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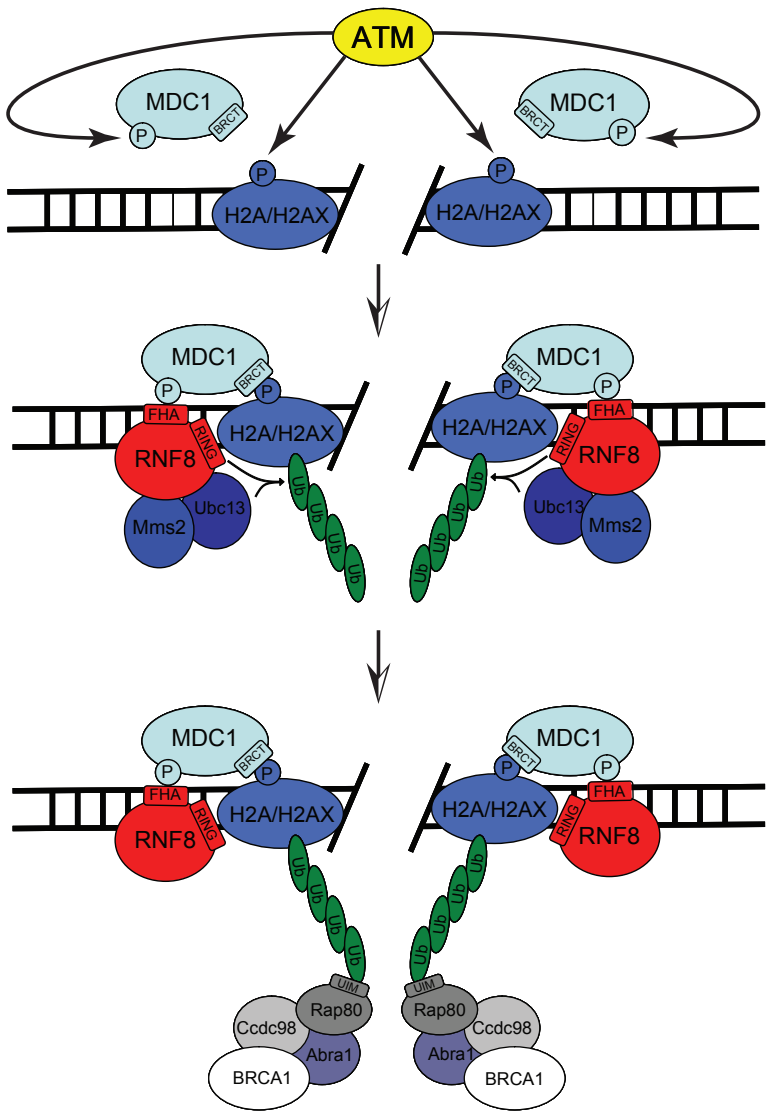

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Cellular and Molecular Life Sciences

Review

FHA-RING ubiquitin ligases in cell division cycle control

L. Brooks, III[a], E. G. Heimsath, Jr.[a], G. L. Loring[b] and C. Brenner[a,*]

[a] Departments of Genetics and Biochemistry and Norris Cotton Cancer Center, Dartmouth Medical School, Rubin 733–HB7937, Lebanon, NH 03756 (USA), Fax: +603 653 9923, e-mail: charles.brenner@dartmouth.edu
[b] Altus Pharmaceuticals, Cambridge, MA 02139 (USA)

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Abstract. Despite the common occurrence of forkhead associated (FHA) phosphopeptide-binding domains and really interesting new gene (RING) E3 ubiquitin ligase domains, gene products containing both an N-terminal FHA domain and C-terminal RING domain constitute a highly distinctive intersection. Characterized FHA-RING ligases include the two vertebrate proteins, Checkpoint with FHA and RING (Chfr) and RING finger 8 (Rnf8), as well as three fungal proteins, Defective in mitosis (Dma1), Chf1 and Chf2. These FHA-RING ligases play roles in negative regulation of the cell division cycle, apparently by coupling protein phosphorylation events to specific ubiquitylation of target proteins. Here, the available data on upstream and downstream regulation of and by FHA-RING ligases are reviewed.

Keywords. Cell cycle, checkpoint, E3 ubiquitin ligase, Ubc4, Ubc13/Mms2.

Introduction

FHA domains are phosphothreonine-binding modules frequently found in checkpoint and DNA repair proteins [1, 2]. RING domains are zinc-containing protein folds that encode one family of E3 ubiquitin ligases [3]. The Ensembl 49 assembly of the human genome contains 33 genes with an FHA domain (Pfam ID PF00498) and 258 genes with a RING domain (Pfam ID PF00097). However, only Chfr and Rnf8 contain both. Each plays a distinct role in induced negative regulation of cell cycle progression. Chfr is a tumor suppressor protein that delays the cell cycle upon microtubule (MT) stress [4], whereas Rnf8 halts the cell cycle when double strand DNA breaks (DSB) are detected [5–8].

