P2Y Receptor Calcium Signaling in Corneal Epithelial Cells

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Corneal Anatomy

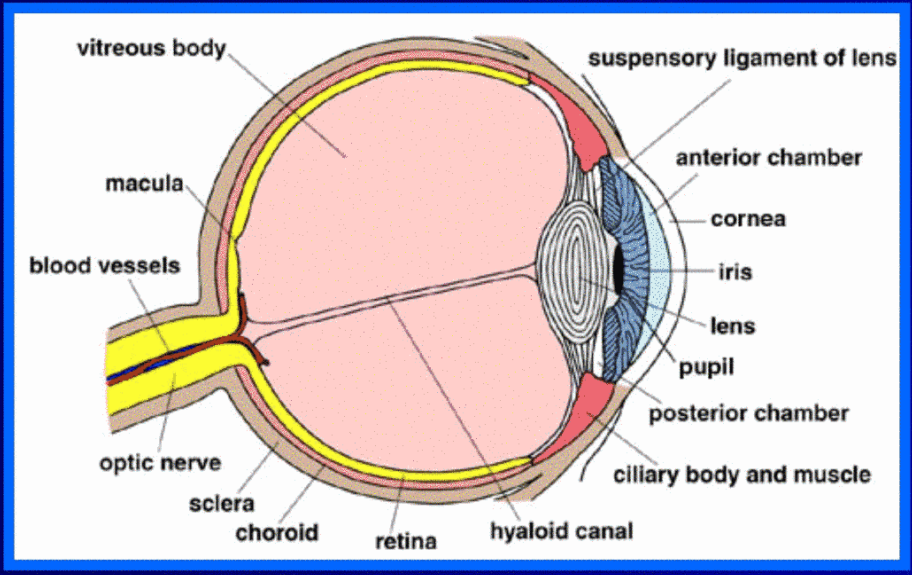
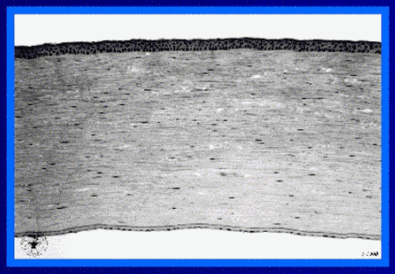


Illustration from: http://www.viahealth.org/disease/eyecare/illustra.htm

Corneal Cross-Section

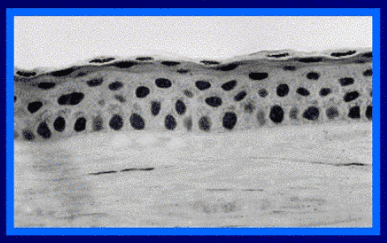


Epithelium

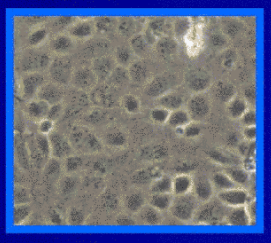
Stroma

Endothelium

Corneal Epithelium



HCE-Ts



Human Corneal Epithelial Cell Line
Established by Araki-Sasaki *et al.*

Light micrographs from:
http://medocs.ucdavis.edu/cha/402/labsyl/98/lab18.htm

Major Functions of the Cornea

- Provide a protective barrier
  - Tight junctions between epithelial cells
- Maintain transparency
  - Avascular tissue
  - Selective entry of ions and water
- Refract light
  - Smooth surface, transparent

