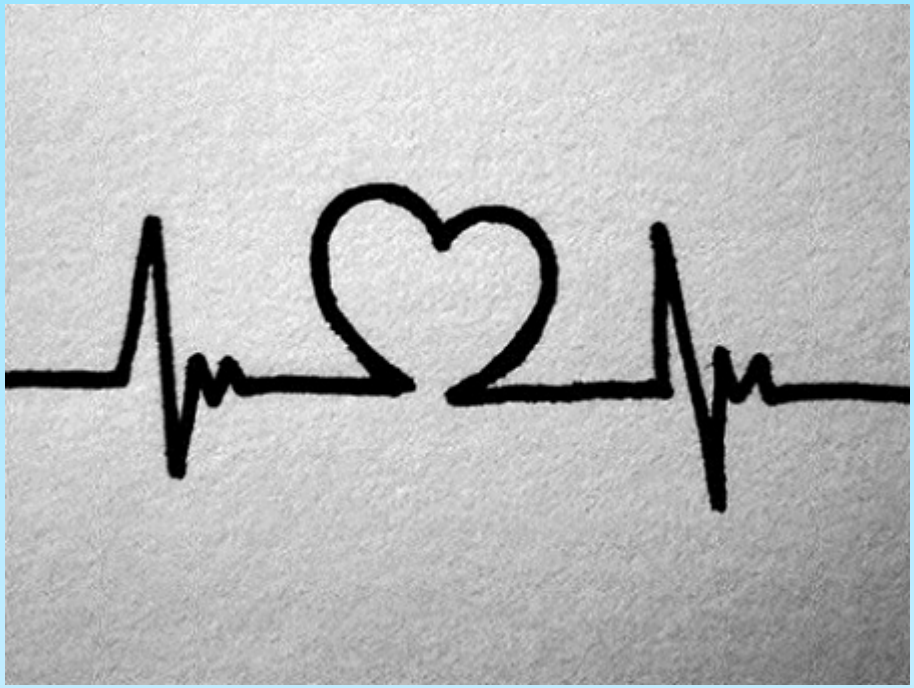
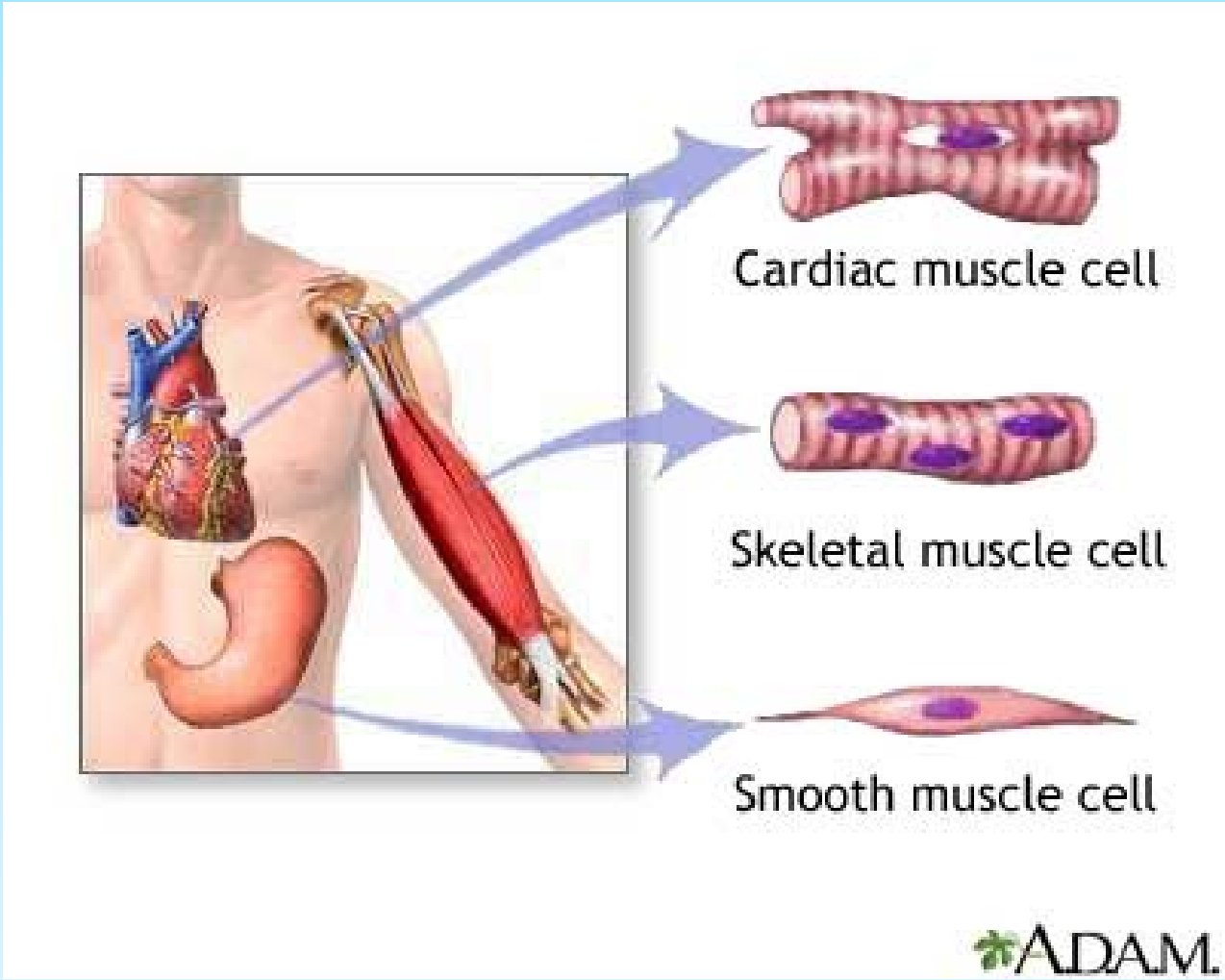
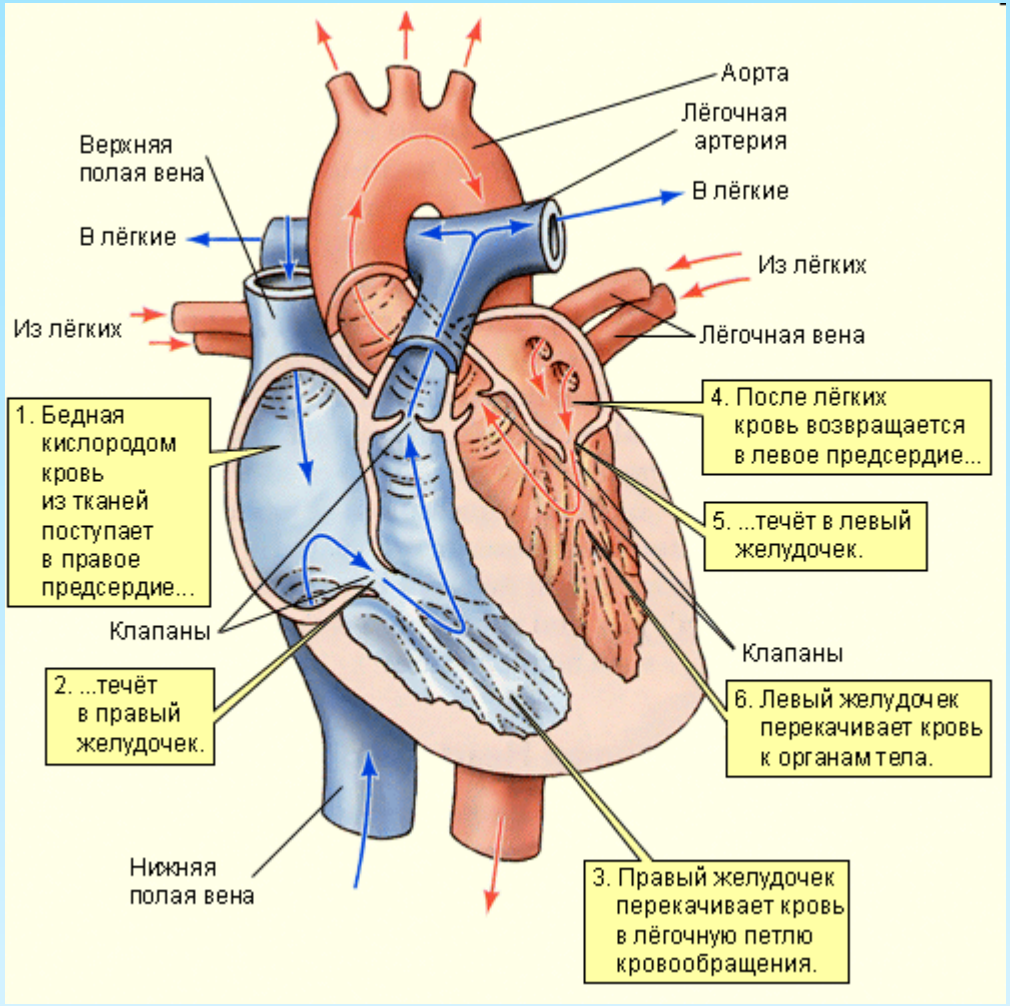


