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OPINION

HEAT repeats – versatile arrays of amphiphilic helices working in crowded environments?

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ABSTRACT

Cellular proteins do not work in isolation. Instead, they often function as part of large macromolecular complexes, which are transported and concentrated into specific cellular compartments and function in a highly crowded environment. A central theme of modern cell biology is to understand how such macromolecular complexes are assembled efficiently and find their destinations faithfully. In this Opinion article, we will focus on HEAT repeats, flexible arrays of amphiphilic helices found in many eukaryotic proteins, such as karyopherins and condensins, and discuss how these uniquely designed helical repeats might underlie dynamic protein–protein interactions and support cellular functions in crowded environments. We will make bold speculations on functional similarities between the action of HEAT repeats and intrinsically disordered regions (IDRs) in macromolecular phase separation. Potential contributions of HEAT–HEAT interactions, as well as cooperation between HEATs and IDRs, to mesoscale organelle assembly will be discussed.

KEY WORDS: HEAT repeat, Karyopherin, Condensin, Molecular crowding, IDR, Phase separation, Hydrogel

Introduction

HEAT repeats, repetitive arrays of short amphiphilic α-helices, are found in a wide variety of eukaryotic proteins with diverse functions. The acronym HEAT comes from four proteins that were originally found to contain this repeat motif, that is Huntingtin, elongation factor 3, the A subunit of protein phosphatase 2A (PP2A) and the signaling kinase TOR1 (Andrade and Bork, 1995). Previous structural and biophysical studies have provided evidence that HEAT repeats undergo highly flexible and elastic conformational changes when they interact with different binding partners or when external forces are applied to them (Grinthal et al., 2010). This high degree of flexibility is based on an unusual hydrophobic core that supports intramolecular helix–helix interactions (Kappel et al., 2010), and therefore has a potential to respond to differential environmental factors, such as ionic strengths and macromolecular crowding. Very little is known, however, about how these unique structural properties of HEAT repeats might be utilized in the various functions of macromolecules and in their specific intracellular contexts. In this Opinion article, we will provide an overview of and discuss two seemingly distinct cellular processes, namely, nucleo-cytoplasmic transport and mitotic chromosome assembly, in which HEAT repeat proteins play crucial roles. For instance, karyopherins, which are involved in nuclear transport, flexibly change their own conformation during nuclear translocation to move across the amphiphilic environment inside the nuclear pore channel. The HEAT subunits of condensin complexes appear to use their flexibility to support the dynamic assembly of chromosome axes in the highly crowded environment of the interior of chromosomes. We argue here that, in both cases, the amphiphilic nature of the HEAT repeats is at the core of these dynamic functions. Finally, we will also draw attention to potential similarities between HEAT-mediated protein dynamics and phase separation, an emerging concept of macromolecular assembly that is driven by proteins containing intrinsically disordered regions (IDRs).

Distribution of HEAT repeats in a wide variety of eukaryotic proteins

A single HEAT motif (~30–40 amino acids long) is composed of a pair of α-helices (referred to as A- and B-helices) connected by a short linker. The motif is highly degenerate at the primary structure level and can only be recognized by a very loose consensus sequence (Fig. 1A) (Neuwald and Hirano, 2000). Despite the degenerate primary structure, the secondary and tertiary structures of the HEAT motif are highly characteristic and well conserved. The two helices are amphiphilic (i.e. one surface is enriched with hydrophilic residues and the other surface with hydrophobic ones), and are arranged in an anti-parallel fashion so that their hydrophobic surfaces are concealed (Fig. 1B). The conserved hydrophobic residues help to define a rotational orientation of the two helices, and proline and aspartate residues are often found in the turn region. An additional unique property of the HEAT motif is the existence of another proline residue within the A-helix. This proline residue often kinks the helix and thereby affects the curvature of the solenoid (Cingolani et al., 1999), although its functional significance is not yet fully understood.

Multiple HEAT motifs occur in a long linear array, and constitute a HEAT repeat. The number of repeating motifs within individual HEAT repeat proteins is variable and ranges from 15 to 50, or even more. Owing to the loose consensus sequence, however, the exact positions and numbers of HEAT motifs are difficult to deduce from the primary sequences alone without any additional information from crystal structures. Based on their overall domain organizations, HEAT repeat proteins can be classified into three groups (Group I–III; Fig. 1C). Proteins in Group I are composed of a long consecutive repeat of HEAT motifs with little or no other discernible domains. This group includes karyopherins, a large family of nucleo-cytoplasmic transport receptors, and the A (scaffold) subunit of protein phosphatase 2A (PP2A), one of the founding members of HEAT repeat proteins (Xu et al., 2006; Cho and Xu, 2007). In Group II, stretches of IDRs divide a HEAT repeat array into several blocks; this group includes the regulatory subunits of condensin I (CAP-D2 and CAP-G, also known as NCAPD2 and NCAPG, respectively) and of cohesin (SA2, also known as STAG2,

