Mechanically-Induced Tissue-like Multicellularity in Archaea

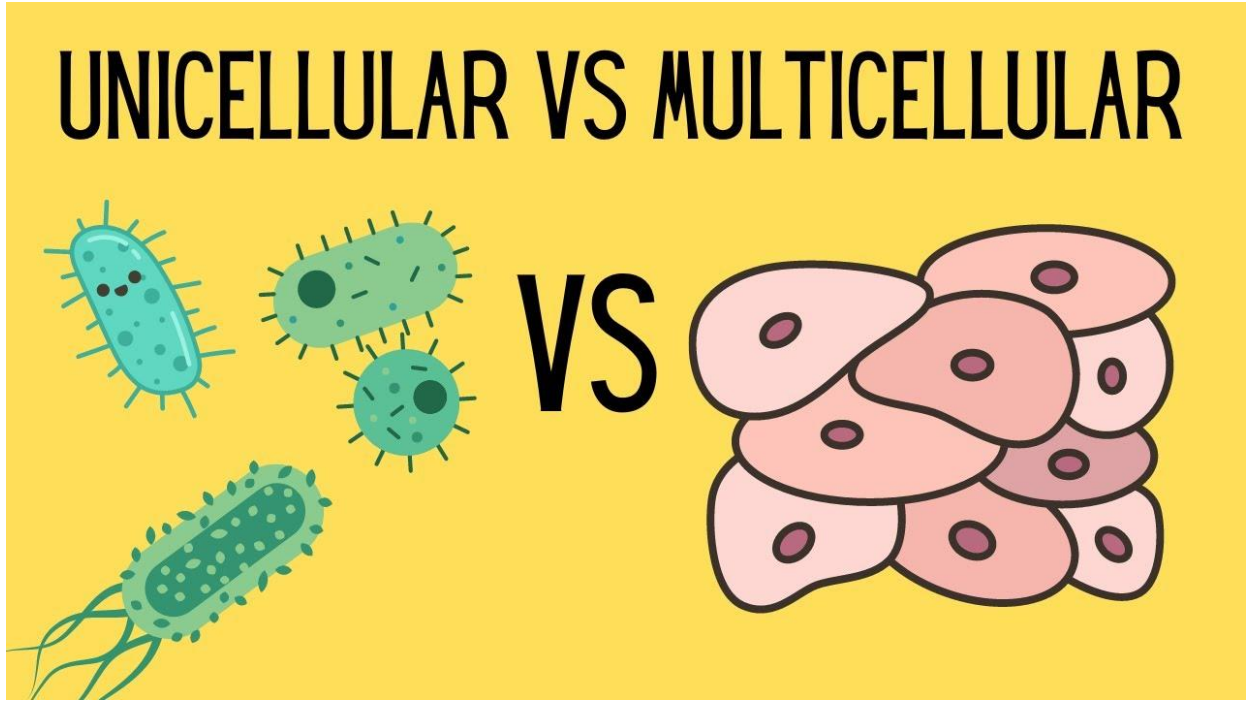
&

Cancer from an Evolutionary Unicellularity-Multicellularity Perspective

Yangyi Zhang & Huimin Cai

May 9, 2025

Introduction: What is Multicellularity?



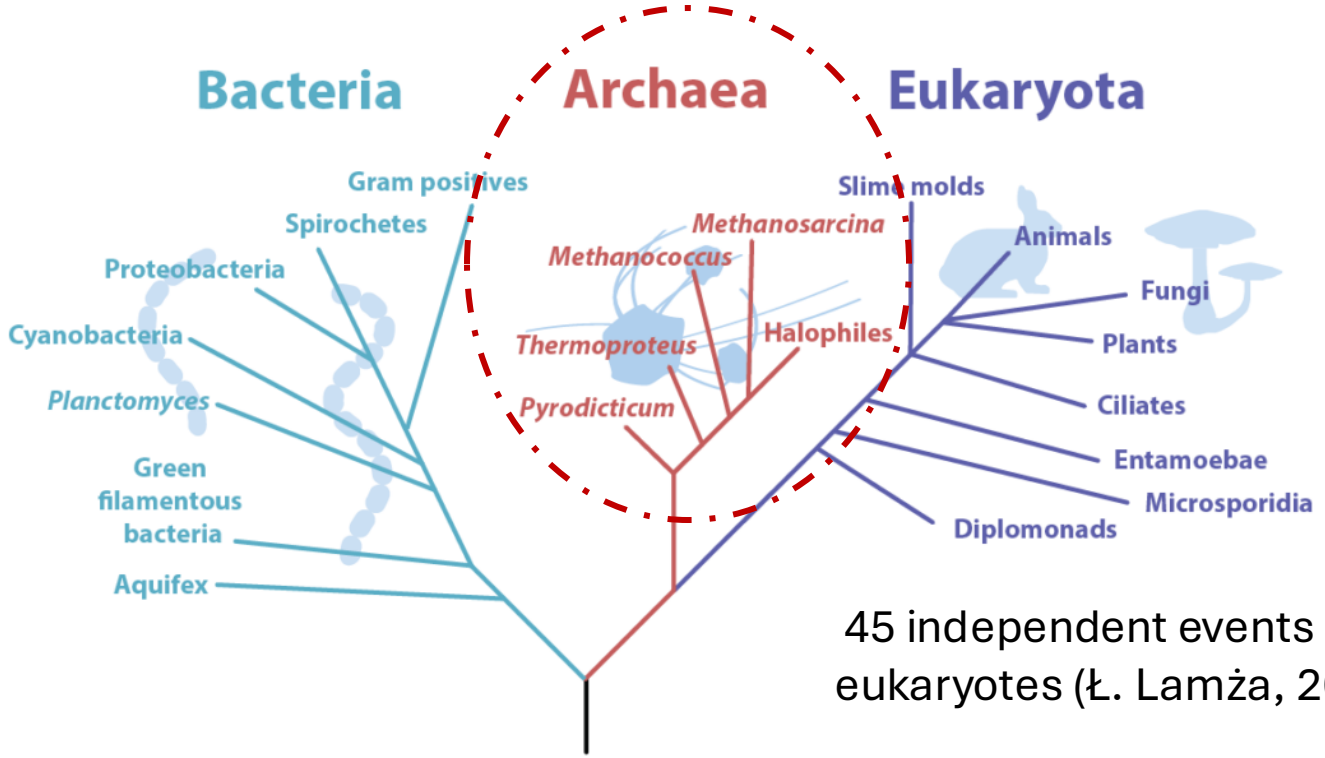
Multicellularity(多细胞性）: is a biological state in which an organism is composed of multiple cells that are integrated and interdependent.

Multicellularity vs aggregations of cells?

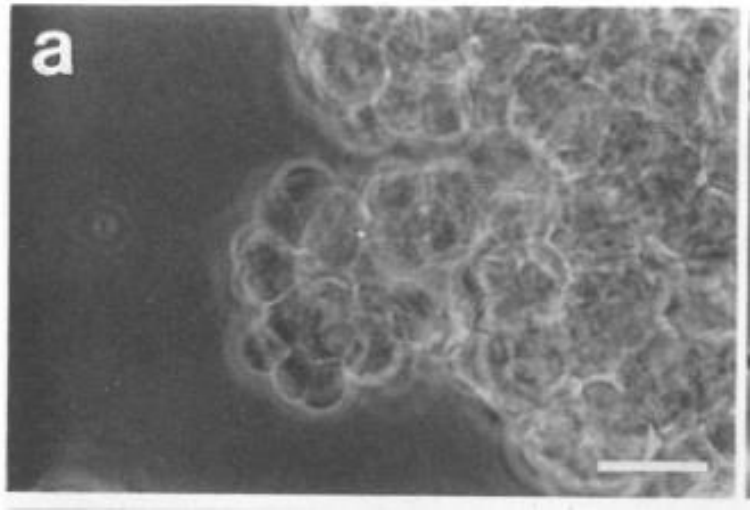
- Cell specialization and differentiation
- Division of labor
- Intercellular communication
- Development from a single cell

…

Why study archaeal multicellularity?



45 independent events in eukaryotes (Ł. Lamża, 2023)

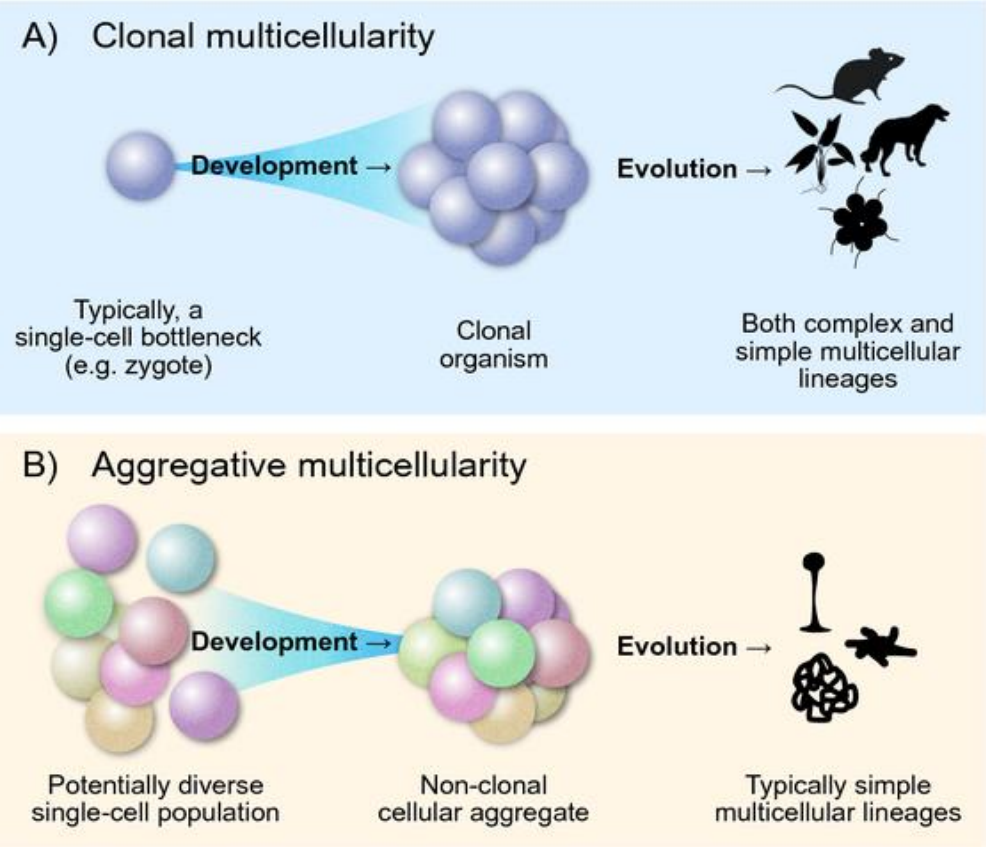


Methanosarcina (甲烷八叠球菌，古菌）form large multicellular aggregates in fresh water （Kevin R. Sowers,1993）

Multicellularity happens across domains of life, but…

- Relatively rare in archaea species…
- As close relatives to eukaryotic ancestor, archaeal multicellularity,offers key insights into the origins of multicellular life on Earth.

Historical Perspectives on the Evolution of Multicellularity



Traditional the origin of multicellularity: genetic regulation and variation.

- Genetic Driver
- Gene Regulatory Networks
- Gradual Evolution

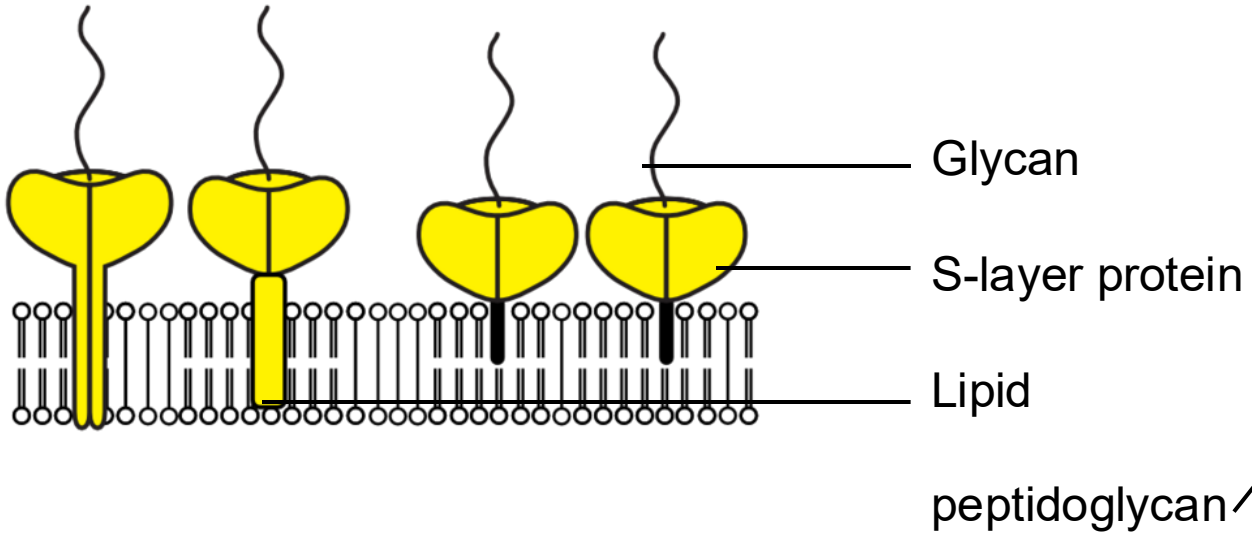
New Perspectives on the Evolution of Multicellularity

Driving Role of Physical Environment: Mechanical pressure, shear forces, and other physical stimuli directly induce cellular aggregation states, thereby promoting the formation of multicellularity.

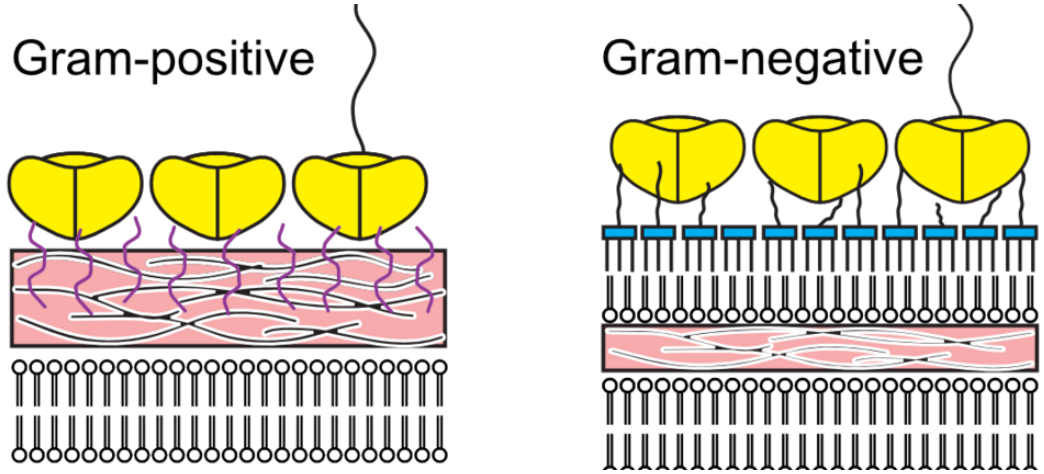
Archaea & Physical stimulation: Surface Layer Structures

S-layer: is a regularly structured crystalline layer present on the surface of many bacteria and archaea.

