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PERSPECTIVES

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Chromosome Territorial Organization Drives Efficient Protein Complex Formation: A Hypothesis

Manindra Bera* and Ramalingam Venkat Kalyana Sundaram

Department of Cell Biology, Yale University School of Medicine, New Haven, CT

In eukaryotes, chromosomes often form a transcriptional kissing loop during interphase. We propose that these kissing loops facilitate the formation of protein complexes. mRNA transcripts from these loops could cluster together into phase-separated nuclear granules. Their export into the ER could be ensured by guided diffusion through the inter-chromatin space followed by association with nuclear baskets and export factors. Inside the ER, these mRNAs would form a translation hub. Juxtaposed translation of these mRNAs would increase the cis/trans protein complex assembly among the nascent protein chains. Eukaryotes might employ this pathway to increase complex formation efficiency.

INTRODUCTION

A protein molecule has to traverse a crowded macromolecular environment inside the cell to find the right binding partners. Stochastic diffusion in highly crowded environments can be an impediment for biological interactions. Non-specific or semi-specific interactions are another major barrier to forming protein complexes at a specific location. In absence of a concerted transport mechanism, a significant percentage of proteins would be unable to find their binding partners. As organismal complexity increased during evolution, different mechanisms have been employed to increase local concentration of binding partners such as active transport, local translation, post-translational modification to anchor onto the membrane and phase-separation. However, maintaining the local stoichiometry of the components of a complex was another hurdle. A variety of mechanisms were developed to overcome this hurdle.

Prokaryotes produce polycistronic mRNA and simultaneous translation helps the nascent chains initiate folding of the cis and/or trans complex at a very early stage. Conversely, in eukaryotes, mRNAs originate from different gene loci and/or different chromosomes. Therefore, eukaryotes need to employ spatiotemporal regulation to the trafficking of both mRNA and individual protein com-

*To whom all correspondence should be addressed: Manindra Bera, Department of Cell Biology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT USA, 06520; Tel: 203-737-3269, Email: manindra.bera@yale.edu, bera.manindra@yahoo.com.

†Abbreviations: TREX, transcription-coupled export; CT, chromosome territories; TAD, Topologically Associating Domain; CIDs, Chromatin Interaction Domains; NHCC, non-homologous chromosomal contacts; FISH, Fluorescence *in situ* hybridization; 3C, Chromosome Conformational Capture; RNA-TRAP, RNA tagging and recovery of associated proteins; OR, Olfactory receptor; ICD, Inter Chromatin Domain.

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ponents to increase the efficiency of the protein complex formation. Eukaryotes developed mRNA transport and localized translation. During evolution, they physically separated their genome from the cytoplasmic translation machinery requiring export of mRNA transcripts into the cytoplasm to complete their translation. In neuronal cells and developmental tissues, localized translation solves this problem [1]. Co-translation and folding of the nascent polypeptide chains trigger protein complex formation which may be an adaptive strategy of the eukaryotic cell [2]. It is still poorly understood how eukaryotes maintain the homeostasis of the protein complex formation.

In this article, a hypothesis for the efficient pathway for protein complex formation has been proposed. Functionally relevant genes inside the nucleus form a phase-separated cluster and their transcription can be regulated in a concerted manner. mRNA transcripts from these genes can colocalize into a phase-separated granule. Export of these mRNAs can be dictated by genome organization which leads to the formation of a translation hub in the ER membrane. Co-translational protein folding and interactions among the nascent chains in these translation hubs may resolve the spatiotemporal barrier of the protein complex formation.