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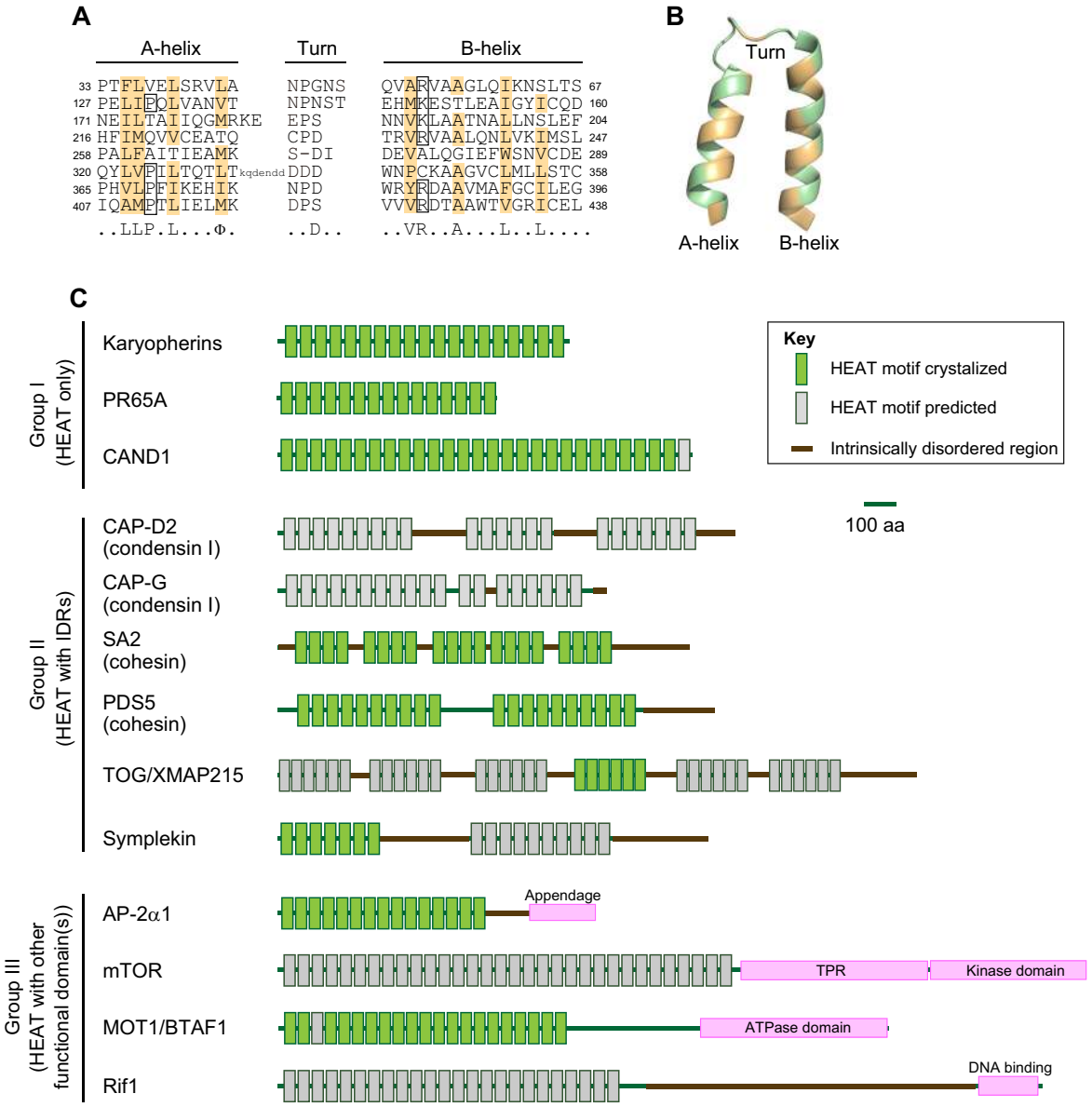

Fig. 1. Overview of HEAT-motif-containing proteins. (A) Sequence alignment of HEAT motifs in mouse importin β. A consensus sequence is shown at the bottom. A HEAT motif is composed of a pair of α-helices (A- and B-helices) that is connected by a short linker (turn). Conserved hydrophobic residues in the helices are marked by an orange background. Proline and positively charged (arginine or lysine) residues conserved in the A- and B-helices, respectively, are boxed. (B) In a HEAT motif, the A- and B-helices are arranged in an antiparallel fashion through hydrophobic interactions. Hydrophobic residues are marked in orange. (C) Domain organizations of the three groups of HEAT-motif-containing proteins in humans. HEAT motifs, IDRs (brown) and other functional domains (pink) are shown. HEAT motifs whose crystal structures have been determined are shown in green, whereas HEAT motifs predicted solely based on their primary sequences are shown in gray.

and PDS5), as well as the tubulin-polymerizing factor TOG (also known as XMAP215 and CKAP5) (Fox et al., 2014). HEAT repeat proteins of Group III possess additional well-defined structural or functional domain(s) within single polypeptides. For instance, in mammalian target of rapamycin (mTOR), a large N-terminal HEAT repeat is followed by a tetratricopeptide-repeat (TPR) and a protein kinase domain (Aylett et al., 2016). Similarly, the TATA-binding protein-associated factor MOT1 (also known as BTAF1) contains a Swi2/Snf2-type ATPase domain (Wollmann et al., 2011). It is also important to note that many HEAT repeat proteins interact with multiple proteins and often function as part of a large protein complex (Fig. 2). From this point of view, many HEAT repeat proteins that belong to group I function as scaffolds that accommodate adaptable interactions with numerous different binding partners. In contrast, HEAT repeat proteins classified into groups II or III have a limited number of binding partners, if any.