Archaea



Bacteria



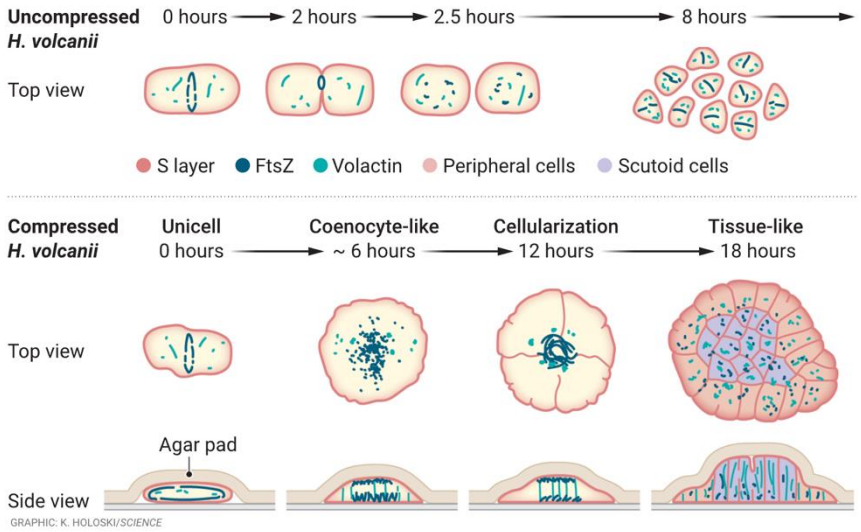
Most archaea lack a rigid cell wall ,Encapsulated by a proteinaceous surface monolayer (S-layer)

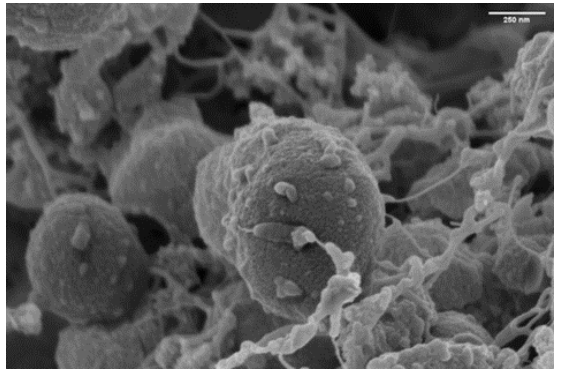
- facilitates close interactions between cells, such as cell-cell contact and fusion
- mechanically vulnerable, potentially sensitive to mechanical stress

Science

Tissue-like multicellular development triggered by mechanical compression in archaea

Theopi Rados[1]†‡, Olivia S. Leland[1]†, Pedro Escudeiro[2], John Mallon[1], Katherine Andre[1], Ido Caspy[3], Andriko von Kügelgen[3], Elad Stolovicki[4], Sinead Nguyen[1], Inés Lucía Patop[1]§, L. Thiberio Rangel[5], Sebastian Kadener[1], Lars D. Renner[6], Vera Thiel[7], Yoav Soen[4], Tanmay A. M. Bharat[3], Vikram Alva[2], Alex Bisson[1]*





Haloferax volcanii
死海嗜盐古菌

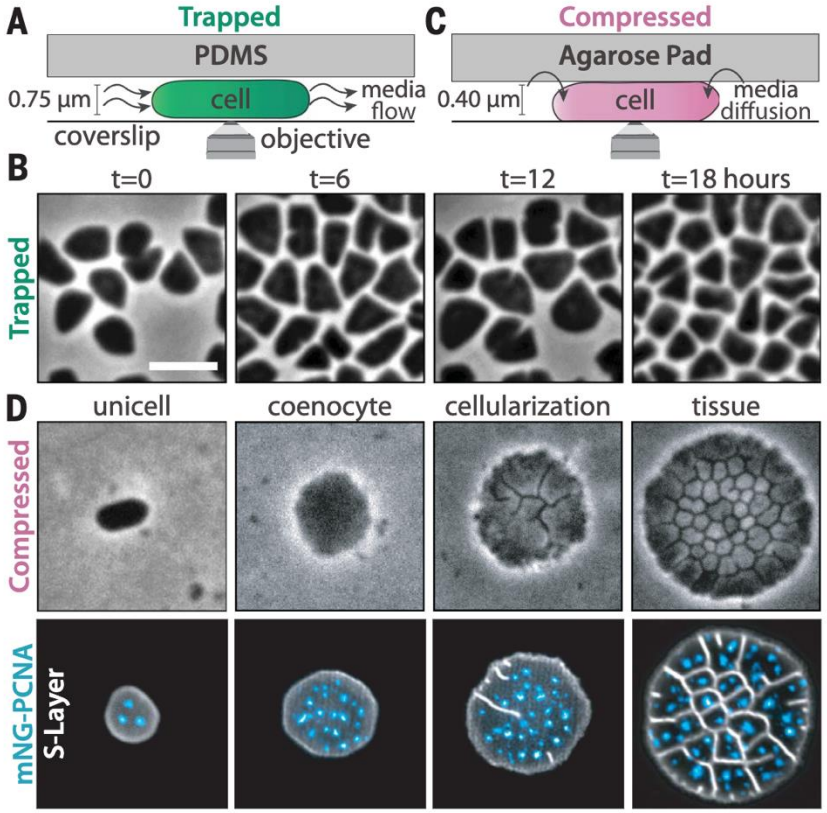
Bisson Lab investigates cellular organization and behavior in Archaea, focusing on mechanisms controlling cell shape, division regulation, and environmental sensing.

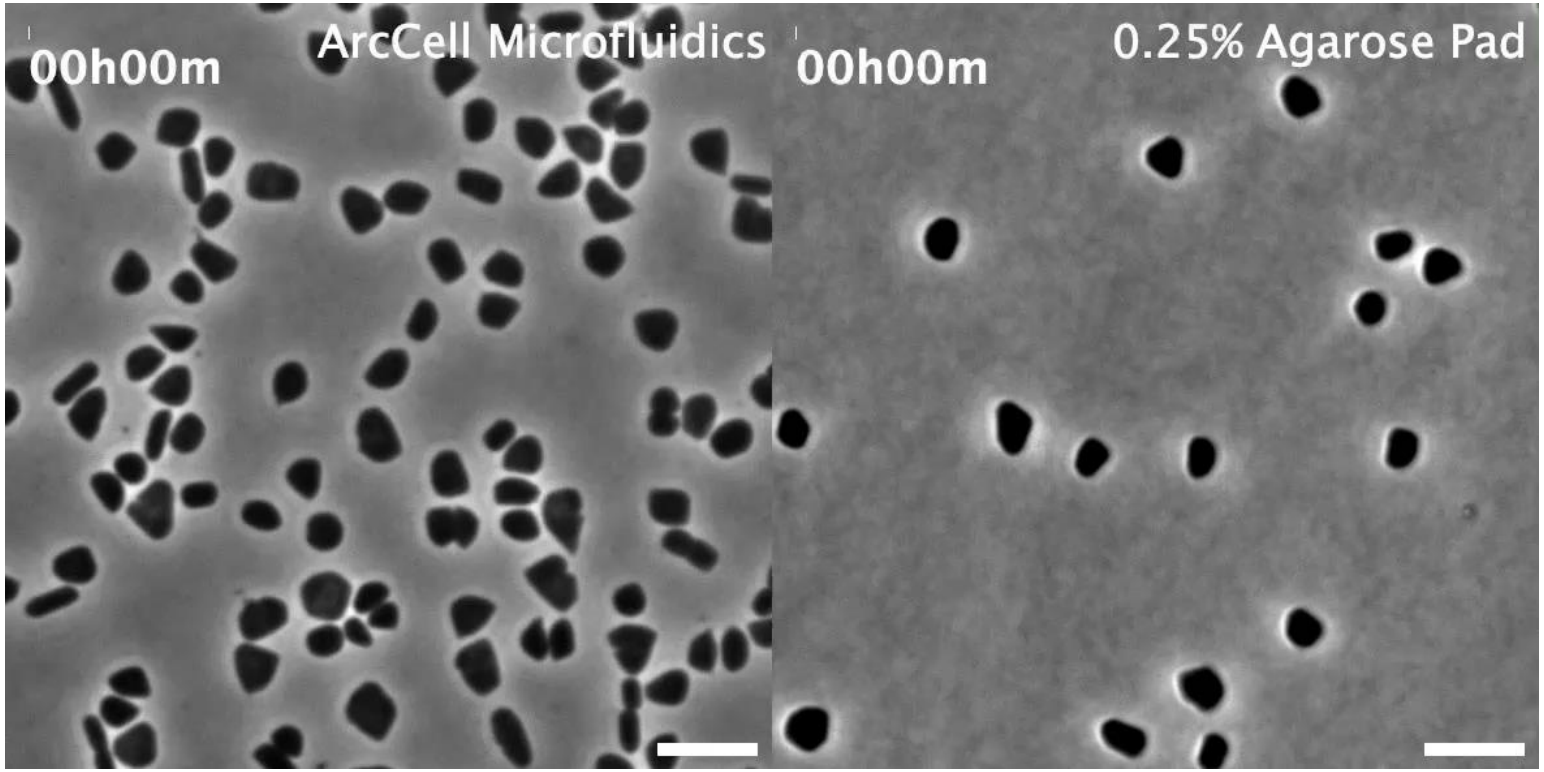
https://www.brandeis.edu/biology/faculty/bisson-alexandre.html

- How do mechanical forces affect archaeal development?
- Is this process evolutionarily conserved across related species?
- What survival benefits does this multicellularity confer?
- What molecular mechanisms govern archaeal cellularization?
- What molecular markers define archaeal tissue organization?

Discovery of Compression-Induced Multicellularity

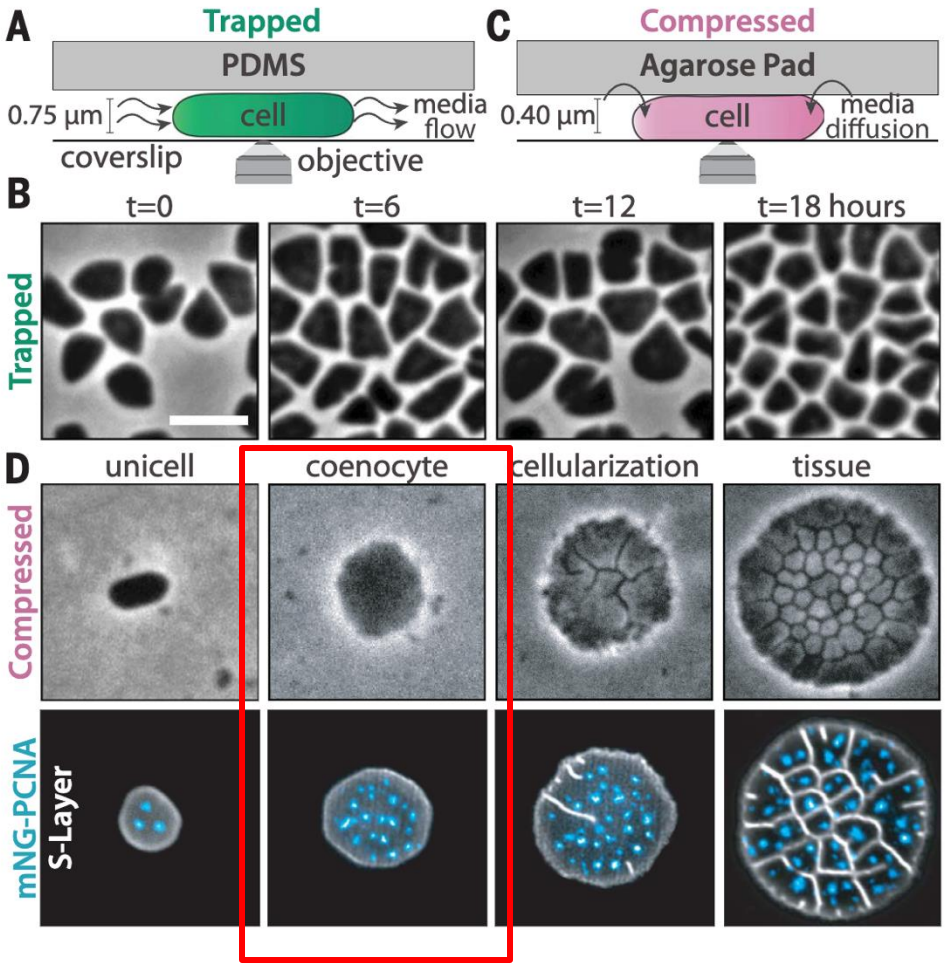
- Agarose pad pressure induces a multicellularity program in *H. volcanii*



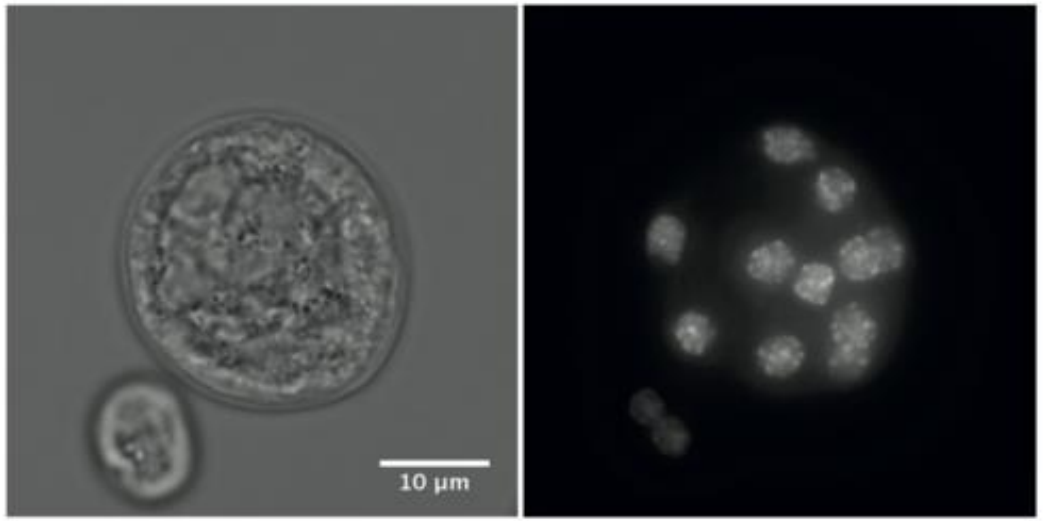


normal conditions vs under compression

Compression Induces Coenocytes



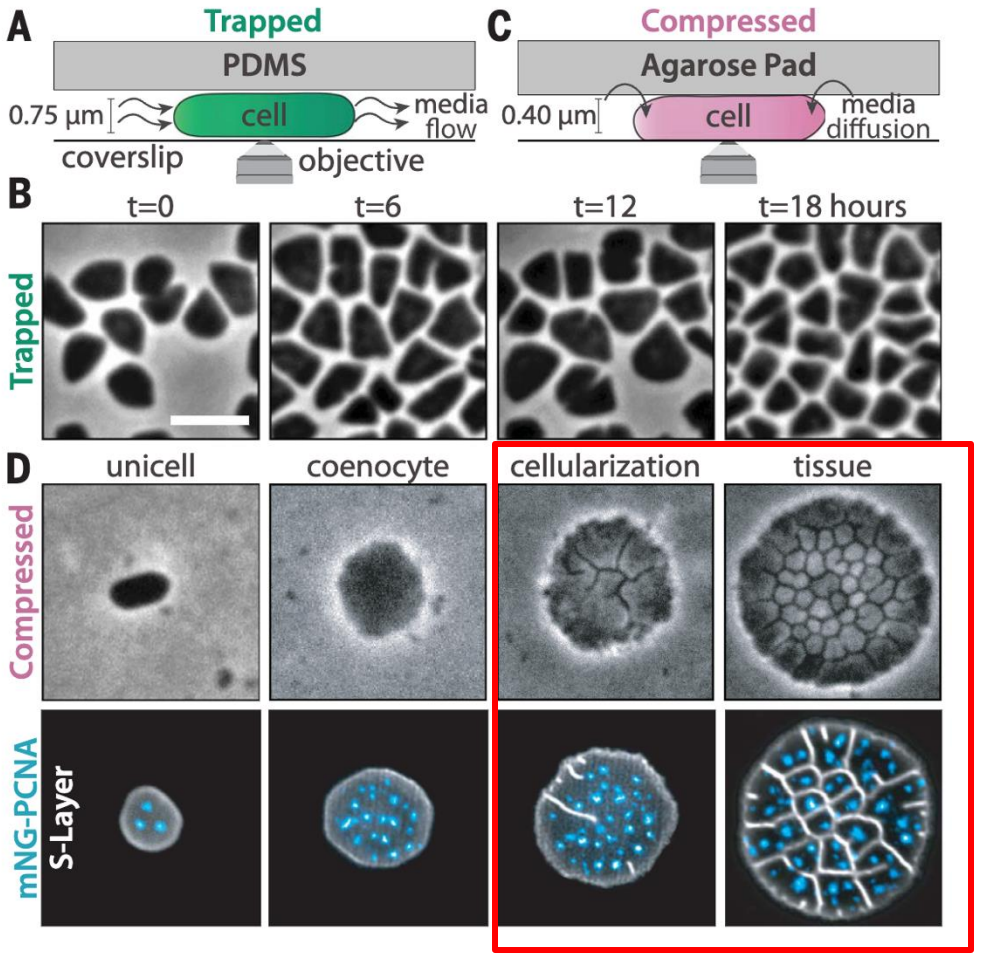
msfGFP-PCNA foci (blue) : DNA replication sites