LIFE OF mRNA INSIDE THE NUCLEUS

Eukaryotic mRNAs are synthesized by the RNA Pol II as precursor mRNA and then capped at the 5' end by capping enzymes. These pre-mRNAs move to interchromatin granule clusters for the final splicing [3]. Several nuclear factors form dynamic clusters such as nuclear speckles in the nucleoplasm which are the potential hub for mRNA splicing [4,5]. These speckles are phase-separated granules with sizes varying from 20 to 25 nm and they are interconnected by thin fibrils [6]. In mammals, nuclear speckles or interchromatin granule clusters consist of several splicing factors including hnRNPs, snRNPs, and mRNPs [7]. The sizes of these speckles vary from one to several microns [8]. These nuclear speckles are concentrated with serine and arginine (SR)-rich proteins [9] and their phosphorylation and dephosphorylation determine the stability of the speckle formation [4,10]. In eukaryotes, the TREX (transcription-coupled export) complex associates with mRNPs and triggers the nuclear export [11,12]. TREX-complex physically interacts with the nuclear baskets and subsequently delivers the mRNA into the central channels of the nuclear pore complex [13,14]. mRNAs are exported to the cytoplasm in the 5' to 3' orientation through the coupled actions of CBP20, TREX complex and ATP-dependent RNA helicase [11,13,15].

TERRITORIAL ORGANIZATION OF THE EUKARYOTIC GENOMES

In diploid nuclei, organization of the chromosomes inside the nucleus is non-random during interphase. This was first proposed by Theodor Boveri in 1909. He also predicted that the territorial organization of chromatins are stably maintained in daughter nuclei as well [16]. Inside the nucleus, chromosomes maintain a spatial separation between active and inactive chromatins where inactive heterochromatins tend to be localized near the nuclear periphery while actively transcribing euchromatins are localized more toward the center of the nucleus [17,18].

These spatial organizations can further be dissected into large multi-Mb compartments containing open chromatin (A-compartments) and inactive, closed chromatin (B-compartments). These compartments can interact with similar compartments like an A-compartment can cluster with other A compartments [19,20]. However, these interactions are dependent on specific cell types and are cell cycle-dependent. Gene-rich chromosome territories (CT) like Ch19 (human) are less compact than the gene-poor chromosome like Ch18 [18,21]. CTs always maintain a structural hierarchy of chromatin consisting of 10 and 30 nm fibers and 60 to 130 nm chromonema fibers [22,23]. Folding of these chromatin fibers is still being debated. These chromosomal organizations regulate gene expressions [24].

Through evolution, cells have also acquired several mechanisms to regulate gene expression. Recent advances in Hi-C techniques revealed that chromosomes can maintain an additional level of compaction, known as Topologically Associating Domain (TAD) [25-27]. From bacteria to humans, genomes are organized into a string of spatially separated domains which maintain preferential interactions. In mammals, these domains range from several hundred kb to 2 Mb in size, while in bacteria, they are about ~200 kb in size. These domains are defined as TADs and Chromatin Interaction Domains (CIDs) in eukaryotes and bacteria respectively. TADs are smaller than the A- or B-type compartments and mostly independent of the cell types [20,28] and they can facilitate transcriptional regulation [20]. TADs can maintain the chromatin partitioning and colocalize with transcription granules [29].

It has also been observed that long-range interactions among the chromosome during transcription can also play an important role [30]. "Intermingling" or "kissing" chromosomes as non-homologous chromosomal contacts (NHCC) was first experimentally observed using laser UV microbeam by Cremer group [31,32]. These NHCCs are the coalescence of several chromosomes. Furthermore, the chromatins with high gene densities can extend into

the interchromosomal space or interchromatin domains (ICDs) from their chromosomal territories [33]. The TAD structure and genome organization are appropriately explained in the "loop-extrusion" model that proposes how the intrachromosomal interactions help to cluster distal regulatory regions into close 3D proximities [34]. More recently, the phase separation model proposed that the interactions of low complexity regions of the transcription regulators with DNA and RNA maintain genome compartmentalization [35,36].

COLOCALIZED GENE TRANSCRIPTION

The movements of the active chromatins within the nucleus are not random. Several actively transcribing genes are localized at the focal points of RNA Pol II which are called "transcription foci" [37-39]. As the number of transcription foci are fairly small compared to actively transcribing genes, it can be suggested that several genes are transcribed together. Indeed, several FISH and 3C experiments have shown convincingly the colocalization of active alleles which are several Mbs apart, either in cis- or trans-chromosomes [37,40,41].

