Stepwise and cooperative assembly of a cytokinetic core complex in yeast Saccharomyces cerevisiae

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Keywords: Cytokinesis, IQGAP, F-BAR proteins, protein protein interaction
Running title: A ternary complex of Iqg1p, Mlc1p and Hof1p
Abstract

Actomyosin ring (AMR) contraction and the synthesis of extracellular material are interdependent pathways of cytokinesis in the yeast *Saccharomyces cerevisiae* and other eukaryotes. How these interdependent pathways are physically connected is central for understanding cytokinesis. The yeast IQGAP (Iqg1p) belongs to the conserved AMR. The F-BAR domain-containing Hof1p is member of a complex that stimulates cell wall synthesis. We report on the stepwise formation of a physical connection between both proteins. The C-terminal IQ-repeats of Iqg1p first bind to the essential myosin light chain before both proteins assemble with Hof1p into the Mlc1p-Iqg1p-Hof1p bridge (MIH). Mutations in Iqg1p that disrupt the MIH alter Hof1p targeting to the AMR and impair AMR contraction. Epistasis analyses of two *IQG1* alleles that are incompatible with MIH formation support the existence and functional significance of a large cytokinetic core complex. We propose that the MIH acts as hinge between the AMR and the proteins involved in cell wall synthesis and membrane attachment.
Introduction

In the budding yeast *Saccharomyces cerevisiae* (from here on yeast) cytokinesis involves the concerted action of membrane ingression and formation of an extracellular septum at the pre-selected division site, the bud neck of the cell. Membrane ingression is achieved through the contraction of the actomyosin ring (AMR). The primary septum (PS) is formed by Chs2p, a chitin synthesizing enzyme that is specifically delivered to the membrane at the division site during cytokinesis. A secondary septum (SS), mainly synthesized by the 1,3-beta-D-glucan synthase Fks1p, is layered on both sides between the forming PS and the membrane (Bi and Park, 2012). Septum formation and AMR contraction are interdependent processes (Schmidt et al., 2002). Deletion of Myo1p, the single type II myosin of yeast, leads to the loss of the AMR and its contraction and to incorrectly formed and mis-oriented septa (Bi et al., 1998; Tolliday et al., 2003). This defect is not caused by the lack of force production as cells expressing only the C-terminal tail domain separate nearly as well as wild type cells (Lord et al., 2005). Conversely, the deletion of *CHS2* affects not only septum formation but also AMR contraction, and plasma membrane ingression (VerPlank and Li, 2005). Both PS- and SS-formation are important for the final abscission of the daughter cell at the end of cytokinesis (Barr and Gruneberg, 2007; Onishi et al., 2013).

The architecture of the cytokinetic apparatus and its coordinated assembly are studied in very diverse organisms and shared components and mechanisms of their assembly are beginning to emerge (Balasubramanian et al., 2004). IQGAP proteins are important for cytokinesis in many organisms (Bielak-Zmijewska et al., 2008; Shannon, 2012; Skop et al., 2004). The single yeast IQGAP protein Iqg1p is part of the AMR. Iqg1p helps to recruit Myo1p and actin to the division site and upon overexpression can even suppress the phenotypes of cells lacking *MYO1* (Fang et al., 2010; Ko et al., 2007; Shannon and Li, 1999). Iqg1p is a multidomain protein where a Calponin homology domain, thought to crosslink actin filaments, is followed by eleven IQ repeats, a GAP related domain (GRD) and a GTPase activating protein (GAP) domain (Fig. 1A) (Shannon, 2012). The IQ repeats bind to the essential Myosin light chain Mlc1p. Binding to Mlc1p is a prerequisite for anchoring Iqg1p to the site of cytokinesis (Boyne et al., 2000; Luo et al., 2004; Shannon and Li, 2000). Cells lacking Iqg1p fail to contract the AMR and do not properly progress through cytokinesis (Epp and Chant, 1997; Shannon and Li, 1999). The separation of these cells can be rescued without restoring a functional AMR by overexpression of either one of the two further members of the cytokinetic apparatus Cyk3p or Hof1p (Korinek et al., 2000). Cyk3p forms a complex with Inn1p and Hof1p that was shown to stimulate the synthesis of the primary septum by binding
to and activating Chs2p (Devrekanli et al., 2012; Labedzka et al., 2012; Nishihama et al., 2009; Oh et al., 2012; Oh et al., 2013). The Hof1p/Cyk3p-induced suppression of the \textit{IQG1} deletion is a strong indication for a role of both proteins downstream of Iqg1p. Hof1p resembles Iqg1p in that its deletion compromises AMR contraction, septum formation, and, together with the loss of Rvs167p, actin ring assembly (Lippincott and Li, 1998a; Meitinger et al., 2011; Oh et al., 2013; Vallen et al., 2000; Nkosi et al., 2013). Hof1p’s postulated involvement in both cytokinetic pathways is further supported by the observed synthetic lethality between \textit{HOF1} and \textit{CYK3} and between \textit{HOF1} and \textit{MYO1} (Korinek et al., 2000; Vallen et al., 2000). Hof1p displays a very dynamic and characteristic cellular distribution (Lippincott and Li, 1998a; Meitinger et al., 2011). In the S-phase of the cell cycle the protein attaches to the septins at the bud neck, separates temporarily into two rings before the onset of cytokinesis to then associate with the AMR shortly before its contraction. The region in Hof1p responsible for its interaction with the septins was mapped to the N-terminal 355 residues including the F-BAR domain and a C-terminal coiled-coil region (CC2, Fig. 1B) (Meitinger et al., 2013; Oh et al., 2013). The region responsible for attaching Hof1p to the AMR was localized between its N-terminal F-BAR domain and its C-terminal SH3 domain (RLS, Fig. 1B) (Meitinger et al., 2011). Members of the F-BAR family are known to bind and bend membranes in very different cellular contexts suggesting that Hof1p might act as one of the molecular linkers that translate the force provided by the AMR contraction to pull the membrane inwards (Frost et al., 2009; Roberts-Galbraith and Gould, 2010).

Understanding the temporal and very dynamic assembly of the cytokinetic apparatus in yeast and other organisms is technically demanding and far from complete (Balasubramanian and Tao, 2013; Pollard, 2010). One of the unresolved issues concerns the existence and composition of a central element of cytokinesis that is proposed to link and coordinate AMR contraction with septum synthesis in yeast or with extracellular matrix-remodelling in case of animal cells (Bi and Park, 2012; Onishi et al., 2013). Iqg1p was tentatively assigned this important regulatory role in yeast (Bi and Park, 2012; Onishi et al., 2013). Starting from a systematic Split-ubiquitin screen we characterize a newly identified interaction between Iqg1p and Hof1p in a mechanistic detail that allows addressing it as a bridge between AMR contraction, membrane ingression, and septum synthesis during cytokinesis.
Results