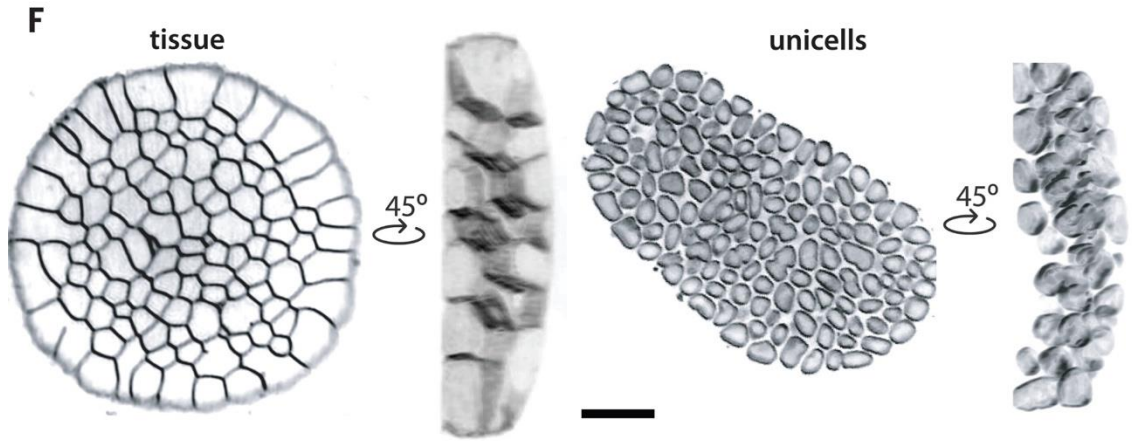


coenocytes (multi-nucleates cell) of ***Sphaeroforma arctica***（球形菌属北极种，真核生物）

Coenocytes: a multinucleate cell which can result from multiple nuclear divisions without their accompanying cytokinesis(多次核分裂,而不伴随胞质分裂)

From Coenocyte to Tissue: Cellularization





3D-SoRa microscopy images of a tissue (left) and unicells (right)

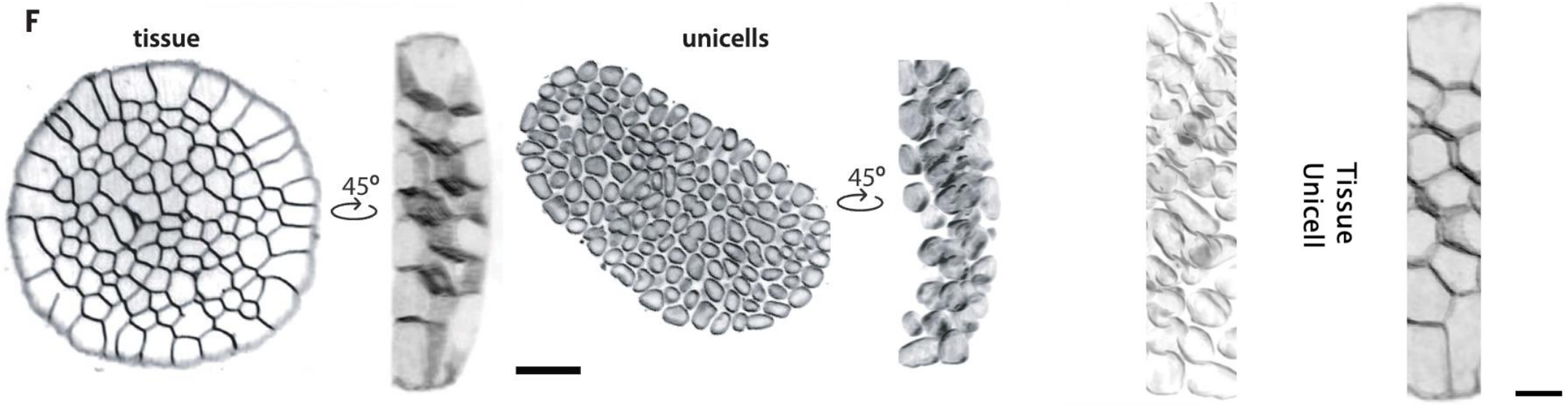
cellularization
↓
'tissue-like' structure

Cellularization : internal membranes grew inwards from the periphery, partitioning the large cell into many smaller, distinct cells

Do archaeal multicellular structures have tissue-like physical properties?

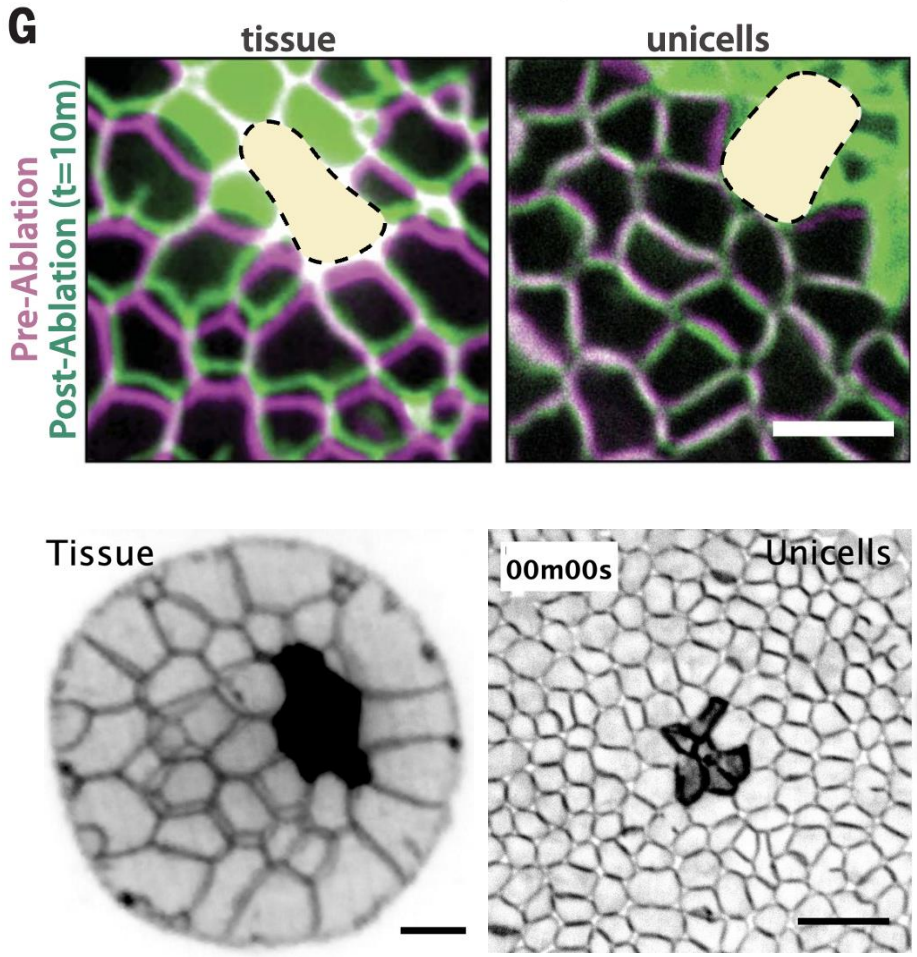
Cells show physical connections within tissues

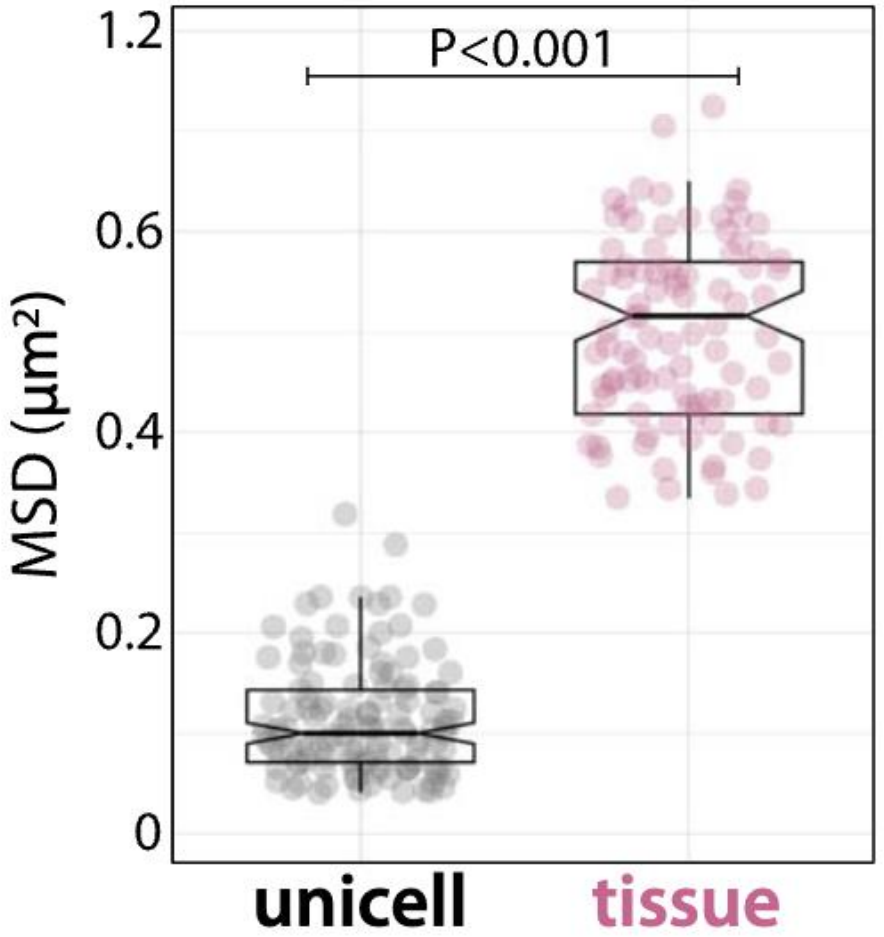
- Stretched and compressed areas comprise large monolayers of **epithelia-like tissues**.



