**Academia**
Molecular Biology and Genomics

10.20935/AcadMolBioGen8672

Review Article

# A liquid–liquid phase separation hypothesis for neutral evolution in endosymbiosis and tumorigenesis

Jessie J. Grazier[1,*]

[1] Independent Researcher, Monroe, LA, USA
* Correspondence: jgrazier7933@icloud.com

**Received:** 2 September 2026 | **Accepted:** 19 November 2026 | **Published:** 24 November 2026

## Abstract

Liquid–liquid phase separation (LLPS) organizes biomolecules into dynamic compartments and may permit evolutionary change without immediate loss of function. Extending an earlier abiogenesis framework, this article proposes that molecular dynamics can buffer mutational effects by preserving functional interactions as conformational ensembles change. Intrinsically disordered proteins and conformationally heterogeneous nucleic-acid regions are proposed to offer the greatest opportunity for such buffering, with LLPS compartmentalizing their dynamic interactions. Structured proteins and nucleic acids can also accommodate mutations through local flexibility and conformational rearrangements. This proposed difference in buffering probability is conditional, not a universal ranking of mutation tolerance or motion rates. Neither disorder nor condensation guarantees preserved function or fitness neutrality. Contemporary mitochondrial nucleoids, mitochondria-associated translation condensates, and bacterial MIC60 homologues motivate, but do not demonstrate, the historical endosymbiotic mechanism. Three genomic tests address sequence versus molecular-feature conservation, phylogenetically controlled nucleotide composition–disorder associations, and disorder-associated endosymbiotic gene transfer. Matched functional assays would separately test dynamics-mediated buffering and any added contribution of LLPS. The suggested adenine/thymine (A/T)–disorder relationship remains unverified. A tumorigenesis analogy considers whether condensate-dependent organization across extracellular, cytoplasmic, and nuclear compartments contributes to heterogeneity, reversible drug tolerance, and invasion. Compartment-specific perturbation and rescue distinguish spatial contributions from bulk signaling. Published breast cancer micrographs provide qualitative perturbation comparisons, not evidence of LLPS, adenosine triphosphate (ATP) compensation, or adhesion turnover. A separate two-state-water interpretation remains speculative and unnecessary for the biological hypothesis. These proposals distinguish contemporary experimental evidence from evolutionary inference and disease analogy; negative results constrain individual mechanistic predictions rather than invalidate all condensate biology.

**Keywords**: liquid–liquid phase separation; biomolecular condensate; mitochondria; neutral evolution; genome reduction; breast cancer

# 1. Introduction

## 1.1. From abiogenesis to eukaryogenesis—extending the LLPS evolutionary framework

Liquid–liquid phase separation (LLPS) can organize biomolecules into condensates through collective interactions in aqueous environments. The earlier LLPS framework proposed a role for condensate-mediated molecular organization in abiogenesis and evolution [1]. Xu et al. review the physical principles of LLPS and discuss possible applications in biomedicine and bioengineering [2]. The emergence of eukaryotic life involved compartmentalized metabolism, specialized membranes, and regulatory networks. Mitochondrial acquisition involved an ancestral archaeal host and a bacterial lineage related to Alphaproteobacteria [3–5]. How their components became integrated remains an active question; contemporary condensates provide candidate mechanisms, not a reconstruction of that event.

The traditional view of evolution emphasizes genetic mutation, selection, and inheritance as the primary drivers of biological innovation [6]. Increasing evidence suggests that cellular organization itself provides an additional evolutionary layer, in which molecular interactions, protein networks, and spatial organization create emergent properties that influence evolutionary trajectories [7]. LLPS is one mechanism capable of producing such organization, because it allows biomolecules to self-assemble into dynamic condensates without membrane boundaries [8].

How biology exploits LLPS is not settled. Relevant questions include physiological concentration regimes, condensate identity, and distinction from high-avidity binding, polymerization, and surface-templated clustering [9–11]. A separate physical interpretation concerns water structure. Studies of local water structures and realistic water models support aspects of a two-state description or a liquid–liquid critical point [12–14]. Palmer et al. used the ST2 molecular model of deeply supercooled water to support a metastable liquid–liquid transition [15]; Chandler presented a corresponding critique [16]. These are different positions. Neither establishes high-density (HD)/low-density (LD) water partitioning in living-cell condensates. That extrapolation is a speculative adjunct, not a premise required for the biological hypothesis.

The proposed water interpretation is that liquid-like, gel-like, and fibrous assemblies differ in hydration and possibly HD/LD contributions (**Figure 1a**) [1, 9, 12]. The fibrous state's most ordered network is a hypothesis schematic, not a measured or simulated hydration structure. Phase behavior also depends on pH, temperature, ionic strength, post-translational modification, adenosine triphosphate (ATP), crowding, and sequence properties. No universal or irreversible maturation sequence is implied. The dynamics-mediated buffering hypothesis is independent of this proposed water-state interpretation.

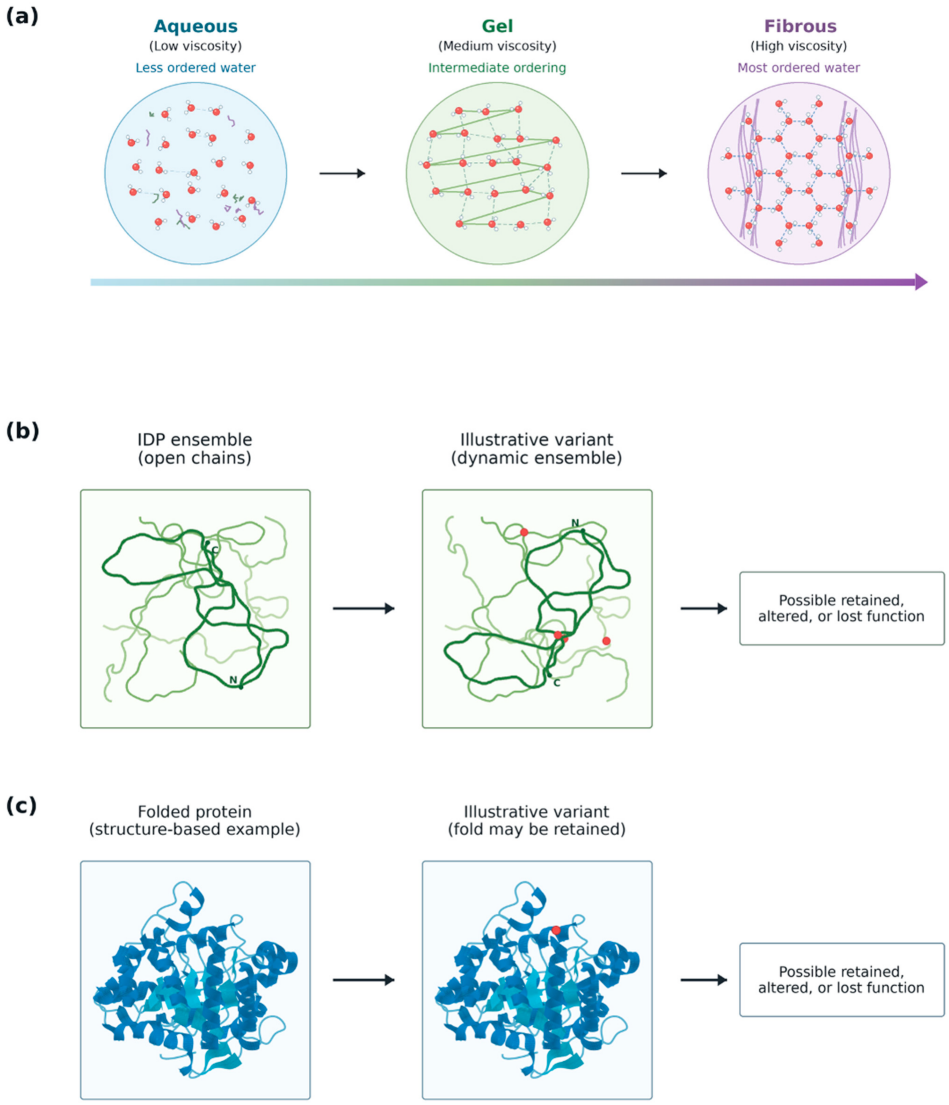


**Figure 1.** Conceptual illustration of condensate organization and alternative mutational outcomes. (**a**) Water ordering and HD/LD assignments are unmeasured hypothesis schematics, with the most ordered network depicted in the fibrous state; no compulsory progression is implied. The aqueous, gel, and fibrous states are depicted with low, medium, and high viscosity, respectively. The proposed assignments are HD-rich, mixed HD/LD, and LD-rich, respectively; these assignments are not measured. Water molecules and proposed hydrogen-bond connections are schematic, not measured structures. (**b**) Synthetic open-chain IDP ensembles, not sequence-specific structural predictions. Each open path represents a conformation; N and C indicate the amino and carboxyl termini, respectively. Red markers denote illustrative sequence changes. (**b**,**c**) Dynamics can accommodate mutations [17, 18], including in folded proteins; neither disorder nor LLPS guarantees preserved function. (**c**) Ribbons rendered from experimental Rev6 phosphotriesterase coordinates (PDB 4PBE, chain A). Based on data reported in [18]. Both boxes in (**c**) use the same coordinates; red markers are schematic sequence changes, not modeled mutants or measured responses. Both classes can retain, alter, or lose function. Loss of function can be beneficial. Selection acts whenever heritable variants affect fitness, not only after innovation. The broader nucleic-acid hypothesis is not depicted. This original composite was created/modified using Academia Co-scientist (underlying model GPT-6.1 Sol) for code assistance. The present protein panels were rendered using custom Python 3.14.4 code with Matplotlib 3.11.2 (mplot3d), NumPy 2.5.4, and SciPy 1.18.1; the composite and annotation revisions used Pillow 13.0.0. Panel (**a**) retains the original author-created schematic. No visual material from Campbell et al. [18] was reproduced. HD: high-density; LD: low-density; IDP: intrinsically disordered protein; PDB: Protein Data Bank; LLPS: liquid–liquid phase separation.

In early evolution, LLPS may have helped concentrate molecules and organize reactions [1]. Contemporary microbial condensate studies and databases provide candidate systems [19, 20], while condensate-mediated organization in innate immunity illustrates regulatory integration in a modern, distinct context [21]. None establishes ancestral endosymbiotic buffering. Extending these organizational principles to eukaryogenesis is a hypothesis: partially integrated components might have persisted within dynamic assemblies long enough for some nonadaptive changes and dependencies to accumulate [22].

Molecular dynamics can buffer the functional consequences of mutations when conformational rearrangements or redistributed interactions preserve a specified output. Intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) are central examples [23, 24]: their accessible ensembles support multivalent interactions, and LLPS can compartmentalize those dynamic interactions [25]. The working hypothesis is that IDPs and conformationally heterogeneous nucleic-acid regions offer the greatest opportunity for this buffering under matched conditions. Here, disordered nucleic-acid regions mean flexible, heterogeneous regions, such as unpaired ribonucleic acid (RNA) or single-stranded segments, rather than a protein-like classification of every nucleic acid. Structured proteins and nucleic acids remain dynamic through local flexibility, transient disorder, and alternative conformations. More constrained ensembles do not imply a universally slower rate of motion or a fixed ranking of mutational tolerance. Folded-protein dynamics can accommodate a changed side chain or alter activity while retaining the overall fold [17, 18]; Campbell et al. demonstrated mutation-dependent shifts in an enzyme's conformational populations during directed evolution [18]. The nucleic-acid buffering extension is a prediction, not a conclusion established by those protein studies. During endosymbiotic integration, such dynamics might have buffered some molecular changes [23, 26, 27]. Molecular buffering means retention of a prespecified output despite mutation under defined conditions; neutral buffering additionally requires unchanged fitness. Neither disorder nor LLPS alone establishes either outcome. **Figure 1b,c** illustrates possible outcomes of this shared mechanism, not a rigid division between IDPs and folded molecules.

Mitochondria provide a worked example of this evolutionary transition. Derived from ancient α-proteobacteria, mitochondria retained bacterial characteristics while becoming deeply integrated into eukaryotic cellular systems [3]. The development of mitochondrial cristae, specialized inner membrane structures required for efficient energy production from the electron transport chain and citric acid cycle, was a major structural innovation [28]. Proteins such as MIC60 within the mitochondrial contact site and cristae organizing system (MICOS) show conserved relationships between bacterial membrane organization and mitochondrial architecture [29, 30].