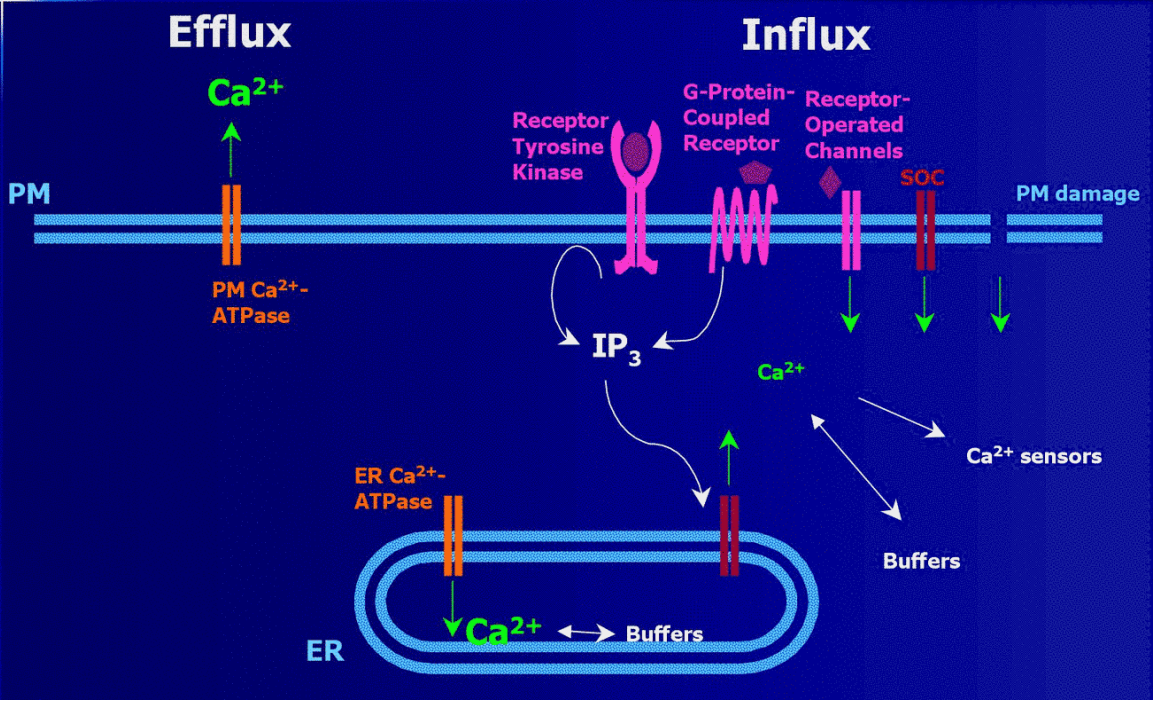
Corneal Injury

- Mechanical forces
  - Shear forces: abrasions
  - Diffuse forces: blunt trauma
  - Lacerations
- Chemicals, UV, heat, lasers, drugs
- Refractive Surgeries
  - LASIK, PRK

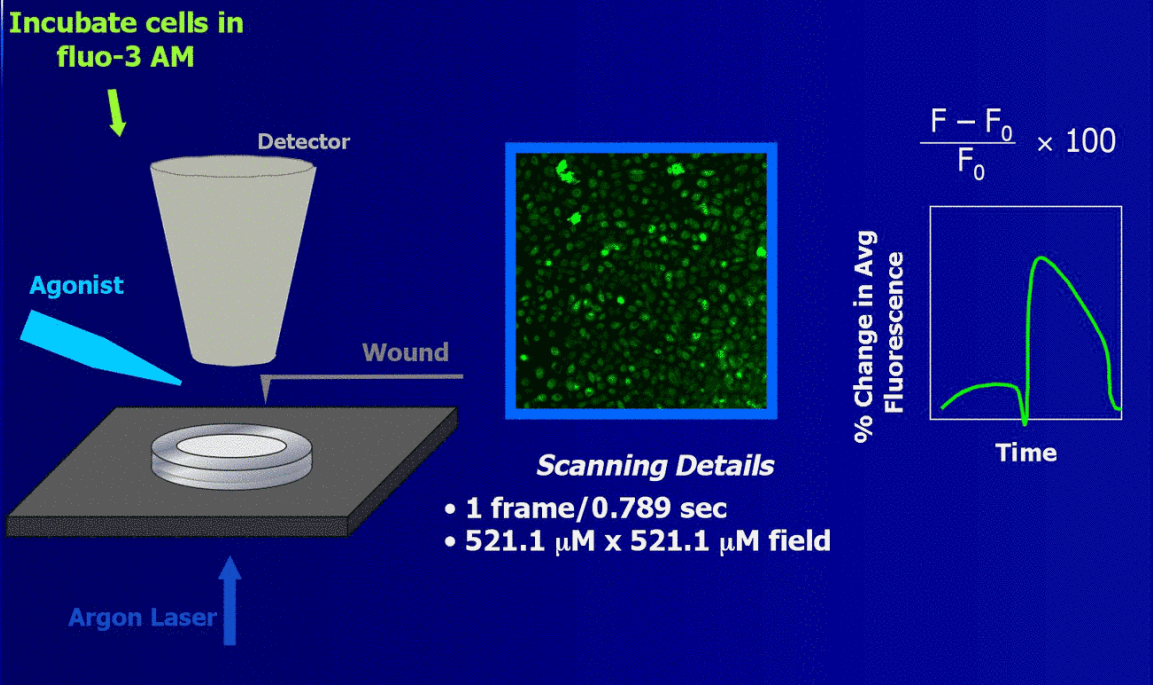
Early Signaling Events Following Injury: Calcium