Wound healing rates similar to animal tissues

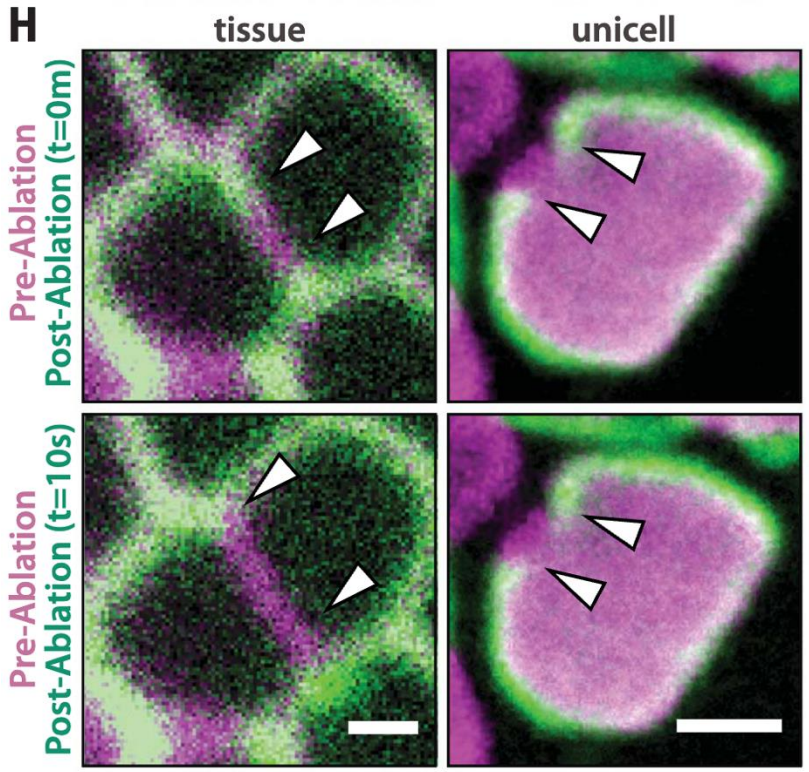
Laser ablation experiments

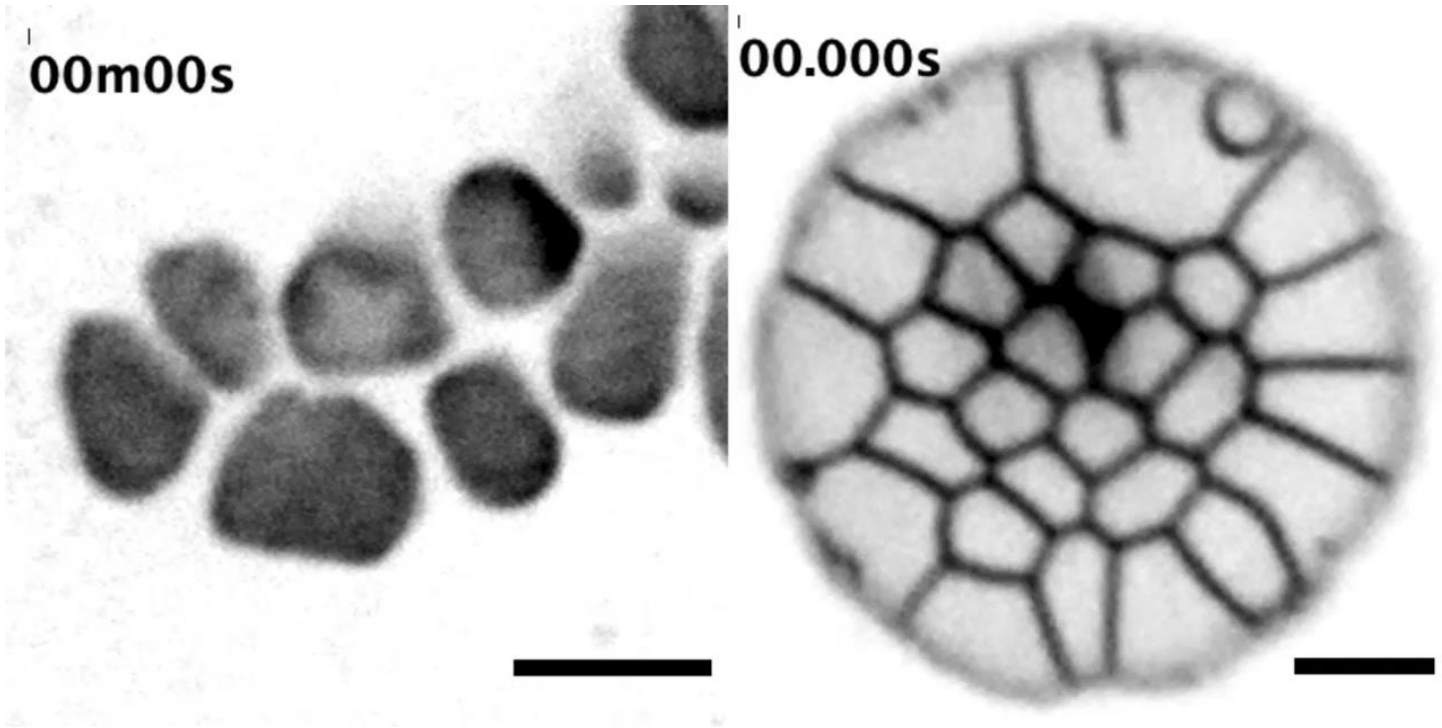




Mean square displacement (MSD) 指的是在一段时间内，粒子（如分子、细胞等）相对于它最初位置的**平均平方位移**。

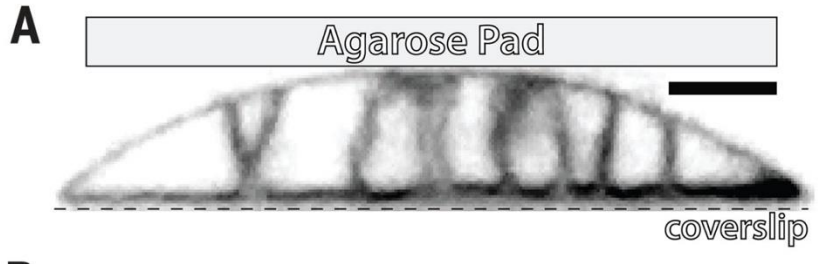
Wound healing rates similar to animal tissues

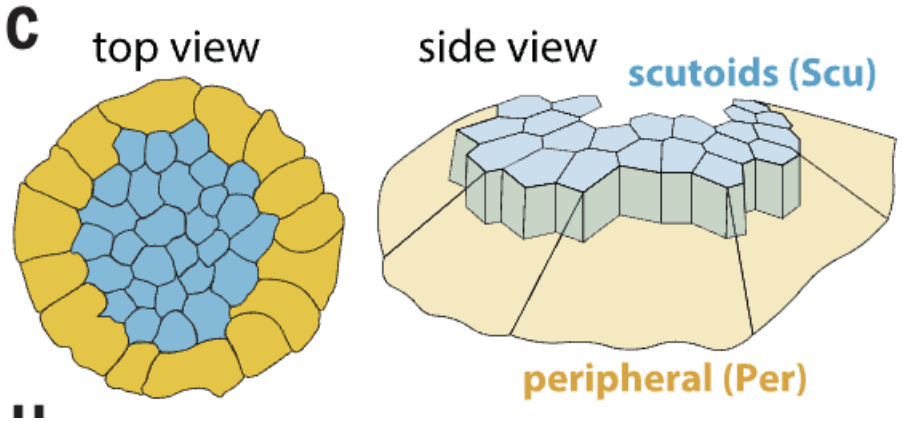


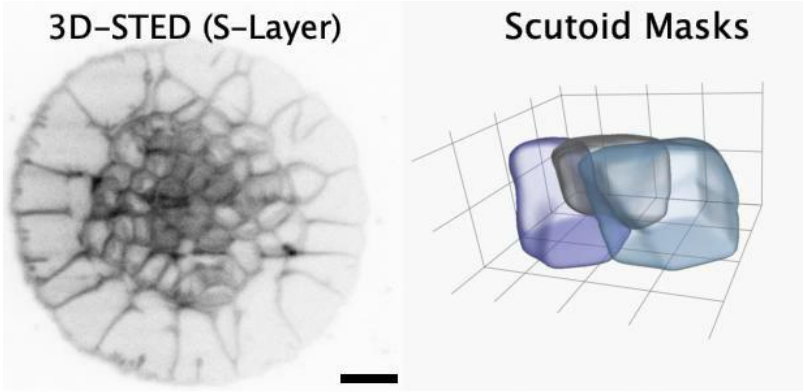


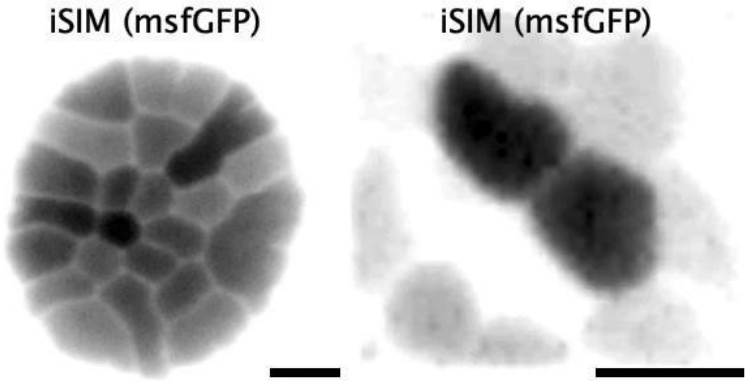
Does pressure-induced multicellularity exhibit tissue-like cellular differentiation?

Cell Differentiation and Specialized Cell Types



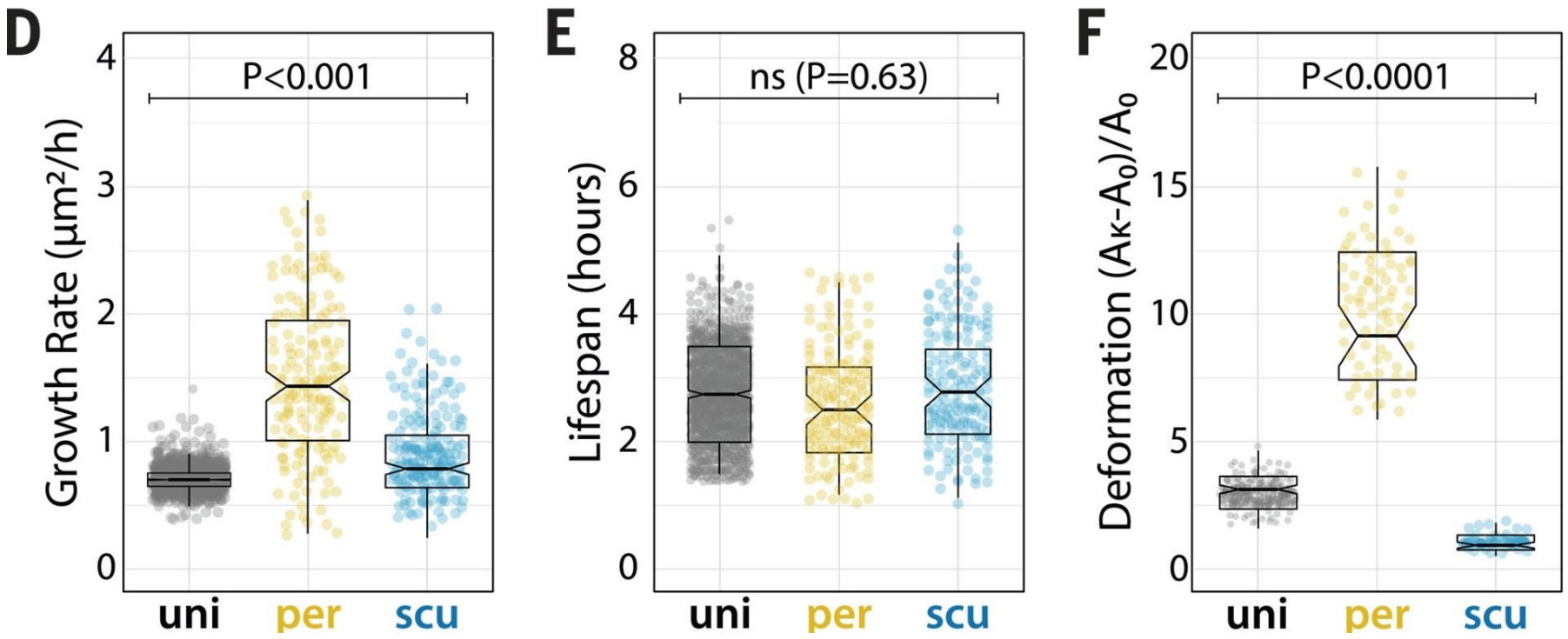






Peripheral (Per) cells: at the edges wider and shorter
Scutoid (Scu) cells: in the center, these cells are taller, narrower, and adopt an irregular 'scutoid' shape

Different Biological and Physical Properties



Both cell types showed higher growth rates and similar life spans relative to unicells.

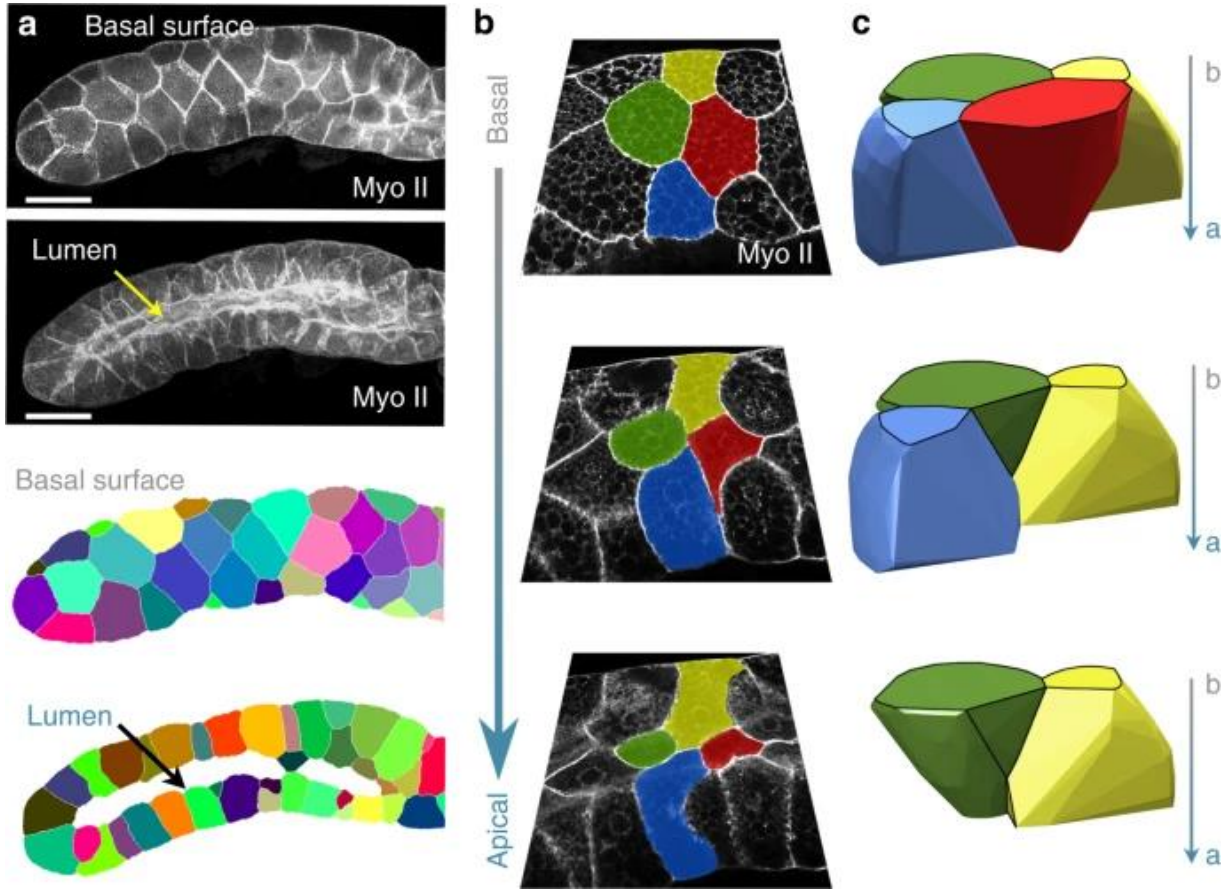
Differential Physical Properties:

- Peripheral cells: 3× more flexible than unicells
- Scutoid cells: 2.5× more rigid than unicells

➢ Scu cells may bear the mechanical load from compression

➢ Per cells are more likely to escape from the pressure.

‘Scutoid’ Shape is Also Seen in Animal Cells



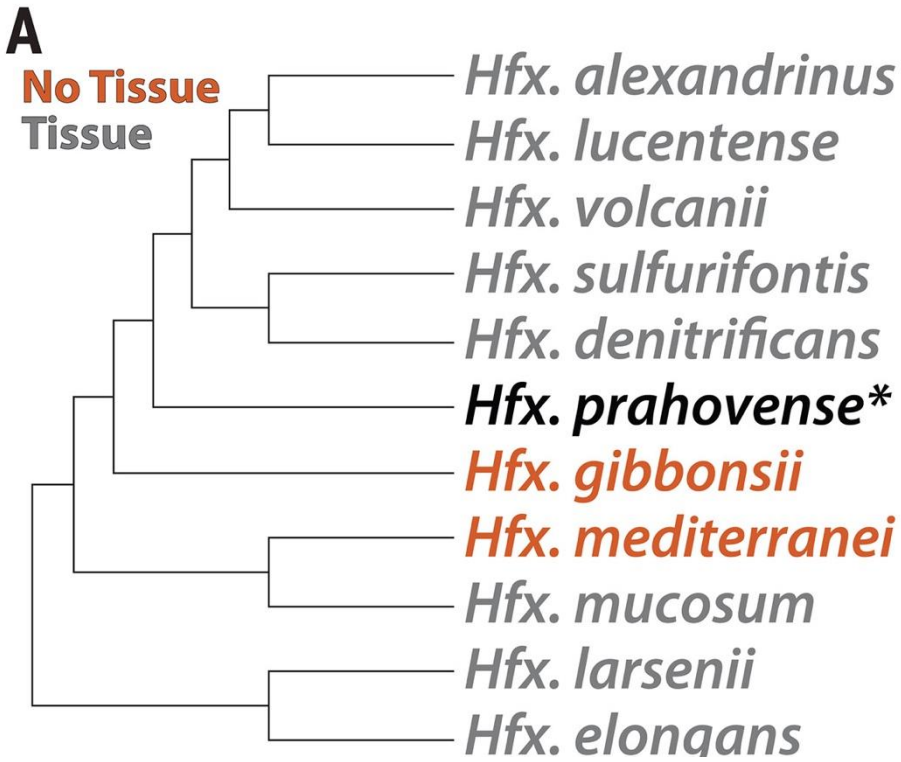
3D tissue packing of scutioid cells（Drosophila salivary gland）