Long-range interactions between genes and their regulatory elements like enhancers have been heavily studied [42]. A number of mechanisms have been proposed to explain long-range gene regulations such as chromatin loop formation linked by large protein complexes [35,43,44]. Expression of the β-globin locus is an example of a well-characterized long-range interaction and chromatin loop formation [45]. The enhancer elements and local control region (LCR) form a loop with the β globin genes (HBB) as experimentally shown by 3C and RNA tagging and recovery of associated proteins (RNA-TRAP) methods [46,47]. Several other long-range interactions between genes and their regulatory loci such as CFTR locus [48,49], c-MYC locus [50], Th2 interleukin cluster [51] have been described subsequently.

Recent Hi-C and computational predictions revealed that very specific long-range interactions between enhancers and promoters can be found within the boundaries of TADs [52,53]. Another example is the olfactory receptor (OR) genes (~1400 genes) that are located across 18 different chromosomes but through a complex choreography, they all congregate into the same interchromatin space called the "olfactosome" at the time of expression [54]. These examples show that although eukaryotes do not possess polycistronic mRNA, the expression of several genes can be controlled through spatial gene clustering mechanisms which are as of yet unclear.

Nuclear pore complexes are the bi-directional gates for communications between nucleoplasm and cytoplasm. Localization of the nuclear pore complex on the nuclear membrane is non-random [55]. The underlying spatial organization of the genome is regulated by the lamin network [56]. In the gene-gating hypothesis, Gunter Blobel proposed that all the transcripts of genes are destined to specific sets of nuclear pore complexes [57]. Eukaryotes organize their genome by keeping each chromosome at a separate location during interphase [58]. Each chromosome maintains its specific neighbors at the interphase stage, forming a chromosomal territory [31,59,60]. Lamin meshwork helps to organize the chromosome territories [61]. Territorial organizations of the chromosomes vary by cell types and even for the same type of chromosome in the diploid cell [21]. During transcription, each gene forms a transcription loop to provide access for the transcription machinery [62]. Several genes inside the nucleus form "kissing loops" with their neighbors and maintain a transcription hub [63,64]. Often, these transcription hubs form a phase-separated state through intrinsically disordered regions of the transcription machinery and nascent mRNAs [65,66].

CT DRIVES THE PROTEIN COMPLEX FORMATION: A PERSPECTIVE

Phase-separation inside the cell is an emerging concept providing a more general mechanism for several compartmentalized biochemical reactions. Recently, several groups proposed a phase-separation model for transcriptional control [35,36,67,68]. The low complexity region of the transcription regulators forms a liquid-liquid phase separation which helps to co-cluster several genes and their enhancers forming a super-enhancer [69]. These condensates provide a platform for co-transcription and regulations of several genes. The RNA-protein ratio in these interchromatin spaces is crucial for the formation of the phase-separated granules [70,71]. The interchromatin spaces are filled with nuclear speckles and mRNPs. The numbers of these speckles vary from 20 to 50 in each nucleus [72,73]. SR-rich proteins maintain these membrane-less granules and several kinases and phosphatases control their dynamicity [74]. The various transcripts from the neighboring chromatin kissing-loops may cluster together through the association of these RNPs. It has been shown earlier by different groups that the diffusion of the individual mRNPs are random [75,76]. It has also been proposed that mRNA transcripts possess a unique zip code which tightly coupled the downstream processes like translation and subsequently protein complex formation [77,78]. However, the mRNA transcripts vary in size and till now, there is no evidence suggesting any mRNA signal code coupled to the translations. Genome-wide analysis has shown that the specific export factors are available to tightly regulate the export of different types of mRNA [79,80].

