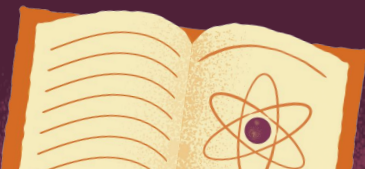


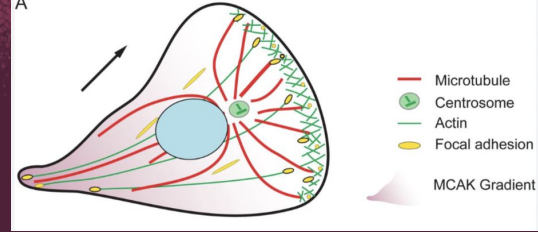


# FAK-Dynein on Cell Polarity in Mouse Fibroblasts

Group 3: Christian Xu, Jeremiah Carrasco, Cemile  
Koseoglu, Denise Soledad Cano



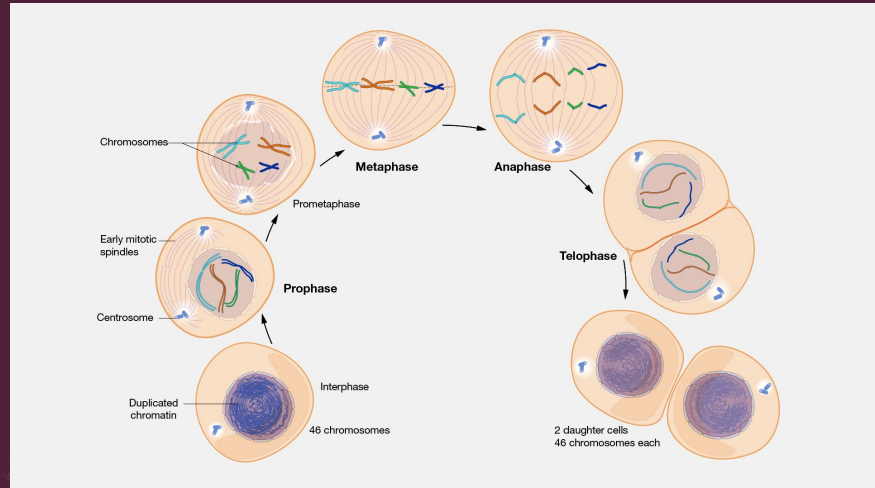
# Intro



- In order for cells to carry out critical adaptive functions to their organization, in response to their micro-environment requires very precise and complex regulation.
- Centrosomes, a vital part in cell division, duplicate themselves and migrate to opposite sides of the cells and separate our chromosomes during cell division.
- This migration requires Dynein, a molecular motor. This motor is thought to pull on microtubules which connect back to Centrosomes, thus moving the Centrosomes into position.
- A question remains of what is Dynein attaching to at the leading edge of the cell.
- In this study evidences are provided suggesting that
- Dynein is attaching to adhesion proteins in focal adhesion points, these proteins are FAK and paxillin.
- FAK is seen as vital in Dynein interactions. Therefore if there is no FAK there is no Dynein and as a result there is no Centrosome Migration.

# FAK(Focal Adhesion Kinase)

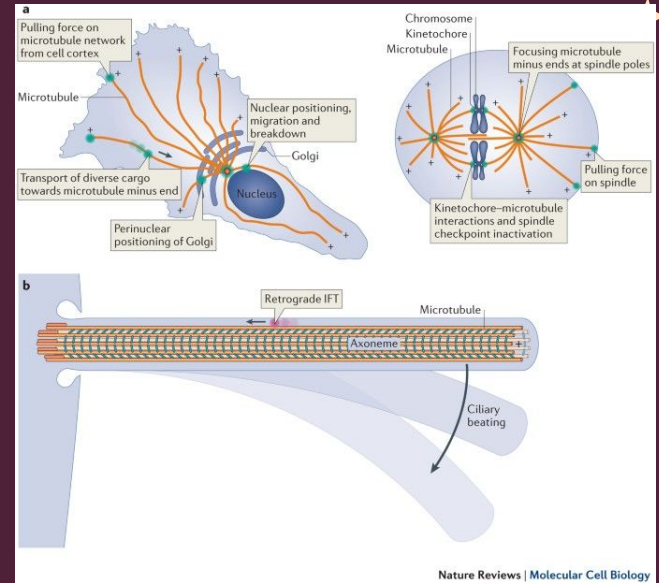
- ❖ FAK are a type of enzyme that help relay important intracellular information such as cell division/survival, gene expression, and gene response.