- Calcium is the most common intracellular signaling molecule
- Oscillations in cytosolic calcium concentrations initiate downstream signaling events
  - Duration
  - Amplitude
  - Frequency
  - Pattern

Calcium Regulation in Nonexcitable Cells



Live Cell Imaging



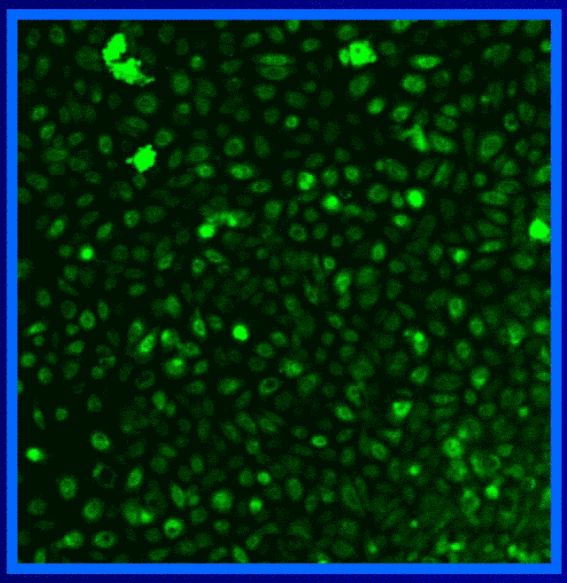
Modeling of Calcium Oscillations and Waves

- Intracellular Calcium Waves
  - Usually originate in one region of the cell and propagate to other regions
  - Coordination and transmission of localized signaling events (puffs, sparks)
- Intercellular Calcium Waves
  - Regenerative Waves
  - Target Waves

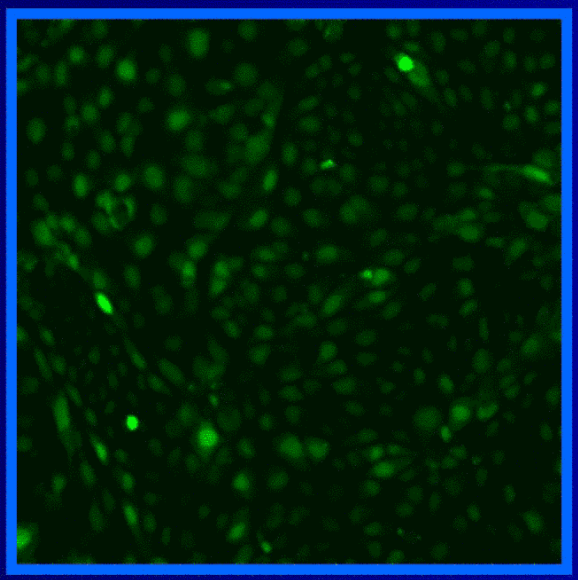
Characterize the Calcium Signaling Events that Occur Immediately Following Injury in Corneal Epithelial Cells

Klepeis et al., 2001, *J. Cell Sci.* 114 (23), 4185-95.

Mechanical Injury Induces a Calcium Wave

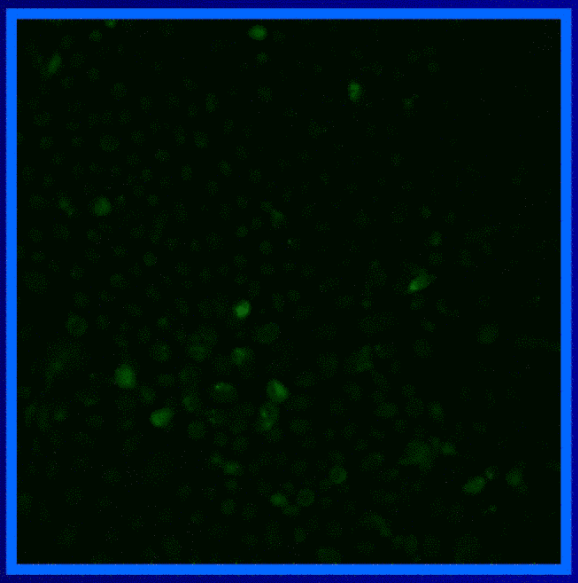


Propagation of the Injury-Induced Calcium Wave Requires Intracellular Calcium



HCE-Ts were incubated with 1 μM thapsigargin to deplete intracellular calcium stores