The observed co-occurrence of FHA and RING domains in these polypeptides has led to the following questions. How many different phosphoproteins bind to the FHA domain? Is the FHA domain a binding site for phosphorylated protein ubiquitylation substrates or a binding site that localizes the FHA-RING ligase for modification of proteins not bound to the FHA domain? Do the FHA-RING ligases work with particular E2 ubiquitin conjugating enzymes? What are the nature of the ubiquitylation sites and linkages catalyzed by FHA-RING ligases and what are the cellular fates of the modified proteins? Is there a meaningful role for FHA-RING ligase autoubiquitylation? How many different substrates are there for a single FHA-RING ligase?

As depicted in Figure 1, in the case of Rnf8, it has become clear that the FHA domain functions to dock the ligase to ataxia-telangiectasia mutated (ATM)-phosphorylated Mdc1 protein, which is bound to chromatin at DSB, thereby orienting the RING domain for Ubc13/Mms2-dependent polyubiquitylation of specialized DNA-damage associated histone substrates [5–8]. Whether all Rnf8 functions are mediated by

* Corresponding author.

ATM as the upstream kinase, Ubc13/Mms2 as the E2 ubiquitin conjugating enzyme, and histones as the essential ubiquitylation substrates is not known. However, there is evidence that Rnf8 may also interact with the E2 enzymes Ube2E2, UbcH6 and Ube2E3 [9] and there is evidence that Rnf8 has ATM-independent functions [10–12]. Additionally, whereas Figure 1 will clearly serve as the basis for thinking about Chfr and the fungal FHA-RING ligases, there are current indications that Cdc123, an FHA-associated protein, may promote turnover of *S. cerevisiae* FHA-RING ligases [13], that Ubc4 may function as a ubiquitin-conjugating enzyme for particular FHA-RING ligase activities [13–16], that autoubiquitylation plays a role in FHA-RING protein regulation [16–18], and that at least some FHA-RING ligases have G1-arresting cell cycle functions [12, 13, 16] in addition to the well documented effects at G2/M.

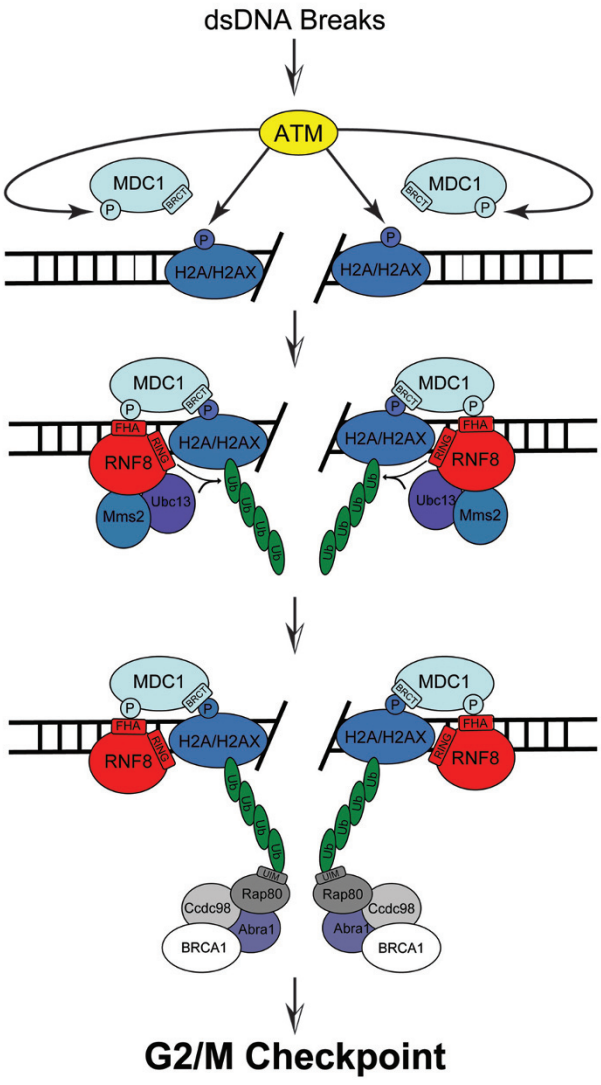

Figure 1. Relationship between FHA and RING defined for Rnf8. Rnf8 catalyzes ATM-signalled, Mdc1-localized, Ubc13/Mms2-mediated polyubiquitylation of histones H2A and H2AX at DSB, thereby localizing additional proteins for G2/M arrest.

