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Review

The control of spindle length by Hsp70 and Hsp110 molecular chaperones

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ABSTRACT

Molecular chaperones are an essential group of proteins required to maintain proper protein homeostasis in the cell and include Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100 among others. Hsp110 proteins form a subfamily of the Hsp70 family and seem to primarily function as nucleotide exchange factors for the Hsp70s. Data to date suggest that Hsp110 together with Hsp70 are required to ensure proper spindle assembly and nuclear distribution during cell division. More specifically, we propose that an Hsp110–Hsp70 complex modulates the activity and directionality of the kinesin-5 motor, Cin8, which is required for spindle elongation. The modulation of spindle length by molecular chaperones might be a mechanism by which cell division can be controlled especially under proteostatic stress.

1. The Hsp70/Hsp110 molecular chaperone family

In response to environmental insults, cells induce the expression of a wide array of genes. Such stress response transcription programs almost certainly include genes that encode heat shock or heat stress proteins (Hsps). Most, if not all, of these proteins are also expressed in the absence of stress and perform critical functions in the cell [1,2]. The functions of these proteins include acting as mo

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preventing the irreversible aggregation of proteins, and refolding of denatured proteins [3]. The major Hsp families are evolutionary conserved and are divided into sequence-related protein families based on their molecular mass. The major families of molecular chaperones are: Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100.

In eukaryotes, the Hsp70 family of molecular chaperones can be divided into three subfamilies: (1) DnaK-like, (2) Hsp110, and (3) Grp170 [4]. *Saccharomyces cerevisiae* (yeast) has 14 Hsp70 paralogs with family members present in the cytoplasm, endoplasmic reticulum (ER), nucleus, and mitochondria [5]. Prototypical Hsp70 proteins have two major domains (Fig. 1A): a nucleotide binding domain (NBD) and a substrate binding domain (SBD) connected by a flexible linker region that contributes to allosteric regulation of NBD and SBD activity [6,7]. SBD can be divided into β-strand rich (SBD β) and α-helical rich (SBDα) subdomains (Fig. 1A). There is also a C-terminal variable domain (Fig. 1A). ATP binding and hydrolysis at the NBD is required to modulate substrate binding and release by SBD in Hsp70. The best studied Hsp70s in yeast are the Ssa and Ssb proteins (stress seventy A or B), encoded by one is required for viability [8]. Both Ssb1 and Ssb2 have been found to be localized to the ribosome as part of the ribosome-associated complex (RAC) involved in cotranslational protein folding [9].

Yeast Sse1 and Sse2 chaperones define the Hsp110 subclass of the Hsp70 chaperones. They have an Hsp110 insertion sequence within SBDβ and an Hsp110 extension at the C-terminus (Fig. 1A) [6,10,11]. The Sse/Hsp110 subclass is only found in eukaryotic cells. These chaperones are not thought to actively assist in protein folding; rather, they are thought to bind unfolded polypeptides and to hold them in a folding-competent state [4]. The Sse chaperones have been implicated in modulating Hsp90, Ssa1, and Ssb1 chaperone activities [12,13]. Yeast lacking *SSE1* are slow growing and slightly temperature sensitive. Deletion of *SSE2* results in no observable growth defects but deletion of both *SSE1* and *SSE2* genes results in synthetic lethality [14,15]. Mammalian Hsp110 does not complement the deletion of *SSE1* in yeast suggesting that these