Extracellular Calcium Enters Cells Along the Immediate Wound Edge

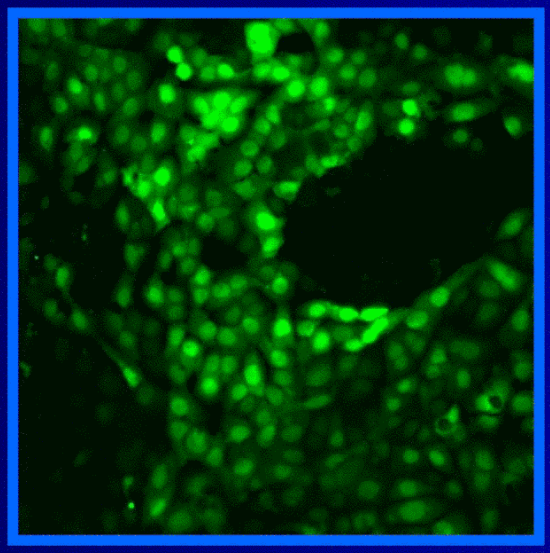


HCE-Ts were incubated with EGTA to deplete extracellular calcium stores

Intercellular Calcium Waves in Other Cell Types

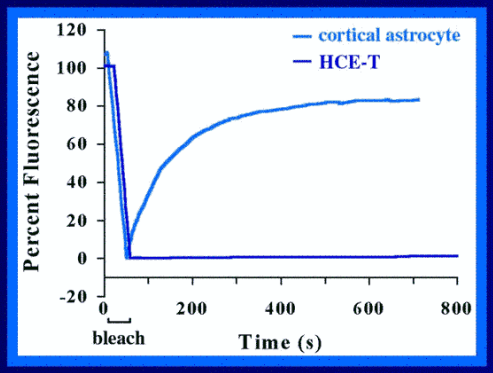
- Described in a wide variety of cell types
  - Coordinate cell activity
- Mediated by:
  - *Intracellular* diffusion of a messenger through gap junctions
    - IP3, Ca2+, cyclic ADP ribose
  - *Extracellular* diffusion of nucleotide signaling molecules
    - Bind P2 nucleotide receptors
  - Both (relative contribution varies with cell type)
    - Gap junctions may play a role in ATP release

Gap junctions are not required for propagation of the injury-induced wave in HCE-Ts

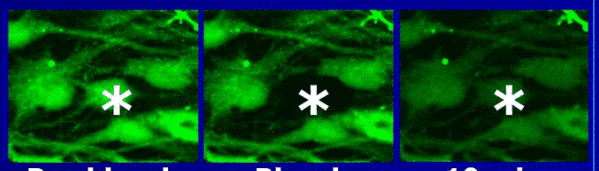


HCE-Ts were incubated with 20 μM α-GA or 2 mM heptanol to inhibit gap junction coupling.

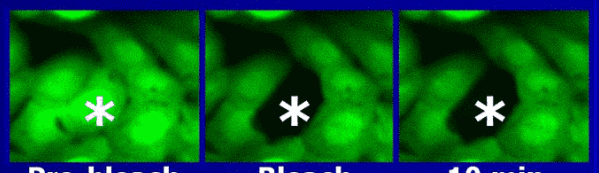
Functional Gap Junctions are not Present in cultured HCE-Ts



