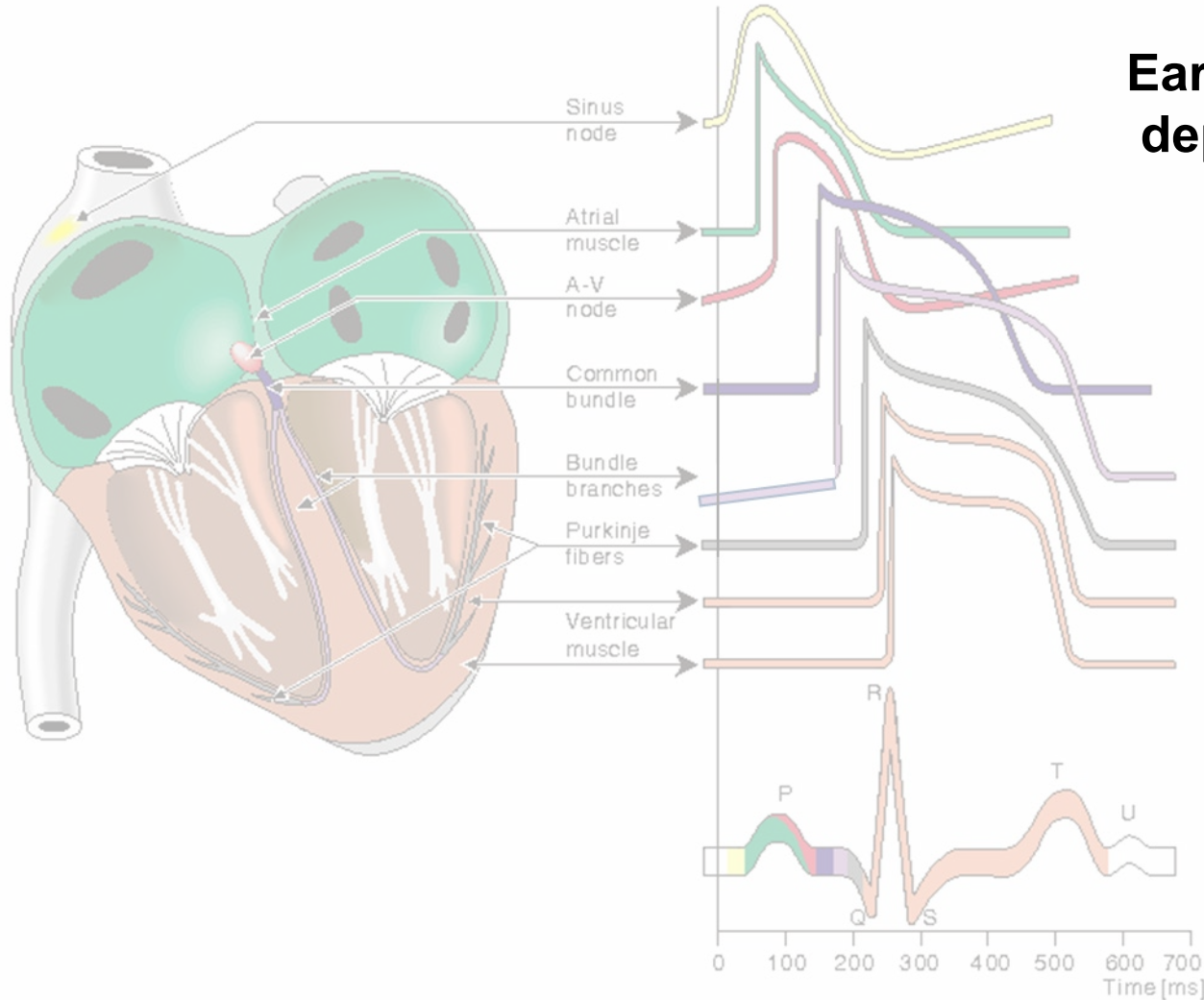
Yan *et al.* Cell. 2017 Jul 27;170(3):470-482.e11.

Mechanism for Nav1.5 Opening



Jiang D *et al.* Cell. 2021 Sep 30;184(20):5151-5162.e11

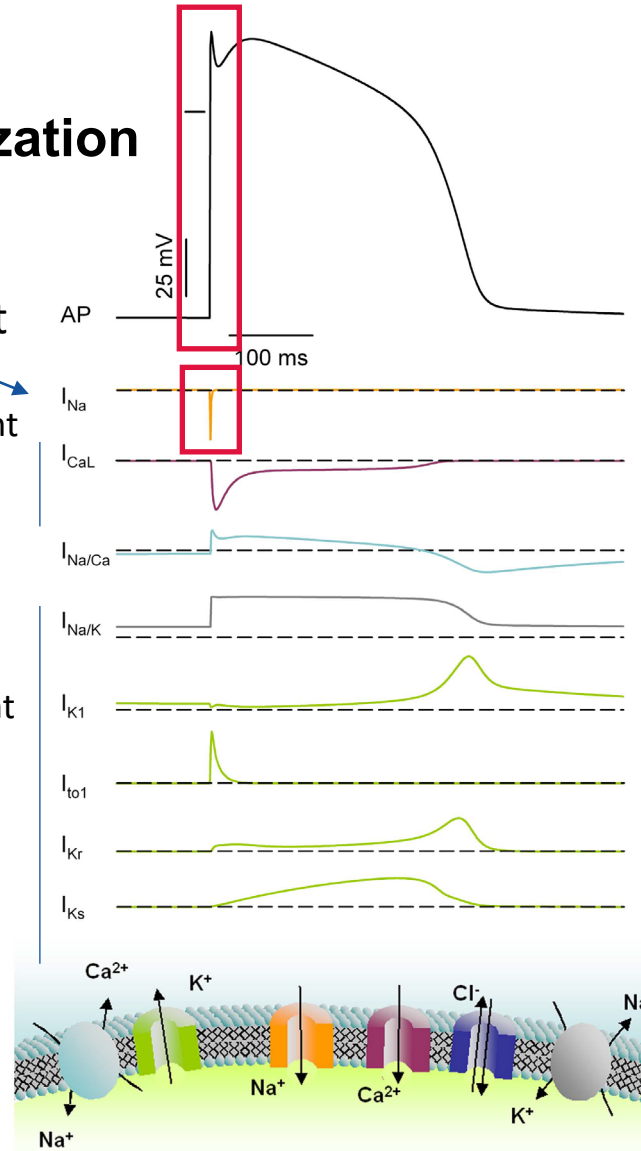
Activité électrique du cardiomyocyte ventriculaire : le potentiel d'action



Early depolarization

courant
entrant

sortant



gène du pore
protéine

SCN5A : Nav1.5

CANAC1C

NCX1

ATP1A1

KCNJ2

KCND3, KCNA4

KCNH2

KCNQ1

hétérogénéité du potentiel d'action (PA)

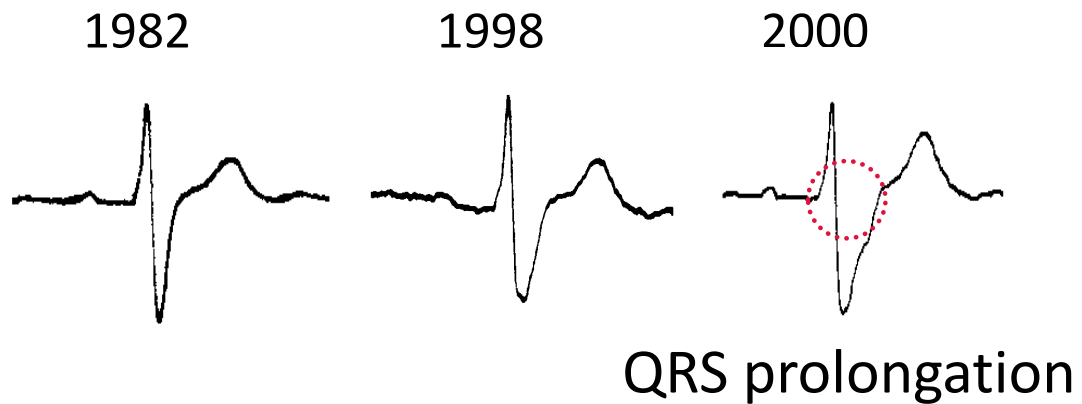
Du modèle cellulaire au modèle murin du syndrome de Lenègre

- 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



Mutation *SCN5A* : canal Nav1.5 Δ exon22 ← causal?

transfected COS-7

$I_{Nav1.5}$

WT

B

WT + h β 1

5 ms

C

Δ exon22

D

Δ exon22 + h β 1

Du modèle cellulaire au modèle murin du syndrome de Lenège

1982

1998

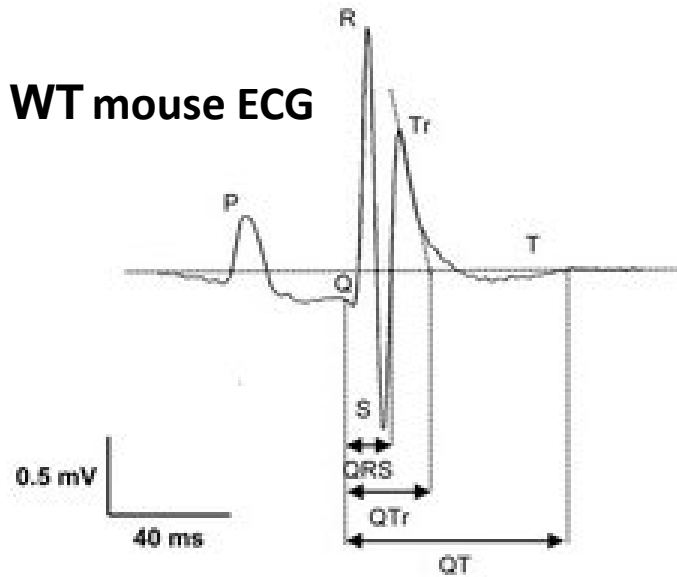
2000

prolongation



SCN5A mut : exon 22 absent

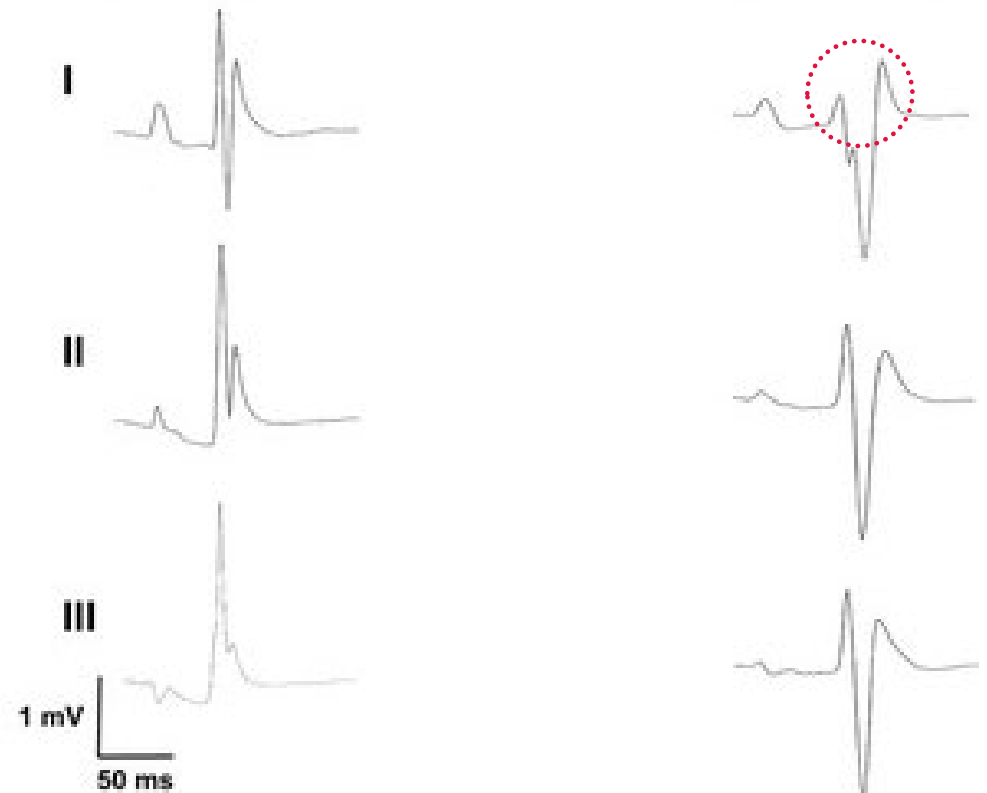
WT mouse ECG



Scn5a^{+/-} mouse

**Z502
(wild-type)**

**Y95
(Scn5a^{+/-})**



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

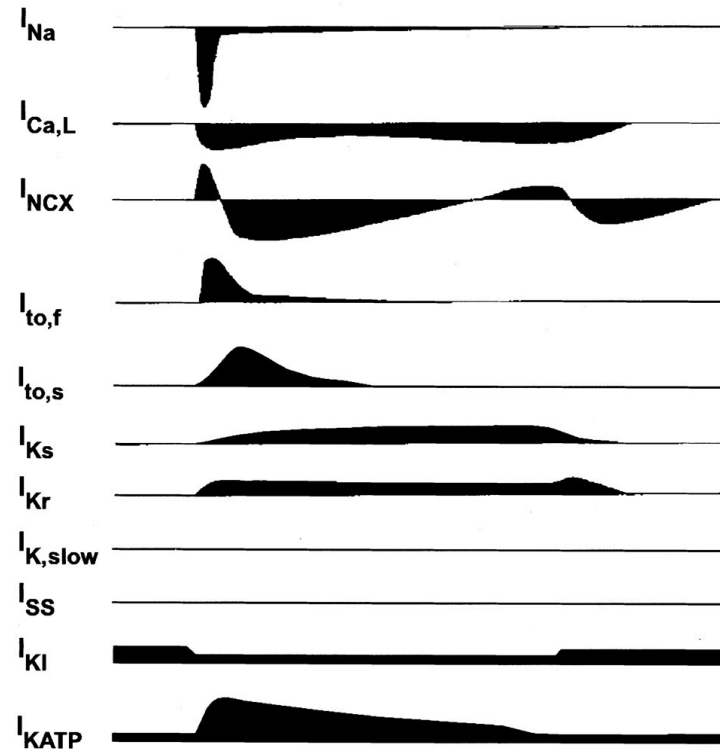
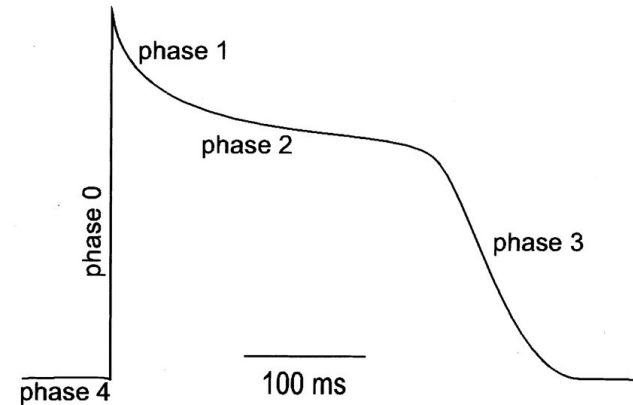
Modèles unicellulaires