Structural properties of HEAT repeats

A number of crystallographic studies have revealed three-dimensional structures of HEAT repeat proteins, often together with their binding partners (for a review, see Stewart, 2007). In these structures, adjacent HEAT motifs are linked by short (inter-unit) turns, and they are successively stacked with each other, forming a two-layered helical array. Owing to twists and tilts between adjacent motifs, the entire repeat forms a right-handed solenoid in which A- and B-helices are aligned on the convex and

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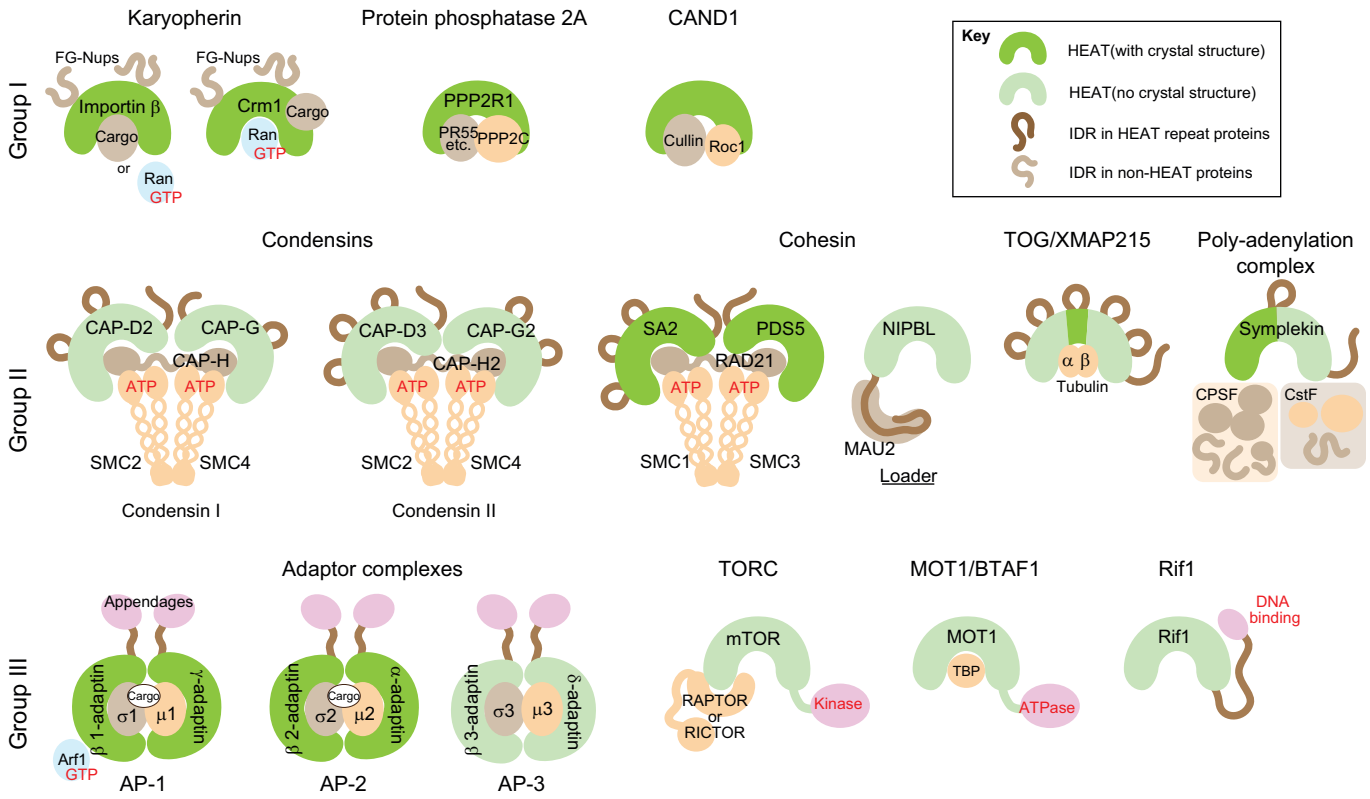

Fig. 2. HEAT repeats as part of large protein complexes. Some HEAT repeat proteins function as intrinsic subunits of large protein complexes, whereas others only temporarily interact with their partners. Many of HEAT repeat proteins that belong to Group I have numerous binding partners. In contrast, HEAT repeat proteins classified into Groups II or III have a limited number of binding partners, or possess specific functional domains (shown in pink) within single polypeptides. IDRs that occur within HEAT repeat proteins or HEAT-repeat-containing complexes (in-cis action) are shown in dark brown, whereas IDRs that occur within non-HEAT protein or complexes (in-trans action) are shown in light brown.

concave surfaces, respectively (Fig. 3A). The overall conformation of the solenoid (i.e. its diameter, curvature and pitch) varies from protein to protein, and is also affected by the interactions formed with their binding partners (Conti et al., 2006; Lee et al., 2000; Forwood et al., 2010). For example, structural comparison of the karyopherin importin β with and without the cargo has revealed substantial differences in the curvature of the solenoid (Cingolani et al., 1999, 2000). These results suggest that each crystal structure represents one snapshot of a number of different conformations that could be found in the entire energy landscape. Such structural flexibility is thought to play an important role in the ability of the protein to simultaneously interact with multiple binding partners. In the case of the PP2A holoenzyme, the HEAT-containing A subunit functions as a flexible scaffold that brings together the catalytic subunit and a wide variety of different regulatory subunits involved in substrate recognition (Xu et al., 2006; Cho and Xu, 2007; Janssens et al., 2008) (Fig. 2).