Phylogenetic analysis of FHA-RING ligases

It has been frequently observed that there are two FHA-RING proteins in *S. cerevisiae* [13, 16, 19, 20] and two in vertebrates [11]. However, it has not been entirely clear whether the two budding yeast FHA-RING proteins represent anciently diverged molecules, which might be distinctly orthologous to Chfr and Rnf8. To explore this question, we performed pairwise identity analysis of *S. cerevisiae* Chf1, Chf2, *S. pombe* Dma1, and human Chfr and Rnf8 FHA and RING domain peptide sequences, which are schematized in Figure 2A. Each protein contains an FHA [2] and a RING [3] domain in the same relative orientation. Chfr is unique in containing a C-terminal poly(ADPribose)-binding zinc finger (PBZ) domain [21].

As shown in Table 1, the most similar sequences are those of *S. cerevisiae* Chf1 and Chf2. Chf1 and Chf2 are, in turn, more similar to *S. pombe* Dma1, the lone FHA-RING protein of fission yeast, than to any vertebrate sequence. These results indicate that, consistent with the genome duplication that predated the emergence of *S. cerevisiae* and closely related budding yeasts [22], Chf1 and Chf2 commonly derive from a single fungal FHA-RING protein similar to *S. pombe* Dma1 and that the appearance in vertebrates of two FHA-RING paralogs is unconnected to the existence of two budding yeast paralogs. Indeed, pairwise comparisons involving the two human FHA-RING ligases indicate that they are more similar to each other than to any of the fungal FHA-RING ring ligases. These data suggest that Chfr and Rnf8 diverged in a vertebrate duplication event independent of the budding yeast duplication. As shown in Figure 2B, two or three FHA-RING paralogs are found in yeast species closely related to *S. cerevisiae*, which emerged after a budding yeast whole genome duplication [22, 23], whereas a single Dma1-homologous protein occurs in *S. pombe*, *K. lactis*, *A. gossypii*, *K. waltii*, *D. hansenii*, *C. albicans* and *Y. lipolytica.* Curiously, the euascomycota *N. crassa*, *F. graminearum*, *M. grisea* and *A. nidulans* contain a duplication of FHA-RING proteins that appears independent of that in *S. cerevisiae.* Additionally, two budding yeasts, *C. glabrata* and *S. castellii*, which are classified as post-whole genome duplication [23], have no FHA-RING ligase. The loss of FHA-RING ligases from these two yeasts is similar to the apparent absence of FHA-RING proteins from the

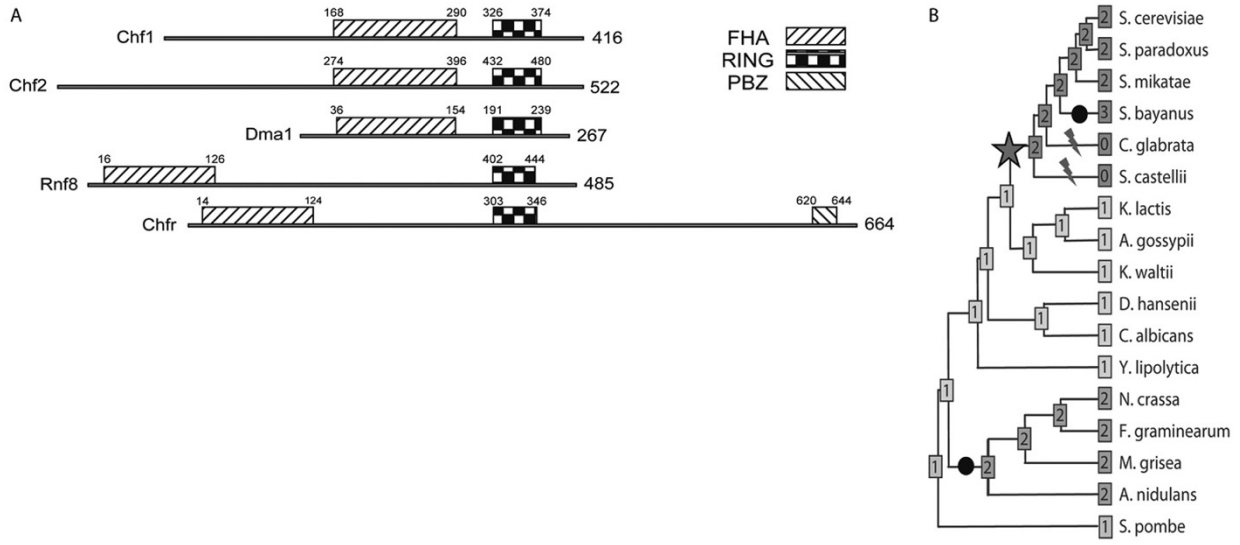

Figure 2. Domain structure of FHA-RING ligases. (*A*) A domain-based alignment of *S. cerevisae* Chf1, Chf2, *S. pombe* Dma1, and human Chfr and Rnf8. (*B*) A phylogram calculated from whole genome sequences [23] depicting the numbers of FHA-RING paralogs in Ascomycota fungi. The star at the base of the hemiascomycete clade maps a whole genome duplication event. Gene gain or loss at each node was inferred from the number of paralogs in each sequenced genome. Lightning bolts indicate apparent gene loss events. Circles indicate apparent gene duplication events.

