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11. Karyoplasts were observed with Zeiss Universal or WL microscopes equipped with phase contrast optics and shuttered illumination pathways. Microscopes were maintained at 37°C in a cardboard box with a proportional temperature control system (Omega Engineering, Stamford, CT). Images were taken with a charge-coupled device (CCD) camera (Orca; Hamamatsu, East Bridgewater, NJ), and video sequences were written to the hard drive of a PC using C-imaging software (Compix Inc., Brandywine, PA).
12. For supplementary figures, see *Science* Online (www.sciencemag.org/cgi/content/full/291/5508/1547/DC1).
13. Within 1 hour after mitosis for the karyoplast of interest, the medium was replaced with DMEM containing BrdU (10 μg/ml) and the preparation was returned to the microscope. When 12 or 28 hours had elapsed since mitosis, the karyoplasts were fixed in methanol for 5 min at −20°C, then immediately transferred to 4 M HCl at room temperature for 2 hours and processed for immunofluorescence microscopy [N. Gunduz, *Cytometry* **6**, 597 (1985)] using a mouse BrdU monoclonal antibody (mAb) (Boehringer Mannheim).
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Centrosome-Dependent Exit of Cytokinesis in Animal Cells

Matthieu Piel,[1] Joshua Nordberg,[2] Ursula Euteneuer,[3] Michel Bornens[1]*

As an organelle coupling nuclear and cytoplasmic divisions, the centrosome is essential to mitotic fidelity, and its inheritance could be critical to understanding cell transformation. Investigating the behavior of the centrosome in living mitotic cells, we documented a transient and remarkable postanaphase repositioning of this organelle, which apparently controls the release of central microtubules from the midbody and the completion of cell division. We also observed that the absence of the centrosome leads to defects in cytokinesis. Together with recent results in yeasts, our data point to a conserved centrosome-dependent pathway that integrates spatial controls into the decision of completing cell division, which requires the repositioning of the centrosome organelle.

The checkpoint concept (*1, 2*) was introduced to describe mechanisms controlling the progression of the cell division cycle at critical steps that impose delays to allow corrections or repairs, or even to trigger cell death. Many of the key genes involved in these processes are not essential but, when defective, can be oncogenic (*3*). In *Saccharomyces cerevisiae*, a checkpoint mechanism monitors the coupling between nuclear and cytoplasmic division and relies on the spatial proximity of interacting components. A guanosine triphosphatase (GTPase) is concentrated at the spindle pole body (SPB), whereas the corresponding exchange factor is concentrated in the bud. Therefore, the migration of one SPB to the neck is necessary to activate the GTPase and to trigger mitotic exit (*4–7*). This ensures that cytokinesis does not take place before the nucleus has entered the bud.

We investigated whether similar spatial controls involving the centrosome (the functional equivalent of the SPB) might operate in vertebrate cells, which are much larger than yeast cells. We made time-lapse recordings of cell division in cell lines stably expressing the centrin protein coupled to the green fluorescent protein (GFP) as a centrosomal marker (*8*). During mitosis, cells possess two centrosomes located at the spindle poles, one of which is inherited by each of the two daughter cells. Each centrosome is made up of a daughter centriole that was assembled during the previous S phase and a mother centriole that was assembled during a previous cycle. The two centrioles can be distinguished ultrastructurally (*9*) and biochemically (*10*). In vivo, the mother centriole is also more strongly labeled by centrin-GFP than is the daughter centriole during the first hours of the G_1 phase (*10*).

After furrow ingression is completed, there is a period (from 1.5 to 5 hours) during which daughter cells are still linked by a cytoplasmic bridge before cytokinesis is complete (abscission). We observed that the first centrosomal event after formation of the cleavage furrow was the separation of the two centrioles in each daughter cell (Fig. 1, A and B, panel b), the daughter centriole being more motile than the mother centriole, which bears the microtubule (MT) aster and sits near the cell center (*10*). Just before abscission, the mother centriole in one (70% of the cases) (Fig. 1) or in both daughter cells [Web movie 3, part 1 (*11*)] transiently left its central position and moved straight to the intercellular bridge [Fig. 1, A and B, panels c and d; Fig. 1C; and Web fig. 1 (*11*)]. This movement was as fast as 10 μm/min (2 μm/min on average). When the mother centriole moved back to the cell center, cytokinesis was completed. This unexpected movement was observed in 45 of 50 HeLa cells. In most cases (75%), the mother centriole stayed near the bridge for <1 hour (15 min on average). Altogether, the asynchrony of abscission and the correlative movement of the mother centriole to the

[1]Institut Curie, Section Recherche, UMR 144 du CNRS, 26 rue d'Ulm, 75248 Paris Cedex 05, France. [2]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01605, USA. [3]Zellbiologie, Adolf-Butenandt-Institut, Ludwig-Maximilians-Universität, D-80336 München, Germany.