The structural flexibility of HEAT repeats has been directly characterized by spectroscopic approaches (Tsytlonok et al., 2013) as well as by small angle X-ray scattering (Forwood et al., 2010). Molecular dynamics simulations have also demonstrated that, when external forces are applied at the ends of the molecule, the HEAT repeats exhibit unique elastic properties similar to a Hookean spring, whereby the extension is proportional to the tension applied (Grinthal et al., 2010; Kappel et al., 2010). This means that the HEAT repeat is highly elastic against external forces (Fig. 3B). Remarkably, such linear extension is completely reversible, and can be observed up to forces of ∼100 pN after which, at a certain point, inter-helical interactions collapse (Grinthal et al., 2010). These findings suggest that the stress imposed on the ends of the HEAT repeats is redistributed along the entire repeat array.

What is the physiological significance of the structural flexibility and elasticity of HEAT repeats? One possibility is that the HEAT array functions as a mechanosensor by sensing and utilizing mechanical force to modify protein function (Grinthal et al., 2010; Viswanathan and Auble, 2011). For example, an external force applied to the HEAT subunit of PP2A could change the mode of inter-subunit interactions, thereby modulating the catalytic activity of the enzyme (Grinthal et al., 2010). Alternatively, even without external forces, structural fluctuations of the array could help expose binding sites for other proteins through a 'fly-casting' mechanism (Tsytlonok et al., 2013). In addition, the convex and concave arrays of the helices display different degrees of elasticity (Grinthal et al., 2010), thereby conferring highly complex elastic properties on the two-layered helical array of HEAT repeats.

HEAT repeats in nucleo-cytoplasmic transport

Karyopherins are among the best-studied classes of HEAT repeat proteins. They are involved in the molecular transport between the cytoplasm and nucleoplasm through the nuclear pore complex (NPC) that is embedded in the nuclear envelope (Peters, 2009). In the case of importins, they bind to their cargos in the cytoplasm and travel through the NPC, before releasing them in the nucleus (Fig. 3C). This catch-and-release mechanism and, hence, the directionality of the transport, is dependent on differential

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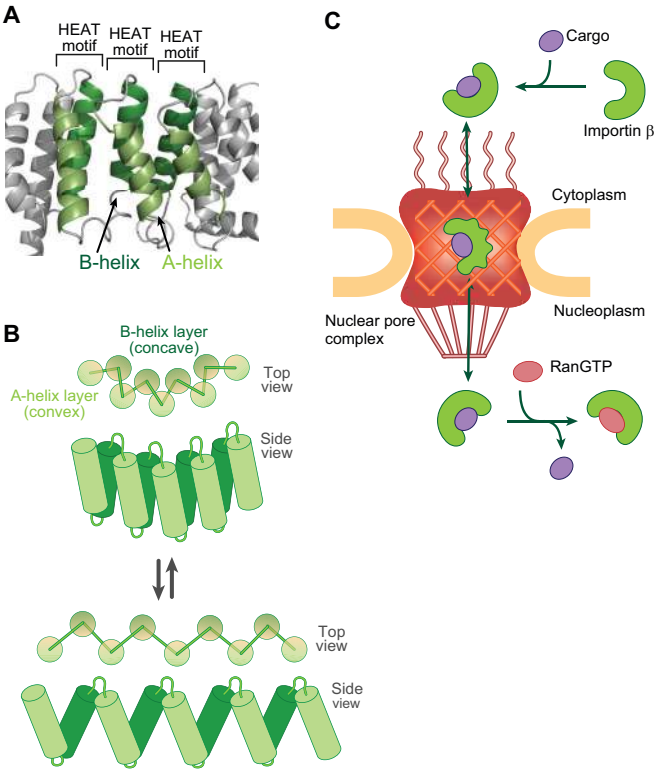

Fig. 3. Structural properties of HEAT repeats and the action of karyopherins. (A) Structure of a HEAT repeat array of yeast importin β (PDB code: 3ND2). Multiple HEAT motifs, each being composed of a pair of α-helices (A- and B-helices), are stacked with each other, forming a two-layered array. (B) Structural flexibility of HEAT repeats. Because the two-layered array of amphiphilic helices are organized by weak hydrophobic interactions, HEAT repeats are highly flexible and elastic; they have the potential to undergo large conformational changes by either interacting with other proteins, or responding to external forces or environmental changes. A-helices present in the convex surface are shown in light green, whereas B-helices present in the concave surface are shown in dark green. (C) Transport model of importin β through the nuclear pore complex. Importin β binds to its cargo in the cytoplasm and travels through the NPC, which is composed of flexible FG-Nups. Conformational changes occurring in the HEAT repeat facilitate the translocation of the importin–cargo complex through the crowded environment of the diffusion barrier. In the nucleoplasm, RanGTP binds to importin β and releases the cargo from importin β.

localization of the GTP- and GDP-bound forms of the small GTPase Ran (Lee et al., 2005; Matsuura and Stewart, 2004).

