

## Phase separation may drive mitochondrial nucleoid compartmentation

## ARTICLE INFO

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The compartmentation is critical for cells in carrying multi-functions. Besides the inner membrane system, which segregates space in cells for assembling reaction centers, phase separation could also concentrate biomolecules. Phase separation is a kind of biomacromolecular condensation driven by intrinsically disordered regions and weak multivalent interactions. Independent of the membrane systems, phase separation greatly expands the compartmentation in cells and reduces the threshold in generating the small reaction centers [1]. Phase separation has been reported to be involved in assembling many kinds of nucleus speckles, membraneless organelles, cell junctions, etc, especially the generation of speckles, functional center and even the architecture of the nucleus [2].

Mitochondria are the powerhouse of eukaryotic cells, which play multiple roles in energy production, metabolism, apoptosis, etc. In mammalian cells, mitochondria are also the only semi-autonomous organelles containing their own DNA (mtDNA), which is compacted into a spherical protein-DNA complex known as nucleoid, carrying multiple roles in mtDNA storage, replication, transcription, etc. Each mitochondrion has several nucleoids, which form discontinuous, well-organized compartments in the mitochondrial matrix. Mitochondrial nucleoids present dynamic forms that undergo fission and fusion. They also share many characteristics with the compartmentation of chromatin, such as heterochromatin, which is driven by phase separation. However, how mitochondrial nucleoid is assembled and, especially, how nucleoid carries multiple roles are still under investigation [3].

Recently, Long Q. et al. has identified that mitochondrial transcription factor A (TFAM), the histone like protein in mitochondria, undergoes phase separation in physiological conditions, and has proposed a novel model of mitochondrial nucleoid assembling and transcription modulation via phase separation (Fig. 1) [4]. They have identified that TFAM, the highly abundant protein in the mitochondrial matrix that compacts mtDNA by bending DNA and shaping the core structure of the nucleoid, could undergo co-phase separation with mtDNA, which drives the self-assembling and compartmentation of the nucleoid [4].

Moreover, the phase separation of nucleoid promotes the recruitment of the transcription initiation complex via co-phase separation, which could also concentrate substrates as nucleoside triphosphates (NTPs) for efficiently transcription. A special multi-phase separation of POLRMT, the polymerase for mtDNA transcription, with nucleoid was also identified. POLRMT is a ring-like structure surrounding mtDNA in vitro and in cells, making a standby status for transcription. The multi-phase separation presents an important role in regulating transcription, for which the shield-structure of POLRMT in multi-phase separation with nucleoid could be broken by mitochondrial transcription factor B2 (TFB2M) in promoter melting or by mitochondrial transcription elongation factor (TEFM) in transcription elongation. A similar multi-phase separation of the mitochondrial transcription termination factor (MTERF1), the termination factor of the transcription, was also observed, which further demonstrates the importance of multi-phase separation in mitochondrial transcription regulation [4].

The theory of phase separation, which may drive both the nucleoid self-assembly and transcription regulation in mitochondria, is proposed for the first time. It may also serve in replication and translation of mtDNA as the compartmentation driven by phase separation is critical for the multi-functional role of the mitochondrial nucleoid. The phase separation in mitochondrial nucleoid greatly expands the compartmentation of nucleoid and reduces the threshold in generate multiple reaction centers, presenting important physiologic and pathophysiologic implications [5-9].

According to the endosymbiotic theory, mitochondria originate from ancestral prokaryotic organism, which were engulfed by a primitive eukaryotic cell about two billion years ago [10]. The number of genes in mitochondrial genomes has shrunk through evolution, for which most were either lost or transferred to the nucleus [11]. Similar to the bacterial nucleoid compacted by the nucleoid-associated protein HU [12], one of the most prominent histone-like proteins in bacteria, mitochondrial genome is packaged by TFAM [13]. So far, HU protein has not been reported to phase separate, while TFAM could undergo phase separation

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**Abbreviations**

mtDNA: mitochondrial DNA  
 Pol II: RNA polymerase II  
 RNAPs: RNA polymerases;  
 TFAM: mitochondrial transcription factor A

mtDNA replication [22], the mtDNA replication system could also function like transcription via a phase separation system, where the TFAM-mtDNA droplet could play an important role [13].