*To whom correspondence should be addressed.

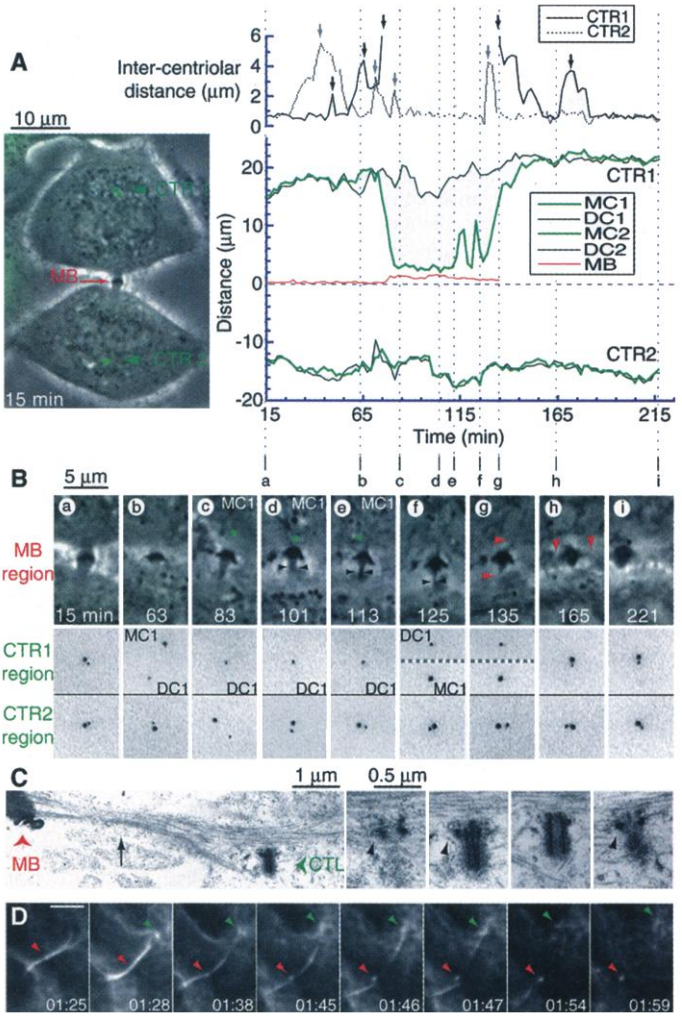


Fig. 1. HeLa cell stably expressing centrin-GFP. (**A**) Cells were video-recorded in phase contrast and fluorescence microscopy (*12*). Fifteen minutes after anaphase onset, furrow ingression is completed (left). Each daughter cell has inherited a centrosome (CTR1 and CTR2, green arrows) containing a mother centriole (MC1 or MC2) and a daughter centriole (DC1 or DC2). The movements of the centrioles and of the midbody (MB, red arrow) were recorded at 30 frames/hour. Their distance to the middle of the bridge is plotted on the right (colored curves). The two black upper curves show the intercentriolar distance for each centrosome. The "a" to "i" labeling on the time axis corresponds to events illustrated in (**B**) as phase contrast of the midbody and GFP signal at the poles (black and white). Centrioles, which are still orthogonal at each pole in point a have split in both centrosomes in point b. MC1 approaches the intercellular bridge (green dot) in c. The midbody has moved toward the approaching centriole, and they are <2 μm away from one another in point d. The bridge narrows from ~1.2 μm wide in d to 0.3 μm wide in f, when MC1 moves back to the pole (the dotted line indicates that the intercentriolar distance is larger than shown), and finally detaches on both sides (red arrowheads) in g, when MC1 is back at the pole. As shown in h and i, the two daughter cells are separated, and the midbody is free in the medium (it has rotated) [see Web movie 1 (*11*)]. (**C**) The left panel shows an electron microscope picture (left) of the midbody and the microtubule bundle, with a pinched region (arrow), emanating from it. CTL, mother centriole. The right panels show serial sectioning through the mother centriole; black arrowheads indicate subdistal appendages, interacting with the microtubule bundle [see Web fig. 1 (*11*)]. (**D**) Time-lapse recording (hour:minutes) of U2OS cells expressing both β-tubulin–GFP and centrin-RFP (red fluorescent protein) [see Web movie 2 (*11*)]. Red arrowhead indicates the midbody; green arrowhead indicates the mother centriole. Bar, 5 μm.