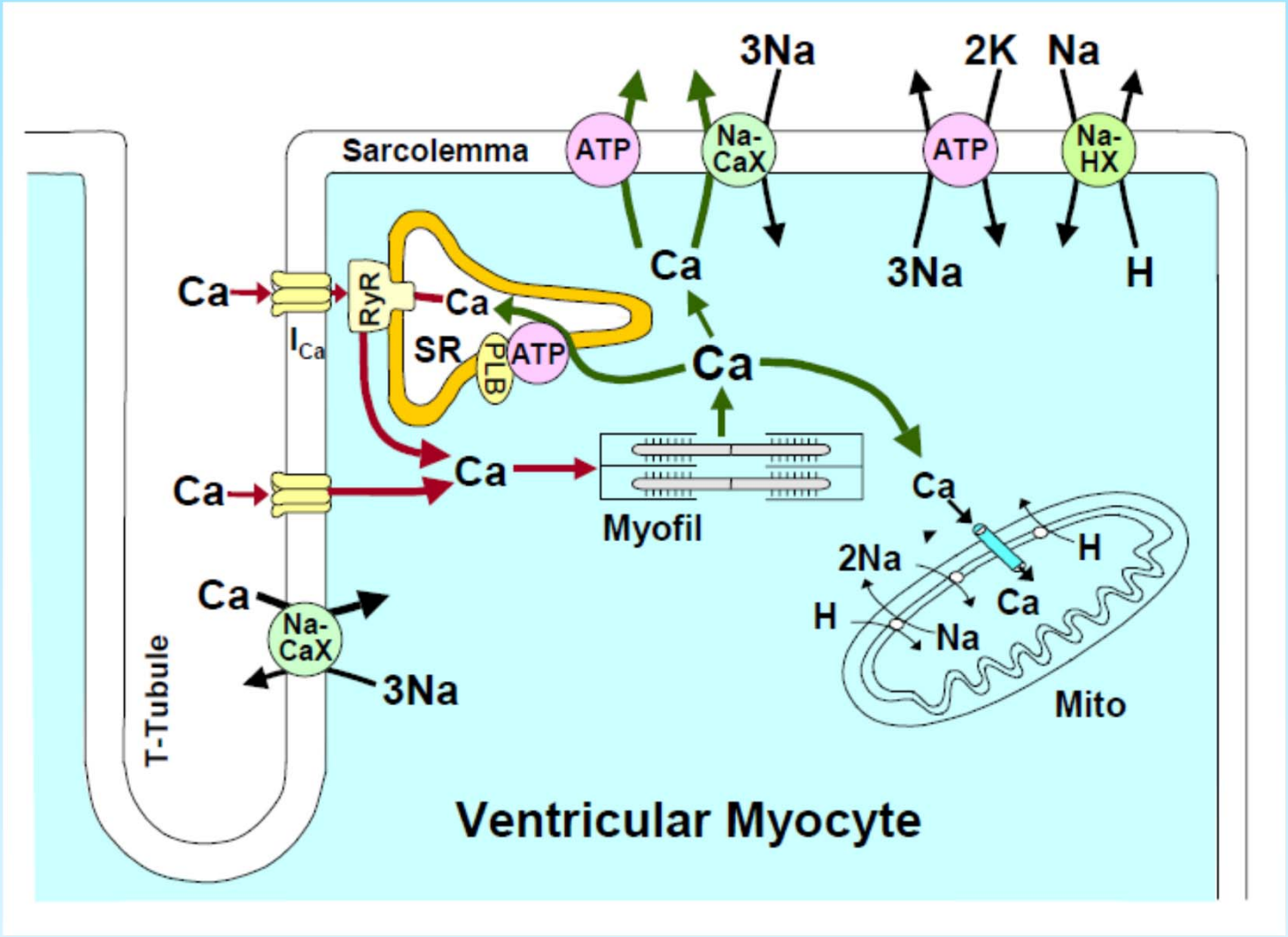
Биофизика возбуждения-сокращения кардиомиоцитов

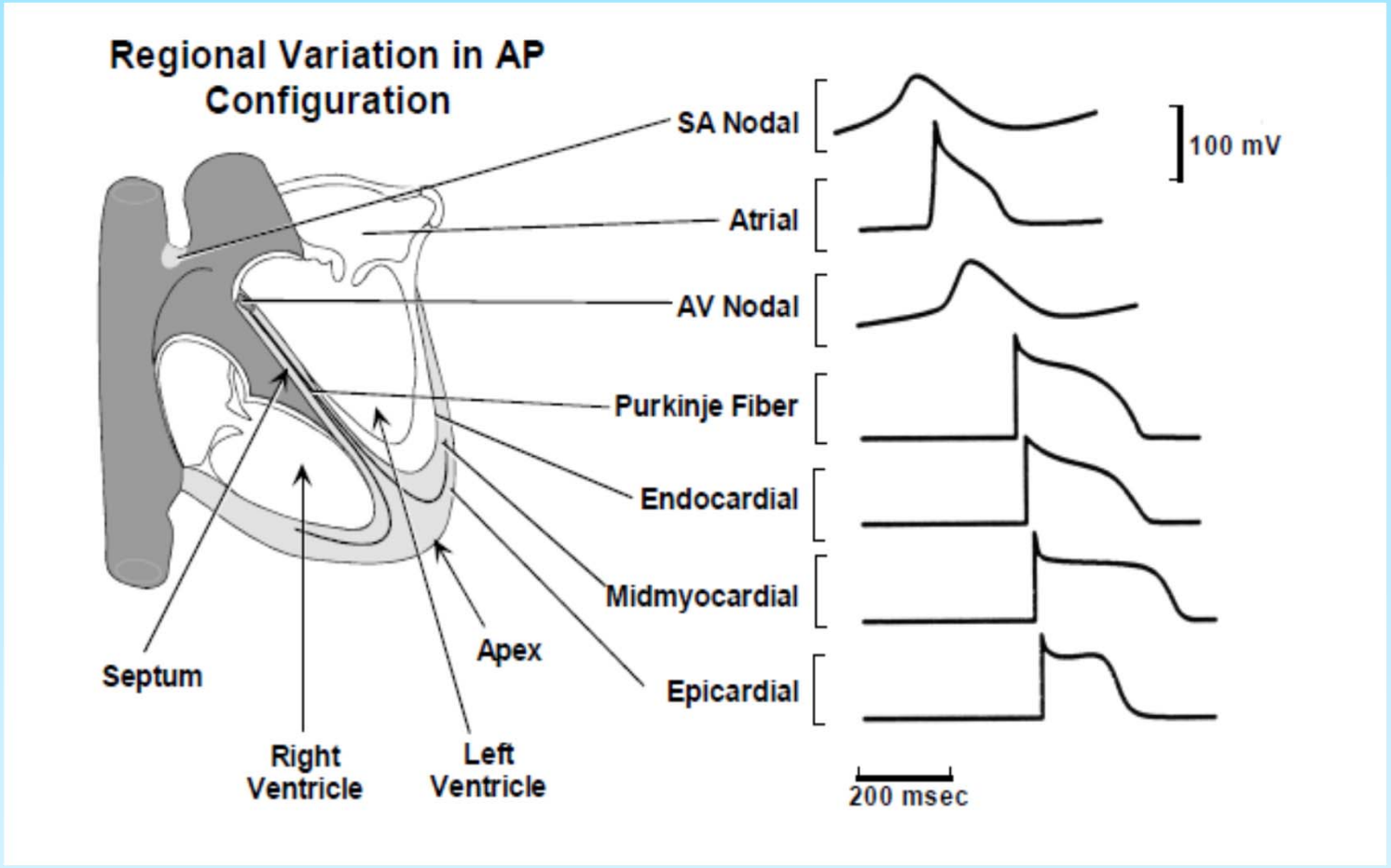
Алексей Вадимович Грищенко

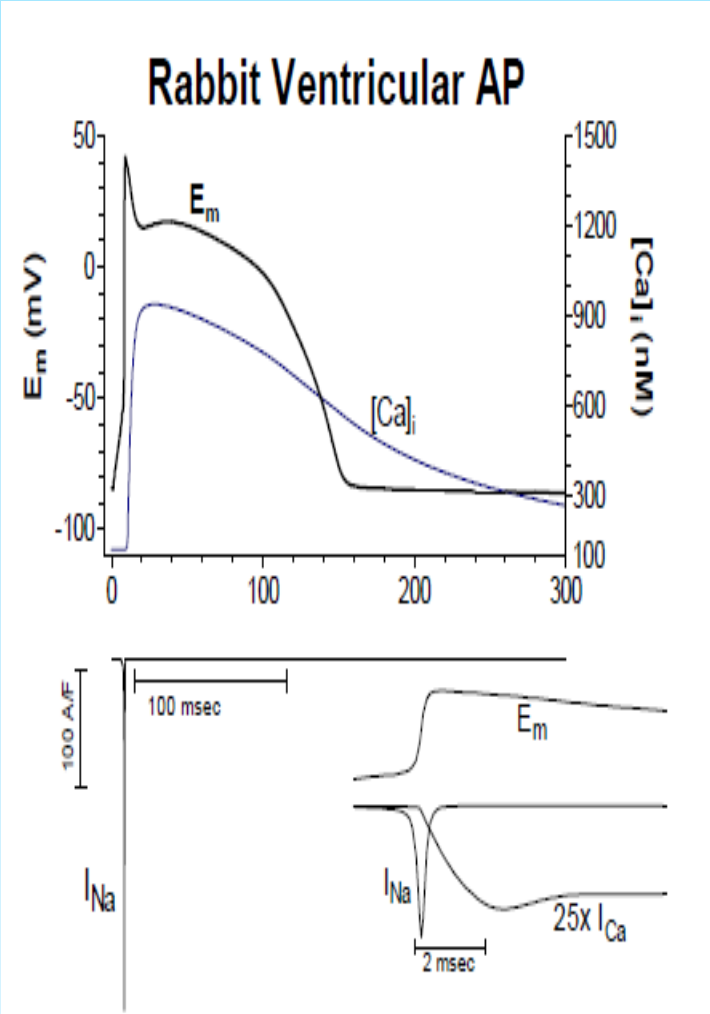
Киев 2015

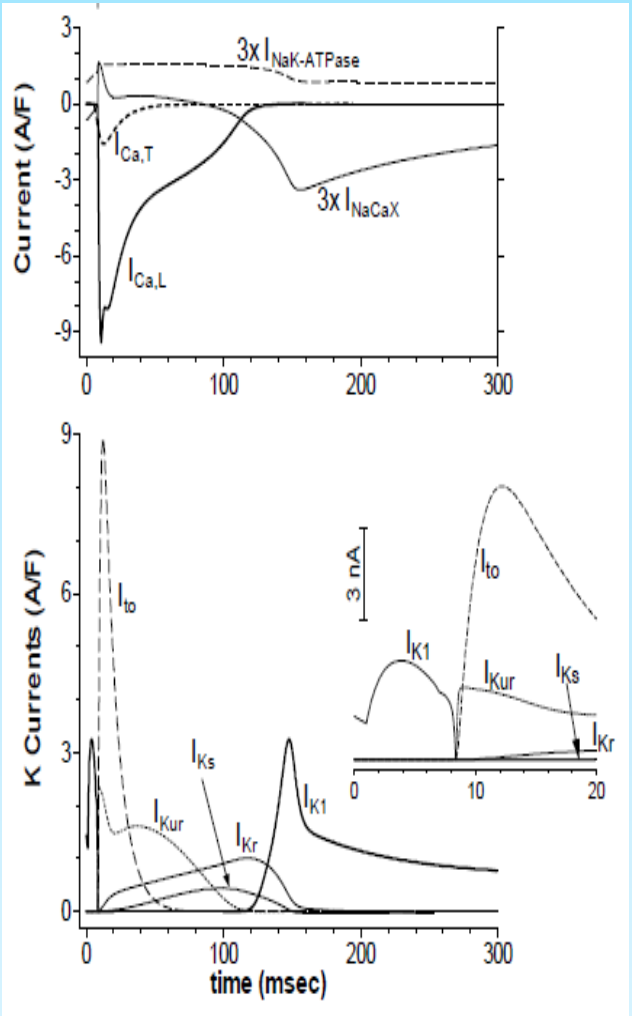

Cardiac muscle cell
Skeletal muscle cell
Smooth muscle cell
A.D.A.M.


Аорта
Лёгочная артерия
В лёгкие
Из лёгких
Лёгочная вена
Верхняя полая вена
В лёгкие
Из лёгких
1. Бедная кислородом кровь из тканей поступает в правое предсердие...
Клапаны
2. ...течёт в правый желудочек.
Нижняя полая вена
4. После лёгких кровь возвращается в левое предсердие...
5. ...течёт в левый желудочек.
Клапаны
6. Левый желудочек перекачивает кровь к органам тела.
3. Правый желудочек перекачивает кровь в лёгочную петлю кровообращения.