The IQ motifs 9-11 of Iqg1p bind to the central region of Hof1p

We performed a Split-Ub interaction assay of Iqg1p. In this assay Iqg1p was coupled to the C-terminal half of Ubiquitin (Cub) that was extended at its C-terminus by the reporter protein Ura3p (Orotidine-5’-phosphate decarboxylase) to create Iqg1CRU. The haploid yeast strain expressing Iqg1CRU was mated against an array of 380 yeast strains each expressing a different yeast protein fused to the N-terminal half of Ubiquitin (Nub). The array was enriched in Nub fusions to proteins of polarity establishment, cytokinesis and stress response (Hruby et al., 2011). In yeast cells expressing Iqg1CRU together with an interacting Nub fusion, the Nub and Cub reassembled to the native like Ub and the Ura3p reporter was cleaved and subsequently degraded. Consequently, cells bearing a pair of interacting Nub and Cub fusions were able to grow on media containing the drug 5-Fluor-Orotic acid that is converted by Ura3p to the toxic 5-Fluor-Uracil (Dünkler et al., 2012). The assay revealed Mlc1p as already known, and Hof1p as well as a fragment of Hof1p missing the FCH-domain (Hof198-669) as novel interaction partners of Iqg1p (Fig. 1A, supplementary material Fig. S1). This in vivo interaction between Hof1p and Iqg1p is corroborated by a recently discovered co-precipitation of both proteins from yeast extracts (Naylor and Morgan, 2014). To further dissect the interactions between Hof1p, Mlc1p and Iqg1p we mapped the binding sites for Mlc1p and Hof1p within the sequence of Iqg1p by screening different fragments of Iqg1p as CRU-fusion against the same Nub-array. A fragment encompassing all eleven IQ repeats of Iqg1p retained the interactions with Mlc1p, Hof1p and Hof198-669 (supplementary material Fig. S1B). Whereas the many potential binding sites for Mlc1p were reflected by strong interaction signals with multiple Iqg1p-fragments spanning different IQ-repeats, the Hof1p-binding site could be clearly restricted to the IQ motifs 9-11 (IQ9-11; Fig. 1A).

To identify the Iqg1p-binding site(s) within the structure of Hof1p we constructed different Nub-fragments of Hof1p using the known borders of its domains as orientation for selecting their start and end points (Fig. 1B). A split-Ub analysis defined the stretch between residue 283 and 592 as binding region of Hof1p for Iqg1p (Fig. 1B). As judged by the growth of the Nub/Cub-expressing yeast cells on 5-FOA containing media, Nub-Hof1283-592 seemed to interact less tightly with Iqg1CRU than Nub-Hof1p or Nub-Hof198-669. However, this difference in growth can not be taken as evidence for a reduced affinity as the N-terminal deletion of 283 residues may change the distance between Nub and Cub and thus the efficiency of Nub/Cub re-association in the measured Hof1p/Iqg1p complex (Johnsson and Varshavsky, 1994; Müller and Johnsson, 2008).
The region between residues 283 and 592 of Hof1p was previously shown to be necessary for the AMR localization of Hof1p, whereas IQ 1-11 of Iqg1p were shown to be important for targeting Iqg1p to the bud neck in a Mlc1p-dependent manner (Meitinger et al., 2011; Shannon and Li, 2000). In agreement with our interaction analysis, Hof1p and Iqg1p co-localize at the AMR during its contraction (supplementary material Fig. S2). To test whether Hof1p binding significantly contributes to recruit Iqg1p to its central position in the AMR we measured the cellular distribution of an Iqg1-GFP fusion that lacks the Hof1p-binding sites (Iqg1\text{\textsuperscript{\Delta}IQ9-11}-GFP). Iqg1\text{\textsuperscript{\Delta}IQ9-11–GFP} could be clearly detected at the bud neck of wild type cells and cells lacking the native copy of \textit{IQG1}. However, in cells lacking native Iqg1p, symmetric contraction of Iqg1\text{\textsuperscript{\Delta}IQ9-11-GFP} was only observed in 7% of the inspected cell separations (n=38), whereas 71% of the cells expressing an additional copy of Iqg1p showed a normal contraction of Iqg1\text{\textsuperscript{\Delta}IQ9-11-GFP} (n=45) (Fig. 1C). Thus, the correct targeting of Iqg1p to the site of cytokinesis occurs independently of IQ9-11 and does not depend on Hof1p, whereas the contraction of the AMR or the tight association of Iqg1p to the AMR during its contraction are affected by the absence of IQ9-11. Accordingly, IQ9-11 alone is not sufficient for bud neck targeting. An ectopically expressed GFP fusion to a fragment of Iqg1p harbouring IQ9-11 (Iqg1\text{\textsuperscript{IQ1-11}}-GFP) did not stain the bud neck but was found to be randomly distributed in the cytoplasm of the cell (Fig. 1D).

**Hof1p-binding to Iqg1p requires myosin light chains**

The interaction between Mlc1p and the IQ repeats 9-11 of Iqg1p could be reconstituted \textit{in vitro} with the bacterially expressed proteins (Fig. 2A). However, neither immobilized fusions of Mlc1p (GST-Mlc1p) nor the isolated IQ9-11 repeats (GST-IQ9-11) were able to precipitate Hof1p or its central element from yeast extracts (Fig. 2B, lanes 2, 4). This observation might suggest that Hof1p binding requires a preformed complex between Mlc1p and Iqg1. To test this hypothesis we first immobilized GST-Mlc1p onto Glutathione-beads and reconstituted the binary IQ9-11/Mlc1p complex by incubating the beads with \textit{E. coli} extracts containing a SNAP-tag fusion to IQ9-11 (IQ9-11-SNAP). Remarkably, the reconstituted GST-Mlc1/IQ9-11-SNAP precipitated Hof1p (Fig. 2B, lane 3), and Hof1\text{\textsuperscript{283-592}} (Fig. 2B, lane 8), but not Hof1\text{\textsuperscript{1-282}} from yeast extracts (Fig. 2B, lane 13). This experiment argues for a stable ternary Mlc1p/Iqg1p/Hof1p complex (MIH). To rule out that MIH formation requires yeast-specific posttranslational modifications or additional factors present in yeast cell extracts, we expressed the central region of Hof1p as a fusion to the maltose binding protein (MBP-Hof1\text{\textsuperscript{283-592}}) in \textit{E.coli} cells. The immobilized GST-Mlc1/IQ9-11-SNAP complex specifically
precipitated the enriched fraction of the MBP-Hof1\textsubscript{283-592} (Fig. 2C, lanes 2, 7), whereas GST-Mlc1p alone failed to bind MBP-Hof1\textsubscript{283-592} (Fig. 2C, lanes 1, 6).

**Mutations in Iqg1p that disrupt its interaction with Hof1p**

At position 2 of their consensus sequence regular IQ-repeats harbour a conserved glutamine (Q2) that is directly involved in binding the C-lobe of Mlc1p (Terrak et al., 2003). Q2 is present in IQ repeats 9 (Q667), 10 (Q695), and 11 (Q725) of Iqg1p. By replacing each of these glutamines by serines individually or in combination we tried to address the influence of each IQ-repeat on the formation of the ternary MIH. Split-Ub analysis revealed that a Q2S exchange in IQ9 (IQ9-11\textsubscript{9Q5CRU}), or IQ10 (IQ9-11\textsubscript{10Q5CRU}), or the corresponding double mutations (IQ9-11\textsubscript{9,10Q5CRU}), abolished the interaction signals with N\textsubscript{ab}-Hof1\textsubscript{98-669} and N\textsubscript{ab}-Hof1\textsubscript{283-592} (Fig. 3A, supplementary material Fig. S3). A similar mutation in IQ11 (IQ9-11\textsubscript{11Q5CRU}) did not measurably interfere with binding to Hof1p (Fig. 3A). The interaction signals with N\textsubscript{ab}-Mlc1p were not significantly reduced by any of the Q2S mutations (Fig. 3A, supplementary material Fig. S3). In vitro reconstitution of the ternary MIH using the mutated SNAP-tag fusions of IQ9-11 complemented the results of the Split-Ub analysis (Fig. 3B).

