Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

An Overview of Liquid-Liquid Phase Separation and Its Mechanisms in Sepsis

Meiling Cao[1,*], Xinyi Zhang[2,*], Xiaohan Wang[2,*], Danyang Zhao[2], Mingyue Shi[2], Jiahui Zou[2], Lei Li[3], Hongkun Jiang[2]

[1]Department of Neonatology, The First Hospital of China Medical University, Shenyang, Liaoning, 110001, People's Republic of China; [2]Department of Pediatrics, The First Hospital of China Medical University, Shenyang, Liaoning, 110001, People's Republic of China; [3]Department of Orthopaedic Surgery, Shengjing Hospital of China Medical University, Shenyang, Liaoning, 110004, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hongkun Jiang, Department of Pediatrics, The First Hospital of China Medical University, No. 155 Nanjing North Street, Heping District, Shenyang, 110001, People's Republic of China, Tel +86 02483282576, Email jianghongkun007@163.com; Lei Li, Department of Orthopedic Surgery, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Heping District, Shenyang, 110004, People's Republic of China, Tel +86 02423896615, Email leilieieil@126.com

Abstract: Sepsis is a systemic inflammatory response syndrome triggered by the invasion of bacteria or pathogenic microorganisms into the human body, which may lead to a variety of serious complications and pose a serious threat to the patient's life and health. Liquid-liquid phase separation (LLPS) is a biomolecular process in which different biomolecules, such as proteins and nucleic acids, form liquid condensates through interactions, and these condensates play key roles in cellular physiological processes. LLPS may affect the development of sepsis through several pathways, such as modulation of inflammatory factors, immune responses, and cell death, by altering the function or activity of biomolecules, which, in turn, affect the cellular response to infection and inflammation. In this paper, we first discuss the mechanism of phase separation, then summarize the studies of LLPS in sepsis, and finally propose the potential application of LLPS in sepsis treatment strategies, while pointing out the limitations of the existing studies and the directions for future research.

Keywords: biomolecular condensate, cGAS-STING, liquid-liquid phase separation, sepsis, inflammatory response

Introduction

Cellular biochemical reactions are the fundamental structural components of living organisms, requiring precise regulation to avoid interference among various reactions that can disrupt normal physiological functions and potentially lead to disease. In addition to the formation of classical organelles via biomembranes, liquid-liquid phase separation (LLPS) is employed in cells to compartmentalize substances into distinct regions, thereby coordinating the orderly progression of various intracellular reactions. LLPS in cells typically involves the formation of discrete droplet-like structures. These structures, known as membraneless organelles or biomolecule condensates, are created through a phase separation mechanism[1] and play an important role in cellular physiological processes such as macromolecular assembly and gene expression.[2] Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. This new definition emphasizes on the impact of an imbalanced immune response with organ dysfunction, rather than straightforward infection.[3] LLPS has been found to affect several cellular processes including intracellular signaling and immune responses by altering the function or activity of biomolecules and thereby modulating the normal function of cells.[4,5] Despite growing interest in LLPS, the specific mechanisms underlying phase separation in sepsis are not well understood. Therefore, this paper focuses on the role of phase separation in the inflammatory response and immunoregulatory mechanisms caused by sepsis. Firstly, we introduce the definition and basic principles of phase separation, then we introduce the mechanism of phase separation in the immune response of sepsis, and we propose the potential therapeutic strategies of phase separation in sepsis, the limitations of the current technology and the direction of future research.

Received: 18 December 2024
Accepted: 22 February 2025
Published: 17 March 2025

An Overview of LLPS

Definition of LLPS

LLPS refers to the process by which multiple liquid mixtures spontaneously segregate into distinct phases under certain conditions. Brangwynne et al were the first to identify that P granules in *Caenorhabditis elegans* (*C. elegans*) exhibited fluid characteristics and were localized on the posterior side of the cell. They proposed that these structures, composed of P granules along with other proteins and RNA, are formed intracellularly through LLPS.[6] Subsequent studies of other membrane-less structures in cells, such as nucleoli[7,8] and stress granules,[9] have revealed that these entities exhibit fluid characteristics similar to those observed in P granules. The proximity of total protein concentration in the cell to that measured in the cytoplasm further suggests that the formation of these membraneless organelles results from the spontaneous segregation of different material phases.[10] LLPS is recognized as a common mechanism underlying the assembly of membraneless organelles. It is generally accepted that the cytoplasm acts as a liquid, serving as a soluble phase, while RNA and proteins undergo phase separation to form a concentrated phase within the cell, resulting in the creation of membrane organelles, also known as biomolecule condensates.[1,11]

Basic Principles

The structure and chemical properties of molecules involved in LLPS play an important role in the process of phase separation. In cells, LLPS can be understood as the process of formation of multiphase droplets that achieve an intracellular equilibrium state due to differences in biophysical properties such as solubility and varying surface tensions, when various components within the cell are mixed.[12] LLPS is a process capable of transformation, allowing liquids to transition into gel-like states. These gel-like substances typically require specific changes in conditions to revert to a liquid state.[13] Research on the role of the Fused in Sarcoma (FUS) protein in amyotrophic lateral sclerosis (ALS) has revealed a shift from liquid to aggregated states in vitro. The mislocalization of FUS leads to abnormal aggregation by disrupting its interactions with RNA-binding proteins, which normally help prevent the aberrant liquid-to-solid phase transition of FUS. Neurodegenerative changes are exacerbated by the dysfunction of RNA-binding proteins, which contribute to abnormal aggregation and phase transitions.[14,15]

For proteins and RNA, the multivalent interactions—arising from their ability to engage in multiple binding interactions—serve as a crucial driving force for LLPS. The presence of LLPS is commonly observed in multivalent systems, indicating that interactions among multivalent molecules are integral to various biological processes.[16] When mixed, these multivalent molecules undergo reactions that generate oligomeric complexes with reduced solubility, thereby promoting phase separation.[17] Macroscopically, the interactions of multivalent macromolecules are characterized by pronounced LLPS, while microscopically, they manifest as the formation of tiny droplets in the cytoplasm. The inherent properties of multivalent molecules highlight the significant role that phase separation plays in cellular activities.[16] The differences arising from the structural characteristics of molecules, as well as intracellular environmental factors, assume importance in the application of LLPS in biomedicine. Key intracellular environmental factors include the concentration of solutions and macromolecules, ambient temperature, the type and concentration of salts and other solutes, pH levels, and cell volume, independent of the influence of other macromolecules. LLPS is concentration-dependent, typically occurring when a substance reaches its saturation threshold. For instance, in nucleolar synthesis, assembly does not take place below the threshold concentration of fibrillin-1; however, once this threshold is exceeded, higher concentrations result in the formation of larger nucleoli. The process of LLPS can be controlled and influenced by regulating the concentration of macromolecules involved in phase separation.[18,19] Banani et al[1] proposed a multilayer model for simulating cellular structures, where scaffold molecules are essential for droplet formation, while client molecules are non-essential components that only associate with the framework under specific conditions.[20] A key feature of the multilayer model is the ability of scaffold molecules to bind to one another through multivalent interactions, thereby facilitating the LLPS process.[13] These scaffold molecules preferentially interact with each other rather than with client molecules, thereby maximizing intermolecular forces among themselves and leading to the formation of more stable structures.[20]

Although various types of membrane-free structures in cells differ, they share common features, such as the specific localization of concentrated components and their fluid-like nature, which can be explained by the LLPS mechanism. Experimental studies have shown that, despite their dynamic properties, these droplets can maintain their overall size and shape for a certain period while also exchanging substances with their surrounding environments.[18,21] (Figure 1).