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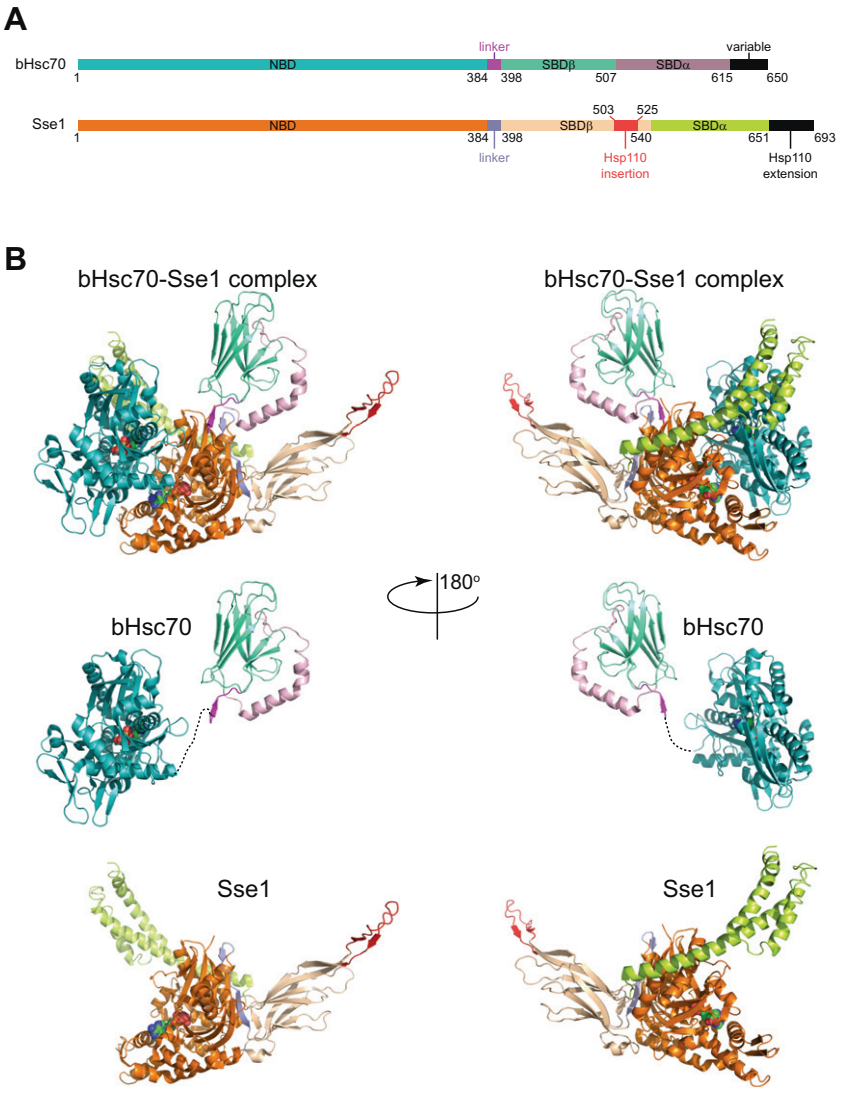

Fig. 1. Domain organization and structure of Hsp70 and Hsp110. (A) Shown are the domain organization of bovine Hsc70 (bHsc70) and yeast Hsp110 (Sse1). The domains are based on the data from Refs [6,11]. (B) Top panel shows the X-ray crystal structure of the bHsc70–Sse1 complex (PDB ID 3C7N [11]). The middle and bottom panels show bHsc70 and Sse1 from the complex, respectively. The different domains and motifs are colored according to (A). Structures on the right are related to those on the left by a 180° rotation around the horizontal axis. The structures were drawn using PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.

two orthologs have different substrate or cofactor specificities [13]. Interestingly, it was demonstrated that point mutants of *SSE1* allele that are defective in ATP binding are not functional, but that mutants which cannot hydrolyze ATP can fully complement the temperature sensitivity of *sse1*Δ cells and the synthetic lethality of *sse1*Δ*sse2*Δ cells [16]. This indicates that Sse1 mechanism of function is distinct from that of the closely related Hsp70 family members. More recently, based on biochemical and structural data, ATP-bound Sse1 and Sse2 have been proposed to act as nucleotide exchange factors (NEF) for the Ssa and Ssb chaperones and, as a result, increase the rate of Hsp70-dependent protein folding [10,11].

The X-ray crystal structure of an Hsp70–Hsp110 complex (bovine Hsc70 with yeast Sse1) is shown in Fig. 1B [11]. Sse1 has to bind nucleotide to fold into a conformation that is capable of associating with the Hsp70 [17,18]. Specifically, upon association of Sse1 with Hsp70, NBD of ATP-bound Sse1 together with SBDα embrace the NBD of Hsp70, inducing opening of NBD and the release of bound ADP from Hsp70. Such functional cooperation between Sse1 and Hsp70 may be further facilitated by direct Sse1-non-native substrate interaction and, hence, Sse1 might assists in the remodeling of the unfolded protein.

Grp170, Lhs1 in yeast, is present in the endoplasmic reticulum (ER) and is not as well studied. However, Lhs1 also seems to act as a NEF for its cognate ER Hsp70, Kar2 in yeast [19]. Lhs1 was found to require ATP to induce nucleotide exchange in Kar2 and to induce similar changes in the conformational dynamics of the Hsp70 as observed for Sse1 upon binding to Hsp70. Inactivation of Lhs1 results in the induction of the unfolded protein response (UPR) and in partial defects in Hsp70-dependent translocation, however, the cells remain viable [20,21].