This article proposes that dynamics-mediated mutation buffering, compartmentalized by LLPS in some IDP–nucleic-acid assemblies, supplied dynamic scaffolding that could have supported mitochondrial integration and some neutral evolutionary changes. IDPs are the main example, not the only biomolecules capable of buffering. Section 4 derives genomic tests concerning chromatin and transcription [31, 32], sequence determinants and their evolution [33–35], and gene loss, replacement, and transfer [36, 37]. Sections 8–10 extend the organizational hypothesis by analogy to tumor-cell plasticity [38, 39]. The analogy concerns physical organization and does not establish reactivation of a specific ancestral program or a shared historical mechanism.

Academia Co-scientist (underlying model GPT-6.1 Sol) was used during preparation of the manuscript for language editing, assembly of reference metadata, and generation of code for schematic illustration and structural visualization in **Figure 1**. All scientific content is based on the author's original synthesis, peer-reviewed literature, and subject matter expertise. The tool assisted in expressing the author's ideas and implementing figure code; it did not independently establish the scientific claims.

## 2. LLPS as a molecular scaffold for endosymbiotic integration

The origin of mitochondria was an evolutionary event in which two previously independent cellular lineages became permanently integrated into a single organism [3]. Current models suggest that an ancestral archaeal host acquired an α-proteobacterium-like endosymbiont, initiating a process that ultimately produced the first eukaryotic cells [4]. The transition from independent prokaryotic organisms to a fully integrated eukaryotic system required more than genetic exchange: it required coordination of membranes, metabolic pathways, protein trafficking systems, and regulatory networks across two distinct evolutionary histories [40, 41]. A major challenge in understanding the emergence of eukaryotes is explaining how these systems remained stable long enough for evolutionary refinement to occur [4].

LLPS offers one way of addressing this challenge, because condensates create dynamic molecular environments that can organize newly integrated cellular components [42]. Unlike membrane-bound compartments, LLPS-generated condensates allow reversible assembly and disassembly of molecular networks in response to environmental conditions [8]. In the proposed model, their contribution is to compartmentalize dynamics-mediated buffering: IDP–nucleic-acid interactions could reorganize around some sequence changes while maintaining selected outputs. This property may have mattered during early endosymbiotic evolution, when cellular systems were undergoing extensive remodeling and genetic exchange [43]. Rather than requiring immediate optimization, partially integrated components could have persisted within dynamic condensate environments that allowed interaction and functional exploration [21]. Structured partners could participate through their own conformational dynamics; buffering is not restricted to wholly disordered molecules.

The acquisition of mitochondria was followed by extensive gene transfer from the endosymbiont genome into the nuclear genome, producing a distribution of genetic information across two cellular compartments [36]. This process required protein import machinery capable of directing nuclear-encoded mitochondrial proteins back into the organelle [40, 41]. Mitochondrial membranes also underwent specialization, producing internal structures such as cristae that increased the efficiency of mitochondrial energetics [28, 44]; this in turn has been linked to the diversification of eukaryotes [45]. These processes are an example of evolutionary integration, in which previously independent systems become dependent on one another [46].

IDPs are candidate adaptable interfaces [17, 23], although multivalent interactions also involve folded domains [25]. Direct contemporary evidence comes from Bai et al. in *Caenorhabditis elegans*: La-related protein 1 (LARP-1) forms mitochondria-associated translation organelles (MATOs) on the cytoplasmic face of the outer mitochondrial membrane through association with the translocase of the outer membrane (TOM) import machinery [47]. These are not matrix-localized RNA assemblies. The study connected LARP-1 to localized translation of *immt-1* (MIC60) and *atp-2* (ATP synthase β subunit) transcripts using nascent-translation/messenger RNA (mRNA) imaging, protein measurements, loss-of-function phenotypes, and rescue experiments. Reduced target protein production accompanied cristae defects and reduced ATP; the evidence is stronger than colocalization alone, but is restricted to the tested organism and conditions [47]. Applying analogous organization to ancient integration is a hypothesis. The metabolic-engineering review [48] provides general context for organizing reactions, not direct evidence for mitochondrial evolution.

MICOS coordinates crista junctions, inner-membrane curvature, and mitochondrial membrane architecture [28]. Primary work identifies bacterial MIC60 homologues and their membrane-organizing function [29, 30]. These findings establish evolutionary and structural continuity, not phase separation of MIC60 or the MICOS complex. The perspective on mitochondrial condensates and bacterial links [49] motivates an organizational connection; the proposed MICOS–LLPS bridge requires direct biophysical testing.

The ability of cellular systems to retain function during intermediate states could have aided endosymbiotic integration; modern host control of endosymbiont division illustrates one form of integration rather than evidence of ancient condensate buffering [50]. Neutrality refers to fitness effects in a specified environment and population-genetic regime. Constructive neutral evolution can establish dependencies through initially nonadaptive changes [22], whereas viral examples illustrate context-dependent evolutionary rates without establishing neutrality in IDRs [26]. Condensate-mediated buffering is proposed, not demonstrated, as one route permitting such changes [17].

In this model, LLPS does not drive evolution through mutation. It provides an organizational landscape that influences which molecular configurations are possible and sustainable [1]. By allowing dynamic assembly of proteins, nucleic acids, and membranes, LLPS may bridge genetic innovation and cellular complexity [8, 42]. The emergence of mitochondria can therefore be viewed not only as a consequence of endosymbiosis [3], but also as a consequence of molecular organization that allowed endosymbiotic components to become integrated into a unified biological system [1, 49].

## 3. Intrinsically disordered proteins and dynamics-mediated mutation buffering during mitochondrial acquisition

The transition from prokaryotic ancestors to eukaryotic cells required extensive restructuring of biological systems [51]. Unlike the compact genomes of bacteria and archaea, eukaryotic organisms developed expanded genomes, complex regulatory networks, and extensive protein interaction systems [52]. This expansion was accompanied by an increased prevalence of IDPs, which provide functional flexibility through their ability to adopt multiple conformations and interact with diverse partners [27]. Disorder content is substantially higher in eukaryotic proteomes than in bacterial or archaeal proteomes [53], and exon elongation has been proposed as one route by which disordered regions were added to the proteins of the last eukaryotic common ancestor [27].

One unverified possibility is that adenine/thymine (A/T)-rich coding sequences alter amino acid composition in ways that favor disorder or particular condensate interactions. No general direction or causal relationship is established here: genome-wide A/T content is not equivalent to coding-sequence A/T content, and the genetic code, codon usage, and protein repertoire can change the association. The mitochondrial phylogeny study [5] addresses ancestry, not an A/T–disorder mechanism; the IDR evolution study [54] identifies conserved molecular features, not greater mutational tolerance of A/T-rich deoxyribonucleic acid (DNA). Accordingly, "tolerate mutation well" is not an intrinsic property of A/T-rich regions. The proposed mechanism is dynamics-mediated retention of function, with the greatest buffering opportunity expected in sufficiently flexible protein and nucleic-acid regions under matched conditions. IDRs may accommodate some substitutions while remaining constrained at motifs, interaction sites, or distributed molecular features [17, 24, 35, 54]. Folded proteins can also accommodate substitutions through conformational reorganization [17, 18]; structured nucleic acids are likewise candidates through their local flexibility and alternative conformations. That extension and the proposed comparative advantage require functional tests. Neutral buffering additionally requires fitness measurements.

During endosymbiosis, such flexibility could have helped coordinate host and bacterial-derived components, although mitochondrial ancestry [5] does not establish an IDR-mediated mechanism. The modern symbiosis of *Pseudoblepharisma tenue* with purple bacteria illustrates coexistence of diverse partners, not ancient mitochondrial integration or LLPS buffering [55]. IDRs are proposed as adaptable interfaces that might permit incremental refinement under some neutral evolutionary scenarios [17, 22].

This extends to mitochondrial architecture, where dynamic remodeling is needed to maintain cellular function [56]. MICOS depends on coordinated interactions between membrane proteins, structural domains, and regulatory factors [28]. A dynamin superfamily-like pseudoenzyme containing IDRs has been proposed to coordinate with MICOS to promote cristae architecture [44]. Such proteins illustrate how flexible molecular components can regulate membrane organization without acting solely through classical enzymatic activity.

Taken together, these observations motivate, but do not establish, a model in which molecular dynamics buffered some mutations and LLPS compartmentalized the relevant interactions during integration. IDPs provide the clearest example of the proposed dynamic scaffolding, while structured partners and nucleic acids are included rather than excluded by nomenclature. Some buffered changes might accumulate with little fitness effect [22], while other changes would be selected positively or negatively at any stage. Energetic diversification [45, 57] does not imply that selection began only after mitochondrial networks were complete.

## 4. Genome-level predictions: chromatin organization, the sequence determinants of phase behavior, and endosymbiotic gene transfer

The argument so far rests on cellular and biophysical phenotype. If LLPS contributed to eukaryogenesis in the way proposed, the model should also make claims at the level of the genome—about how condensates relate to chromatin and transcription, about how phase behavior is encoded in sequence and how that encoding evolves, and about what should be observed during genome reduction and gene transfer in an endosymbiosis. This section sets out those links and the predictions that follow from them.

### 4.1. Condensates and the organization of the genome

Phase separation is implicated in the organization of eukaryotic chromatin and in the control of transcriptional output. Heterochromatin protein 1a forms liquid-like domains that compact underlying chromatin, providing a physical route to gene silencing that does not depend on a fixed stoichiometric complex [32]. Reconstituted nucleosome arrays phase separate under physiological conditions, and their material state is tuned by histone modification and by linker histone binding [58]. At the active end, coactivators concentrate at super-enhancers in condensates that compartmentalize the transcription apparatus [31], and the activation domains of sequence-specific transcription factors—themselves disordered—drive that concentration [59]. The nucleolus, the most-studied nuclear condensate, is a multiphase structure whose subcompartments arise from differences in the surface tension of coexisting liquids [60]. Phase separation also operates at the nuclear periphery, where it contributes to nuclear envelope reformation after mitosis [61].

These contemporary nuclear systems show that collective assembly can regulate genome organization without requiring a new catalytic reaction for each organizational state. Their existence does not demonstrate that they were present during eukaryogenesis. Mechanistic studies of prion-like RNA-binding protein domains show how aromatic valence and residue patterning alter phase behavior [33, 34]; “prion-like” describes sequence properties and does not mean that these studies establish infectious prions or nuclear genome control. Their relevance here is an experimentally grounded interaction grammar that might be evolvable. Extrapolation from that grammar to the origin of nuclear regulation remains a hypothesis. Molecular specificity can depend on both folded recognition interfaces and disordered sequence features.

Mitochondrial nucleoids provide a related contemporary example. Mitochondrial transcription factor A (TFAM)-containing multicomponent nucleoids self-assemble by phase separation in vitro, with cellular experiments supporting condensate-like organization [62]. Reconstituted mitochondrial transcriptional condensates exhibit structure–function relationships that affect transcription [63]. TFAM is nuclear-encoded and contains structured high-mobility group (HMG) DNA-binding domains and flexible regions; it is not a wholly disordered protein. These mixed protein–nucleic-acid systems illustrate why dynamics, rather than an all-or-none disorder label, is the proposed buffering variable. Flexible nucleic-acid segments may sample diverse conformations, while base-paired DNA and folded RNA retain local motions and alternative states. Whether those dynamics preserve output after a sequence change must be tested independently of condensation. The cited studies support physical regulation of mitochondrial genome organization, not universal mutation buffering or proof of the evolutionary route proposed here.

## 4.2. Sequence determinants of phase behavior and how they evolve

Phase behavior depends on sequence, composition, residue patterning, molecular partners, and conditions. In experimentally studied prion-like domains, aromatic "sticker" valence and spacing, together with intervening "spacer" residues, tune phase boundaries [33, 34]. Charge patterning parameters such as $\kappa$ (which describes the segregation of oppositely charged residues) and sequence charge decoration (SCD), net charge per residue, hydropathy, and short linear motifs can also be relevant. Composition alone is not a sufficient or universal code for condensation.

The evolutionary possibility is that some substitutions preserve function by maintaining distributed molecular features even when sequence identity declines. Zarin et al. examined feature conservation in diverged yeast IDRs [35]; Singleton and Eisen analyzed IDRs across 33 *Drosophila* species and found widespread conservation of features including composition, charge, charge patterning, hydropathy, complexity, and motif-related properties [54]. These are the specific features intended here, not unspecified "correct features." Neither study implies that every IDR forms condensates, that exact sequence never matters, or that feature conservation establishes fitness neutrality.