Here, IQ9-11\textsubscript{9Q5}-SNAP retained a residual interaction to Hof1p whereas no ternary complex formation was observed for IQ9-11\textsubscript{10Q5}-SNAP and IQ9-11\textsubscript{9,10Q5}-SNAP (Fig. 3B). The latter two mutants showed a strong reduction in Mlc1p-binding that was not observed for IQ9-11\textsubscript{9Q5}-SNAP. IQ9-11\textsubscript{9,10Q5}-SNAP was barely seen in the Coomassie-stained gel of the GST-Mlc1p-eluate, but could still be detected by antibody staining (Fig. 3B). Our data thus do not allow unequivocally distinguishing whether the mutations affect the binding between Iqg1p and Hof1p directly, or indirectly through disrupting the interaction between Iqg1p and Mlc1p.

To confirm aspects of the Iqg1p-Hof1p interaction in vivo, we fused IQ9-11-CHERRY to the t-SNARE Sso1p (Schneider et al., 2013). The Sso1-moiety in the IQ9-11-CHERRY-Sso1p fusion relocated IQ9-11 homogenously to the plasma membrane (Fig. 3C). A co-expressed Hof1-GFP strongly stained the bud neck but could occasionally also be observed at spots of concentrated IQ9-11-CHERRY-Sso1p below the membrane of large-budded cells. Such an irregular Hof1-GFP staining of the plasma membrane was neither detected in wild type cells nor in cells co-expressing IQ9-11\textsubscript{9,10Q5}-CHERRY-Sso1p (Fig. 3C). This finding thus offered independent in vivo evidence for the interaction between Hof1p and Iqg1p. Finally, we introduced the MIH-interfering mutations into the CRU constructs of *IQG1* and confirmed their negative impact on the binding of the full-length Iqg1p to Hof1p by Split-Ub interaction analysis (Fig. 3D).
Reduced affinity between Iqg1p and Hof1p impairs Hof1p recruitment and contraction of the AMR

Does the experimental dissolution of the MIH affect Hof1p localization and AMR contraction during cytokinesis? In wild type cells Hof1-GFP shifts its position to the central AMR shortly after the arrival of Iqg1p but before the septin rings split (Fig. 4A upper panel, supplementary material movie S1)(Meitinger et al., 2011). In cells carrying the mutants Iqg1∆IQ9-11, or Iqg1IQ9, 10QS as their sole IQG1 allele, Hof1-GFP was retained as a double ring at the bud neck for a prolonged period of time before merging into the gap between the two septin-rings (Fig. 4A middle and lower panel, supplementary material movies S2, S3). Compared to cells expressing the native Iqg1p, the appearance of Hof1p at the position of the AMR was delayed by 5 min in iqq1IQ9, 10QS cells and by 6.5 min in iqq1∆IQ9-11-cells (Fig. 4D). As both Iqg1-mutants lacked the binding site to Hof1p, the emergence of Hof1p at this position was unexpected and invoked the presence of an alternative and Iqg1p-independent targeting region within Hof1p. Indeed, the isolated N-terminal F-BAR domain (Hof11-282) is known to remain associated with the AMR during contraction (Oh et al., 2013). Time-lapse analysis of wild type cells revealed that Hof11-282-GFP appeared at the position of the AMR later than full length Hof1p and only after the septin rings have split (Fig. 4B upper panel, 4D). Hof11-282-GFP acquired this position independently of Iqg1p as it was clearly seen between the split septins in ∆iqq1, iqq1∆IQ9-11, or iqq1IQ9,10QS cells (Fig. 4B, E). In contrast, the central region of Hof1p, Hof1283-592-GFP, harbouring the binding site to Iqg1p, appeared shortly after Iqg1p in wild type cells and assumed the position at the AMR approximately 8 min before the full length Hof1-GFP normally arrives at this site (Fig. 4C, D). Contrary to the full length protein and its N-terminal Hof11-282-GFP fragment, Hof1283-592-GFP was almost never observed at the AMR when the IQ repeats 9-11 of Iqg1p were either deleted or carried the Q2S exchanges (Fig. 4E).

After being finally localized at the AMR, the contraction of Hof1-GFP and Hof11-282-GFP was significantly impaired in all cells carrying mutations that disturb the binding between Hof1p and Iqg1p (Fig. 4F; supplementary material movies S2, S3, S4).

The MIH-interfering mutations in IQG1 also affected the constriction of Myo1-GFP during cytokinesis. Roughly half of the inspected iqq1∆IQ9-11 cells and a quarter of the iqq1IQ9,10QS-cells displayed Myo1-GFP rings that were either arrested or contracted asymmetrically during cytokinesis (Fig. 4G; supplementary material movies S5, S6, S7). ∆hof1- and iqq1IQ9,10QS-cells affected Myo1-GFP contraction to a very similar extent (Fig. 4G).
Genetic interactions of \(IQG1\) alleles support the role of the MIH as coordinator of cytokinesis

The results of our interaction analysis together with already published experiments support the existence of a large cytokinetic core complex (Fig. 5A). The proposed architecture of this multi-protein assembly implies that the MIH connects Hof1p to the AMR and furthermore physically and functionally links the core proteins of the AMR Myo1p/Iqg1p to Chs2p and the stimulators of its activity, Cyk3p and Inn1p (Fig. 5A). Although many data including our own support or are compatible with the existence of this central cytokinetic core complex, direct evidences for many of the postulated physical interactions are still lacking. For example, others and we have shown that Cyk3p binds to Hof1p and Hof1p binds to Iqg1p (Jendretzki et al., 2009; Labedzka et al., 2012; Nishihama et al., 2009) (Figs. 1, 2). As the Hof1p binding sites for Cyk3p and Iqg1p are located in different regions of the molecule it is suggestive but not proven that both ligands interact with Hof1p at the same time (Fig. 1). Depending on stability, geometry and \textit{in vivo} duration, the detection of such indirect interactions as the one between Cyk3p and Iqg1p may exceed the limits of current technologies (Johnsson, 2014). To test key predictions derived from the proposed existence of the cytokinetic core complex by an alternative approach we measured genetic interactions between the MIH-interfering alleles of \(IQG1\) and other selected members of the cytokinesis pathway. A stringent interpretation of these experiments is based on the presumption that the investigated \(IQG1\) allele(s) affect only the binding to Hof1p and thus display no negative genetic interaction with a \(HOF1\) deletion. The comparison between the phenotypic consequences of the \(iqg1_{\Delta IQ9\text{-}11}\) and \(iqg1_{IQ9\text{-}10QS}\) alleles in wild type and \(\Delta hof1\)-cells strongly suggested that \(iqg1_{IQ9\text{-}10QS}\) impaired cytokinesis by exclusively interfering with MIH formation, whereas \(iqg1_{\Delta IQ9\text{-}11}\) caused additional phenotypes on top of disrupting the MIH (Fig. 5B). The deletions of \(MYO1\) or \(CYK3\) are tolerated in wild type cells but are synthetic lethal to each other and to a deletion of \(HOF1\) (Korinek et al., 2000; Vallen et al., 2000). \(\Delta cyk3\)-cells display defects in PS formation and thus depend on a functional AMR for survival (Meitinger et al., 2010; Onishi et al., 2013) (Fig. 5C complex 1). Deleting \(CYK3\) in \(iqg1_{IQ9\text{-}10QS}\) cells resulted in a strong impairment of growth arguing for a physical Hof1p-Iqg1p interaction that significantly contributes to proper AMR-contraction (Fig. 5B, C complex 4). Contrary to \(\Delta cyk3\)-cells, \(\Delta myo1\)-cells fail to assemble a functional AMR (Bi et al., 1998; Tolliday et al., 2003) (Fig. 5C, complex 3). Mutations in Iqg1p that further impair cytokinesis in \(\Delta myo1\)-cells will thus most likely point to a role for Iqg1p in septum synthesis and perhaps other functions that are not directly related to the AMR. Indeed, \(iqg1_{IQ9\text{-}10QS}\);
\(\Delta myo1\)-cells were not viable thus supporting a complementary role for the MIH in septum synthesis and the existence of an indirect interaction between Cyk3p/Inn1p and Iqg1p in the cytokinetic core complex (Fig. 5B, C complex 6).

