

*Methods for studying signalling  
in osteoclasts*



**Aymen I. Idris MSc. PhD.**

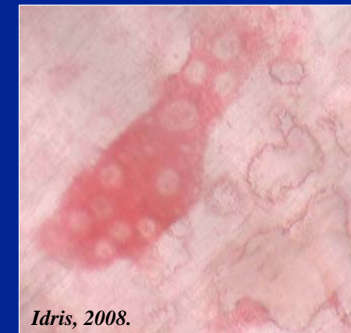
**Institute of Genetics and Molecular Medicine  
University of Edinburgh**

# Bone Metabolism:

## *method for studying signalling in osteoclasts*

### × Signalling pathways

- × **NFκB pathway**
- × **MAPK & AP-1 pathway**
  - × ERK1/2, P38, JNK
  - × cFOS/cJUN
- × **PI3K**
- × **cAMP activation**
- × **Intracellular Calcium**
- × **G-protein coupled receptors**
- × **Smad activation**
- × **Caspase-3 activation**
- × **etc...**

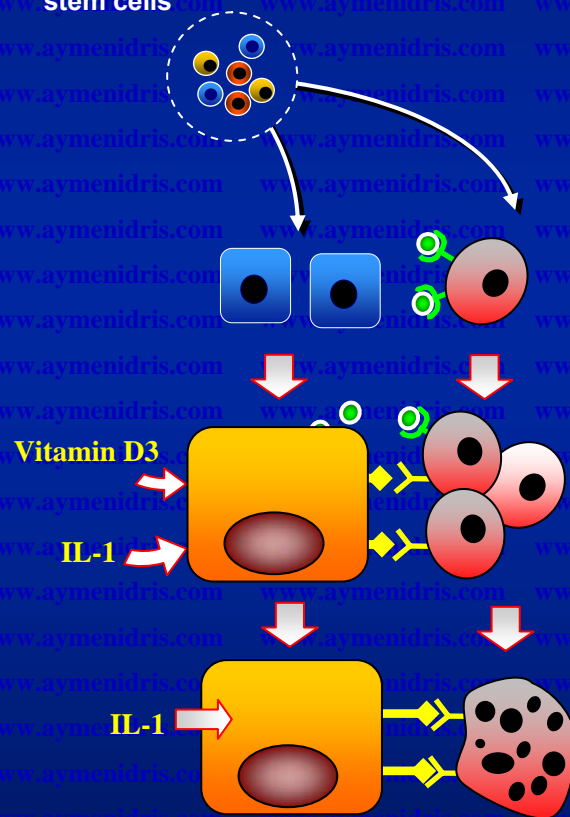


Idris, 2008.

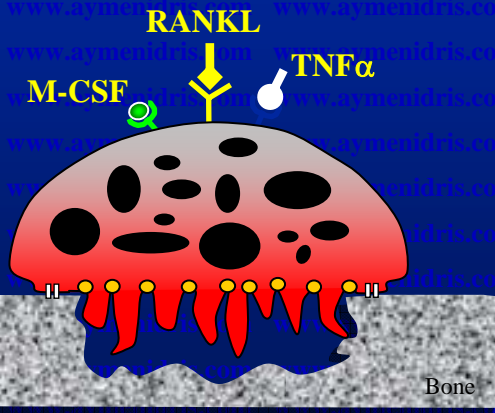
*osteoclasts*

# The bone resorbing osteoclasts originate from a precursor cell of a monocyte-macrophage origin

Bone marrow stem cells



-  BMC
-  OB precursors
-  Mature OB
-  M-CSF
-  M-CSF R
-  RANK-L
-  RANK
-  OC precursors
-  Mature OC



Copyright Aymen Idris (2008)