The first operational prediction is relative sequence divergence with retention of experimentally relevant molecular features in selected condensate-forming IDRs. It tests the proposed greater opportunity for dynamics-mediated buffering, not an assumption that structured comparators cannot buffer mutations. Define orthologous protein families, validated IDR/folded-domain boundaries, and a priori phase-relevant features. Compare nonsynonymous/synonymous substitution ratios ($d_N/d_S$) and feature-conservation statistics between aligned IDRs and folded domains within the same proteins, using phylogenetic models and controls for alignment uncertainty, synonymous saturation, codon bias, protein length, and expression where available. Do not require $d_N/d_S \approx 1$: selection on motifs and distributed properties can keep IDRs under purifying selection. Include only supported homologues; bacterial homologues must not be presumed for TFAM or LARP-1. Folded-domain comparators remain dynamic and can reorganize after mutation [17, 18]. Measure mutation-dependent conformational changes alongside stability and activity; test candidate variants in phase diagrams and functional assays. **Figure 1b,c** show schematic alternatives, not measured mutant responses or a rule that folded-interface mutations abolish function [17, 18]. Failure to find the relative pattern would weaken the proposed disorder-associated advantage, not every dynamics-mediated or LLPS contribution.

A complementary direct test would compare the fraction of defined mutations that preserve a prespecified output in disordered versus structured protein or RNA regions, while measuring their conformational

responses. Match mutation classes, expression or abundance, and environmental conditions; assess fitness separately where applicable. Compare validated condensation-competent and condensation-impaired states while controlling binding, localization, and soluble concentration. Measure phase behavior independently rather than treating exchange rate as a proxy for molecular flexibility. The proposed greater buffering probability in disordered ensembles and any extra contribution from LLPS are separate predictions. These assays test RNA directly; they do not assume that protein $d_N/d_S$ measures apply to noncoding nucleic acids.

The second operational prediction treats A/T composition as an exploratory hypothesis rather than an established causal input. Across a prespecified set of independent lineages, quantify genome-wide A/T content and coding A/T content separately; quantify proteome disorder fraction with fixed predictors and experimentally supported annotations where available. Use phylogenetic comparative models with codon/amino acid composition, protein length, gene repertoire, and taxonomic structure as competing explanations. Analyze predicted condensate propensity separately from disorder and validate selected proteins experimentally. A/T enrichment need not favor disorder; a null or opposite association would reject the proposed positive relationship, while correlation alone would not demonstrate mutation buffering or ancient condensate capacity.

## 4.3. What the model predicts for genome reduction and endosymbiotic gene transfer

Endosymbiont genome reduction can involve genuine gene deletion when a function becomes dispensable, functional replacement by a host or other gene, and endosymbiotic gene transfer (EGT) followed by nuclear expression and, where required, protein import [36, 37]. These outcomes must not be equated: most missing genes cannot be assumed to have transferred to the nucleus. A productive transfer requires an appropriate expression context and a functional route for the product to reach its site of action [40, 41]. Condensate organization might buffer some interactions, but cannot substitute for essential import or rescue a protein that never reaches its required compartment.

The framework predicts that this window is survivable to the extent that molecular dynamics preserve the product's relevant functions and interactions. Disordered interfaces are proposed to offer a greater buffering opportunity, with LLPS compartmentalizing some interactions, but structured proteins and nucleic acids are not excluded. Three specific expectations follow.

(1) Transfer bias—conditional prediction. Use gene trees and taxonomic distribution to distinguish supported EGT from host-gene recruitment, unrelated replacement, and deletion. Model supported transfer occurrence, or branch-supported relative order where resolvable, against measured/predicted disordered-interface properties. Control for membrane hydrophobicity, protein importability, expression, complex membership, and phylogeny. Ask whether disorder adds explanatory value beyond these alternatives. Retention of hydrophobic respiratory genes is not itself evidence for a disorder-dependent transfer bias; unavailable historical order must remain unresolved.

(2) Targeting-sequence accessibility—separate import and assembly tests. Mitochondrial presequences often have positively charged, amphipathic properties and may form helices during recognition; they are not equivalent to condensate-forming sticker-and-spacer sequences [36]. Test randomized or repurposed N-terminal sequences for import efficiency, then independently test assembly and function of the imported product. This separates accessible targeting solutions from the additional hypothesis that disordered interfaces improve tolerance of altered assembly.

(3) Population-genetic permissiveness—conditional scenario. Reduced effective population size can weaken selection against mildly deleterious changes and has been proposed to contribute to genome expansion [52]; constructive neutral evolution describes one route to nonadaptive dependencies [22]. Neither the magnitude nor timing of an ancestral population-size change is established here. Compare the buffering/transfer predictions across plausible population-genetic regimes rather than assume a single historical demographic event. Phenotypic tolerance and drift are distinct proposed contributions.

Together, these are bounded tests of feature-preserving sequence divergence, an unverified composition–disorder relationship, and disorder-associated transfer after controlling for alternatives. Disorder is an indicator of a proposed opportunity for dynamics-mediated buffering, not its definition; measured function and fitness distinguish buffering from mere flexibility. Phylogenetic correlation or a positive assay would not uniquely identify an ancient LLPS mechanism; failure of one prediction limits that component of the hypothesis.

## 5. MICOS, MIC60, and the evolutionary origin of mitochondrial cristae

The evolution of mitochondrial architecture was one of the structural innovations associated with the emergence of eukaryotic life. Following acquisition of an α-proteobacterial ancestor, mitochondria transformed from a free-living bacterium into an organelle producing chemical energy through oxidative phosphorylation (OXPHOS) [41]. A defining feature of this transition was the development of cristae, organized invaginations of the inner mitochondrial membrane that increase the surface area available for electron transport chain activity and ATP production while positioning membrane-bound complexes where citric acid cycle enzymes are active [30, 64]. MICOS is central to the formation and maintenance of these structures, regulating crista junction formation, membrane curvature, and the spatial organization of respiratory complexes [28]. Conservation of MICOS-related proteins, particularly MIC60, indicates that mitochondrial cristae did not arise entirely de novo within eukaryotes but may have evolved from membrane-organizing systems already present in α-proteobacterial ancestors [29, 30].

MIC60 is the most conserved MICOS component and is required for inner membrane organization [28]. A homolog, αMIC60, is involved in intracytoplasmic membrane development in α-proteobacteria, which suggests an evolutionary connection between bacterial membrane remodeling and mitochondrial crista formation and places the origin of MICOS before eukaryotic endosymbiosis [30].

In photosynthetic and metabolically specialized α-proteobacteria, intracytoplasmic membranes function as platforms for energy conversion [30]. These bacterial membrane systems are a plausible evolutionary precursor to the folded mitochondrial inner membrane, and the compositional evolution of that membrane has been traced independently [64]. The transition may therefore be an example of evolutionary repurposing, in which existing molecular machinery was co-opted to support new cellular functions [64].

The presence of mitochondrial IDRs motivates a role for dynamic interactions [65]. Both folded domains and disordered regions can bind multiple partners and respond to cellular conditions [17, 23]. Within the proposed model, IDPs are the clearest examples of dynamics-mediated buffering because their interactions can be compartmentalized by LLPS. Folded domains can also accommodate substitutions through conformational reorganization [17, 18], and associated structured nucleic acids remain candidates through local flexibility and alternative conformations. The expected advantage of broader accessible ensembles is conditional, not a fixed ranking of motion rates. Reviews discuss LLPS as a candidate regulator of mitochondrial metabolism [66], but disorder alone establishes neither phase separation nor mutation buffering nor a role in MICOS evolution.

The proposed MICOS–LLPS connection has a specific evidential bridge: bacterial MIC60 function supports membrane-organizing continuity [30], whereas LARP-1-dependent MATOs support contemporary localized synthesis of IMMT-1/MIC60 [47]. Neither shows that MIC60 or the assembled MICOS complex phase separates. The condensate perspective [49] and mitochondrial-metabolism review [66] provide synthesis, not direct demonstration of that link. Testing the proposed connection would require biophysical analysis of MICOS-associated assemblies and perturbations that separate condensation from membrane binding and protein-complex assembly. Cristae remodeling is established [67]; condensate-dependent coordination is the additional hypothesis [25, 47].

A related case is the interaction between mitochondrial translation and membrane organization. Synthesis of respiratory chain components requires coordination between mitochondrial DNA (mtDNA) expression, nuclear-encoded proteins, and membrane insertion pathways [68]. LLPS-associated hubs may allow these processes to proceed efficiently by concentrating RNA molecules, ribosomal components, and protein factors involved in mitochondrial biogenesis [63, 69].

The evolution of mitochondrial cristae can therefore be read as a continuation of an ancient bacterial organizational strategy rather than a wholly novel eukaryotic invention [29, 30]. MICOS, MIC60, and associated regulatory proteins show how systems can be modified through gradual integration [22], with LLPS and IDP-mediated interactions offering a candidate source of the flexibility that complex cellular architecture requires [66, 69].

## 6. Mitochondrial energetics and the expansion of eukaryotic complexity

The acquisition of mitochondria altered the energetic landscape of eukaryotic evolution [45, 57]. Before mitochondrial integration, early cells were limited by the energetic constraints of prokaryotic metabolism [57]. OXPHOS provided a more efficient route to ATP production, which has been argued to allow support for larger genomes, greater cellular complexity, and expanded regulatory networks [45]. The magnitude and direction of that advantage remain debated, and alternative accounts hold that genome expansion followed from reduced effective population size rather than from an energetic release [52].

The citric acid cycle and electron transport chain appear to have become coupled within mitochondria early in eukaryotic evolution, before the diversification of the last eukaryotic common ancestor [51]. This created a metabolic platform capable of supporting processes that distinguish eukaryotes from prokaryotes [45]. Increased available energy may have enabled cytoskeletal complexity, intracellular trafficking, membrane compartmentalization, and eventually multicellularity [57]. On this view mitochondria were not only energy-producing organelles but evolutionary catalysts that widened the range of possible cellular morphologies and tissue architectures [57, 70].

The relationship between mitochondrial energetics and evolutionary diversification is most visible when aerobic and anaerobic eukaryotic lineages are compared [57]. Some modern eukaryotes have reduced or modified mitochondria, but their evolutionary history reflects a period in which mitochondrial metabolism enabled ecological exploration [45, 57]. Enhanced respiratory capacity allowed organisms to occupy new environments, develop specialized structures, and evolve greater complexity [45].

LLPS might have contributed by organizing selected reactions, as discussed generally in metabolic engineering [48] and mitochondrial reviews [66, 69]. Mitochondrial biogenesis involves enzymes, membranes, nucleic acids, and regulatory networks [71]; it must not be assumed that all operate in one condensate. Bai et al. directly tested mitochondria-associated LARP-1 condensates in *C. elegans* [47]. Separately, Aththawala Gedara et al.

used engineered mitochondria-targeted optogenetic IDR constructs in human HeLa cells to show that tubular mitochondrial geometry restricted condensate growth and mobility, whereas enlarged regions produced by fission inhibition permitted coarsening [72]. This establishes a geometry-dependent effect in that system, not that membranes universally prevent coarsening of all endogenous mitochondrial condensates.

The emergence of mitochondria can therefore be interpreted as both an energetic and an organizational transition. The endosymbiotic event supplied a metabolic engine [45, 57]; LLPS-mediated organization is proposed as dynamic scaffolding that could compartmentalize mutation-buffering interactions during integration [1, 47]. IDPs exemplify this proposal, while structured proteins and nucleic acids can contribute through their own dynamics.

## 7. LLPS, mitochondrial homeostasis, and cellular adaptation

The success of mitochondria required not only their initial integration but also mechanisms that maintain their function across changing cellular environments [3]. Modern mitochondria undergo continuous fusion, fission, protein turnover, and membrane remodeling, so that mitochondrial form and function are continuously coupled [56, 73, 74]. These processes require coordinated regulation among mitochondrial genomes, nuclear-encoded proteins, metabolic pathways, and signaling networks [71]. LLPS offers a mechanism by which such interactions could be organized in space and time without additional membranes [47, 69].