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

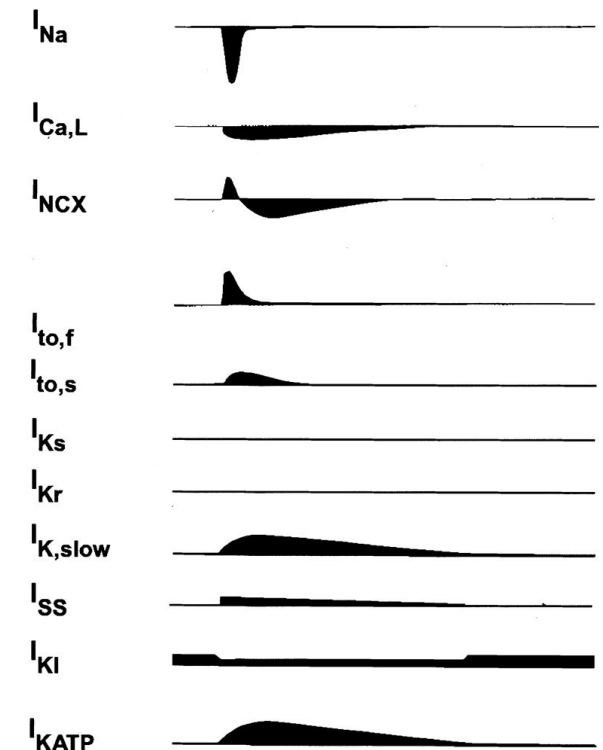
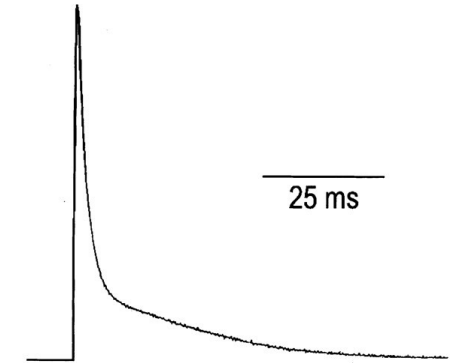
Modèles informatiques

- environnement fonctionnel ?

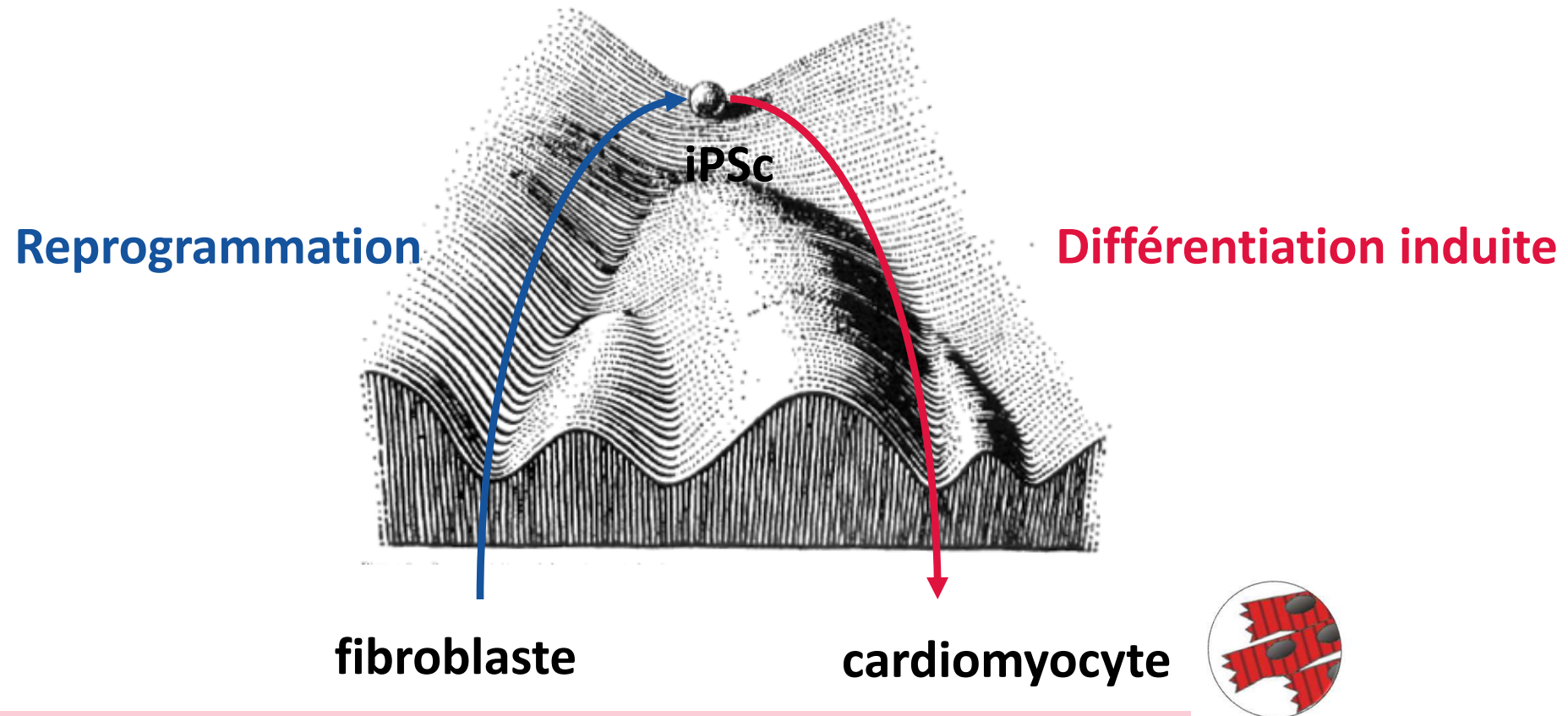
Human AP



mouse AP



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)

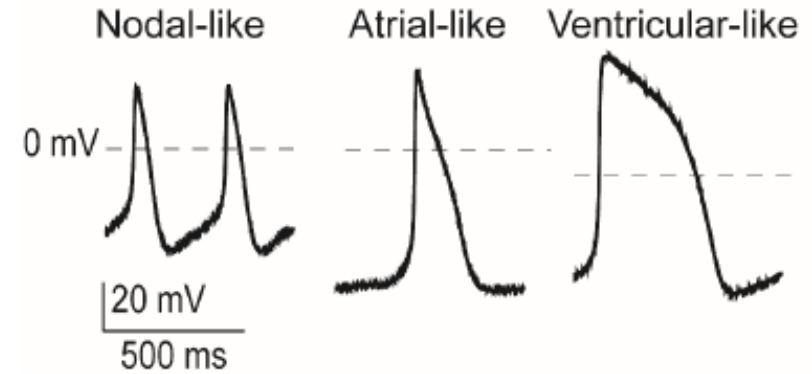
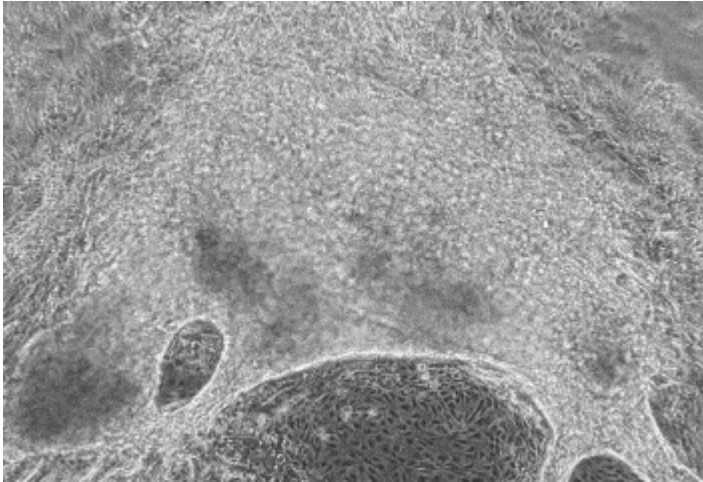
Cellules d'origine :

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

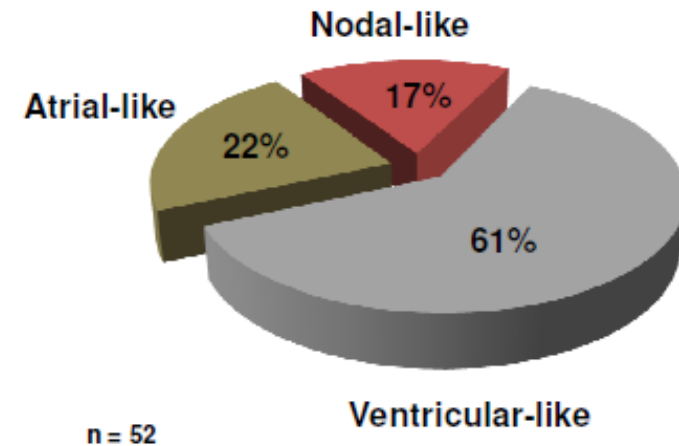
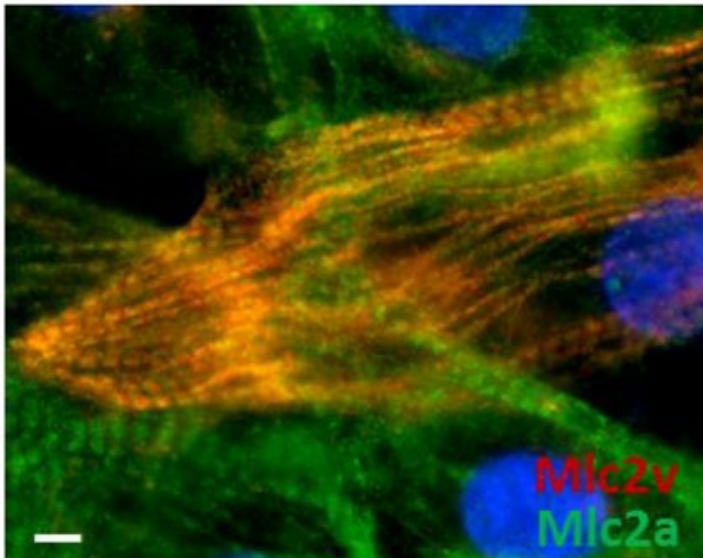
Cardiomyocytes issus d'iPSc

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

hiPS-cardiomyocytes



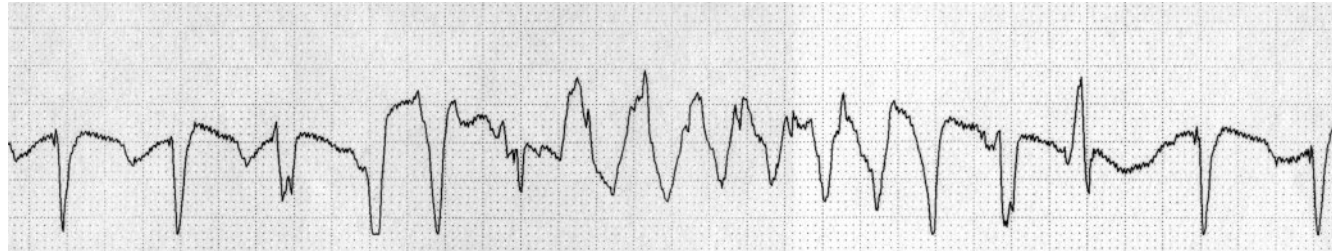
Hétérogénéité phénotypique



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



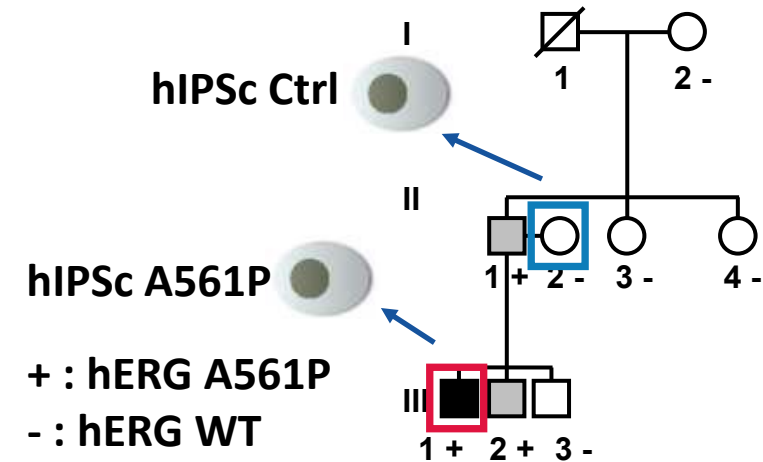
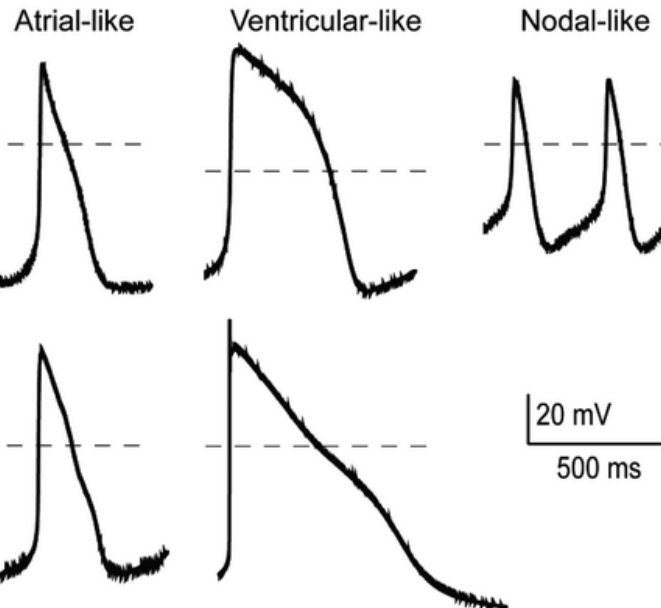
• re-programmation

urinary cells

hiPSc

hiPS-CM

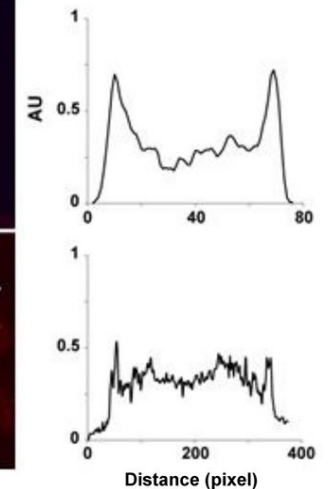
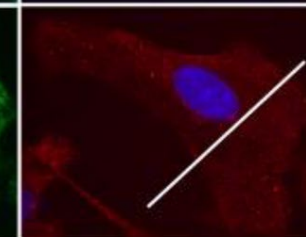
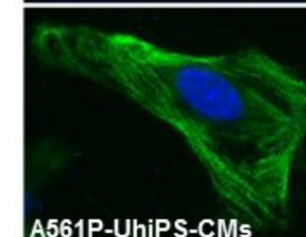
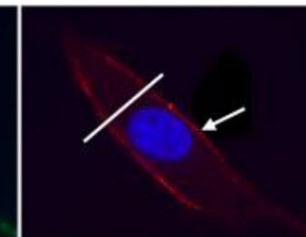
• current-clamp