A number of crystallographic studies have revealed that the structural flexibility of importin β has important roles in its interactions with both its cargo and RanGTP. A structural comparison of different importin β molecules that are bound to cargo, RanGTP or nucleoporins, has revealed conformational differences not only in specific HEAT motifs, but also in the entire molecule (Fukuhara et al., 2004; Lee et al., 2005). An important implication here is that each of these distinct conformations of importin β might not so much represent a particular metastable structure in the entire energy diagram, but rather corresponds to one snapshot of a wide array of possible flexible conformations, as has been demonstrated by force-applying molecular dynamics simulations (Grinthal et al., 2010; Kappel et al., 2010) (Fig. 3B). Indeed, when importin β translocates through the NPC with its cargo, it needs to interact with a number of different nucleoporins (Nups) at its convex surface, while simultaneously holding onto the cargo at its concave surface. This challenging task requires dynamic and flexible conformational changes of the HEAT repeat of importin β.

Recent studies have focused on the interaction between karyopherins and intrinsically disordered Nups that contain phenylalanine-glycine motifs (collectively referred to as FG-Nups) (Milles et al., 2015; Bestembayeva et al., 2015; Zahn et al., 2016). Such hydrophobic residues are believed to crosslink the flexible polypeptide chains and to form a hydrogel, a meshwork structure that prevents cellular macromolecules from passively diffusing through the nuclear pore. Karyopherins interact with the FG motifs and other hydrophobic residues of FG-Nups through a hydrophobic pocket that is formed by adjacent A-helices of their HEAT repeat (Bayliss et al., 2000, 2002; Liu and Stewart, 2005). Our recent spectroscopic analysis combined with molecular dynamics simulation of importin β has demonstrated that the structural flexibility of HEAT repeats plays a crucial role in allowing the migration through the crowded space of the nuclear pore channel and is mediated through interactions with FG motifs (Yoshimura et al., 2014). Here, a number of weak interactions between multiple FG motifs and importin β induce temporary conformational changes in both the HEAT repeat and the matrix of FG-hydrogels, which enable karyopherins to migrate through the hydrogel-like environment of the nuclear pore channel (see below for more detailed discussion).

HEAT repeats in mitotic chromosome dynamics

Condensins are large protein complexes that play a fundamental role in chromosome organization and segregation (Hirano, 2016). Most eukaryotes have two different types of condensin complexes (condensins I and II), each of which is composed of five subunits (Fig. 2). The two complexes share the same pair of structural maintenance of chromosomes (SMC) ATPase subunits, but have distinct sets of non-SMC regulatory subunits. Among these, condensins I and II have different pairs of HEAT subunits, CAP-D2 and CAP-G, and CAP-D3 (NCAPD3) and CAP-G2 (NCAPG2), respectively. Although condensin-like complexes are also found among most bacterial and archaeal species, the HEAT-containing subunits are unique to eukaryotic condensins, implying that the HEAT subunits might be involved in eukaryote-specific aspects of large-scale chromosome organization.

However, exactly how this type of elaborate protein machine works to organize mitotic chromosomes is not fully understood. A recent study using *Xenopus* cell-free egg extracts has provided evidence that the HEAT subunits of condensin I have crucial roles in the dynamic assembly of chromosome axes (Kinoshita et al., 2015). Interestingly, the two HEAT subunits appear to have distinct roles in this process and are possibly involved in both construction and deconstruction of chromosomes that occur upon mitotic entry and exit, respectively. These findings raise the possibility that regulated HEAT–HEAT interactions between different condensin complexes underlie the organization of chromosome axes. At present, however, there is no direct evidence that supports this idea. To provide evidence for such a mechanism, several issues need to be taken into account. Firstly, if the predicted HEAT–HEAT interactions take place, then they would not involve stereospecific, stable interactions. Rather they would consist of an ensemble of multivalent, weak interactions that reflect the flexible and elastic nature of HEAT repeats (Kappel et al., 2010). Secondly, such interactions would be highly dynamic; condensins turn over rapidly under the control of their SMC ATPase activity, as has been implied from experiments using mutant complexes in cell-free extracts (Kinoshita et al., 2015) or from fluorescence recovery after

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photobleaching (FRAP) experiments *in vivo* (Gerlich et al., 2006). Thirdly, these postulated interactions would occur only on (or inside) chromosomes, and would not take place when the condensin complexes are not bound to chromosomes. In fact, no physical interaction between purified condensin complexes has been detected thus far. Furthermore, the molecular environment surrounding and constituting mitotic chromosomes might also be crucial as discussed below.