The body of evidence presented thus far support the

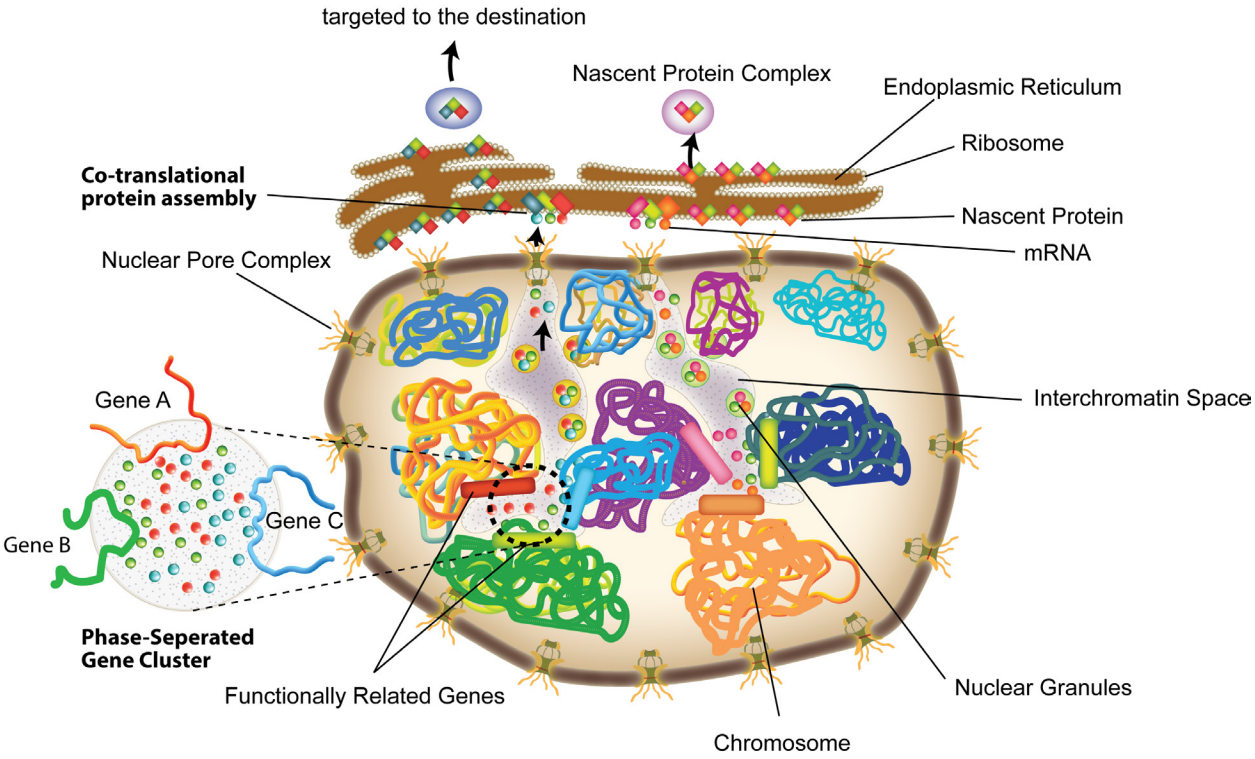

Figure 1. A model for chromosome loop driving the protein complex formation. Functionally related genes (Gene A, B, and C) from different chromosomes extend their loops into the inter chromosome space and form phase-separated clusters (zoomed out). Their transcriptions are tightly coupled through the interactions of the transcription factors and mRNAs synthesized from these transcription loops in these clusters can be recruited to the specific nuclear granules. The movements of these granules are guided through the interchromatin channels and their exits are destined to the nearby nuclear pore complex and finally exported into the ER. On the ER membrane, these mRNAs form a translational hub and local translation of these mRNAs helps to form the co-translational protein assembly (ABC- Nascent Protein Complex). On the ER membrane, translation hub increases concentrations of the nascent chains which induce cis-trans-complex formation. The pre-assembled protein complex travels to their corresponding destination via the protein transport vesicles (circular box containing protein complex). These chromosomal loop formations increase the efficiency of nascent chain complexes formation. This proposed pathway could be the adaptive strategy of the eukaryotes to avoid the futile protein production.