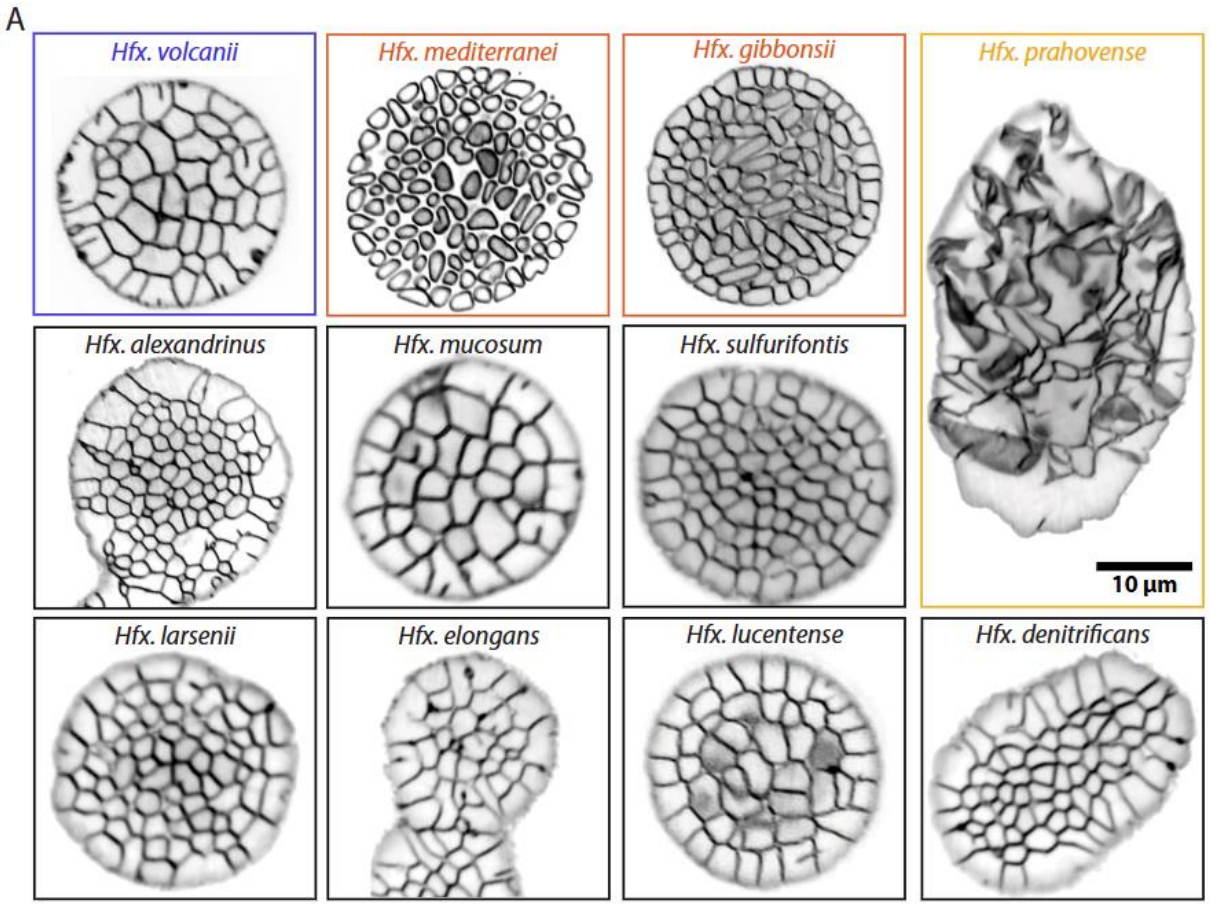
- animal scutoid cells is minimizing energy by distributing membrane tension
- helps tissues curve and pack efficiently

➢ hypothesis of archaea tissue: Scu cells bear the mechanical load from compression, potentially shielding the peripheral cells

Is this process evolutionarily conserved across related species?

Distribution of Multicellularity Across Haloarchaea



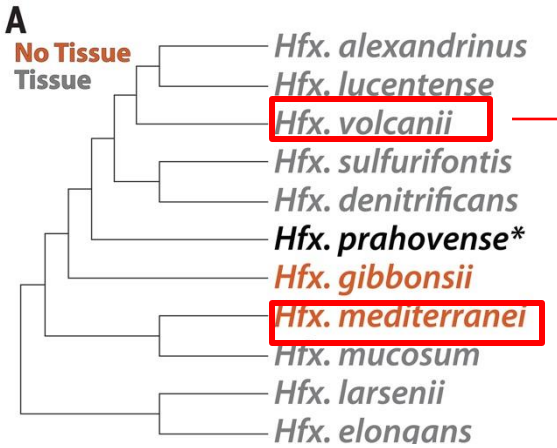


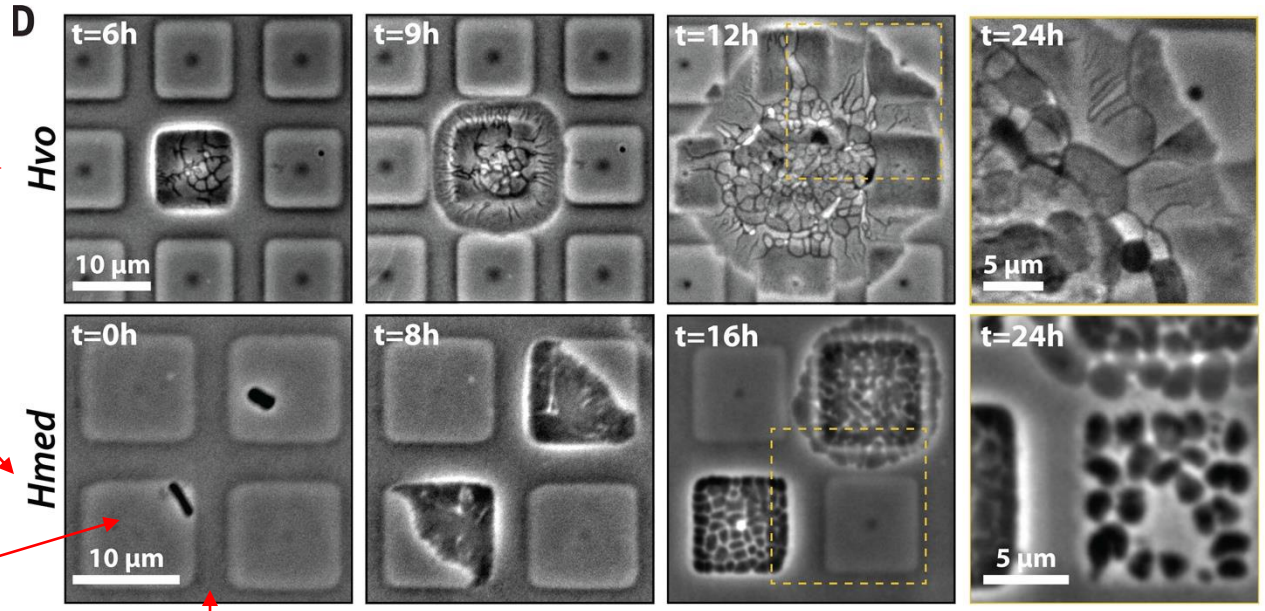
- Examined 52 species across 14 genera, 61.6% of haloarchaeal species can form tissues

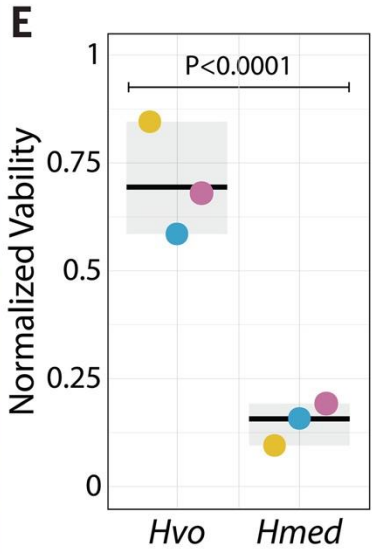
Hfx. Prahovense:* develops to considerably larger, deformed tissues

What survival benefits does this multicellularity confer?

Tissue-forming archaea show higher survival rates



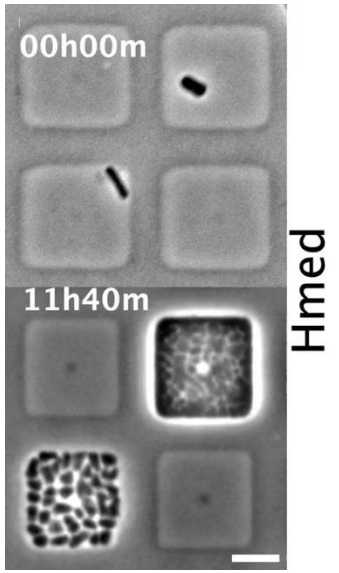


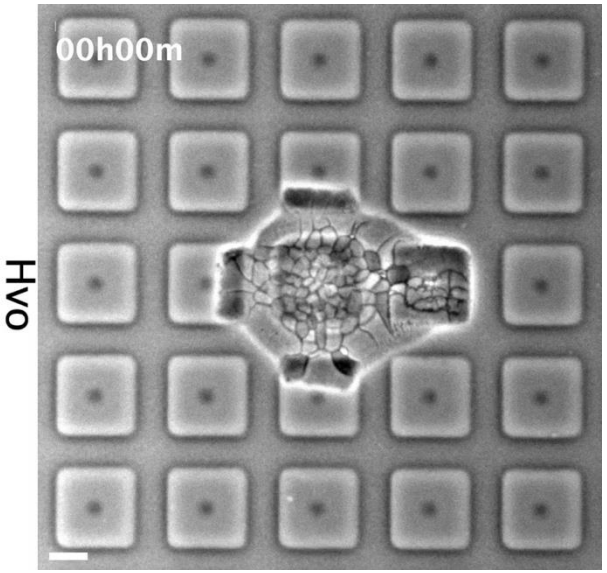


microfabricated pillars
(have compression)

"relief" zones

"escape room" challenges experiment
mimicking natural habitat



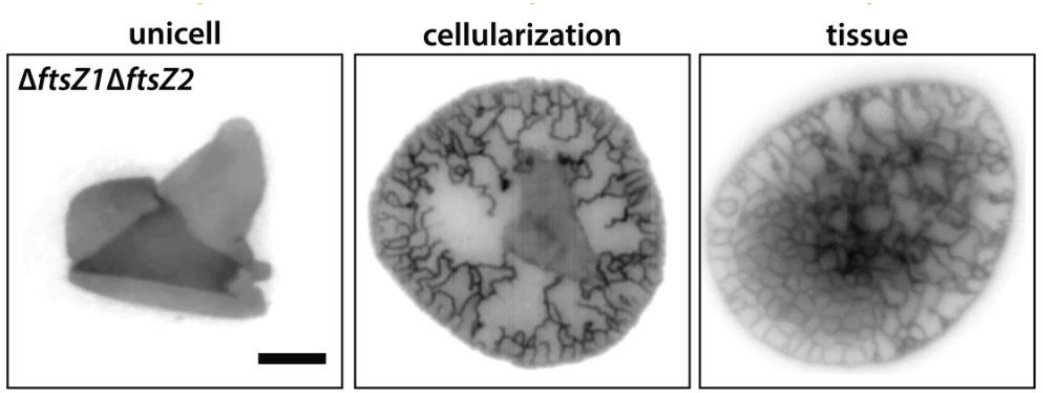


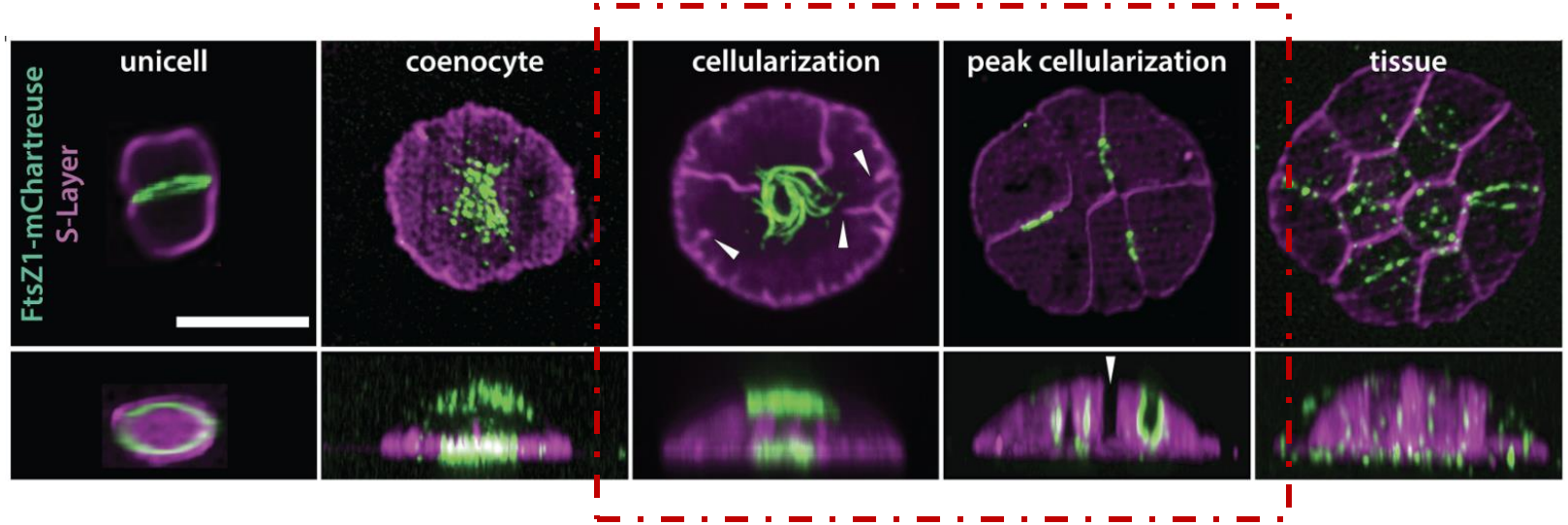
- How do mechanical forces affect archaeal development?
- Is this process evolutionarily conserved across related species?
- What survival benefits does this multicellularity confer?
- What molecular mechanisms govern archaeal cellularization?
- What molecular markers define archaeal tissue organization?

Cellularization does not require FtsZ

- **FtsZ**

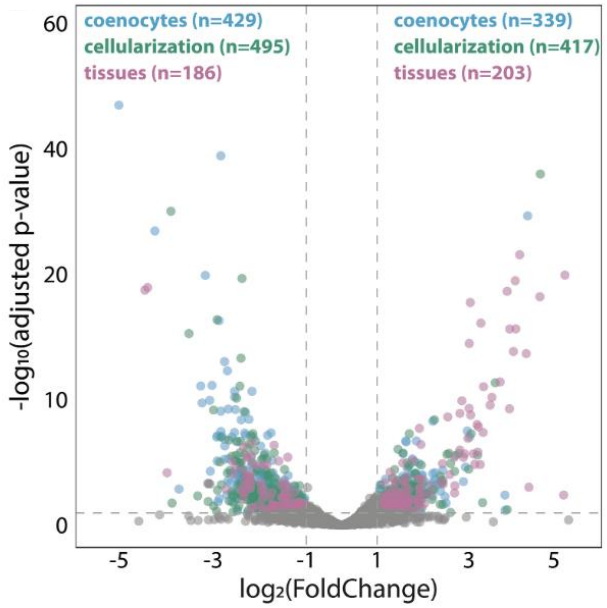
In unicells, cell division relies on the functions of an essential protein, FtsZ. FtsZ polymerizes at the future division site to form a ring-like structure, termed the Z-ring, that serves as a scaffold to recruit all other division proteins, and possibly generates force to constrict the cell.