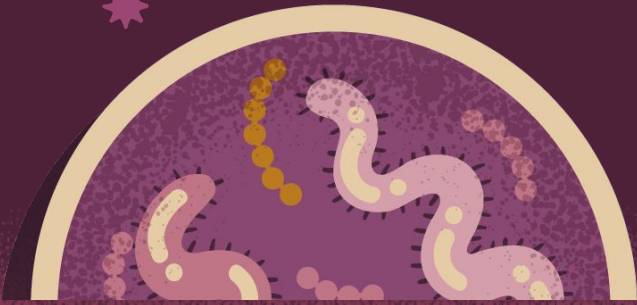
Source: *Mitosis*. Genome.gov. (n.d). Retrieved February 6, 2023, from <https://www.genome.gov/genetics-glossary/Mitosis>

# Dynein

- ❖ Dynein are also proteins and they are responsible for proper cellular division
- ❖ Responsible for transporting cargo intracellularly
- ❖ And rearranging microtubules/organelles, such as the Golgi Apparatus, during cell division

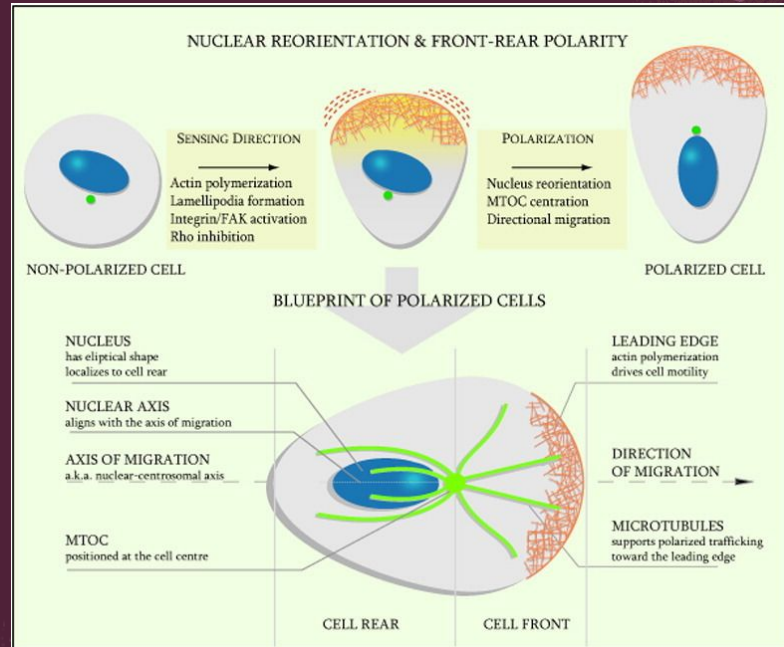
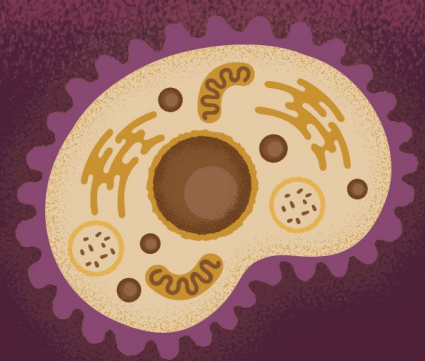


Source: Roberts, A., Kon, T., Knight, P. et al. Functions and mechanics of dynein motor proteins. *Nat Rev Mol Cell Biol* 14, 713-726 (2013). <https://doi.org/10.1038/nrm3667>