Deletions of other Hof1p-interacting proteins like Nba1p, Sho1p or Spa2p did not further impair the growth of \(iqg1_{IQ9,10QS}\)-cells (Fig. 5B) (Labedzka et al., 2012; Moreno et al., 2013). We conclude that the genetic interactions of \(iqg1_{IQ9,10QS}\) do not reflect a general reduction in fitness but are very specific and restricted to a certain subclass of cytokinetic genes. Similar to \(HOF1\) and in contrast to \(SHO1\) and \(SPA2\), a deletion of \(NBA1\) displayed an allele specific synthetic lethality with \(iqg1_{\Delta IQ9,11}\). Nba1p interacts with Hof1p as well as with Cyk3p (Labedzka et al., 2012). The genetic interaction assigns this complex a distinct yet unknown role in cell separation.
Discussion

Iqg1p is synthesized late during the cell cycle when its interaction with Mlc1p targets the protein immediately to the bud neck (Fig. 5A) (Lippincott and Li, 1998b). Once attached to the site of cytokinesis the Mlc1p/Iqg1p complex recruits Myo1p to then form a scaffold for the subsequent incorporation of Hof1p into the MIH (Fig. 5A) (Fang et al., 2010). While the timely transfer of the septin-associated Hof1p to the C-terminal IQ-repeats of Iqg1p is initiated by cell cycle-specific phosphorylations of Hof1p (Meitinger et al., 2011), the formation of the ternary MIH ensures that Hof1p only associates with the AMR once Iqg1p and Mlc1p are already in place. Two alternative mechanisms could account for this directionality: Mlc1p and Iqg1p may provide a shared interface for the interaction with Hof1p, or Hof1p binding may depend on the helical conformation of IQ repeats 9-11 that is only formed upon prior docking to Mlc1p (Terrak et al., 2003). The in vitro assembly of the ternary complex showed a nearly stoichiometric binding of Hof1p to IQ9-11. We propose that the formation of the Mlc1p/IQ9-11 complex limits the amount of MIH assembly in this experiment. Once formed, Mlc1p/IQ9-11 associates tightly and stoichiometrically with Hof1p. We further learned from our in vitro experiment that the binding of Hof1p to Mlc1p/IQ9-11 does not require any post-translational modifications. We thus propose that the cell cycle specific phosphorylations of Hof1p that initiate its transfer to the AMR might instead be needed to either dissolve the connection between Hof1p and the septins or to render the otherwise hidden central region of Hof1p accessible for binding to Mlc1p/Iqg1p (Meitinger et al., 2011). Our model for the role of the IQ9-11 repeats of Iqg1p can not explain why the deletion of this stretch is more deleterious for the cells than the point mutations that disrupt the interaction with Hof1p. The activity that makes Iqg1p essential for cytokinesis is still unknown. This activity resides in the region of Iqg1p situated C-terminally to its binding site for Hof1p. Although other explanations cannot be excluded at this point we favour the interpretation that the altered and thus non-optimal distance of the essential GRD/GAP domains in Iqg1pΔIQ9-11 to other binding partners of the molecule might further impair the activity of Iqg1p.

Once the central cytokinetic complex is assembled the simultaneous contraction of the AMR and the synthesis of the septum will lead to the ingression of the plasma membrane and ultimately to its fusion. Our model of the cytokinetic core complex stipulates that a physical connection between Myo1p and Hof1p through the Mlc1/Iqg1-complex although not essential in wild type cells might become especially important in cells with impaired septum synthesis (Fig. 5). The observed growth defect of Δcyk3 cells upon disruption of the MIH complex...
seems to support this prediction but may alternatively be caused by the failure of Inn1p/Sho1p or other binding partners of Hof1p to link to Iqg1p (Fig. 5B, C). The latter interpretation would predict that a HOF1 allele that disrupts these interactions should be lethal in Δcyk3 cells. As Δcyk3-cells expressing a HOF1 allele lacking its SH3 domain (Δcyk3; hoflΔSH3) were already found to be viable (Fig. 5C, complex 7) (Labedzka et al., 2012; Meitinger et al., 2013; Oh et al., 2013), we conclude that the ultimate defect in complex 4 and its functional difference to complex 7 is due to the failure of Iqg1IQ9,10QS to directly contact Hof1p. Accordingly, alleles of HOF1 lacking a functional F-BAR domain fail to support growth of Δcyk3-cells (Labedzka et al., 2012) indicating that in the absence of proper septum synthesis the connection between the F-BAR domain and Iqg1p/Myo1p becomes essential for a productive AMR/membrane constriction. Two further observations seem to support this role of Hof1p as a tether between the membrane and the cytokinesis machinery (Meitinger et al., 2011; Nishihama et al., 2009): 1. The isolated F-BAR domain of Hof1p binds to the area between the split septin rings independently of Iqg1p (Fig. 4). Whether the F-BAR domain is attached to the exposed membrane, and if yes, directly or indirectly through a further protein, is presently unknown and deserves further investigation. 2. Deletion of HOF1 and the MIH-interfering mutations seem to have quantitatively similar effects on the contraction of Myo1-GFP (Fig. 4G).

Following a similar mode of argumentation we propose that the lethality of complex 6 expressing cells (iqg1IQ9,10QS; Δmyo1) proves the functional importance of the physical connection between Iqg1p and Cyk3p/Inn1p (Fig. 5C). This interpretation predicts that a deletion of SH3Hof1 should be detrimental in Δmyo1-cells. Indeed it was shown by several groups that complex 8 of Δmyo1; hoflΔSH3-cells does not support cell growth (Fig. 5C) (Labedzka et al., 2012; Meitinger et al., 2013; Oh et al., 2013). We surmise that the intimate connection between Iqg1p and Cyk3p/Inn1p is the important feature of complex 3 (Fig. 5C). A reported co-precipitation of Iqg1p with Inn1p may be seen as independent evidence for the existence of a physical link between Iqg1p and Inn1p through Hof1p/Cyk3p (Sanchez-Diaz et al., 2008).