3Na
2K
Na
Sarcolemma
ATP
Na-CaX
ATP
Na-HX
Ca
3Na
H
Ca
RyR
Ca
SR
PLB
ATP
Ca
I_{Ca}
Ca
Ca
Myofil
Ca
H
2Na
Ca
H
Na
Ca
Na-CaX
3Na
T-Tubule
Mito
Ventricular Myocyte


Regional Variation in AP Configuration
SA Nodal
Atrial
AV Nodal
Purkinje Fiber
Endocardial
Midmyocardial
Epicardial
100 mV
200 msec
Septum
Right Ventricle
Apex
Left Ventricle

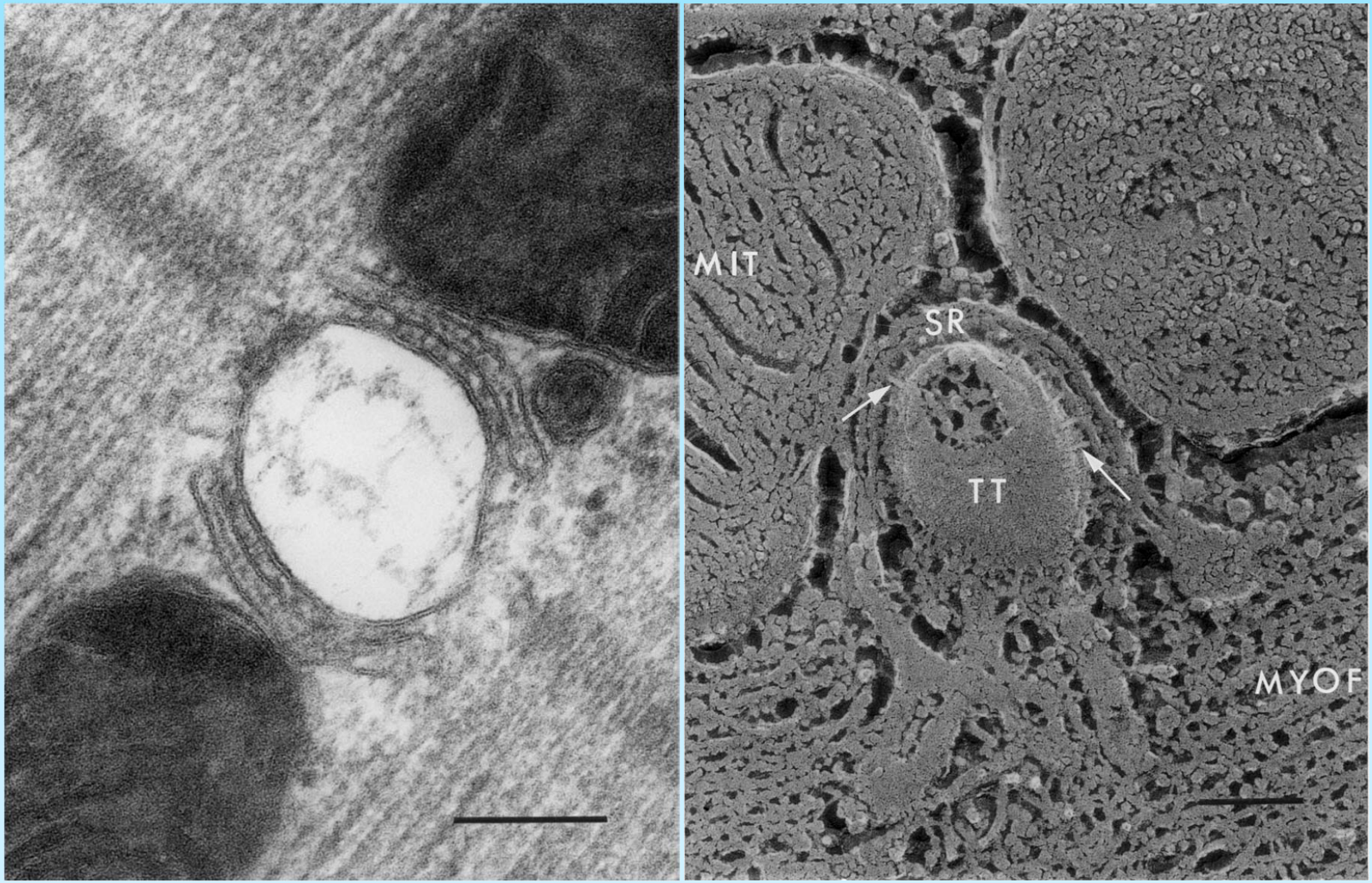

Rabbit Ventricular AP
E_m (mV)
$[Ca]_i$ (nM)
E_m
$[Ca]_i$
100 msec
100 A/F
I_{Na}
E_m
I_{Na}
25x I_{Ca}
2 msec


Current (A/F)
3x $I_{NaK-ATPase}$
$I_{Ca,T}$
3x I_{NaCaX}
$I_{Ca,L}$
K Currents (A/F)
I_{to}
I_{Ks}
I_{Kur}
I_{Kr}
I_{K1}
3 nA
time (msec)

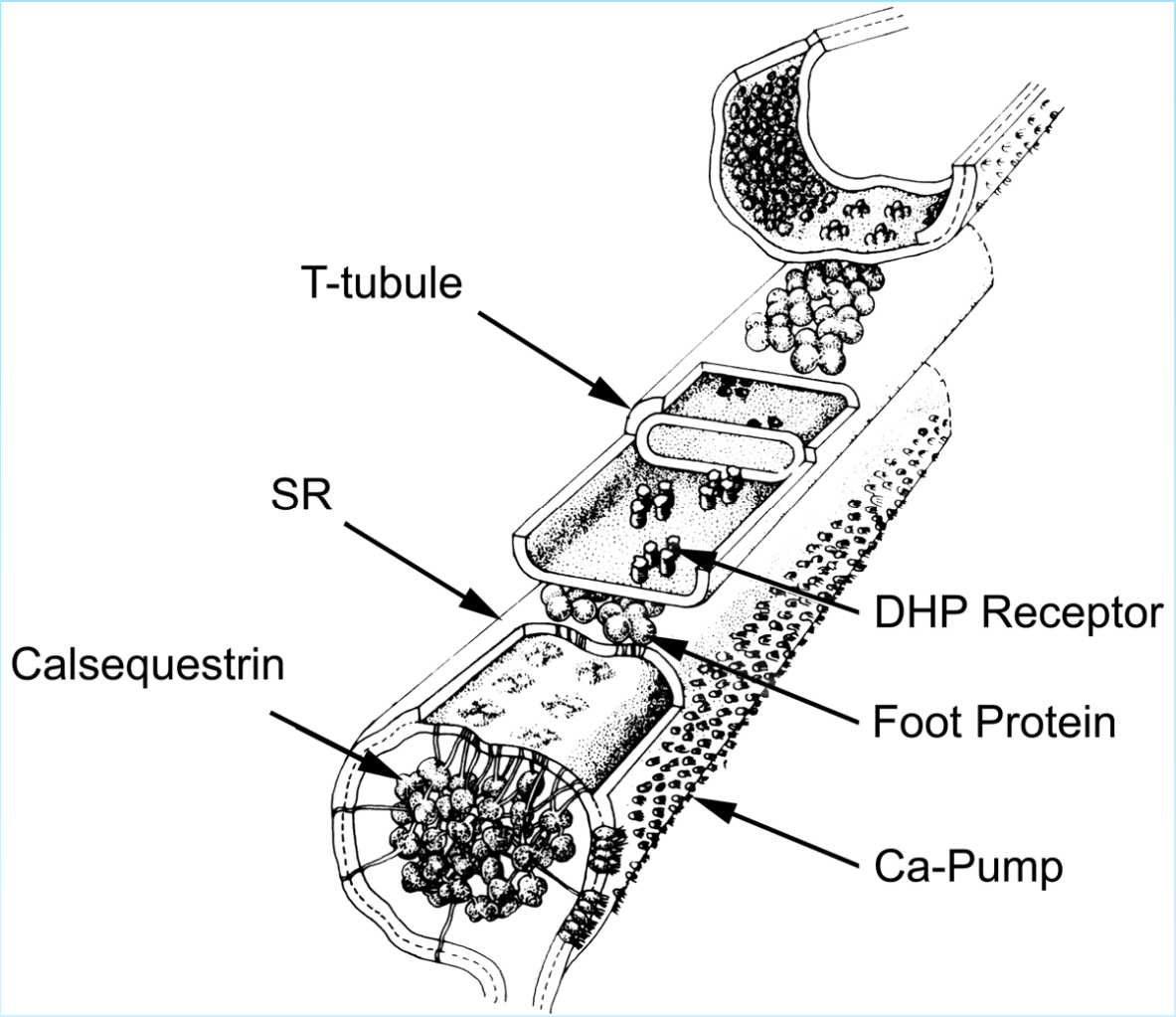
Cardiac Ion Channels

Current	Candidate Gene	Acti-vation	Inacti-vation	Role in AP	Subunits?	Blockers
Voltage gated Channels						
I_{Na}	SCN5A	VVF	VF	Rapid Depol.	β	TTX,STX
$I_{Ca,L}$	α_{1C}, α_{1D}	VF	M	Depol & Plat	$\alpha_2\delta$, β	DHP, ΦAA
$I_{Ca,T}$	α_{1G}, α_{1H}	VF	F	Depol-PMK	β	Mibefradil, Ni
$I_{to,fast}$	Kv4.2, 4.3	VF	F	Early Repol	β	4-AP, 2,3-DAP
$I_{to,slow}$	Kv1.4	VF	M	Early Repol	β	4-AP, 2,3-DAP
I_{Kr}	HERG	M	VF	Plat-Repol	MirP1	Dofetilide, E-4031
I_{Ks}	KvLQT1	VS	x	Plat-Repol	MinK	Chromanol 293
I_{Kur}	Kv1.5	F	x	Plat-Repol		μM 4-AP
I_{Kp}	Kv1.5?	F	x	Plat-Repol		Ba
$I_{K,slow}$	Kv1.2	F	VS	Plat-Repol		TEA
I_{K1}	Kir2.1 (IRK1)	VF	x	Rest E_m		Ba
I_f	HCN2, HCN4	MS	x	PMK		
Ligand Gated Channels						
$I_{K(ACh)}$	Kir 3.1:3.4	ACh		↓ PMK		
$I_{K(ATP)}$	Kir6.2	Pinacidil		↓APD & PMK	SUR	Glibenclamide
$I_{Cl(Ca)}$	?	$[Ca]_i$		Early Repol		DIDS,niflumate
$I_{Cl(cAMP)}$	CFTR	cAMP		↑Repol.		9-AC, DNDS
Mechanosensitive Channels						
$I_{Cl(Swell)}$	ClC-3	Swelling		↓APD?		Gd, DIDS
$I_{NS(stretch)}$	?	Stretch		PMK?		Gd