2. Interaction of Hsp70/Hsp110 with microtubules

Multiple links between the Hsp70/Hsp110 chaperones and the microtubule cytoskeleton were previously observed [22–25]. Hsp70 molecular chaperones were shown to bind to the carboxy-terminal domain of polymerized tubulin subunits; this is the same domain which is involved in interacting with other microtubule binding proteins [26–28]. Furthermore, the putative tubulin binding domain within Hsp70 contains basic sequence motifs similar to those required for tubulin interaction in the microtubule-associated protein MAP1B [29]. A role for Hsp70 in folding tubulin has

also been suggested [30]. Hsp110, was found to colocalize with tubulin under stress [24]. Both Hsp70 and Hsp110 were found to co-purify with the spindle pole body (SPB) components in yeast and mammalian cells [23,31,32]. Indeed, our analysis of complexes associated with N- and C-terminally tagged Hsp70/Hsp110 chaperones supported this notion and uncovered, beside tubulin, many components of the SPB, kinetochore, proteins with spindle midzone organization roles, and microtubule-based motors [33]. Furthermore, data to date from our group and others [33–36] suggest that Hsp70/Hsp110 are required for normal spindle function starting from S phase until anaphase.

3. Mitotic spindle assembly and regulation in yeast: the role of different kinesin motors

It is known that chromosome segregation in eukaryotes is orchestrated by the microtubule (MT)-based spindle [37], which provides the structural and mechanical framework for chromosome attachment and requires proper assembly and positioning. The mitotic spindle is composed of three sets of microtubules (Fig. 2A): kinetochore microtubules (kMTs) that attach chromosomes to the spindle; astral or cytoplasmic microtubules (aMTs) that mediate spindle interaction with the cell cortex and, hence, are responsible for spindle positioning; and interpolar microtubules (iMTs) that form antiparallel lattice, also called spindle midzone, between SPBs. Stable overlap of antiparallel microtubules at the spindle midzone is important for proper SPB separation and chromosome segregation.

Assembly of the bipolar spindle, an early mitotic event, occurs by the separation of the duplicated spindle poles that function as microtubule organizing centers in yeast (functionally equivalent to the centrosome in higher eukaryotes) and the formation of an antiparallel microtubule array between them. In diverse eukaryotic cell types, spindle assembly and pole separation require the action of members of a conserved subfamily of kinesin-related motor proteins. In budding yeast, *S. cerevisiae*, two members of this family, kinesin-5 plus-end directed motors Cin8 and Kip1, act redundantly in mitosis (Fig. 2B). These motors have been shown to localize to the spindle midzone [38] and to fulfill their mitotic roles by cross-linking and sliding iMTs [39]. Although neither motor is essential for viability, the function of at least one is required during spindle assembly to separate the spindle poles and prevent the inward collapse of the separated poles until anaphase is reached. However, Cin8 makes the major contribution to spindle assembly because *cin8*Δ mutants exhibit defects in spindle assembly and activate the spindle checkpoint, while *kip1*Δ mutants have no or less detectable phenotypes [40,41]. The assembled pre-anaphase bipolar spindle is a metastable structure. A force generated by Cin8/Kip1 pushing the spindle poles apart appears to be counterbalanced by a force that is pulling them towards each other; the latter force is generated by the minus-end directed kinesin motor Kar3 (Fig. 2B). Therefore, the yeast mitotic spindle length is a product of the balancing forces of the different types of kinesin-related motors pushing the SPBs in opposite directions [42–44].

Spindle positioning in *S. cerevisiae* begins during spindle assembly, G1 and S phases, and is accomplished exclusively by the cytoplasmic MTs. The anaphase spindle and associated nucleus is pulled into the bud neck between the mother and daughter cells such that the subsequent mitotic division equally segregates the nuclear contents between the two cells. In budding yeast, nuclear migration is accomplished by the cytoplasmic dynein motor (Fig. 2B). Activities of dynein and its nuclear counterparts Cin8 and Kip1 have been shown to be required for spindle elongation during anaphase. Mutants lacking dynein and Cin8 are not viable and combining a temperature sensitive allele of *cin8* with the deletion of either *kip1* or *dyn1* results in anaphase arrest [45].