Table 1. Pairwise domain identity (FHA% / RING%) between FHA-RING ligases.

	Human Rnf8	*S. pombe* Dma1	*S. cerevisiae* Chf1	*S. cerevisiae* Chf2
Human Chfr	28.2/40.9	22.4/23.5	19.0/26.8	21.0/31.6
Rnf8	–	23.4/28.6	26.3/26.5	24.6/26.4
Dma1	–	–	43.3/42.9	45.7/44.9
Chf1	–	–	–	82.1/83.7

genomes of invertebrates *D. melanogaster* and *C. elegans*, despite the general conservation of one ancestral FHA-RING ligase throughout fungal-metazoan lineages.

FHA Domains in FHA-RING Ligases

FHA is a phosphothreonine-binding domain frequently found in DNA repair and checkpoint proteins [1, 2]. Presence of an FHA domain implies that one or more protein kinases function upstream of these proteins. An intact FHA domain is required for checkpoint function of all characterized FHA-RING ligases. In the case of *S. cerevisiae* Chf1 and Chf2, point mutations in the FHA domain produce alleles that are loss of function, yet which are greatly increased in protein abundance, suggesting that the FHA domain may play a role in Chf protein destruction [13]. Ablation of the FHA domain of Chfr disrupts checkpoint function, such that a Chfr allele in which the FHA domain is deleted behaves as a dominant negative [4]. The FHA domain of Chfr is also important for proper subnuclear localization of Chfr to promyelocytic leukemia (PML) protein bodies [24]. The FHA domain of Rnf8 is similarly required for the subnuclear localization of Rnf8 to chromatin and is necessary for function [5–8, 12]. The FHA domain of Dma1 is required for association with spindle pole body and the division site [25].

RING Domains in FHA-RING Proteins

E3 ubiquitin ligases function as scaffolding or specificity factors in catalyzing the transfer of monomeric ubiquitin [3] or polyubiquitin chains [26] from E2 ubiquitin-conjugating enzymes, thereby facilitating autoubiquitylation and transfer of ubiquitin to external substrates [27]. Indeed, it has been demonstrated that Chfr and *S. cerevisiae* Chf proteins possess RING domain-dependent ubiquitin ligase activity *in vitro* with Ubc4, Ubc5 and Ubc13/Mms2 acting as the E2 [14–17]. The linkage topology of the assembled polyubiquitin chains differs according to the E2 provided. Lys63 linked chains are assembled when

Ubc13/Mms2 acts as E2 and a mixture of Lys48-linked and Lys63-linked polyubiquitin is produced when Ubc4 or Ubc5 acts as E2 [16, 17]. Rnf8 has been reported to interact with E2 enzymes Ube2E2, UbcH6 and Ube2E3 [9] and to catalyze autoubiquitylation [28] and histone H2A ubiquitylation [7] with the Lys63 linkage-forming Ubc13/Mms2 ubiquitin conjugating enzyme.

The RING domain is critical for function of FHA-RING ligases. An intact RING domain is required for Chfr checkpoint function [14, 17, 18, 29], Rnf8 checkpoint function [5, 7], Chf protein checkpoint function [13, 16] and Dma1 checkpoint fuction [25]. Though loss of Rnf8 function by mutation of the RING domain is correlated with loss of ubiquitin ligase activity, the RING domain of Rnf8 is also required for constitutive nuclear localization during interphase [12] and for Rnf8 association with retinoid X receptor alpha [10].

Because RING domain ubiquitin ligases do not form covalent ubiquitylated intermediates, it is easier to demonstrate the autoubiquitylated products of E3 ubiquitin ligases than to identify and characterize external substrates as direct E3 targets. For example, overexpression of a RING E3 ligase may lead to increased formation of an ubiquitylated protein species, which might be identified either in a hypothesis-based experiment or a proteomic survey of modified tryptic peptides. However, to demonstrate that the RING protein is directly responsible for modification requires knocking down the RING protein *in vivo* plus evidence that *in vivo* sites and linkages formed *in vivo* are the same as those produced in direct *in vitro* reactions. Demonstration of autoubiquitylation products is comparatively simpler because one can simply determine whether a tagged E3 ligase becomes modified *in vivo* in a RING-dependent manner and test whether the same sites and linkages are formed in purified *in vitro* reactions. Though autoubiquitylation of FHA-RING ligases is straightforward to demonstrate, the *in vivo* significance of autoubiquitylation is open to question. In the case of Chfr, the deubiquitylating enzyme Usp7/Hausp has been shown to deubiquitylate Chfr protein *in vitro* and overexpression of Usp7/Hausp stabilizes Chfr [30]. In budding yeast, evidence suggests that FHA-dependent Cdc123 association with Chf1 and Chf2 promotes RING-dependent Chf protein turnover [13]. E2 ubiquitin conjugating enzymes Ubc4 and Ubc13/Mms2 have distinct roles in mediating Chf protein function *in vivo* and each E2 has been shown to promote Chf protein autoubiquitylation *in vitro* [16]. However, available data indicate that Ubc4-dependent autoubiquitylation is responsible for Chf protein turnover [16].