• immuno-cytofluorimétrie

troponin I

hERG

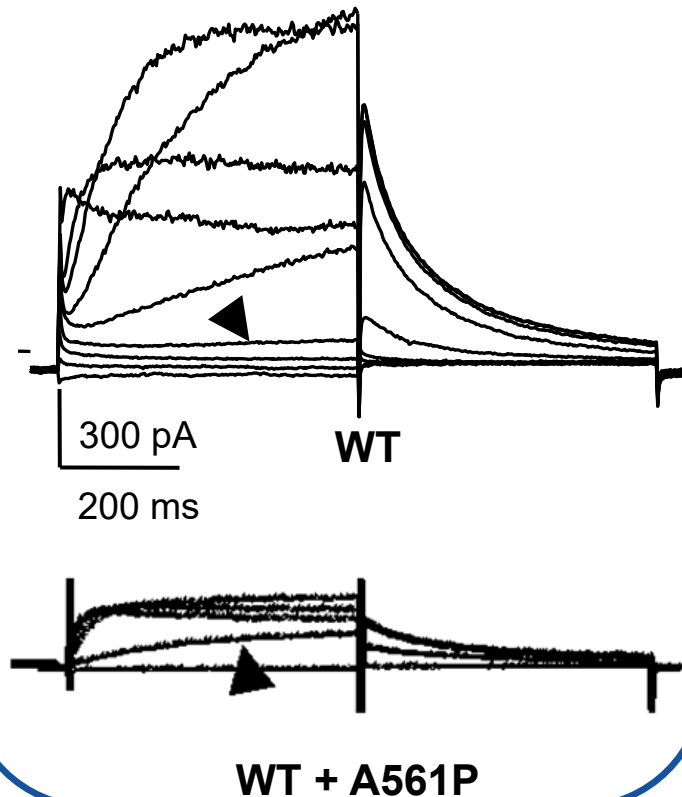


DAPI

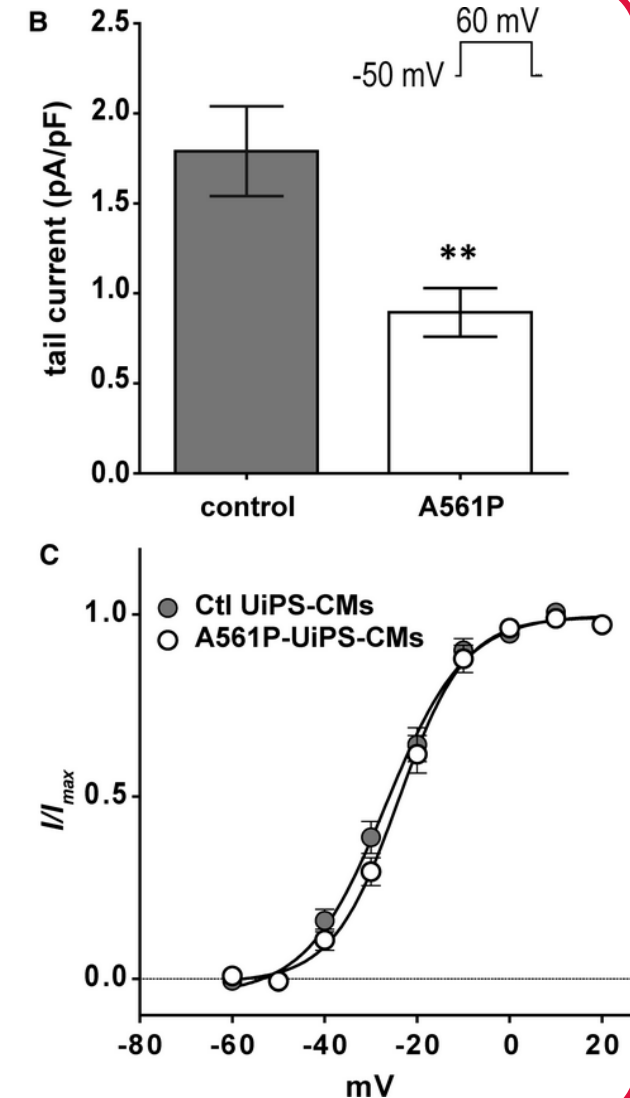
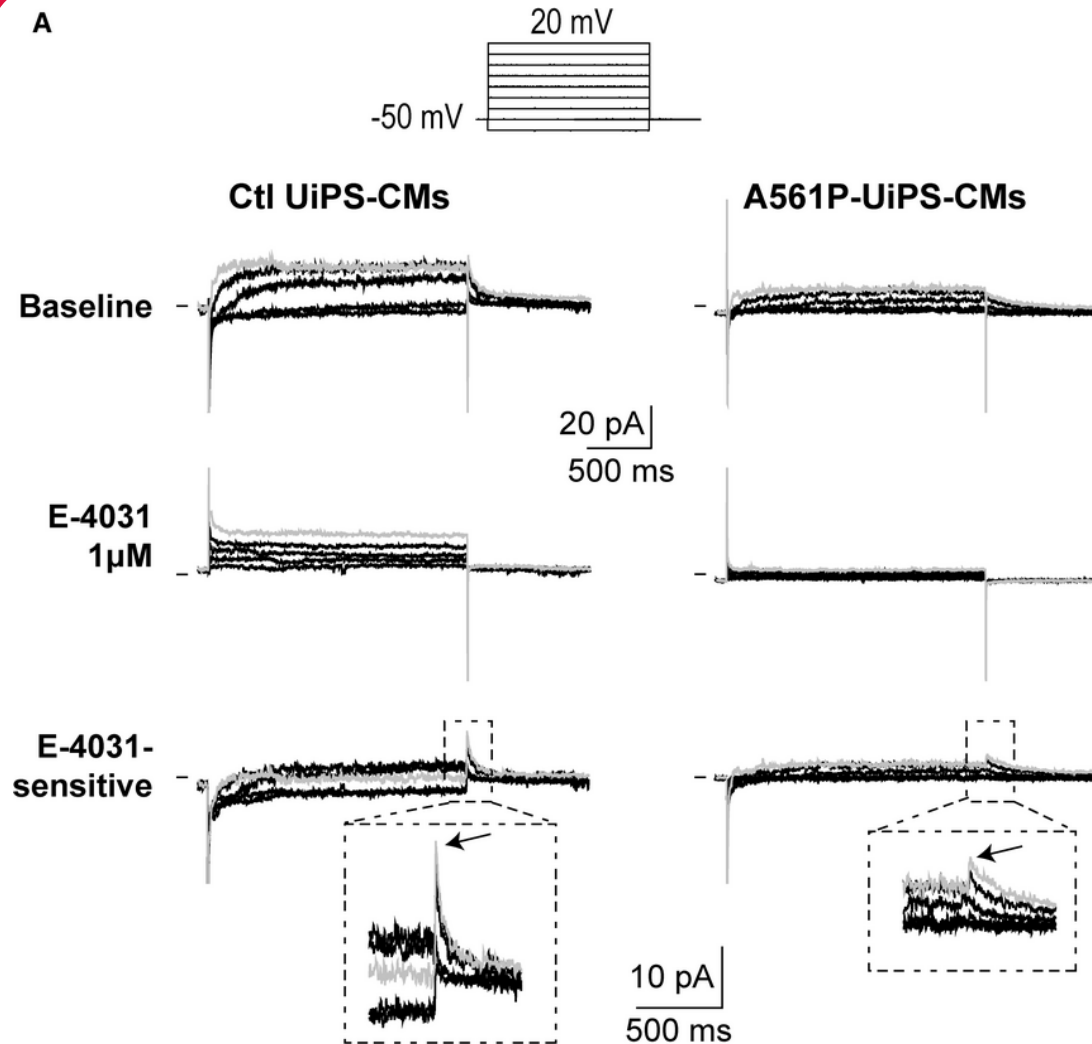
Transfected cells vs hiPS-cardiomyocytes

- voltage-clamp

transfected COS-7 cells



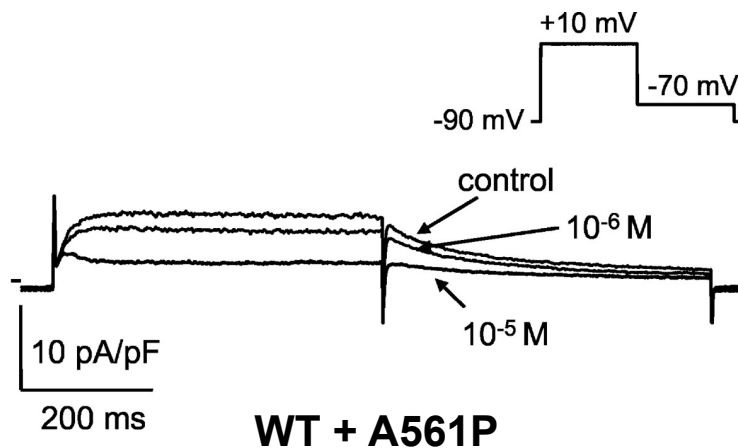
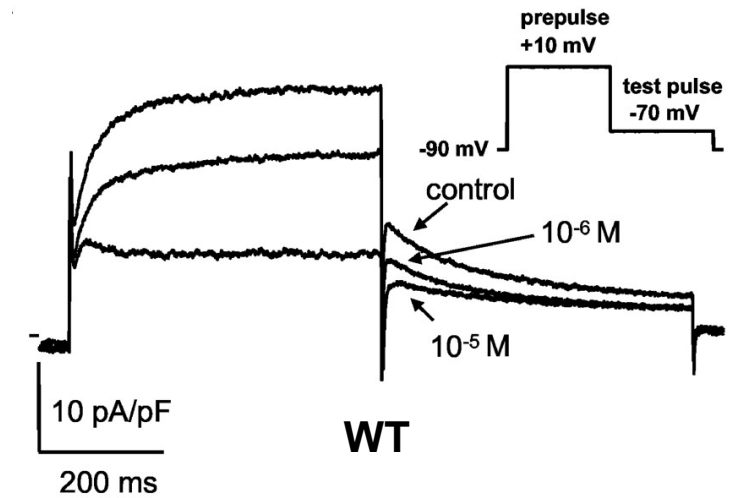
Bellocq C *et al.* Mol Pharmacol.
2004 Nov;66(5):1093-102



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

Transfected cells vs hiPS-cardiomyocytes

+ clobutinol (I_{Kr} inhib.)



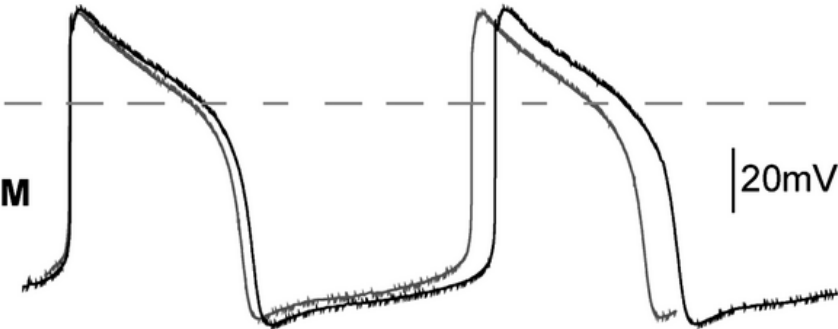
Belloq C *et al.* Mol Pharmacol. 2004 Nov;66(5):1093-102

+ E4031 (I_{Kr} inhib.)