Myo1p binds weakly to Hof1p (Oh et al., 2013). Accordingly, cells lacking MYO1 fail to localize the central element of Hof1p to the AMR (Oh et al., 2013). The Myo1p-Hof1p interaction can be incorporated into our model by proposing that Iqg1p and Myo1p bind Hof1p simultaneously and that both proteins recruit Hof1p synergistically to the AMR. However, a dominant contribution of the Myo1p-Hof1p interaction in connecting the AMR with septum synthesis is difficult to reconcile with the relatively mild growth defects of cells.
expressing complex 3 and with the lethal transition of complex 3 into complex 6 upon introducing MIH interfering mutations (Fig. 5C). The difference in the fitness of cells expressing complex 4 and 7 is also not compatible with a central role of Myo1p in attaching Hof1p to the AMR. Myo1p was previously shown to support the stable assembly of most members of the contractile ring during cytokinesis (Wloka et al., 2013). It is therefore equally possible that alternatively to a synergistic binding a general scaffolding function of Myo1p ties Iqg1p and indirectly its binding partner Hof1p to the AMR (Fig. 5A).

The binding to Myo1p and Iqg1p renders Mlc1p essential for cytokinesis (Boyne et al., 2000; Luo et al., 2004; Shannon and Li, 2000). We have shown that in addition to targeting Iqg1p to the AMR Mlc1p also helps to recruit Hof1p. Our MIH interfering Iqg1p mutations could clearly distinguish between these two separate functions. Iqg1p is correctly localized at the AMR although it lacks the binding sites for Mlc1p that promote the interaction of Iqg1p with Hof1p. Taken together our findings on the formation of the MIH may lead to a more general model of the interactions of IQ repeats with their targets. It will be therefore important to distinguish through future experiments whether Mlc1p contacts Hof1p directly or induces an interaction-compatible conformation within the IQ repeats.
Materials and Methods

Construction of fusion genes and other molecular manipulations. GFP-, CHERRY-, and CRU-fusion genes were constructed by homologous recombination in the yeast genome as described (Dünkler et al., 2012; Hruby et al., 2011). A list of the constructs used in this study can be found in the supplementary material Table S1. All plasmid maps and sequences, as well as the sequences of the used primers can be obtained upon request. Briefly, the plasmid pCRU 303, an integrative plasmid for construction of full-length CRU fusions, was cut with EagI and SalI and a PCR fragment of IQG1 covering the sequence 4040-4485 was cut with the same restriction sites and inserted in frame to the C\textsubscript{ub}-RURA3 module. The primers used for the IQG1 amplification read: CGGCCGCCGTCACAAAAGTAAA and GTCGACCCCAAGCGCTCTTTTATAGAA. The EagI and SalI sites are underlined respectively. For recombination in yeast the obtained plasmid was cut at the single XbaI site in the ORF of IQG1 and transformed into competent yeast cells. Successful homologous recombination at the IQG1 locus was verified by colony PCR using a primer annealing in the sequence of IQG1 upstream of position 4040 and a primer annealing within the sequence of C\textsubscript{ub}. Insertion of the PMET17 promoter in front of the genomic IQG1CRU was achieved by a one-step replacement of the IQG1 upstream sequence (Janke et al., 2004). N\textsubscript{ub}-fusion genes of HOFI fragments harbouring the N-terminal 282 residues or residue 283 till 592 were obtained by inserting the coding sequences in frame behind the PCUP1-Nub module on a centromeric plasmid (Dünkler et al., 2012). The same gene fragments were inserted between the sequences of the PMET17 promoter and GFP or 9MYC to create PMET\textsubscript{17}Hof1\textsubscript{1-282}\textsubscript{-GFP/9MYC} fusions on the centromeric plasmids pRS313 or pRS315 (supplementary material Table S1) (Sikorski and Hieter, 1989). To delete residues 650-750 in IQG1 a 5’-fragment of IQG1 till position 1947 and a 3’ fragment starting with position 2251 were amplified through PCR. The forward primer for the 3’ fragment contained 40 bp of identical sequence to the 3’ end of the 5’ fragment. The sequence of the reverse primer for the PCR of the 5’fragment reads: TGACTGTACCTTATGTCTTACAGC. The sequence of the forward primer for the PCR of the 3’ fragment reads: CTGTATACGCGAAACGCTGTACAGCAGTACA GTCAAAAAATTTCGCTATTTCG. Underlined is the shared sequence of both fragments. The subsequent overlapping PCR reaction with both fragments yielded the ORF of IQG1 lacking the codons for residues 650-750. Mutations in IQG1 were introduced accordingly by overlapping PCR using primers annealing at the respective sites but carrying a mismatch to change the codons. The glutamines at position 667, 695 and 725 were exchanged each by a
serine. For complementation tests and genetic interaction assays, the ORFs of \textit{IQG1} and its alleles were flanked by 300 base pairs of their genomic up- and down-stream region and inserted into the centromeric plasmids pRS314, pRS315 or pRS316 (Sikorski and Hieter, 1989). To yield the plasmids pIQ9-11-CHERRY-SSO1 or pIQ9-11QS-CHERRY-SSO1 the sequences coding for the residues 650 to 750 of \textit{IQG1} or \textit{iqg1IQ9,10QS} were inserted in frame between the \textit{P}_{\text{MET17}} promoter and the \textit{CHERRY}-SSO1 coding sequence. GST- fusions were obtained by placing the ORF of the respective gene or gene fragment behind the \textit{E. coli} GST sequence on the pGEX-2T vector (GE Healthcare, Freiburg, Germany). The SNAP-tag fusions were expressed from the vector pAGT-Xpress, a pET15b derivative, where the native or the mutation-containing sequences of IQ-repeats 9-11 of \textit{IQG1} were inserted in frame into a multi-cloning site located between the upstream 6XHIS-tag-coding sequence and the downstream SNAP-coding sequence (Merck-Millipore, Darmstadt, Germany).