Astrocytes

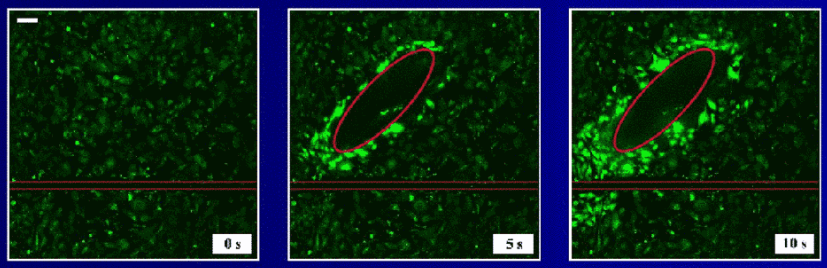


Epithelial



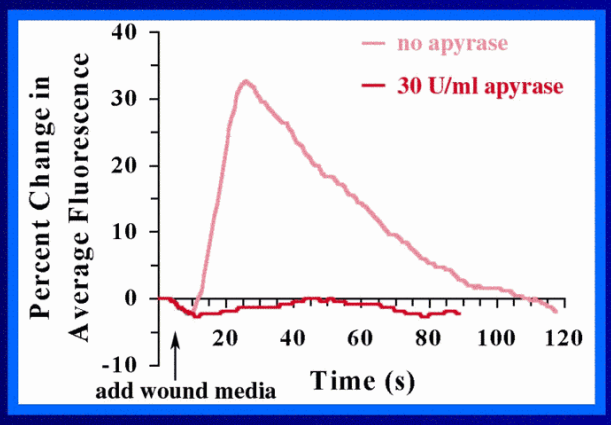
HCE-Ts were incubated with CFDA, one cell was photobleached and fluorescence recovery was followed. Astrocytes were used as a positive control for gap junction expression.

The calcium wave propagates across an acellular space



A linear wound was made and the cells allowed to recover for 1 hr. Cells were incubated with fluo-3 AM, imaged, and a circular wound was made near the original linear wound.

Calcium waves are mediated by extracellular nucleotides



Release of Extracellular Nucleotides

- Unknown mechanism for release in epithelial cells
  - Passive leakage across damaged cells
  - Exocytotic release
    - Neuronal cells
  - Actively released through channels in the plasma membrane
    - connexin hemichannels; CFTR-like

ATP release produces a target wave rather than a regenerative wave

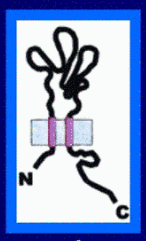
- Limited time and duration of wave propagation
- Propagation rate decreases with increase distance from origin
- Wave intensity decreases with increasing distance from the origin

Nucleotides as Extracellular Signaling Molecules

- Short-term events:
  - Neurotransmission
  - Secretion
- Long-term roles:
  - Proliferation and growth
  - Migration

P2 Nucleotide Receptors

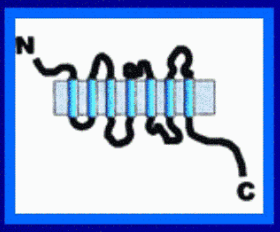
P2X



Ion channel receptors

- 2 transmembrane domains
- 7 subtypes in humans
- Ca^{2+} influx across PM

P2Y



G-protein coupled receptors

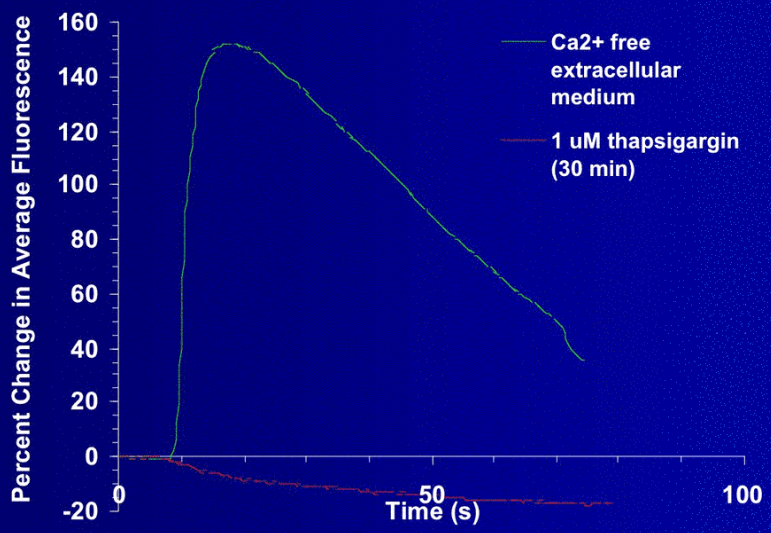
- 7 transmembrane domains
- 7 subtypes in humans
- PLCβ-/IP_3-mediated Ca^{2+} influx
- Range from 41 to 53 kDA after glycosylation (308 to 377 residues)
- Couple to $G_{q/11}$ (PTX insensitive) or $G_{i/o}$ (PTX sensitive)