Both condensins I and II are enriched at the axial core of metaphase chromosomes, and their cooperative actions have crucial roles in determining the shape and physical properties of eukaryotic chromosomes (see Box 1). Condensin II associates with chromosomes in prophase earlier than condensin I, and is found more internally than condensin I in metaphase chromosomes (Fig. 4A). Why and how the different condensins are enriched at these chromosomal regions is unknown. Although the interior of mitotic chromosomes is highly crowded (Hancock, 2012; Wachsmuth et al., 2008), at the same time, it is also a network of well-solvated chromatin that is held together by noncovalent crosslinking proteins (Poirier and Marko, 2002) and is readily accessible by macromolecules (Hihara et al., 2012). Thus, the interior of chromosomes could share some of the physico-chemical properties of a hydrogel. In fact, micromechanical experiments using micropipettes have shown that mitotic chromosomes are highly elastic objects that return to their native lengths even after five-fold extensions (Marko, 2008). Compared with the interior of chromosomes, their periphery is expected to be less dense and to behave like a liquid, as has been predicted for interphase chromatin (Maeshima et al., 2016). Along these lines, an intriguing observation from an early study is that the condensin subunit SMC2 first appears at the surface of condensing chromosomes in middle prophase, and then suddenly translocates into the interior of chromosomes where axial structures are formed by late prophase (Kireeva et al., 2004) (Fig. 4A). We speculate that this relocalization is accompanied by conformational changes in condensin subunits, in particular, the HEAT-containing subunits. HEAT-mediated condensin–condensin interactions could then occur in a

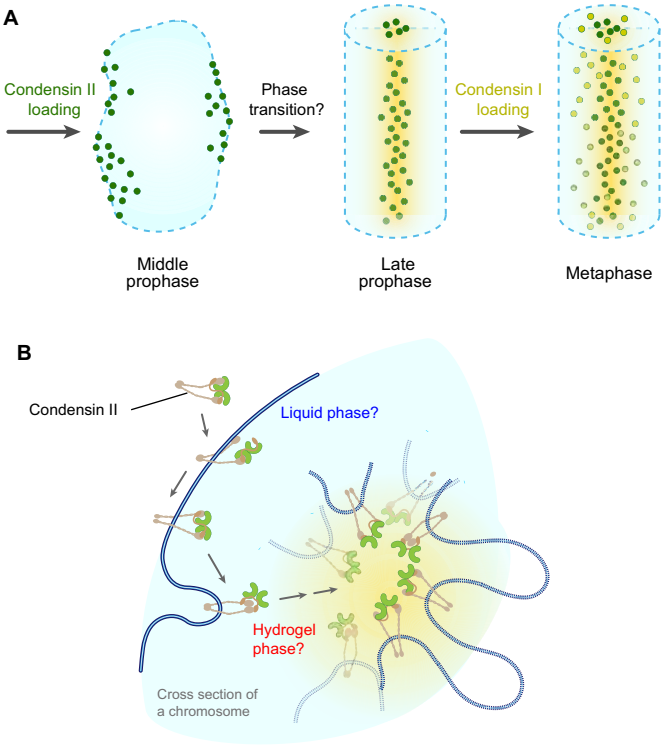
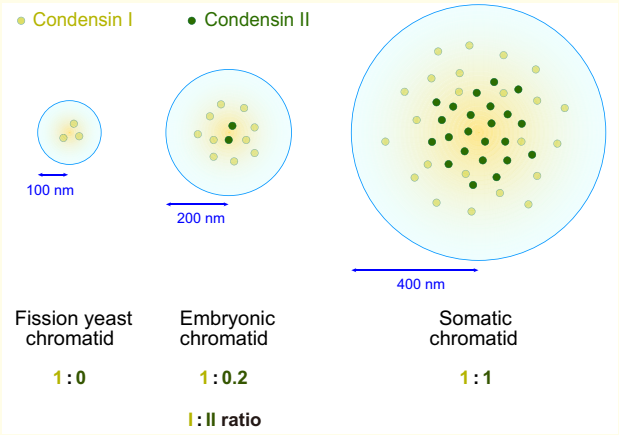


Fig. 4. Dynamic behaviors of condensins during mitosis. (A) Architecture and subunit composition of the eukaryotic condensin complexes are shown in Fig. 2. Condensins I and II have different pairs of HEAT subunits, CAP-D2–CAP-G and CAP-D3–CAP-G2, respectively. Condensin II (dark green) first appears on the surface of chromatin in middle prophase, and then translocates into the interior of chromatids (represented by the dashed cylinder) to form their central axes by late prophase. Upon nuclear envelope breakdown in prometaphase, condensin I (light green) gains access to chromatids and accumulates around the condensin-II-positive chromosome axes by metaphase. (B) Hypothetical actions of condensin II. Condensin II binds to chromosomes at their periphery, possibly through an ATP-dependent entrapment mechanism. The ATPase cycle of the SMC subunits could further modulate any conformational changes of the HEAT subunits and also trigger HEAT-mediated condensin–condensin interactions in the interior of chromosomes (Kinoshita et al., 2015). The translocation of condensin II from the exterior to the interior of chromosomes and the resulting assembly of chromosome axes could steer a phase transition of chromatin from a liquid-like structure to a hydrogel.

Box 1. Chromosome size and shape – relevance of two condensin complexes



Most eukaryotic species have both condensins I and II, but some lack condensin II (Hirano, 2016). Shown here are cross-sections of three different chromatids with different diameters. For instance, small chromatids, such as those in fission yeast, contain condensin I only (shown on the left). In vertebrates, embryonic (middle) and somatic (right) chromatids have different ratios of condensins I and II, and display different shapes; the embryonic chromatids are thin and long, whereas the somatic ones are thick and short. Experiments using *Xenopus* cell-free egg extracts have provided evidence that the relative ratio between condensin I and II determines chromosome shape (Shintomi and Hirano, 2011). Moreover, condensin II is located more internally than condensin I, which is found along the axial core of chromatids (Ono et al., 2003). On the basis of these and other data, condensin II has been proposed to contribute to lengthwise shortening, especially of large and thick chromatids (Shintomi and Hirano, 2011), and to confer their physical rigidity (Houlard et al., 2015). Thus, if condensins indeed act as crosslinkers for chromatin networks as has been predicted (Marko, 2008), condensin II would be a more robust crosslinker than condensin I. This raises the question of whether the different pairs of HEAT repeat subunits present in the two condensin complexes confer different crosslinking properties. To that end, it will be of interest to carefully compare their biochemical properties in order to understand how they might differentially contribute to assembly and maintenance of chromosomes with characteristic physico-chemical properties. Critical comparisons with non-biological, amphiphilic materials that self-assemble to form a rod-shaped structure (Qiu et al., 2015) will be another exciting direction of future research.