LLPS-driven organization can combine persistent molecular structure with reversible association [1]. The shared proposed mechanism is dynamics-mediated mutation buffering, not a division between rigid folded proteins and dynamic IDPs. Stable folded complexes sample conformational states, and mutations can redistribute these states within a retained fold [17, 18]. IDP-mediated condensates compartmentalize ensemble-dependent, multicomponent interactions [25], making IDPs the central example of the proposed greater buffering opportunity. Structured proteins and nucleic acids can also preserve outputs through local flexibility or alternative conformations; their dynamics are more constrained in some respects, not universally slower. Functional measurements must distinguish successful accommodation from altered or lost activity, and fitness neutrality is a separate endpoint. For mitochondria, structural stability must coexist with continuous remodeling [56]. Cristae architecture [67], respiratory chain organization [68], mitochondrial translation [63], and metabolic regulation [66] all require adaptable molecular systems.

A possible evolutionary interpretation is that related organizational principles contributed both to integration and to modern homeostasis [49, 66]. Contemporary evidence for mitochondrial IDRs, condensate-associated regulation, and environmental adaptation [65, 69, 75] cannot by itself establish ancestral conservation of an LLPS mechanism. That historical connection remains an inference requiring comparative and functional tests; modern LARP-1 condensates provide a tractable example rather than a historical reconstruction [47].

The relationship between mitochondrial organization and evolutionary adaptation is also visible in organisms with unusual mitochondrial histories. Studies of diverse microbial eukaryotes show that mitochondrial genomes and structures have undergone extensive diversification, indicating that mitochondria are continuously adapting systems rather than static evolutionary remnants [43]. The persistence of this diversity points to flexible regulatory mechanisms capable of supporting different cellular environments [3].

The general principle proposed is that complex biological systems emerge not only through genetic innovation but through the ability to organize molecular interactions dynamically [1, 7]. This becomes relevant when examining pathological states in which evolutionary cellular programs are altered, such as cancer [38, 39].

## 8. From evolutionary innovation to tumorigenesis: mitochondrial remodeling in cancer

Cancer can be viewed as a disease of altered cellular organization, in which regulatory networks are reprogrammed to support uncontrolled growth, survival, and adaptation [76]. This echoes ancient single-cell hypermutation programs that enable growth during stress [77]. Many features of tumorigenesis resemble evolutionary processes, including genetic diversification, metabolic adaptation, and selection of cellular states capable of surviving changing conditions [78–80]. Because mitochondria were central to the emergence of complex eukaryotic metabolism, their dysfunction and remodeling are a major component of cancer biology [81, 82].

Cancer metabolism has historically been associated with the Warburg effect, in which tumor cells preferentially use glycolysis despite the presence of oxygen [83, 84]. Current work shows that cancer metabolism is flexible, and that many tumors maintain active mitochondrial respiration alongside glycolysis [85, 86]. Rather than a simple loss of mitochondrial function, tumorigenesis often involves mitochondrial adaptation and metabolic reprogramming to the local tumor microenvironment [85].

This parallels mitochondrial evolution. During endosymbiosis, mitochondria expanded the energetic capabilities of early eukaryotes [45, 57]. During tumorigenesis, altered mitochondrial function may similarly increase adaptability, allowing cells to survive metabolic stress, invade new environments, and develop aggressive phenotypes [85, 86]. Spatial mapping of mitochondrial networks in lung tumors [87] and the identification of metabolically distinct mitochondrial subpopulations set by local ATP demand [88] support the view that mitochondrial heterogeneity within a tumor is functional rather than incidental.

Polyploid giant cancer cells (PGCCs) are associated with stress adaptation and aggressive tumor behavior, and their origins and consequences are reviewed across cancers [89]. Genomic profiling in undifferentiated pleomorphic sarcoma addresses PGCC evolutionary histories [90]. Neither reference establishes a universal increase in mitochondrial abundance or organelle expansion in PGCCs. Mitochondrial expansion could be a context-dependent adaptation to biosynthetic or energetic demand, but testing it requires mitochondrial mass/volume normalized to cell size, mtDNA copy number, and respiration or ATP-flux measurements. The lung-tumor and local-ATP-demand studies [87, 88] support mitochondrial heterogeneity in their own systems, not a PGCC-specific generalization.

The formation of such plastic cellular states may involve mechanisms similar to those that supported evolutionary transitions [90]. Just as early eukaryotic cells required flexible systems to integrate new genetic and metabolic components, cancer cells exploit flexible regulatory networks to generate diverse phenotypes [91]. LLPS may contribute by organizing signaling molecules, transcriptional regulators, RNA-binding proteins, and metabolic enzymes into functional condensates [39, 92]. Cancer-associated condensates are increasingly recognized as regulators of gene expression, signaling, and metabolism [93, 94]. Through multivalent interactions, LLPS can assemble signaling hubs that influence survival, proliferation, and differentiation [39], which would allow cancer cells to explore alternative cellular states in a manner analogous to how evolutionary systems explore new configurations [95].

The parallel between mitochondrial evolution and tumorigenesis is a structural analogy, not evidence of a shared ancestral mechanism. Both involve adaptation and molecular reorganization [79, 85, 89], but evolutionary relatedness, present-day physical organization, and cancer-cell fitness are separate propositions. The mutation-buffering hypothesis concerns dynamics preserving outputs despite sequence changes, with IDP-containing condensates as a central example; structured protein and nucleic-acid partners may also

contribute. Reversible cell-state plasticity without a mutation is not itself evidence for that mechanism. The added cancer question is whether condensate-dependent spatial coupling explains phenotypic variation beyond measured genotype and bulk pathway activity. Sections 9 and 10.1 make that question experimentally discriminable; it is not an assertion that existing cancer models cannot explain heterogeneity.

## 9. Galectins, metastasis, and condensate-based regulation of cancer phenotypes

The relationship between cellular organization, metabolism, and disease progression extends beyond mitochondria to signaling networks, as seen in hubs such as mechanistic target of rapamycin (mTOR) [96]. Among the proteins that illustrate this connection are galectins, a family of carbohydrate-binding proteins that regulate cell adhesion, extracellular matrix interactions, immune signaling, and metastatic progression [97, 98]. Galectin-3 is a multifunctional β-galactoside-binding lectin capable of forming higher-order assemblies through its carbohydrate recognition domain (CRD) and intrinsically disordered N-terminal domain (NTD) via LLPS [99]. These features allow galectin-3 to participate in multivalent interactions and to act as a regulator of LLPS-associated processes across cellular compartments [99, 100].

Galectin-3 has several properties associated with condensate-forming proteins: intrinsic disorder, oligomerization capacity, and the ability to interact with diverse partners [99, 101]. Through these, galectin-3 binding to *N*-glycans on glycoproteins can influence the organization of extracellular and intracellular signaling environments [38, 102]. In metastatic cancer, this contributes to changes in cell adhesion, migration, invasion, and communication between tumor cells and the microenvironment [38, 103].

From an evolutionary perspective, complex multicellular organisms required new mechanisms for organizing cells into functional tissues [104]. In the same way that mitochondria provided an energetic foundation for increased cellular complexity [45, 57], proteins capable of coordinating extracellular and intracellular organization would have enabled specialized tissue architectures through adhesion and matrix remodeling [102, 104]. Galectin-mediated interactions may be one such system, allowing cells to communicate with their environment and integrate signaling inside and outside the cell [99, 100]. Galectin-3 has been mapped to functional glycoprotein counterreceptors during syncytialization [105] and promotes cytotrophoblast fusion during implantation [106], and it modulates epithelial adaptation to stress at the endoplasmic reticulum (ER)–mitochondria interface [107].

The hexosamine biosynthetic pathway (HBP) provides uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) for glycosylation and links nutrient availability to signaling [108–111]. ER–mitochondrial coordination, calcium homeostasis, protein quality control, and galectin-3-associated stress responses provide related but distinct components [107, 112]. Changes in glycan branching can alter extracellular galectin binding; intracellular *O*-GlcNAcylation is a separate modification system, not an interaction directly mediated by extracellular galectin lattices [109, 110]. Calcium-dependent ER function and metabolic signaling could connect these processes, but a single causal HBP–calcium–galectin–LLPS circuit has not been established here. Plant work illustrates pathway interconnection in a different biological context and is not direct evidence for human breast cancer [113].

Remodeling of mitochondrial morphology echoes the integration of ancestral α-proteobacteria and has been proposed to represent an inherited mitochondrial defense strategy against cellular stress [114]. These morphologies allow mitochondrial structure to favor ATP production for survival or biosynthesis for proliferation and invasion [87, 88]. IDPs that undergo spontaneous LLPS have been associated with bacterial cell-pole

organization [115] and with mitochondrial morphology [65], which is consistent with a role for this protein class across the evolutionary interval in question.

During tumorigenesis, galectin-mediated integration of hexosamine flux can be exploited to support malignant progression [109, 111]. Metastatic cancer cells must overcome physical barriers, reorganize interactions with cytoskeletal and extracellular matrix proteins, and adapt to new microenvironments [38]. Galectin-3 contributes by regulating adhesion molecules, matrix interactions, and pathways associated with invasion and survival [102, 103, 116, 117].

The relationship between LLPS and galectin-3 biology suggests one mechanism by which cancer cells generate flexible signaling environments [99, 118]. Condensate formation can concentrate signaling proteins locally and alter their interactions [39]. Galectin-3 combines structured and disordered regions and may organize context-dependent signaling networks [101, 118, 119]. It therefore exemplifies the proposed continuum: an IDR can support compartmentalized dynamics while a folded recognition domain retains its own conformational motions. Whether either contribution buffers specific mutations is a separate question from whether the assembly changes signaling or morphology.

This parallels the evolutionary role proposed for LLPS during eukaryogenesis [1, 49]. In both cases, dynamic molecular organization would enable adaptation: during evolution, by facilitating integration of bacterial and archaeal components [3, 49]; during cancer progression, by allowing tumor cells to adapt to environmental stress, alter metabolism, and acquire invasive properties [38, 39]. Neutral evolution has been identified across cancer types and is one source of intratumoral heterogeneity [95].

Breast cancer offers a context for testing this distinction [38, 120]. Cell fusion and plasticity can contribute to tumor heterogeneity [121, 122], but neither establishes LLPS. The proposed cross-compartment model concerns coupling among extracellular galectin–glycan/receptor organization, cytoplasmic signaling and RNA/stress assemblies, and nuclear transcriptional assemblies [99–102, 116, 118]. Galectin-3 need not physically span these compartments or use the same partners in each. Extracellular lattices must also be distinguished from genuine phase-separated liquids. A change in one compartment could alter concentration thresholds or residence times in another through receptor signaling, trafficking, or transcription; coordinated coupling is the hypothesis, not a demonstrated universal property of galectin-3.

Primary cancer studies show why spatial organization merits testing. Tulpule et al. showed that ALK/RET fusion-protein granules organize cytoplasmic RAS signaling [123]; some granules were predominantly solid-like, so the finding does not establish simple liquid droplets in every case. Klein et al. demonstrated partitioning of therapeutics into selected nuclear condensates [124]. These studies provide direct evidence for particular spatial mechanisms, not proof that the proposed breast-cancer model explains adaptive resistance or metastasis. The additional hypothesis is that reversible, concentration-dependent coupling across compartments contributes to different cell states despite similar genotypes or bulk pathway output. Altered drug partitioning or residence time could support reversible drug tolerance, while receptor/matrix and cytoskeletal organization could support invasion. These remain distinguishable hypotheses, and invasion is not itself evidence of successful metastasis.

**Figure 2a–h** supplies previously published qualitative illustrations of MDA-MB-231 morphology under sucrose-associated hyperosmotic stress plus serum withdrawal versus serum withdrawal alone, alongside swainsonine (**Figure 2a,b**) and β-lactose (**Figure 2e,f**) perturbation controls [38]. Separate preparations show cytoskeletal organization or galectin-3 staining. Enlarged multinucleated cells remain PGCC-like: morphology and 4′,6-diamidino-2-phenylindole (DAPI) do not establish ploidy, fusion origin, or mitochondrial mass. Cell spreading,

actin organization, and staining differences are qualitative observations, not measurements of adhesion turnover or ATP flux.