PBZ Domain of Chfr

Chfr contains a unique cysteine-rich C-terminus containing a PBZ domain, which binds poly(ADPribose) with a K_D of 0.5 nM [21]. An intact PBZ domain and the ability to bind poly(ADPribose) are required to establish the Chfr checkpoint upon MT stress. Additionally, the PBZ domain is required for the dominant negative activity of the ΔFHA allele, though the PBZ is not required for ubiquitylation activity nor is binding to poly(ADPribose) dependent on the ubiquitylation state of Chfr. Because the Chfr checkpoint can be abrogated by treatment with the poly(ADPribose) polymerase (PARP) inhibitor, KU-0058948, Chfr may be the key protein target of PARP activity in times of mitotic stress [21].

Chfr Mediates the MT Stress-Induced Axis of the Antephase Checkpoint

Upon exposure to a variety of stresses during interphase, cells exhibit the capacity to arrest the cell division cycle prior to mitotic entry. The window of arrest competence before prophase is termed antephase [31]. Treatment with radiation [32], low temperature [33], fluoride [34], and MT-destabilizing agents such as nocodazole [35] activate the antephase checkpoint, transiently arresting cells at G2/M. MT-hyperstabilization with compounds such as Taxol also appears to be sufficient to induce antephase arrest [4, 14, 36–38], although this not universally accepted [39, 40].

Data indicate that Chfr is required for MT stress-induced but not DNA damage-induced antephase checkpoint [14, 18, 38]. The DNA damage response is mediated by the ATM/ATR kinases and, therefore, can be abrogated by caffeine, a specific inhibitor of ATM/ATR activities [41]. If cells are exposed to caffeine prior to nocodazole, the ability of cells to arrest at antephase is not compromised [35]. Thus, the ATM/ATR-mediated antephase DNA damage response is not Chfr-dependent and, conversely, the Chfr-dependent antephase response is not dependent upon ATM/ATR.

Although it is clear that MT stress-induced antephase arrest and DNA damage-induced antephase arrest are activated by different upstream surveillance mechanisms, both pathways result in transient arrest at the same point in the cell cycle, potentially mediated by p38 stress activated protein kinases [42]. Treatment of cells under MT stress with p38 inhibitors blocks antephase arrest, whereas microinjection of activated recombinant p38 or treatment with the p38 kinase activator anisomycin during early prophase causes

