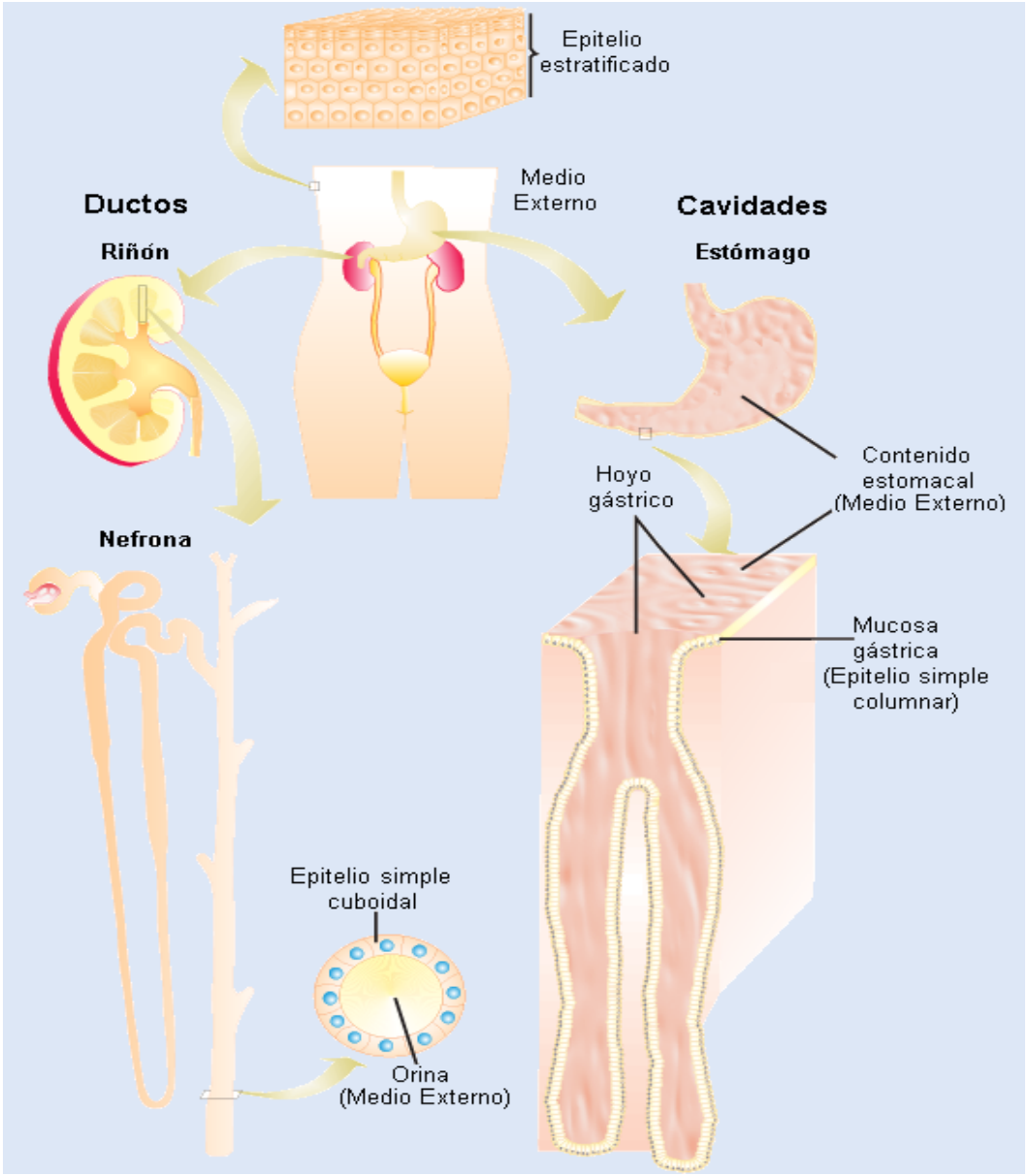
La unión estrecha y los trabajos muy citados del laboratorio de la Dra Lorenza González-Mariscal

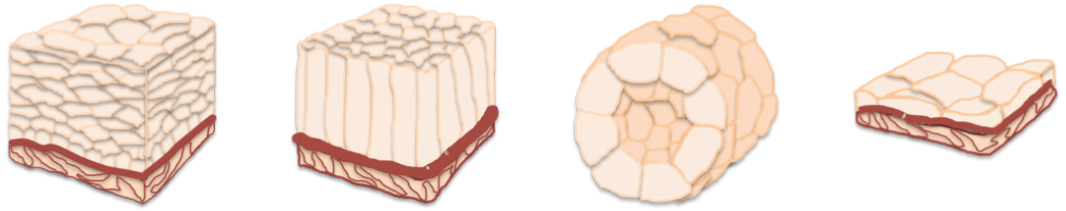
Cinvestav,
México, Octubre 2012

Los epitelios son la frontera entre el organismo y el medio ambiente



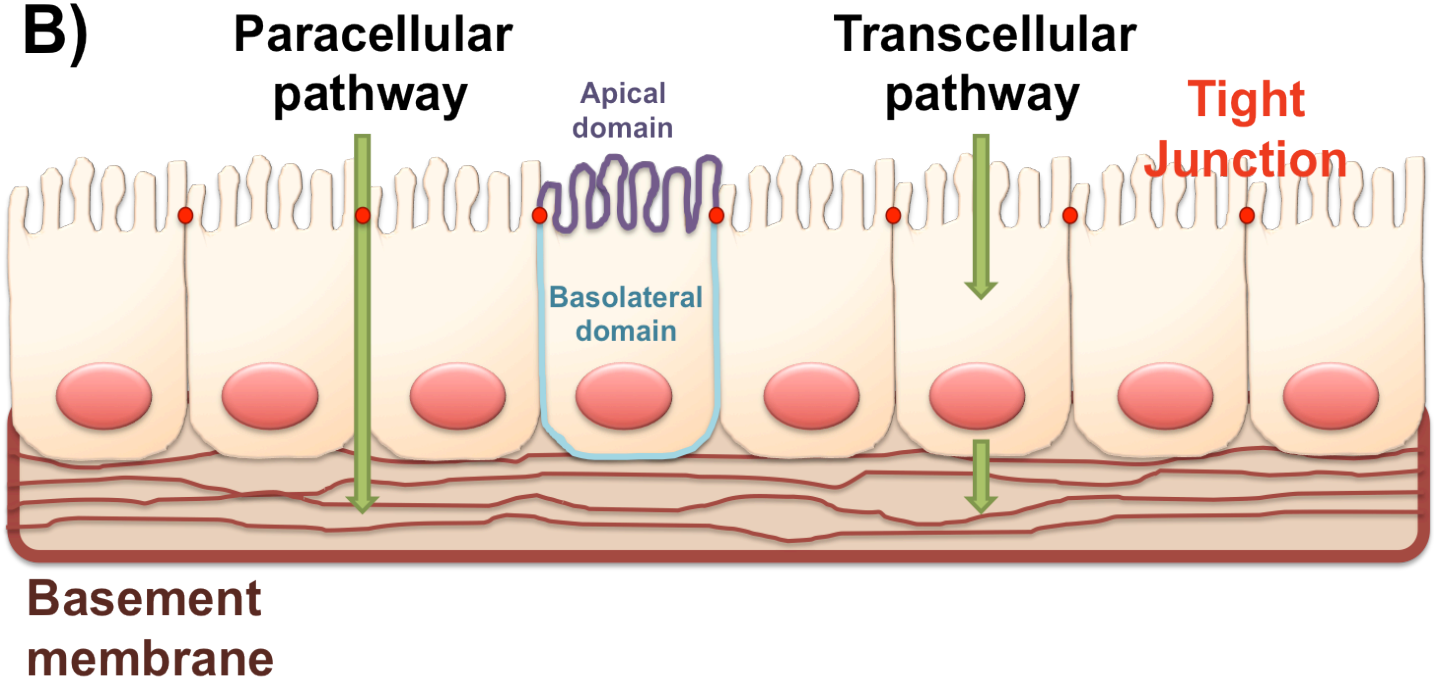
Las células epiteliales están polarizadas y tienen uniones estrechas

A)