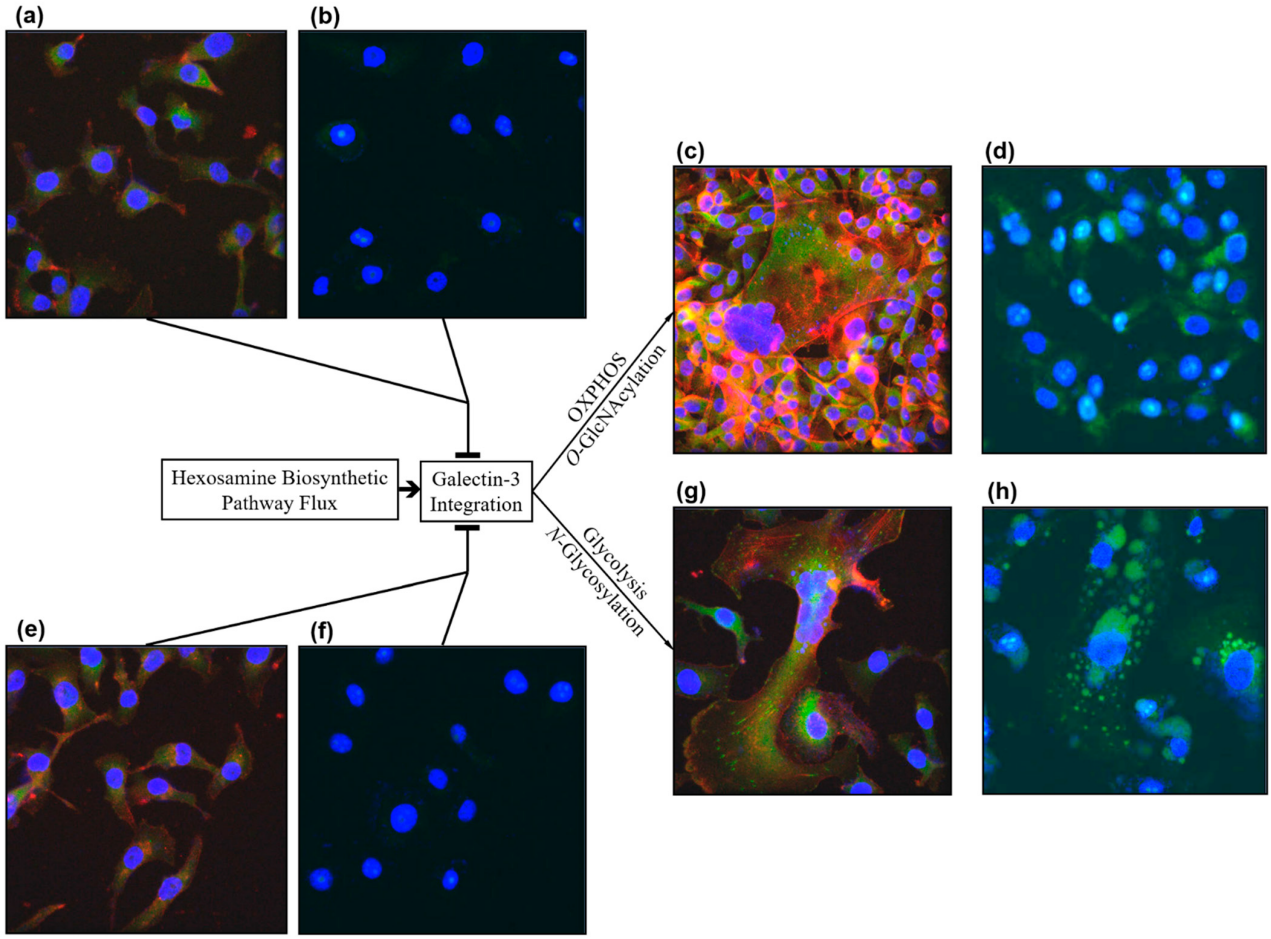


**Figure 2.** Qualitative morphology and galectin-3 staining with glycan-processing and galectin-binding controls. Previously published MDA-MB-231 preparations after four days [38]. (**a**,**b**) Swainsonine; (**c**,**d**) 200 mM sucrose plus serum withdrawal and 10 ng/mL EGF; (**e**,**f**) β-lactose; (**g**,**h**) serum withdrawal and 10 ng/mL EGF without added sucrose. (**a**,**c**,**e**,**g**) Vinculin (green), F-actin (red), and DAPI (blue). (**b**,**d**,**f**,**h**) Galectin-3 (green) and DAPI (blue), in separate parallel preparations. DAPI is a blue nuclear counterstain in all panels. Swainsonine alters complex *N*-glycan processing through Golgi α-mannosidase II inhibition; β-lactose competitively inhibits galectin–glycan binding, not glycosylation. The controls provide qualitative perturbation comparisons supporting a glycan-dependent contribution to galectin-3-associated organization, not galectin-3-specific or condensation-specific proof. Cell spreading, actin organization, and staining differences are illustrative and unquantified. The schematic arrows and inhibition bars depict proposed regulatory relationships, not measured pathway flux, OXPHOS/glycolysis, or *O*-GlcNAcylation. These images do not establish ploidy, fusion, LLPS, mitochondrial mass, ATP compensation, or adhesion turnover. See Grazier and Sylvester [38] for original methods and treatment details. Micrographs were acquired at 40–63× magnification to include complete cellular morphology. Adapted from [38]. EGF: epidermal growth factor; DAPI: 4′,6-diamidino-2-phenylindole; OXPHOS: oxidative phosphorylation; *O*-GlcNAcylation: *O*-linked *N*-acetylglucosamine modification; LLPS: liquid–liquid phase separation; ATP: adenosine triphosphate.

The swainsonine and β-lactose panels provide perturbation-based context for regulation of galectin-3-associated cellular organization [38]. Swainsonine inhibits Golgi α-mannosidase II-dependent complex *N*-glycan

processing [125]; β-lactose competitively inhibits galectin–glycan binding, not glycosylation. Differences between treated and inhibitor-free preparations provide qualitative support for a glycan-dependent component of "galectin-3 integration"—the proposed coupling of galectin–glycan interactions to cytoskeletal and adhesion organization. These comparisons add perturbation context but do not quantify effect sizes or establish a galectin-3-specific mechanism. Both treatments have broader effects, and the displayed conditions are not a complete factorial or osmolarity-matched design. They do not isolate condensation or establish cross-compartment coupling; those claims require the selective perturbation/rescue and quantitative assays in Section 10.1.

A proposed energetic explanation is that hyperosmotic stress changes glucose handling and glycolytic ATP supply, with compensatory OXPHOS and altered adhesion dynamics. Neither this sequence, absence of extracellular sucrase activity, sucrose utilization, nor galectin-3-dependent adhesion turnover was measured by the images. These possibilities require metabolic flux/ATP assays, appropriate osmotic and nutrient controls, live adhesion measurements, and galectin-3 perturbation/rescue. They must not be read as results of **Figure 2** [38, 85, 102, 116].

Cancer progression can therefore be understood as a disruption of organizational principles that are otherwise adaptive. Mechanisms that supported biological complexity and survival can be redirected toward uncontrolled growth and invasion [39, 76]. Examining LLPS, IDPs, mitochondrial remodeling, and galectin-mediated organization together may reveal shared molecular principles underlying both evolutionary innovation and pathological transformation [1, 38, 39, 66].

## 10. A comparative hypothesis: LLPS as a candidate organizer of evolutionary and disease states

The origin of eukaryotic life and the development of cancer are different biological processes, but both involve cellular adaptation, molecular reorganization, and the emergence of new phenotypic states, and both have neutral components [22, 95]. The transition from prokaryotes to eukaryotes required integration of previously independent biological systems; tumor progression involves reorganization of existing cellular regulation. In both, dynamic molecular organization is required [126].

LLPS is proposed here as one scaffold that could mediate these processes [1, 126]. During early eukaryogenesis, condensate-based organization may have enabled the coexistence and integration of archaeal and bacterial components [49]. IDP–nucleic-acid assemblies could compartmentalize dynamic interactions within functional networks [25]. The central hypothesis is that those dynamics buffer some mutational effects, with the greatest opportunity expected in broadly accessible disordered ensembles under matched conditions. Structured proteins and nucleic acids remain capable candidates through more constrained but still adaptable conformations; the proposed difference is probabilistic, not categorical.

The mitochondrial endosymbiotic event illustrates the proposal [28–30]. Incorporation of an α-proteobacterium-derived organelle required coordination of metabolism, membrane structure, and genetic information [40, 41]. MIC60 and other MICOS components show how ancient bacterial systems were transformed into mitochondrial structures [30], and the persistence of dynamic regulatory mechanisms suggests that flexibility mattered for the transition [47].

Following mitochondrial integration, increased energetic capacity has been linked to expansion in eukaryotic size, complexity, and ecological diversity [45, 57]. OXPHOS supplied the energetic foundation for cellular innovation;

IDP-mediated organization is proposed here as part of the regulatory framework for coordinating the resulting molecular systems [23, 25].

The same classes of physical interaction can be relevant to adaptation and disease without implying a single conserved causal pathway [39, 92]. Cancer metabolism and condensate-associated regulation provide contemporary contexts [85, 93, 94], and PGCC plasticity illustrates additional diversity [89, 90]. Neutral tumor-evolution patterns [95] do not establish condensate-mediated buffering, and somatic selection remains active whenever states affect fitness [79]. The comparative hypothesis adds dynamic scaffolding as a measurable spatial variable, not a replacement for genomic or signaling mechanisms.

## 10.1. What is established, what is proposed, and what would falsify the model

The claims in this article are not all of the same status, and it is worth separating them.

Direct contemporary experimental evidence: Primary studies characterize phase behavior or condensate-associated function in the nucleolus [60], heterochromatin [32], transcriptional assemblies [31, 59], reconstituted chromatin [58], mitochondrial nucleoids/transcriptional systems [62, 63], and selected prion-like domains [33, 34]. Each finding is system- and assay-specific; microscopy, rapid exchange, or fluorescence recovery after photobleaching (FRAP) alone is not a diagnosis of LLPS [10, 11]. *C. elegans* LARP-1/MATO experiments support localized translation and mitochondrial homeostasis [47]. Galectin-3 studies directly test phase behavior under specified biochemical or cellular conditions [99, 101, 118]. Engineered human mitochondrial condensates establish geometry-dependent coarsening constraints [72]. ALK/RET granules and nuclear drug partitioning establish particular cancer spatial mechanisms [123, 124]. None is direct evidence for ancient endosymbiotic condensate buffering.

Comparative/evolutionary inference: Bacterial MIC60 homology and function support continuity in membrane organization [28–30]; proteome comparisons support relative disorder enrichment [53]; gene transfer and genome reduction are documented but distinct outcomes [36, 37]. IDR feature conservation supports constrained molecular properties despite sequence divergence [35, 54]. The broader proposal is dynamics-mediated mutation buffering, with IDPs and flexible nucleic-acid regions offering the greatest buffering opportunity and LLPS compartmentalizing selected interactions. The comparative advantage and nucleic-acid extension remain hypotheses; protein feature conservation does not establish them. Structured proteins can accommodate mutation-dependent conformational changes [17, 18], but neither those examples nor condensation establish universal functional rescue or fitness neutrality. Connecting these observations to ancient endosymbiotic buffering remains a historical hypothesis.

Illustration and proposed disease mechanism: **Figure 2** includes previously published swainsonine and β-lactose perturbation comparisons that support a glycan-dependent component of galectin-3-associated morphology. This is qualitative perturbation evidence, not a quantitative demonstration of galectin-3 integration, condensation, ATP compensation, or adhesion turnover. The controls are not galectin-3-specific and do not establish cross-compartment coupling. Cross-compartment galectin-dependent coupling, reversible tolerance, and invasion remain hypotheses to test separately. The endosymbiosis–cancer parallel is heuristic: it may suggest useful experiments but does not uniquely predict them or prove a shared evolutionary mechanism.

Contested or untested physical extrapolation: Water models and local-structure studies provide support for aspects of two-state behavior [12–15], including Palmer et al.'s metastable ST2 transition [15], whereas Chandler critiques that interpretation [16]. Assigning HD/LD contributions and relative water ordering to cellular

condensate material states is an additional untested extrapolation. **Figure 1a**'s most ordered network in the fibrous state illustrates that proposal, not a measured hydration structure or a compulsory maturation sequence. The biological hypothesis in Sections 2–9 does not require it. Alternative assembly mechanisms must remain in consideration when evaluating LLPS [10, 11].

Operational genomic tests and failure criteria: Section 4 specifies units, response variables, comparisons, and confounders for three genomic predictions and matched functional tests. Absence of feature-preserving relative sequence divergence in validated condensate IDRs would weaken the predicted disorder-associated advantage; a null/opposite controlled A/T–disorder relationship would reject that association; and no incremental disorder-associated EGT signal would weaken transfer bias. A matched mutational comparison finding equal or greater buffering in structured molecules would limit the proposed probability ranking without excluding dynamics-mediated buffering. An absent LLPS-dependent increment after validated assembly-selective perturbation would limit the compartmentalization claim. Alignment limitations, unresolved ancient order, and predictor error can make tests inconclusive. Ordinary purifying selection in IDRs would not alone falsify LLPS-mediated organization, and positive associations would not uniquely establish its ancient role.