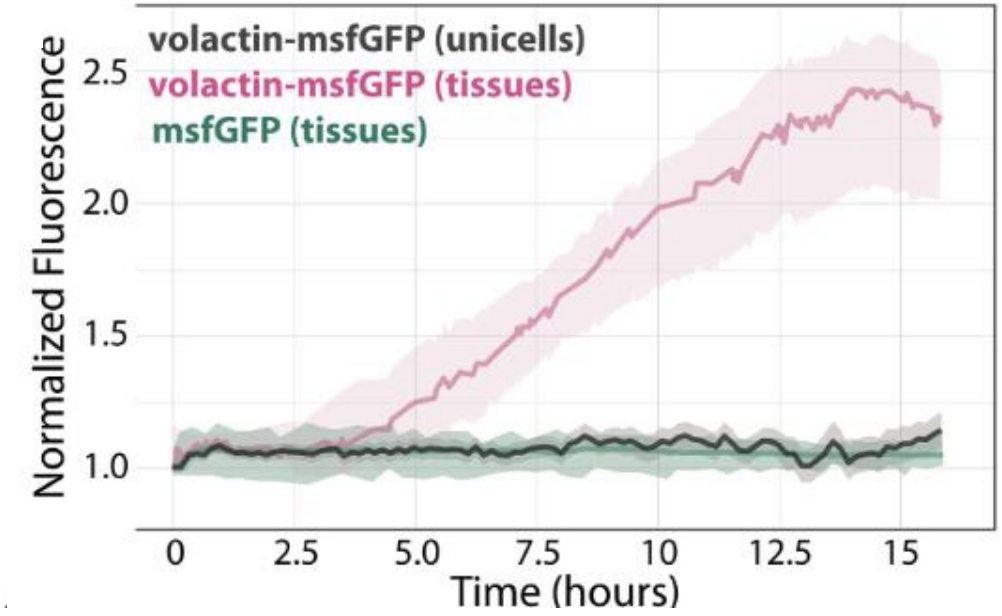




What molecular markers define archaeal tissue organization?

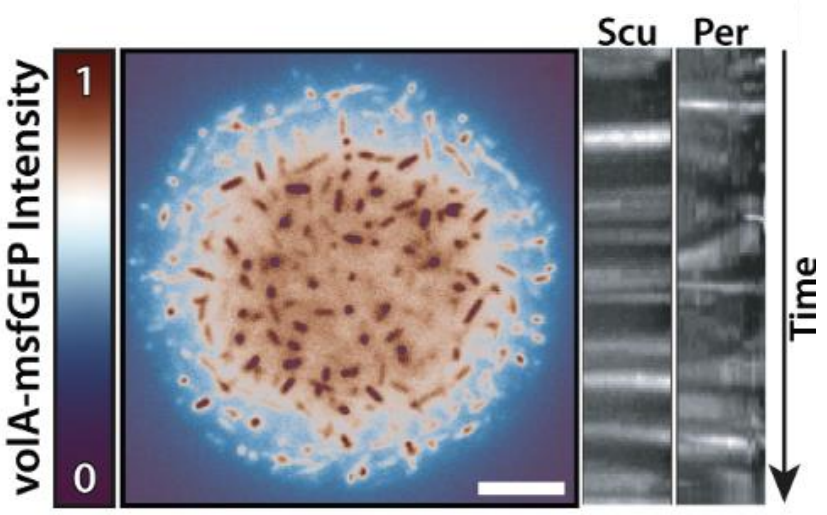
- RNA Sequencing Reveals: Upregulation of VolA (Volactin, archaeal actin) during multicellular development

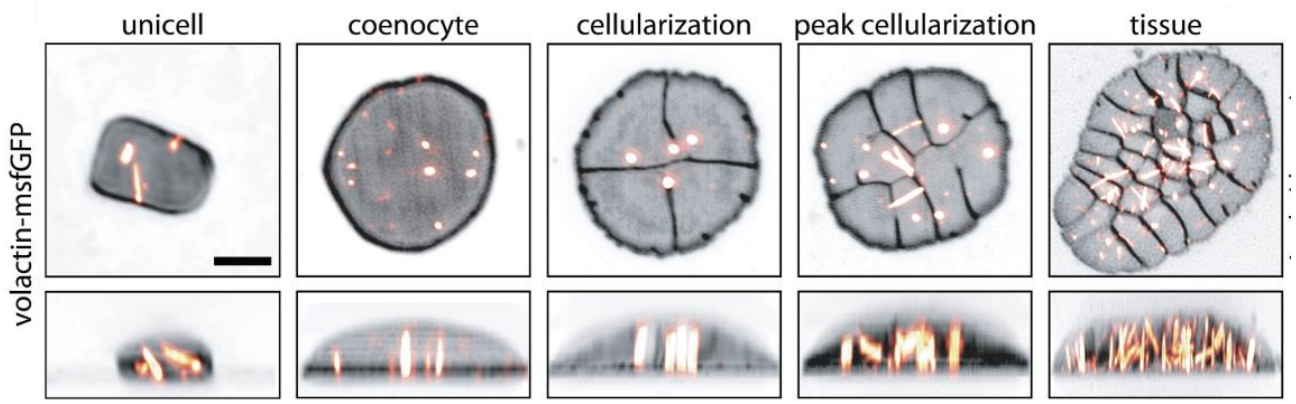




What molecular markers define archaeal tissue organization?

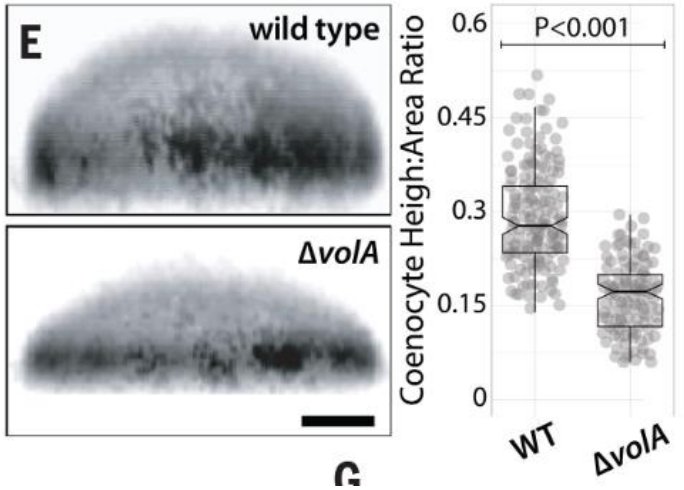
- VolA also displayed changes in structural patterns

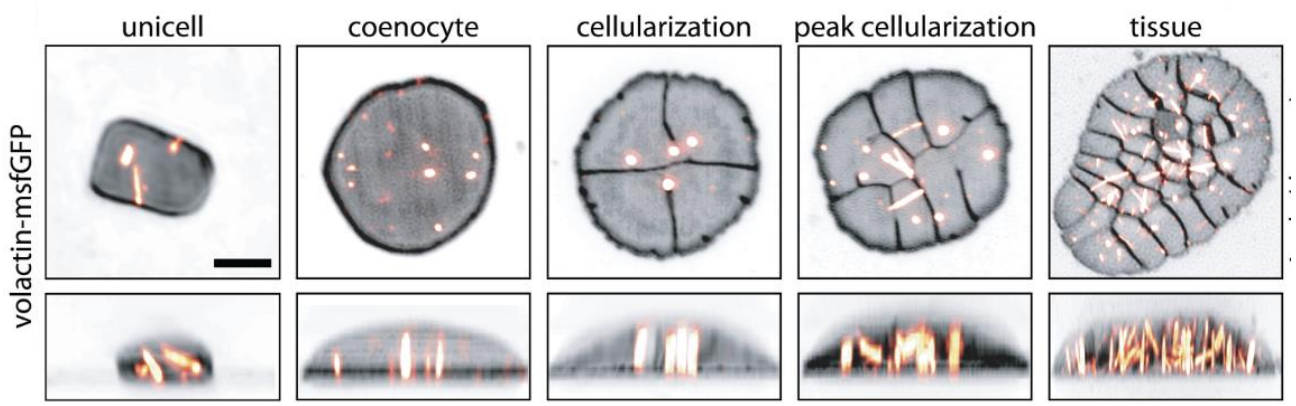




What molecular markers define archaeal tissue organization?

- Compression of ΔvolA cells resulted in shorter coenocytes than the wild-type counterpart





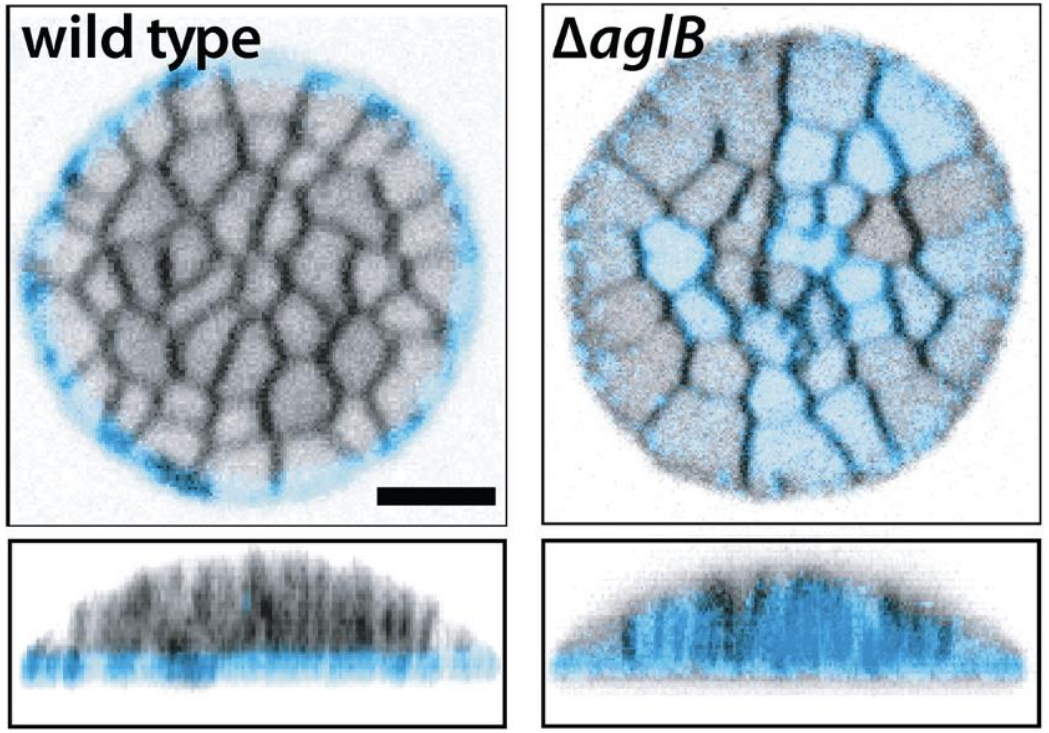
What molecular markers define archaeal tissue organization?

- Differential N-glycosylation Patterns: indicate cell polarity within tissues

Whether N-glycosylation, the hallmark of archaeal cell surfaces, is also involved in tissue organization

(In eukaryotes, N-glycosylation is crucial for cell identity, polarity, junctions, and adhesion)

- In wild type, AglB ensures N-glycosylation tags form a ring pattern, marking cell polarity
- In ΔaglB, the pattern becomes scattered



Conclusions and Implications

- **Complexity may evolve more readily than previously thought, potentially triggered by simple physical forces**
  - Physical pressure induces organized, tissue-like structures in archaea.
  - Tissue formation does not require complex genetic changes.
  - Structures show layering, polarity, and cell specialization, similar to eukaryotic tissues.
  - Process is independent of traditional FtsZ-based division; mechanism remains unknown.

Cancer from an Evolutionary Unicellularity-Multicellularity Perspective

The atavism(返祖) hypothesis of cancer

- Cancer cells undergo a ‘reverse evolution’ process from a well-organized multicellular state towards an unrestricted state resembling unicellular organisms

- This atavistic shift explains not only the destructive potential of tumors but also their remarkable ability to adapt, invade, and survive under stressful microenvironments—behaviors akin to unicellular organisms.

❑ **Often interpreted as representing a simple loss of regulation of unicellular genes in cancer.**

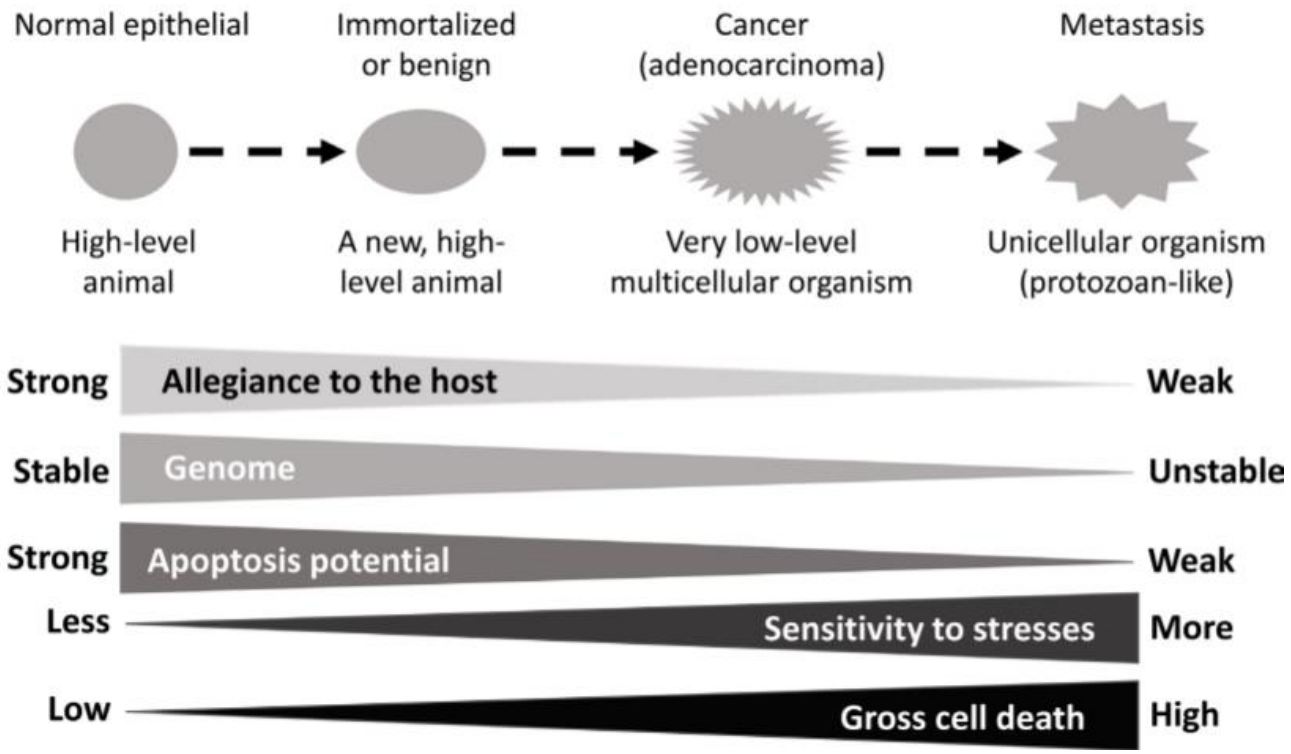


Illustration of stepwise carcinogenesis as an atavistic process of a somatic cell in animals, with epithelial carcinogenesis as an example.