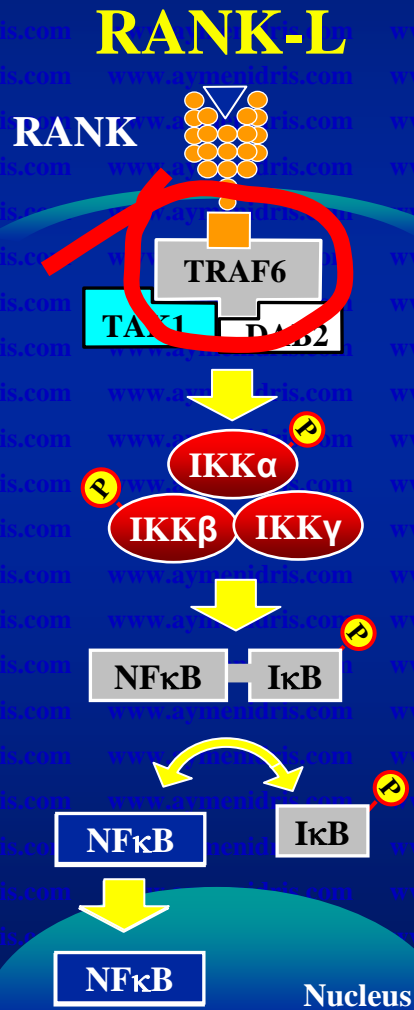
[aymen.idris@ed.ac.uk](mailto:aymen.idris@ed.ac.uk) or [www.aymenidris.com](http://www.aymenidris.com)

---

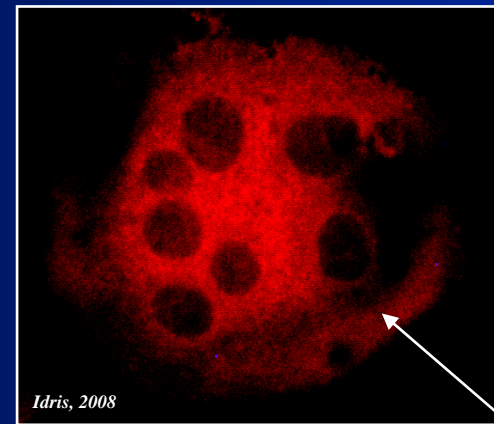
# The role of NF $\kappa$ B activation in osteoclast formation and function

---

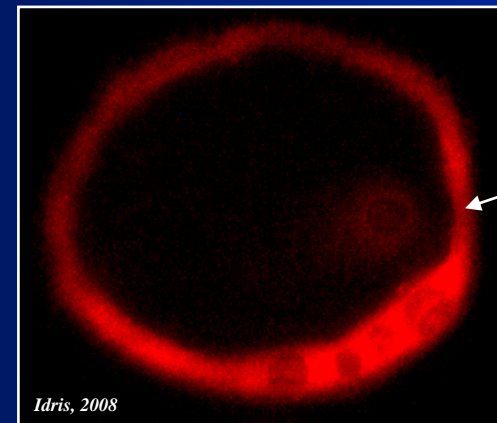
# RANKL induces TRAF6 membrane localization in osteoclasts