C Control UhiPS-CM

Baseline

+E-4031 1 μ M



A561P-UhiPS-CM

Baseline

+E-4031 1 μ 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



Original Article

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



Zeineb Es-Salah-Lamoureux ^{a,1}, Mariam Jouni ^{a,1}, Olfat A. Malak ^a, Nadjat 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}

^a l'institut du thorax, Inserm, CNRS, Université de Nantes, Nantes, France

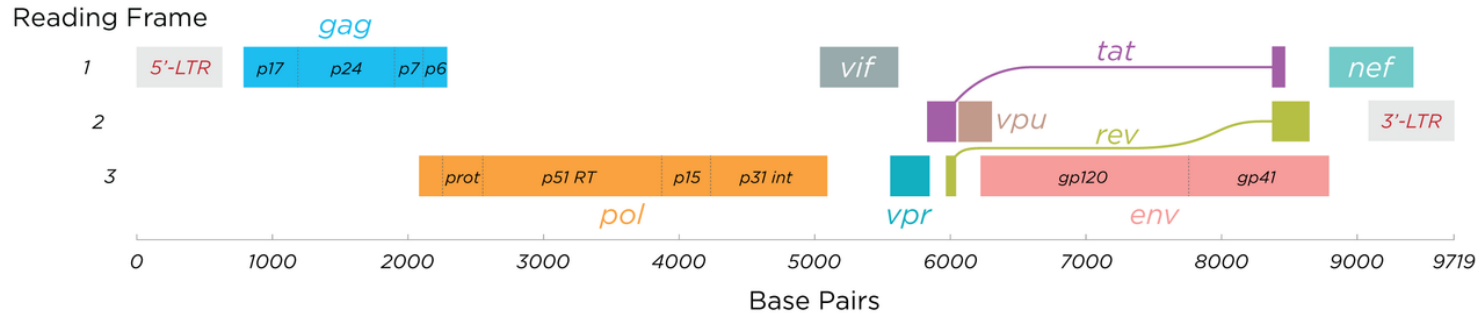
^b l'institut du thorax, Inserm, CNRS, Université de Nantes, CHU Nantes, Nantes, France

^c ERO45, PRASE, Laboratory of stem cells, Lebanese university, Beirut, Lebanon

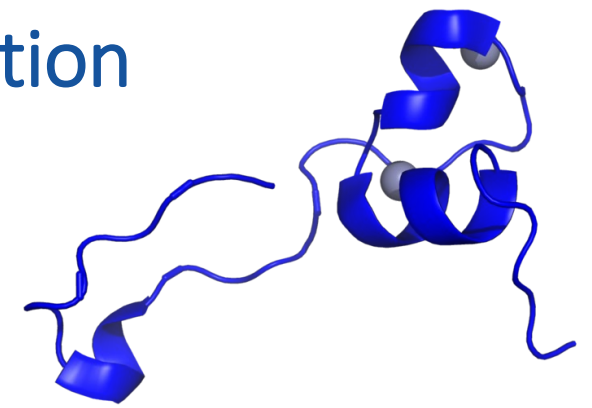
^d Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé, CNRS, Université de Montpellier, Montpellier, France

- 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 des anti-viraux)

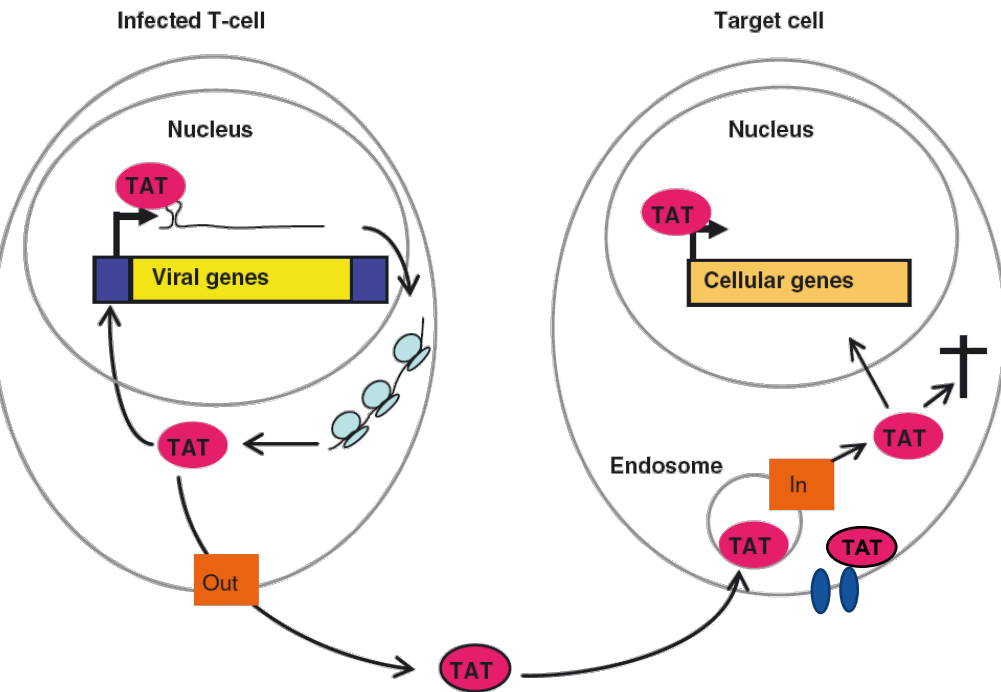
Tat = Trans-Activator of Transcription



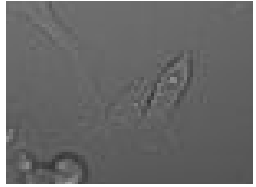
<https://commons.wikimedia.org/w/index.php?curid=33943759>



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



- 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?



cellules
COS-7

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

- Courant I_{hERG}

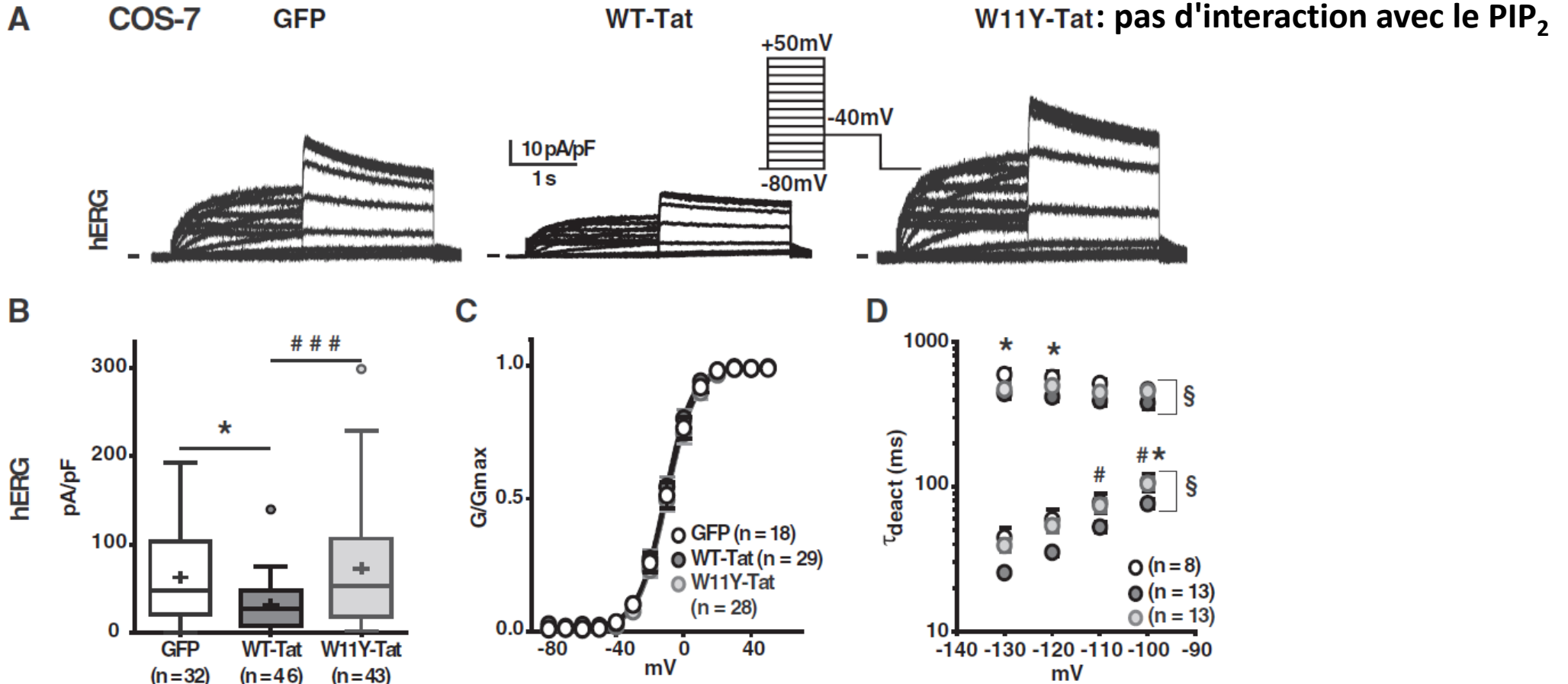
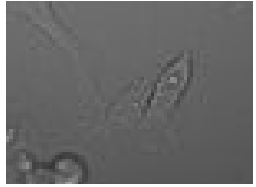