A discriminating cancer experiment: In lineage-matched or isogenic MDA-MB-231 cells, perturb experimentally validated galectin-3 condensation determinants while matching expression and assessing CRD glycan binding, secretion, localization, and relevant partner interactions. Compare wild-type rescue, condensation-impaired rescue, and compartment-restricted rescue, supported by an orthogonal perturbation with distinct off-target risks. First establish concentration-dependent assembly/partitioning, reversibility, and material properties; FRAP or 1,6-hexanediol sensitivity alone is insufficient. If separation-of-function controls cannot be validated, the perturbation cannot isolate condensation.

In the same tracked cells, quantify extracellular receptor clustering/residence and fibronectin organization, cytoplasmic signaling/stress assemblies, and nuclear transcriptional assembly states or transcriptional output. Measure respiration and ATP flux separately rather than infer them from morphology. Compare spatial state coupling and cell-to-cell heterogeneity after matching bulk pathway activity, receptor abundance, and genotype. Follow drug survival through treatment and washout to distinguish reversible tolerance from stable resistance, and measure invasion separately from metastatic competence. Include viability, cell-cycle/ploidy, glycan-binding, and osmolarity controls, with independent biological replicates and prespecified single-cell endpoints.

The predicted added value is coordinated, reversible, threshold-dependent state changes across compartments, with restoration by the appropriate condensation-competent/compartmental rescue despite comparable bulk signaling. Thresholds and heterogeneity alone are not unique to LLPS. If validated condensation-selective perturbations do not alter the proposed coupling, or if all differences disappear after accounting for ordinary signaling and toxicity, this spatial hypothesis gains no explanatory support in that setting. Standard cancer biology can also motivate this experiment; the analogy cannot honestly supply an experiment uniquely predicted only by an evolutionary synthesis. Even a positive result would support a contemporary organizing mechanism, not demonstrate ancient endosymbiosis or a universal mechanism of metastasis.

Importantly, this framework does not claim that LLPS alone causes evolution or cancer. It claims that LLPS provides an organizational mechanism influencing how biological systems assemble, adapt, and respond to environmental pressure—a possible bridge between genetic information and cellular architecture, and a possible substrate for reprogramming disease states [126].

## 11. Conclusions

The emergence of eukaryotic life required integration of genetic, metabolic, and spatial organization. Mitochondrial acquisition expanded metabolic possibilities, although the extent of its energetic contribution to complexity remains debated. The central proposal is that molecular dynamics can buffer mutations by preserving outputs while conformations and interactions reorganize. IDPs and conformationally heterogeneous nucleic-acid regions are proposed to offer the greatest opportunity for such buffering, with LLPS compartmentalizing selected dynamic interactions. Structured proteins and nucleic acids can also buffer some substitutions through local flexibility and alternative conformations. IDPs are the main example, not an exclusive category; the proposed advantage requires matched tests rather than an assumed universal ranking.

The evolutionary history of mitochondria shows that biological complexity often emerges through integration of existing systems rather than creation of entirely new components. MICOS, MIC60, mitochondrial translation systems, and membrane-organizing proteins point to continuity between bacterial ancestors and modern eukaryotic organelles.

Cancer provides a separate setting in which these organizational ideas can be tested. Tumor cells can combine metabolic adaptation, mitochondrial plasticity, and condensate-associated regulation with genomic and signaling changes. Galectin-3 is a candidate context-dependent organizer, not proof that disruption of ancestral LLPS causes cancer. The proposed cross-compartment tests ask whether spatial coupling adds explanatory value for heterogeneity, reversible drug tolerance, and invasion beyond established pathway effects.

Together these observations motivate a testable hypothesis linking molecular organization, evolutionary integration, and disease by comparison rather than an established shared mechanism. The three genomic predictions and matched functional comparisons in Section 4, together with the perturbation/rescue experiment in Section 10.1, define where its components can be supported, limited, or left unresolved. Dynamics-mediated mutation buffering and LLPS-dependent compartmentalization are separable claims: neither disorder nor condensation alone demonstrates retained output or fitness neutrality. Such dynamic scaffolding may have contributed to complexity and may contribute to tumor-cell plasticity; neither historical contribution nor general disease causality is demonstrated by this synthesis.

## Acknowledgments

I sincerely thank Paul W. Sylvester and the University of Louisiana Monroe School of Pharmacy for providing the mentorship, equipment, materials, and research facilities that enabled me to develop as an independent scientist. The author acknowledges the use of the AI tool Academia Co-scientist (underlying model GPT-6.1 Sol) for language editing, as well as for assembly of reference metadata and generation of code for schematic illustration and structural visualization in **Figure 1**, as declared in the Introduction section. The tool was applied to enhance clarity and scholarly tone, and all edits were carefully reviewed and validated by the author to ensure accuracy, compliance with academic standards, and research integrity. The author fully supports Academia.edu Journals' adherence to COPE guidelines on AI in publication ethics and confirms that this use has been managed responsibly and ethically.

## Funding

This research received no external funding.

## Conflict of interest

The author declares that they have no competing interests.

## Data availability statement

No new data was created or analyzed in this study. Data sharing is not applicable to this article.

## Publisher's note

Academia.edu Journals stays neutral regarding jurisdictional claims in published maps and institutional affiliations. All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Copyright

© 2026 copyright by the author. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

## References

1. Grazier JJ, Sylvester PW. Liquid–liquid phase separation as a driver of abiogenesis and evolution. Acad Mol Biol Genom. 2026; 3 (1). doi: 10.20935/acadmolbiogen8175
2. Xu Z, Wang W, Cao Y, Xue B. Liquid-liquid phase separation: fundamental physical principles, biological implications, and applications in supramolecular materials engineering. Supramol Mater. 2023; 2: 100049. doi: 10.1016/j.supmat.2023.100049
3. Roger AJ, Muñoz-Gómez SA, Kamikawa R. The origin and diversification of mitochondria. Curr Biol. 2017; 27 (21): R1177–92. doi: 10.1016/j.cub.2017.09.015
4. Vosseberg J, van Hooff JJE, Köstlbacher S, Panagiotou K, Tamarit D, Ettema TJG. The emerging view on the origin and early evolution of eukaryotic cells. Nature. 2024; 633 (8029): 295–305. doi: 10.1038/s41586-024-07677-6
5. Muñoz-Gómez SA, Susko E, Williamson K, Eme L, Slamovits CH, Moreira D, et al. Site-and-branch-heterogeneous analyses of an expanded dataset favour mitochondria as sister to known Alphaproteobacteria. Nat Ecol Evol. 2022; 6 (3): 253–62. doi: 10.1038/s41559-021-01638-2
6. Xia S, Chen J, Arsala D, Emerson JJ, Long M. Functional innovation through new genes as a general evolutionary process. Nat Genet. 2025; 57 (2): 295–309. doi: 10.1038/s41588-024-02059-0

7. Grazier JJ, Sylvester PW. Chaos to coherence: a path to molecular organization. In: Ashrafuzzaman M, Tuszynski JA, editors. Quantum mechanics of biological systems. Boca Raton, FL, USA: CRC Press; 2026. p. 66–84. doi: 10.1201/9781003455714-4
8. Antifeeva IA, Fonin AV, Fefilova AS, Stepanenko OV, Povarova OI, Silonov SA, et al. Liquid–liquid phase separation as an organizing principle of intracellular space: overview of the evolution of the cell compartmentalization concept. Cell Mol Life Sci. 2022; 79 (5): 251. doi: 10.1007/s00018-022-04276-4
9. Spruijt E. Open questions on liquid–liquid phase separation. Commun Chem. 2023; 6 (1): 23. doi: 10.1038/s42004-023-00823-7
10. Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid–liquid phase separation and biomolecular condensates. Cell. 2019; 176 (3): 419–34. doi: 10.1016/j.cell.2018.12.035
11. McSwiggen DT, Mir M, Darzacq X, Tjian R. Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. Genes Dev. 2019; 33 (23–4): 1619–34. doi: 10.1101/gad.331520.119
12. Li L, Zhong J, Zhang J, Wang Z, Zeng XC. Evidence for the generic existence of two local structures in liquid water. Nat Phys. 2026; 22 (7): 1144–51. doi: 10.1038/s41567-026-03301-8
13. Debenedetti PG, Sciortino F, Zerze GH. Second critical point in two realistic models of water. Science. 2020; 369 (6501): 289–92. doi: 10.1126/science.abb9796
14. Nilsson A, Pettersson LGM. The structural origin of anomalous properties of liquid water. Nat Commun. 2015; 6 (1): 8998. doi: 10.1038/ncomms9998
15. Palmer JC, Martelli F, Liu Y, Car R, Panagiotopoulos AZ, Debenedetti PG. Metastable liquid–liquid transition in a molecular model of water. Nature. 2014; 510 (7505): 385–8. doi: 10.1038/nature13405
16. Chandler D. Metastability and no criticality. Nature. 2016; 531 (7593): E1–2. doi: 10.1038/nature16539
17. Tokuriki N, Tawfik DS. Protein dynamism and evolvability. Science. 2009; 324 (5924): 203–7. doi: 10.1126/science.1169375
18. Campbell E, Kaltenbach M, Correy GJ, Carr PD, Porebski BT, Livingstone EK, et al. The role of protein dynamics in the evolution of new enzyme function. Nat Chem Biol. 2016; 12 (11): 944–50. doi: 10.1038/nchembio.2175
19. Yusuf A, Usman A, Isah MB, Dang M, Zhang X. Liquid–liquid phase separation in microorganisms: insights into existence, functions, and applications. Microbiol Res. 2025; 292: 128026. doi: 10.1016/j.micres.2024.128026
20. Rodriguez CB, Tunque Cahui RR, Demitroff N, Hirsh L, Devos DP, Boccaccio G, et al. BAV-LLPS: a database of bacterial, archaea and virus liquid–liquid phase separation proteins. Bioinformatics. 2025; 41 (10): btaf550. doi: 10.1093/bioinformatics/btaf550
21. Liu D, Yang J, Cristea IM. Liquid–liquid phase separation in innate immunity. Trends Immunol. 2024; 45 (6): 454–69. doi: 10.1016/j.it.2024.04.009
22. Muñoz-Gómez SA, Bilolikar G, Wideman JG, Geiler-Samerotte K. Constructive neutral evolution 20 years later. J Mol Evol. 2021; 89 (3): 172–82. doi: 10.1007/s00239-021-09996-y
23. Kulkarni P, Bhattacharya S, Achuthan S, Behal A, Jolly MK, Kotnala S, et al. Intrinsically disordered proteins: critical components of the wetware. Chem Rev. 2022; 122 (6): 6614–33. doi: 10.1021/acs.chemrev.1c00848
24. Uversky VN. Intrinsically disordered proteins. Cham, Switzerland: Springer International Publishing; 2014. doi: 10.1007/978-3-319-08921-8
25. Holehouse AS, Alberti S. Molecular determinants of condensate composition. Mol Cell. 2025; 85 (2): 290–308. doi: 10.1016/j.molcel.2024.12.021
26. Frost SDW, Magalis BR, Kosakovsky Pond SL. Neutral theory and rapidly evolving viral pathogens. Mol Biol Evol. 2018; 35 (6): 1348–54. doi: 10.1093/molbev/msy088

