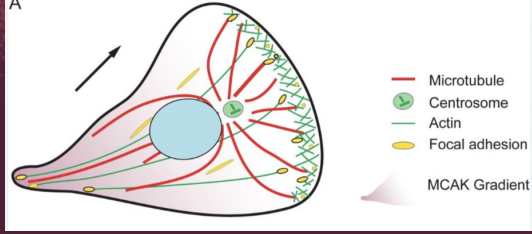
FAK-Dynein on Cell Polarity in Mouse Fibroblasts

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

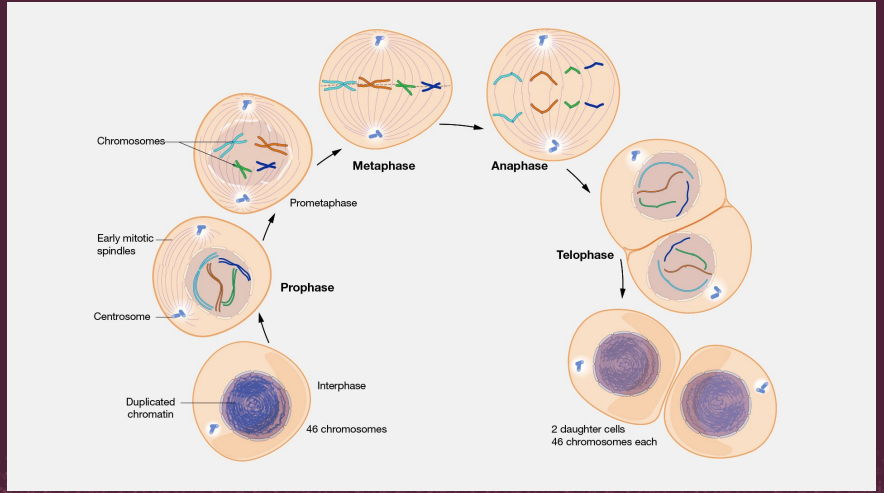
Intro



- 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 is there is no FAK there is no Dynein and as a result there is no Centrosome Migration.

FAK(Focal Adhesion Kinase)

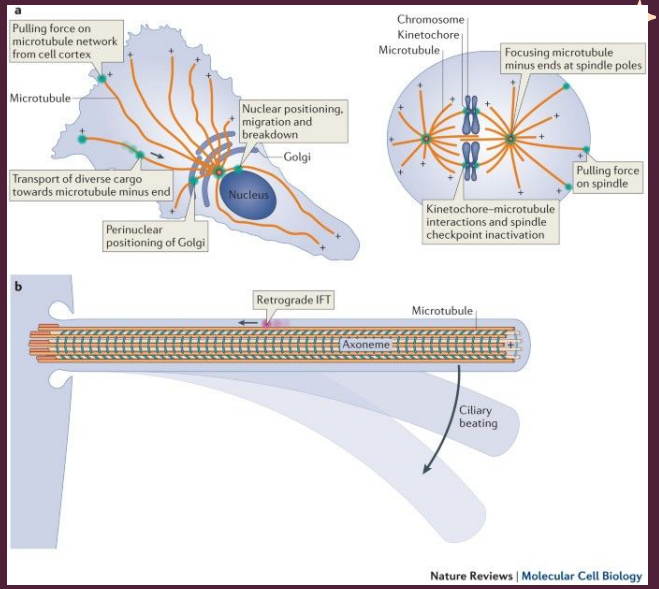
- FAK are a type of enzyme that help relay important intracellular information such as cell division/survival,gene expression, and gene response.



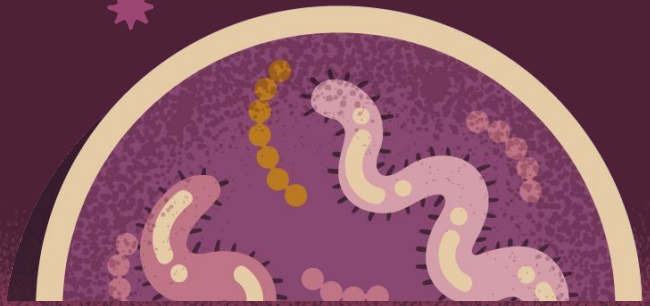
Source: *Mitosis*. Genome.gov. (n.d.). Retrieved February 6, 2023, from https://www.genome.gov/genetics-glossary/Mitosis