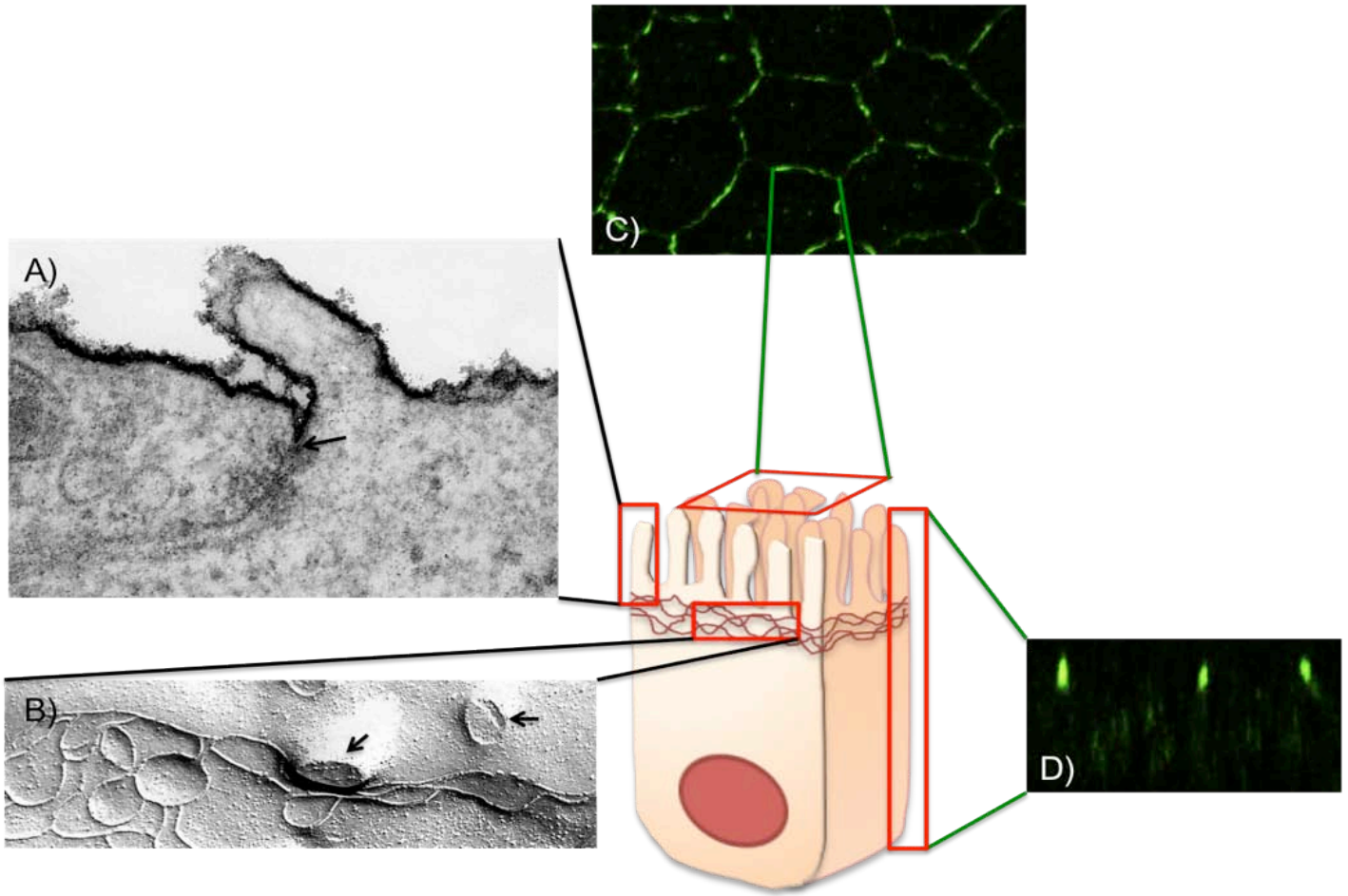


Stratified Columnar Tubular Squamous

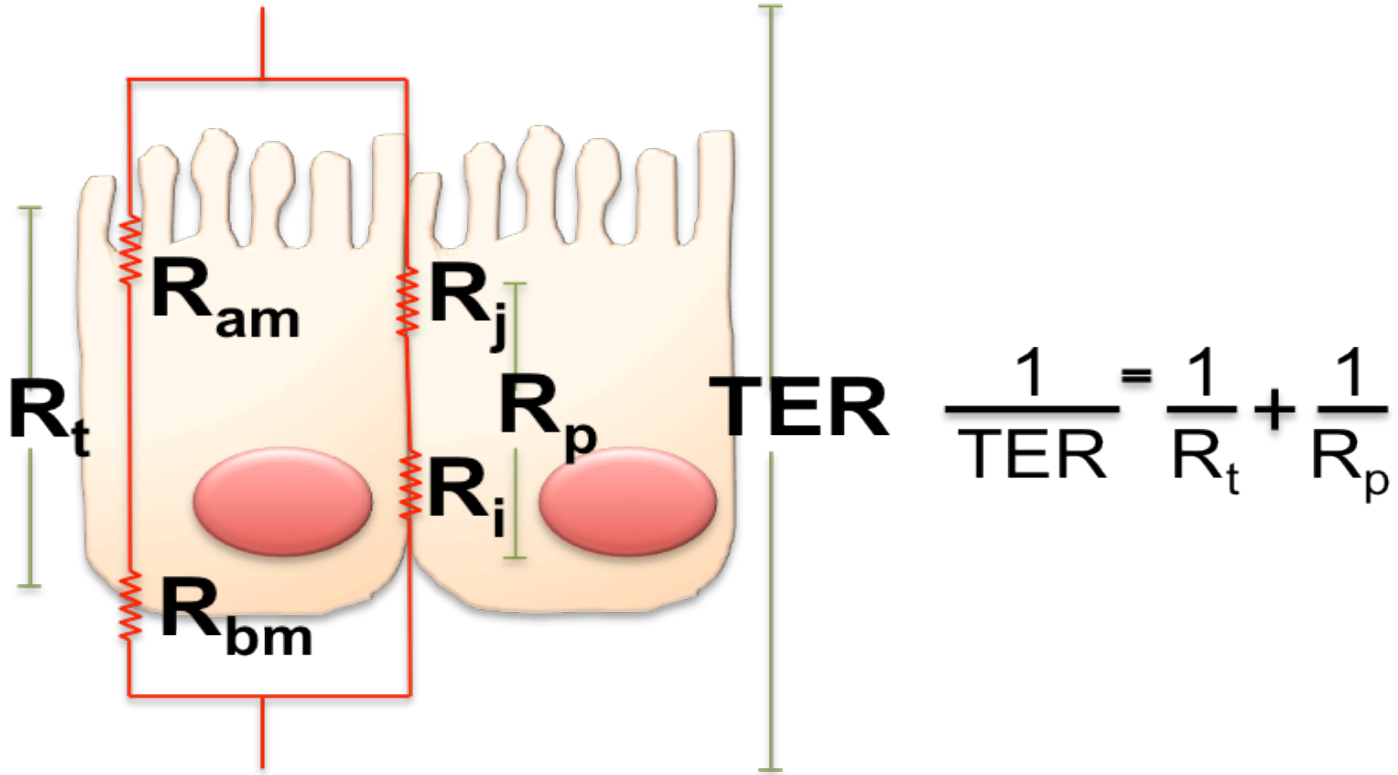
B)



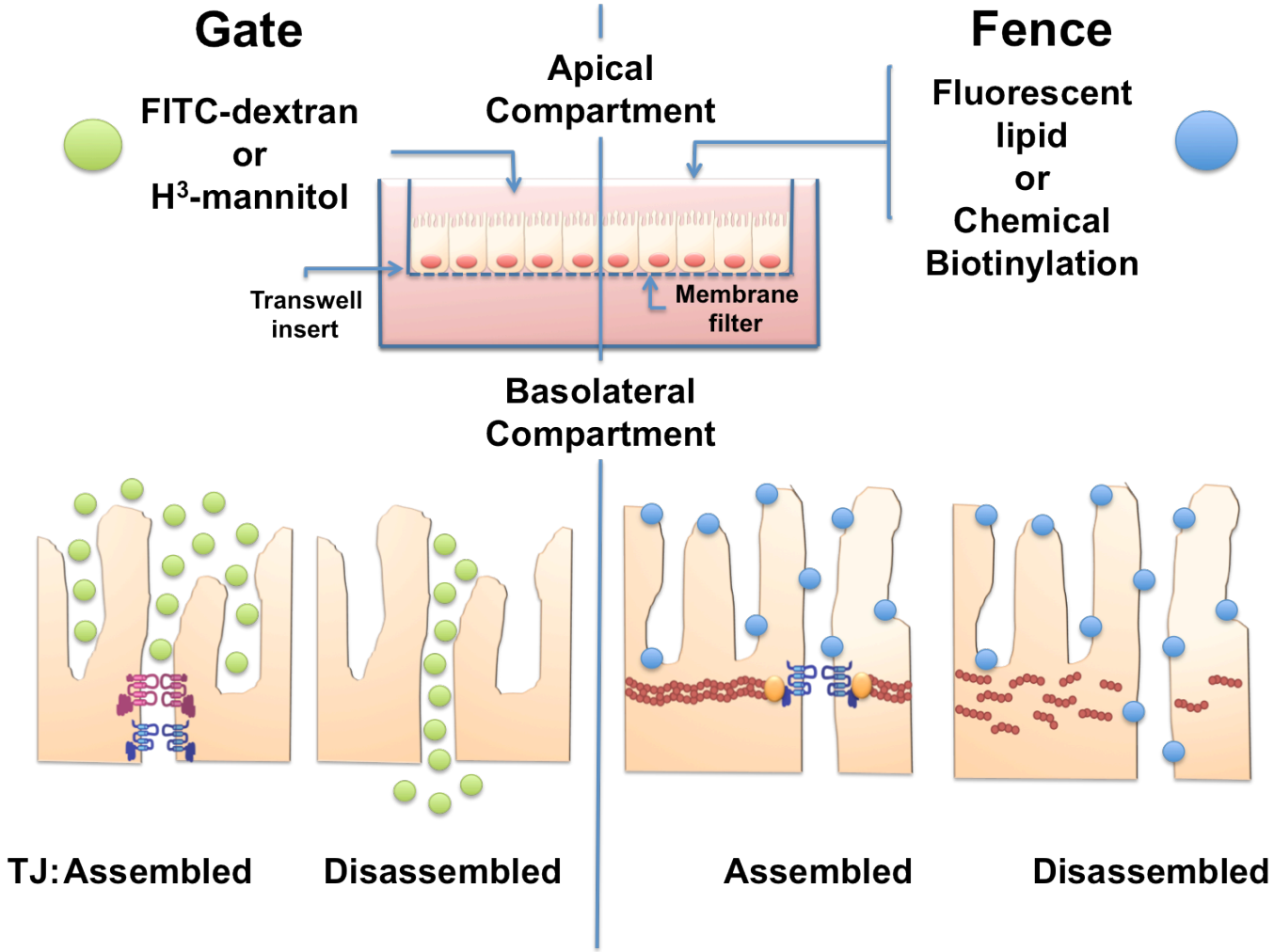
Las uniones estrecha forman una red que circunda a la célula en el límite apico-basal



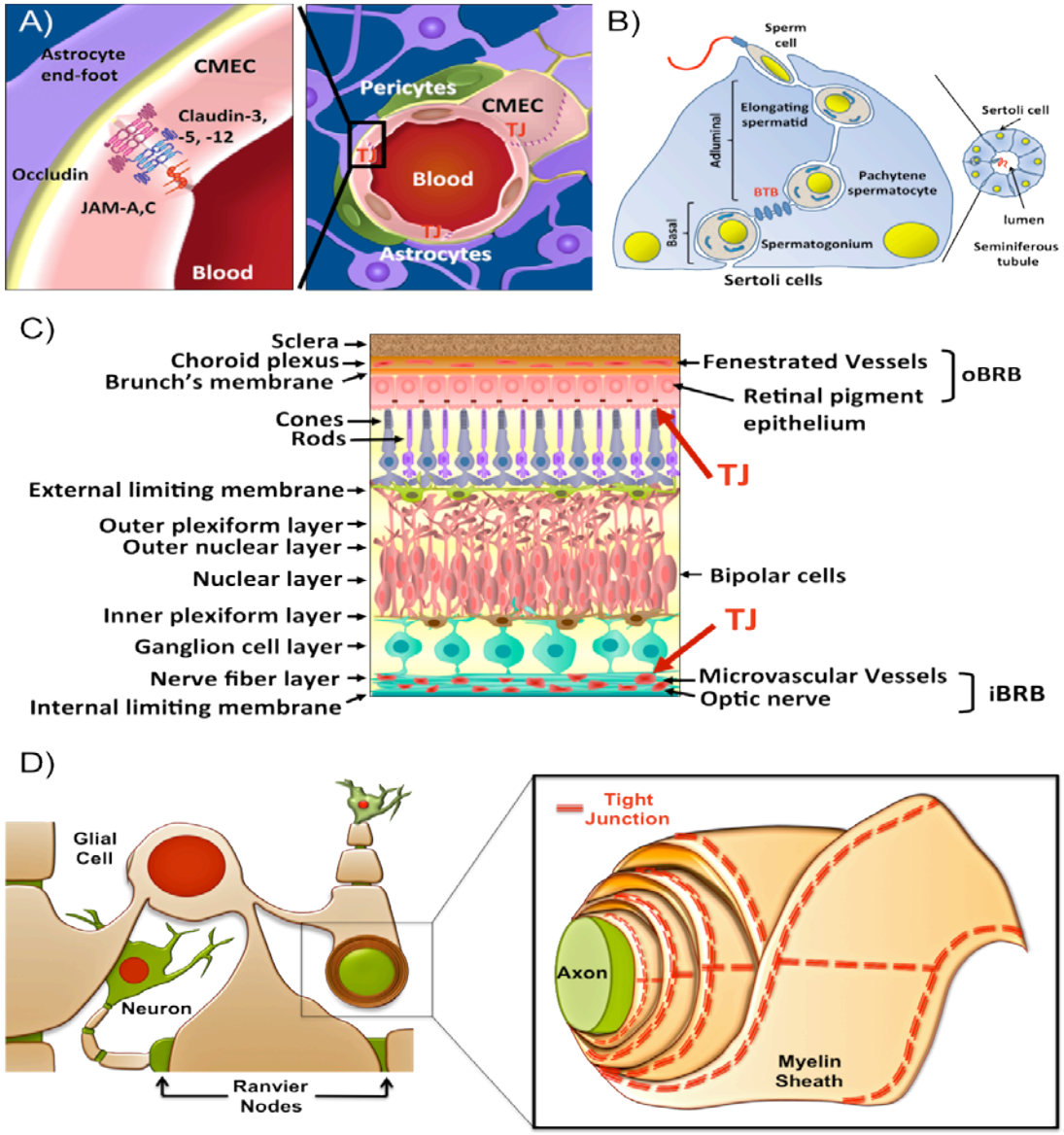
Las UEs definen la resistencia eléctrica transepitelial ya que regulan el tránsito de iones y moléculas por la ruta paracelular



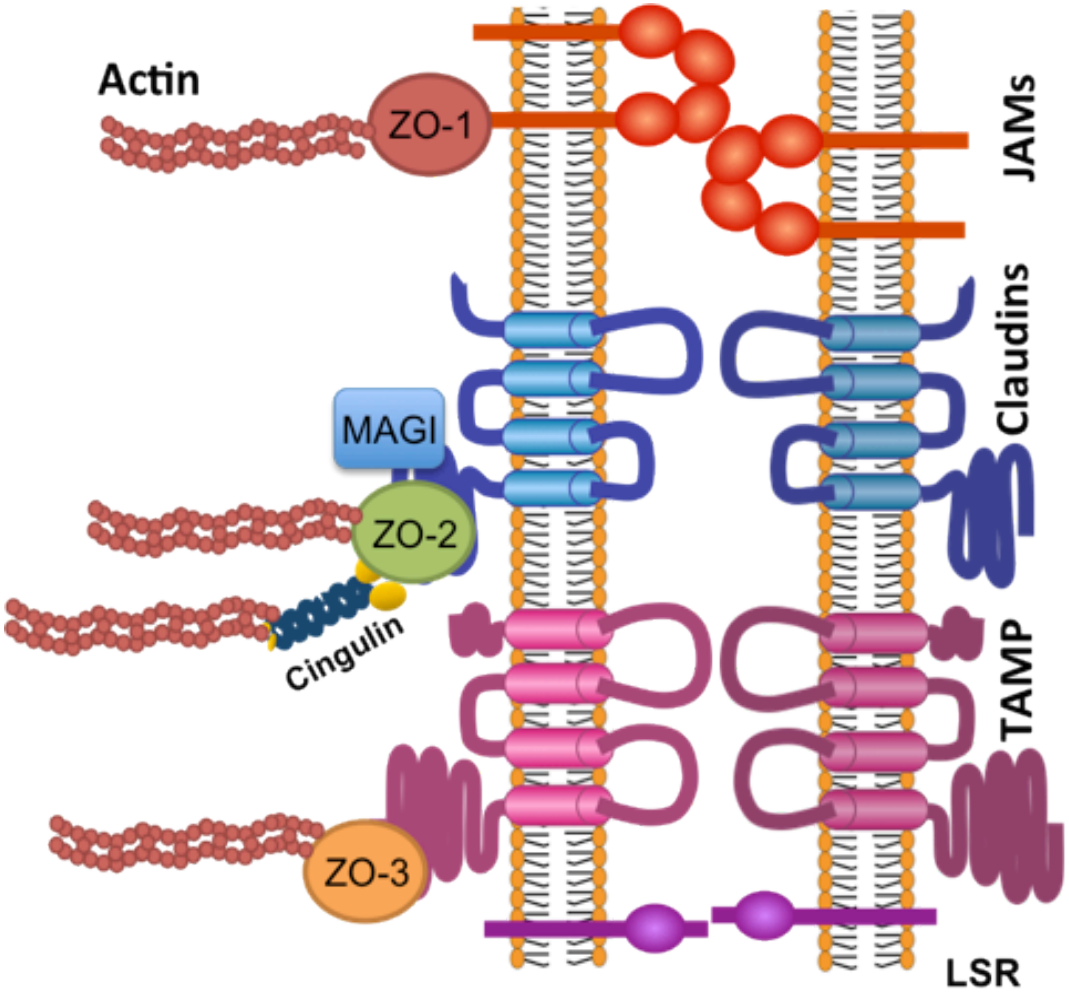
Las UES funcionan como cerca y compuerta