4. Role of Hsp70/Hsp110 chaperones in spindle organization

Functional Hsp70/Hsp110 chaperones have been shown to be required for proper spindle assembly and nuclear distribution [23,33]. *S. cerevisiae* cells with an *ssa1* temperature-sensitive

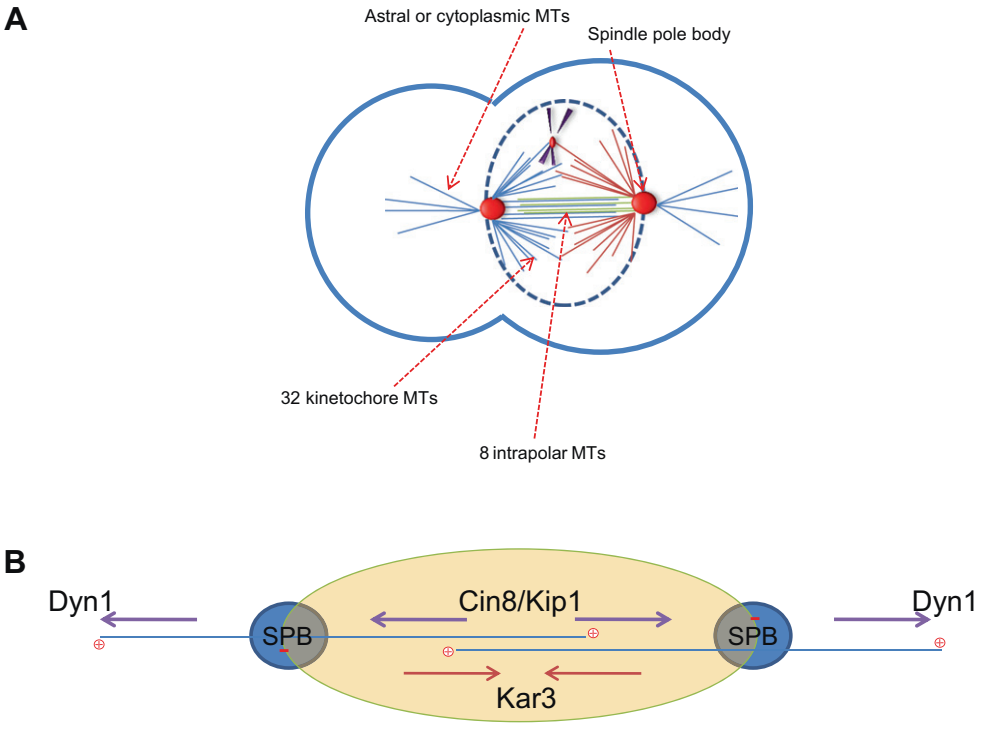

Fig. 2. The yeast spindle. (A) Cartoon representation showing the spindle of a large-budded yeast cell. In yeast, there are 8 interpolar MTs, 32 kinetochore MTs (16 from each SPB), and a number of aMTs. (B) Schematic representation of the effect of different motors on spindle length. The polarity of each MT is indicated by + and −.

mutant were defective in microtubule function and formed aberrant microtubule structures at the restrictive temperature suggesting the presence of defects in the assembly/disassembly of microtubules [23]. The authors suggested that Ssa1, together with cognate Hsp40 (Ydj1), have a critical role in regulating microtubule formation in M phase.

In a more recent effort, our analysis of the protein interaction network we obtained for the yeast cytoplasmic Hsp70/Hsp110 chaperones prompted us to investigate spindle assembly processes in different chaperone mutants [33]. We found that only in *sse1*Δ strain spindle morphology was altered such that the spindle MTs were generally longer while the cytoplasmic MTs were shorter than that of the wildtype (WT) strain in S phase. Importantly, the nucleotide exchange activity of Sse1 towards Ssa1/2, which requires ATP binding to Sse1 but not ATP hydrolysis, was requisite for acquiring the proper spindle length in S phase. Deletion of both *SSA1* and *SSA2* resulted in a shorter spindle than that of WT, which is the opposite effect on spindle length compared to that seen for the *sse1*Δ mutant. The data suggest that the unperturbed cycle of ATP binding and hydrolysis by the multi-chaperone complex Sse1–Ssa1/2 is essential for proper spindle assembly and function.