**control**



**RANKL**



**TRAF6  
(red)**

# RANK ligand stimulates IKK $\alpha/\beta$ phosphorylation in osteoclast

**RANK-L**

**RANK**

**TRAF6**  
**TAK1** **DAB2**

**IKK $\alpha$**   
**IKK $\beta$**  **IKK $\gamma$**

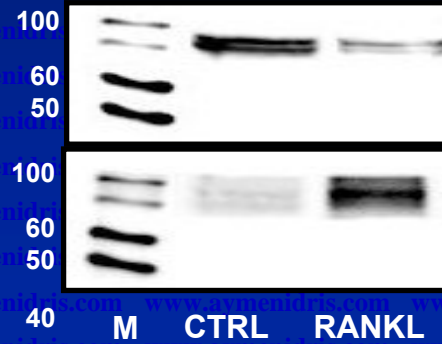
**NF $\kappa$ B** **I $\kappa$ B**

**NF $\kappa$ B** **I $\kappa$ B**

**NF $\kappa$ B**

**Nucleus**

kD

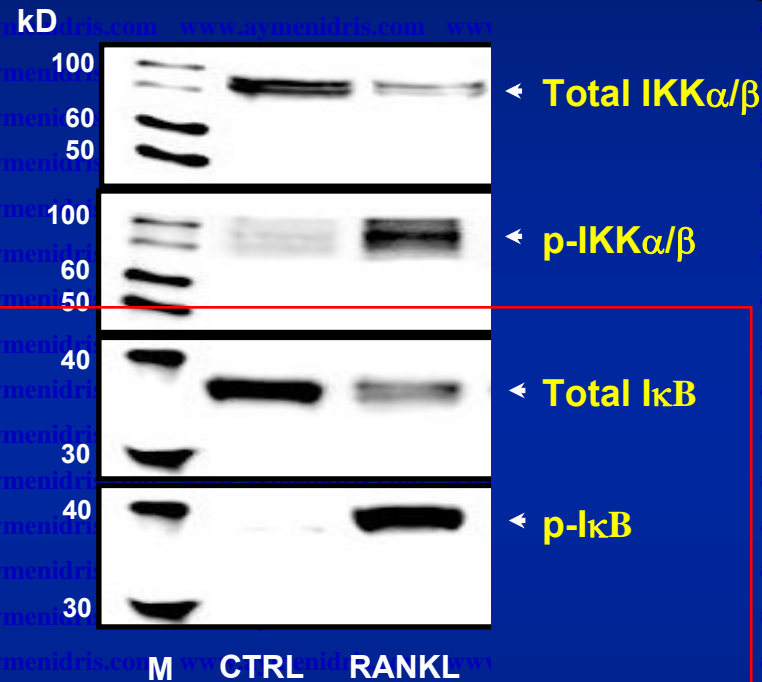
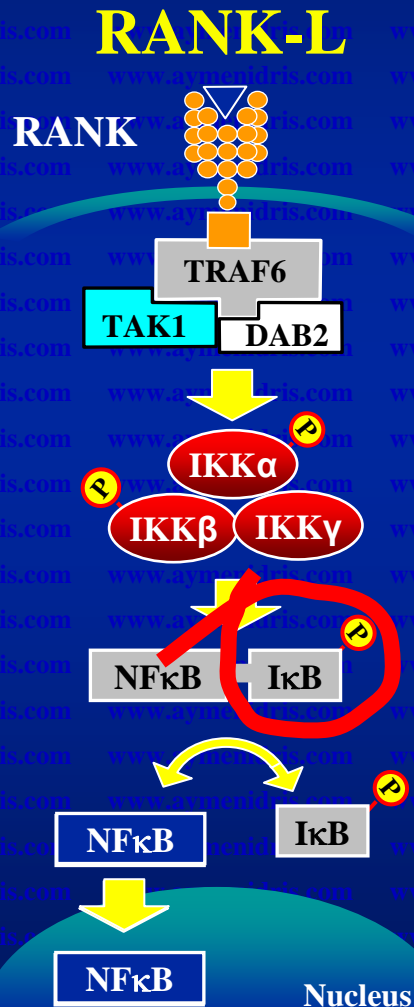