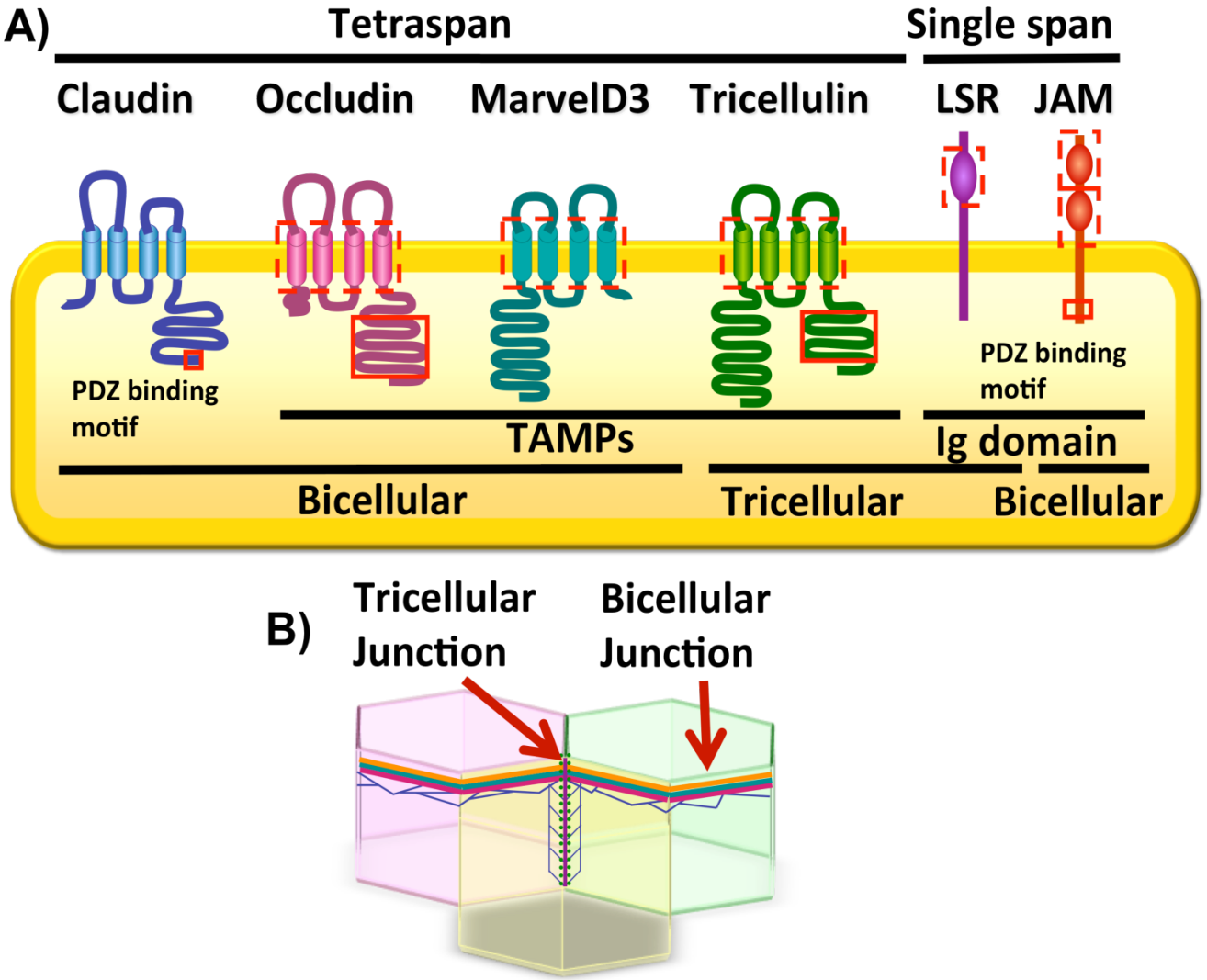


Las barreras hemato-encefálica (A), hemato-testicular (B), hemato-retiniana (C) y las coberturas de mielina de los axones dependen de las UEs

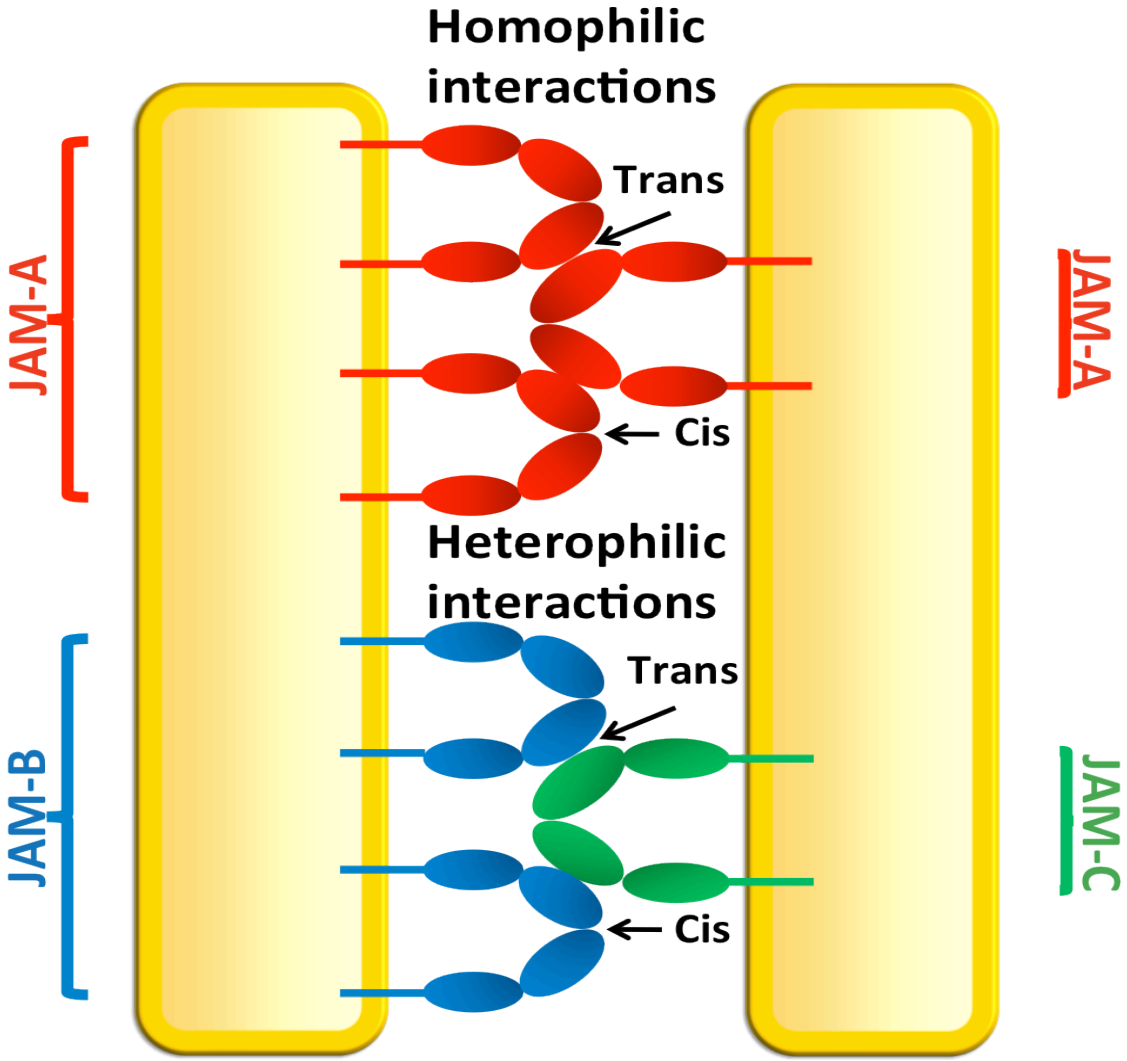


Las UEs están constituidas por proteínas integrales y periféricas

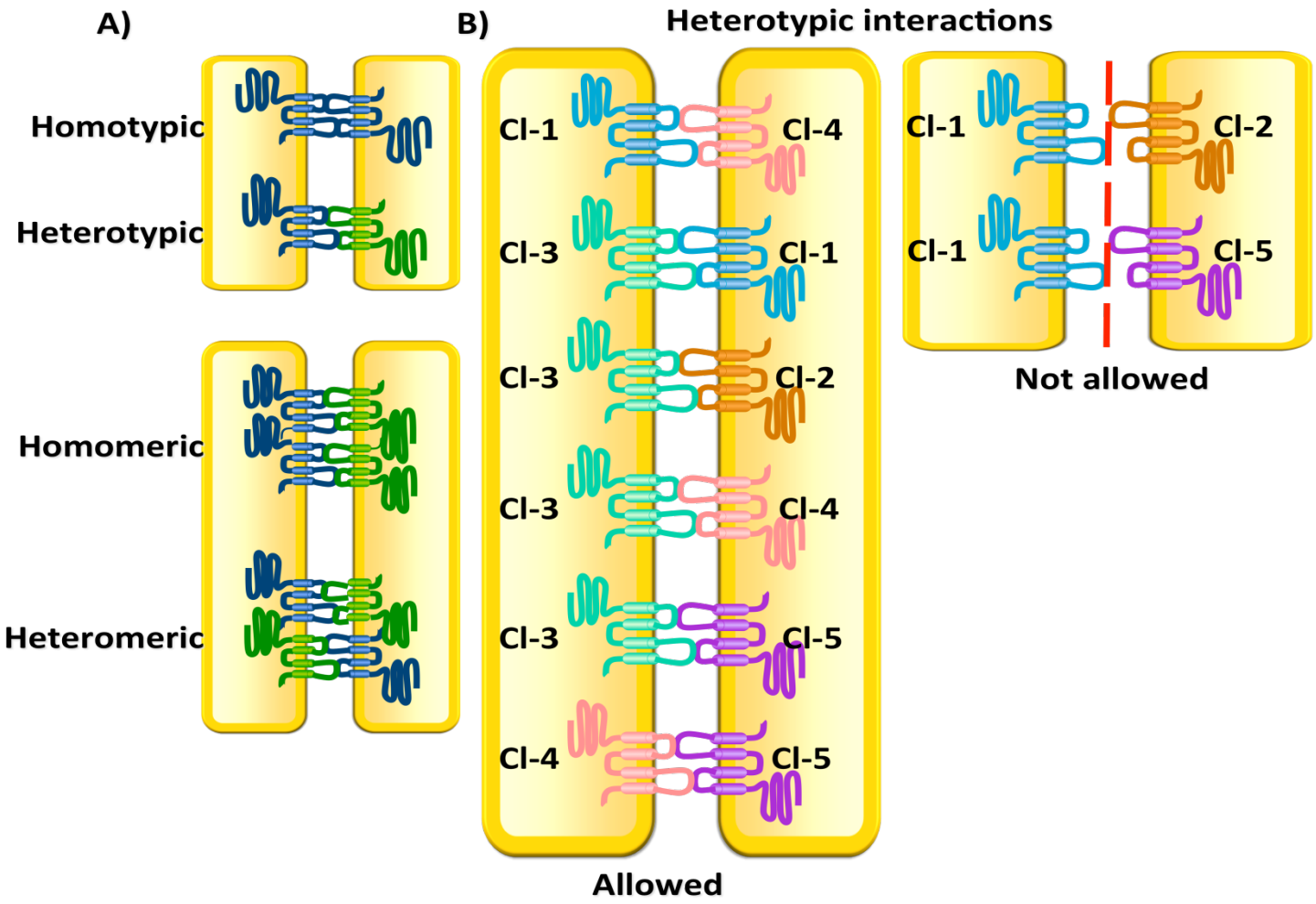



A)
Tetraspan
Single span
Claudin
Occludin
MarvelD3
Tricellulin
LSR
JAM
PDZ binding motif
PDZ binding motif
TAMPs
Ig domain
Bicellular
Tricellular
Bicellular
B)
Tricellular Junction
Bicellular Junction