Characterize P2Y Receptor Expression in Corneal Epithelial Cells

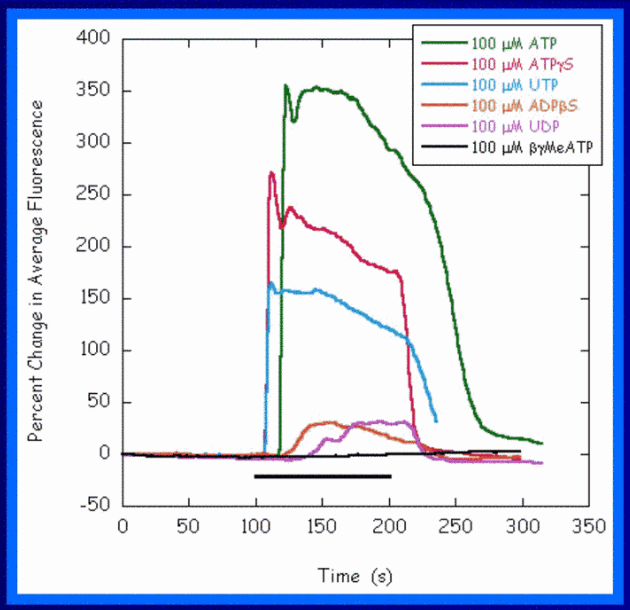
P2Y Receptor agonist selectivities

	$P2Y_1$	$P2Y_2$	$P2Y_4$	$P2Y_6$	$P2Y_{11}$	$P2Y_{12}$
Agonist Sensitivity						
ADPβS	✓					
ADP	✓					✓
ATPγS		✓				
UTPγS		✓				
UTP		✓	✓			
UDP				✓		
UDPβS				✓		
ATP	(✓)	✓			✓	
2MeSATP	(✓)				✓	
2MeSADP	✓					✓
Antagonist Sensitivity						
Suramin		✓		✓	✓	
PPADS	✓		✓	✓		
Reactive Blue2	✓			✓	✓	

ATP-induced Calcium Response Requires Intracellular Calcium



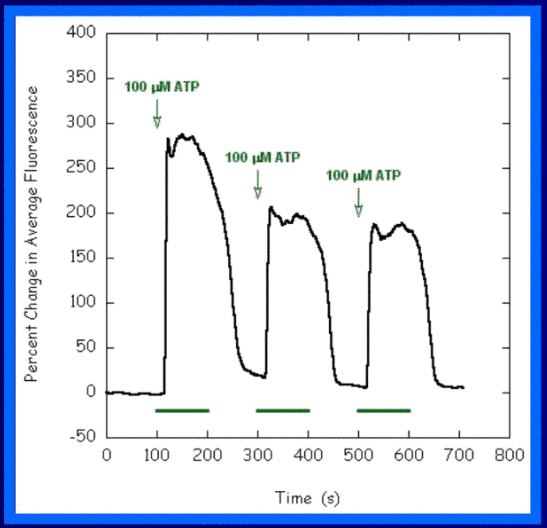
Dose Response to Different P2Y agonists

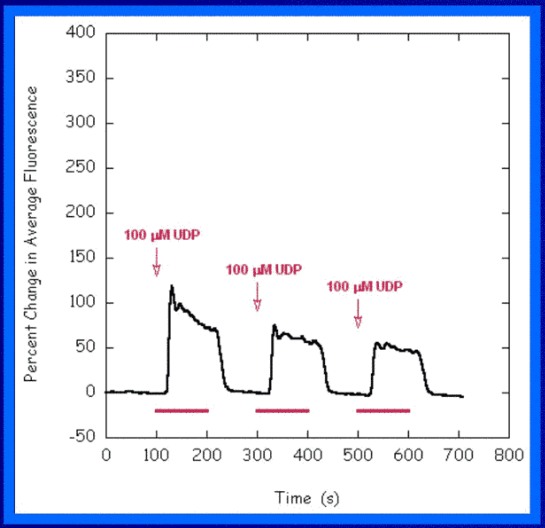


Expression has been verified by RT-PCR
Similar results for primary corneal epithelial cells