27. Fukuchi S, Noguchi T, Anbo H, Homma K. Exon elongation added intrinsically disordered regions to the encoded proteins and facilitated the emergence of the last eukaryotic common ancestor. Mol Biol Evol. 2023; 40 (1): msac272. doi: 10.1093/molbev/msac272
28. Muñoz-Gómez SA, Slamovits CH, Dacks JB, Baier KA, Spencer KD, Wideman JG. Ancient homology of the mitochondrial contact site and cristae organizing system points to an endosymbiotic origin of mitochondrial cristae. Curr Biol. 2015; 25 (11): 1489–95. doi: 10.1016/j.cub.2015.04.006
29. Muñoz-Gómez SA, Wideman JG, Roger AJ, Slamovits CH. The origin of mitochondrial cristae from alphaproteobacteria. Mol Biol Evol. 2017; 34 (4): 943–56. doi: 10.1093/molbev/msw298
30. Muñoz-Gómez SA, Cadena LR, Gardiner AT, Leger MM, Sheikh S, Connell LB, et al. Intracytoplasmic-membrane development in alphaproteobacteria involves the homolog of the mitochondrial crista-developing protein Mic60. Curr Biol. 2023; 33 (6): 1099–111.e6. doi: 10.1016/j.cub.2023.02.059
31. Sabari BR, Dall'Agnese A, Boija A, Klein IA, Coffey EL, Shrinivas K, et al. Coactivator condensation at super-enhancers links phase separation and gene control. Science. 2018; 361 (6400): eaar3958. doi: 10.1126/science.aar3958
32. Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH. Phase separation drives heterochromatin domain formation. Nature. 2017; 547 (7662): 241–5. doi: 10.1038/nature22989
33. Wang J, Choi JM, Holehouse AS, Lee HO, Zhang X, Jahnel M, et al. A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. Cell. 2018; 174 (3): 688–99.e16. doi: 10.1016/j.cell.2018.06.006
34. Martin EW, Holehouse AS, Peran I, Farag M, Incicco JJ, Bremer A, et al. Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. Science. 2020; 367 (6478): 694–9. doi: 10.1126/science.aaw8653
35. Zarin T, Strome B, Nguyen Ba AN, Alberti S, Forman-Kay JD, Moses AM. Proteome-wide signatures of function in highly diverged intrinsically disordered regions. eLife. 2019; 8: e46883. doi: 10.7554/elife.46883
36. Timmis JN, Ayliffe MA, Huang CY, Martin W. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet. 2004; 5 (2): 123–35. doi: 10.1038/nrg1271
37. McCutcheon JP, Moran NA. Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol. 2012; 10 (1): 13–26. doi: 10.1038/nrmicro2670
38. Grazier JJ, Sylvester PW. Role of galectins in metastatic breast cancer. In: Mayrovitz HN, editor. Breast cancer. Brisbane, Australia: Exon Publications; 2022. p. 115–30. doi: 10.36255/exon-publications-breast-cancer-galectins
39. Mehta S, Zhang J. Liquid–liquid phase separation drives cellular function and dysfunction in cancer. Nat Rev Cancer. 2022; 22 (4): 239–52. doi: 10.1038/s41568-022-00444-7
40. von Känel C, Muñoz-Gómez SA, Oeljeklaus S, Wenger C, Warscheid B, Wideman JG, et al. Homologue replacement in the import motor of the mitochondrial inner membrane of trypanosomes. eLife. 2020; 9: e52560. doi: 10.7554/elife.52560
41. Gabaldón T, Huynen MA. From endosymbiont to host-controlled organelle: the hijacking of mitochondrial protein synthesis and metabolism. PLoS Comput Biol. 2007; 3 (11): e219. doi: 10.1371/journal.pcbi.0030219
42. Chen L, Zhuang Z, Fan Y, Jiang C, Sun T. Liquid–liquid phase separation mediated order and pathological chaos: from cellular organization to precision modulating. J Control Release. 2026; 390: 114516. doi: 10.1016/j.jconrel.2025.114516
43. Macher JN, Coots NL, Poh YP, Girard EB, Langerak A, Muñoz-Gómez SA, et al. Single-cell genomics reveals the divergent mitochondrial genomes of Retaria (Foraminifera and Radiolaria). mBio. 2023; 14 (2): e00302-23. doi: 10.1128/mbio.00302-23

44. Kumar A, Gok MO, Nguyen KN, Connor OM, Reese ML, Wideman JG, et al. A dynamin superfamily-like pseudoenzyme coordinates with MICOS to promote cristae architecture. Curr Biol. 2024; 34 (12): 2606–22.e9. doi: 10.1016/j.cub.2024.04.028
45. Schavemaker PE, Muñoz-Gómez SA. The role of mitochondrial energetics in the origin and diversification of eukaryotes. Nat Ecol Evol. 2022; 6 (9): 1307–17. doi: 10.1038/s41559-022-01833-9
46. Bennett GM, Kwak Y, Maynard R. Endosymbioses have shaped the evolution of biological diversity and complexity time and time again. Genome Biol Evol. 2024; 16 (6): evae112. doi: 10.1093/gbe/evae112
47. Bai Y, Ma T, Zhao S, Li S, Wang X, Li J, et al. Mitochondria-associated condensates maintain mitochondrial homeostasis and promote lifespan. Nat Aging. 2025; 5 (10): 1983–2002. doi: 10.1038/s43587-025-00942-x
48. Liu W, Tian L, Xie J, Wu L, Hu L, Ge R, et al. Liquid–liquid phase separation in metabolic engineering: mechanistic insights, emerging applications, and future challenges. Biotechnol Adv. 2026; 91: 108931. doi: 10.1016/j.biotechadv.2026.108931
49. Calcagnile M, Alifano P, Damiano F, Pontieri P, Del Giudice L. A perspective on the role of mitochondrial biomolecular condensates (mtBCs) in neurodegenerative diseases and evolutionary links to bacterial BCs. Int J Mol Sci. 2025; 26 (17): 8216. doi: 10.3390/ijms26178216
50. Maurya AK, Kröninger L, Ehret G, Bäumers M, Marson M, Scheu S, et al. A nucleus-encoded dynamin-like protein controls endosymbiont division in the trypanosomatid *Angomonas deanei*. Sci Adv. 2025; 11 (12): eadp8518. doi: 10.1126/sciadv.adp8518
51. Richards TA, Eme L, Archibald JM, Leonard G, Coelho SM, de Mendoza A, et al. Reconstructing the last common ancestor of all eukaryotes. PLoS Biol. 2024; 22 (11): e3002917. doi: 10.1371/journal.pbio.3002917
52. Lynch M, Conery JS. The origins of genome complexity. Science. 2003; 302 (5649): 1401–4. doi: 10.1126/science.1089370
53. Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF, Jones DT. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J Mol Biol. 2004; 337 (3): 635–45. doi: 10.1016/j.jmb.2004.02.002
54. Singleton MD, Eisen MB. Evolutionary analyses of intrinsically disordered regions reveal widespread signals of conservation. PLoS Comput Biol. 2024; 20 (4): e1012028. doi: 10.1371/journal.pcbi.1012028
55. Muñoz-Gómez SA, Kreutz M, Hess S. A microbial eukaryote with a unique combination of purple bacteria and green algae as endosymbionts. Sci Adv. 2021; 7 (24): eabg4102. doi: 10.1126/sciadv.abg4102
56. Fenton AR, Jongens TA, Holzbaur ELF. Mitochondrial dynamics: shaping and remodeling an organelle network. Curr Opin Cell Biol. 2021; 68: 28–36. doi: 10.1016/j.ceb.2020.08.014
57. Muñoz-Gómez SA. Energetics and evolution of anaerobic microbial eukaryotes. Nat Microbiol. 2023; 8 (2): 197–203. doi: 10.1038/s41564-022-01299-2
58. Gibson BA, Doolittle LK, Schneider MWG, Jensen LE, Gamarra N, Henry L, et al. Organization of chromatin by intrinsic and regulated phase separation. Cell. 2019; 179 (2): 470–84.e21. doi: 10.1016/j.cell.2019.08.037
59. Boija A, Klein IA, Sabari BR, Dall'Agnese A, Coffey EL, Zamudio AV, et al. Transcription factors activate genes through the phase-separation capacity of their activation domains. Cell. 2018; 175 (7): 1842–55.e16. doi: 10.1016/j.cell.2018.10.042
60. Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, Richardson TM, et al. Coexisting liquid phases underlie nucleolar subcompartments. Cell. 2016; 165 (7): 1686–97. doi: 10.1016/j.cell.2016.04.047
61. Maccaroni K, La Torre M, Burla R, Saggio I. Phase separation in the nucleus and at the nuclear periphery during post-mitotic nuclear envelope reformation. Cells. 2022; 11 (11): 1749. doi: 10.3390/cells11111749
62. Feric M, Demarest TG, Tian J, Croteau DL, Bohr VA, Misteli T. Self-assembly of multi-component mitochondrial nucleoids via phase separation. EMBO J. 2021; 40 (6): e107165. doi: 10.15252/embj.2020107165

63. Feric M, Sarfallah A, Dar F, Temiakov D, Pappu RV, Misteli T. Mesoscale structure–function relationships in mitochondrial transcriptional condensates. Proc Natl Acad Sci USA. 2022; 119 (41): e2207303119. doi: 10.1073/pnas.2207303119
64. Venkatraman K, Lipp NF, Budin I. Origin and evolution of mitochondrial inner membrane composition. J Cell Sci. 2025; 138 (9): jcs263780. doi: 10.1242/jcs.263780
65. Ito M, Tohsato Y, Sugisawa H, Kohara S, Fukuchi S, Nishikawa I, et al. Intrinsically disordered proteins in human mitochondria. Genes Cells. 2012; 17 (10): 817–25. doi: 10.1111/gtc.12000
66. Gao C, Ding P, Zhang C, Gao J. Phase separation regulates metabolism, mitochondria, and diseases. MedComm. 2025; 6 (7): e70283. doi: 10.1002/mco2.70283
67. Caron C, Bertolin G. Cristae shaping and dynamics in mitochondrial function. J Cell Sci. 2024; 137 (1): jcs260986. doi: 10.1242/jcs.260986
68. Vercellino I, Sazanov LA. The assembly, regulation and function of the mitochondrial respiratory chain. Nat Rev Mol Cell Biol. 2022; 23 (2): 141–61. doi: 10.1038/s41580-021-00415-0
69. Chen Q, An S, Wang C, Zhou Y, Liu X, Ren W. Phase separation in mitochondrial fate and mitochondrial diseases. Proc Natl Acad Sci USA. 2025; 122 (21): e2422255122. doi: 10.1073/pnas.2422255122
70. Holt KB, Winter J, Manley S, Koslover EF. Spatiotemporal modeling of mitochondrial network architecture. PRX Life. 2024; 2 (4): 043002. doi: 10.1103/prxlife.2.043002
71. Pfanner N, Warscheid B, Wiedemann N. Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol. 2019; 20 (5): 267–84. doi: 10.1038/s41580-018-0092-0
72. Aththawala Gedara SVBD, Penna ST, Feric M. Membranes arrest the coarsening of mitochondrial condensates in human cells. Commun Biol. 2026; 9 (1): 867. doi: 10.1038/s42003-026-10085-3
73. Tábara LC, Segawa M, Prudent J. Molecular mechanisms of mitochondrial dynamics. Nat Rev Mol Cell Biol. 2025; 26 (2): 123–46. doi: 10.1038/s41580-024-00785-1
74. Ferree A, Shirihai O. Mitochondrial dynamics: the intersection of form and function. In: Kadenbach B, editor. Mitochondrial oxidative phosphorylation: nuclear-encoded genes, enzyme regulation, and pathophysiology. New York, NY, USA: Springer; 2012. p. 13–40. doi: 10.1007/978-1-4614-3573-0_2
75. Sokolova I. Mitochondrial adaptations to variable environments and their role in animals' stress tolerance. Integr Comp Biol. 2018; 58 (3): 519–31. doi: 10.1093/icb/icy017
76. Hanahan D. Hallmarks of cancer—then and now, and beyond. Cell. 2026; 189 (8): 2254–77. doi: 10.1016/j.cell.2025.12.049
77. Bridges BA. Hypermutation under stress. Nature. 1997; 387 (6633): 557–8. doi: 10.1038/42370
78. Zhang S, Xiao X, Yi Y, Wang X, Zhu L, Shen Y, et al. Tumor initiation and early tumorigenesis: molecular mechanisms and interventional targets. Signal Transduct Target Ther. 2024; 9 (1): 149. doi: 10.1038/s41392-024-01848-7
79. Ciriello G, Magnani L, Aitken SJ, Akkari L, Behjati S, Hanahan D, et al. Cancer evolution: a multifaceted affair. Cancer Discov. 2024; 14 (1): 36–48. doi: 10.1158/2159-8290.cd-23-0530
80. Zhu X, Zhao W, Zhou Z, Gu X. Unraveling the drivers of tumorigenesis in the context of evolution: theoretical models and bioinformatics tools. J Mol Evol. 2023; 91 (4): 405–23. doi: 10.1007/s00239-023-10117-0
81. Wang SF, Tseng LM, Lee HC. Role of mitochondrial alterations in human cancer progression and cancer immunity. J Biomed Sci. 2023; 30 (1): 61. doi: 10.1186/s12929-023-00956-w
82. Atlante A, Valenti D. Mitochondria have made a long evolutionary path from ancient bacteria immigrants within eukaryotic cells to essential cellular hosts and key players in human health and disease. Curr Issues Mol Biol. 2023; 45 (5): 4451–79. doi: 10.3390/cimb45050283