**Growth conditions, yeast strains, and genetic methods.** Culture media and yeast genetic methods were performed following standard protocols (Guthrie and Fink, 1991). SD-media for the Split-Ubiquitin interaction assay contained 1 mg/ml 5-fluoro-orotic acid (5-FOA, Fermentas, Heidelberg, Germany). All yeast strains used were derivatives of JD51 and are listed in Table S2 of the supplementary material (Dohmen et al., 1995). One-step gene deletions were performed by PCR based methods as described (Janke et al., 2004; Wach et al., 1997). In order to generate haploid $\Delta$\textit{iqg1} cells containing \textit{IQG1} on a centromeric plasmid, we sporulated diploid $\Delta$\textit{iqg1}/\textit{IQG1} cells containing an extra copy of pIqg1 316. Haploid $\Delta$\textit{iqg1} containing plasmid-borne \textit{IQG1} were verified by colony PCR ($\Delta$\textit{iqg1}/\textit{IQG1}:pRS316). Occasionally very slow growing colonies were obtained that carried the deletion of \textit{IQG1} but no plasmid-borne \textit{IQG1} ($\Delta$\textit{iqg1}). These cells were transformed to generate $\Delta$\textit{iqg1}-cells expressing \textit{HOF1-GFP}, GFP-labelled fragments of \textit{HOF1}, or \textit{MYO1-GFP} from centromeric plasmids. $\Delta$\textit{iqg1}-cells expressing different \textit{IQG1} alleles or their GFP-fusions were obtained by transformation of $\Delta$\textit{iqg1}/\textit{IQG1}:pRS316 cells followed by growth on SD media selecting for the presence of the \textit{IQG1} alleles and containing 5-FOA, or alternatively by directly transforming $\Delta$\textit{iqg1}-cells.

**Split-Ub interaction analysis.** Large scale Split-Ubiquitin assays were performed as described (Dünkler et al., 2012; Hruby et al., 2011). SD media contained no methionine to fully express Iqg1CRU and the CRU-fusions of the IQ-repeats. Measuring interactions between individual N\textsubscript{ub}- and C\textsubscript{ub}-fusion proteins by spotting yeast cells expressing both fusions onto 5-FOA containing SD media selecting for the presence of the N\textsubscript{ub}- and C\textsubscript{ub} fusions, was essentially as described (Eckert and Johnsson, 2003).
Genetic interaction assay. The genes of interest were deleted in a $\Delta iqq1$ strain harbouring $IQG1$ on a centromeric $URA3$ plasmid by a one step PCR-based method as described (Janke et al., 2004). The obtained strains were subsequently transformed with centromeric $LEU2$ or $TRP1$ harbouring plasmids expressing $Iqg1p$, $Iqg1_{\Delta IQ9-11}$, or $Iqg1_{IQ9,10QS}$. Independent transformants were grown overnight in SD Trp-, or SD Leu-, and 4.5 $\mu$l culture of OD$_{600}$=3 were spotted in 10 fold serial dilutions onto SD medium lacking Trp or Leu and either containing or lacking 5-FOA. Growth was documented after two days at 30°C.

Fluorescence Microscopy. Yeast strains were grown overnight in liquid selective SD media, diluted 1:10 in 3-5 ml fresh media the next day, and grown with or without methionine to mid-log phase at 30°C. Cells were washed twice in 1×PBS and 3-4 $\mu$l of a cell suspension was spotted on a microscope slide, covered with a glass coverslip and immediately observed under the microscope. For time-lapse microscopy, the suspension was spread over a 1.7% agarose pad containing SD complete. Cells were inspected with a Delta Vision fluorescence microscopy system (GE Healthcare, Freiburg, Germany) provided with a steady state heating chamber and equipped with a mercury arc lamp and a charge-coupled-device (CCD) camera CoolSNAP HQ2-ICX285 (Photometrics, Munich, Germany). In all cases a 100x NA 1.4 UPlanSApo oil immersion objective (Olympus, Hamburg, Germany) was used. Images were acquired and analysed with the softWoRx software of the Delta Vision System, and processed using ImageJ software and Adobe Photoshop. Unless otherwise stated, 4-section z stacks with 0.6 $\mu$m intervals were collected every 2 or 3 min. Fluorescent proteins were visualized using a live cell filter set (Chroma Technology, Bellow Falls, USA) for EGFP ($\lambda$ex470, $\lambda$em525) and mCHERRY ($\lambda$ex572, $\lambda$em632) respectively. The CCD capture time was adapted to the intensity of GFP and CHERRY signal in every construct to reduce bleaching and phototoxicity. All live cell imaging experiments were performed at 30°C in humidified atmosphere that was held constant by a Delta Vision System-supplied temperature chamber for up to 5 hours.

For defining the localization of Hof1-GFP and its GFP-labelled fragments at the bud neck, Shs1-CHERRY expressing cells with large buds were monitored for green fluorescence at the neck and additionally inspected by time-lapse analysis. For measuring the contraction of Myo1-GFP, Iqg1-GFP, Iqg1$_{\Delta IQ9-11}$-GFP, Hof1-GFP and its GFP-labelled fragments, time-lapse analysis of cells co-expressing Shs1-CHERRY were performed, and the contraction of the GFP fluorescence between the split-septin rings classified as either symmetric, asymmetric, or arrested. Cells were observed at least 40 min after septin splitting before being classified as arrested. The fragments of Hof1-GFP were expressed from centromeric plasmids.
under the control of the P_{MET17}-promoter, Myo1-GFP was expressed from a centromeric plasmid under control of the native P_{MYO1}-promoter, and the GFP-fusions of all other full-length proteins were expressed from their native genomic locations. If not stated otherwise, expression from the P_{MET17}-promoter was induced by omitting methionine in the medium.

**Statistical tests.** Data evaluation and statistical analyses were performed using Prism. T-tests were used to evaluate the significance of the times of appearances of Hof1-GFP and its different GFP-labelled fragments at the AMR.

**Preparation of yeast cell extracts.** For pull-down experiments, yeast cell cultures were grown in SD media selecting for the presence of the fusion proteins to an OD_{600} of 1.5, pelleted, washed once in ice-cold water, and then transferred into liquid nitrogen. The cell pellets were ground in liquid nitrogen using a mortar. The cell powder was collected in yeast protein extraction buffer (50 mM Hepes pH 7.5, 150 mM NaCl, 1 mM EDTA) containing 1x protease inhibitor cocktail Complete (Roche Diagnostics, Mannheim, Germany), 1 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF). 0.1% Triton X-100 was added, incubated for 10-20 min on ice, and extracts were clarified by centrifugation.

**Expression and immobilization of GST-, MBP- and SNAP-tagged proteins**

*E. coli* cells (BL21, Amersham, Freiburg, Germany) expressing MBP-, SNAP- or GST-fusions were grown at 37°C to an OD_{600} of 0.6 in SB- (GST-Mlc1, SNAP-fusions, MBP-Hof1_{238-592}) or LB-medium (GST, MBP). The expression of the GST- and SNAP- fusion proteins were induced by 0.1 mM IPTG for four hours at 30°C. The expression of the MBP-fusion proteins were induced by addition of 0.5 mM IPTG for 4 h at 37°C (MBP) or by addition of 0.5 mM IPTG followed overnight incubation at 18°C (MBP-Hof1_{283-592}). 200 ml of the cells were chilled on ice and harvested by centrifugation at 4°C. Cells expressing MBP or MBP-Hof1_{283-592} were resuspended in 12 ml 20 mM Tris-Cl pH 7.4 containing protease inhibitor cocktail, 0.4 mM PMSF and 1mg/ml lysozyme (Sigma-Aldrich Chemie, Steinheim, Germany). Cells were sonicated for three min on ice, and the lysates were clarified by centrifugation at 13,000 rpm for 30 min at 4°C. The extract was incubated for one hour at 4°C with 0.15 ml amylose beads (NEB, Frankfurt, Germany). The beads were washed four times with CW Buffer and the bound protein was eluted in 0.15 ml CW buffer containing 10 mM maltose. Cells expressing GST- or SNAP-fusions were resuspended in 4 ml PBS containing protease inhibitor cocktail, 0.4 mM PMSF and 1mg/ml lysozyme. To keep the GST- or SNAP-labelled IQ repeats 9-11 soluble, 0.5% Triton X-100 and 1.5% Na-N-laurylsarcosine were added to the extract. To immobilize the GST-fusions, the extracts were incubated with PBS-equilibrated glutathione-sepharose 4B beads (GE Healthcare, Freiburg, Germany) for
one hour at 4°C under rotation. Finally, bound material was washed four times in 1x PBS.