Formation of Intracellular Membrane-Free Structures

Membrane organelles function as closed reaction compartments, allowing for the enrichment of the same substances at designated locations and ensuring that reactions proceed correctly in both spatial and temporal contexts.[1,22] The mechanisms of LLPS offer advantages such as compartmentalization, selective partitioning, and the concentration of various substances, which are essential for regulating the precise and effective execution of biological reactions within the nucleus.[23] The process of compartmentalization creates a closed environment for reactions, minimizing unwanted molecular interactions while increasing the concentration of functional proteins, thereby enhancing reaction efficiency. During transcription within the nucleus, components such as Polymerase II, transcription factors, and elongation factors utilize a phase separation mechanism to form droplets. This organization not only facilitates the correct progression of the transcription process but also effectively isolates these molecules from other reaction systems, thereby preventing interference from other reactions.[24–26]

Additionally, cellular responses can also be facilitated by the exclusion of negative regulators. For instance, T-cell receptor (TCR) transduction in T-cells occurs through interconnected modules. The LAT complex can activate multiple downstream modules, leading to calcium mobilization, mitogen-activated protein kinase (MAPK) activation, and actin polymerization.[27] Upon TCR activation, LAT becomes phosphorylated, and this enhances the responsiveness of downstream factors. Multivalent assembly with partner molecules and condensation into micron- or submicron-sized clusters at the plasma membrane occur through LLPS.[28] These LAT cluster aggregates exclude repressors through phase separation, thereby creating an environment that perpetuates the phosphorylation state, promoting the prolongation of the TCR activation process.[29] (Figure 2). However, abnormalities such as changes in composition and agglutination in the absence of membrane structures can contribute to disease development. These dysfunctional mechanisms result in the loss of normal function including

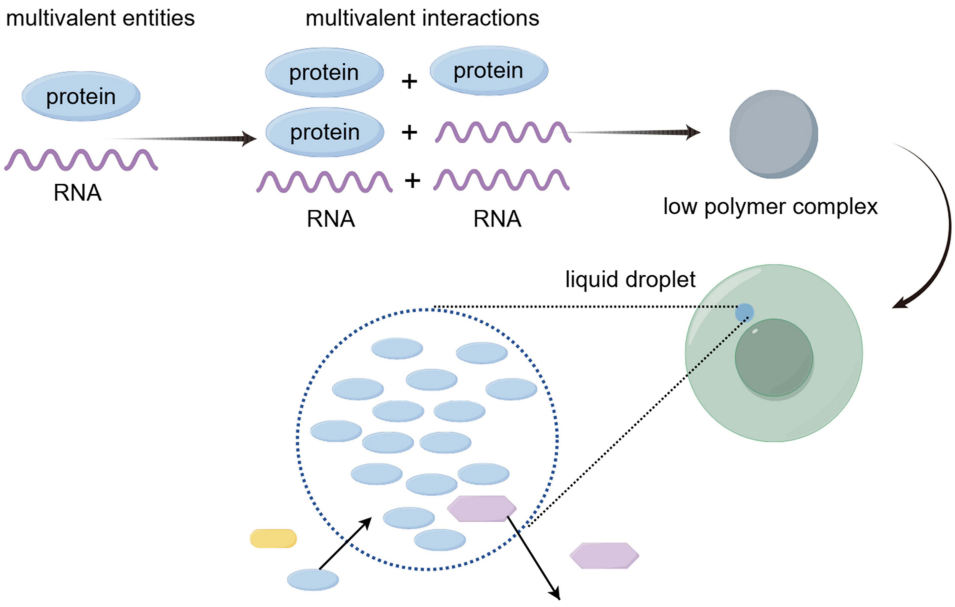

Figure 1 Polyvalent proteins and RNAs engage in polyvalent interactions either among themselves or with identical molecules, leading to the formation of oligomeric complexes. These interactions reduce the solubility of these species in the cytoplasm, thereby facilitating LLPS. In addition to the multivalency of the substances involved in the reaction, the distinct physical and chemical properties of different substances can also drive spontaneous liquid separation, resulting in the formation of multiphasic droplets, or membrane-less organelles, within the cell—commonly referred to as biomolecular aggregates. The LLPS process is characterized by a dynamic reassembly mechanism, whereby specific molecules (the blue ellipses in the figure) within the cell become concentrated at designated sites while others (the yellow and purple polygons in the figure) are selectively excluded. This figure is drawn by Figfraw(www.figdraw.com).