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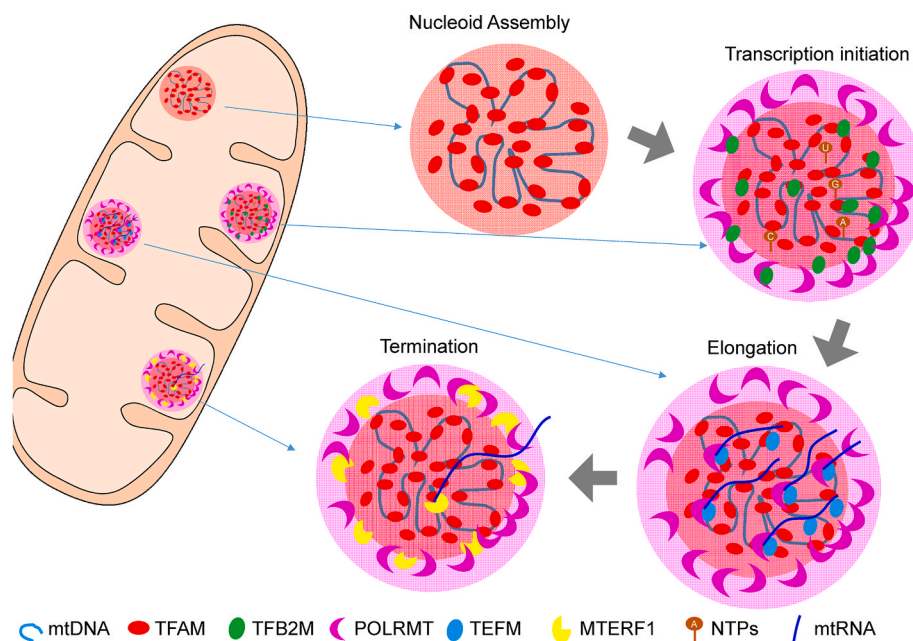


Fig. 1. Phase separation in organization and transcription of mitochondrial nucleoids.

and assemble mitochondrial nucleoids [4,14]. Surprisingly, mitochondrial transcription is carried out by a mitochondrial RNA polymerase (mtRNAP or POLRMT) that is more similar to single-subunit RNA polymerases (RNAPs) in bacteriophages [15], but is not related to the multi-subunit RNAPs, such as bacterial RNAP or eukaryotic RNA polymerase II (Pol II), both exhibiting phase behavior [16–18]. In contrast to the self-sufficient bacteriophage T7 RNAPs, POLRMT requires the assistance of additional protein factors for each step of the transcription cycle [3]. Long Q et al. has proposed a multi-phase separation model of the mitochondrial nucleoid in transcription [4]. POLRMT and other auxiliary protein factors did not display any phase separation individually or with added DNA. Mitochondria have evolved in a unique manner to orchestrate the nucleoid organization and transcription. TFAM presents dual roles in transcription: 1) TFAM-mtDNA can undergo phase transition to recruit transcriptional substrates and transcription machineries, resulting in dramatically concentrating these enzymes and substrates for efficient transcription; 2) TFAM is a component of the transcription initiation complex [19].

Chloroplasts, another semi-autonomous organelle, have two types of RNA polymerases, including the bacterial type plastid-encoded RNA polymerase and the phage-type nuclear-encoded RNA polymerase [20]. Nevertheless, the nucleoid of chloroplasts is packaged by an HU-like protein [21], not the TFAM homolog. It is interesting to investigate whether the organization and transcription of chloroplasts nucleoid is mediated by phase separation.

In summary, the study by Long Q et al. presented evidence that mitochondrial nucleoid assembly may be driven by phase separation, proposing a model of recruitment of the mitochondrial transcription machinery through phase separation. Since mitochondrial transcription produces not only RNAs for protein synthesis but also primers for

**Declaration of competing interest**

None.

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