**Pull-down and in vitro binding assays.** Sepharose slurries of the immobilized GST-fusions were incubated for 1 h at 4°C under rotation with either 1 ml of yeast cell extract containing the MYC-tagged proteins, with 1 ml of *E.coli* extracts containing SNAP-fusion proteins or with 0.8 ml of CW buffer containing 0.04 ml of the enriched MBP-fusion protein in a concentration of 5 mg/ml. Beads with bound proteins were separated by 2 min centrifugation at 6000 rpm and washed 4 times with yeast protein extraction buffer. The bound material was specifically eluted through incubation with 100μl of 50 mM TRIS pH 7.0, 20 mM reduced glutathione for 10 min at 4°C. After a brief centrifugation, the supernatant was transferred to a fresh tube, and analysed by SDS-PAGE and immunoblotting as described (Hruby et al., 2011). To reconstitute the binary IQ9-11/Mlc1p complex onto sepharose beads, GST-Mlc1p sepharose slurry was incubated for 1 h at 4°C under rotation with 1 ml of *E.coli* extracts containing IQ9-11-SNAP or its mutants. Beads with bound proteins were separated by 2 min centrifugations at 6000 rpm and washed four times with PBS and two times with yeast protein extraction buffer before using them for the *in vitro* binding and pull-down assays.

**Acknowledgements**

We like to thank Dr. J. Müller for comments on the manuscript. We thank Ute Nussbaumer and Steffi Timmermann for excellent technical assistance.

**Competing interests**

The authors declare no competing interests.

**Author contribution**

C.T. performed the experiments shown in Figures 1, and 3-5. Y.W. and C.T. performed the *in vitro* binding studies shown in Figures 2 and 3. C.T. and N.J. devised the experiments and wrote with the help of Y.W. the manuscript.

**Funding**

The work was supported by a DFG Research Grant to N.J. (JO 187/5-1).
References


Figure Legends:

Figure 1
The IQ-repeats 9-11 of Iqg1p interact with the central region of Hof1p. (A) Upper panel: domain structure of Iqg1p. Lower panel: results of Split-Ub interaction assays of Iqg1CRU and different fragments of Iqg1p tested against a panel of 383 Nub-fusions. Shown are the cutouts of the N_{ab}- and CRU-expressing yeast strains in media containing 5-FOA. The cutouts displaying the interactions of a certain CRU fusion are always derived from the same 5-FOA plate. Growth of a quadruplet of yeast cells on plates containing 5-FOA indicates interaction of the co-expressed fusion proteins. (B) Upper panel: domain structure of Hof1p. Lower panel: Split-Ub assay of Iqg1CRU and the CRU-fusion of the IQ-repeats 9-11 of Iqg1p tested against the N_{ab}-fusion of Hof1p, and of different Hof1-fragments, N_{ab}-Mlc1p and N_{ab}-fusions of proteins that were assumed not to interact with Iqg1p. Shown is the growth of serial dilutions of the N_{ab}- and CRU-fusion co-expressing yeast strains on media containing 5-FOA. Growth indicates interaction between the co-expressed fusion proteins. Cutouts belonging to the Iqg1IQ9-11CRU assay were derived from the same 5-FOA plate. (C) Time-lapse analysis of wild type- (upper panel) or ∆iqg1-cells (lower panel) both expressing Iqg1\_IQ9-11-GFP. (D) Fluorescence microscopy of Iqg1IQ1-11-GFP-expressing cells. The expression of the GFP fusion was under control of the P_{MET17}-promoter. Left panel shows cells grown in medium containing no methionine resulting in high expression levels, and right panel in medium containing 70 µM methionine resulting in moderate expression levels. Scale bars 5 µm. See also supplementary material Figs. S1, S2.

Figure 2
A ternary complex consisting of Iqg1p, Mlc1p, and Hof1p. (A) Extracts of E.coli cells expressing IQ9-11 coupled to a HIS-tagged SNAP (IQ9-11-SNAP, lane 3) were incubated with Glutathione beads carrying GST (lane 1) or GST-Mlc1p (lane 2). The bound fractions were eluted and analysed by coomassie staining (upper panel) and immune-detection with anti-HIS antibody after SDS-PAGE (lower panel). (B) Upper panel: extracts of yeast cells (lanes 5, 10, 15) expressing MYC-tag fusion of Hof1p (lanes 1-5), Hof1_{1283-592} (lanes 6-10), or Hof1_{1-282} (lanes 11-15) were incubated with Glutathione beads carrying GST-Mlc1p (lanes 2, 7, 12), GST-IQ9-11 (lanes 4, 9, 14), GST-Mlc1p pre-incubated with IQ9-11-SNAP (lanes 3, 8, 13), or GST pre-incubated with IQ9-11-SNAP (lanes 1, 6, 11). The bound fractions were analysed by SDS-PAGE and subsequent immuno-detection with anti-MYC antibody. Lower panel: coomassie-stained gel of the eluates of the glutathione beads incubated with yeast.
extract expressing Hof1\textsubscript{283-592}-MYC from the experiment of the upper panel of (B). Beads were incubated with GST and IQ9-11-SNAP (lane 16, corresponds to lane 6 of the upper panel), with GST-Mlc1p and IQ9-11-SNAP (lane 17, corresponds to lane 8 of the upper panel), or with GST-IQ9-11 (lane 19, corresponds to lane 9 of the upper panel). The enriched fraction of IQ9-11-SNAP used for incubation with GST- (lane 16) and GST-Mlc1p-beads (lane 17) is shown in lane 18. (C) MBP-Hof1\textsubscript{283-592} (lanes 1, 2, 6, 7, 11, 12) or MBP (lanes 3, 8, 13) were incubated with Glutathione-beads carrying GST-Mlc1p (lanes 1, 6, 11), or GST-Mlc1p pre-incubated with IQ9-11-SNAP (lanes 2, 3, 7, 8, 12, 13). The bound fractions were analysed by SDS-PAGE and either coomassie staining (lanes 1-3), or immuno-detection with anti-MBP (lanes 6-8) or anti-HIS antibodies (lanes 11-13). Enriched MBP-Hof1\textsubscript{283-592} (lanes 4, 9, 14) and MBP (lanes 5, 10, 15) used as input for the binding assay were analyzed by coomassie-staining (lanes 4, 5) or immuno-detection with anti-MBP (lanes 9, 10) or anti-HIS antibodies (lanes 14, 15).