https://doi.org/10.2147/JIR.S513098

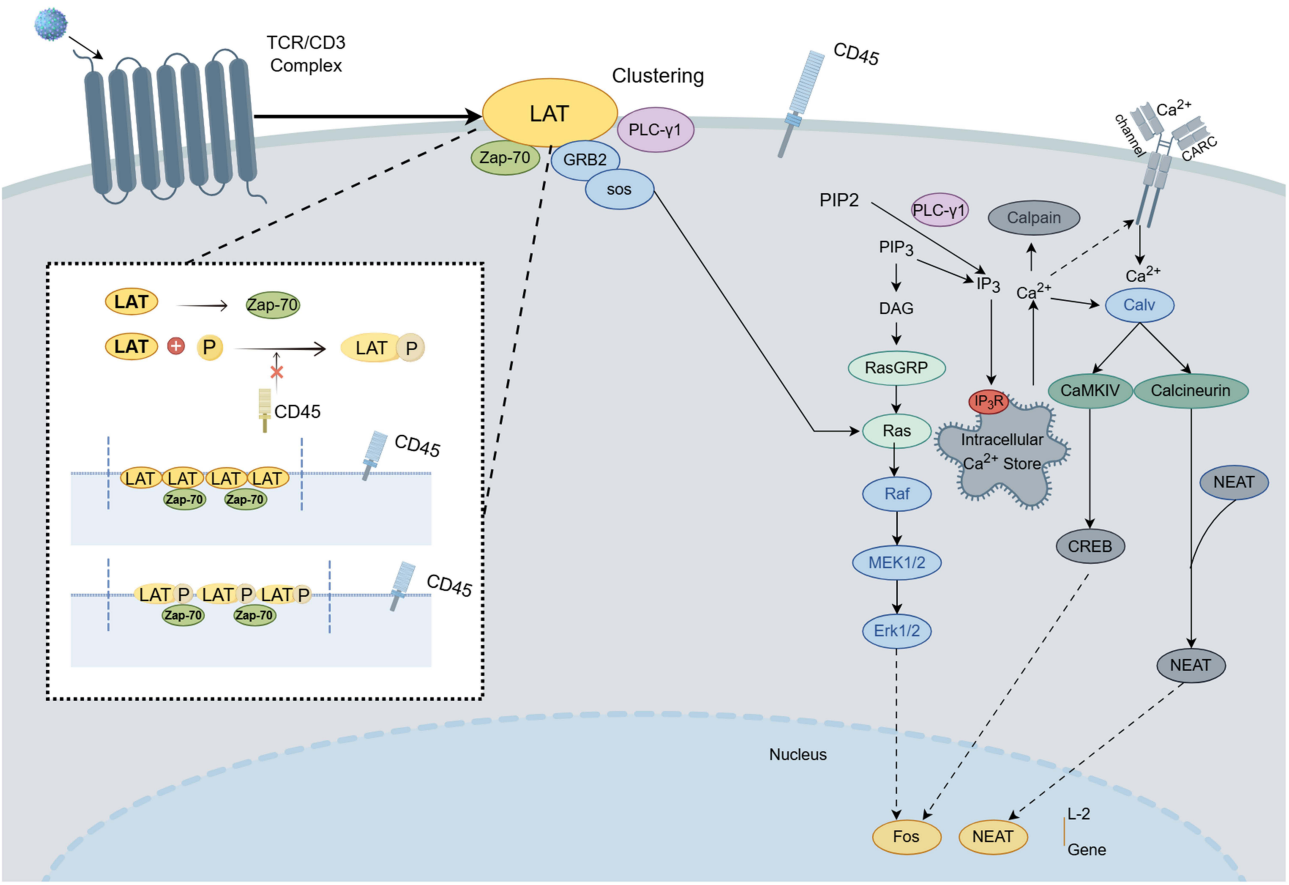

Figure 2 LAT in the TCR pathway enhances signal transduction by sequestering inhibitory factors through a LLPS mechanism. Upon antigen activation, the T-cell receptor (TCR) promotes the phosphorylation of LAT protein, mediated by its interaction with Grb2 and SOS proteins. This activation triggers the Ras signaling pathway and recruits PLC-γ1, ultimately enhancing cytokine production and calcium release from T-cell stores. Inhibition of LAT phosphorylation by the phosphatase CD45 results in the suppression of downstream signaling events. To ensure effective signal propagation, LAT forms clusters via the LLPS mechanism with its cooperative proteins, selectively enriching ZAP-70 while excluding a portion of CD45, effectively isolating the inhibitors of the reaction. Consequently, this creates a microenvironment that preserves a sustained phosphorylation state, promoting prolonged signaling and the release of calcium (Ca^{2+}) ions following TCR activation. This figure is drawn by Figfraw(www.figdraw.com).

disrupting protein localization, signal transduction, and gene expression, which leads to the development of various diseases.[30,31]

Studies on LLPS in Sepsis

The Role of LLPS in Inflammatory Response

Sepsis is usually caused by activation of the innate immune system, in which Toll-like receptors (TLRs) play an important role. In particular, TLR4 on the cell membrane recognizes the presence of lipopolysaccharide (LPS) and triggers an inflammatory response. Dimerization of TLR4 activates the NF-κB signaling pathway, which increases the transcription of pro-inflammatory cytokines, leading to inflammation and tissue damage, especially in the context of lung injury.[32,33]

Recent studies have shown that expression of the fusion suppressor Sufu is significantly reduced during the early stages of lipopolysaccharide (LPS)-induced acute inflammation in mouse lung and peritoneal macrophages. Sufu deficiency, exacerbates LPS- and cecum ligation puncture (CLP)-induced lung injury and increases mortality in mice. In addition, Sufu deficiency amplified LPS-induced expression of pro-inflammatory genes in macrophages. Sufu deficiency further enhanced TLR4 signaling as evidenced by increased phosphorylation of downstream kinases (IKKα/β, Jnk, and Erk), which augmented the inflammatory response and TLR-induced NF-κB signaling in macrophages. Tumor necrosis factor receptor-associated factor 6 (TRAF6) has an important role in the physiological and pathological processes of sepsis.[34]

In the context of inflammation triggered by lipopolysaccharide (LPS), TRAF6 experiences phase separation. Studies have demonstrated that Sufu protein directly impedes the formation of TRAF6 phase-separated droplets. This inhibition occurs by blocking the oligomerization and self-ubiquitination of TRAF6, consequently curtailing the signaling activity mediated by TRAF6 during the inflammatory response. In a model of infectious shock, TRAF6 depletion attenuated the exacerbation of the inflammatory phenotype in myeloid-specific Sufu-deficient mice.[35] (Figure 3)

Excessive inflammatory response and cytokine storm during sepsis have been recognized as key factors contributing to high mortality. In patients with sepsis, the antigen-presenting capacity of macrophages is significantly reduced and macrophages are categorized into two main phenotypes: classically activated (M1) and selectively activated (M2). It has been shown that under different regulation by pro- and anti-inflammatory stimuli, changes in the phase separation of macrophage lipid membranes reach a critical point, prompting macrophages to enter two different activation states, M1 or M2, respectively. IFN-γ, LPS, and Kdo 2-Lipid A (KLA) were able to increase the critical temperature of the macrophage plasma membrane phase separation, activating macrophages and causing them to shift to the M1 phenotype. In contrast, IL-4 was able to decrease this critical temperature, prompting macrophages to shift to the M2 type.[36] The imbalance between M1 and M2 polarization has a significant impact on the inflammatory response, especially the increase in uncontrolled M1-type macrophages, which may trigger severe inflammatory diseases such as gastroenteritis, pyelonephritis, neonatal meningitis, and sepsis.[37] Previous studies have found that cytotoxic necrosis factor 1 (CNF1) - a key toxin secreted by urinary tract pathogenic Escherichia coli (UPEC) - induces urinary tract inflammation and reduces the phagocytosis of UPEC by macrophages. Macrophages can phagocytose UPEC and eliminate neutrophils, but