bridge region suggested the existence of a control that depended on the repositioning of the centrosome.

Recording both phase contrast and epifluorescence images (*12*) revealed that narrowing of the bridge on both sides of the midbody, which always preceded abscission (*13*–*15*), took place within a few minutes after mother centriole repositioning to the bridge (Fig. 1, A and B, panels d through f), when the MT bundles were released from the midbody (Fig. 1D). We noted, however, that the final cut of the bridge always occurred after the mother centriole moved from the bridge back to the cell center, indicating that narrowing of the bridge and abscission were distinct events. For example, the mother centriole could stay near the bridge for >1 hour after narrowing of the bridge (25% of the cases; 90 min on average), and abscission occurred only when the mother centriole had moved away. Although the bridge could be cut on both sides of the midbody, abscission occurred preferentially on the side where the mother centriole came close to the bridge. In addition, we investigated whether the daughter centrioles could also be involved in that process because, in a fixed population of L929 cells, we often observed daughter centrioles close to the intercellular bridge. However, a time-lapse recording revealed that abscission occurred only after a mother centriole had also moved toward the bridge. Therefore, it is the mother centriole behavior that matters. We then demonstrated that, contrasting with the actomyosin-dependent movements of the daughter centriole (*10*), the transient repositioning of the mother centriole depended on a remodeling of the post-anaphase MT network; when we perturbed the MT cytoskeleton by short treatment (15 min) with the MT-disrupting agent nocodazole (ND) applied <1 hour after mitotic shake (mitotic cells collected by a shake-off), a rapid repositioning of mother centrioles at the cell periphery opposite to the midbody was observed (Fig. 2, A and D). By contrast, when

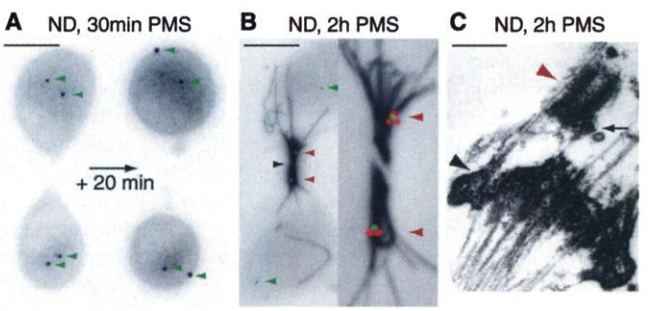
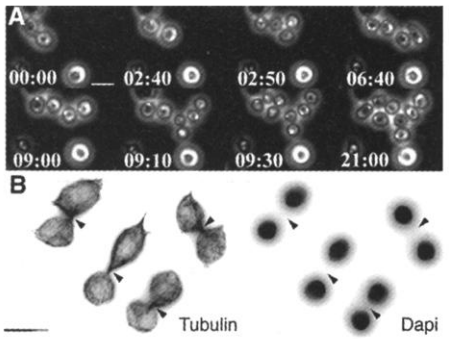


Fig. 2. Repositioning of the mother centriole depends on post-anaphase microtubule network remodeling. Throughout these images of post–mitotic shake (PMS) L929 cells, centrioles are indicated by green or red arrowheads when identified as mother centrioles. The midbody is indicated by a black arrowhead. (**A**) Thirty minutes after mitotic shake, L929 cells stably expressing centrin-GFP (black and white) were treated with 5 μM ND and time-lapse recorded. After 20 min, one centriole in each daughter cell had relocated toward the cell periphery. Bar, 10 μm. (**B**) ND was added 2 hours after mitotic shake, and cells were fixed 15 min later and stained for α-tubulin (black and white) and ninein (red). The GFP signal is shown in green. The two mother centrioles, identified by the antibody to ninein (*10*), are relocated inside the bridge (fourfold magnification is shown on the left). Bar, 10 μm. (**C**) Electron microscope observation of the midbody region of HeLa cells treated as in (B). The arrow indicates an appendage typical of a mother centriole. Bar, 0.5 μm. (**D**) After mitotic shake, cells were seeded on coverslips with (20 μg/ml) or without fibronectin (Fibro) and fixed at various times after ND treatment (*29*). The numbers are the percentages of paired daughter cells containing at least one centriole in the intercellular bridge region. Between brackets are the percentages of daughter cells in which the mother centriole was found near the cell periphery. For each case, 1000 cells were counted, and at least three independent experiments were performed.