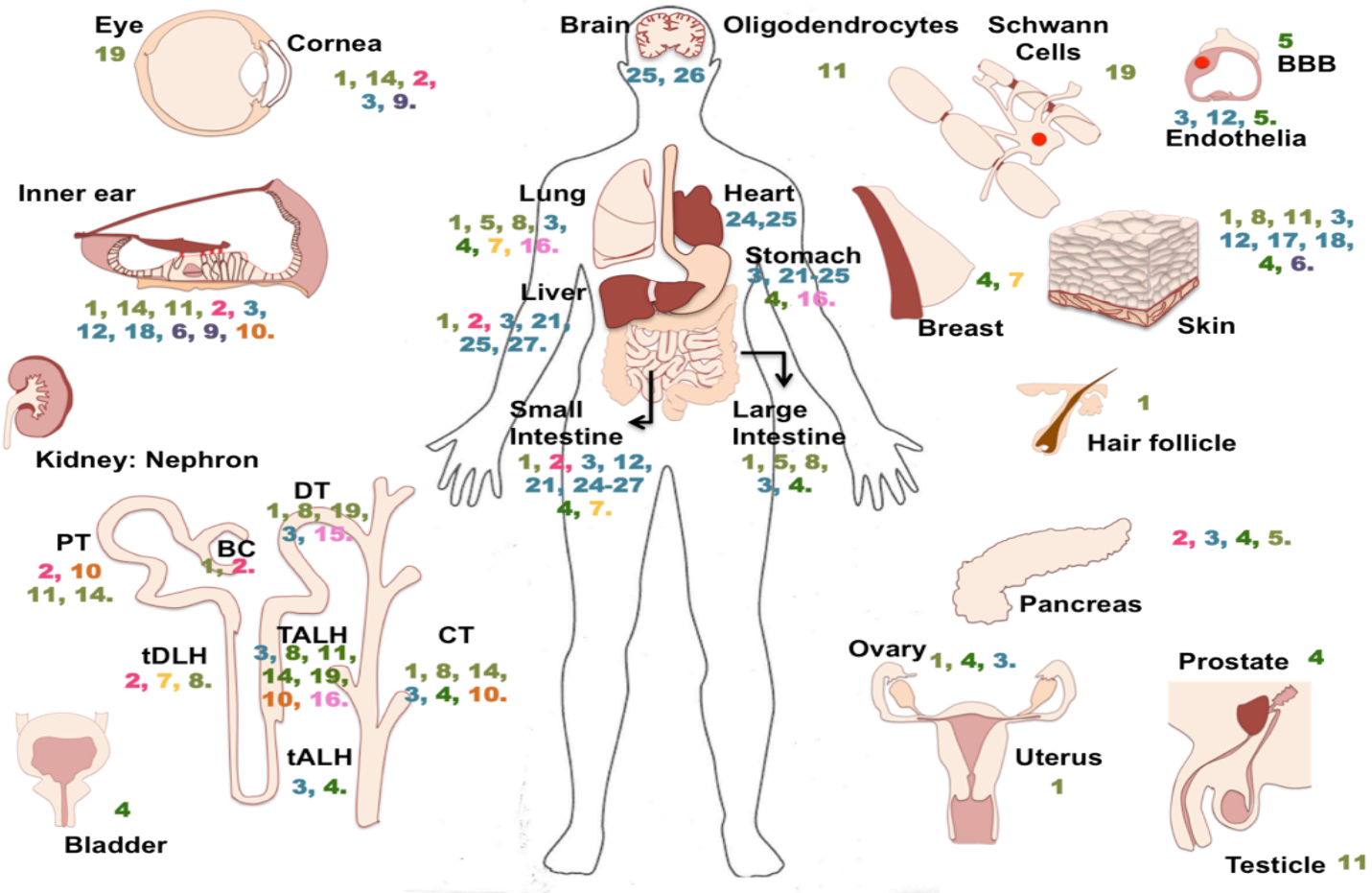
Las JAMs establecen interacciones homo y heterotípicas



Las claudinas interactúan de manera homo y heterotípica



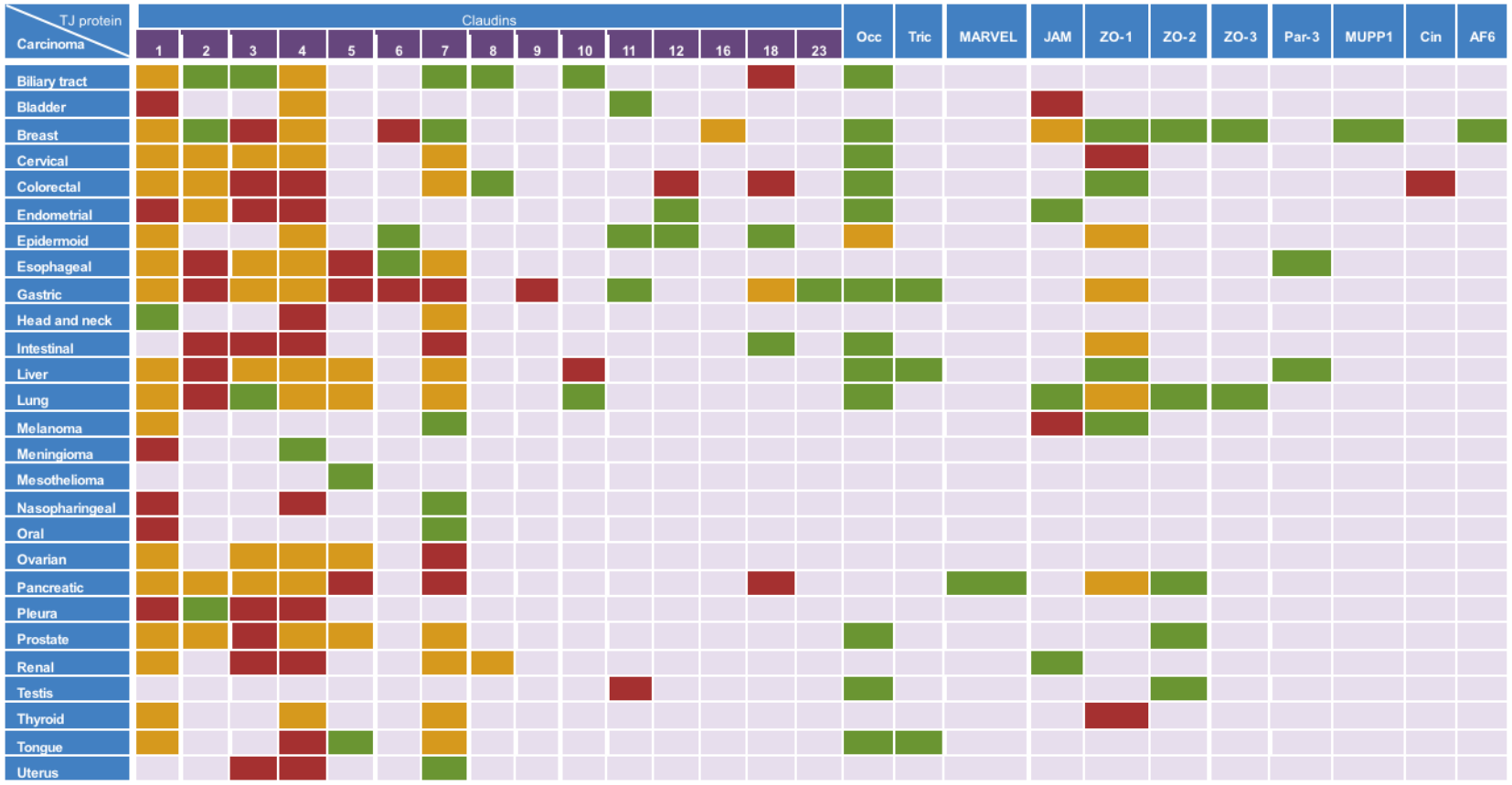
Cada tejido expresa a un conjunto particular de claudinas



El fenotipo de los animales KO, KD, transgénicos y con mutaciones sirve para entender la función de las claudinas

Claudin	KO, KD, TG and MT mice phenotype	Hereditary human/bovine diseases
1	KO: lethal, loss of skin barrier	H: 2 bp deletion → absence of Cl-1 → neonatal sclerosing cholangitis
2	KO: Defective reabsorption of NA^+,Cl^- and H_2O at proximal tubule	ND
3	NA	ND
4	NA	ND
5	KO: lethal, permeable BBB	ND
6	TG: permeable skin barrier	ND
7	KO: lethal, renal salt wasting and dehydratation	ND
8	NA	ND
9	MT: Deafness	ND
10a	NA	ND
10b	NA	ND
11	KO: Male sterility, hind limb weakness and deafness.	ND
12	NA	ND
14	KO: deafness	H: Single nucleotide deletion → loss of half of predicted protein → DFNB29 H: T254A/V85D → disrupts secondary structure in 2nd TMD → DFNB29
15	KO: Megaintestine	ND
16	KD: FHHNC, no accumulation of Cl-19 at TAL	H: FHHNC B: chronic interstitial nephritis (↑ blood urea nitrogen and creatinin, ↑ urinary proteins)
17	NA	ND
18	NA	ND
19	KO: disorganized Schwann cells TJs, abnormal animal behavior and peripheral neuropathy KD: FHHNC, no accumulation of Cl-16 in TAL	H: FHHNC and severe visual impairment. H: Mut G20D → disturbance of signal peptide sequence → perinuclear protein H: Mut Q57E (within W-GLW-C-C signature) → dimerization disruption H: Mut L90P → disrupts α-helix in 2nd TMD

En el cáncer la expresión de algunas proteínas de las UEs disminuye (verde), aumenta (rojo) o ambas cosas suceden (amarillo)



Cada tipo de tumor, incluso dentro de un mismo órgano, expresa a un grupo particular de claudinas

Stomach

Tumor / TJ protein	IM	Displasia	IAC	Difusse	PDAC
Cl 2	-	++	+++		
Cl 4				++	++
Cl 7	++	++	++	+	

IAC, intestinal adenocarcinoma; IM, intestinal metaplasia; PDAC, poor differentiated adenocarcinoma.

Lung

Tumor / TJ protein	NSCC			SCC	Met
	SQ	AC	LCC		
Occ	-		-	-	
ZO-1	+	+			
Cingulin	-				
Cl 1	+	-	-		+
Cl 2	+	+			+
Cl 3	-	+			+
Cl 4	+	+	+		
Cl 5	-	+			-
Cl 7	+	+	-		+

AC, adenocarcinoma; LCC, large cell carcinoma; Met, metastasis; NSCC, non-small cell carcinoma; SCC, small cell carcinoma; SQ, aquamous cell carcinoma.

Breast

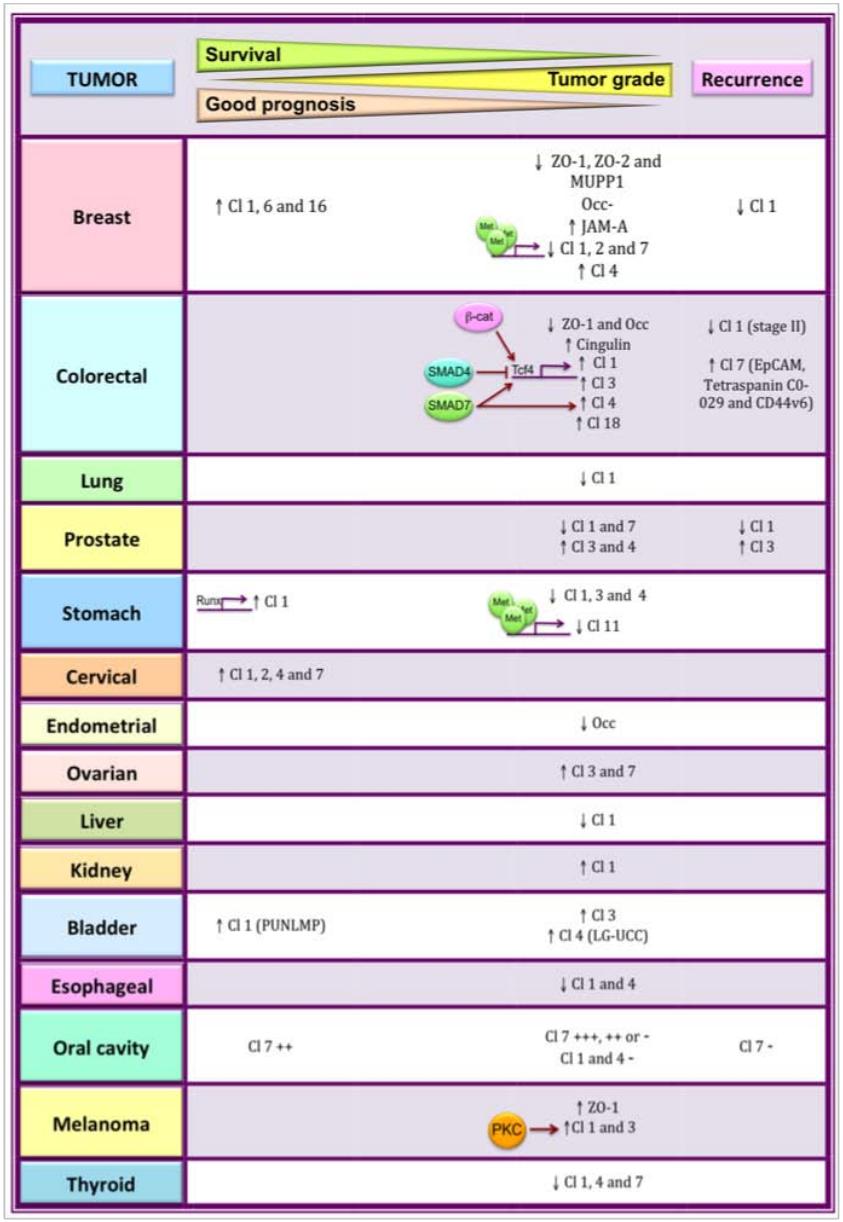
Tumor / TJ protein	Her2 overexpressing (ER-, PR-,Her2+)	Basal-like (ER-, PR-, Her2-)
ZO-1	+++	
ZO-2	+++	
PAR3	+++	
Cl 1		+++
Cl 4		+++
Cl 16	+++	+++