5. Sse1–Ssa1/2 regulate Cin8 activity: a working model

Interestingly, the effect of chaperones on spindle length was predominantly dependent on Cin8 activity. We found that in *sse1*Δ cells Cin8 and to a lesser extent Kip1 were either asymmetrically distributed within the spindle or mislocalized to the spindle midzone there they can execute sliding of anti-parallel iMT and, hence, induce premature spindle elongation in S phase.

As mentioned above, kinesin-5 motors are key determinants of spindle length as they bundle MTs and slide apart iMT to exert outwards forces [46,47]. In addition, kinesin-5 motors have been implicated in MT depolymerization, which was shown to be critical for proper anaphase initiation and chromosome segregation [48]. Thus, according to the current model, kinesin-5 motors bind kMT, move to kMT plus end, and upon arrival at a growing plus-end promote net kMT plus-end disassembly that ultimately drives chromosome congression during mitosis. This disassembly-promoting activity is not specific to kMTs since it was demonstrated that Cin8 also promotes disassembly of cytoplasmic aMT. These new findings make kinesin-5 motors extremely important for proper execution of mitosis and, hence, the motors need to be tightly regulated both spatially and temporally [38,47,49,50].

Another level of regulation was proposed for Cin8 based on the alteration between plus- and minus-end directed motility for this motor. Cin8 functions as a tetrameric unit [51]. Two different mechanisms have been proposed that allow Cin8 to switch the direction of its motility. One group found that switching directionality depended on ionic strength and on the binding geometry of Cin8 to MTs [52]. On single MTs in vitro, low ionic strength (non-physiological) induced plus-end directed motility of individual Cin8 tetramers, while high ionic strength (near physiological) induced minus-end directed motion [52]. Furthermore, at high-salt conditions, Cin8 tetramers crosslinking antiparallel MTs switch from fast minus-end directed motion to plus-end force generation when they reach the overlap zone, while single motors between parallel MTs kept moving in the minus-end direction [52]. Another group found that the direction of motility of Cin8 relied on whether Cin8 acts as a single tetramer or assembles into large teams of tetrameric motors [50]. According to this study, Cin8 teams have a net plus-end directed motility, while individual Cin8 tetramers have minus-end directed motility.