D

Cell type	Hela (%)		L929 (%)	
Time	t ≤1h	t ≥2h	t ≤1h	t ≥2h
Control	1,5	10	0,2 (0)	2 (0)
ND	3,5	50	2,5 (60)	30 (0)
Fibro, ND			1 (60)	15(20)



C

Phenotype	Control (n=20)	Karyoplast (n=32)
	1 (5%)	13 (41%)
	0 (0%)	3 (9%)
	11 (55%)	10 (31%)
	8 (40%)	6 (22%)

Fig. 3. Cytokinesis defect of acentrosomal cells. (**A**) Cells from the *Drosophila melanogaster* 1182-4 cell line were video-recorded in phase contrast microscopy over 2 days (hour:minutes). Cells stay in pairs during the entire cell cycle. They separate during the following mitosis. Bar, 10 μm. (**B**) Unsynchronized 1182-4 cells, which were fixed and stained for microtubules and DNA, display a MT bridge (arrowhead) and no aster. DNA staining shows that abscission was not inhibited by the presence of a DNA bridge (*15*). Bar, 10 μm. (**C**) Control BSC1 cells and karyoplasts obtained by microsurgical removal of centrosomes were video-recorded (*18*). Various postmitotic phenotypes were observed: furrow regression leading to binucleated cells (first row), no furrow regression and no abscission leading to two cells linked by a cytoplasmic bridge (second row), full separation leading to two independent daughter cells (third row), and cells too close to one another to enable a clear observation of abscission (fourth row).

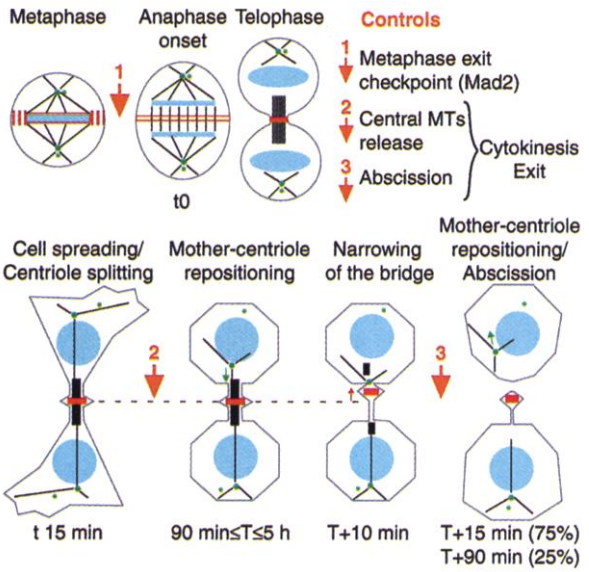


Fig. 4. Model. The metaphase exit checkpoint controls chromosome segregation and cyclin-dependent kinase 1 inactivation, which is necessary for assembly of the centrally located MTs, the telophase disk in the midzone, and the cleavage furrow (*30*). Once furrow ingression is complete, daughter cells demonstrate opposite ruffling activities. During this period, the centrosome in each daughter cell is retained on the leading-edge side. Each mother centriole is under tension exerted by the interaction of its associated MTs with the cell periphery and with the midbody [see Web fig. 2 (*11*)]. A progressive change in the MT network occurs (Fig. 2), the duration of which depends on contact with neighboring cells or on substrate adhesion. This period reflects the need for daughter cells to integrate information from their periphery before proceeding. When conditions are met, ruffling activity would be actively suppressed in one or both cells, and one or both mother centrioles would reposition to the bridge, possibly through the activity of kinesin Eg5 (*31*). The repositioning of a mother centriole would be instrumental in triggering the release of the central spindle MTs, because its proximity with the midbody activates a signaling pathway. Finally, the disassembly of the actin ring and the sealing of the plasma membrane occur only when the mother centriole has moved back within the cell body, indicating that the mother centriole is also participating in this phase. *T*, time of centrosome repositioning.