hypothesis that chromosome kissing loops in the active A-compartments can efficiently drive protein complex formation. Chromosome kissing loops are often a conglomerate of several chromosomal contacts extended into the interchromatin space. These loops may contain functionally related genes and their regulators that form a phase-separated granule through the interaction of the low complexity regions of the transcription regulators. Although each loop can be very long and dynamic, the downstream products of these kissing loops will participate in the forming protein complexes in the cytoplasm. The mRNA transcripts from these loops can cluster together into the ICDs with the association of the splicing machinery, mRNPs and several nuclear export factors. These mRNPs and nuclear export factors can furthermore act as sorting machinery for the functionally related mRNAs. Although the diffusion of the individual mRNPs is random, these diffusions can be guided via the narrow channels of interchromatin space. These channels can be gated to specific nuclear pores as proposed by Gunter Blobel and interaction between the nuclear baskets and TREX-complex can help to nucleate various mRNPs containing functionally related mRNAs. Coupled exports of these mRNPs can help form a translation hub in the ER and the co-clustering of these translation hubs can ensure the high local concentration of the nascent polypeptide chains which leads them to form co-translational cis and/or trans-protein complex (illustrated in Figure 1).

The proposed hypothesis is the following.

1. Chromatin kissing loops in the active compartments are enriched with the genes which participate in protein complex formation in the cytoplasm.

2. Transcriptions of these genes are regulated in a concerted manner by forming a phase-separated state maintained by the disordered domains of the transcription factors.

3. The mRNA transcripts from these kissing loops can cluster into the nuclear granules or speckles via the association of mRNPs, splicing machinery and different classes of the export factors thus forming phase-separated condensates.

4. Diffusion of these granules is guided through narrow channels in the interchromatin spaces towards specific nuclear pore complexes.

5. Association of the nuclear baskets and nuclear export factors nucleate the mRNPs and couples their export into the cytosol.

6. Juxtaposed translations of these mRNAs onto the ER membrane ensure co-translational cis/trans protein complex formation.

CONCLUSION

Maintaining the local concentration and stoichiometry of the individual components are fundamental to all biological interactions. Co-translation and movements of the mRNAs may facilitate protein complexes formation. The pathway of complex formation can stem from the genome organization as proposed above. Concerted transcriptional regulation of gene clusters can ensure the stoichiometry of the individual components. Chromatin loops are transient and their dynamic association could lead to the formation of protein complexes.

It has been shown that the RNA to protein ratio in nuclear granules is crucial to maintaining phase-separated granules, hence loading of mRNAs into granules could be highly regulated [81]. The ubiquitous examples of formation of co-translational protein complexes in eukaryotes [2,82,83] supports this protein complex formation hypothesis.

Certainly, not all protein complexes formed within cells follow these sequence of events but when they do, the efficiency of the complex formation may be several orders of magnitude higher. ER-mediated co-translational membrane insertion is the fundamental step for the membrane protein maturation. Hence, eukaryotes can employ this proposed pathway to increase the efficiency of membrane protein complex formation. However, there is no experimental evidence to prove this hypothesis yet. In fact, there have been observations that movements and exports of the mRNPs are random [84]. Hi-C data from the single-cell analysis failed to show clusters of functionally related genes. However, since the loop structures are transient and cell cycle-dependent, these inter-chromatin contacts were probably difficult to detect under these experimental conditions.

Over the past few decades, certain protein complexes have been studied extensively for their heteromeric co-translational protein assembly in eukaryotes. These include membrane-bound voltage-gated K+ channel [85], hERG ion channel [86], D1 protein of the photosystem II [87], IgE high-affinity receptor [88], soluble histone methyltransferase [89], and acetyltransferase [90]. Studying their mRNA movements and gene clustering in the transcription foci and tracking individual RNPs can be useful in proving this hypothesis. This can be done by using fluorescence *in situ* hybridization (FISH) probes specific to these functionally related genes and followed by co-translation immunoprecipitation of these proteins. The hypothesis predicts the co-movement of mRNAs from functionally related genes as long as they originate from the same chromosome kissing loop. However, a systematic investigation is needed to further confirm the hypothesis.

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