Figure 3
Mutations at conserved positions of the IQ repeats interfere with Hof1p binding to the Iqg1p/Mlc1p complex. (A) Split-Ub assay of IQ9-11CRU harbouring QS exchanges in the indicated IQ-repeats as in (1B). Shown are cutouts from the same 5-FOA plate. (B) Beads carrying GST-Mlc1p (lane 1) or GST-Mlc1p pre-treated with IQ9-11\textsubscript{9,10QS}-SNAP (lane 2), IQ9-11-SNAP (lane 3), IQ9-11\textsubscript{9QS}-SNAP (lane 5), or IQ9-11\textsubscript{10QS}-SNAP (lane 6), were incubated with yeast extracts expressing Hof1\textsubscript{283-592}MYC (lane 4). Shown are the Glutathione-eluates of the beads after SDS-PAGE and anti-MYC immuno-detection (upper panel), or after coomassie-staining of the gel (middle panel), or after anti-HIS immuno-detection (lower panel). (C) Fluorescence microscopy of two cells each co-expressing Hof1-GFP and IQ9-11-CHERRY-Sso1p (left panel) or Hof1-GFP and IQ9-11\textsubscript{9,10QS}-CHERRY-Sso1p (right panel). Experiments were done in otherwise wild type cells expressing Hof1-GFP from its genomic location and the CHERRY fusions from a centromeric plasmid. (D) Split-Ub assay of full length Iqg1CRU as in (1B) but harbouring the indicated QS exchanges in the IQ repeats 9 and 10 of Iqg1p. Shown are cutouts from the same 5-FOA plate. Scale bars 5 μm. See also supplementary material Fig. S3.

Figure 4
The choreography of Hof1 targeting to the AMR and AMR contraction are altered by MIH-interfering mutations. (A) Time-lapse analysis of cells carrying different I\textsubscript{G}I\textsubscript{G}I alleles and co-
expressing Hof1-GFP and Shs1-CHERRY. Top frame: GFP. Middle frame: CHERRY. Bottom frame: merge of GFP and CHERRY. (B) Same as in (A) but with cells expressing Hof1_283-592-GFP. (C) As in (A) but with wild type cells expressing Hof1_283-592-GFP. (D) Time of appearance of Iqg1-GFP, Hof1-GFP and its GFP-labelled fragments at the position of the AMR in wild type cells and cells of the indicated IQG1 genotypes. Time 0 marks the splitting of the septin rings. Error bars, standard error of the mean (s.e.m.). ns indicates that the measured differences are not significant. * P ≤ 0.016, *** P ≤ 0.0001. See also supplementary material Fig. S2 and movies S1-S4. (E) Co-staining of CHERRY-labelled septin subunit Shs1p and GFP-labelled Hof1p or fragments of Hof1p in cells of the indicated IQG1 genotypes. Left panel: shown from left to right are the GFP-, CHERRY-, and the merged channels of Hof1_283-592-GFP expressing cells. Right panel: quantitative analysis of static images of the localization of GFP-labelled Hof1p, Hof1_1-282 and Hof1_283-592 in IQG1-cells (n= 60, 48, 95), iqq1_1-282-IQ9-11-cells (n= 71, 87, 60), iqq1 IQ9,10QS-cells (n= 240, 139, 200), and Δiqg1-cells (n= 297, 37, 40). The fractions of cells containing correctly positioned GFP-fusions of Hof1p or its fragments between the split septin rings are indicated. Scale bars 5 µm. (F) Quantification of time-lapse analyses of cells during cytokinesis. Shown are the fractions of cells displaying no, asymmetric or symmetric contraction of GFP-labelled Hof1p, Hof1_1-282 or Hof1_283-592 in IQG1-cells (n= 23, 26, 41), iqq1_1-282-IQ9-11-cells (n= 40, 70, 38), iqq1 IQ9,10QS-cells (n= 227, 130, 175), and Δiqg1-cells (n= 39, 21, 23). (G) AMR-contraction followed by time-lapse analysis of Myo1-GFP- and Shs1-CHERRY-expressing cells of the indicated genotypes. Left panel: shown is the symmetric contraction of Myo1-GFP in a wild type cell contrasted with its asymmetric contraction in iqq1_1-282-IQ9-11, iqq1 IQ9,10QS- and Δhof1-cells. The overlays of the GFP- and the CHERRY-signals are shown. Right panel: quantitative analysis of the experiments. Shown are the fractions of cells displaying no, asymmetric or symmetric contraction of Myo1-GFP in IQG1-cells (n= 42), iqq1_1-282-IQ9-11-cells (n= 47), iqq1 IQ9,10QS-cells (n= 86), and Δhof1-cells (n= 74). See also supplementary material movies S1-S7. Red arrowheads indicate the splitting of the septin rings.

**Figure 5**

Genetic interactions of the iqq1 IQ9,10QS allele. (A) Model of the assembly and architecture of the cytokinetic core complex. Note that Mlc1p is only depicted as part of the MIH complex. The well-described interaction between Mlc1p and Myo1p is not shown. The presentation divides Hof1p into a N-terminal region (Hof1ΔSH3) and its SH3 domain (SH3Hof1). The positional shift of SH3Hof1 should highlight that the interactions with its ligands occur late
during core complex assembly. (B) Upper panel: serial dilutions of yeast cells carrying \textit{IQG1}, \textit{iqg1}_{M09-11}, or \textit{iqg1}_{9,10QS} were incubated at 37°C for two days. Lower panel: yeast cells of the indicated genotypes and carrying in addition \textit{IQG1} on a \textit{URA3}-harboring plasmid were spotted in serial dilutions onto plates containing uracil and 5-FOA and incubated at 30°C for two days. Non-growth of the cells indicates a negative genetic interaction between the introduced \textit{IQG1} allele and the respective gene deletion. (C) Proposed architecture of the cytokinetic core complex in the indicated genetic backgrounds. Numbers specify the different complexes, open and closed circles indicate growth or non-growth of cells expressing these protein complexes. For simplicity the symbol representing Mlc1p stays bound to complex 4, 5, 6.
A

\[ \text{Mlc1} \quad \text{Hof1ΔSH3} \quad \text{SH3}_{\text{Hof1}} \quad \text{Cyk3} \]

\[ \text{Iqg1} \quad \text{Myo1} \quad \text{Sho1} \quad \text{Inn1} \]

B

\[ \text{Δcyk3} \quad \text{Δhof1} \quad \text{Δmyo1} \]

\[ \text{Δnba1} \quad \text{Δspa2} \quad \text{Δsho1} \]

\[ 5 \text{ FOA} \]

C

\[ \text{Iqg1ΔIQ9-11} \quad \text{Iqg1}_{\text{IQ9, 10QS}} \]

\[ \text{Δcyk3} \quad \text{Δhof1} \quad \text{Δmyo1} \]

\[ 1^{\circ} \quad 2^{\circ} \quad 3^{\circ} \]

\[ \text{Iqg1}_{\text{IQ9, 10QS}} \]

\[ 4^{\circ} \quad 5^{\circ} \quad 6^{\circ} \]

\[ \text{Iqg1}_{\text{IQ9, 10QS}} \quad \text{ΔSH3} \]

\[ 7^{\circ} \quad 8^{\circ} \]

\[ \text{Iqg1}_{\text{IQ9, 10QS}} \quad \text{ΔSH3} \]

\[ \text{Growth promoting} \quad \text{non promoting} \]