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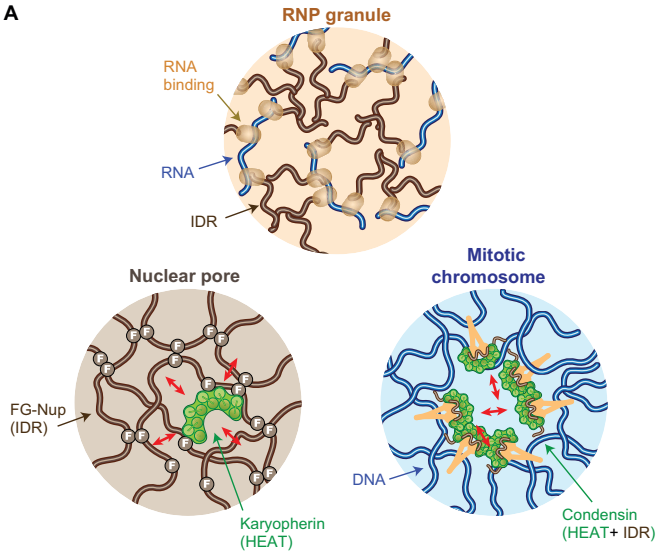
cooperative manner and support the dynamic assembly of chromosome axes. Such interactions could be further favored by the newly created, crowded environment at the interior of chromosomes. Thus, this step of large-scale reorganization of chromosomes is reminiscent of a phase transition from a liquid phase to a hydrogel phase (Fig. 4B). It is certainly possible that condensin I, which localizes to chromosomes later, is attracted to them by the environment that is created by condensin II (Box 1).

Potential similarities and functional cooperation between HEAT repeats and IDRs?

A recent series of studies has uncovered a hitherto-unexpected cellular phenomenon, known as phase separation, in which promiscuous interactions among IDRs underlie the dynamic assembly of intracellular membrane-less organelles (Hyman and Brangwynne, 2011; Weber and Brangwynne, 2012). For instance, ribonucleoprotein (RNP) granules such as P granules are composed of RNAs and RNA-binding proteins that have IDRs (Fig. 5). It has been proposed that IDRs, which often contain so-called low-complexity sequences (LCSs), contribute to multivalent weak interactions, and so help to assemble a liquid droplet that separates them from the surrounding nucleoplasm (Bergeron-Sandoval et al., 2016). Although the molecular mechanism by which phase separation is achieved is not fully understood, it is believed that the underlying protein–protein interactions differ from conventional stereospecific interactions, and depend on the different amino acid compositions of the IDRs involved (either electrostatic, hydrophobic or both) (Pak et al., 2016). It has been shown that NPCs also use a similar molecular principle to form a hydrogel in the central channel, although its composition is completely different from that of RNP granules (Schmidt and Görlich, 2016). In the case of NPCs, the FG-Nups, which are largely composed of IDRs, act as the major polymer component of the meshwork (Fig. 5). IDRs are generally rich in polar and charged residues, but what distinguishes FG-Nups from other IDRs is the inclusion of repetitive hydrophobic residues such as phenylalanine
(i.e. FG motif). Hydrophobic interactions between phenylalanine residues crosslink non-structured hydrophilic polypeptides, thereby forming a hydrogel-like meshwork in the pore channel (Frey et al., 2006) (Fig. 5). By taking advantage of the flexible array of the amphiphilic helices, karyopherins disengage hydrophobic Nup–Nup interactions by simultaneously binding to multiple FG motifs (Hülsmann et al., 2012). In this way, karyopherins transiently open the meshwork and allow their rapid migration through the crowded environment. In fact, the translocation kinetics of importin β through the NPC depends on the concentration of importin β itself (Ribbeck and Görlich, 2001; Yang and Musser, 2006; Ma et al., 2016), implying that the HEAT repeat actively participates in the transient deconstruction and reconstruction of the hydrogel phase of the pore channel. In this sense, karyopherins themselves could be considered as a temporal component of the flexible hydrogel rather than a mere traveler migrating through the rigid meshwork structure (Fig. 5).