83. Bose S, Zhang C, Le A. Glucose metabolism in cancer: the Warburg effect and beyond. In: Le A, editor. The heterogeneity of cancer metabolism. Cham, Switzerland: Springer International Publishing; 2021. p. 3–15. doi: 10.1007/978-3-030-65768-0_1
84. Jaworska M, Szczudło J, Pietrzyk A, Shah J, Trojan SE, Ostrowska B, et al. The Warburg effect: a score for many instruments in the concert of cancer and cancer niche cells. Pharmacol Rep. 2023; 75 (4): 876–90. doi: 10.1007/s43440-023-00504-1
85. Du H, Xu T, Yu S, Wu S, Zhang J. Mitochondrial metabolism and cancer therapeutic innovation. Signal Transduct Target Ther. 2025; 10 (1): 245. doi: 10.1038/s41392-025-02311-x
86. Fadavi P, Taghizadeh-Hesary F. Mitochondrial metabolism and dynamics in cancer cells. In: Ashique S, Taghizadeh-Hesary F, Debnath B, Madan A, editors. Cancer metabolism: molecular mechanisms and therapeutic strategies. Cham, Switzerland: Springer Nature Switzerland; 2026. p. 109–20. doi: 10.1007/978-3-032-21861-2_6
87. Han M, Bushong EA, Segawa M, Tiard A, Wong A, Brady MR, et al. Spatial mapping of mitochondrial networks and bioenergetics in lung cancer. Nature. 2023; 615 (7953): 712–9. doi: 10.1038/s41586-023-05793-3
88. Ryu KW, Fung TS, Baker DC, Saoi M, Park J, Febres-Aldana CA, et al. Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. Nature. 2024; 635 (8039): 746–54. doi: 10.1038/s41586-024-08146-w
89. Manickasamy MK, Aswani BS, Banerjee R, Abbas M, Alqahtani MS, Sethi G, et al. Etiology of polyploid giant cancer cells: a new frontier in cancer biology. Cancer Cell Int. 2026; 26 (1): 84. doi: 10.1186/s12935-025-04079-0
90. Bowes AL, Waise S, Lesluyes T, Butters T, English C, Yan H, et al. Profiling the genomic landscape and evolutionary history of polyploid giant cancer cells in undifferentiated pleomorphic sarcomas. Cancer Lett. 2026; 639: 218173. doi: 10.1016/j.canlet.2025.218173
91. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. Somatic mutations in early metazoan genes disrupt regulatory links between unicellular and multicellular genes in cancer. eLife. 2019; 8: e40947. doi: 10.7554/elife.40947
92. Tong X, Tang R, Xu J, Wang W, Zhao Y, Yu X, et al. Liquid–liquid phase separation in tumor biology. Signal Transduct Target Ther. 2022; 7 (1): 221. doi: 10.1038/s41392-022-01076-x
93. Zheng LW, Liu CC, Yu KD. Phase separations in oncogenesis, tumor progressions and metastasis: a glance from hallmarks of cancer. J Hematol Oncol. 2023; 16 (1): 123. doi: 10.1186/s13045-023-01522-5
94. Zhao ZH, Li T, Shi ZZ. Liquid–liquid phase separation in cancer: oncogenic roles, therapeutic potential, and epigenetic regulation. Mol Biol Rep. 2026; 53 (1): 663. doi: 10.1007/s11033-026-11842-7
95. Williams MJ, Werner B, Barnes CP, Graham TA, Sottoriva A. Identification of neutral tumor evolution across cancer types. Nat Genet. 2016; 48 (3): 238–44. doi: 10.1038/ng.3489
96. Zhang H, Xiao X, Pan Z, Dokudovskaya S. mTOR signaling networks: mechanistic insights and translational frontiers in disease therapeutics. Signal Transduct Target Ther. 2025; 10 (1): 428. doi: 10.1038/s41392-025-02493-4
97. Verkerke H, Dias-Baruffi M, Cummings RD, Arthur CM, Stowell SR. Galectins: an ancient family of carbohydrate binding proteins with modern functions. In: Stowell SR, Arthur CM, Cummings RD, editors. Galectins: methods and protocols. 2nd ed. New York, NY, USA: Humana; 2022. p. 1–40. doi: 10.1007/978-1-0716-2055-7_1
98. Zhang N, Liu Q, Wang D, Wang X, Pan Z, Han B, et al. Multifaceted roles of galectins: from carbohydrate binding to targeted cancer therapy. Biomark Res. 2025; 13 (1): 49. doi: 10.1186/s40364-025-00759-1

99. Chiu YP, Sun YC, Qiu DC, Lin YH, Chen YQ, Kuo JC, et al. Liquid–liquid phase separation and extracellular multivalent interactions in the tale of galectin-3. Nat Commun. 2020; 11 (1): 1229. doi: 10.1038/s41467-020-15007-3
100. Voss PG, Wang JL. Liquid–liquid phase separation: galectin-3 in nuclear speckles and ribonucleoprotein complexes. Exp Cell Res. 2023; 427 (1): 113571. doi: 10.1016/j.yexcr.2023.113571
101. Zhao Z, Xu X, Cheng H, Miller MC, He Z, Gu H, et al. Galectin-3 N-terminal tail prolines modulate cell activity and glycan-mediated oligomerization/phase separation. Proc Natl Acad Sci USA. 2021; 118 (19): e2021074118. doi: 10.1073/pnas.2021074118
102. Lagana A, Goetz JG, Cheung P, Raz A, Dennis JW, Nabi IR. Galectin binding to Mgat5-modified N-glycans regulates fibronectin matrix remodeling in tumor cells. Mol Cell Biol. 2006; 26 (8): 3181–93. doi: 10.1128/mcb.26.8.3181-3193.2006
103. Ruvolo PP. Galectin 3 as a guardian of the tumor microenvironment. Biochim Biophys Acta Mol Cell Res. 2016; 1863 (3): 427–37. doi: 10.1016/j.bbamcr.2015.08.008
104. Moreno Á, Peretó J. Multicellular agents: the challenge of combining body organization and behavior. In: An evolutionary story of agency. Cham, Switzerland: Springer Nature Switzerland; 2026. p. 57–75. doi: 10.1007/978-3-032-05644-3_5
105. Reeves AE, Yang GS, Baboo S, Diedrich JK, Bedekar P, Farhadi SA, et al. Mapping the placental galectin-3 interactome identifies CD9 and ITGB1 as functional glycoprotein counterreceptors during syncytialization. Proc Natl Acad Sci USA. 2025; 122 (45): e2511042122. doi: 10.1073/pnas.2511042122
106. Ohara T, Horie A, Nakamura M, Imakita S, Ikeda A, Shitanaka S, et al. Galectin-3 in endometrial small extracellular vesicles promotes cytotrophoblast cell fusion during the implantation phase. Cell Commun Signal. 2026; 24 (1): 41. doi: 10.1186/s12964-025-02586-1
107. Coppin L, Jannin A, Ait Yahya E, Thuillier C, Villenet C, Tardivel M, et al. Galectin-3 modulates epithelial cell adaptation to stress at the ER–mitochondria interface. Cell Death Dis. 2020; 11 (5): 360. doi: 10.1038/s41419-020-2556-3
108. Chiaradonna F, Ricciardiello F, Palorini R. The nutrient-sensing hexosamine biosynthetic pathway as the hub of cancer metabolic rewiring. Cells. 2018; 7 (6): 53. doi: 10.3390/cells7060053
109. Mukherjee MM, Biesbrock D, Hanover JA. Galectin-3: integrator of signaling via hexosamine flux. Biomolecules. 2025; 15 (7): 1028. doi: 10.3390/biom15071028
110. Paneque A, Fortus H, Zheng J, Werlen G, Jacinto E. The hexosamine biosynthesis pathway: regulation and function. Genes. 2023; 14 (4): 933. doi: 10.3390/genes14040933
111. Itano N, Iwamoto S. Dysregulation of hexosamine biosynthetic pathway wiring metabolic signaling circuits in cancer. Biochim Biophys Acta Gen Subj. 2023; 1867 (1): 130250. doi: 10.1016/j.bbagen.2022.130250
112. An G, Park J, Song J, Hong T, Song G, Lim W. Relevance of the endoplasmic reticulum–mitochondria axis in cancer diagnosis and therapy. Exp Mol Med. 2024; 56 (1): 40–50. doi: 10.1038/s12276-023-01137-3
113. Chen YH, Cheng WH. Hexosamine biosynthesis and related pathways, protein N-glycosylation and O-GlcNAcylation: their interconnection and role in plants. Front Plant Sci. 2024; 15: 1349064. doi: 10.3389/fpls.2024.1349064
114. Hong W, Long S, Ashrafizadeh M, Sethi G, Duan C. From symbiosis to immunity: the evolutionary revival of mitochondrial defense programs in inflammatory diseases. Cell Commun Signal. 2026; 24 (1): 219. doi: 10.1186/s12964-026-02736-z
115. Aldadah R, Lekic D, Sabanovic K, Kovalenko B, Adilovic M, Hromic-Jahjefendic A, et al. Looking at bacterial cell poles from a liquid–liquid phase separation of intrinsically disordered proteins perspective. Curr Res Struct Biol. 2025; 10: 100175. doi: 10.1016/j.crstbi.2025.100175

116. Boscher C, Nabi IR. Galectin-3- and phospho-caveolin-1-dependent outside-in integrin signaling mediates the EGF motogenic response in mammary cancer cells. Mol Biol Cell. 2013; 24 (13): 2134–45. doi: 10.1091/mbc.e13-02-0095
117. Li S, Pritchard DM, Yu LG. Galectin-3 promotes secretion of proteases that decrease epithelium integrity in human colon cancer cells. Cell Death Dis. 2023; 14 (4): 268. doi: 10.1038/s41419-023-05789-x
118. Sun YC, Hsieh TL, Lin CI, Shao WY, Lin YH, Huang JR. A few charged residues in galectin-3's folded and disordered regions regulate phase separation. Adv Sci. 2024; 11 (41): 2402570. doi: 10.1002/advs.202402570
119. Lupu (Surlea) A, Negreanu-Pirjol T, Olariu L, Negreanu-Pirjol BS, Jurja S, Lepadatu AC, et al. Galectin-3—a multifunctional molecule and a key player in health and disease. Molecules. 2026; 31 (10): 1694. doi: 10.3390/molecules31101694
120. Niang DGM, Gaba FM, Diouf A, Hendricks J, Diallo RN, Niang MDS, et al. Galectin-3 as a biomarker in breast neoplasms: mechanisms and applications in patient care. J Leukoc Biol. 2022; 112 (5): 1041–52. doi: 10.1002/jlb.5mr0822-673r
121. Shultes PV, Weaver DT, Tadele DS, Barker-Clarke RJ, Scott JG. Cell-cell fusion in cancer: the next cancer hallmark? Int J Biochem Cell Biol. 2024; 175: 106649. doi: 10.1016/j.biocel.2024.106649
122. Melzer C, von der Ohe J, Luo T, Hass R. Spontaneous fusion of MSC with breast cancer cells can generate tumor dormancy. Int J Mol Sci. 2021; 22 (11): 5930. doi: 10.3390/ijms22115930
123. Tulpule A, Guan J, Neel DS, Allegakoen HR, Lin YP, Brown D, et al. Kinase-mediated RAS signaling via membraneless cytoplasmic protein granules. Cell. 2021; 184 (10): 2649–64.e18. doi: 10.1016/j.cell.2021.03.031
124. Klein IA, Boija A, Afeyan LK, Hawken SW, Fan M, Dall'Agnese A, et al. Partitioning of cancer therapeutics in nuclear condensates. Science. 2020; 368 (6497): 1386–92. doi: 10.1126/science.aaz4427
125. Tulsiani DR, Harris TM, Touster O. Swainsonine inhibits the biosynthesis of complex glycoproteins by inhibition of Golgi mannosidase II. J Biol Chem. 1982; 257 (14): 7936–9. doi: 10.1016/s0021-9258(18)34274-1
126. Kosara S, Biswas A, Yadav AK, Solanki R, Bhatia D. DNA-driven liquid–liquid phase separation from cellular condensates to programmable synthetic systems. ACS Appl Bio Mater. 2026; 9 (4): 1837–58. doi: 10.1021/acsabm.5c02505