← **Total IKK $\alpha/\beta$**

← **p-IKK $\alpha/\beta$**

**M** **CTRL** **RANKL**

# RANK ligand stimulates I $\kappa$ B phosphorylation in osteoclasts



# RANK ligand frees NFκB to translocate to the nucleus in osteoclasts

**RANK-L**

**RANK**

**TRAF6**  
**TAK1** **DAB2**

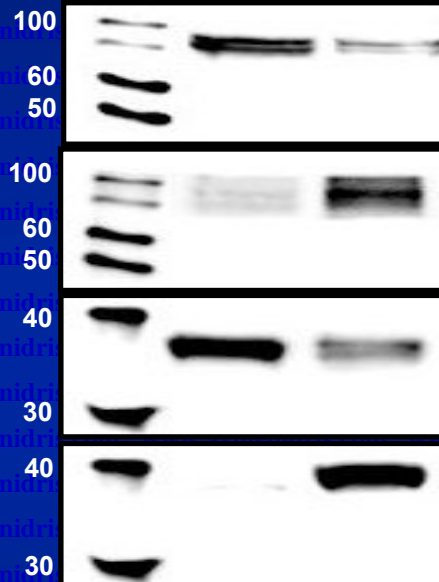
**IKKα**  
**IKKβ** **IKKγ**

**NFκB** **IκB**

**NFκB**

**Nucleus**

kD



← **Total IKKα/β**

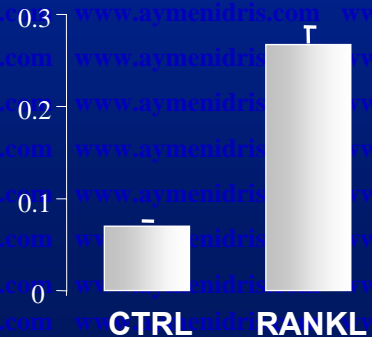
← **p-IKKα/β**

← **Total IκB**

← **p-IκB**

**M** **CTRL** **RANKL**

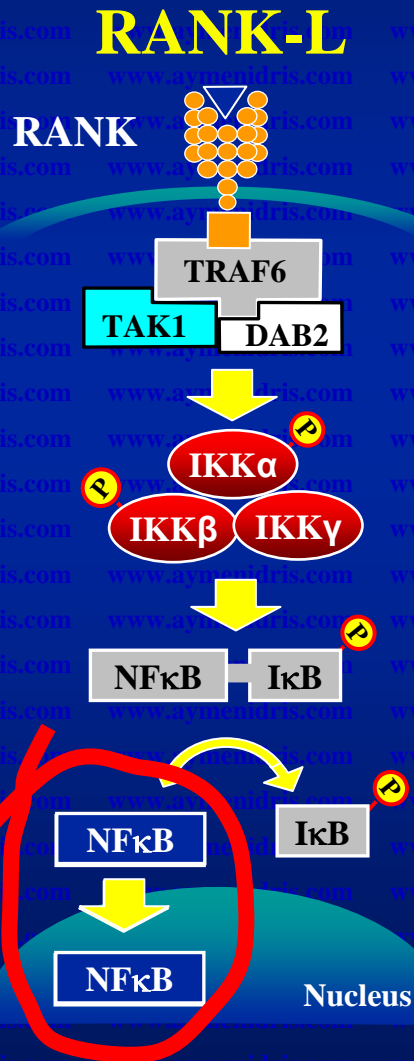
**NFκB DNA Binding**



**CTRL** **RANKL**



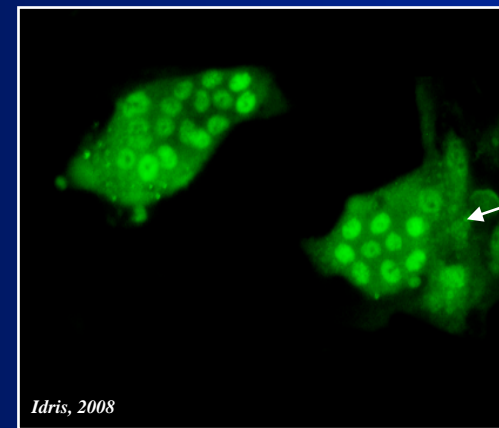
# RANK ligand induces NFκB nuclear Translocation in osteoclasts



control

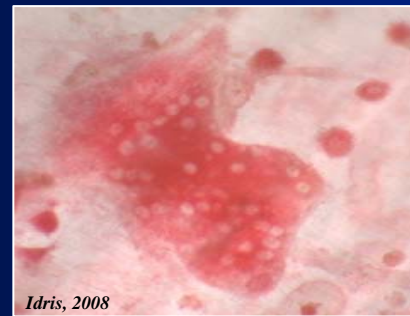
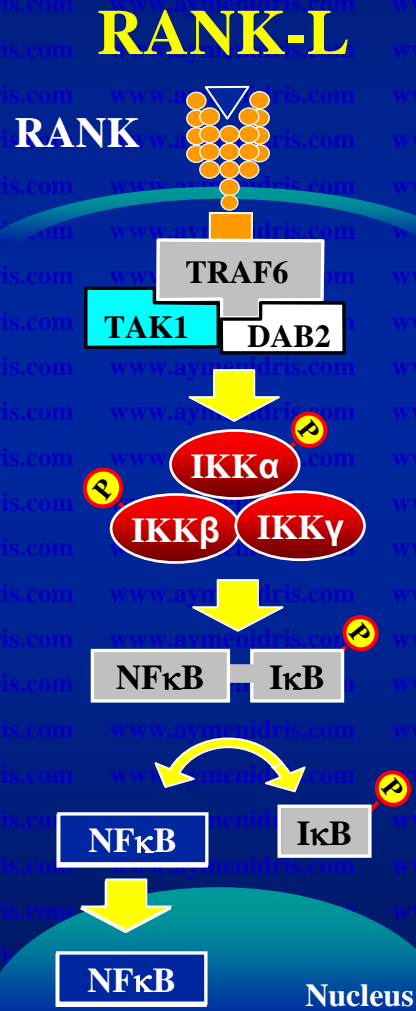