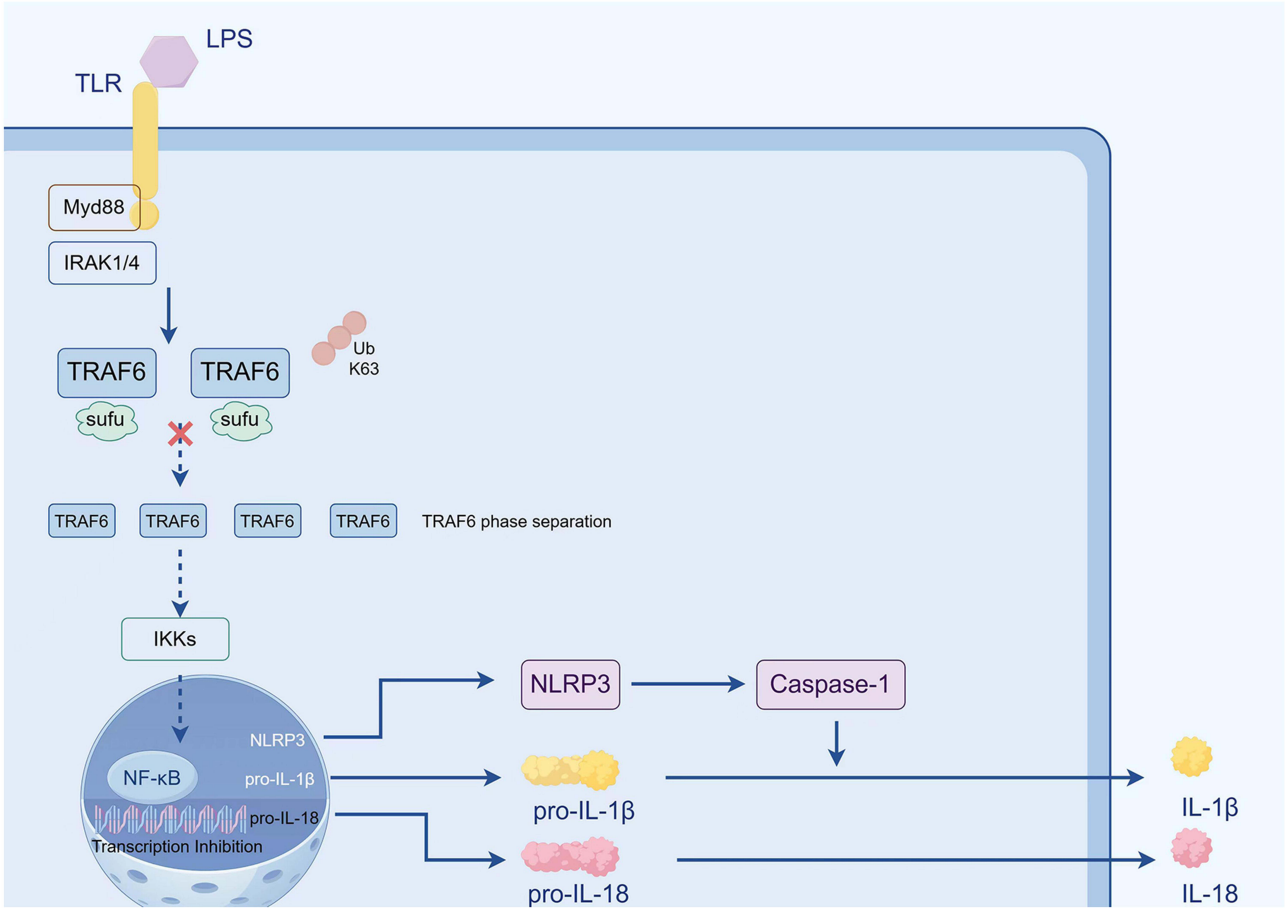

Figure 3 Sufu plays an important regulatory role in Toll-like receptor (TLR)-mediated inflammatory responses. It inhibits the process of TRAF6 oligomerization and self-ubiquitination by directly interacting with TNF receptor-associated factor 6 (TRAF6). In LPS-induced inflammation, phase separation of TRAF6 is a key link in the activation of its ubiquitination and NF-κB signaling pathway, and Sufu effectively prevented the formation of phase-separated droplets of TRAF6, which inhibited the activation of the LPS-induced NF-κB signaling pathway. This figure is drawn by Figfraw(www.figdraw.com).

https://doi.org/10.2147/JIR.S513098

their excess leads to increased inflammation and tissue damage.[38,39] UPEC's α-hemolysin induces macrophage accumulation and exacerbates renal injury. CNF1 promotes M1-type macrophage polarization in the early phase of acute urinary tract infection (UTI) by modulating the NF-κB and JAK1/2-STAT1 signaling pathways in the kidney. In addition, CNF1 not only activated the NF-κB and JAK-STAT1 signaling pathways, but also directly interacted with JAK1 and JAK2 via liquid-liquid phase separation (LLPS) to form protein complexes, which further promoted M1-type macrophage polarization and triggered renal inflammatory responses in the early stage of acute UTI.[40]

Studies suggest that liquid-liquid phase separation may also be involved in the regulation of inflammatory vesicle assembly. NLRP6 inflammatory vesicles are implicated in a variety of host defense mechanisms by inhibiting TLR-induced MAPK and classical NF-κB signaling to suppress proinflammatory cytokine and chemokine production during bacterial infections. Activation of NLRP6 results in an increased susceptibility to a number of both Gram-positive (Listeria monocytogenes) and negative (Escherichia coli and Salmonella typhimurium) bacterial pathogens with increased susceptibility.[41,42] It was experimentally found to undergo liquid-liquid phase separation upon interaction with dsRNA and to promote its integration of multiple signaling stimuli in response to gut microbiota stimulation, participating in antimicrobial defense in mice.[43]

The biomolecular agglomerates produced by phase separation enhance the survival and adaptability of bacteria, enabling them to survive in the face of stresses such as antibiotic treatment, starvation, oxidative stress, heat shock or phage infection. These structures are found not only in E. coli, but also in other Gram-negative bacteria, which are capable of accumulating key proteins such as HslU (a component of the HslVU protease), Kbl (an enzyme that degrades threonine in the serine biosynthesis pathway), and AcnB (a cis-aconuclease involved in central metabolism). These structures sequester proteins critical to cellular function, shutting down related processes and forcing the cell into a state of dormancy,[44,45] and in addition, due to the physical properties of biomolecular condensates, they are able to respond to external pressures and stimuli, a strategy that viruses may utilize to evade the host's innate immune response. For example, viruses can be induced to form STING phase separators by LLPS. In DNA virus-infected cells, excess 2′3′-cGAMP prompts STING to form biomolecular condensates, and STING biocondensates limit the activities of STING and TBK1, preventing excessive activation of innate immunity.[46]

The Role of LLPS in Metabolic Regulation

Prolonged sepsis may lead to suppression of immune system function, triggering massive immune cell dysfunction. RCD (regulated cell death) in addition to functioning as a built-in effector of physiological programs of development or tissue renewal, on the other hand, in response to a variety of noxious factor stimuli other forms such as necrotizing necrosis, pyroptosis, autophagy, and iron necrosis occur.[47] Regulated by multiple interrelated signaling pathways and molecular mechanisms, when adaptive processes in response to stress fail, either intracellularly or exogenous perturbations in the extracellular microenvironment can trigger RCD.[48] Apoptosis is a key component in maintaining the homeostasis of the immune system, and its rapid clearance of apoptotic cells is essential for building immune tolerance and preventing inflammatory responses.[49] It is mainly disrupted by cysteine-dependent aspartate-specific proteases (caspases), a ribonucleoprotein (RNP) granule type that is stress granules (SGs), which are dynamic and reversible cytoplasmic assemblies formed in response to stress in eukaryotic cells, and it has been found that the formation of SGs is triggered by G3BP1 acting as a molecular switch to respond to the increase in intracellular free G3BP1 is a tunable switch that triggers phase separation to assemble stress granules). Cytoplasmic SGs segregate caspase-3 and caspase-7, inhibit caspase activity, and ultimately prevent apoptosis by removing harmful substances through autophagy. Therefore, regulating the formation of SGs may help to improve the process of cysteine-mediated apoptosis.[50,51] Autophagy, as a stress defense mechanism, is crucial for defense against harmful substances such as bacteria.[52] The heat shock protein HSPA8 plays a key role in the autophagic degradation of proteins, and it contains intrinsically disordered regions (IDRs) that promote liquid-liquid phase separation, concentrating RHOB and BECN1 into the liquid-phase droplets formed by HSPA8 to form the HSPA8-RHOB-BECN1 complex, which induces autophagy to remove intracellular bacteria.[53] Various types of regulated cell death have also been reported in sepsis-induced organ dysfunction, and in sepsis, in addition to apoptosis and autophagy, the types of cell death include pyroptosis,[54] the latter of which usually requires bacterial or viral stimulation of inflammatory responses and activation through pathways such as NF-κB.

The Role of LLPS in Tissue and Organ Dysfunction

The cyclic GMP-AMP synthase (cGAS) of cytoplasmic DNA has been found to be essential for recognizing the immune response to pathogen infection.[55] cGAS contains two key structural domains: a C-terminal nucleotidyl transferase (NTase) structural domain and an N-terminal structural domain. These structural domains enable cGAS to bind to double-stranded DNA (dsDNA) through multivalent interactions, inducing phase separation (LLPS) and the formation of membrane-free cellular compartments. High concentrations of cGAS-DNA complexes increase the activity of cGAS, leading to increased synthesis of cGAMP, a key signaling molecule involved in activating the immune response.[56] cGAMP binds to the ER-localized connexin interferon gene stimulator (STING), which causes a conformational change in STING, and promotes the production of type I IFN.