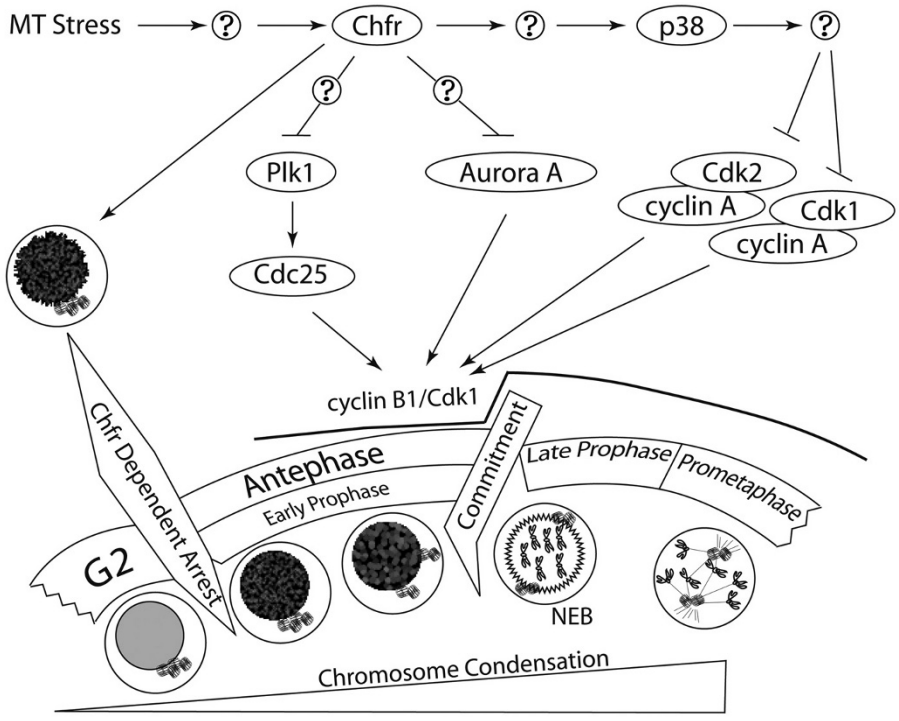

Figure 3. A model for Chfr checkpoint function. Chfr is required for establishment of the MT stress axis of the antephase checkpoint, which delays cells with a distinctive ruffled nuclear morphology and decondensed chromosomes. The execution point of Chfr is prior to commitment to late prophase/prometaphase and nuclear envelope breakdown (NEB). Aurora A and Polo-like kinase 1 (Plk1) are possible direct substrates. Downstream effector molecules include the p38 stress activated protein kinases, the protein phosphatase Cdc25, and Cdk1/Cyclin B1, which remains inactive during Chfr arrest.

chromosome decondensation, which is a component of antephase checkpoint arrest [18]. This effect has been observed in Chfr-deficient cell lines such as HeLa and U2OS, and also in cells expressing the dominant negative ChfrΔFHA allele, implying that the p38 kinases are downstream of Chfr in the MT stress response pathway [18]. The accumulated data indicate that the antephase DNA damage response and MT stress response may converge on p38 kinases as downstream effectors [18, 40, 42].

Antephase arrest has been characterized morphologically and in terms of biochemical markers [42, 43]. As depicted in Figure 3, antephase begins in early prophase when chromosome condensation becomes apparent by light microscopy and terminates in midprophase with nucleolar breakdown, which coincides with translocation of Cdk1/cyclin B1 into the nucleus [44] and activation of kinase activity [45]. If cells are exposed to mitotic stress during G2 or antephase, chromosomes decondense and transiently arrest at G2/M, thereby reducing mitotic index. During Chfr-dependent antephase arrest, centrosomes are unseparated [4] and cells exhibit a ruffled nuclear envelope morphology [38]. However, if mitotic stress is introduced after antephase, the cell cycle cannot arrest and cells progress into prometaphase with kinetics similar to those of untreated cells.

Inactive Cdk1/Cyclin B1 is a feature of the Chfr-dependent arrest state [15, 18, 46]. Further, cells cannot invoke Chfr arrest if MT poisons are applied after nucleolar breakdown, which is the morphological hallmark of antephase termination and Cdk1/cyclin B1 activation [18]. Available data suggest that Chfr-arrested cells do contain active Cdk1/cyclin A, however [38]. Moreover, the Chfr checkpoint can be overcome by injecting the cell with cyclin A bound to a form of Cdk2, which is refractory to inhibition [18]. The major problem in dissecting the cell biology of Chfr function is identifying the direct protein targets of Chfr that account for antephase arrest. Supporting the view that nuclear localization of cyclin B1 is specifically blocked at the Chfr checkpoint, forced nuclear expression of cyclin B1 overrides nocodazole-induced delay [38]. Accordingly, there has been an intense focus on mechanisms that connect Chfr activation with blocking nuclear accumulation of cyclin B1.

In Xenopus extracts, Chfr has been shown to ubiquitylate Plk1, leading to proteasome-dependent proteolysis of Plk1 [15], a known mediator of cyclin B1 localization. Cyclin B1 protein contains a nuclear export motif that can be phosphorylated by Plk1, causing nuclear retention of cyclin B1 during midprophase [47]. There is evidence that Plk1 levels may be reduced by Chfr activity *in vivo* [48]. However, consistent differences in Plk1 levels have not been observed as a function of Chfr status [38, 46], such that it is not clear that Plk1 is the key *in vivo* target.

Aurora A also mediates cyclin B1 localization [49] and, based on high levels of Aurora A in *chfr-/-* knockout mice, has been identified as a potential substrate of Chfr [48]. However, in cell lines, no

change in Aurora A protein levels nor localization pattern has been observed during antephase arrest [38].

Rnf8 as a DNA-Damage Inducible E3 Ubiquitin Ligase

The function of Rnf8 as a mediator of repair complex assembly at DSB sites has been recently defined [5–8]. GFP-tagged Rnf8 transfected into U2OS cells accumulates at ionizing radiation-induced foci (IRIF) in a pattern identical to that of the DNA damage-associated histone, γ-H2AX [7]. In 293T cells, Rnf8 not only colocalizes with γ-H2AX, but also with Mdc1, Nbs1, 53bp1, Brca1, phosphorylated ATM and Mcph1 [5] at DSB sites following irradiation. Rnf8 acts upstream of Nbs1, 53bp1, Brca1, Rap80 and Abraxas such that, when Rnf8 is mutated or depleted, none of these proteins form IRIF at DSB sites. In HEK293T cells, endogenous Mdc1 is associated with endogenous and FLAG-tagged Rnf8 in an FHA-dependent manner [6, 7].