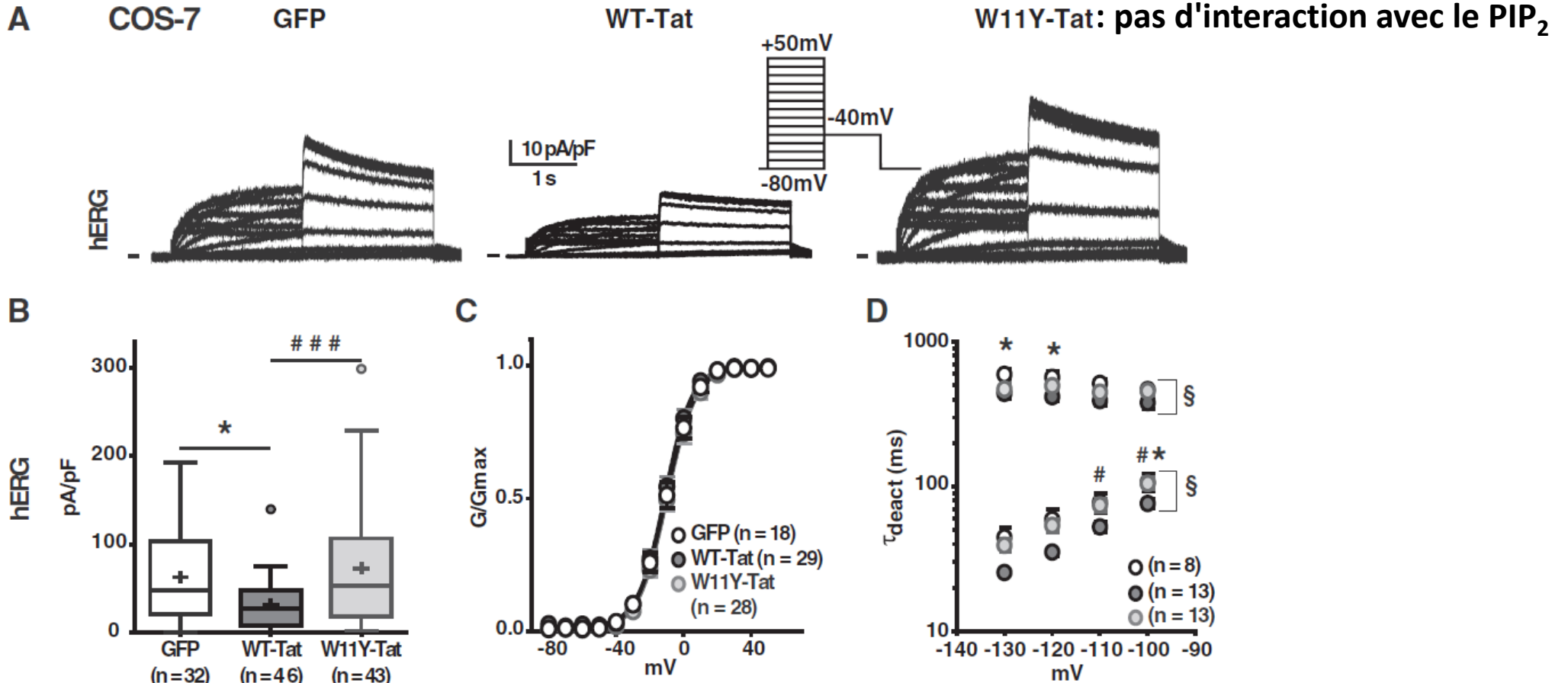
Figure 1



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

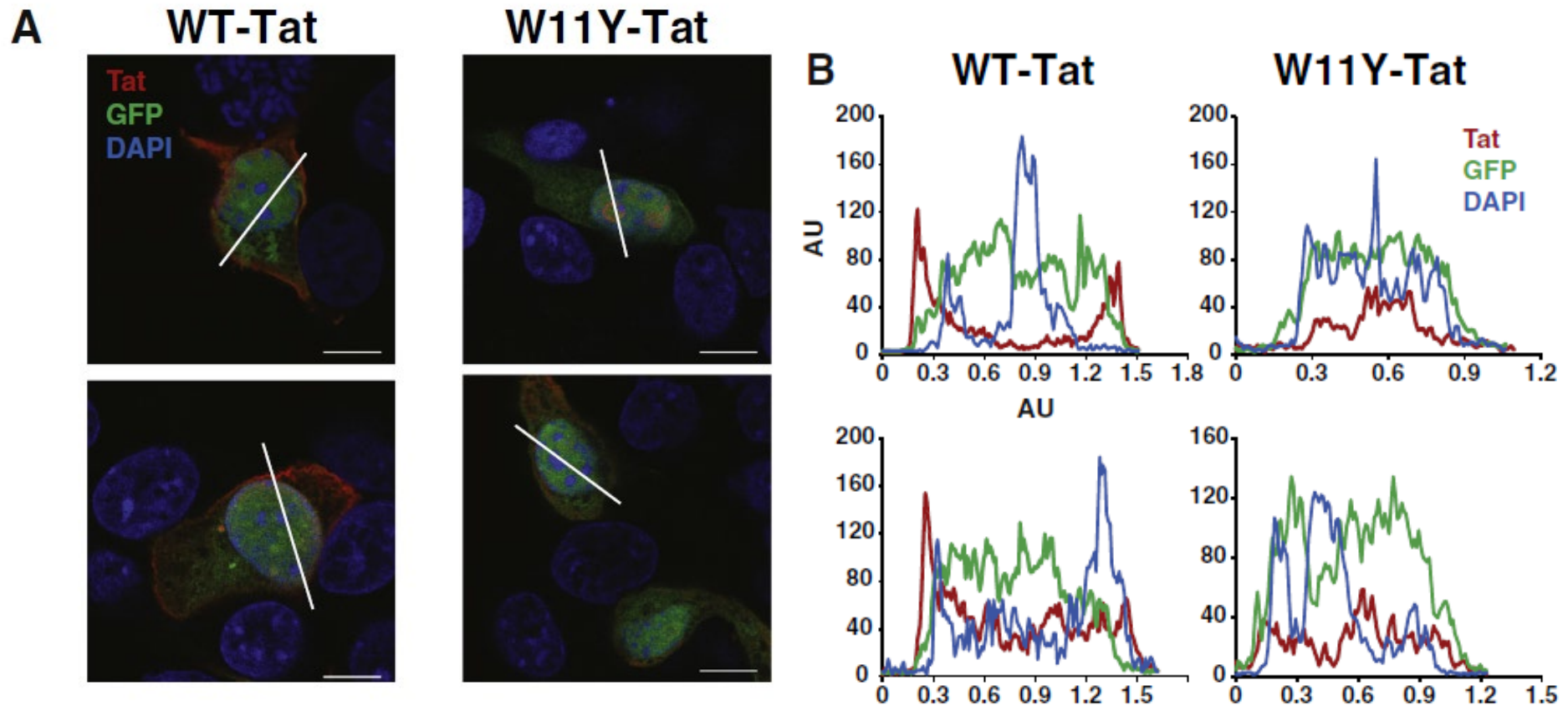


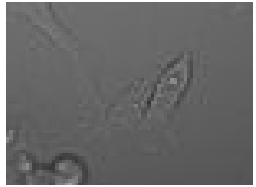
cellules
COS-7

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



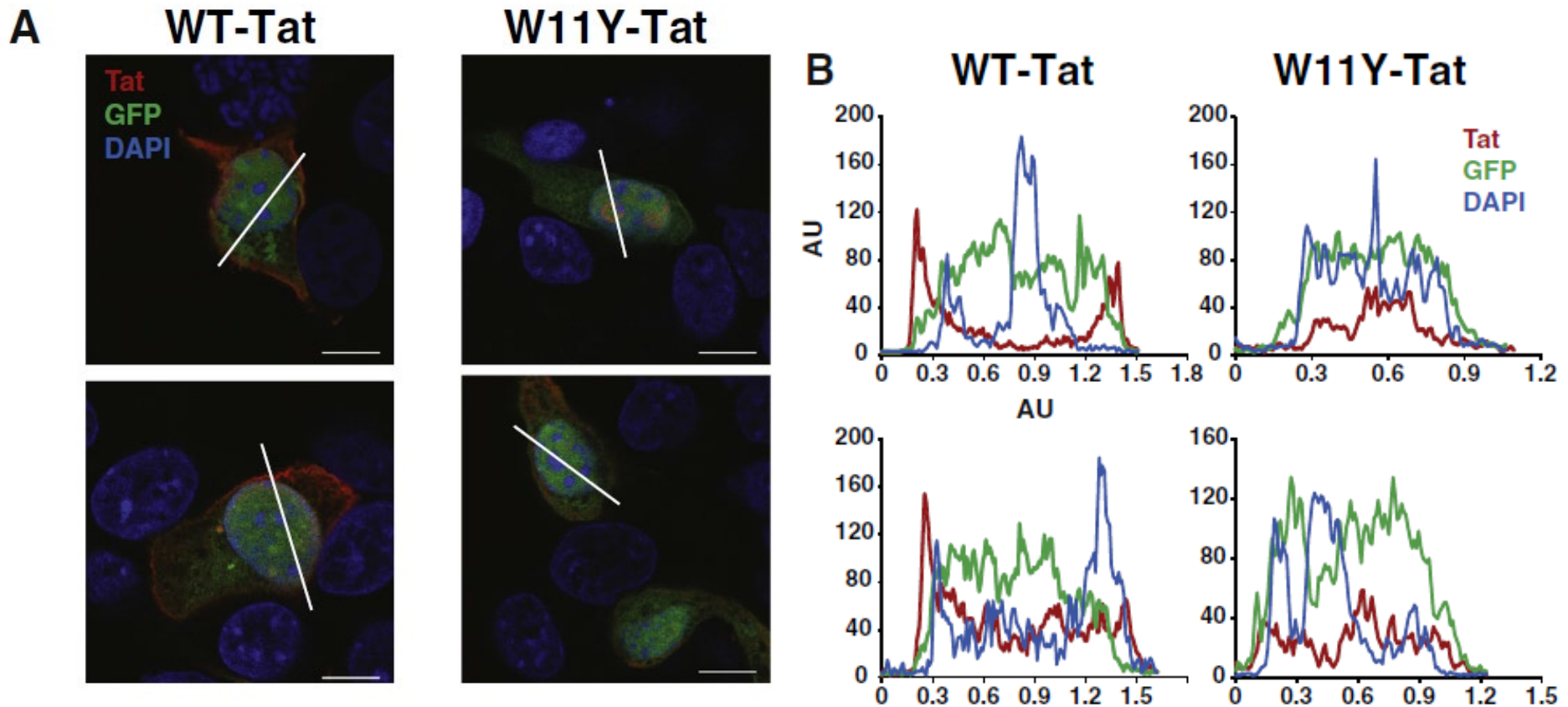


cellules
COS-7

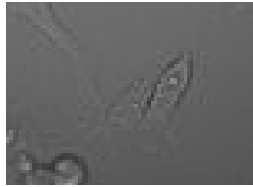
Condition : sur-expression du canal hERG et de la protéine Tat (WT et mutée) après transfection (plasmides)