Acute lung injury (ALI) and acute kidney injury (AKI) are common complications during sepsis. NLRP3 is an important mediator of immune response initiation and inflammatory vesicle formation, and once activated by a dangerous stimulus, NLRP3 inflammatory vesicles promote the maturation and secretion of pro-inflammatory cytokines (eg, Caspase1, IL-1β, and IL-18) and initiate cellular cell death. In sepsis, inflammasome-induced cellular pyroptosis disrupts the cell membrane and leads to the release of multiple inflammatory factors.[57] NLRP3 inflammasome activation was found to be significantly correlated with sepsis-induced acute kidney injury, with the cGAS- STING axis being affected by cytoplasmic mtp and the cGAS- STING axis being affected by cytoplasmic mtp. STING axis was triggered by cytoplasmic mtDNA to promote acute kidney injury through activation of NLRP3 inflammasome, whereas reduction of cytoplasmic mtDNA accumulation or inhibition of the cGAS-STING axis may be a potential therapeutic target for the treatment of sepsis-induced progressive kidney injury.[58] Similarly, cGAS or STING defects may prevent LPS-induced ALI by inhibiting inflammation, oxidative stress, and cellular injury.[59] (Figure 4).

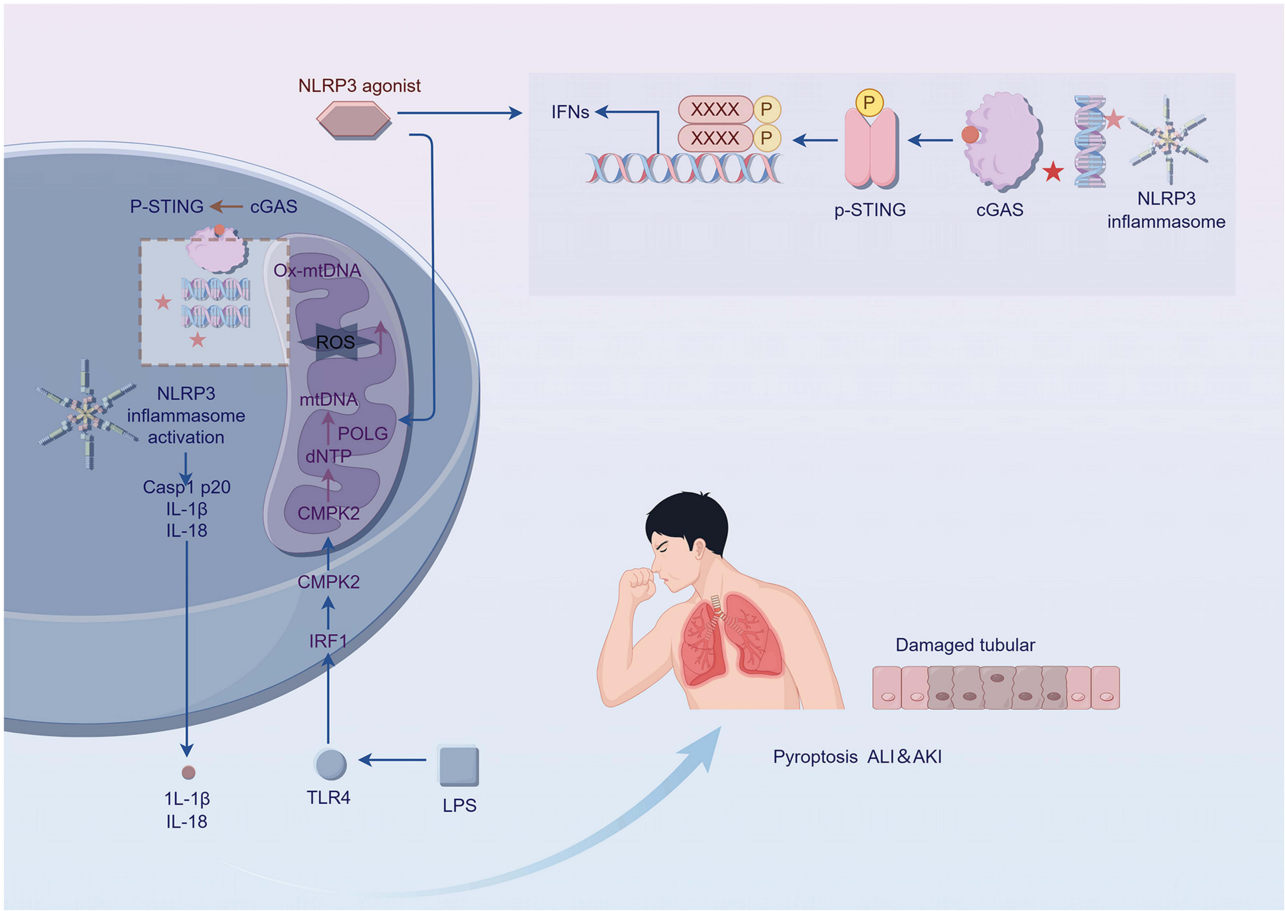

Figure 4 Innate immunity and inflammation are thought to be key factors in sepsis-induced acute lung injury (ALI), acute kidney injury (AKI). cGAS binds to dsDNA, induces phase separation (LLPS), promotes cGAMP synthesis, and induces inflammatory insults and type I interferon production via STING phosphorylation. LPS stimulation leads to ROS and ASC induction in lung and kidney tissues. cGAS-STING axis is triggered by cytoplasmic mtDNA and activates NLRP3 inflammatory vesicles. This figure is drawn by Figfraw(www.figdraw.com).

https://doi.org/10.2147/JIR.S513098

Challenges and Prospects for LLPS in Sepsis

Potential therapeutic strategies and future research directions of liquid-liquid phase separation in sepsis.

Potential Therapeutic Strategies

In the absence of targeted therapies to modulate the host immune response, traditional sepsis treatment involves the timely implementation of supportive therapies such as fluid resuscitation, vasopressors, oxygen, mechanical ventilation, prompt source control of infection, and antibiotics.[48] Research into the phase separation process is beneficial for developing more effective targeted treatments for sepsis, thereby reducing the release of inflammatory factors and organ damage and dysfunction.[60] Drugs selectively partition into condensates, which can occur through their physical and chemical properties independent of their molecular targets. Targeting biomolecular condensates has the potential to enable the development of novel and diverse therapeutic approaches.[61]

Current intracellularly regulated phase separation processes that occur using the principle of multivalent molecular interactions in phase separation processes, such as optogenetic techniques and chemical tools artificially modulate the onset or cessation of phase separation processes within the cell.[62] Through the use of near-infrared nanoparticles, infrared light with deep tissue penetration and low phototoxicity can be converted into visible light compatible with current optogenetic tools. This reduces the side effects on cells of applying less transmissive and phototoxic visible light in experiments.[63] In terms of chemical tools, for example, a newly constructed YK peptide can bind ATP in mammalian cells to form reversible amyloid-like fibers in biomolecular condensates. YK peptide has homotypic assembly properties, has the ability to bind multiple substances to build complex biomolecular condensates, and can be altered by changing the length of the YK peptide to regulate the mobility of the product, thus realizing the precise regulation, providing a potential therapeutic target for regulating the phase separation process in the cellular response to sepsis. The YK peptide provides a potential therapeutic target for regulating the phase separation process in the immune process of cellular response to sepsis.[64] In addition to affecting phase separation by participating in multivalent interactions in the molecule, the most direct way in which small molecules can have an impact on biomolecular condensates is by targeting and promoting the degradation of proteins that serve as scaffolds in the condensate, eg, protease-targeted chimeric (PROTAC) technology directly reduces the concentration of key proteins in the formation of the condensate by linking proteins to E3 ligases and affects biomolecular condensate formation, thereby inhibiting the development of associated diseases. This inhibits the development of the associated disease.[63,65] Targeting key proteins in the phase-separated processes of the inflammatory response and immunoregulatory pathways associated with sepsis by similar technologies, thereby reducing the adverse effects caused by sepsis complications, is also a potential therapeutic direction. Since biomolecular condensates in the nucleus usually function in concert with chromosomes, phase separation processes in the nucleus may have implications for chromosome assembly and gene expression processes.[66] For example, the Casdrop system can guide RNA to undergo phase separation at specific locations in the genome, thus allowing the phase separation regulatory process to proceed in a highly ordered manner,[67] and this type of technology provides a new way of thinking about regulating the immune response to sepsis at the genetic level, among other things. Cells can develop resistance to drugs through mechanisms that alter the condensates. These findings also impact the development of effective disease therapies in the future; the involvement of effective targets will depend on measurable factors, such as drug distribution within the condensates.[68]