# FAK-Dynein

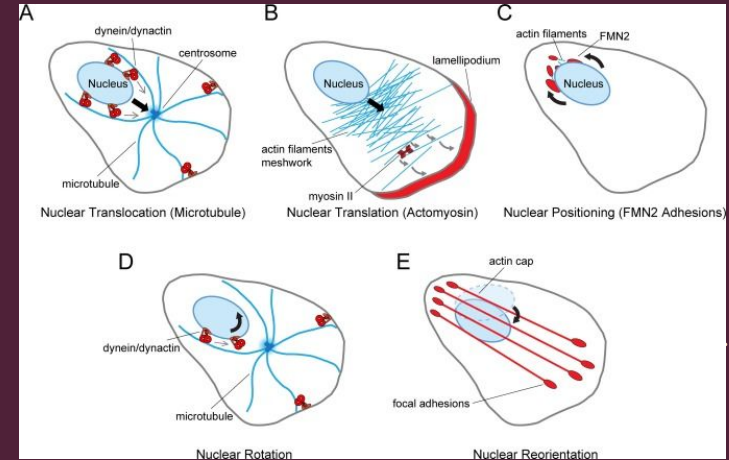
- ❖ Together FAK-Dynein contribute to the intracellular polarization (cell reorganization to form its shape)



Source: Maninová, M., Klímová, Z., Parsons, J. T., Weber, M. J., Iwanicki, M. P., & Vomastek, T. (2013). The reorientation of cell nucleus promotes the establishment of front-rear polarity in migrating fibroblasts. *Journal of Molecular Biology*, 425(11), 2039–2055. <https://doi.org/10.1016/j.jmb.2013.02.034>

# Research & Purpose

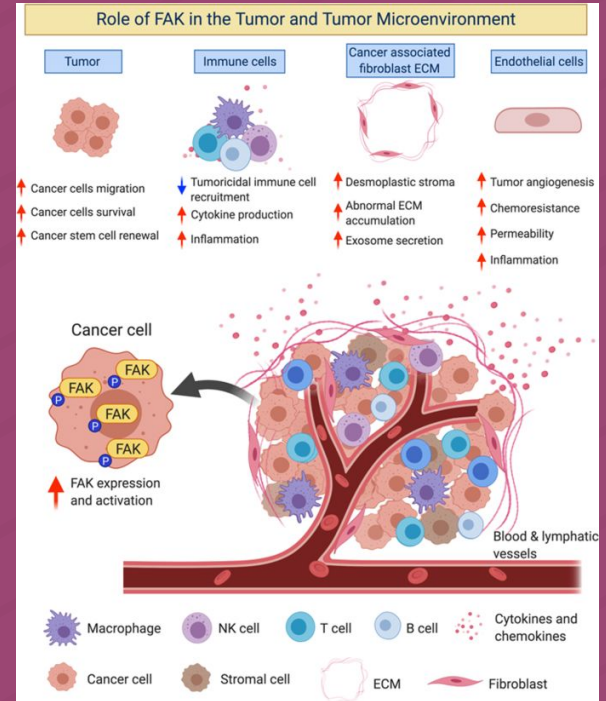
- ❖ Researchers in this study experimented using FAK-Dynein protein on mouse fibroblast (cells that form connective tissue).
- ❖ The purpose was to determine how much of an effect the nucleus (nuclear movements) of mouse fibroblasts has on centrosome positioning during polarization.