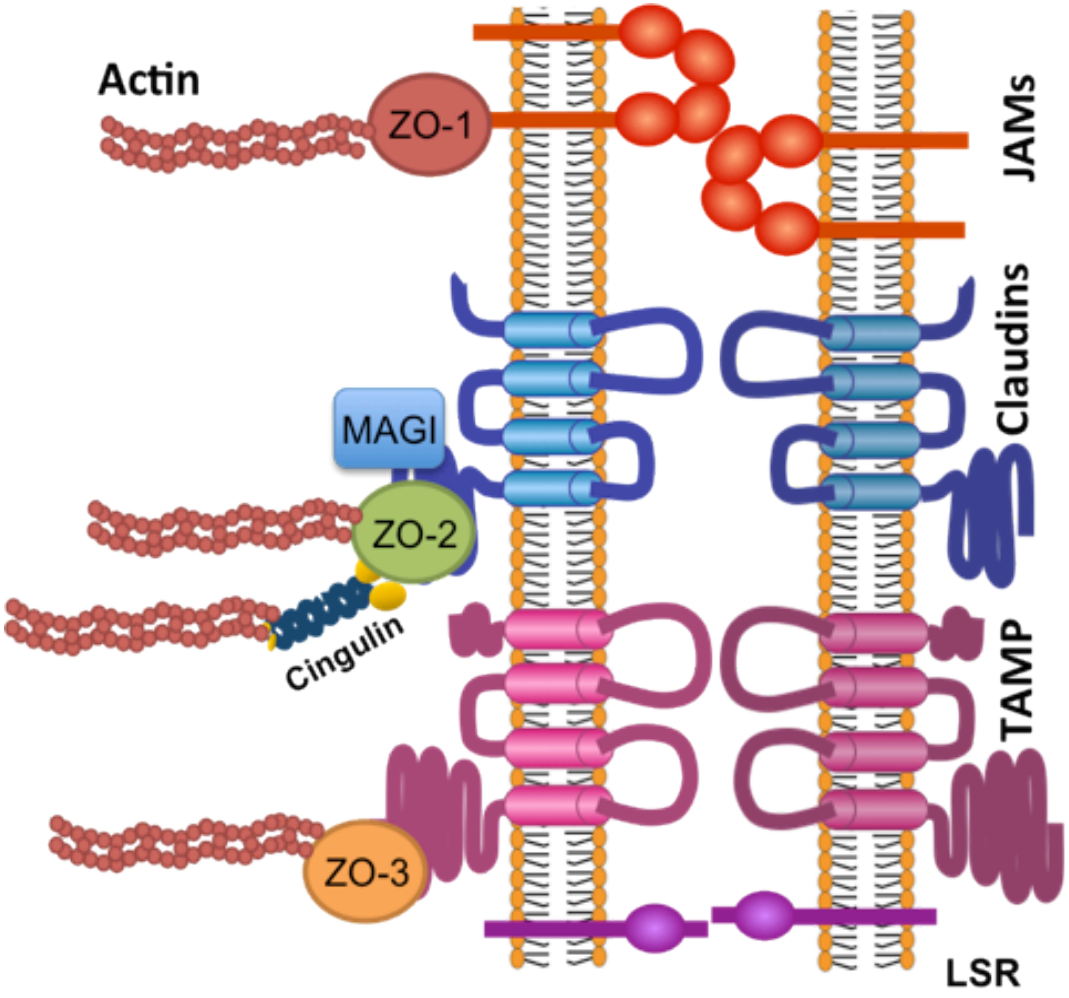
Pancreas

Tumor / TJ protein	IPMN	IIPMN	Endocrine	Exocrine			
				AC	SPT	PB	ACC
Cl 1			-	+			
Cl 2			-	+	+		
Cl 3			+		-		
Cl 4	+	++	-	+	-		
Cl 5					+		
Cl 7			+	+		+	+

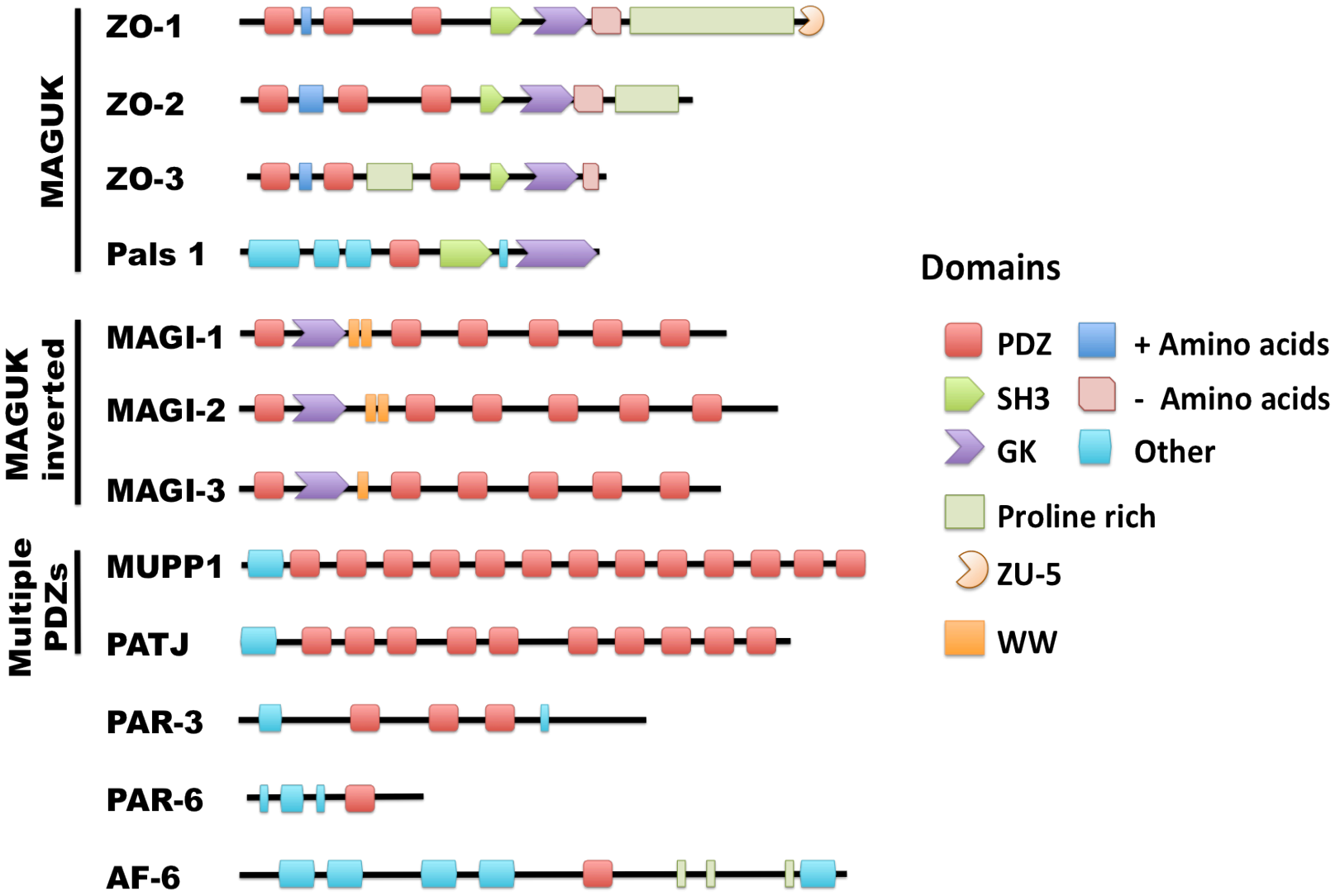
AC, ductal adenocarcinoma; ACC, undifferentiated carcinoma with osteoclastic-like giant cells and acinar cell carcinomas; IPMN, intraductal papillary mucinous neoplasm; IIPMN, intestinal type IPMN; PB, pancreatoblastoma; SPT, solid pseudopapillary tumor.

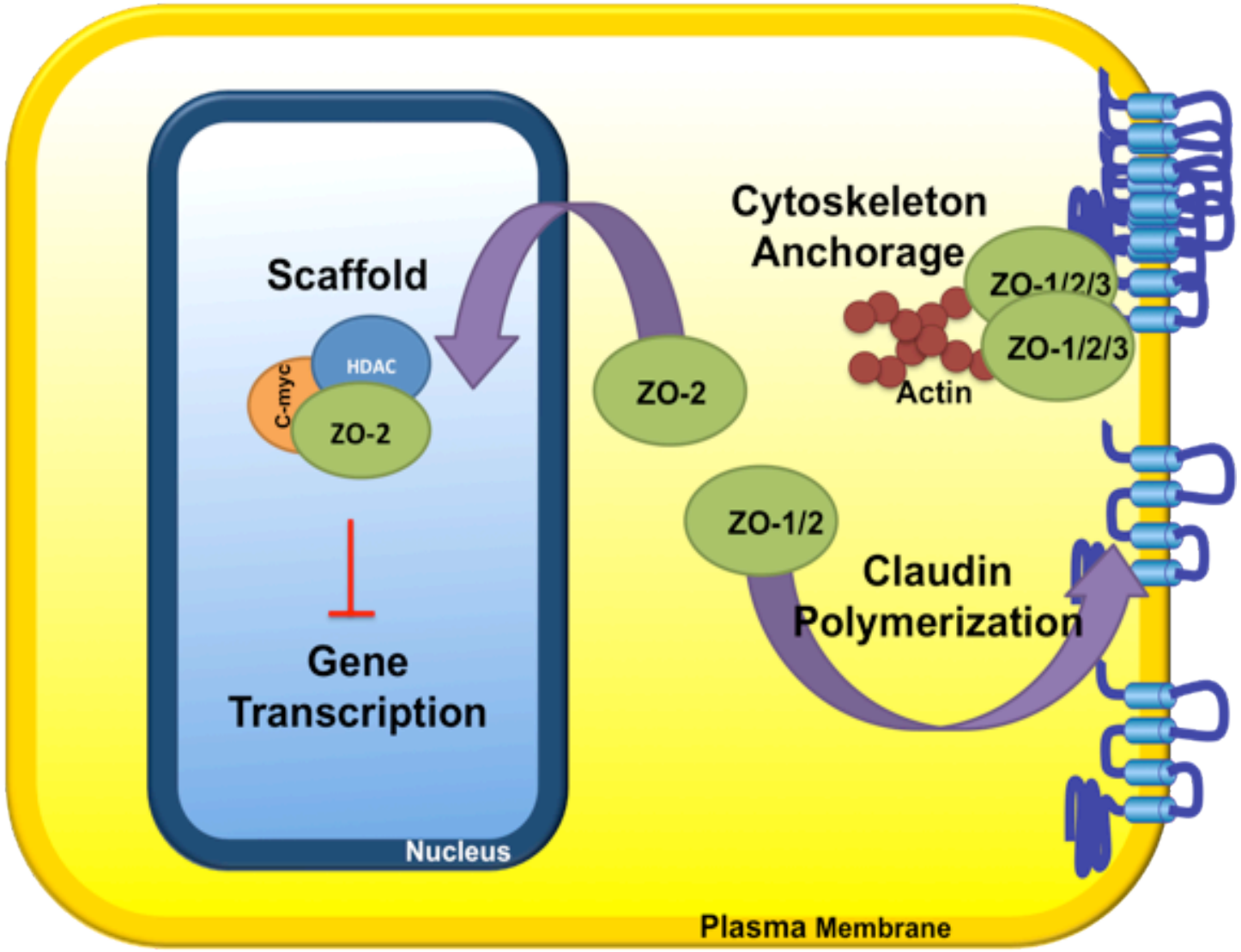
La expresión de claudinas tiene un valor pronóstico en el cáncer.