Mdc1 contains three Thr residues in Thr-Gln-X-Phe motifs, which are responsible for the FHA-dependent and phosphorylation-dependent interaction with Rnf8 [5–7]. Although a direct interaction was demonstrated between Rnf8 and phosphorylated Mdc1 *via* the Rnf8 FHA domain, Mdc1 is not a substrate for ubiquitin modification mediated by Rnf8. Instead, DSB-associated Rnf8 catalyzes the ubiquitylation of H2A and γ-H2AX [5,7]. Thus, Mdc1 as an FHA domain-binding protein for Rnf8, serves as a docking protein for FHA-RING ligase activity.

A screen of 13 candidate E2 ubiquitin-conjugating enzymes revealed that Ubc13 acts as the conjugating enzyme in the Rnf8-dependent ubiquitylation of histones H2A and γ-H2AX [6], presumably modifying these histone substrates with Lys63-linked polyubiquitin. There is an early report that Rnf8 is degraded in a proteosome-dependent manner, suggesting that Rnf8 may also be modified by Lys48-linked polyubiquitin [9], but it can also clearly form Lys63-linked polyubiquitin [28]. Rap80 contains two ubiquitin-interacting motifs that bind to Lys63-linked polyubiquitin chains to localize functional Brca1 complexes in response to DNA damage [50]. Ubc13 and the Rnf8 ligase, containing functional FHA and RING domains, are required for irradiation-induced association of Rap80 with γ-H2AX and for Brca1 localization [5]. Thus, there is excellent evidence for the cellular mechanism of Rnf8 summarized in Figure 1.

Fission Yeast Dma1 Enforces the Septation Initiation Network (SIN)

In fission yeast, the SIN is a spindle pole body associated kinase signaling cascade, fully activated at the end of mitosis, which couples mitotic exit with cytokinesis. Once Cdk1 activity is fully depressed at anaphase, the SIN invokes formation of the contractile actin ring (CAR) and deposition of the septum at the midbody. Constriction of the cellular midbody is facilitated by the coordinated action of the CAR and the septum, as both are required for completion of cytokinesis. Fission yeast Dma1 acts on components of the SIN and prevents cytokinesis in times of MT stress [51].

Dma1 was originally isolated as a multicopy suppressor of *cdc16–116* [19], an allele of the fission yeast homolog of budding yeast *BUB2*, required for proper function of the SIN [52]. Cdc16 and Byr4 function as GTPase activating proteins regulating the activity of the Spg1 GTPase, the central signaling protein in the SIN. The Cdc16/Byr4 complex is active during interphase, maintaining Spg1 in the GDP-bound state. Thus, *cdc16–116* mutants allow Spg1 and SIN activation at restrictive temperature, allowing cells with multiple or no nuclei to accumulate. Dma1 overexpression rescues this phenotype in a manner that depends on intact FHA and RING domains [19].

Dma1 function was also characterized by deletion. Like other FHA-RING ligases, Dma1 is nonessential. Though deletion does not confer a differential growth rate nor grossly disturb cytoskeleton, Dma1 is required for the spindle assembly checkpoint. With loss of Dma1, cold-sensitive mutants in beta tubulin, which normally arrest without a septum, septate in the absence of a spindle [25]. Dma1 protein is localized to the spindle pole body in an FHA domain-dependent manner. One potential mechanism by which Dma1 may inhibit cytokinesis is by controlling localization of the SIN activator, Polo-like kinase, Plk1 [25].

Budding Yeast Chf1 and Chf2 Function at Both Major Cell Cycle Arrest Points

Though budding yeast do not divide by binary fission, some components of the SIN are conserved. The budding yeast mitotic exit network (MEN) is responsible for ensuring correct positioning of the nucleus at the bud neck prior to cytokinesis [53]. Budding yeast Chf1 and Chf2 function in spindle positioning and septin ring assembly in the MEN in a manner that suggests Bub2 antagonism [20]. Additionally, Chf1 and Chf2 were identified as two-hybrid interactors with Cdc123, an essential and conserved positive regulator of the cell