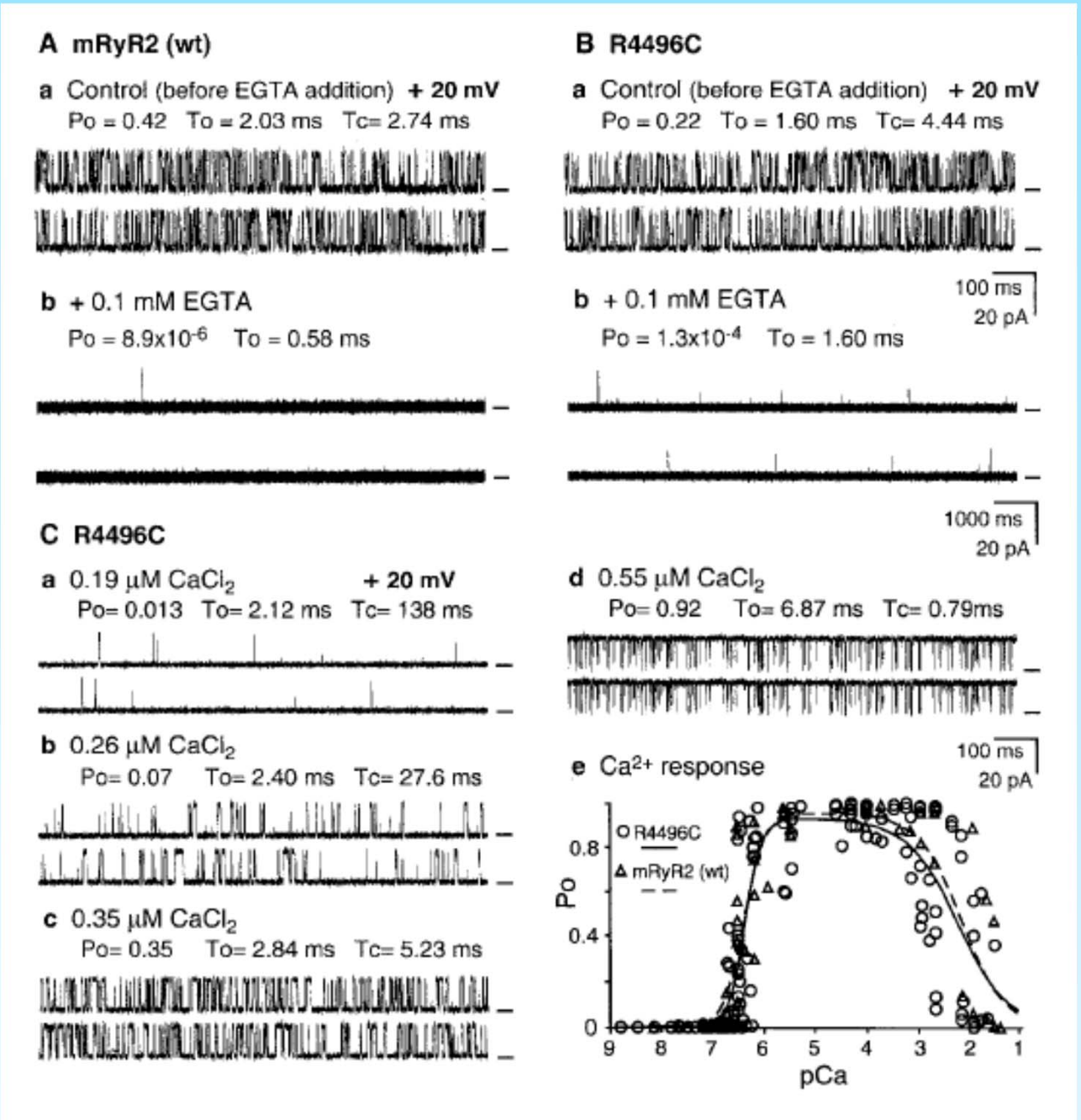
Abbreviations: F=fast, S=slow, M=moderate, V=very and x=none. Depol=depolarization, Repol= repolarization, Plat= plateau, PMK= pacemaker, TTX = tetrodotoxin, STX= saxitoxin, DHP= dihydropyridine, ΦAA=phenylalkylamine, TEA= tetraethylammonium, 4-AP= 4-aminopyridine, 2,3-DAP = 2,3-diaminopyridine, DIDS= 4,4´-diisothiocyanatostilbene - 2,2´-disulphonic acid, DNDS= 4,4´-dinitrostilbene-2,2´-disulphonic acid, 9-AC= 9-aminoacridine, ACh= acetylcholine. The nomenclature for E_m-dependent K channels (Kv) is based on homology to Drosophila gene families referred to as *Shaker*, *Shab*, *Shaw* and *Shal* for Kv1.x, Kv2.x, Kv3.x and Kv4.x (Jan & Jan, 1992; Pongs, 1992).

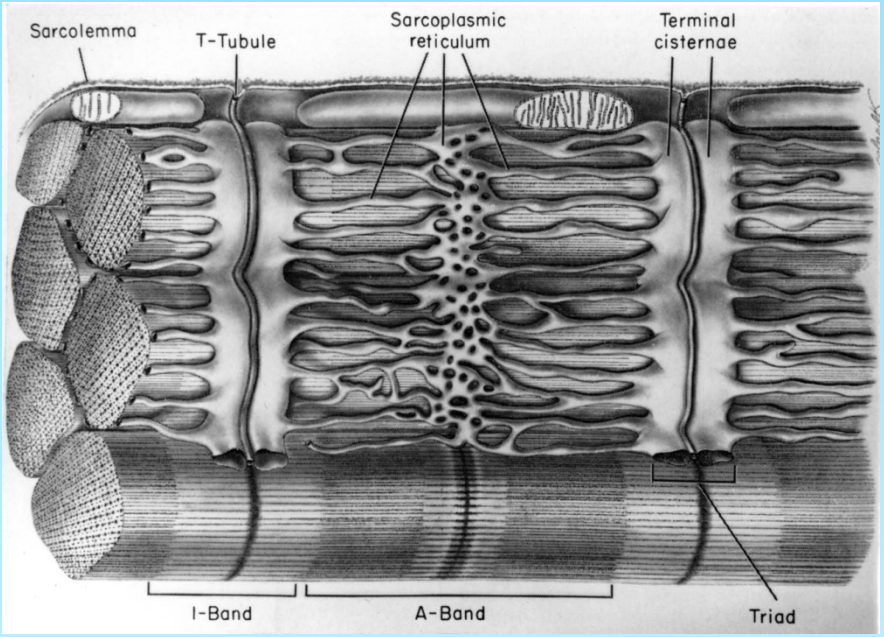


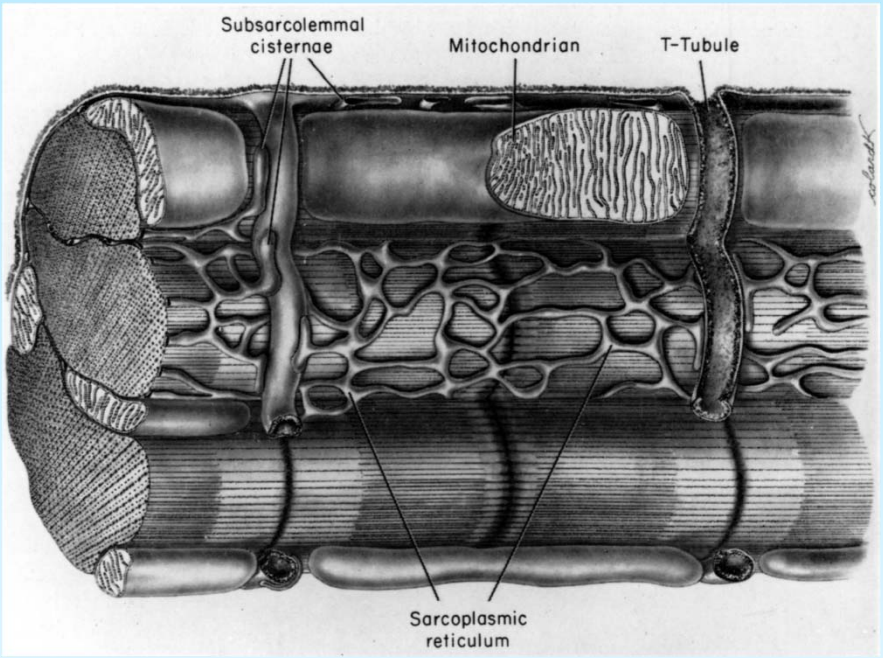
Rat papillary muscle in a thin section electron micrograph (left) and freeze-etched electron microscopy after ultra-rapid freezing without fixation (right). Junctional "feet" between the SR and Ttubule (TT) can be seen to periodically span the gap. Bar=0.2 µm. (From Frank, 1990 with permission).

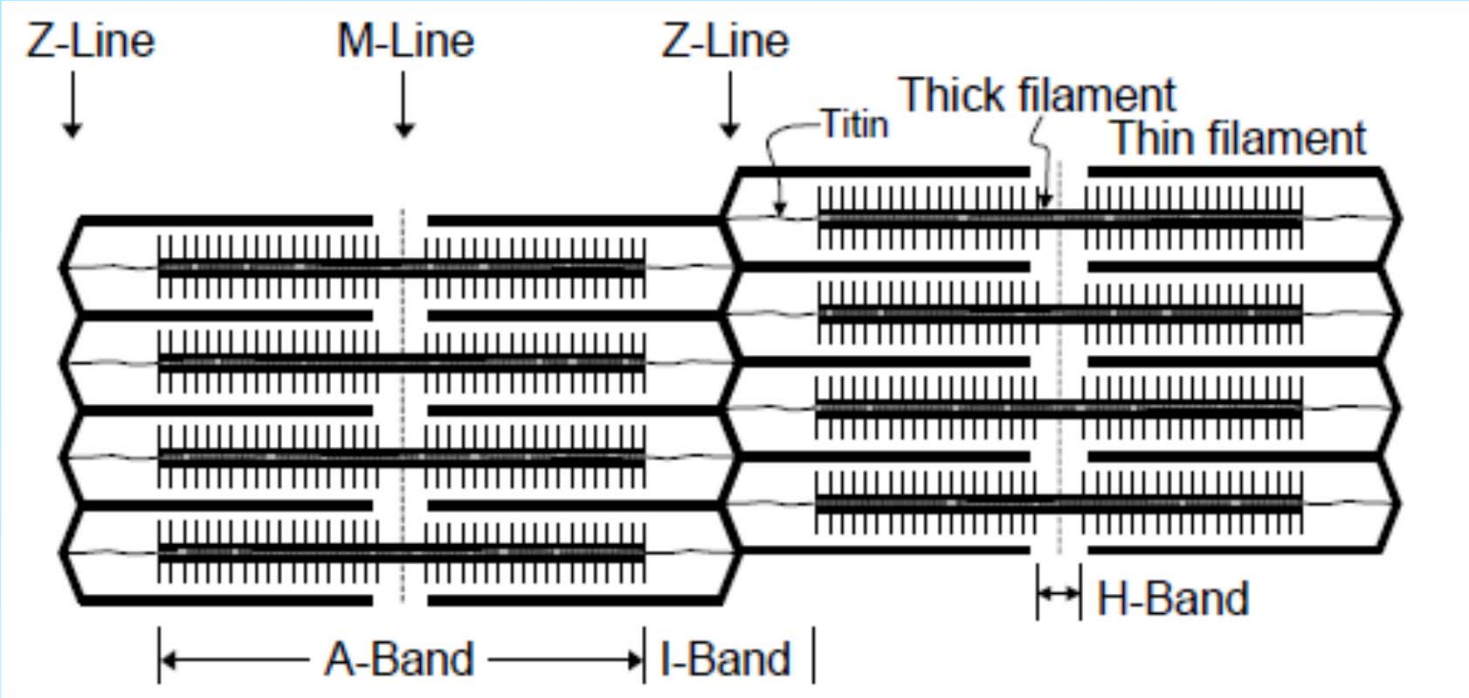


Three-dimensional reconstruction of the relative positions of key proteins at the skeletal muscle triad. The SR is filled with calsequestrin and the non-junctional surface is covered with the Ca-pump protein.