Technical Challenges

A challenging area of research in the field of phase separation is the determination of the internal organization and structure of condensate components at atomic resolution. The intracellular structures of prokaryotes, such as bacteria, and eukaryotes are themselves small, the biomolecular condensates formed within them are even smaller, and the presence of biological membranes complicates the process of observation.[69]

The use of neighbouring molecular labelling techniques in combination with higher resolution microscopy or electron microscopy allows for the visualisation of finer structures than conventional methods. In-situ cryo-electron chromatography allows the visualisation of proteomics without the need for labelling to detect the structure of the biomolecule.[62,70] Although electron microscopy and crystallography have been used in other contexts to answer such structural questions, their

application to liquid objects remains limited. In addition to the difficulty of static observation of the internal organization and structure of biomolecular condensates, many biomolecular condensates are dynamic structures that can rapidly compose and dissolve, a dynamic process that is difficult to accurately observe using existing techniques for the study of macromolecular substances, and thus the processes by which such condensates form and function are not well studied.[65,71] Microfluidic system is a particularly attractive tools for the analysis of supramolecular structures of biomolecules, which one of the benefits is superior control of the reactive environment on the micron scale. The technology has a great prospects for application to the chemical as well as kinetic properties of biomolecular condensates in phase separation processes.[72] At present, although the computer-constructed all-atom model has made great progress and can display accurate images of protein structure, dynamics and interactions at Egmont resolution,[73] it is still not possible to accurately construct the specific aggregation process of molecules in phase separation, and multidisciplinary related collaborations and new development of technologies are still needed to solve the problems in this area. Understanding the internal components of biomolecular aggregates in the cell will facilitate the regulation of the expression of the relevant molecules and the design of antagonists and agonists specific for the phase separation process, thus regulating the phase separation process.[73]

Prospects and Future Research Directions

Since its first discovery, the phenomenon of liquid-liquid phase separation has been widely recognized and valued by researchers. Currently, the role of phase separation in the activation of immune signaling pathways, such as TCR, BCR, cGAS, RIG-I, and NF-κB, has been confirmed.[71] However, there is less direct evidence regarding the process of infection by pathogens such as bacteria and the complications of organ damage caused by sepsis. Further research is still needed to fully explain the role of phase separation in the process of sepsis.

The fundamental role of LLPS in membrane-free compartmentalization has attracted intense interest, and new questions and hypotheses about the molecular mechanisms and biological processes associated with these microbial condensates have been raised. The discovery of additional phase separation systems and the dissection of their functions through new tools and methods are essential to advance the general understanding of how these membrane-free compartments help build biochemical structures.[74,75] Future research should firstly focus on in-depth studies of the role and modes of regulation of the liquid-liquid phase separation process and the resulting biomolecular condensates in the pathogenesis of sepsis, which is crucial for a comprehensive understanding of sepsis pathogenesis and for the development of targeted interventions. The next step is to develop more effective and targeted therapeutic measures by combining the characteristics of the liquid-liquid phase separation process and the biomolecular condensates, so as to reduce the inflammatory response and organ damage caused by sepsis. The field of targeting phase separation processes and biomolecular condensates in the activation of immune responses and organ damage due to sepsis is in its infancy and still requires technological development and physiological exploration, but it has already demonstrated good prospects and great potential for advances through multidisciplinary collaborations and other means, and has the promise of becoming a new therapeutic target for the treatment of sepsis.

Conclusion and Prospects

Sepsis is a systemic inflammatory response syndrome caused by infection and characterized by dysregulated immune responses and multi-organ failure. Biomolecular condensates play key roles in specific biological processes within the cell, and the role of LLPS in sepsis may influence the production and release of inflammatory factors, the activation and migration of immune cells, and affect cell death and organ dysfunction in a number of ways. Liquid-liquid phase separation opens a new field of research on sepsis, and LLPS not only plays a key role in the pathophysiological process of sepsis, but also may become a new target for the treatment of sepsis in the future. The traditional treatment of sepsis is supportive therapy, and some targeting of key proteins involved in the phase separation process in the relevant inflammatory response and signaling pathways in sepsis is also a potential therapeutic approach through the study of phase separation. The role of LLPS in signaling pathways has been widely recognized, and future research needs to focus on deepening the understanding of the complex mechanism of the role of LLPS in the infectious process of bacterial or other pathogens and the Future research needs to focus on deepening the understanding of the complex mechanism of action of LFP in the process of infection by bacteria or other pathogens and in sepsis, which is important for the development of new therapeutic approaches and the improvement of sepsis outcomes.

Abbreviations

GFP, Green Fluorescent Protein; FRAP, Fluorescence Recovery after Photobleaching; FLIP, Fluorescence Lose in Photobleach; RNA, Ribonucleic Acid; FUS, Fused in Sarcoma; IDR, Intrinsically Disordered Region; LCD, Low-complexity Domain; SG, Stress Granules; LLPS, Liquid-Liquid Phase separation; CNF1, Cytotoxic Necrotizing Factors 1; NF-κB, Nuclear Factor kappa-B; JAK-STAT, Janus Kinase-Signal Transducer and Activator of Transcription; UPEC, Uropathogenic Escherichia Coli; TCR, T-cell Receptor; MAPK, Mitogen-Activated Protein Kinase; LAT, Linker for Activation of T cell; BMC, Biomolecular Molecular Condensates; HSF, Heat Shock Factor 1; GMP AMP, Guanosine Monophosphate Adenosine monophosphate; cGAS, Cyclic GMP–AMP Synthase; PAMP, Pathogen-Associated Molecular Patterns; dsDNA, Double-stranded DNA; IB, Inclusion Body; cGAS-STING, Cyclic GMP–AMP Synthase-Stimulator of interferon Genes; KSHV, Kaposi's Sarcoma Associated-Herpesvirus; PTM, Post-Translational Modification.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

We are particularly grateful to all the people who have given us help on our article.

Funding

National Natural Science Foundation of China (No.81300130). Science and Technology Projects for People's Livelihood of Liaoning Province (2021JH/10300008). Basic Research Projects of Liaoning Province (JYTMS20230073). Provincial Natural Science Foundation Joint Fund of Liaoning (2023-BSBA-363).

Disclosure

The authors declare that they have no competing interests.