Actin
ZO-1
JAMs
Claudins
MAGI
ZO-2
Cingulin
TAMP
ZO-3
LSR

Proteínas periféricas de la UE con dominios PDZ




Scaffold
HDAC
C-myc
ZO-2
Gene
Transcription
Nucleus
ZO-2
Cytoskeleton
Anchorage
ZO-1/2/3
ZO-1/2/3
Actin
ZO-1/2
Claudin
Polymerization
Plasma Membrane

Enfermedades en las que se altera la expresión de proteínas de las UEs

Tissue	Disease	TJ disruption	
		Upregulation	Downregulation
Brain	Alzheimer's disease and vascular dementia	Occ, Cl-2, -5 and -11	ND
	Cerebral aneurysm		Occ and ZO-1
	Chronic inflammatory pain	Cl-3 and -5	Occ
	Edema		Occ, Cl-5 and ZO-1
	Epilepsy		Cl-8 and ZO-1
	Massive intracraneal hemorrhage and congenital cataracts.		JAM-C mut
	Multiple sclerosis model (autoinmune encephalomyelitis)		JAM-A and ZO-1
	Multiple sclerosis	Cl-1 and -5 *	Occ, Cl-5 and ZO-1
	Rehumatoid arthritis (RA)		Occ
Ear	Age related nonsyndromic deafness (DFNA51)	ZO-2 genomic duplicarion	
	Nonsyndromic deafness (DFNB49)		Tricellulin mut
Esophagus	Barrett's esophagus	Cl-18	
	Reflux esophagitis	Occ, Cl-1, JAM-A and ZO-1	Cl-3
Eye	Diabetic retinopathy	Cl-1 **	Occ, Cl-5 and ZO-1 and -2
	Dry eye		Occ
	Gelatinous drop-like corneal dystrophy		Occ, Cl-1 and ZO-1
	Retina pigmentosa	ZO-1	
Intestine	Celiac disease	Cl-2, -3 and -4	Occ and ZO-1
	Collagenous colitis		Occ and Cl-4
	Crohn's disease	Cl-2	Occ, Cl-1, -5 and -8, JAM and ZO-1
	Non alcoholic fatty liver disease		ZO-1
	Obstructive jaundice	Cl-4	Occ, Cl-1 and -7
	Pouchitis	Cl-2	Cl-1
	Type I diabetes	Cl-2	Cl-1
	Ulcerative colitis	Cl-2	Occ, Cl-1, JAM and ZO-1
Liver	Intrahepatic cholestasis		7HG
	Necrotizing enterocolitis		Cl-3
	Primary biliary cirrhosis		7HG
	Primary sclerosing cholangitis		7HG
Lung	Acute lung inflammation		ZO-1, Cl-2, -4 and -5
	Acute lung injury	Cl-4	
	Asthma		Occ, Cl-1 and ZO-1
	Chronic alcoholic ingestion	Cl-5	Cl-1 and -7
	Sepsis		Occ, Cl-4 and -18
Spinal cord	Amyotrophic lateral sclerosis (ALS)		Occ and ZO-1
	ALS-mutant mice		Occ, Cl-5 and ZO-1
Vessels	Atherosclerosis		ZO-1

¿Qué es lo que nosotros aportamos
al conocimiento de las uniones estrechas?

Protocolo del Ca^{2+} Switch

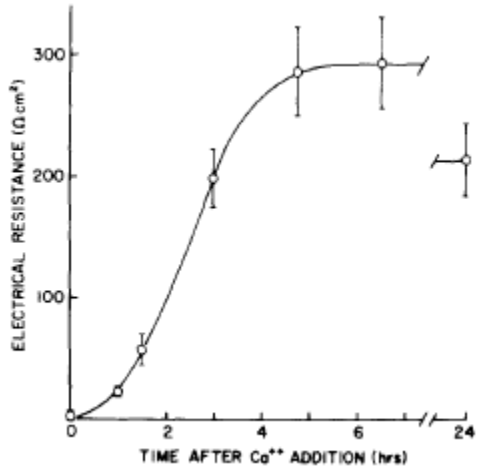


Table. Sealing of tight junctions between MDCK cells, as evaluated by the electrical resistance across the monolayer[a]

Conditions after plating	Electrical resistance, 20 hr (Ω cm^2)[b]	Conditions during incubation	Electrical resistance, 24–26 hr (Ω cm^2)
CDMEM	346 ± 51 (7)	CDMEM	331 ± 38 (20)
DMEM	275 ± 13 (8)	DMEM	238 ± 15 (10)
Ca-free MEM	2.5 ± 0.5 (49)	CDMEM	289 ± 18 (66)
		DMEM	359 ± 28 (9)
		15 min CDMEM → 4.45 hr Ca-free MEM	66 ± 31 (6)
		CDMEM + Cycloheximide	329 ± 18 (12)
		CDMEM + Tunicamycin	334 ± 48 (14)
		CDMEM + Monensin	252 ± 58 (10)
		CDMEM + Cytochalasin B	4 ± 1 (8)
		CDMEM + Colchicine	250 ± 35 (15)
		CDMEM 5°C	11 ± 1 (8)
		CDMEM 25°C	29 ± 3 (7)
CDMEM + Tunicamycin			365 ± 72 (5)
CDMEM + Monensin			194 ± 54 (5)

González-Mariscal el al., J Membrane Bio. 86:113-125, 1985
Gonzalez-Mariscal et al., Am. J. Physiol. 259: C978-C986, 1990

Two for Tango

Table 5. Theoretical *vs.* experimental TER in monolayers of mixed cell types

Cell line	% of cells in the mixture		TER	
	Plated	Experimental[a]	Theoretical[b]	Experimental[c]
			($\Omega \cdot cm^2$)	
PtK_2	50	47 ± 1 (6)	127 ± 8	119 ± 15 (7)
MDBK	75	72 ± 3 (4)	22 ± 1	35 ± 2 (8)
	50	54 ± 1 (8)	28 ± 2	31 ± 2 (19)
	25	34 ± 2 (4)	47 ± 2	82 ± 9 (7)
MK_2	50	45 ± 1 (8)	36 ± 5	31 ± 3 (8)
LLC-RK_1	50	55 ± 1 (8)	53 ± 8	84 ± 4 (8)
MA-104	75	72 ± 3 (4)	47 ± 5	35 ± 2 (8)
	50	54 ± 1 (8)	58 ± 5	31 ± 2 (19)
	25	34 ± 2 (4)	80 ± 6	82 ± 9 (7)
LLC-PK_1	75	77 ± 3 (4)	174 ± 10	184 ± 16 (21)
	50	51 ± 2 (4)	186 ± 9	185 ± 16 (22)
	25	23 ± 2 (4)	202 ± 8	239 ± 22 (15)
CPA_{52}	50	51 ± 2 (6)	15 ± 3	9 ± 3 (8)
VERO	75		7 ± 3	20 ± 6 (9)
	50	55 ± 3 (6)	10 ± 2	36 ± 5 (15)
	25		18 ± 1	55 ± 9 (10)

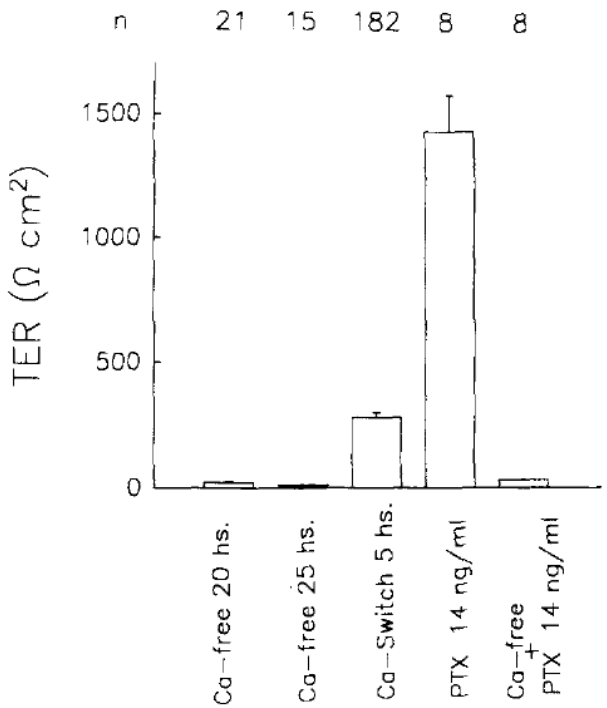
[a] Measured with ^{35}S-methionine at the moment of TER determination

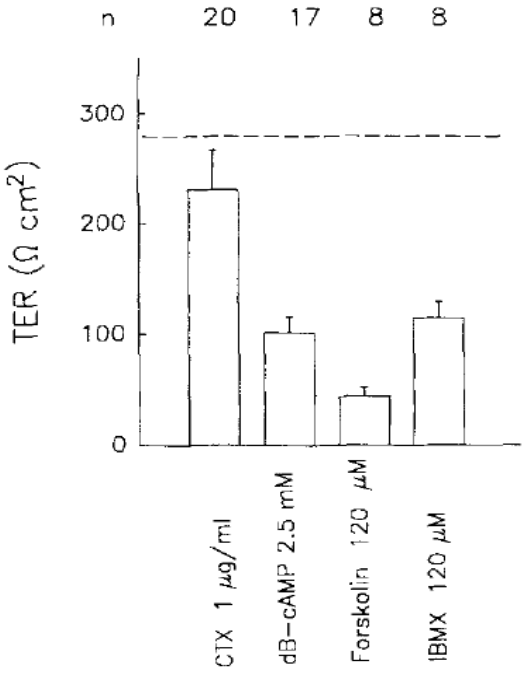
[b] $\frac{1}{R_T} = \frac{f_1}{R_1} + \frac{f_2}{R_2}$

[c] Resistance was measured as in Table 1

Gonzalez-Mariscal et al., 107:43-56, 1989

El ensamble de las UEs se regula por segundos mensajeros: Protenas G y AMPc





Balda et al. J. Membrane Biol. 122:193-202, 1991

La aparición de ZO-1 en la membrana se induce por activación de la PKC

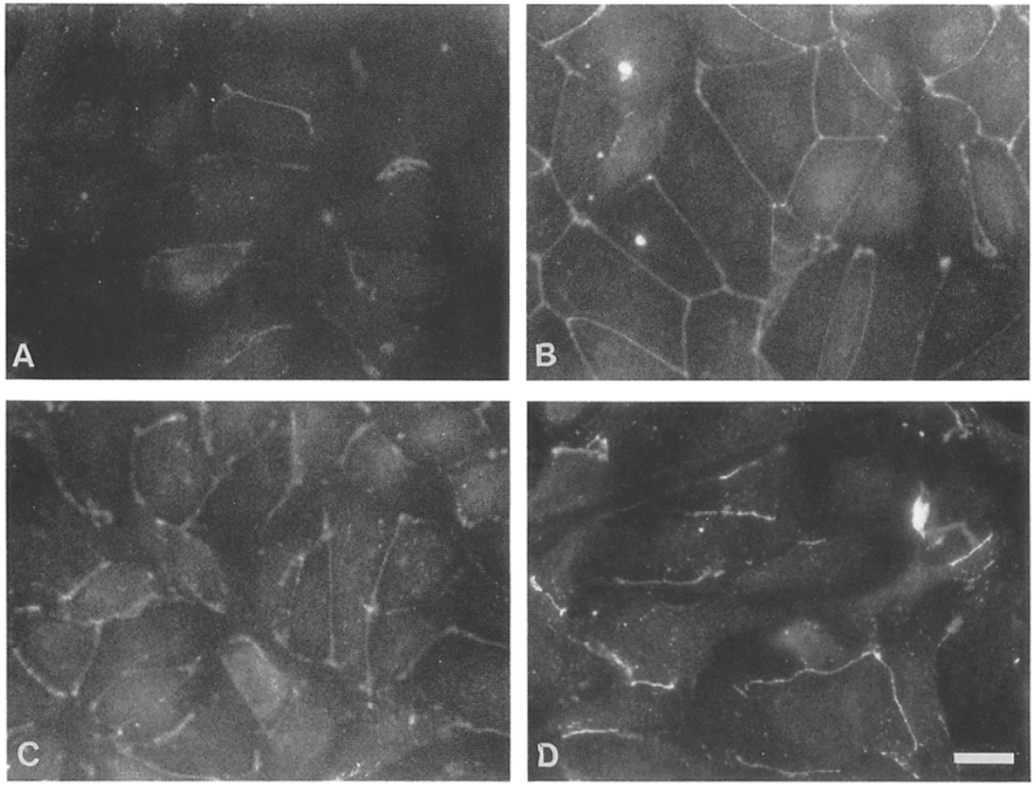


Figure 1. diC8 induces translocation of ZO-1 to the plasma membrane. MDCK cells were incubated in LC for 20 h, and then transferred to LC (*A*), NC (*B*), LC + 0.5 mM diC8 (*C*), or LC + 50 nM staurosporine + diC8 (*D*) for 2 h. Indirect immunofluorescent of ZO-1 was performed as described in Materials and Methods. Bar, 6 μm.