- 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



cellules
COS-7

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

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

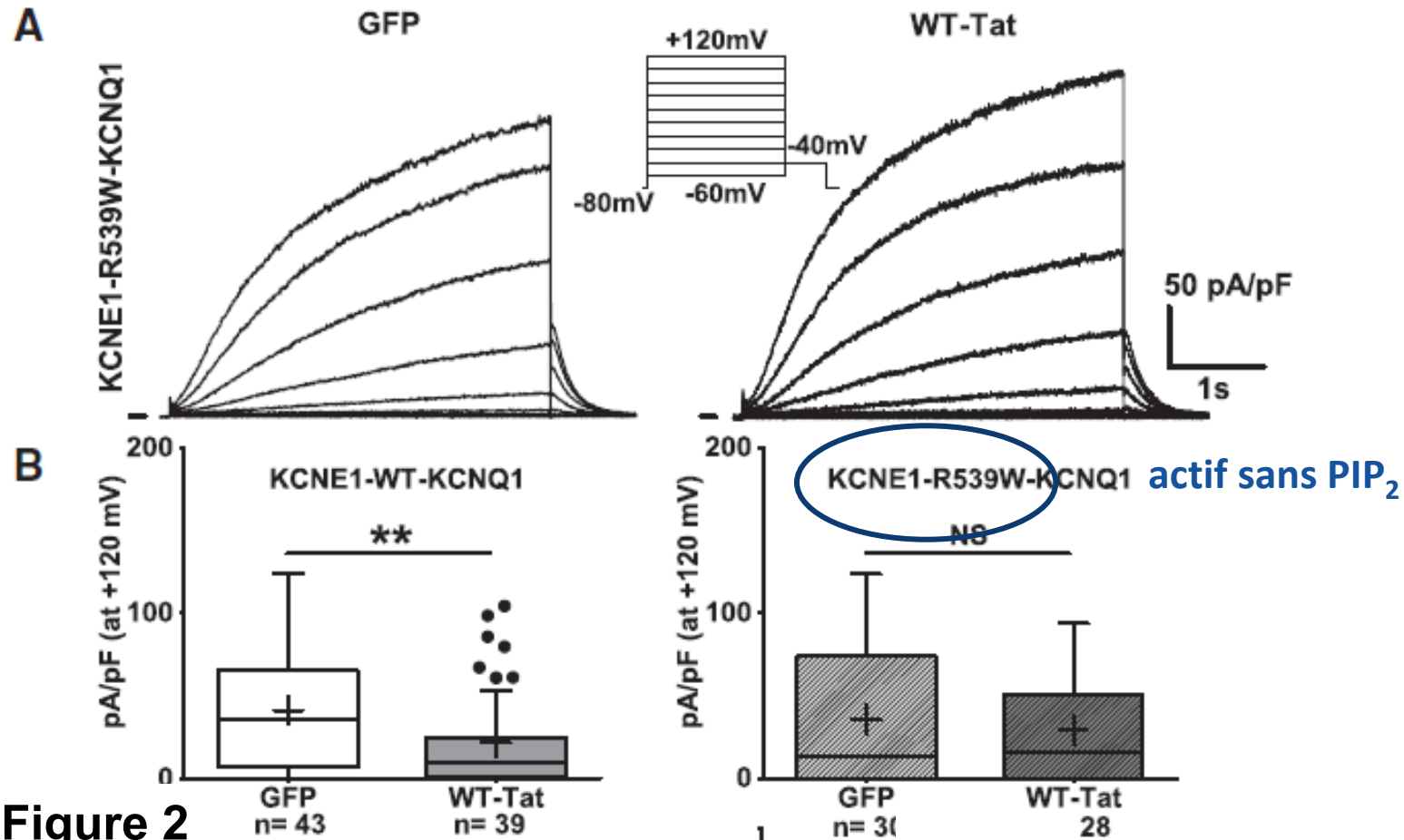


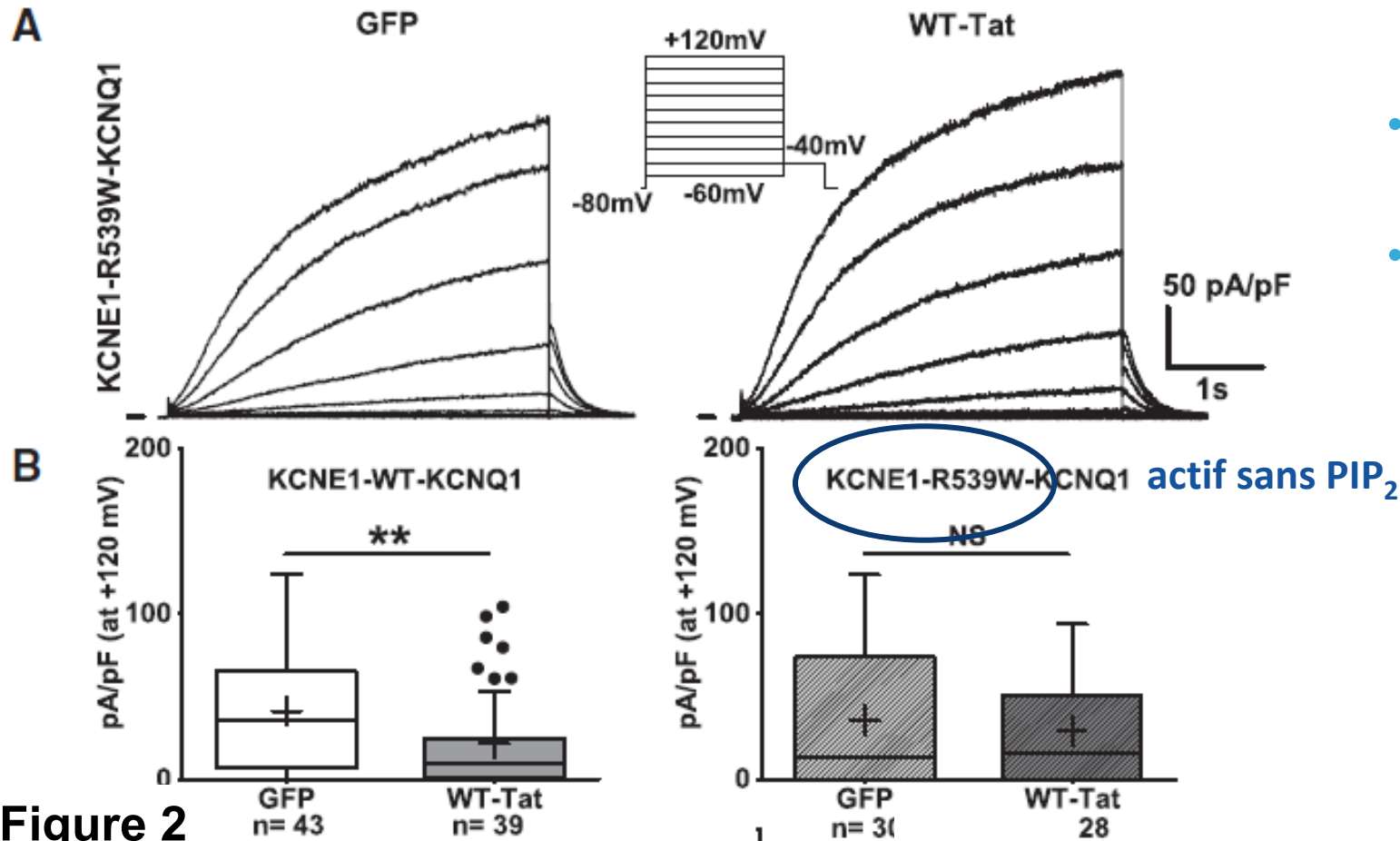
Figure 2



cellules
COS-7

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

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



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

Figure 2

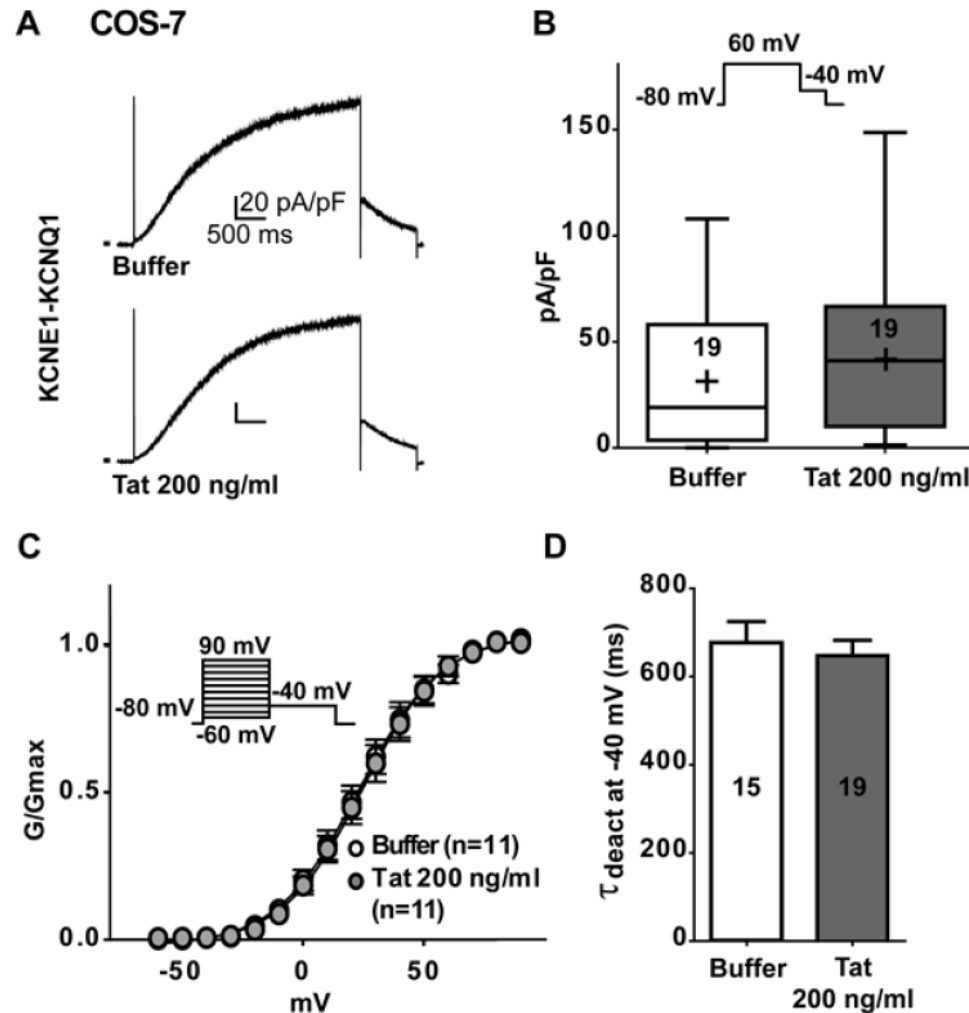


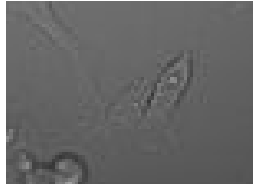
cellules
COS-7

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

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

Suppl.
Figure 4



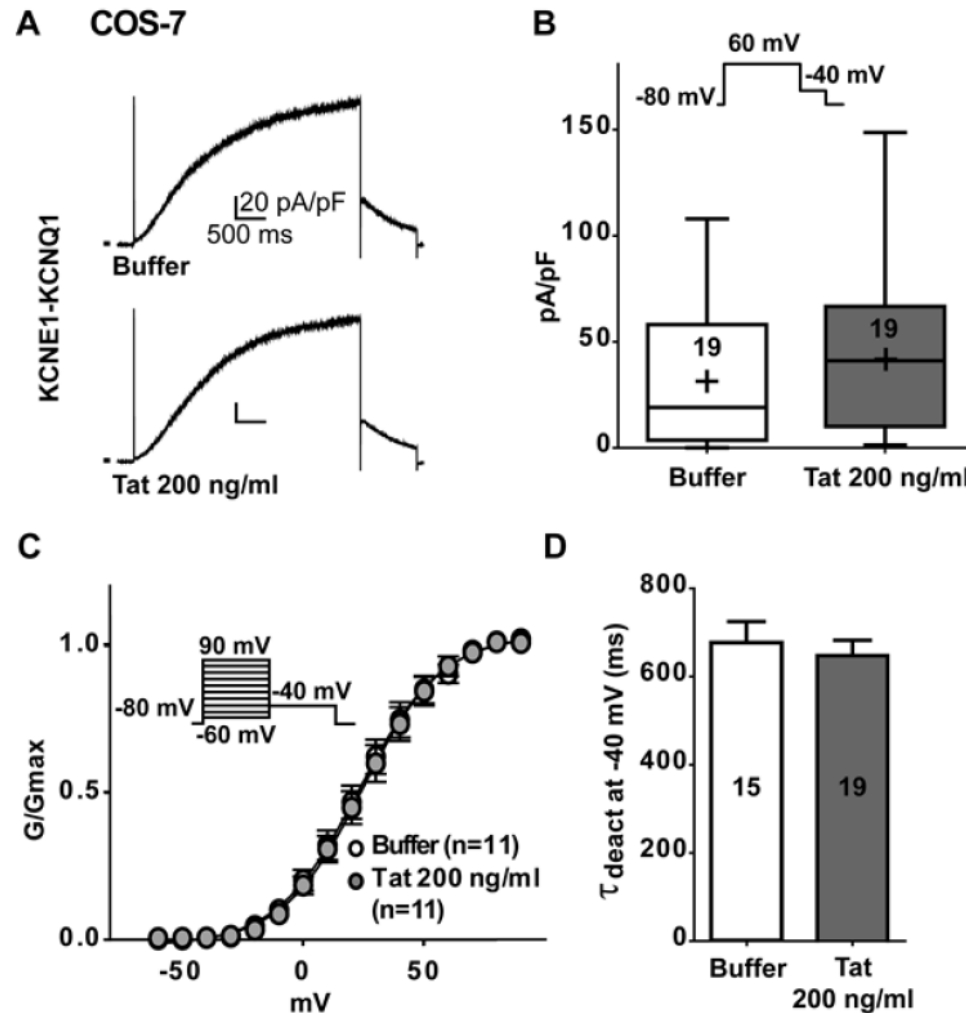


cellules
COS-7

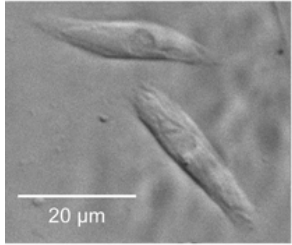
Condition : sur-expression du canal KCNE1-WT- KCNQ1 après transfection (plasmides), **application extracellulaire de la protéine Tat**

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

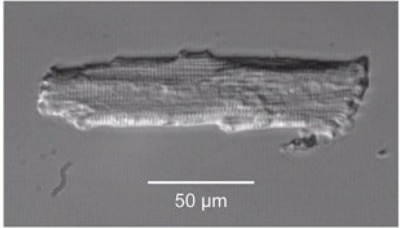
Suppl.
Figure 4



- application de la protéine Tat : pas d'effet



cardiomyocytes
issus de cellules hiPS

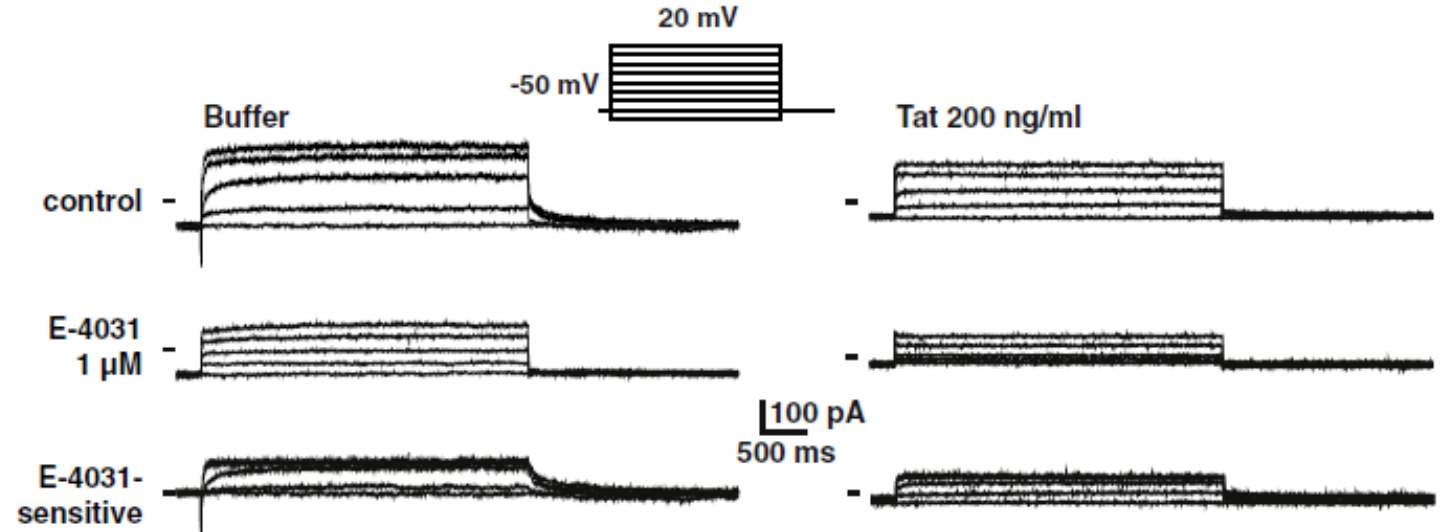


cardiomyocytes humains
fraîchement isolés

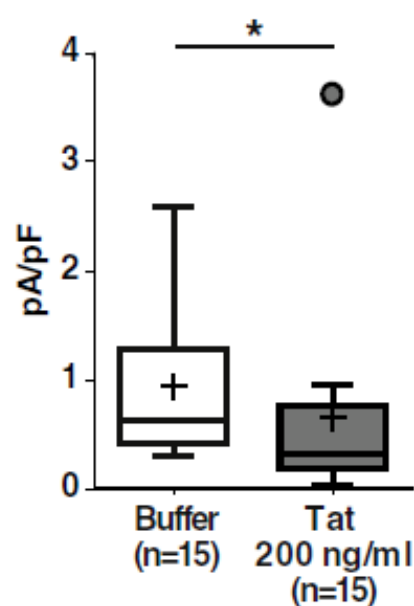
Condition : application extracellulaire de la protéine Tat

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

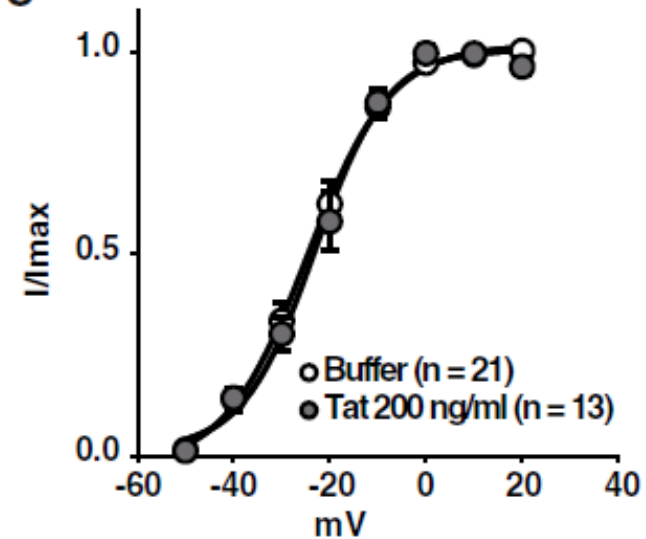
A hiPS-CMs



B



C



D

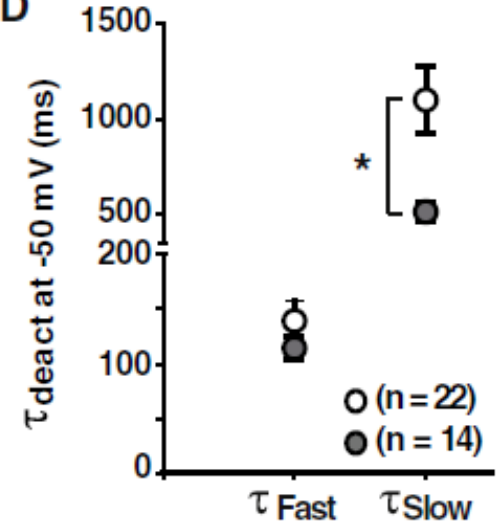
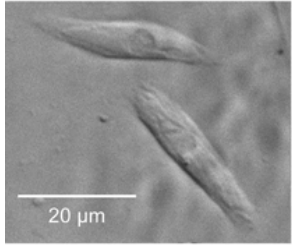
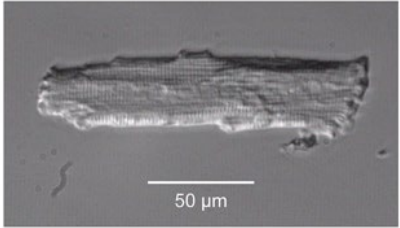


Figure 4



cardiomyocytes
issus de cellules hiPS



cardiomyocytes humains
fraichement 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)

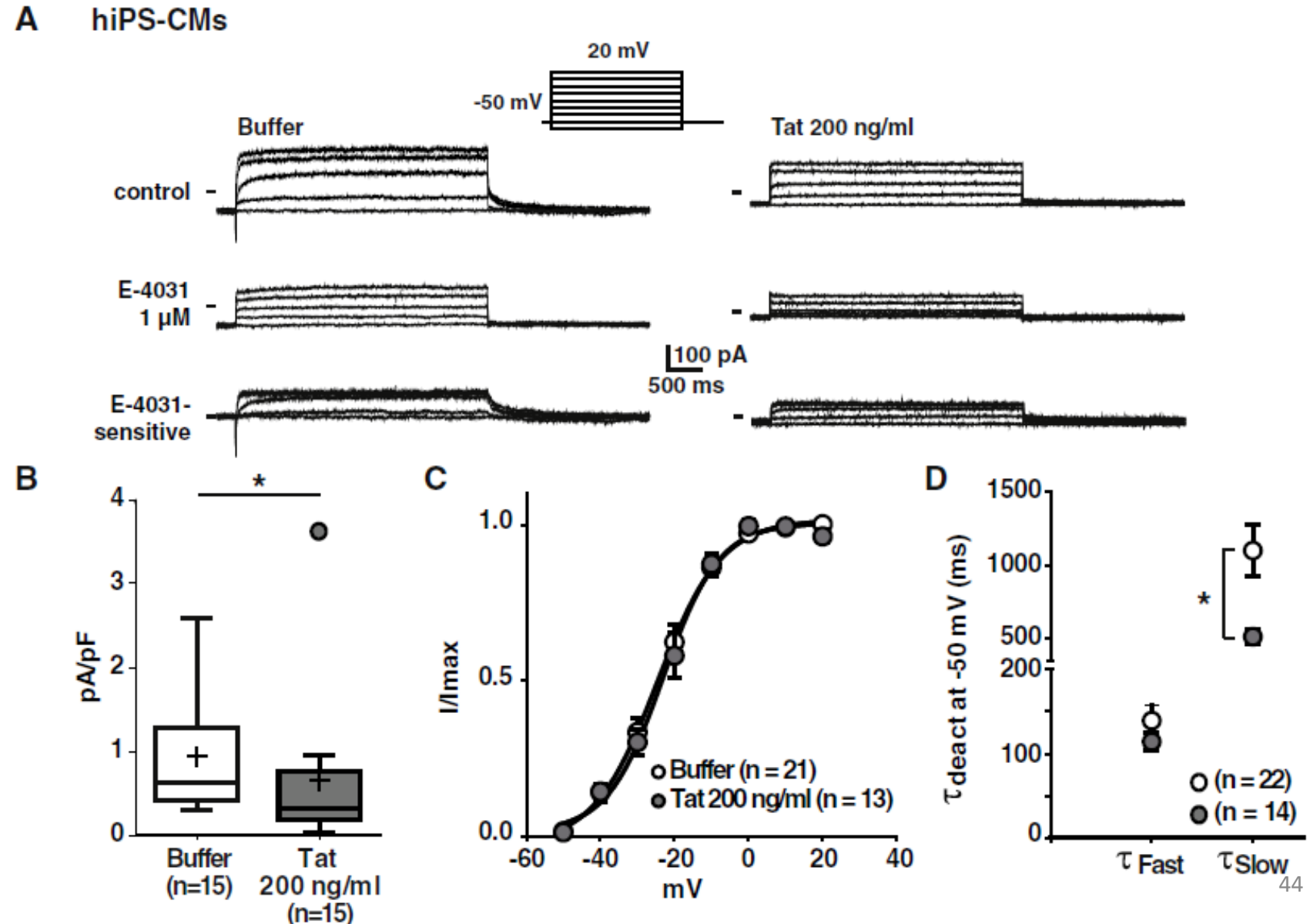
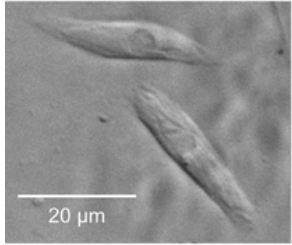