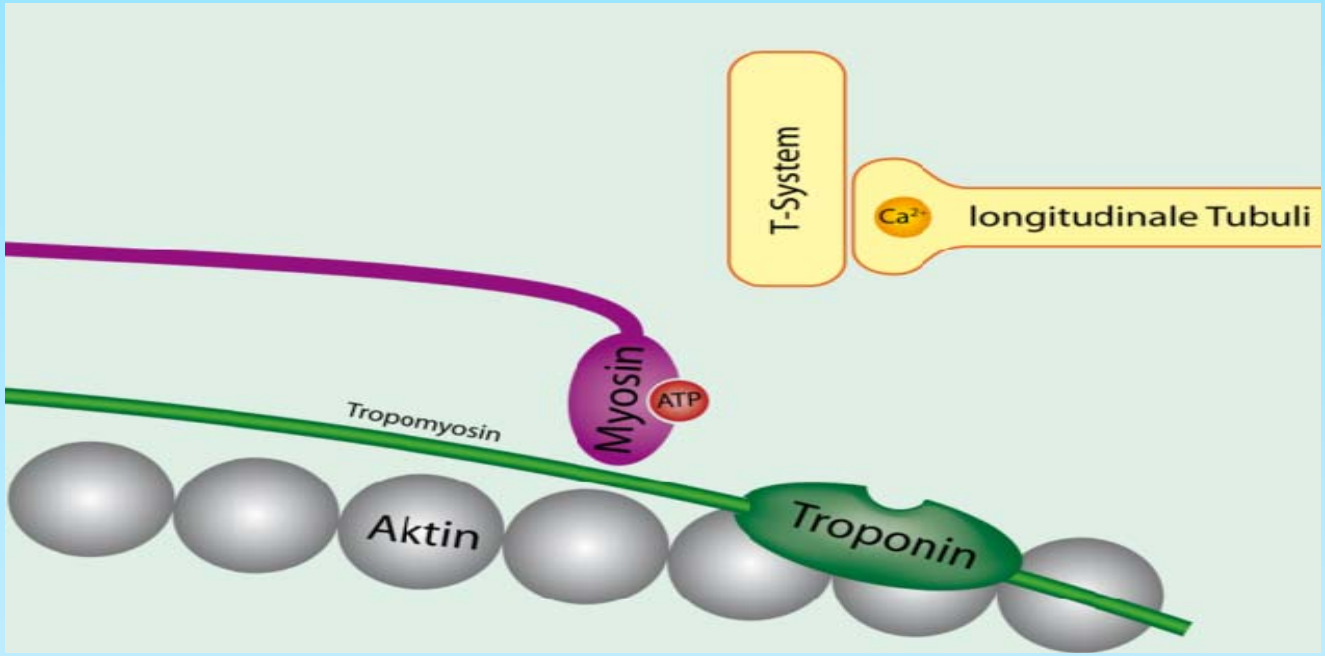

A mRyR2 (wt)
a Control (before EGTA addition) + 20 mV
Po = 0.42 To = 2.03 ms Tc= 2.74 ms
b + 0.1 mM EGTA
Po = 8.9x10-6 To = 0.58 ms
B R4496C
a Control (before EGTA addition) + 20 mV
Po = 0.22 To = 1.60 ms Tc= 4.44 ms
b + 0.1 mM EGTA
Po = 1.3x10-4 To = 1.60 ms
100 ms
20 pA
1000 ms
20 pA
C R4496C
a 0.19 μM CaCl2 + 20 mV
Po= 0.013 To= 2.12 ms Tc= 138 ms
b 0.26 μM CaCl2
Po= 0.07 To= 2.40 ms Tc= 27.6 ms
c 0.35 μM CaCl2
Po= 0.35 To= 2.84 ms Tc= 5.23 ms
d 0.55 μM CaCl2
Po= 0.92 To= 6.87 ms Tc= 0.79ms
e Ca2+ response
100 ms
20 pA
R4496C
mRyR2 (wt)
Po
0.8
0.4
0
9 8 7 6 5 4 3 2 1
pCa

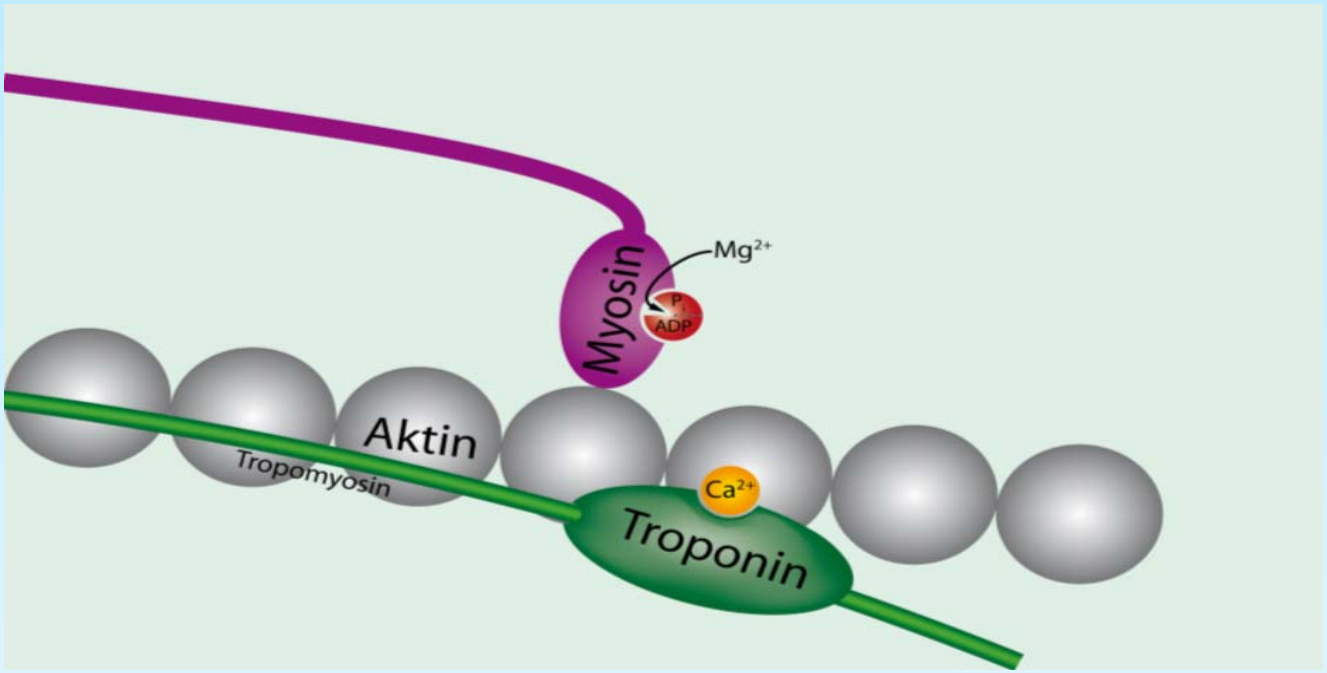

Sarcolemma
T-Tubule
Sarcoplasmic reticulum
Terminal cisternae
I-Band
A-Band
Triad