Balda et al., J. Cell Biol, 123:293-302, 1993

La expresión de ZO-1, ZO-2 y ocludina aumenta conforme aumenta la RET del túbulo renal

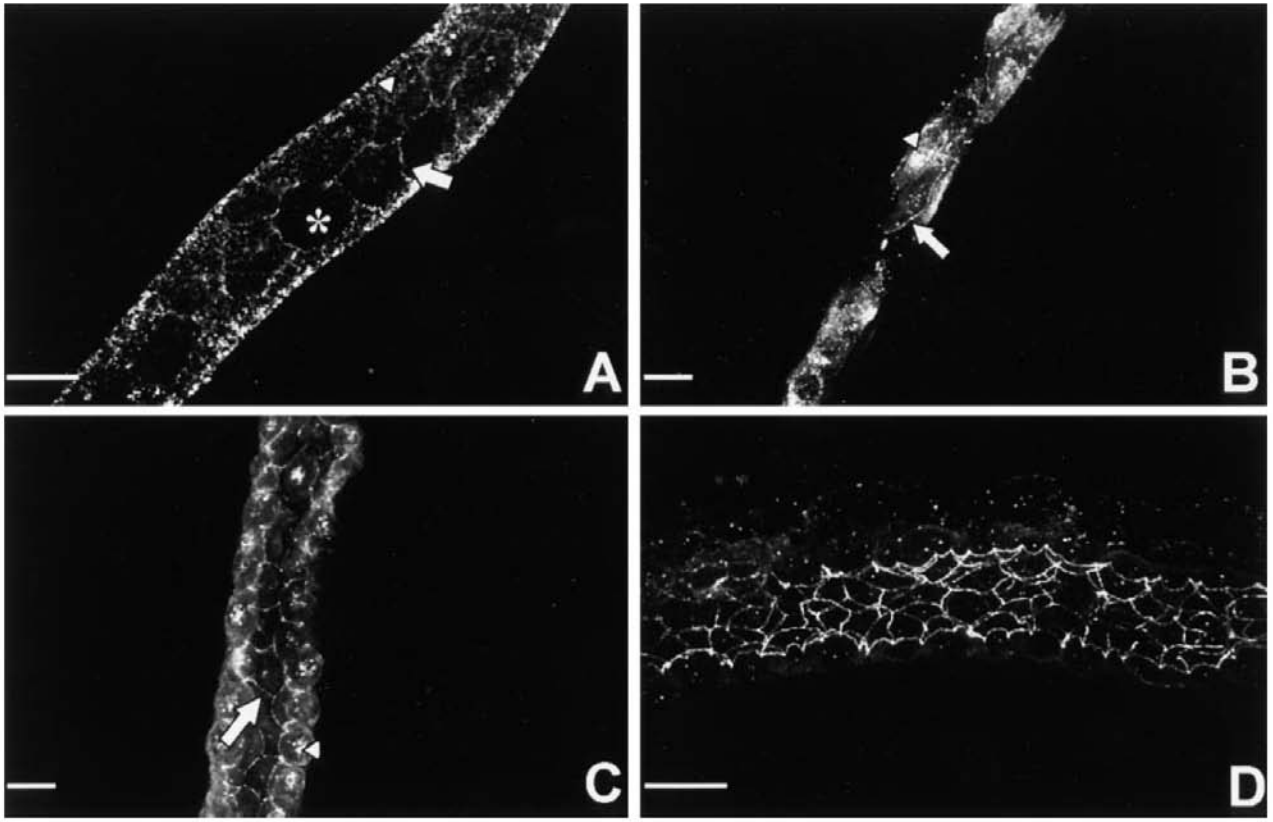
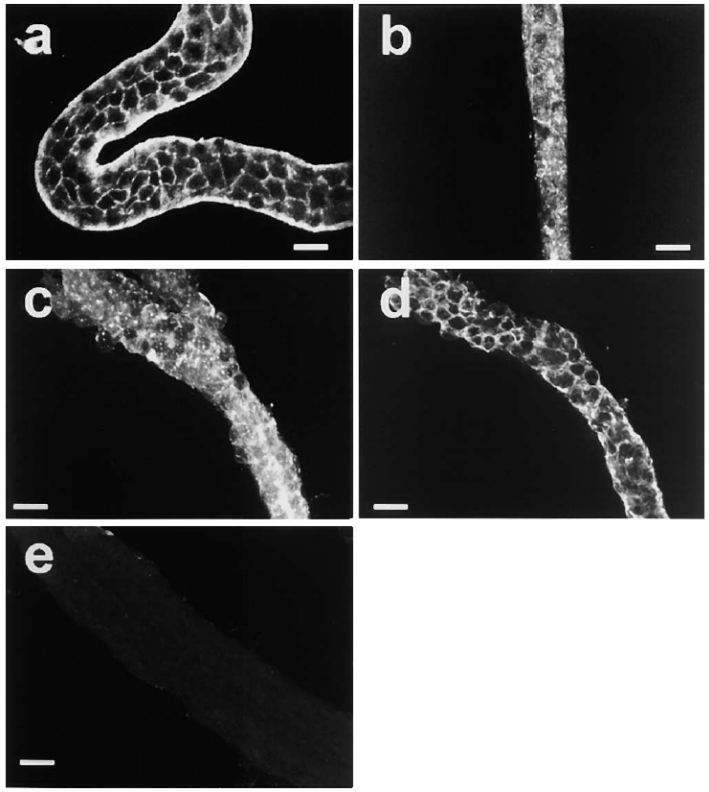


Fig. 6. Localization of ZO-2 in renal tubules. Tubules were stained with rabbit anti–ZO-2 polyclonal antibodies. ZO-2 staining along the cell borders is very faint in proximal tubules (*A*, arrow), while several cells display a punctuated cytoplasmic staining (arrowhead), others are free of it (asterisks). In Henle's loop (*B*), ZO-2 clearly stains cell borders (arrow) as well as nuclei (arrowhead). In distal tubules (*C*), ZO-2 is concentrated at cell borders (arrow) and depicts a dense stain at cell nuclei (arrowhead). In collecting tubules (*D*), ZO-2 forms a network sharply defined. In contrast to distal and Henle's loop, fluorescence is less intense in the nuclei of the collecting tubules.

Gonzalez-Marical et al., Kidney Int. 57:2386:2402, 2000

La expresión de claudina-2 varía a lo largo de los túbulos renales

Claudina 2



Proximal

Henle

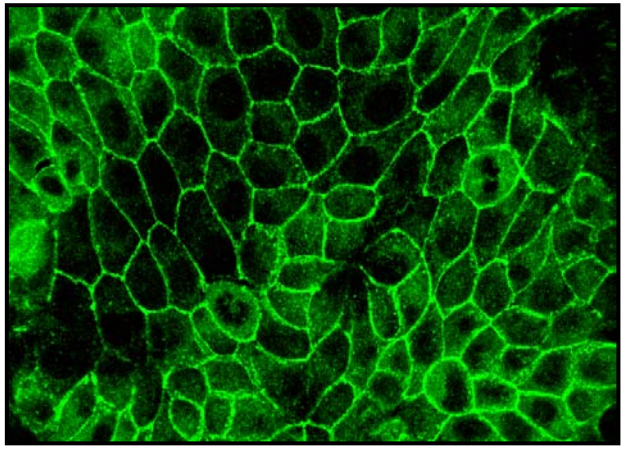
CK8

Distal

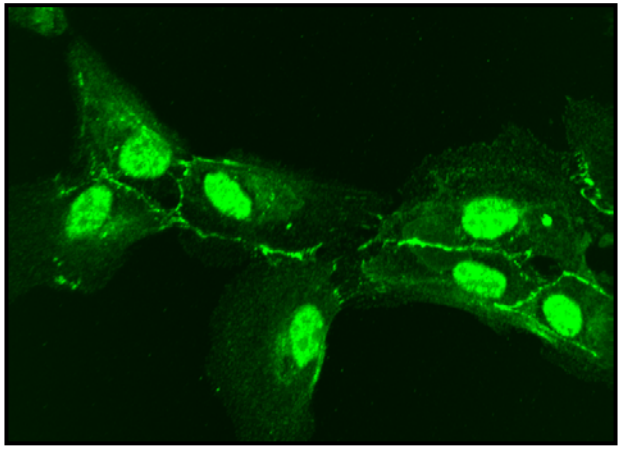
Colector

Reyes et al., Kidney Int. 62:476-487,2002

La proteína de la UE ZO-2 se localiza en el núcleo de las células subconfluentes



Confluent



Sparse

Islas et al., Exp. Cell Res 274:138-148, 2002

ZO-2 se asocia a factores de transcripción

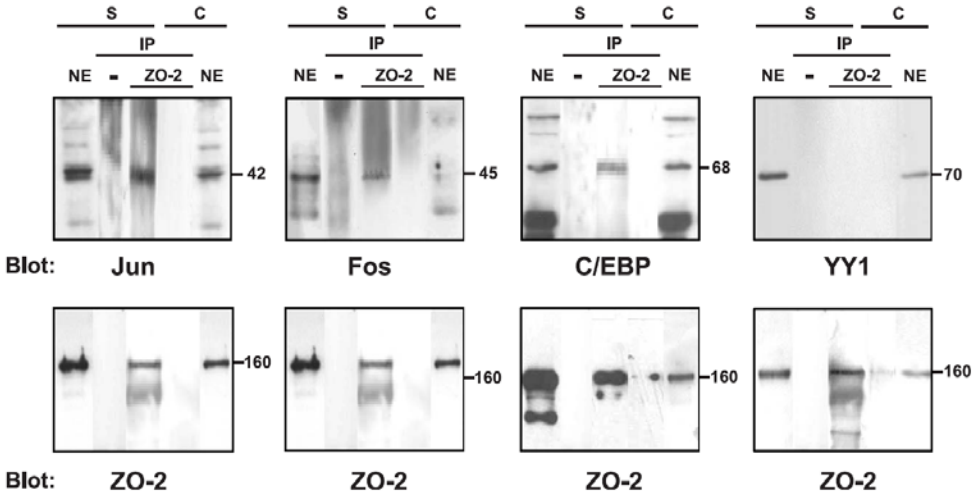
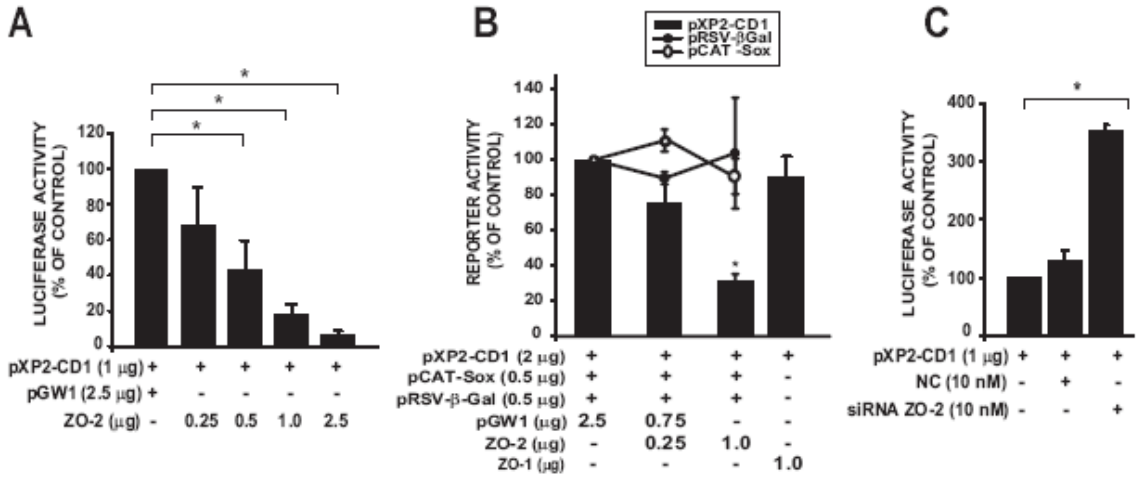


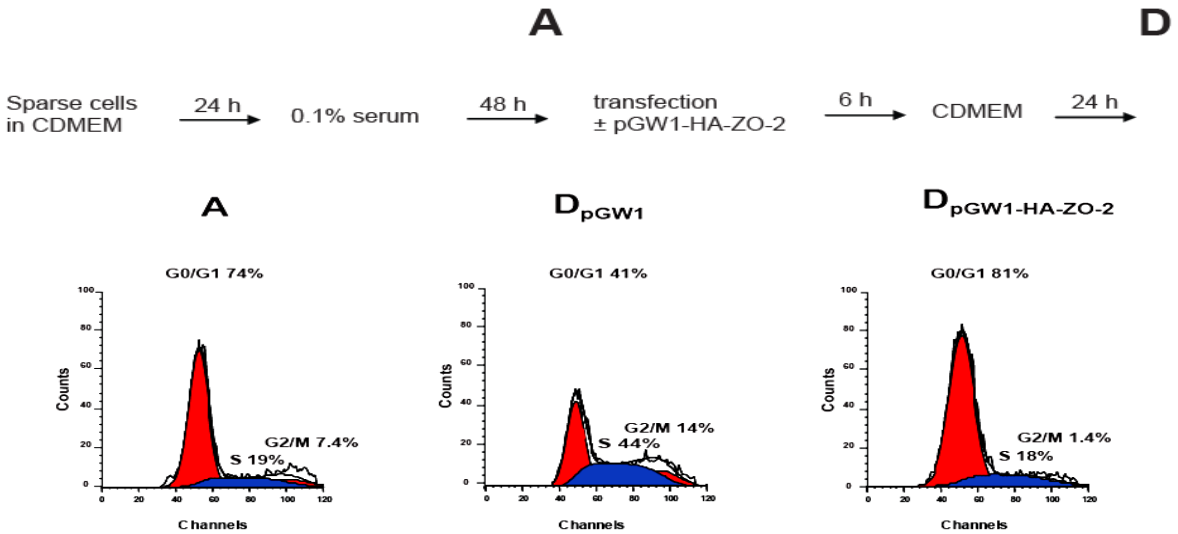
Fig. 4. Jun, Fos and C/EBP co-immunoprecipitate with ZO-2 derived from the nuclei of sparse cultures. From the nuclei of sparse (S) and confluent (C) cultures of MDCK cells, an immunoprecipitation was performed employing anti ZO-2 antibodies or a pre-immune serum (−). The immunoprecipitates were run in a SDS-PAGE and blotted with specific antibodies against Jun, Fos, C/EBP and YY1 (upper panel). The same membranes were then stripped and blotted against ZO-2 (lower panel). NE, indicates the input of nuclear extract derived from sparse and confluent cultures. Numbers to the right indicate the molecular sizes in kilodaltons.