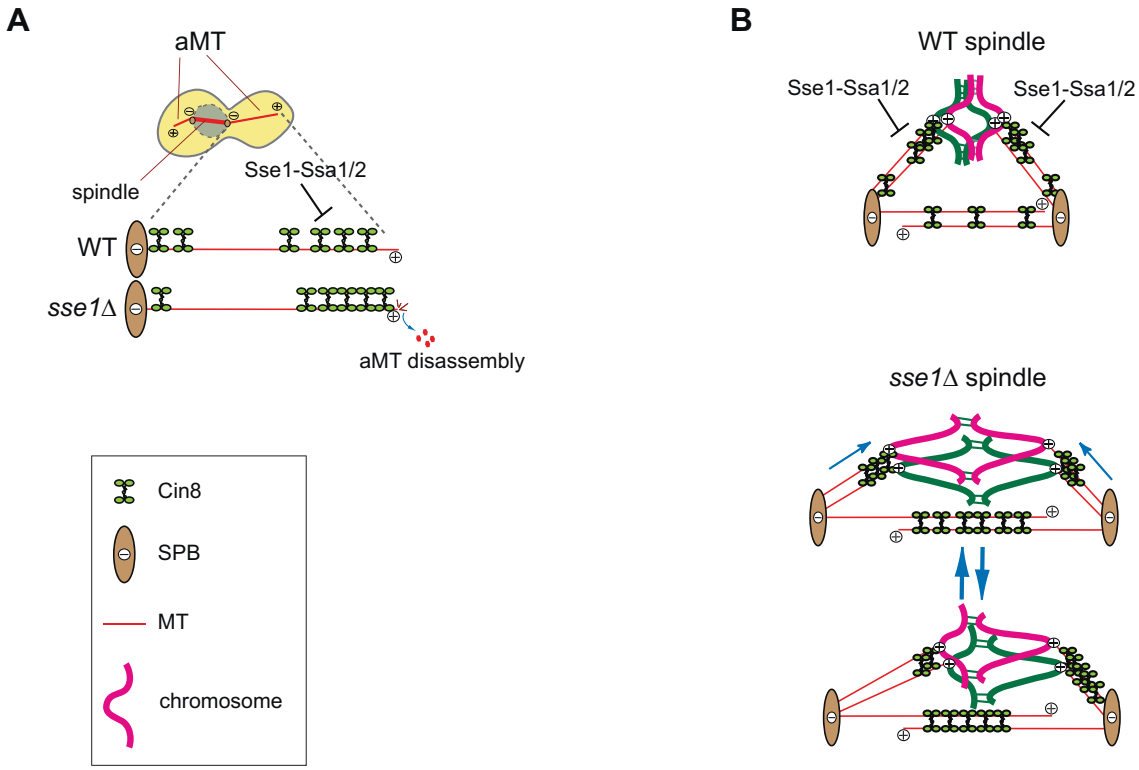

Fig. 3. Model of the regulation of Cin8 motility on MTs by Hsp110–Hsp70. The model is based on the assumption that individual Cin8 tetramers exhibit mainly minus-end motility (i.e., they move towards the SPB), while Cin8 teams move mainly towards the plus-end of MTs. The Sse1–Ssa1/2 complex is proposed to interact with Cin8 tetramers and reduce their ability to form large multi-oligomeric teams. (A) Shows the effect of Sse1–Ssa1/2 on Cin8 (or Cin8ΔNLS) on aMTs, while (B) shows the effect of the chaperones on Cin8 within the spindle. Refer to the text for further details. The figure is modified from Ref. [33].

To investigate the effect of Sse1–Ssa1/2 on spindle length in vivo, we examined Cin8 movement on individual MTs in the cytoplasm by using a Cin8 mutant that lacks the nuclear localization signal (NLS) [50,53]. In WT cells, Cin8ΔNLS was mainly found near the minus-end of aMTs, but in *sse1*Δ background, there was increased clustering of Cin8ΔNLS near aMT plus ends (Fig. 3A). Cin8 motors that accumulated at the plus ends were functional since their presence at the plus-end stimulated aMT disassembly and resulted in shorter aMT. Using these findings, we can propose the following as a potential scenario for the effect of Sse1–Ssa1/2 on Cin8 motility in the nucleus (Fig. 3B). Cin8 is recruited to both kMT and iMT within the spindle in S phase. The Sse1–Ssa1/2 chaperones regulate Cin8 directional movement by modulating the assembly of Cin8 tetramers into motor teams. If Sse1–Ssa1/2 stabilize Cin8 tetramers and reduce their assembly into teams, then this would favor the accumulation of Cin8 near minus ends of kMTs prior to anaphase. *SSE1* deletion enables increased mechanical coupling between Cin8 tetrameric motors, which leads to the assembly of Cin8 motor teams and, hence, triggers the switching of the net movement of Cin8 from minus- to plus-end directed motility. As Cin8 motors move towards the plus-end of kMTs, this results in the uncontrolled disassembly of kMTs and asymmetric distribution of chromatin along the spindle. At the same time, this induces Cin8 redistribution to iMTs at the spindle midzone. Increased binding of Cin8 at the midzone stimulates sliding of iMTs and causes premature spindle elongation in S phase arrested cells. We cannot ignore the possibility the Sse1–Ssa1/2 might also affect the local ionic strength around Cin8 motors also causing switching in the direction of motility. However, given the known activity of molecular chaperones, at this time, we would favor the model whereby the chaperones affect the oligomeric assembly of the motor (Fig. 3). Further studies are needed to elucidate such a proposed model and to determine the biochemical basis by which Sse1–Ssa1/2 might affect this kinesin-5 motor.

It is interesting to consider the physiological role of such regulation of Cin8 by chaperones. Since heat shock proteins are induced under stress, it is plausible to suggest that Cin8 mechanical coupling and thus plus-end directed motility would be inhibited under such unfavorable growth conditions. This should ultimately cause shortening of the spindle due to decreased motor concentration at the midzone. Indeed, when the spindle length was measured in WT cells under heat shock stress, significant spindle shortening was observed compared to normal growth conditions [33]. Spindle shortening was partially dependent on Sse1 indicating that additional, yet to be identified, proteins or chaperones might play a role in this regulation. Nevertheless, the data suggest that the Hsp110–Hsp70 chaperone system is being used by the cell to actively adopt favorable spindle length based on environmental cues. This highlights a novel role for molecular chaperones in the cell in addition to their classical role in protein folding.

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