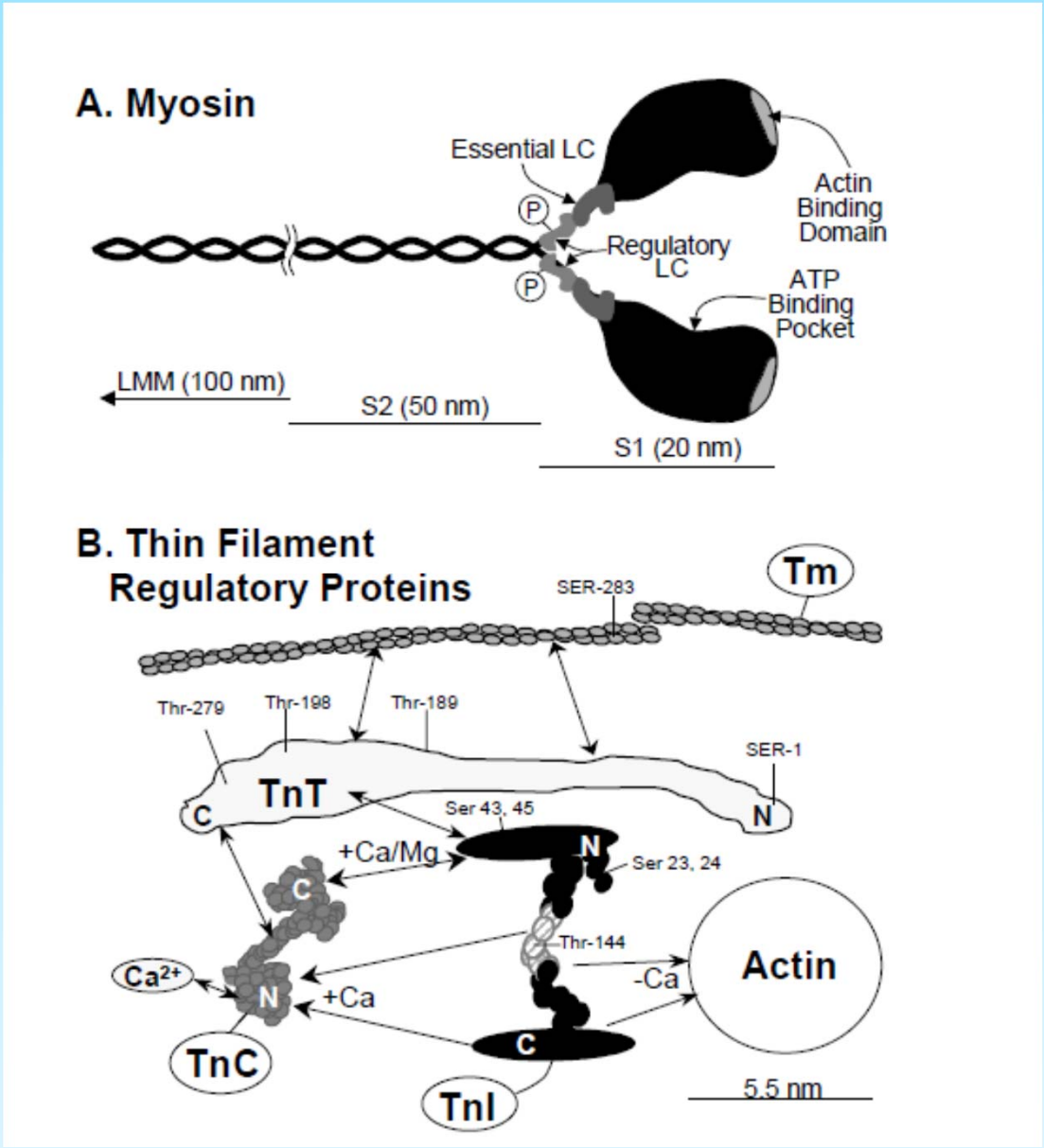

Subsarcolemmal cisternae
Mitochondrian
T-Tubule
Sarcoplasmic reticulum

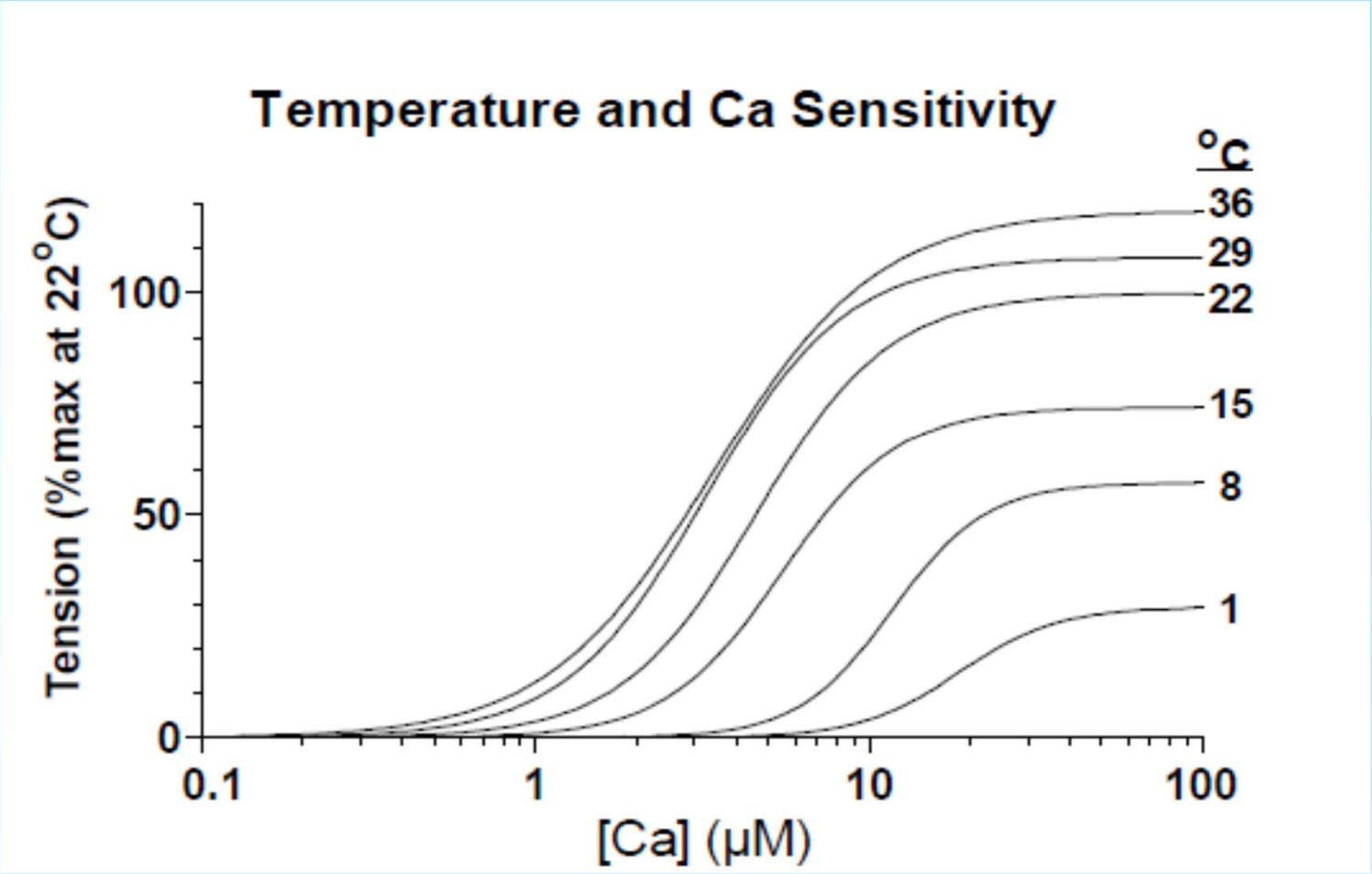


The organization of the sarcomere. The thin filaments meet at the Z-lines and the center of the thick filaments is known as the M-line. The I-band (or isotropic band) is the area where there are only thin filaments and the A-band (or anisotropic band) is the length of the thick filaments. The region of the thick filament where there is no overlap with thin filaments is known as the H-band (or H-zone).