Zhang, Ju, et al. Oncoscience 1.6 (2014)

A study integrating expression data from multiple tumor types

Trigos *et al. Genome Biology* (2024) 25:110
https://doi.org/10.1186/s13059-024-03247-1

Genome Biology

RESEARCH Open Access

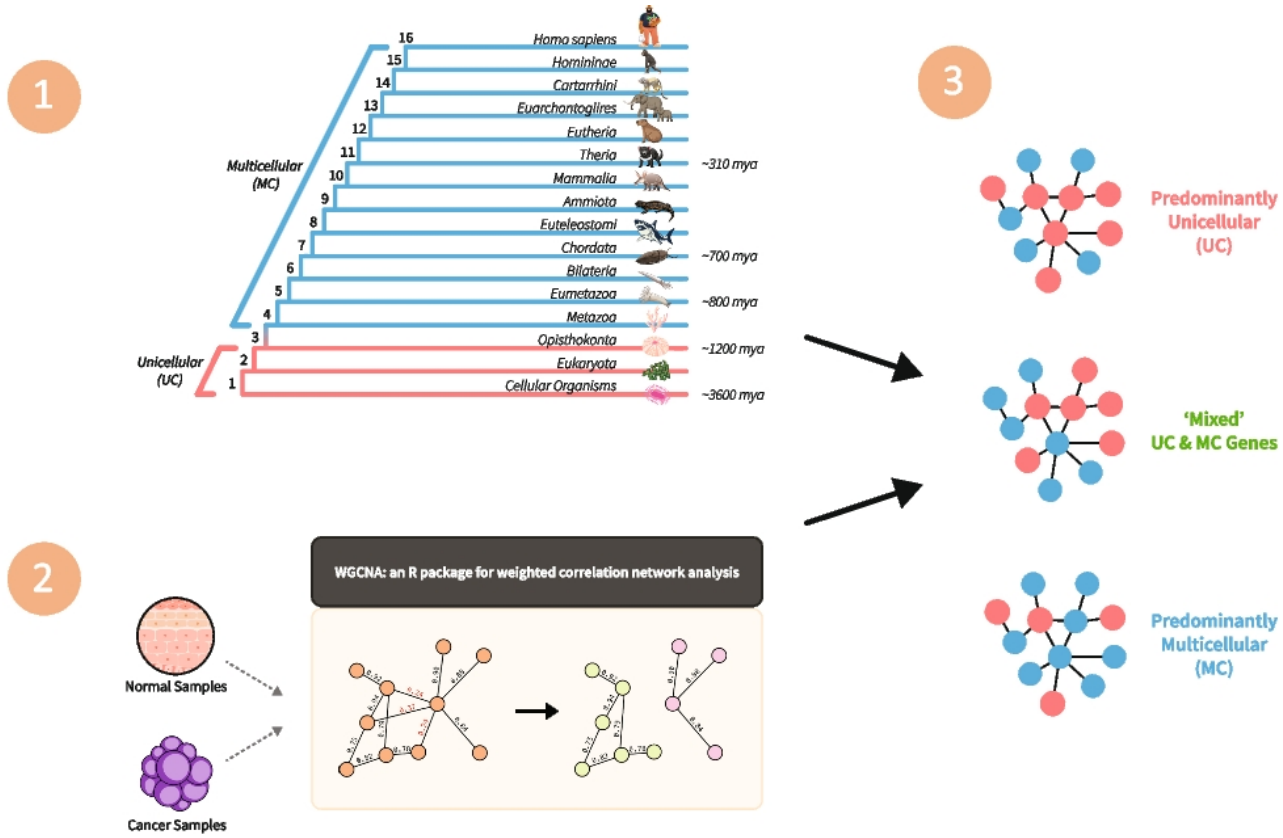
Disruption of metazoan gene regulatory networks in cancer alters the balance of co-expression between genes of unicellular and multicellular origins

Anna S. Trigos[1,2,3*], Felicia Bongiovanni[1,2], Yangyi Zhang[1,2], Maia Zethoven[1,2], Richard Tothill[4], Richard Pearson[1,2,3,5], Anthony T. Papenfuss[1,2,6] and David L. Goode[1,2*]

- Metazoan gene regulatory networks (GRNs) serve to strike a balance in the co-expression of unicellular and multicellular genes.
- When regulatory mechanisms enforcing this integration break down, cancer can emerge.

- Do cancer cells alter the balance between multicellular (MC) and unicellular (UC) gene networks?
- How do genetic variations rewire tumor gene co-expression networks?
- How does this imbalance change as cancer progresses?

Integrating gene co-expression networks with evolutionary conservation analysis

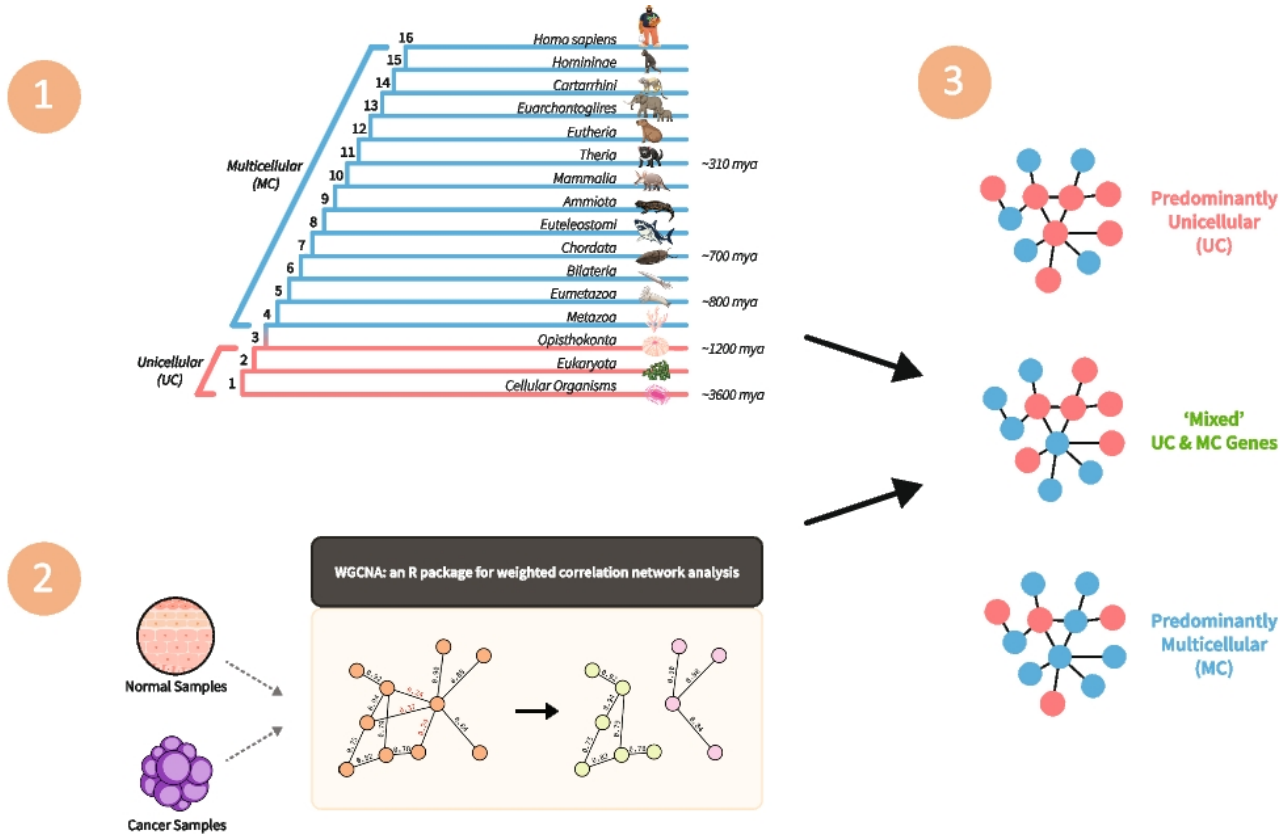


1) **Gene Classification**: human genes were classified as unicellular (UC) or multicellular (MC) origin based on when they first appeared in the evolutionary timeline from cellular organisms to Homo sapiens.

2) **Gene Co-expression Network Analysis**: WGCNA (Weighted Gene Correlation Network Analysis) was applied to both normal and cancer samples

3) **Module Classification**: co-expression modules were categorized as predominantly unicellular (UC), predominantly multicellular (MC), or mixed UC-MC based on their gene composition.

Integrating gene co-expression networks with evolutionary conservation analysis

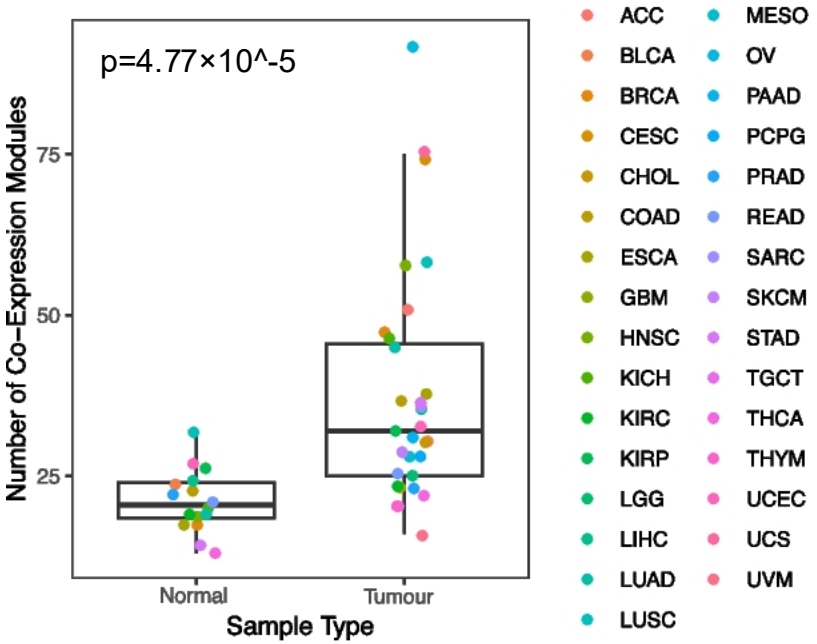


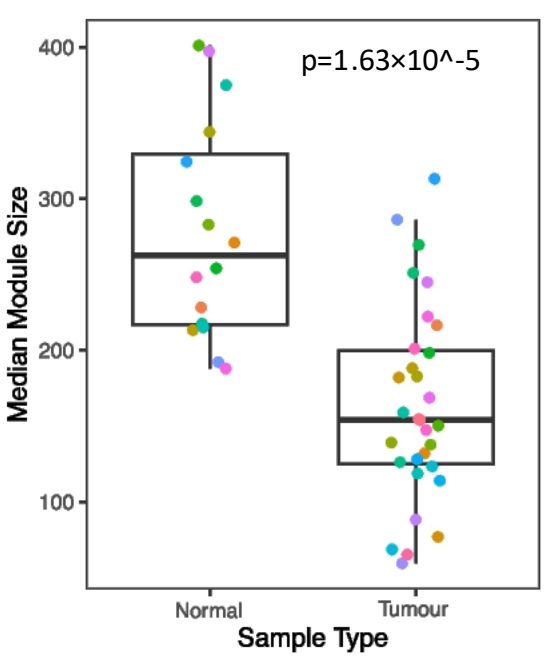
The study incorporates TCGA (The Cancer Genome Atlas) cohort datasets spanning diverse organs and body systems.

System/系统	Organs
Urogenital System 泌尿生殖系统	Bladder (膀胱), Kidney (肾脏), Prostate (前列腺), Uterus (子宫), Ovary (卵巢), Testis (睾丸)
Digestive System 消化系统	Esophagus (食管), Stomach (胃), Colon (结肠), Rectum (直肠), Liver (肝脏), Pancreas (胰腺), Bile Duct (胆管)
Respiratory System 呼吸系统	Lung (肺), Pleura (胸膜), Thymus (胸腺)
Endocrine System 内分泌系统	Thyroid (甲状腺), Adrenal gland (肾上腺), Paraganglia (副神经节)
Nervous System 神经系统	Brain (大脑), Eye (眼)
Other Systems 其他系统	Breast (乳腺), Head and Neck (头颈部), Skin (皮肤), Soft Tissue (软组织)

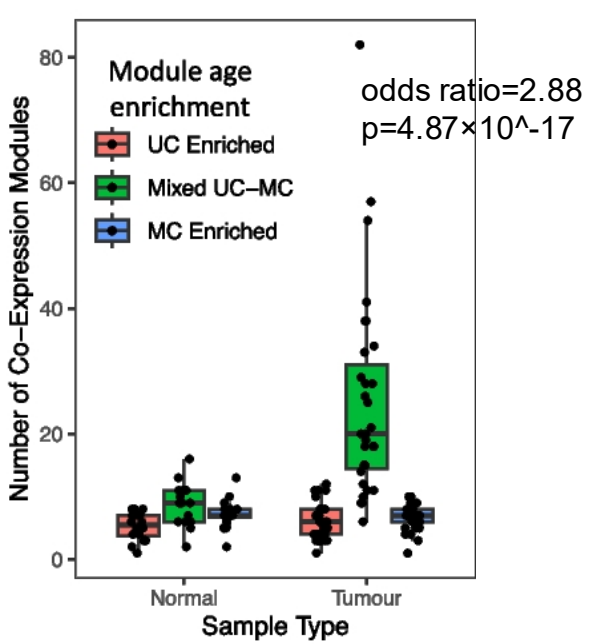
Overview of co-expression module changes in cancer