References

1. Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular molecular condensates: organizers of cellular biochemistry. *Nat Rev mol Cell Biol*. 2017;18(5):285–298. doi:10.1038/nrm.2017.7
2. Lyon AS, Peeples WB, Rosen MK. A framework for understanding functions of biomolecular condensates on molecular to cellular scales. *Nat Rev mol Cell Biol*. 2021;22(3):215–235. doi:10.1038/s41580-020-00303-z
3. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801–810. doi:10.1001/jama.2016.0287
4. Cheng X, Case LB. Phase separation in chemical and mechanical signal transduction. *Curr Opin Cell Biol*. 2023;85:102243. doi:10.1016/j.ceb.2023.102243
5. Case LB, Ditlev JA, Rosen MK. Regulation of transmembrane signaling by phase separation. *Annu Rev Biophys*. 2019;48:465–494. doi:10.1146/annurev-biophys-052118-115534
6. Brangwynne CP, Eckmann CR, Courson DS, et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science*. 2009;324(5935):1729–1732. doi:10.1126/science.1172046
7. Brangwynne CP, Mitchison TJ, Hyman AA. Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. *Proc Natl Acad Sci U S A*. 2011;108(11):4334–4339. doi:10.1073/pnas.1017150108
8. Lafontaine DLJ, Riback JA, Bascetin R, Brangwynne CP. The nucleolus as a multiphase liquid condensate. *Nat Rev mol Cell Biol*. 2021;22(3):165–182. doi:10.1038/s41580-020-0272-6
9. Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R, Pelkmans L. Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. *Cell*. 2013;152(4):791–805. doi:10.1016/j.cell.2013.01.033
10. Hyman AA, Brangwynne CP. Beyond stereospecificity: liquids and mesoscale organization of cytoplasm. *Dev Cell*. 2011;21(1):14–16. doi:10.1016/j.devcel.2011.06.013
11. Mehta S, Zhang J. Liquid-liquid phase separation drives cellular function and dysfunction in cancer. *Nat Rev Cancer*. 2022;22(4):239–252. doi:10.1038/s41568-022-00444-7
12. Feric M, Vaidya N, Harmon TS, et al. Coexisting liquid phases underlie nucleolar subcompartments. *Cell*. 2016;165(7):1686–1697. doi:10.1016/j.cell.2016.04.047
13. Phair RD, Misteli T. High mobility of proteins in the mammalian cell nucleus. *Nature*. 2000;404(6778):604–609. doi:10.1038/35007077
14. Marrone L, Drexler HCA, Wang J, et al. FUS pathology in ALS is linked to alterations in multiple ALS-associated proteins and rescued by drugs stimulating autophagy. *Acta Neuropathol*. 2019;138(1):67–84. doi:10.1007/s00401-019-01998-x
15. Levone BR, Lenzken SC, Antonaci M, et al. FUS-dependent liquid-liquid phase separation is important for DNA repair initiation. *J Cell Biol*. 2021;220(5):e202008030. doi:10.1083/jcb.202008030

16. Li P, Banjade S, Cheng HC, et al. Phase transitions in the assembly of multivalent signalling proteins. *Nature*. 2012;483(7389):336–340. doi:10.1038/nature10879
17. Banjade S, Rosen MK. Phase transitions of multivalent proteins can promote clustering of membrane receptors. *Elife*. 2014;3:e04123. doi:10.7554/eLife.04123
18. Shin Y, Brangwynne CP. Liquid phase condensation in cell physiology and disease. *Science*. 2017;357(6357):eaaf4382. doi:10.1126/science.aaf4382
19. Weber SC, Brangwynne CP. Inverse size scaling of the nucleolus by a concentration-dependent phase transition. *Curr Biol*. 2015;25(5):641–646. doi:10.1016/j.cub.2015.01.012
20. Kim HJ, Kim NC, Wang YD, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature*. 2013;495(7442):467–473. doi:10.1038/nature11922
21. McSwiggen DT, Mir M, Darzacq X, Tjian R. Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. *Genes Dev*. 2019;33(23–24):1619–1634. doi:10.1101/gad.331520.119
22. Kato M, Han TW, Xie S, et al. Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell*. 2012;149(4):753–767. doi:10.1016/j.cell.2012.04.017
23. Lin Y, Fang X. Phase separation in RNA biology. *J Genet Genomics*. 2021;48(10):872–880. doi:10.1016/j.jgg.2021.07.012
24. Pei G, Lyons H, Li P, Sabari BR. Transcription regulation by biomolecular condensates. *Nat Rev mol Cell Biol*. 2024. doi:10.1038/s41580-024-00789-x
25. Shao W, Bi X, Pan Y, et al. Phase separation of RNA-binding protein promotes polymerase binding and transcription. *Nat Chem Biol*. 2022;18(1):70–80. doi:10.1038/s41589-021-00904-5
26. Sabari BR, Dall'Agnese A, Young RA. Biomolecular molecular condensates in the nucleus. *Trends Biochem Sci*. 2020;45(11):961–977. doi:10.1016/j.tibs.2020.06.007
27. Courtney AH, Lo WL, Weiss A. TCR signaling: mechanisms of initiation and propagation. *Trends Biochem Sci*. 2018;43(2):108–123. doi:10.1016/j.tibs.2017.11.008
28. Bilal MY, Houtman JCD. GRB2 nucleates T-cell receptor-mediated LAT clusters that control PLC-γ1 activation and cytokine production. *Front Immunol*. 2015;6:141. doi:10.3389/fimmu.2015.00141
29. Lyon AS, Peeples WB, Rosen MK. A framework for understanding the functions of biomolecular molecular condensates across scales. *Nat Rev mol Cell Biol*. 2021;22(3):215–235. doi:10.1038/s41580-020-00303-z
30. Zhang S, Pei G, Li B, Li P, Lin Y. Abnormal phase separation of biomacromolecules in human diseases. *Acta Biochim Biophys Sin*. 2023;55(7):1133–1152. doi:10.3724/abbs.2023139
31. Y L, Liu Y, Yu XY, et al. Membraneless organelles in health and disease: exploring the molecular basis, physiological roles and pathological implications. *Signal Transduct Target Ther*. 2024;9(1):305. doi:10.1038/s41392-024-02013-w
32. Płóciennikowska A, Hromada-Judycka A, Borzęcka K, Kwiatkowska K. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. *Cell mol Life Sci*. 2015;72(3):557–581. doi:10.1007/s00018-014-1762-5
33. Wang L, Wang Y, Ke Z, et al. Liquid-liquid phase separation: a new perspective on respiratory diseases. *Front Immunol*. 2024;15:1444253. doi:10.3389/fimmu.2024.1444253
34. Chiffoleau E, Kobayashi T, Walsh MC, et al. TNF receptor-associated factor 6 deficiency during hemopoiesis induces Th2-polarized inflammatory disease. *J Immunol*. 2003;171(11):5751–5759. doi:10.4049/jimmunol.171.11.5751
35. Li Y, Peng J, Xia Y, et al. Sufu limits sepsis-induced lung inflammation via regulating phase separation of TRAF6. *Theranostics*. 2023;13(11):3761–3780. doi:10.7150/thno.83676
36. Cammarota E, Soriani C, Taub R, et al. Criticality of plasma membrane lipids reflects activation state of macrophage cells. *J R Soc Interface*. 2020;17(163):20190803. doi:10.1098/rsif.2019.0803
37. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*. 2012;122(3):787–795. doi:10.1172/JCI59643
38. Bottek J, Soun C, Lill JK, et al. Spatial proteomics revealed a CX3CL1-dependent crosstalk between the urothelium and relocated macrophages through IL-6 during an acute bacterial infection in the urinary bladder. *Mucosal Immunol*. 2020;13(4):702–714. doi:10.1038/s41385-020-0269-7
39. Abraham SN, Miao Y. The nature of immune responses to urinary tract infections. *Nat Rev Immunol*. 2015;15(10):655–663. doi:10.1038/nri3887
40. Sun X, Yang J, Deng X, et al. Interactions of bacterial toxin CNF1 and host JAK1/2 driven by liquid-liquid phase separation enhance macrophage polarization. *MBio*. 2022;13(4):e01147–22. doi:10.1128/mbio.01147-22
41. Anand PK, Malireddi RKS, Lukens JR, et al. NLRP6 negatively regulates innate immunity and host defense against bacterial pathogens. *Nature*. 2012;488(7411):389–393. doi:10.1038/nature11250
42. Hara H, Seregin SS, Yang D, et al. The NLRP6 inflammasome recognizes lipoteichoic acid and regulates gram-positive pathogen infection. *Cell*. 2018;175(6):1651–1664.e14. doi:10.1016/j.cell.2018.09.047
43. Shen C, Li R, Negro R, et al. Phase separation drives RNA virus-induced activation of the NLRP6 inflammasome. *Cell*. 2021;184(23):5759. doi:10.1016/j.cell.2021.09.032
44. Persistent bacterial infections and persister cells - PubMed. Available from: https://pubmed.ncbi.nlm.nih.gov/28529326/. Accessed December 16, 2024.
45. Monterroso B, Margolin W, Boersma AJ, Rivas G, Poolman B, Zorrilla S. Macromolecular crowding, phase separation, and homeostasis in the orchestration of bacterial cellular functions. *Chem Rev*. 2024;124(4):1899–1949. doi:10.1021/acs.chemrev.3c00622
46. Yu X, Zhang L, Shen J, et al. The STING phase-separator suppresses innate immune signalling. *Nat Cell Biol*. 2021;23(4):330–340. doi:10.1038/s41556-021-00659-0
47. Galluzzi L, Vitale I, Aaronson SA, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ*. 2018;25(3):486–541. doi:10.1038/s41418-017-0012-4
48. Santagostino SF, Assenmacher CA, Tarrant JC, Adedeji AO, Radaelli E. Mechanisms of regulated cell death: current perspectives. *Vet Pathol*. 2021;58(4):596–623. doi:10.1177/03009858211005537
49. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. *Biosci Rep*. 2019;39(1):BSR20180992. doi:10.1042/BSR20180992
50. Ramirez MG, Salvesen GS. A primer on caspase mechanisms. *Semin Cell Dev Biol*. 2018;82:79–85. doi:10.1016/j.semcdb.2018.01.002