RANKL



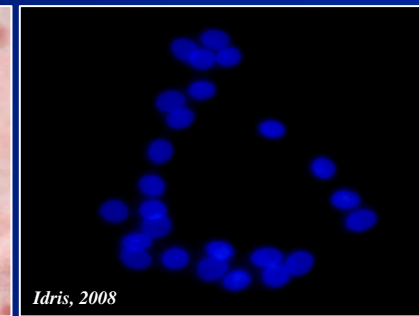
NFκB  
(green)

# RANKL induces NFκB activation and osteoclast formation in bone marrow cultures



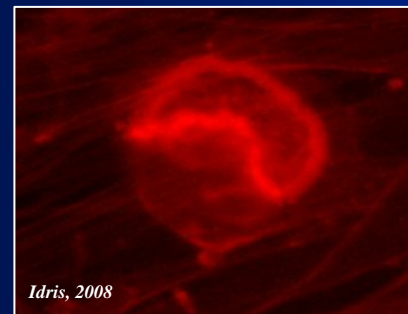
*Idris, 2008*

**TRAcP<sup>+</sup>  
osteoclast**



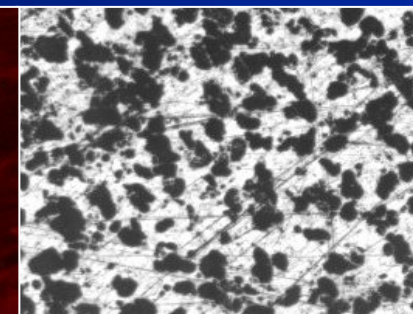
*Idris, 2008*

**DAPI stained  
osteoclast**

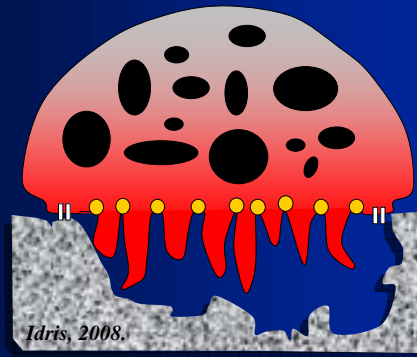


*Idris, 2008*

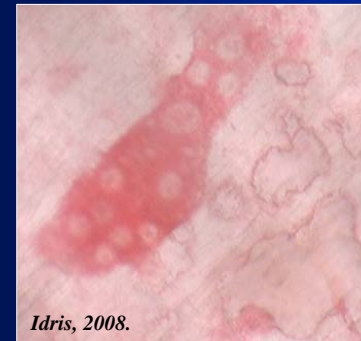
**Actin Rings**



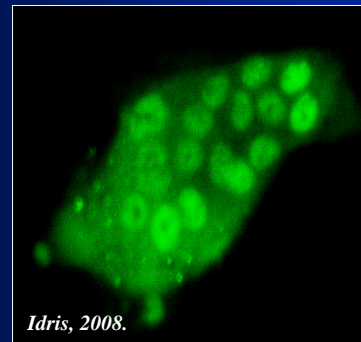
**Resorption pits**



*Functioning  
osteoclast*



*Resorbing  
osteoclast*



*Active  
osteoclast*



*Active  
osteoclast*

Copyright Aymen Idris (2008)

[aymen.idris@ed.ac.uk](mailto:aymen.idris@ed.ac.uk) or [www.aymenidris.com](http://www.aymenidris.com)