Figure 4



cardiomyocytes
issus de cellules hiPS

Condition : **application extracellulaire de la protéine Tat**

- expression de hERG ARNm et protéine

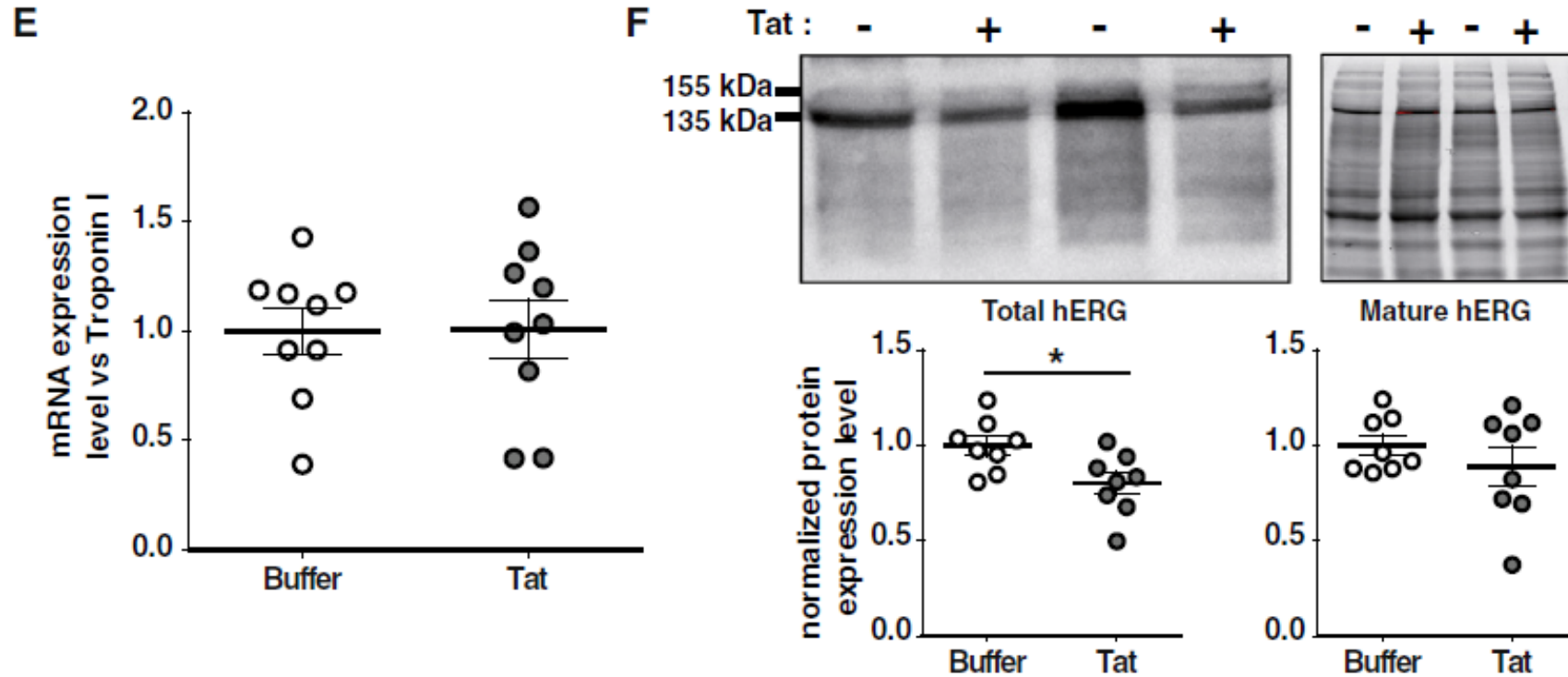
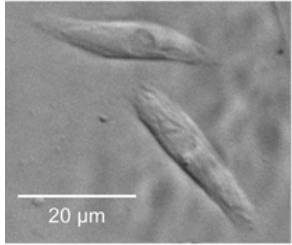


Figure 4



cardiomyocytes
issus de cellules hiPS

Condition : application extracellulaire de la protéine Tat

- expression de hERG ARNm et protéine

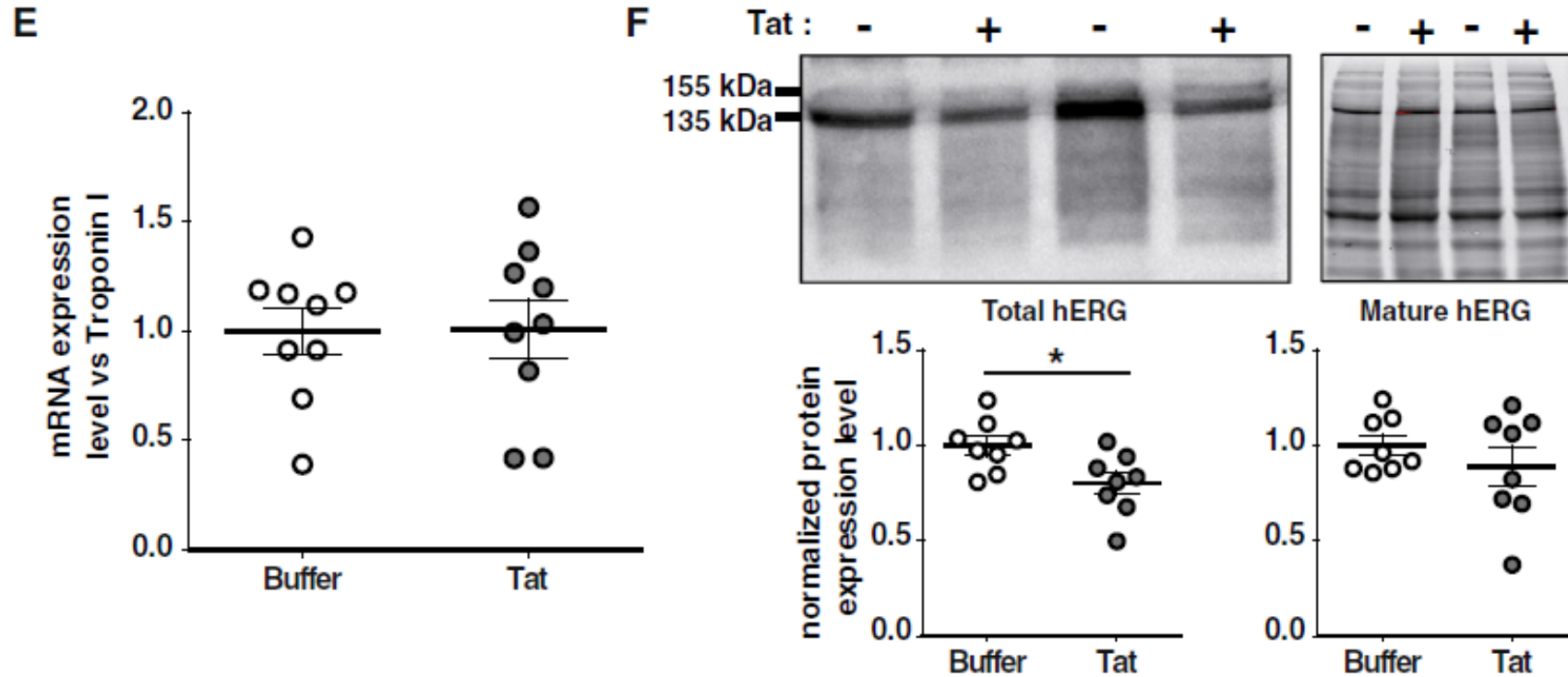


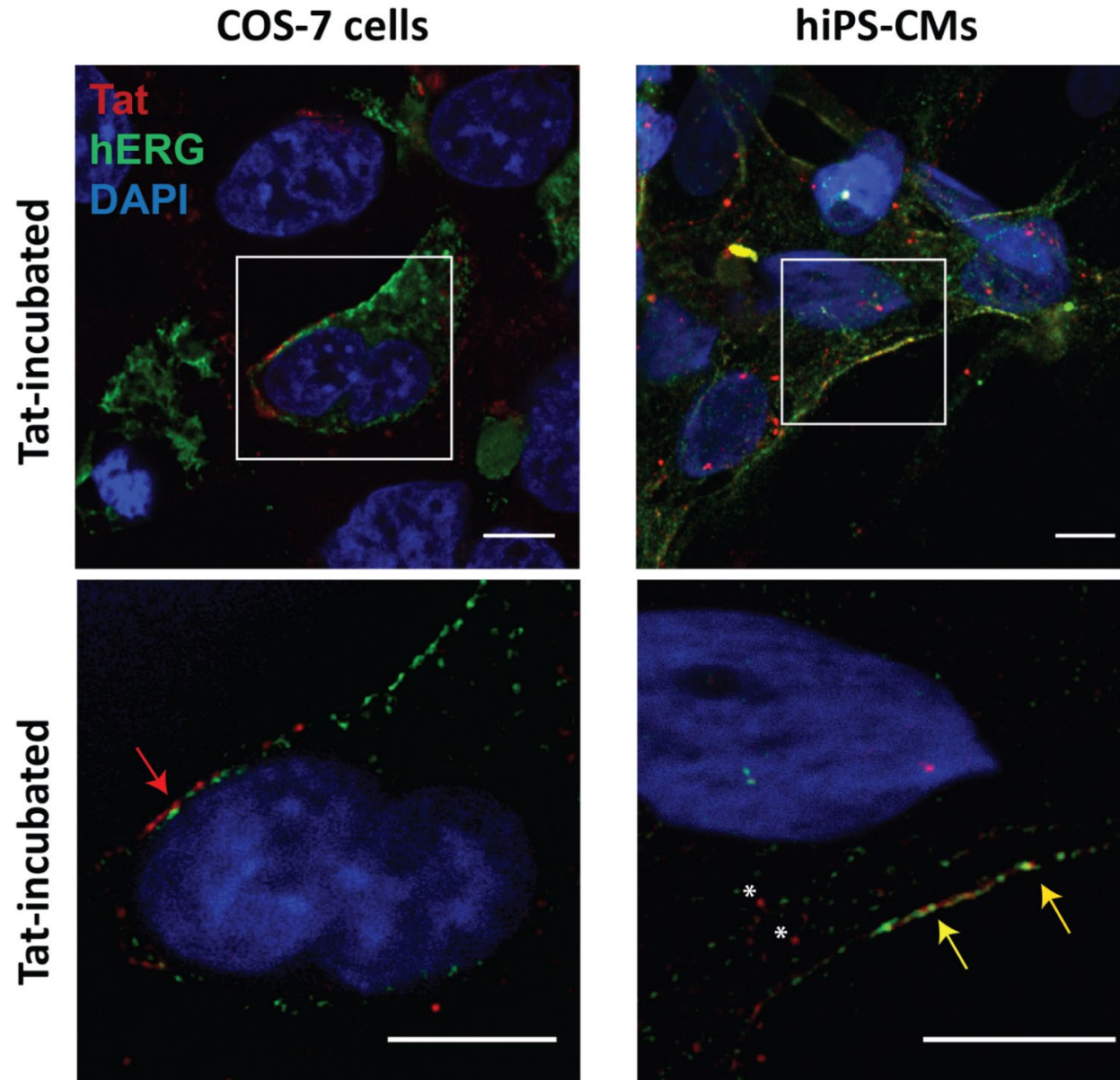
Figure 4

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

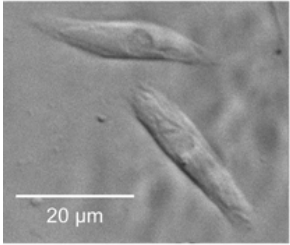
Condition : **application extracellulaire de la protéine Tat**

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

Figure 5



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



cardiomyocytes
issus de cellules hiPS

Condition : **application extracellulaire de la protéine Tat**

- **potentiel d'action**

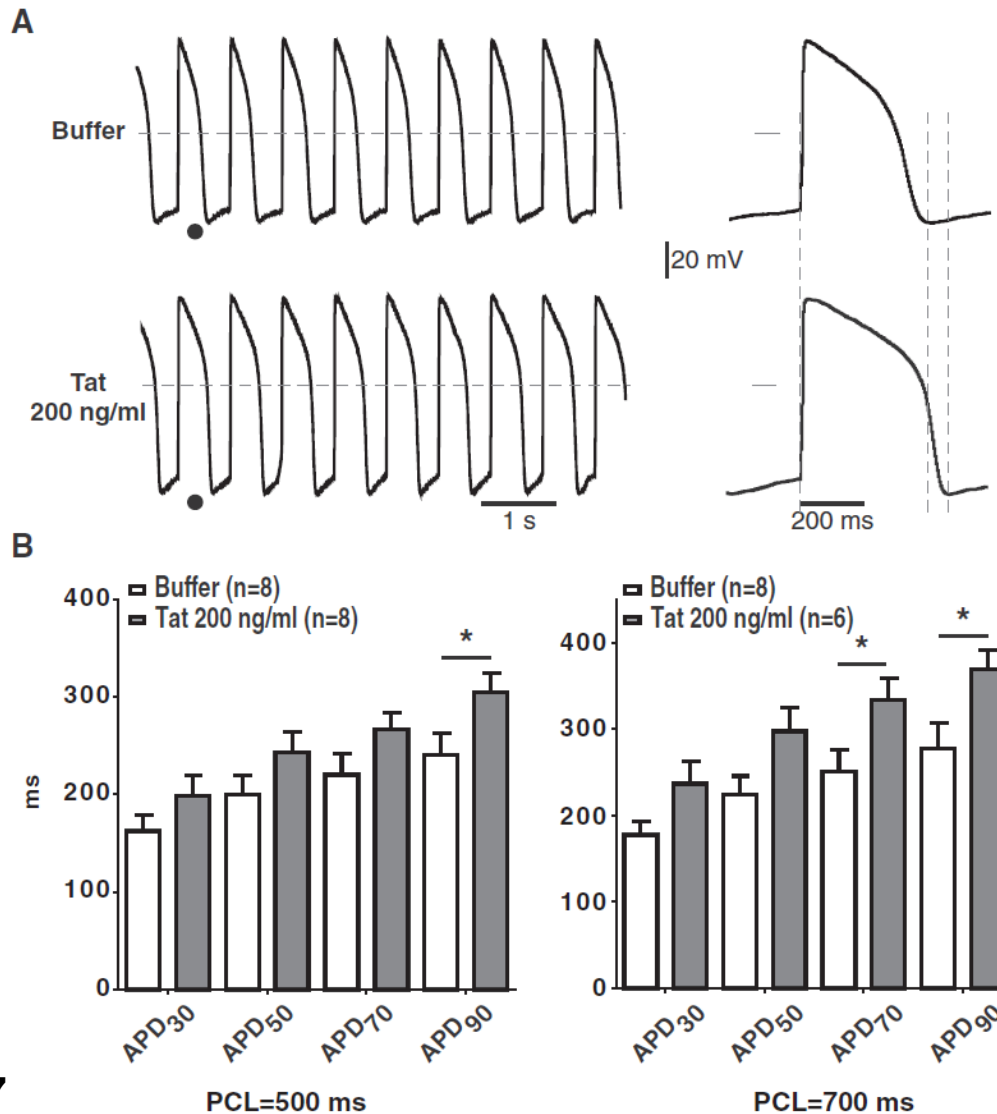
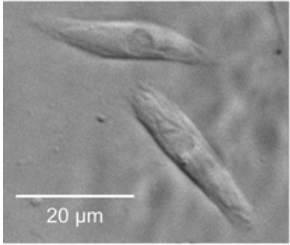