ATPγS: $P2Y_2$
UTP: $P2Y_4$
UDP: $P2Y_6$
ADP: $P2Y_1$

Homologous Desensitization of P2Y receptors

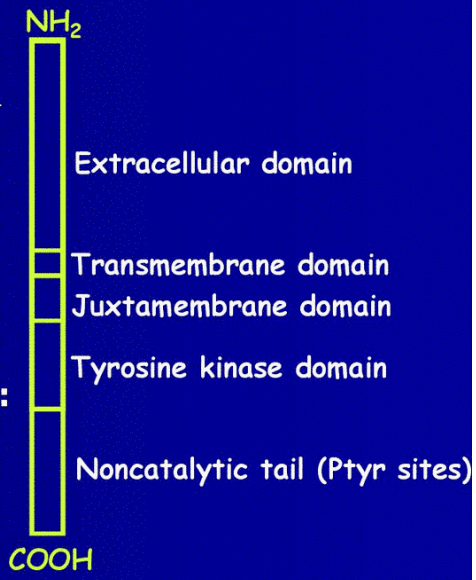




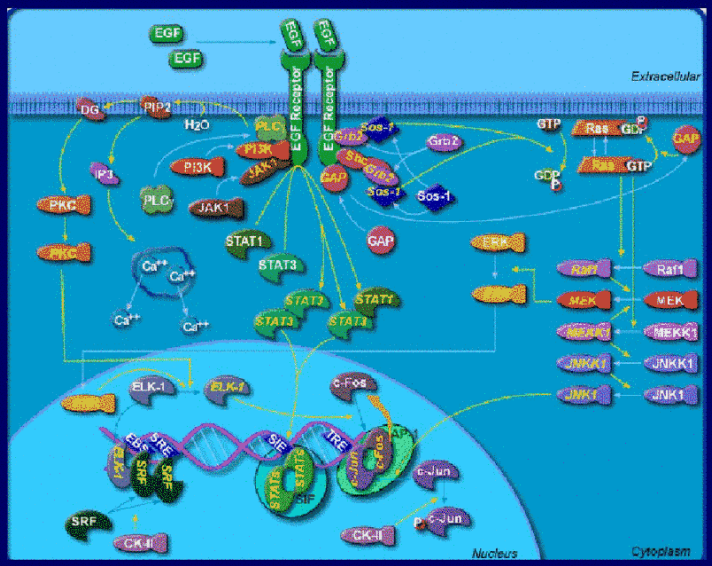
Investigate the Possible Role of P2Y Receptors in Epithelial Wound Healing

Role of the EGF Receptor in Corneal Epithelial Wound Healing

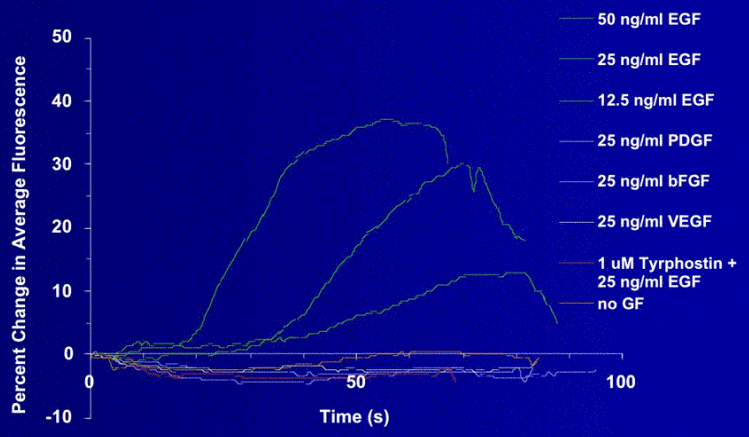
- Member of receptor tyrosine kinase family (170 kDa)
- Regulator of proliferation, migration, differentiation, matrix deposition
- 4 receptor types
  - ErbB1, ErbB2, ErbB3 (no kinase activity), ErbB4
  - 50% homology
- Ligands
  - EGF, TGFα, HB-EGF, amphiregulin: bind ErbB1
  - neuregulins: bind ErbB3 and ErbB4
  - ErbB2 is the preferred heterodimerizing partner