Substantial fragmentation of GRNs in cancer



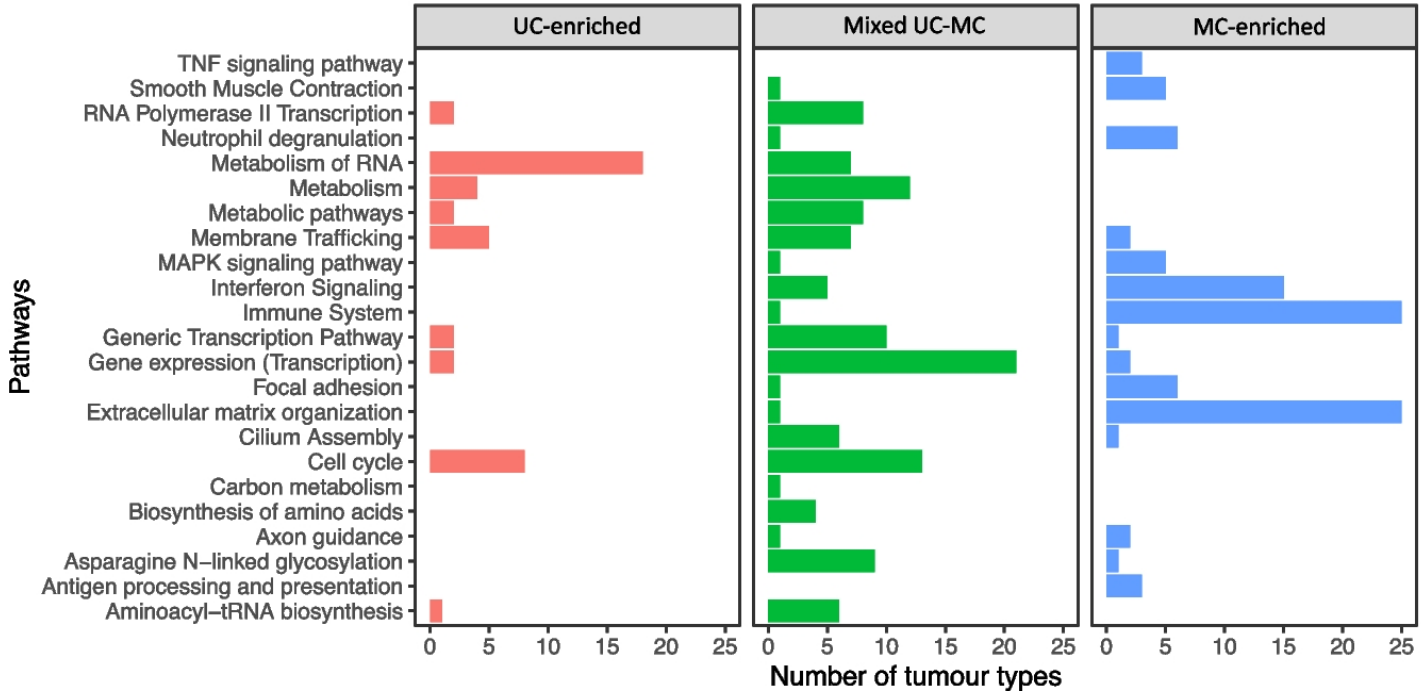


More hybrid modules in cancer

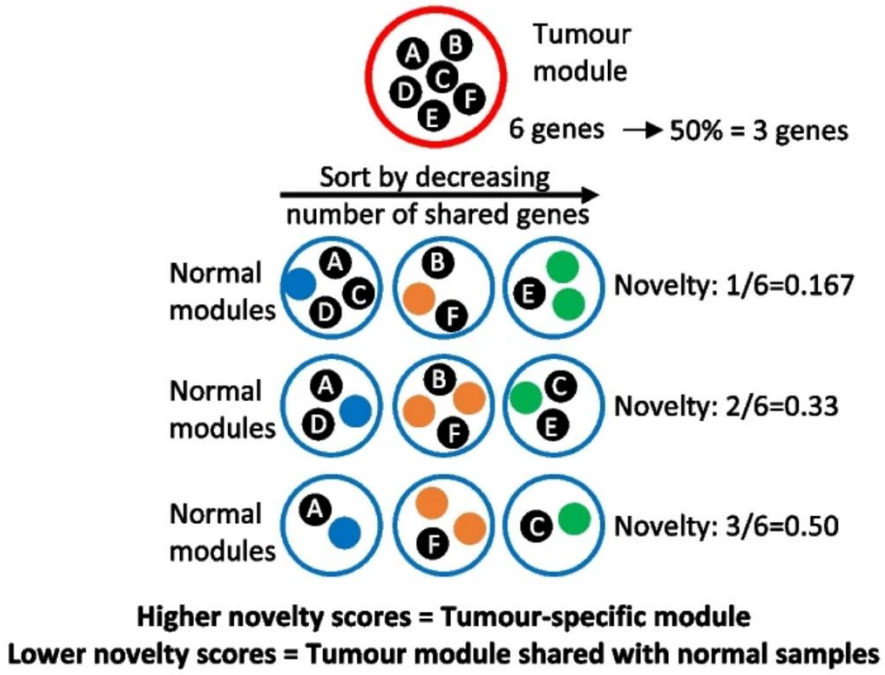


Overview of co-expression module changes in cancer

- **UC-enriched modules** contain fundamental cellular processes (translation, metabolism, cell cycle)
- **MC-enriched modules** encompass specialized multicellular functions (immunity, extracellular matrix)
- **Mixed UC-MC modules** uniquely combine transcriptional regulation and mRNA processing with core cellular processes

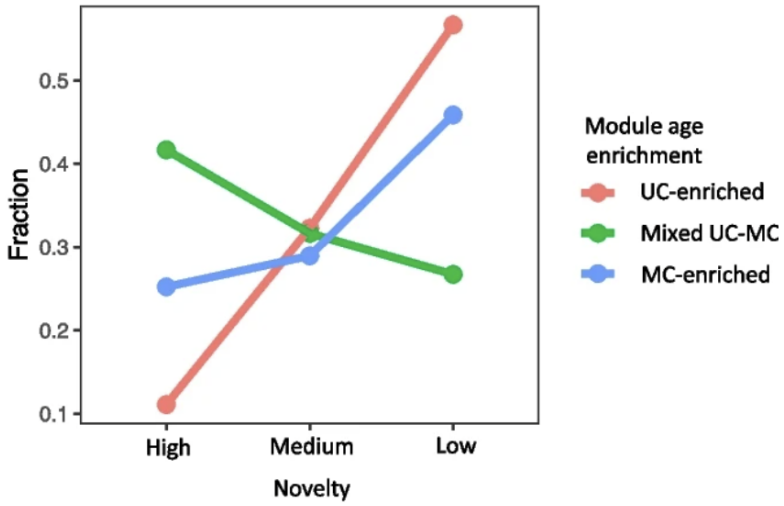


Substantial rewiring of regulatory links between unicellular and multicellular genes in cancer

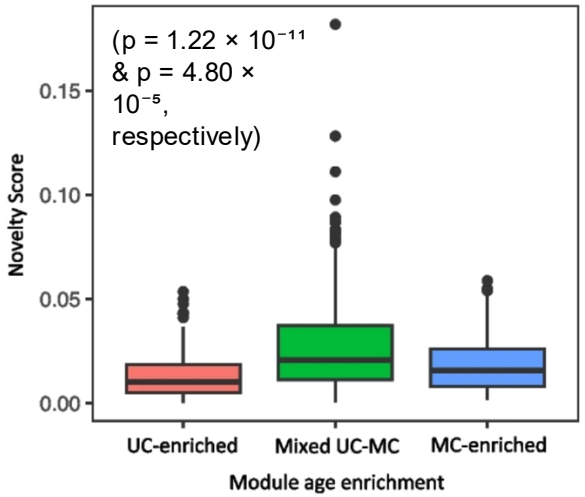


- To quantify the extent of rewiring between UC and MC genes in tumors, they developed a “novelty” score.
- The novelty score measures the relative amount of normal tissue modules needed to 'piece together‘ at least half of the genes in a tumor module.
- Modules were subsequently classified as being of high, moderate, or low novelty by using the 1/3 and 2/3 quantiles as cutoffs.

Substantial rewiring of regulatory links between unicellular and multicellular genes in cancer



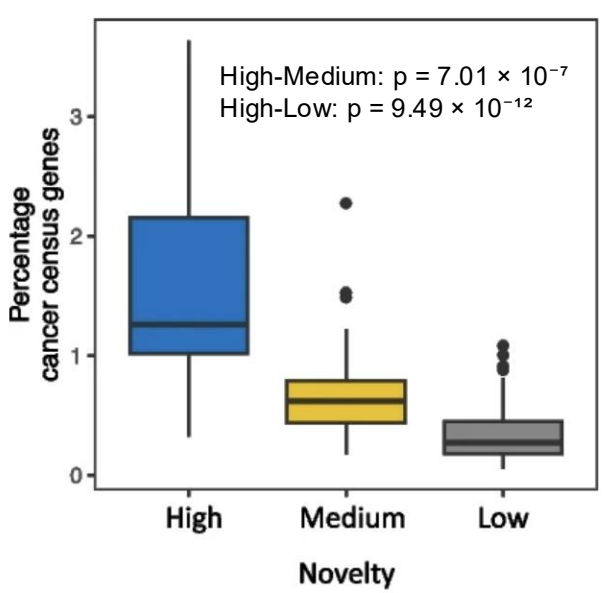
- Mixed UC-MC modules show greater regulatory rewiring when compared to UC-enriched (56.66% expression consistence) and MC-enriched (45.85% expression consistence) tumor modules.

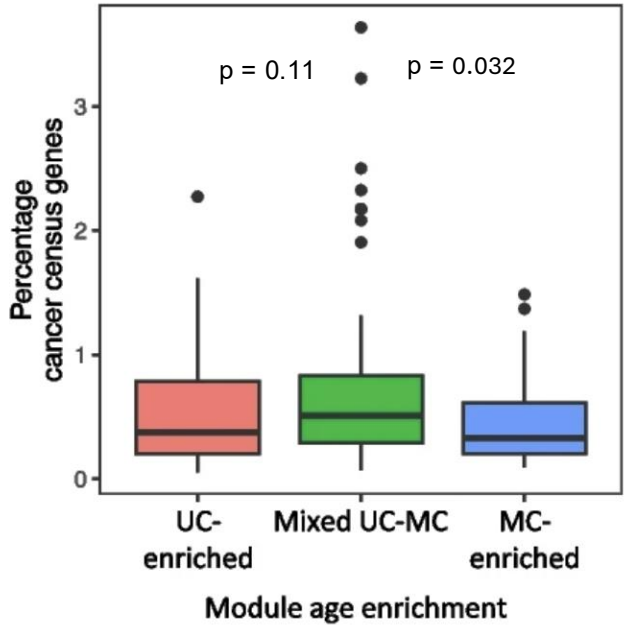


- Mixed UC-MC modules exhibit significantly higher novelty scores than either UC-enriched or MC-enriched modules (one-sided Wilcoxon test)

- **Mix UC-MC modules undergo the most dramatic reorganization in cancer, highlighting their key role in the altered gene regulatory landscape of tumors.**

Somatic variations in cancer genes play a crucial role in the gene network rewiring in cancer

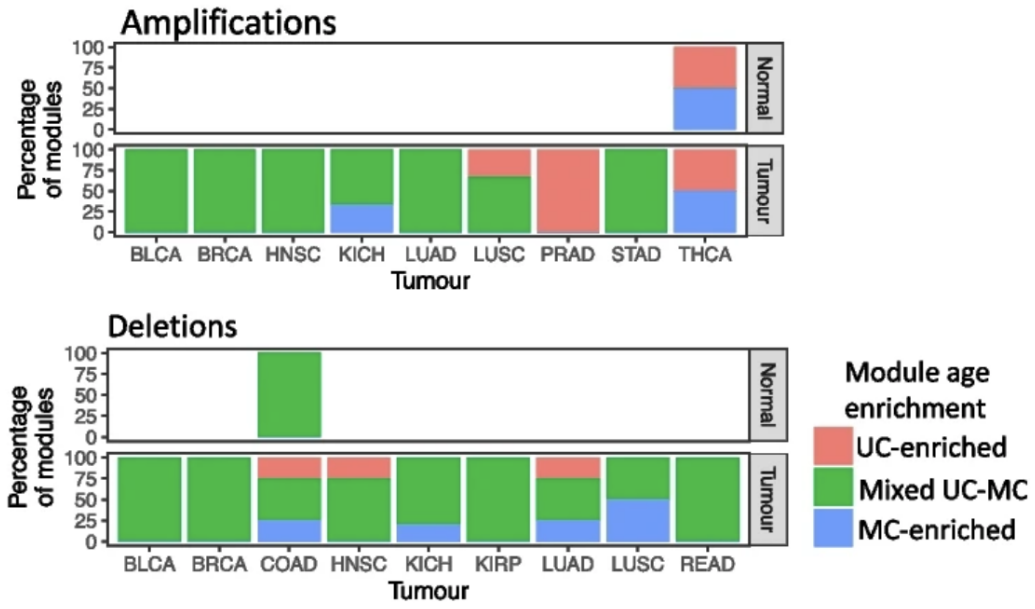




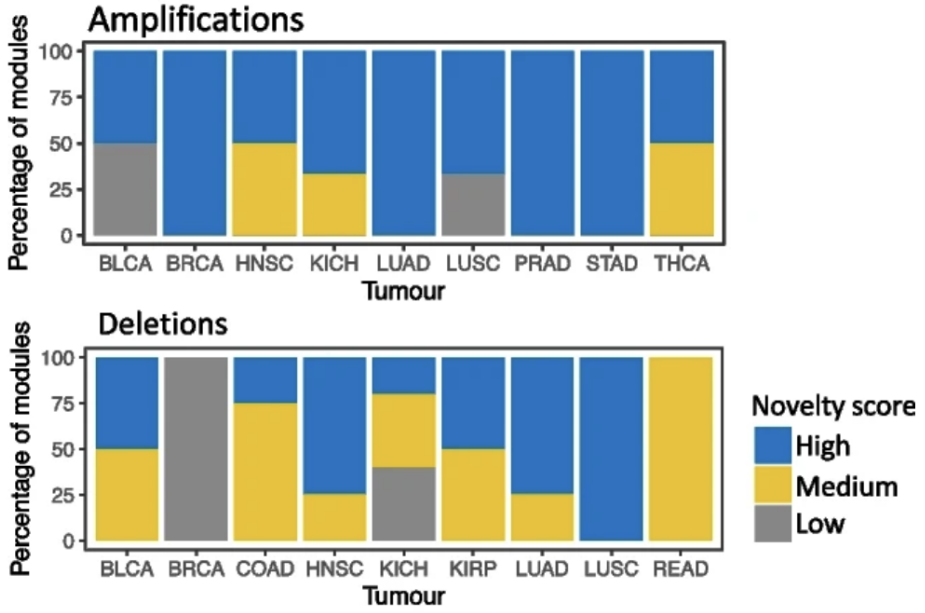
- Novelty scores positively correlate with the proportion of known cancer driver genes.
- Gene age enrichment categories are also associated with the presence of known cancer genes.

- **Somatic variations may actively contribute to the formation of new regulatory links between unicellular and multicellular genes**

CNAs (copy number aberrations) drive high-novelty hybrid modules and regulatory innovation in cancer



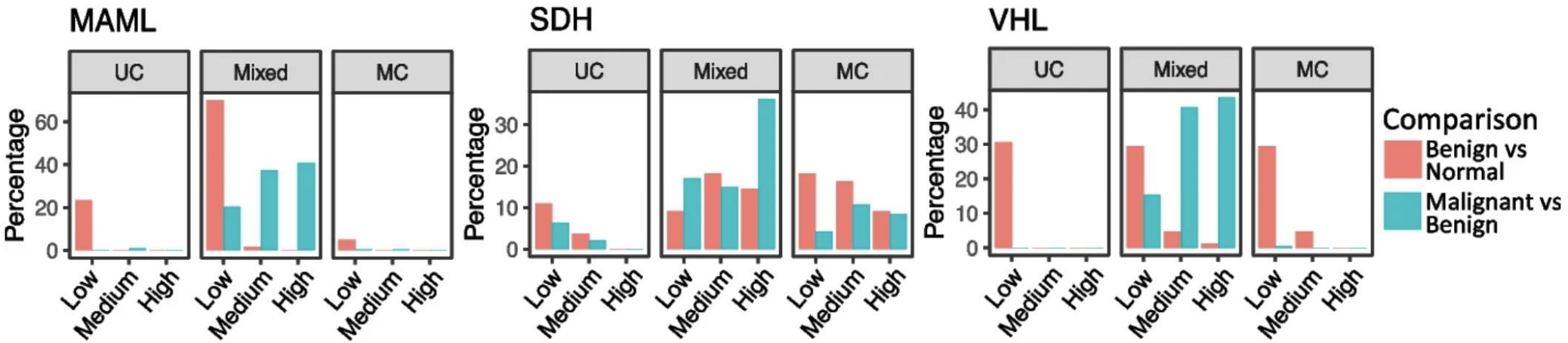
- Recurrently copy number aberrations are most common in high-novelty mixed UC-MC modules.



- Recurrently copy number aberrations are also more prevalent in high-novelty modules.

Increasing imbalance during tumor progression

- Gene co-expression changes across the complete spectrum of normal tissue → benign tumor（良性肿瘤） → malignant tumor（恶性肿瘤）progression in pheochromocytomas（嗜铬细胞瘤）.



- This pattern was consistent across all subtypes regardless of driving mutations, demonstrating that the most significant disruption of evolutionary gene regulatory relationships occurs during the transition to malignancy rather than during initial tumor formation.

Insights & Limitations

Key Insights

- **Evolutionary Perspective:** the study highlights how cancer cells create novel hybrid regulatory modules to coordinate unicellular (UC) and multicellular (MC) gene functions.
- **Approach to quantify the extent of gene network rewiring**

Limitations & Considerations

- **A more precise examination of the deep biological significance of mixed UC-MC Modules**

THANKS