Betanzos et al., Exp. Cell Res 292:51-66, 2004

ZO-2 inhibe la transcripción de CD1 y para el ciclo celular en G1

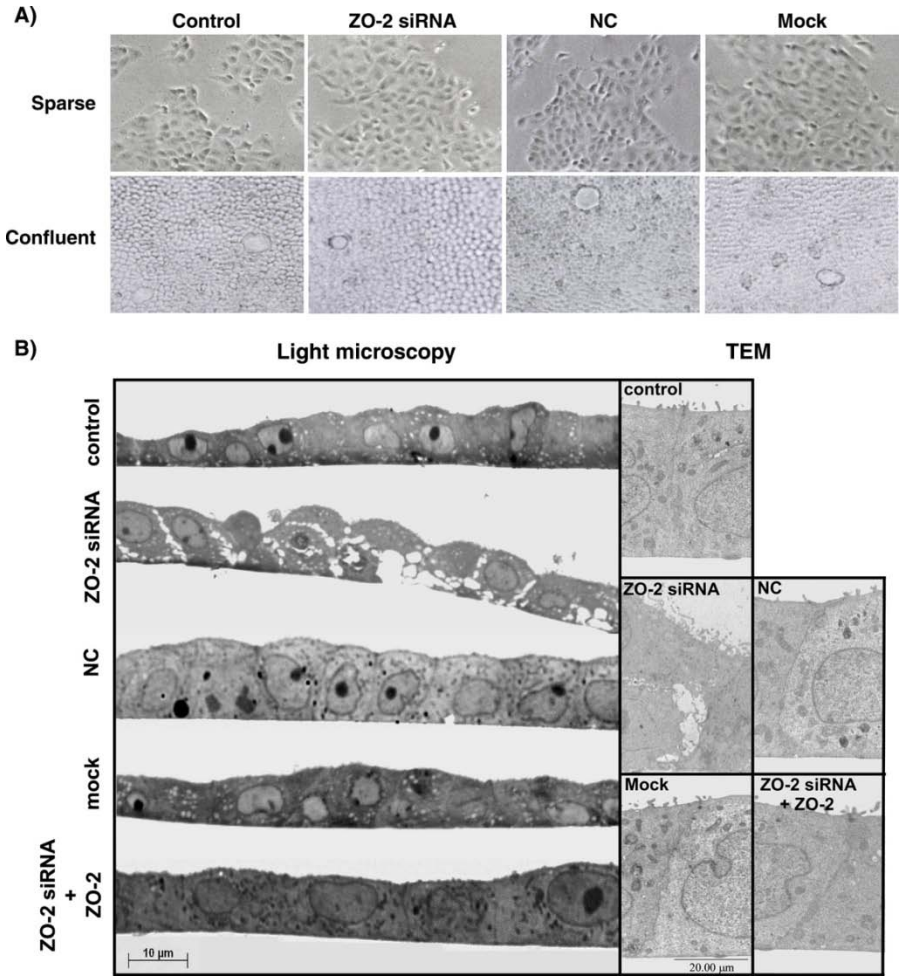


Huerta et al., Mol. Biol Cell 18:4826-4836,2007



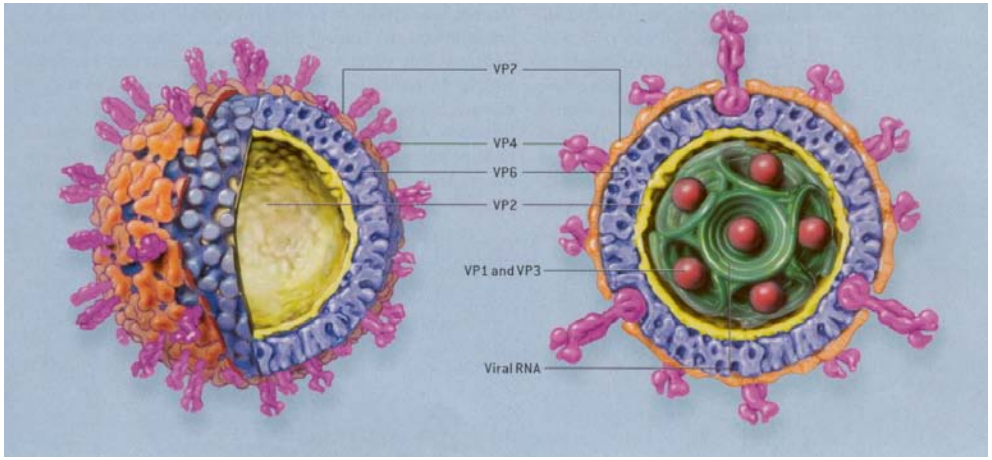
Tapia et al., 2009, Mol. Biol. Cell. 20:1102-1117

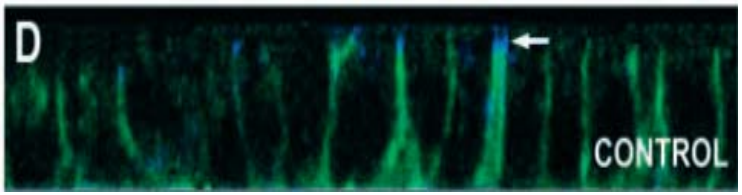
ZO-2 es necesaria para mantener la citoarquitectura epitelial



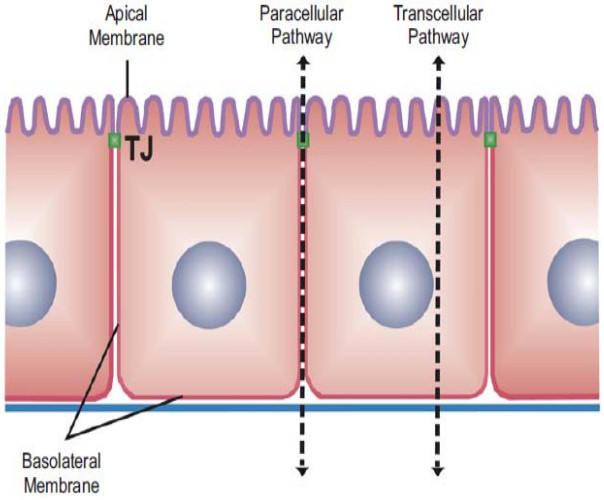
González-Mariscal el al., J Membrane Bio. 86:113-125, 1985

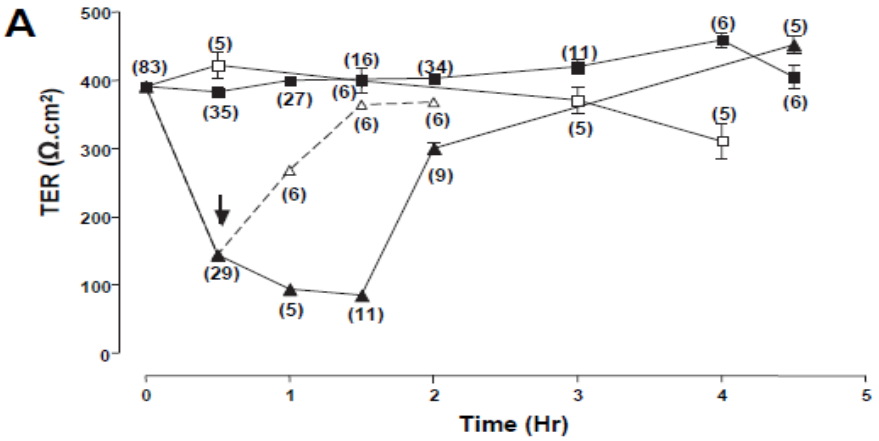
La proteína del rotavirus VP8 abre las UEs





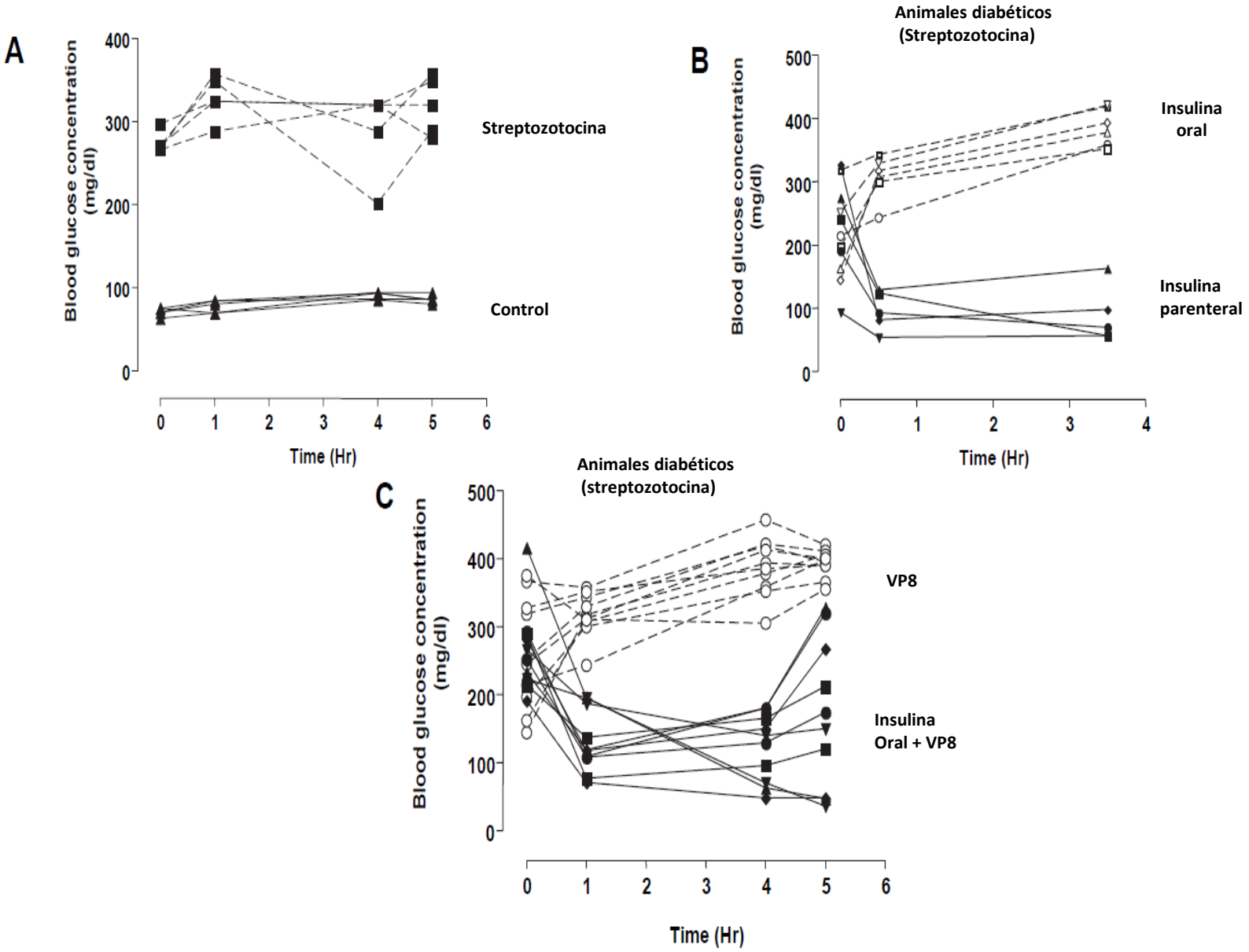
Integrina αvβ3, occludina





Nava, et al., J. Cell Science. 117: 5509-5519 (2004)

VP8 permite la absorción de insulina oral



Nava, et al., J. Cell Science. 117: 5509-5519 (2004).