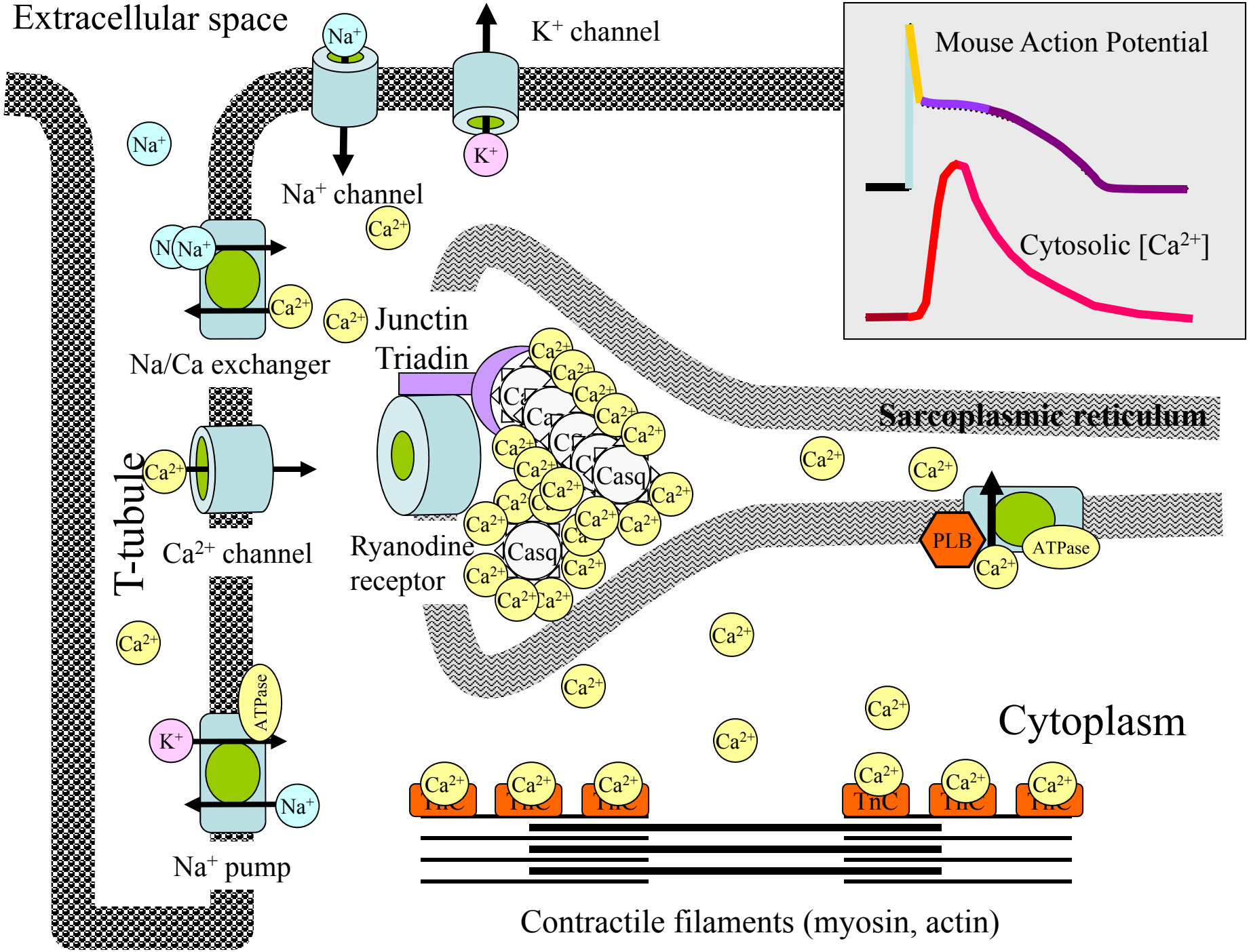

T-System
Ca2+
longitudinale Tubuli
Myosin
ATP
Tropomyosin
Aktin
Troponin

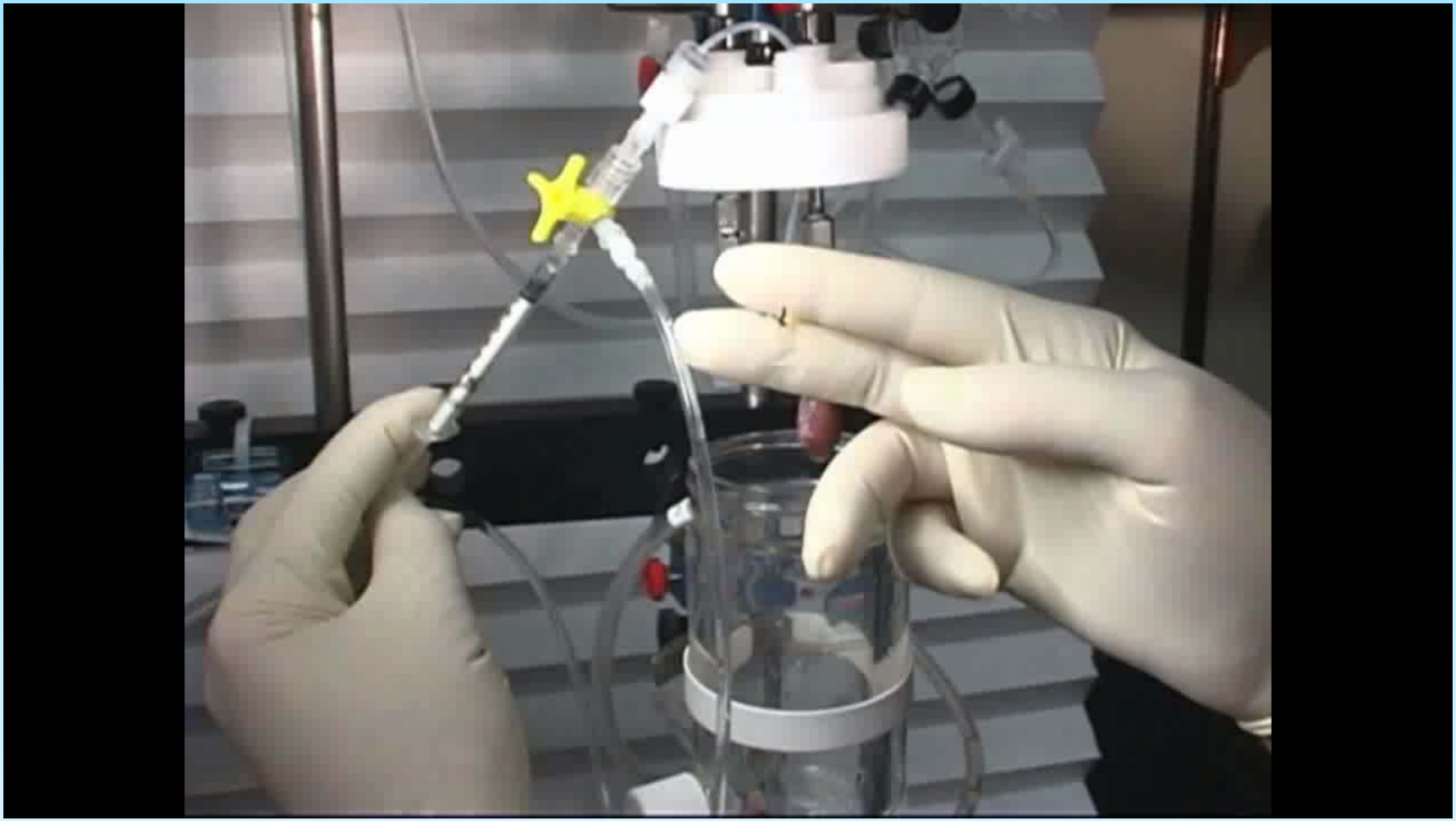

Mg2+
Myosin
P
ADP
Aktin
Tropomyosin
Ca2+
Troponin

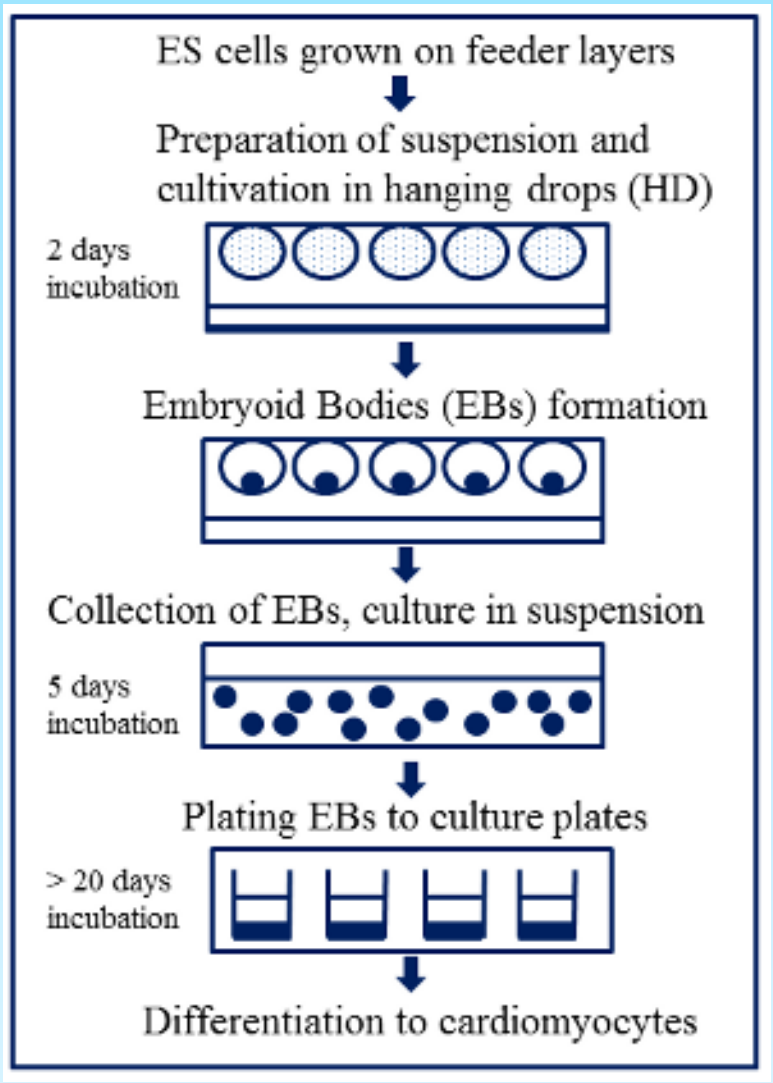

A. Myosin
Essential LC
Actin Binding Domain
P
Regulatory LC
P
ATP Binding Pocket
LMM (100 nm)
S2 (50 nm)
S1 (20 nm)
B. Thin Filament Regulatory Proteins
Tm
SER-283
Thr-279
Thr-198
Thr-189
SER-1
TnT
C
N
Ser 43, 45
+Ca/Mg
N
Ser 23, 24
C
Thr-144
Actin
Ca2+
N
+Ca
-Ca
TnC
C
TnI
5.5 nm

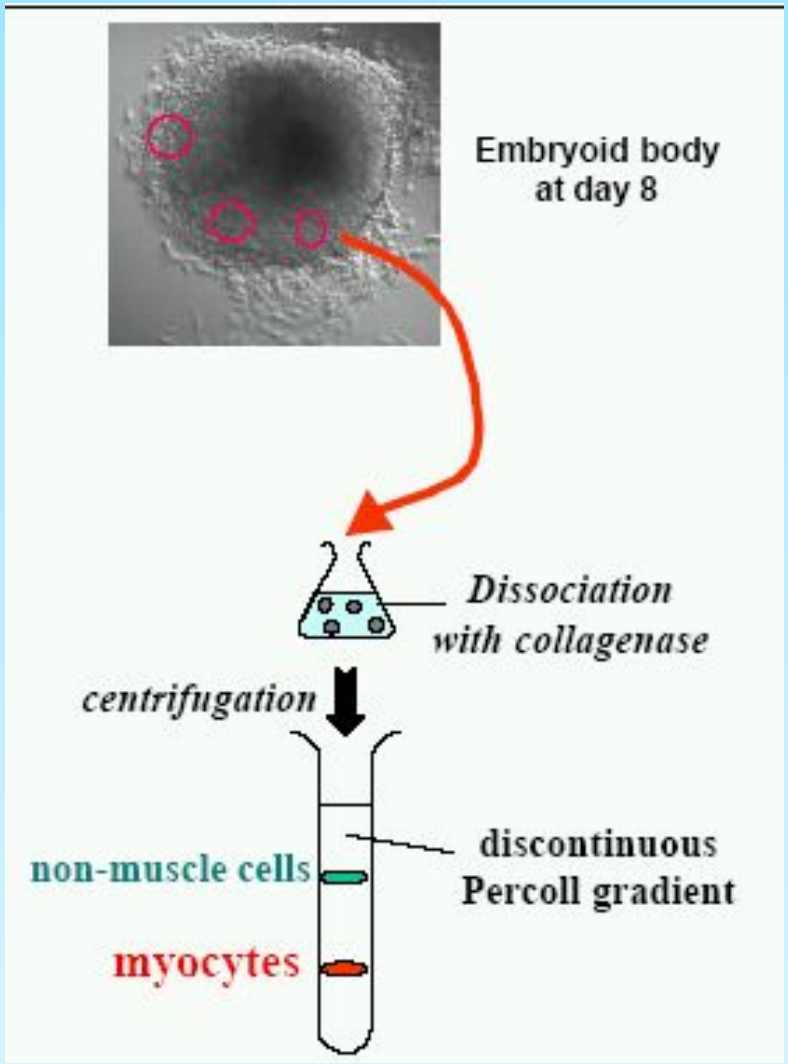


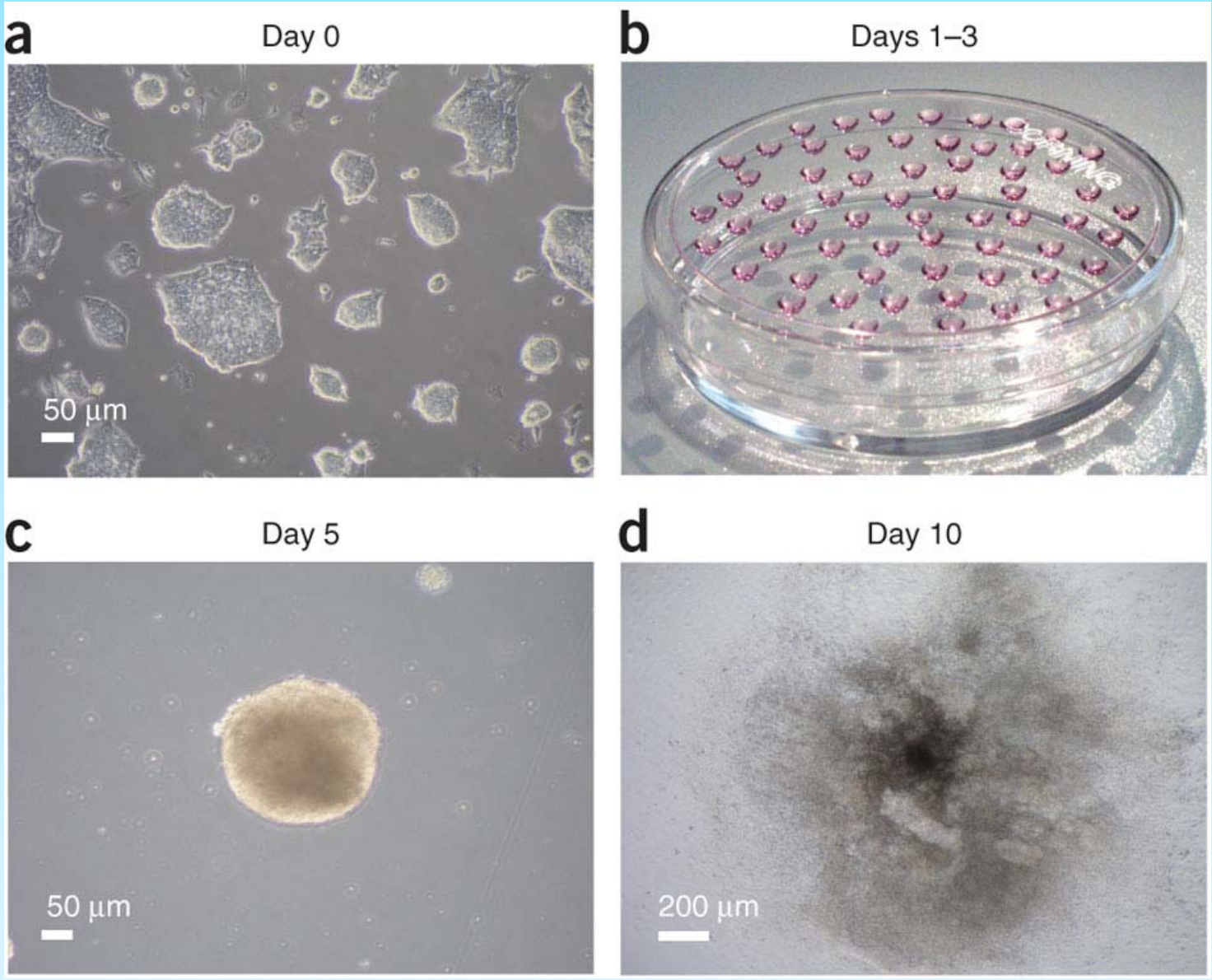
The influence of temperature on the Ca sensitivity of chemically "skinned" rabbit ventricular muscle (data from Harrison and Bers, 1989a have been redrawn). Both the Ca sensitivity and the maximum force are reduced at lower temperatures