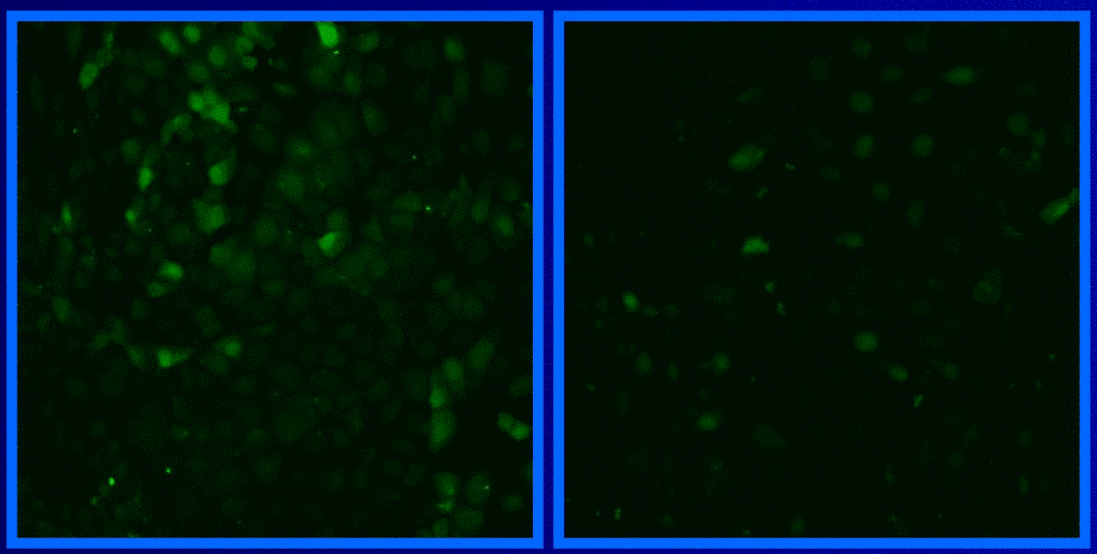
EGF Receptor Activation Mediates many Signaling Pathways



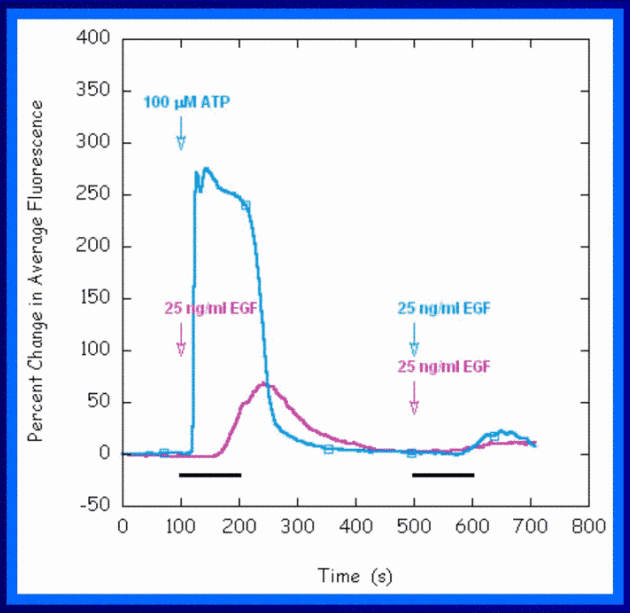
Calcium Response to EGF is Dose-Dependent



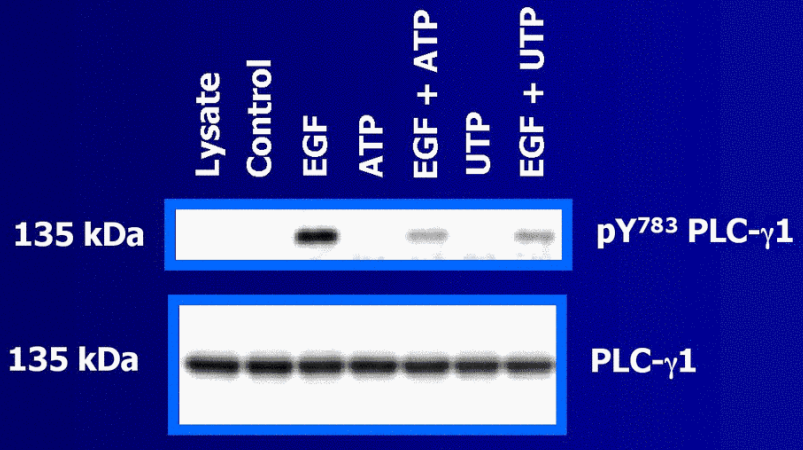
Calcium Response to EGF is Delayed Compared to ATP



Homologous and Heterologous Desensitization of EGFR Occurs



ATP attenuates EGFR activation of PLC-γ1



Western blot analysis of active and total PLC-g1 following stimulation of HCE-Ts with EGF in the presence of absence of nucleotides

Conclusions

- Propagation of injury-induced calcium waves in cultured HCE-Ts and primary corneal epithelial cells
  - Require intracellular calcium stores
  - Do not require gap junctions
  - Are mediated by the release of extracellular nucleotides that bind to P2Y receptors on the cell surface
  - Can be characterized as target waves
- Corneal Epithelial cells express a variety of P2Y receptors on the cell surface
- Nucleotides can attenuate the EGF-induced Calcium response by inhibiting EGFR activation of PLC-γ

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