51. Fujikawa D, Nakamura T, Yoshioka D, et al. Stress granule formation inhibits stress-induced apoptosis by selectively sequestering executioner caspases. *Curr Biol*. 2023;33(10):1967–1981.e8. doi:10.1016/j.cub.2023.04.012
52. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D. Selective autophagy and xenophagy in infection and disease. *Front Cell Dev Biol*. 2018;6:147. doi:10.3389/fcell.2018.00147
53. Miao C, Zhang Y, Yu M, et al. HSPA8 regulates anti-bacterial autophagy through liquid-liquid phase separation. *Autophagy*. 2023;19 (10):2702–2718. doi:10.1080/15548627.2023.2223468
54. Liu D, Huang SY, Sun JH, et al. Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. *Mil Med Res*. 2022;9:56. doi:10.1186/s40779-022-00422-y
55. Zhou W, Mohr L, Maciejowski J, Kranzusch PJ. cGAS phase separation inhibits TREX1-mediated DNA degradation and enhances cytosolic DNA sensing. *Mol Cell*. 2021;81(4):739–755.e7. doi:10.1016/j.molcel.2021.01.024
56. Wei W, Bai L, Yan B, et al. When liquid-liquid phase separation meets viral infections. *Front Immunol*. 2022;13:985622. doi:10.3389/fimmu.2022.985622
57. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011;469(7329):221–225. doi:10.1038/nature09663
58. Luo X, Zhao Y, Luo Y, et al. Cytosolic mtDNA-cGAS-STING axis contributes to sepsis-induced acute kidney injury via activating the NLRP3 inflammasome. *Clin Exp Nephrol*. 2024;28(5):375–390. doi:10.1007/s10157-023-02448-5
59. Ning L, Wei W, Wenyang J, Rui X, Qing G. Cytosolic DNA-STING-NLRP3 axis is involved in murine acute lung injury induced by lipopolysaccharide. *Clin Transl Med*. 2020;10(7):e228. doi:10.1002/ctm2.228
60. Tong X, Tang R, Xu J, et al. Liquid–liquid phase separation in tumor biology. *Signal Transduct Target Ther*. 2022;7:221. doi:10.1038/s41392-022-01076-x
61. Vincent J-L. Current sepsis therapeutics. *EBioMedicine*. 2022;86:104318. doi:10.1016/j.ebiom.2022.104318
62. Bracha D, Walls MT, Brangwynne CP. Probing and engineering liquid-phase organelles. *Nature Biotechnol*. 2019;37(12):1435–1445. doi:10.1038/s41587-019-0341-6
63. Li K, Xie X, Gao R, et al. Spatiotemporal protein interactome profiling through condensation-enhanced photocrosslinking. *Nat Chem*. 2024. doi:10.1038/s41557-024-01663-1
64. Miki T, Hashimoto M, Takahashi H, et al. De novo designed YK peptides forming reversible amyloid for synthetic protein condensates in mammalian cells. *Nat Commun*. 2024;15(1):8503. doi:10.1038/s41467-024-52708-5
65. Chen Y, Tandon I, Heelan W, Wang Y, Tang W, Hu Q. Proteolysis-Targeting Chimera (PROTAC) delivery system: advancing protein degraders towards clinical translation. *Chem Soc Rev*. 2022;51(13):5330–5350. doi:10.1039/d1cs00762a
66. Ruan K, Bai G, Fang Y, et al. Biomolecular condensates and disease pathogenesis. *Sci China Life Sci*. 2024;67(9):1792–1832. doi:10.1007/s11427-024-2661-3
67. Klein IA, Boija A, Afeyan LK, et al. Partitioning of cancer therapeutics in nuclear condensates. *Science*. 2020;368(6497):1386–1392. doi:10.1126/science.aaz4427
68. Drobot B, Iglesias-Artola JM, Le Vay K, et al. Compartmentalised RNA catalysis in membrane-free coacervate protocells. *Nat Commun*. 2018;9 (1):3643. doi:10.1038/s41467-018-06072-w
69. Boeynaems S, Chong S, Gsponer J, et al. Phase separation in biology and disease; current perspectives and open questions. *J mol Biol*. 2023;435 (5):167971. doi:10.1016/j.jmb.2023.167971
70. Asano S, Engel BD, Baumeister W. In situ cryo-electron tomography: a post-reductionist approach to structural biology. *J mol Biol*. 2016;428(2 Pt A):332–343. doi:10.1016/j.jmb.2015.09.030
71. Polyansky AA, Gallego LD, Efremov RG, Köhler A, Zagrovic B. Protein compactness and interaction valency define the architecture of a biomolecular condensate across scales. *Elife*. 2023;12:e80038. doi:10.7554/eLife.80038
72. Linsenmeier M, Kopp MR, Stavrakis S, de Mello A, Arosio P. Analysis of biomolecular condensates and protein phase separation with microfluidic technology. *Biochim Biophys Acta mol Cell Res*. 2021;1868(1):118823. doi:10.1016/j.bbamcr.2020.118823
73. Gao Z, Zhang W, Chang R, Zhang S, Yang G, Zhao G. Liquid-liquid phase separation: unraveling the enigma of biomolecular condensates in microbial cells. *Front Microbiol*. 2021;12:751880. doi:10.3389/fmicb.2021.751880
74. Zhang JZ, Mehta S, Zhang J. Liquid-liquid phase separation: a principal organizer of the cell's biochemical activity architecture. *Trends Pharmacol Sci*. 2021;42(10):845–856. doi:10.1016/j.tips.2021.07.003
75. Wang B, Zhang L, Dai T, et al. Liquid-liquid phase separation in human health and diseases. *Signal Transduct Target Ther*. 2021;6(1):290. doi:10.1038/s41392-021-00678-1