What about mitotic chromosomes? It is tempting to speculate that the mechanism of chromosome assembly discussed above might share some common elements with that of phase separation. Like RNP granules, mitotic chromosomes are membrane-less organelles that are composed of nucleic-acid-based polymers (i.e. chromatin fibers) and protein components that function as flexible crosslinkers (i.e. condensins) (Fig. 5). In both structures, there is a rapid exchange of protein components between the bound pool and the free pool that is present in the surrounding environment (Kinoshita et al., 2015; Gerlich et al., 2006; Li et al., 2012). It has been hypothesized that IDR-driven liquid droplet formation might represent a primordial mechanism of macromolecular self-assembly (Brangwynne et al., 2009). Because structural properties of HEAT repeats are intermediate between those of IDRs and conventional well-structured proteins (Kappel et al., 2010), it is possible that HEAT repeats themselves constitute and function as part of a hydrogel-like structure. Hydrophobic surfaces of HEAT repeats, which are hidden in aqueous solution, could be partially exposed in the crowded environment of the interior of chromosomes, and be involved in the predicted HEAT–HEAT

A



B

	Polymer	Crosslinker	Navigator	Property
RNP granule	RNA	RNA-binding protein (IDR)	?	Liquid droplet
NPC	FG-Nup (IDR)	FG motif	Karyopherin (HEAT)	Hydrogel
Mitotic chromosome	DNA	Condensin (HEAT+ IDR)	Condensin? (HEAT+ IDR)	Hydrogel?

Fig. 5. Comparison between RNP granules, nuclear pore channels and mitotic chromosomes. (A) Top, RNP granules are formed by RNA (blue) and RNA-binding proteins that contain IDRs (brown and dark brown). RNA–protein interactions, as well as multivalent and weak interactions between IDRs are believed to mediate the formation of a liquid droplet-like structure. Bottom-left, a class of nucleoporins, collectively referred to as FG-Nups, is composed of FG repeats and IDRs (brown). Phenylalanine (F) residues in the FG motifs associate with each other through hydrophobic interaction, thereby forming a hydrogel-like structure in the nuclear pore channel. HEAT-rich karyopherins (green) together with their binding partners are able to change their own conformations and so can migrate through the gel matrix. Bottom-right, in mitotic chromosomes, condensins containing HEAT subunits (green) could crosslink looped DNA strands (blue) in a highly dynamic manner and so form a hydrogel-like structure to generate central chromosome axes. Condensins also contain various IDRs (dark brown), which could functionally collaborate with the HEAT repeats. (B) Summary of the constituents and properties of the three cellular structures. Condensins could not only act as crosslinkers but might also share a 'navigator' character with karyopherins, which helps deliver a specific protein function to a specific intracellular location. Our attempt here to deduce molecular principles that might be shared among the three intracellular organelles is admittedly incomplete, calling for further elaboration of ideas and future investigations.

interactions. Thus, formation of mitotic chromosomes might represent a highly sophisticated version of macromolecular self-assembly in which the amphiphilic nature of HEAT repeats has a crucial role. It should also be added that, like in NPCs, HEAT repeats and IDRs could also functionally cooperate in mitotic chromosome assembly. For instance, the HEAT subunits of condensins interact with conserved hydrophobic patches present in the central IDR of the kleisin subunits (Piazza et al., 2014). The HEAT subunits themselves also contain IDRs. Interestingly, some of their IDRs are post-translationally modified (Kimura et al., 1998; Abe et al., 2011), which has also been implicated in the regulation of phase separation (Yang et al., 2006; Wang et al., 2014). Although functional cooperation between HEAT repeats and IDRs needs to be investigated in future studies, it will become increasingly important to view and study mitotic chromosomes as a dynamic physico-chemical entity. From this perspective, an interesting recent study has shown that Ki-67, a peripheral chromosome component, acts as a steric and electrostatic charge barrier that helps to 'individualize' and disperse mitotic chromosomes within the crowded cytoplasm (Cuylen et al., 2016).

Conclusions and perspectives

HEAT repeats occur in a wide variety of eukaryotic proteins with diverse functions. If related motifs, such as armadillo and ankyrin repeats, are included, α-helical repeats are found in ∼5% of total eukaryotic proteins (Kajander et al., 2005). These repeats were traditionally classified as protein–protein interaction domains, and only limited efforts had been made to investigate how their unique structural properties might regulate protein functions. In this current Opinion article, we have emphasized that the flexible array of amphiphilic helices that constitute HEAT repeats undergoes dynamic conformational changes not only upon binding to specific partners but also upon responding to intracellular environments such as those in the nuclear pore channel. We also hypothesize that the multivalent interaction surfaces HEAT repeats possess could have the potential to generate mesoscale intracellular structures, such as mitotic chromosomes. We still do not know whether there is a common principle of action that can be applied to all HEAT repeat proteins. It is nonetheless highly likely that their amphiphilic nature, which allows them to quickly adapt to both hydrophilic and hydrophobic environments, is at the heart of the action of HEAT repeat proteins. Although the ideas proposed here are admittedly highly speculative at present, we believe that they are worthy of further investigations. Potential difficulties in studying the function of HEAT repeat proteins lie in the fact that they often function as part of a dynamic macromolecular assembly under highly crowded conditions. It is therefore insufficient to employ conventional biochemical methods in which protein–protein interactions and their activities are assayed when highly diluted in buffer. New-generation reconstitution assays, possibly combined with microfluidics and microfabrication, as well as advanced imaging techniques measuring the proximity of macromolecules in real time and at high resolution, will be required to address the question of exactly how HEAT repeat proteins might work in crowded environments in the cell. Equally important, we will need fearless spirit and imagination to tackle these challenging yet fundamental questions left in the field of cell biology.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.H.Y. and T.H. designed and wrote the manuscript.

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