ND was applied 2 hours after mitotic shake, mother centrioles were found inside the intercellular bridge, close to the midbody (Fig. 2, B through D). We took advantage of this effect to confirm the role of the centrosome in the control of abscission, as short ND treatment enabled us to control the moment at which the mother centriole moved toward and away from the bridge; when mother centrioles repositioned deep inside the bridge after ND treatment, the intercellular bridge did not cut as long as ND was present (up to 2 hours), and the mother-centriole was maintained in the bridge. A few minutes after ND removal, the centriole moved away from the bridge, and abscission immediately followed (7 out of 10 cells).

We conclude that an important requirement for abscission to occur is to bring together a mother centriole and the midbody. This could reflect the need for spatial proximity, reminiscent of the yeast SPB-dependent pathway for cytokinesis.

To test more directly whether the centrosome plays a role in the completion of cytokinesis, we carried out centrosome-removal experiments. Although centrosome-deprived cells (karyoplasts) have been reported to be unable to enter mitosis (*16*), this result is at variance with another method of centrosome disruption in HeLa cells (*17*). Microsurgical ablation of centrosomes in BSC1 cells was reinvestigated, coupled with video-microscopy observations (*18*). Most of the centrosome-free cells went through an apparently normal mitosis (*18*), but a highly significant number of them had cytokinesis defects (Fig. 3C). We also reinvestigated the division process of the acentriolar *Drosophila* cell line 1182-4 (*19, 20*). This cell line was reported to become rapidly polyploid and has to be subcloned regularly. We observed that 1182-4 cells have an incomplete cytokinesis (Fig. 3, A and B), leading to daughter cells permanently linked by a MT-containing bridge from where the MT network seemed to be organized (73% of cells showed MT-containing bridges, 14% were individual mononucleated cells, and 13% were multinucleated). No MT aster was visible in these cells, consistent with the absence of a centrosome. Paired cells often separated at the next mitosis, although four connected cells or larger aggregates could be observed. Time-lapse recording revealed that the cell division cycle was also severely affected: its duration varied from 6 to 24 hours, not only from one cell to another but also from one cycle division cycle to the next. Paired cells could eventually fuse to form binucleated cells that usually did not divide anymore. Altogether, these results suggest that the centrosome organelle has a direct role in the process of abscission.

We attempted to identify conditions modifying the timing of abscission by varying culture conditions for three cell lines [Web movie 3 (*11*)]. In HeLa cells grown in small clones (like in Fig. 1), abscission occurred ~2 hours after the onset of anaphase, in a 1-hour window, and coincided with the contact of spreading daughter cells with their neighbors. However, when cells were seeded at low density, abscission was more asynchronous and sometimes occurred as late as 5 hours after the onset of anaphase. L929 cells, when seeded on a low concentration of fibronectin (1 μg/ml solution; 10 cells), were poorly adherent, and their abscission process was similar to that of HeLa cells at low density. When seeded on a high concentration of fibronectin (20 μg/ml solution; 10 cells), these cells flattened extensively, and split centrioles seemed to be attracted toward lamellipodia [this could be demonstrated by brief ND treatment (Fig. 2D)]. Under these conditions, abscission was delayed (taking twice as long on average) or even failed to take place. Intercellular bridges could be very long and eventually torn under the traction exerted by the two daughter cells, without repositioning of a centriole in the bridge (4 out of 10 cells). Finally, in strongly adherent flat 3T3 cells seeded on fibronectin (5 μg/ml), centriole movement toward the midbody was completely inhibited (5 out of 5 cells).

Abscission occurred in a markedly different manner: A very extensive stretching of the intercellular bridge between the daughter cells could be observed. In these cells, the midbody itself apparently never disassembled [Web movie 3, part 3 (*11*)]. We conclude that a key parameter for the movement of the mother centriole, and for abscission, is cellular adhesion to the substrate or contact to neighboring cells.