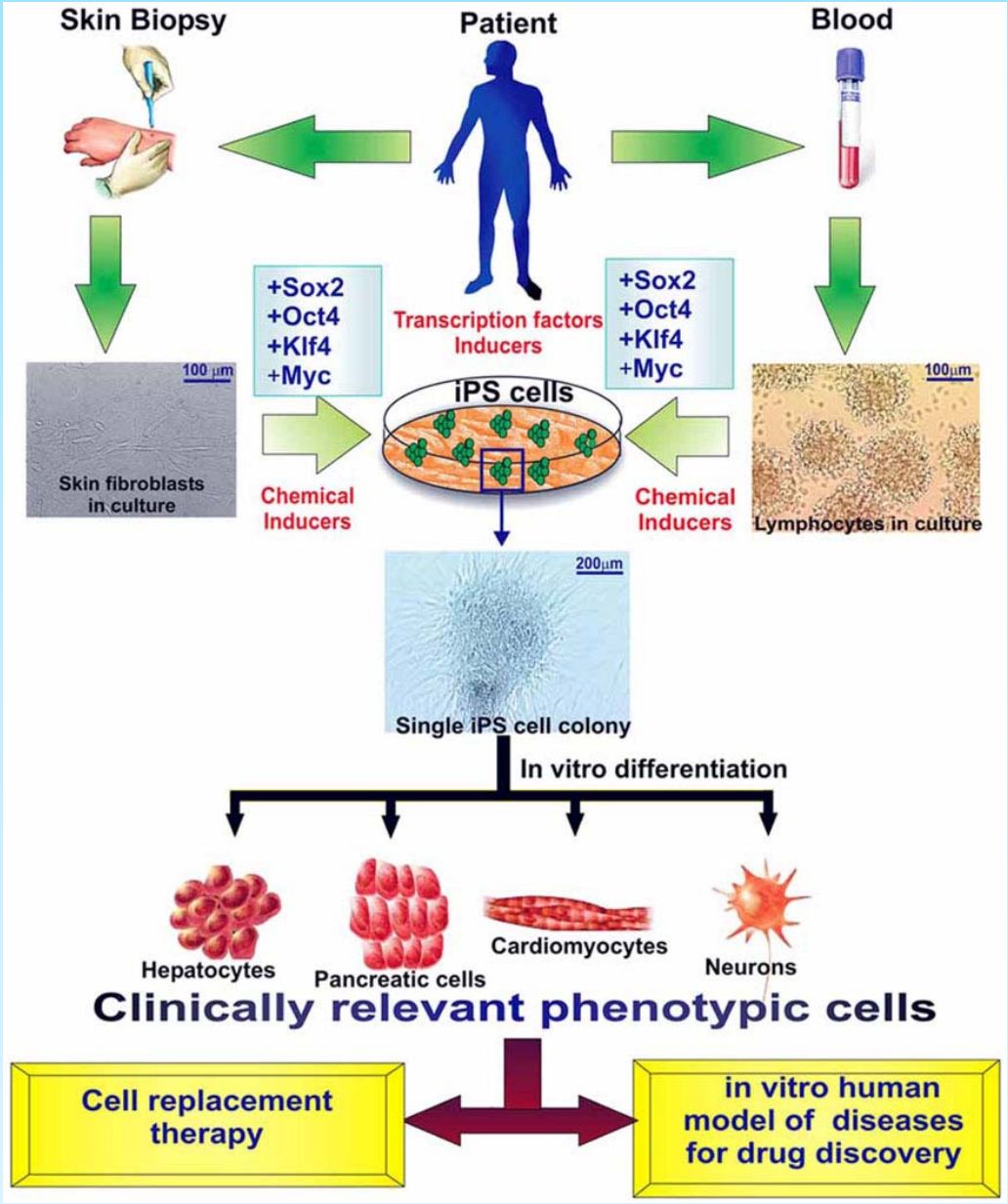

Extracellular space
Na+
K+ channel
Mouse Action Potential
Na+
Na+ channel
K+
Ca2+
Cytosolic [Ca2+]
N
Na+
Ca2+
Ca2+
Junctin
Triadin
Na/Ca exchanger
Sarcoplasmic reticulum
T-tubule
Ca2+
Ca2+ channel
Casq
Ryanodine
receptor
PLB
ATPase
Ca2+
K+
ATPase
Na+
Cytoplasm
TnC
Na+ pump
Contractile filaments (myosin, actin)

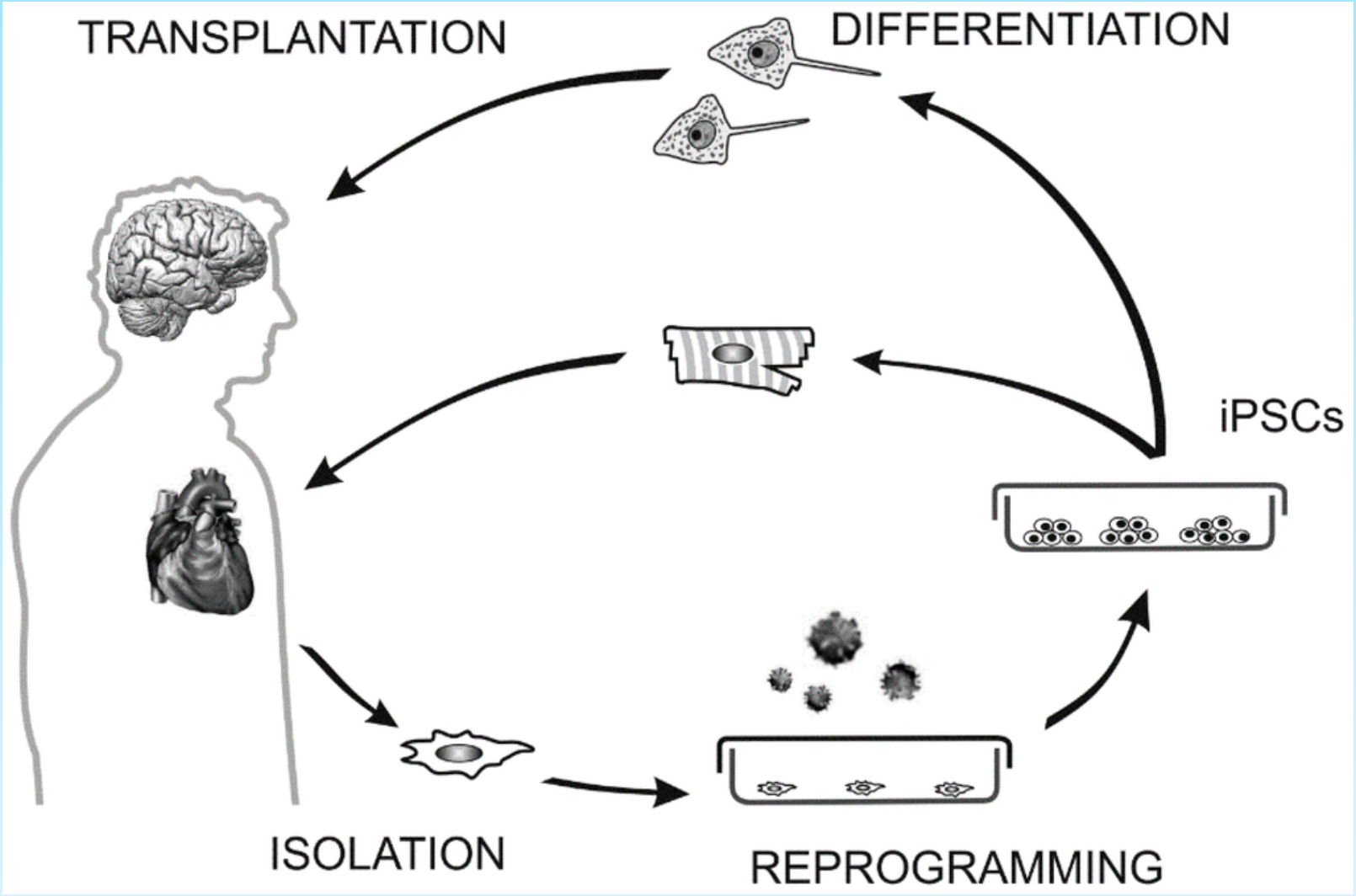



ES cells grown on feeder layers
Preparation of suspension and cultivation in hanging drops (HD)
2 days incubation
Embryoid Bodies (EBs) formation
Collection of EBs, culture in suspension
5 days incubation
Plating EBs to culture plates
> 20 days incubation
Differentiation to cardiomyocytes


Embryoid body at day 8
Dissociation with collagenase
centrifugation
discontinuous Percoll gradient
non-muscle cells
myocytes


a
Day 0
50 µm
b
Days 1–3
c
Day 5
50 µm
d
Day 10
200 µm


Skin Biopsy
Patient
Blood
+Sox2
+Oct4
+Klf4
+Myc
Transcription factors
Inducers
+Sox2
+Oct4
+Klf4
+Myc
100 µm
Skin fibroblasts
in culture
iPS cells
100µm
Lymphocytes in culture
Chemical
Inducers
Chemical
Inducers
200µm
Single iPS cell colony
In vitro differentiation
Hepatocytes
Pancreatic cells
Cardiomyocytes
Neurons
Clinically relevant phenotypic cells
Cell replacement
therapy
in vitro human
model of diseases
for drug discovery


TRANSPLANTATION
DIFFERENTIATION
iPSCs
ISOLATION
REPROGRAMMING