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

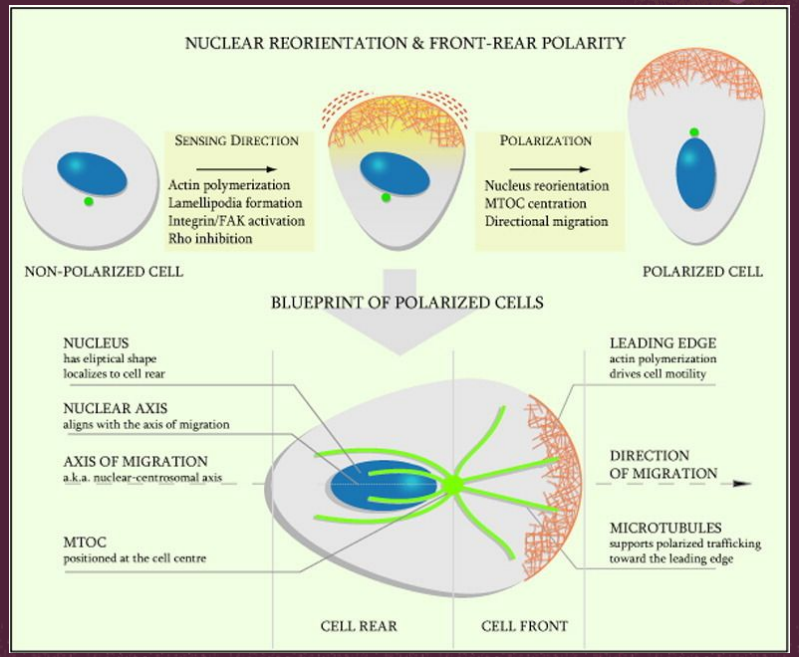


Source: Roberts, A., Kon, T., Knight, P. *et al.* Functions and mechanics of dynein motor proteins. *Nat Rev Mol Cell Biol* 14, 713–726 (2013). https://doi.org/10.1038/nrm3667



FAK-Dynein

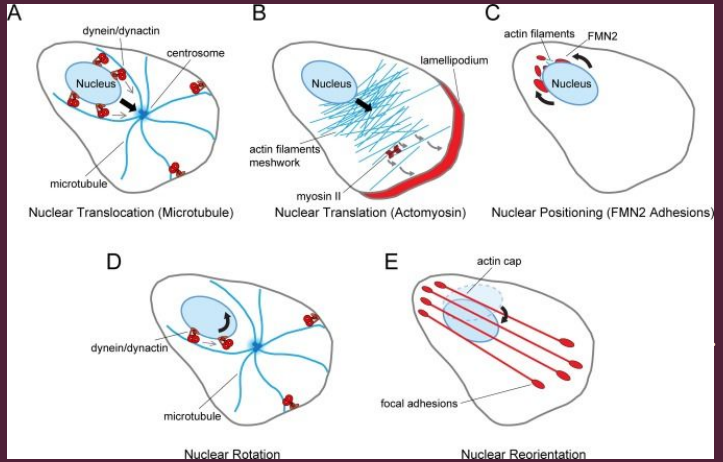
- Together FAK-Dynein contribute to the intracellular polarization(cell reorganization to form its shape)



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

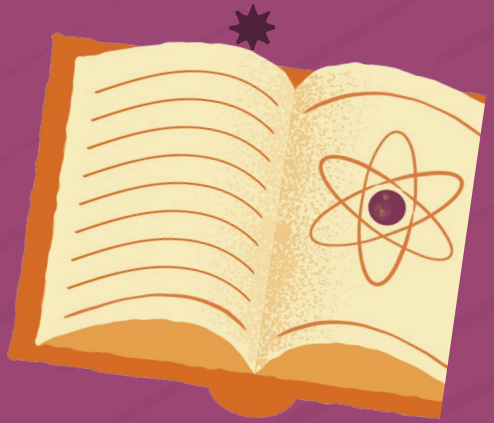
Research & Purpose

- 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.

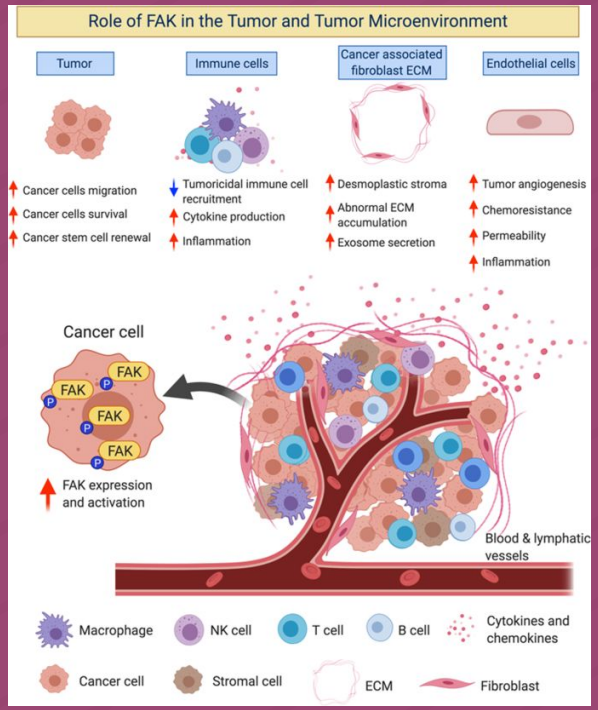


Source: Zhu, R., Liu, C., & Gundersen, G. G. (2018). Nuclear positioning in migrating fibroblasts. *Seminars in Cell & Developmental Biology*, *82*, 41–50. https://doi.org/10.1016/j.semcdb.2017.11.006

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 a 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. & Schlaepfer, D. FAK in cancer: mechanistic findings and clinical applications. *Nat Rev Cancer* 14, 598–610 (2014). https://doi.org/10.1038/nrc3792

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.