Source: Zhu, R., Liu, C., & Gundersen, G. G. (2018). Nuclear positioning in migrating fibroblasts. *Seminars in Cell & Developmental Biology*, 82, 41–50. <https://doi.org/10.1016/j.semcdb.2017.11.006>

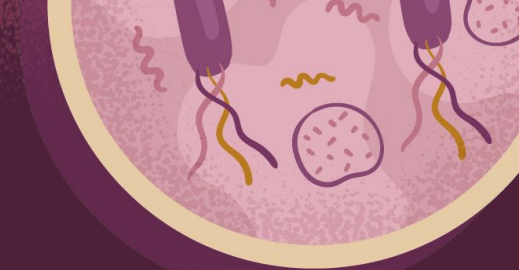
# Importance

- ❖ Studying FAK and dynein's subsequent implications on cellular mitosis/division can help scientists better understand how cancer cells metastasize



Source: Murphy, J.M., Rodriguez, Y.A.R., Jeong, K. *et al.* Targeting focal adhesion kinase in cancer cells and the tumor microenvironment. *Exp Mol Med* 52, 877-886 (2020). <https://doi.org/10.1038/s12276-020-0447-4>

# Discussion



- Literature review: gathers all of the current discussions going on between scientists and researchers regarding this topic.
- Ezratty, Kaverina, Stehbens, and Wittmann:
  - FAK controls the alignment of the centrosome and its associated Golgi apparatus in mouse fibroblasts
- Park and Rosse:
  - Lessening the interaction between dynein and FAK damaged Golgi polarisation.
- Robinson
  - The position centrosome inside the cell is determined by the opposing dynein forces, pulling it in opposite directions.
- Plotnikov, Prager-Khoutorsky, Wang
  - Focal adhesions and FAK allow cells to orient the centrosome to sense the physical characteristics of the microenvironment to better regulate cell polarity.





# Materials and Methods

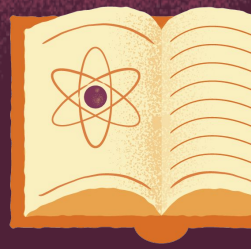


- Plasmids
  - All of the different cells were constructed and amplified by Qiagen Hispeed Maxiprep kits and were analysed by sequencing them
- Cell culture and transfection
  - The cells were scattered for them to convert in 48 hours to be ready for cell extract
  - There was a transfection that was performed with the six-well dishes
  - The flowing cells were placed on coverslips with a pipet tip
  - They were either placed in the incubator for 4 hours or they were directly placed on a microscope to be analysed
- Immunocytochemistry and immunological reagents
  - The coverslips were washed in 37 degree celsius buffer
  - Then they were incubated for about 30 min and then were incubated in protein concentration for another 30 min

# Materials and Methods (pt.2) ✨

- ✨ ● Immunoprecipitation and western blotting
  - There is a standard protein analysis to check for DIC and FAK
  - Six well-dishes were washed twice and then were recovered with a cell scraper
- Microscopy and live cell imaging
  - The live cell samples were later analysed through a specific microscope that was equipped with a 37 degree celsius temperature and CO2 concentration
- Cell polarity analysis and statistics
  - The polarity angle known as  $\alpha$  is being represented by a line passing through the center of the nucleus and the center of the Golgi as well as the centrosomes, perpendicular to the polarity axis ✨
  - There is is a watershed mask placed to separate nuclei from the surrounding

# Work Cited



Roberts, A., Kon, T., Knight, P. *et al.* Functions and mechanics of dynein motor proteins. *Nat Rev Mol Cell Biol* 14, 713–726 (2013).

<https://doi.org/10.1038/nrm3667>

Sulzmaier, F., Jean, C. & Schloepfer, D. FAK in cancer: mechanistic findings and clinical applications. *Nat Rev Cancer* 14, 598–610 (2014).

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Vicente-Manzanares, M., & Sánchez-Madrid, F. (2000). Cell polarization: A Comparative Cell Biology and immunological view. *Developmental Immunology*, 7(2-4), 51–65. <https://doi.org/10.1155/2000/70801>

Fructuoso M, Legrand M, Mousson A, Steffan T, Vauchelles R, De Mey J, Sick E, Rondé P, Dujardin D. FAK regulates dynein localisation and cell polarity in migrating mouse fibroblasts. *Biol Cell*. 2020 Feb;112(2):53-72. doi: 10.1111/boc.201900041. Epub 2020 Jan 9. PMID: 31859373.

# Work Cited cont.