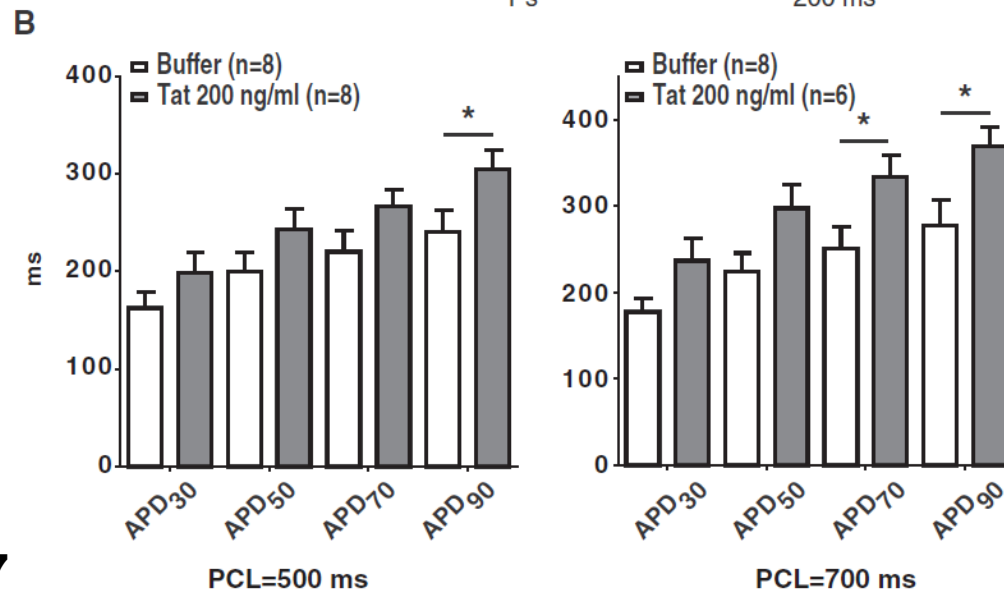
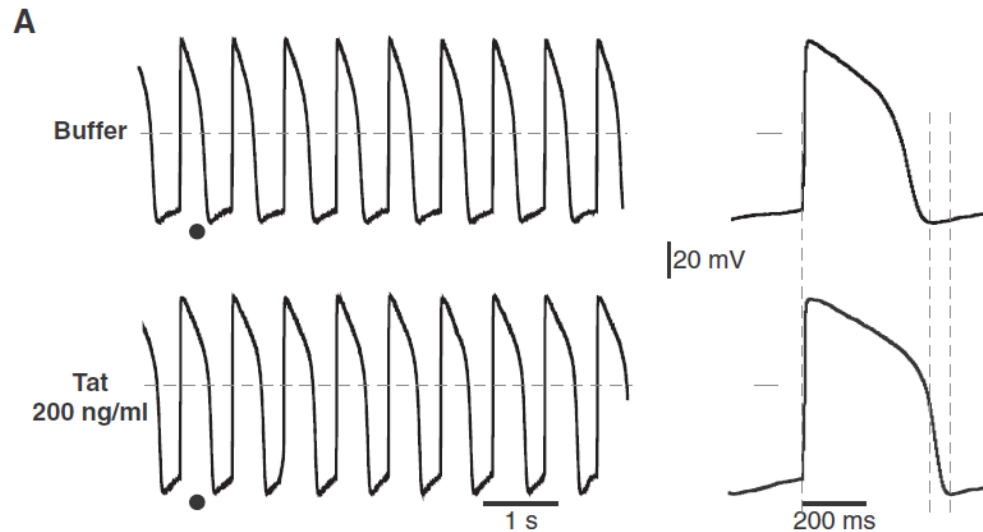
Figure 7



cardiomyocytes
issus de cellules hiPS

Condition : application **extracellulaire de la protéine Tat**

- **potentiel d'action**

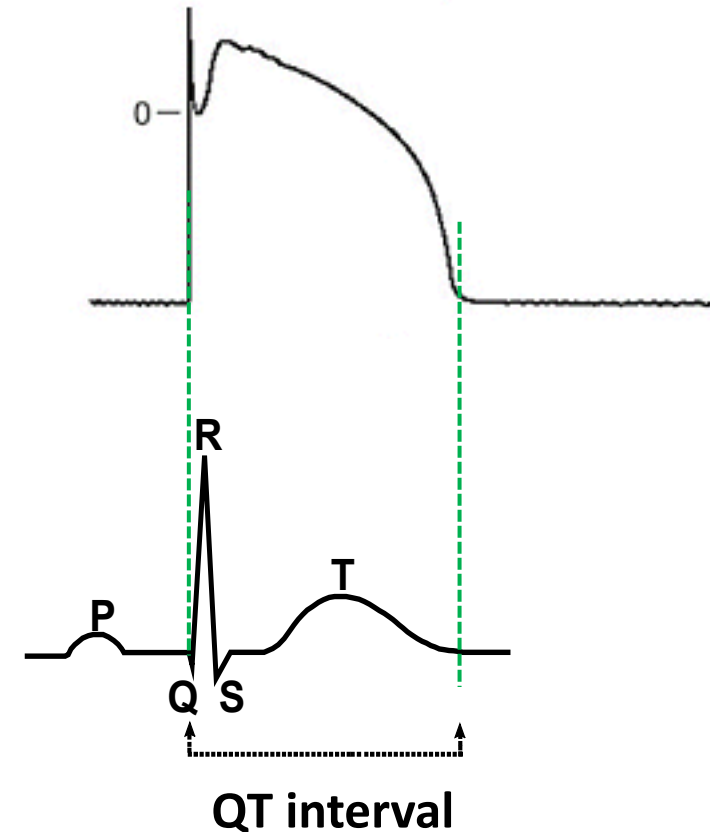
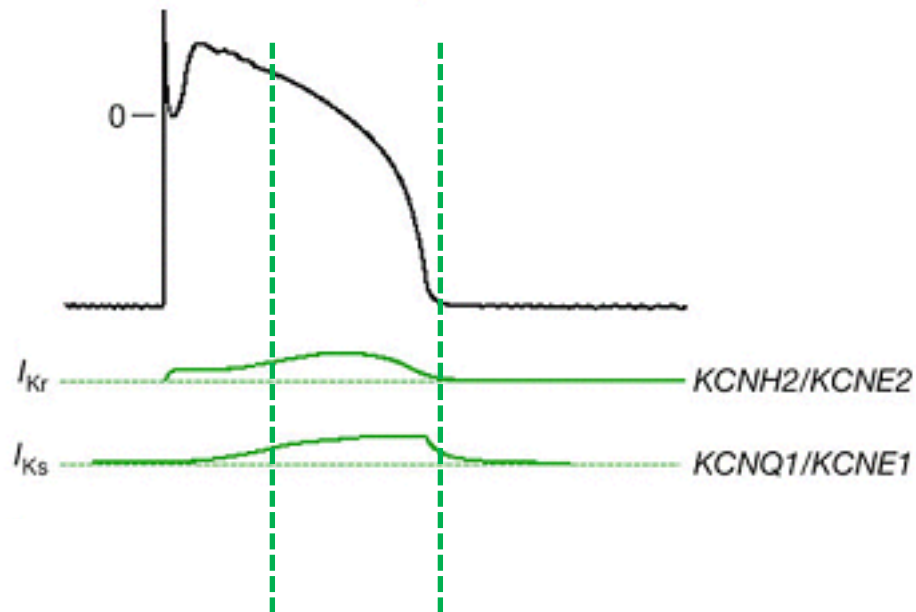


- **allongement de la durée du potentiel d'action induit par la Tat (phases tardives)**

Figure 7

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."

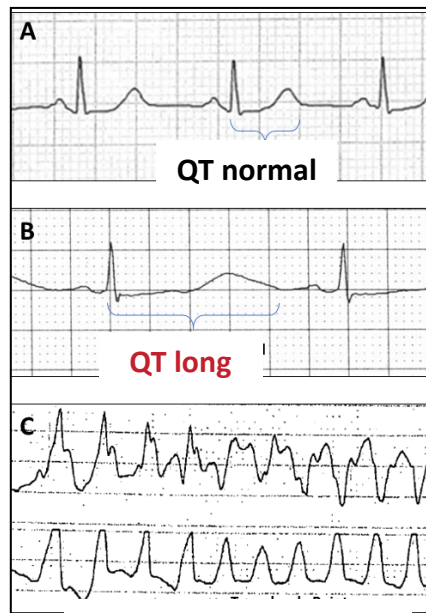


Syndrome du QT long congénital : variants de *KCNH2*

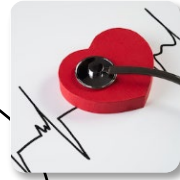
Contexte du projet

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

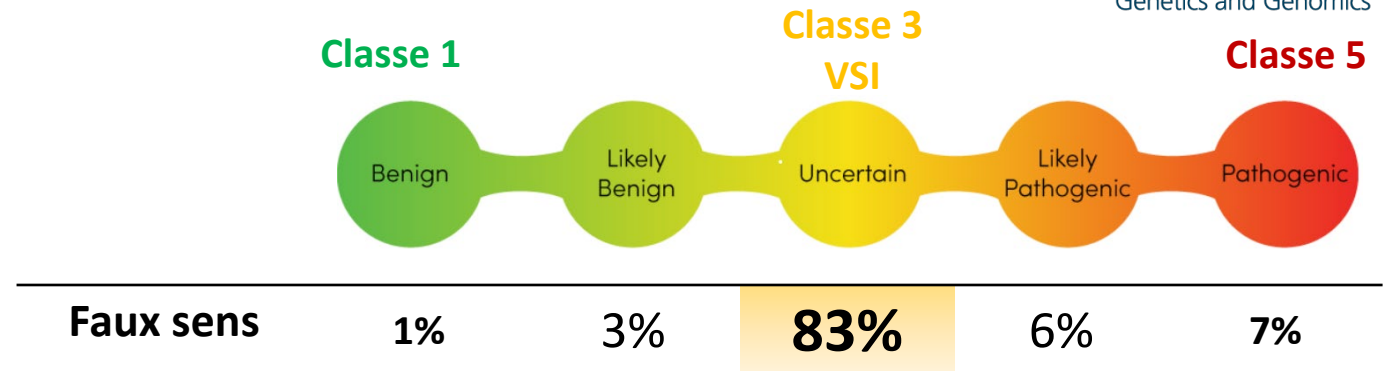
ECG



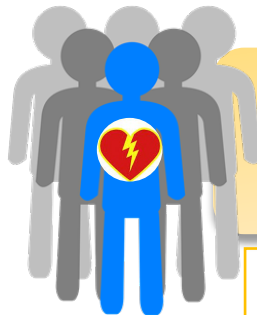
Syncopes et mort subite



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



VSI: Variants de Signification Inconnue



Besoin clinique: caractérisation fonctionnelle à haut débit de variants VSI

Stratification optimale du risque rythmique des patients

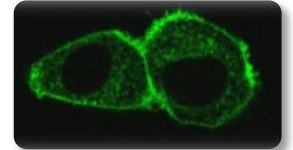
Caractérisation de l'ensemble des variants du gène *KCNH2*

Caractérisation multiparamétrique

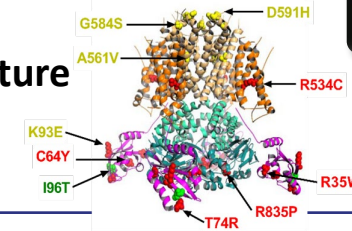


patch-clamp automatisé à haut débit
(384 cellules en simultanément)

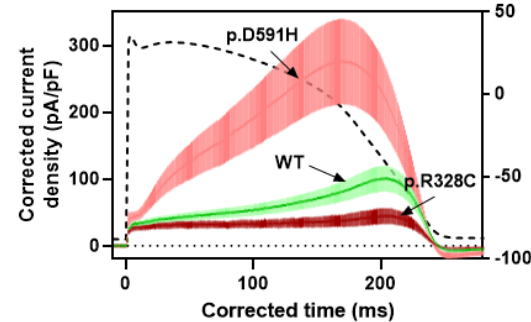
trafic à la membrane



structure



Score de pathogénicité moléculaire



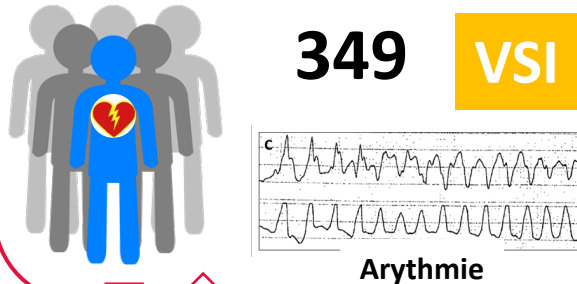
pouvoir de repolarisation



Variants bénins et pathogènes

Score final de pathogénicité pour le patient

= score moléculaire + score génétique



Informations
cliniques

Diagnostic
complet

Base de données
internationale
dynamique



Informations fonctionnelles complètes + génétiques + cliniques

Oliveira-Mendes BBR *et al.* Clin Transl Med. 2023
May;13(5):e1266.

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iPSC core facility

Libanese University
Team 045: Stem cells
Kasem Zibara

l'institut du thorax CIC
Vincent Probst
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Fundings



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