Strongly adherent cultured cells can apparently divide by traction-mediated "cytofission" (*21*). Some mammalian cells can divide without actomyosin rings when attached to a solid substrate (*22*), a behavior reminiscent of *Dictyostelium discoidum* cytokinesis mutants (*23*). Traction-mediated cytofission is unlikely to occur in a tissue where cells are not free to move far away from one another and thus could be an artifact due to cell culture on too-adhesive substrates rather than a genuine alternative pathway. We noted, for example, that 3T3 or L929 cells grown on a very adherent substrate show many binucleated cells, suggesting frequent cytokinesis defects. Moreover, several recent reports have shown that cytokinesis can reverse after furrowing, leading to the formation of binucleated cells, or it can be blocked at a late stage, the two cells being linked by a cytoplasmic bridge (*24, 25*). In agreement with other reports (*26–28*), our data strongly support the idea that abscission is a regulated process.

Our observations on living cells can be interpreted by the model shown in Fig. 4. At the exit of metaphase, cells assemble three structures: the so-called central spindle, the cleavage furrow, and the telophase disk/midbody. We propose that the disassembly of the central spindle and the cleavage furrow, both necessary for abscission, are distinct events that, like metaphase spindle disassembly, are under tight control; we also propose that these controls would involve the repositioning of the centrosome with respect to the midbody.

This control implies that the centrosome, which normally maintains itself at the cell center, moves transiently to the cell periphery. In addition to its relevance for cell division control, such a behavior could reveal a more general function of the centrosome, namely to integrate spatial constraints, for example, during cell locomotion and cell differentiation.

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12. Time-lapse recordings were performed as described (*10*). To record post-anaphase movements of the centriole, we acquired a z-series every 2 min with a ×100 objective in both phase contrast and epifluorescence, enabling us to follow centriole movements and intercellular bridge morphology at a high resolution without losing the focus, which was crucial for determining the rupture of the bridge. The illumination device was a 100-W halogen lamp, with the potentiometer set under 8 V to avoid overillumination. In these conditions, cells could enter mitosis and exit metaphase without any delay, as compared to cells recorded in phase contrast only.
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Structure of a Bag/Hsc70 Complex: Convergent Functional Evolution of Hsp70 Nucleotide Exchange Factors

Holger Sondermann,[1] Clemens Scheufler,[1] Christine Schneider,[1] Jörg Höhfeld,[2] F.-Ulrich Hartl,[1] Ismail Moarefi[1]*

Bag (Bcl2-associated athanogene) domains occur in a class of cofactors of the eukaryotic chaperone 70-kilodalton heat shock protein (Hsp70) family. Binding of the Bag domain to the Hsp70 adenosine triphosphatase (ATPase) domain promotes adenosine 5′-triphosphate–dependent release of substrate from Hsp70 in vitro. In a 1.9 angstrom crystal structure of a complex with the ATPase of the 70-kilodalton heat shock cognate protein (Hsc70), the Bag domain forms a three-helix bundle, inducing a conformational switch in the ATPase that is incompatible with nucleotide binding. The same switch is observed in the bacterial Hsp70 homolog DnaK upon binding of the structurally unrelated nucleotide exchange factor GrpE. Thus, functional convergence has allowed proteins with different architectures to trigger a conserved conformational shift in Hsp70 that leads to nucleotide exchange.

The evolutionary conserved members of the Hsp70 family play essential roles in preventing misfolding and aggregation of newly synthesized or unfolded proteins (*1–3*). Coordinated binding and release of substrates by these molecular chaperones is strictly dependent on their ATPase activity. Nucleotide binding to the NH_2-terminal ATPase domain of Hsp70 regulates the substrate binding properties of its COOH-terminal peptide-binding domain by an unknown mechanism (*4, 5*). Hsp70 binds adenosine 5′-triphosphate (ATP) with high affinity and slowly hydrolyzes it to adenosine 5′-diphosphate (ADP). ATP-bound Hsp70 has low affinity for substrate, whereas the ADP-bound form has high affinity. Substrate binding to Hsp70/ATP stimulates ATP hydrolysis (*6*), resulting in a more stable complex of Hsp70/ADP with bound substrate. ATP hydrolysis is also stimulated by Hsp40 proteins, an evolutionary

[1]Department of Cellular Biochemistry, Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany. [2]Institut für Zellbiologie, Rheinische Friedrich-Wilhelms-Universität Bonn, D-53121 Bonn, Germany.

*To whom correspondence should be addressed. E-mail: ismail@moarefi.com