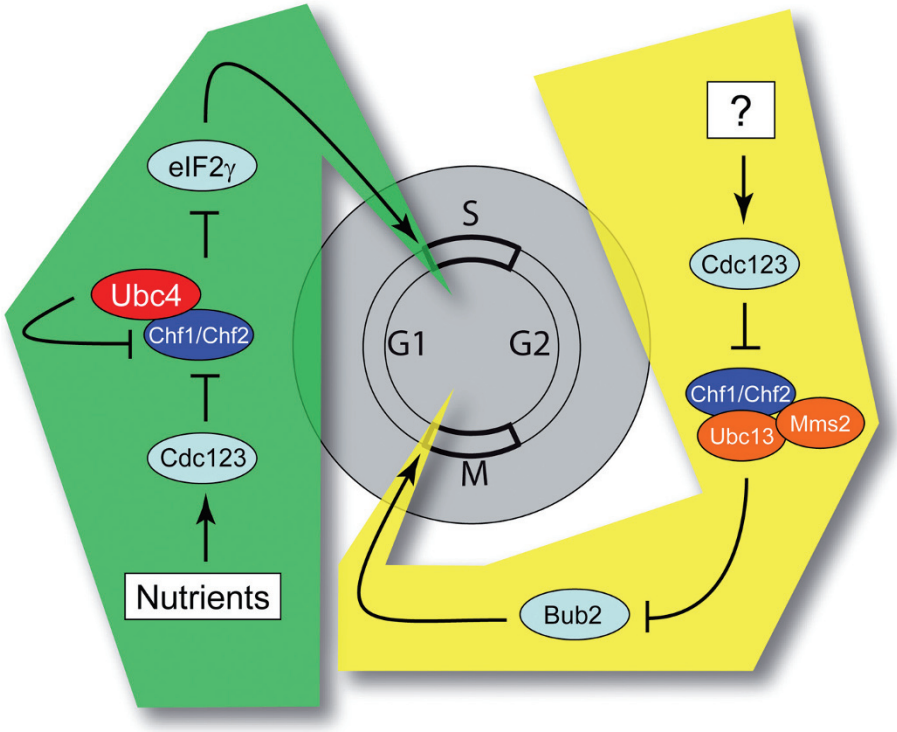

Figure 4. Budding yeast FHA-RING ligases, Chf1 and Chf2, elicit cell cycle delays at G1 and mitotic exit. The FHA-interacting Cdc123 protein, the E2 ubiquitin conjugating enzyme Ubc4, and the eukaryotic translational initiation factor 2γ Gcd11 constitute an axis of nutritional control of the cell cycle by Chf proteins at G1 phase. The model indicates that Cdc123 and Ubc4 mediate Chf protein downregulation and external target polyubiquitylation. Cdc123 activity is also required late in the cell cycle, where it may promote Chf protein and Ubc13/Mms2-dependent downregulation of Bub2.

division cycle [13]. *cdc123–4* mutants have a G2 delay phenotype at permissive temperature and arrest as large, unbudded (late G1) cells at the restrictive temperature [13]. Overexpression of either Chf protein delays the cell cycle in a manner dependent on intact FHA and RING domains, whereas deletion of Chf proteins prevents the G2 delay and G1 arrest phenotypes of *cdc123–4* [13]. Though one might have guessed that expression of Chf proteins, as negative regulators of the cell cycle, might block the positive cell cycle effects of Cdc123 by Cdc123 ubiquitylation, Chf protein overexpression does not change the abundance or the mobility of Cdc123. In contrast, Cdc123 depletion, and mutation of Chf protein RING domains and FHA domains lead to accumulation of Chf proteins. These data suggest that Cdc123, a candidate FHA domain-binding protein, may promote Chf protein turnover. Cdc123 protein abundance is positively regulated by nutrients in an unknown manner [13].

Overexpression of Chf2 extends the G1 phase and also extends the cell cycle at a later point [16]. As depicted in Figure 4, Ubc4 mediates the G1 delay and Ubc13/Mms2 mediate Chf2 activity late in the cell cycle [16]. There are two likely Chf functions mediated by the ubiquitin conjugating enzyme, Ubc4. First, because Chf proteins are stabilized by *ubc4* deletion and by Lys to Arg substitutions at sites of *in vitro* Chf protein autoubiquitylation, Ubc4 appears to function in promoting Chf protein turnover [16]. However, because *ubc4* mutants exhibit stabilized Chf proteins but fail to delay G1 phase, it is likely that there is an external G1 target [16]. Gcd11, which encodes eukaryotic initiation factor 2γ, is an essential Cdc123-associated protein and a candidate G1 target [13]. Though it is not known, one would predict that the ability of Chf proteins to antagonize the MEN-acting Bub2 protein would depend on Ubc13/Mms2 and Lys63-linked polyubiquitylation.

Conclusions

Cdc123 and Mdc1 have emerged as two proteins that appear to function upstream of FHA-RING ubiquitin ligases. In the case of Cdc123, association with the FHA-RING ligase appears to drive Chf protein degradation, which is FHA and RING-dependent [13] and Ubc4-dependent [16]. In the case of Mdc1, association with Rnf8 depends on DSB signaling through ATM/ATR kinases, and this association leads to Ubc13/Mms2-dependent Lys63-linked polyubiquitylation of DSB-associated histone substrates [5–8]. Though definitive ubiquitylation targets of Chfr and fungal FHA-RING ligases have not been identified, Ubc4-dependent mechanisms should not be excluded and may co-exist with Ubc13/Mms2-dependent mechanisms. Frequent loss of Chfr in epithelial malignancies [4] suggests that synthetic lethal strategies may be important for chemotherapies targeted to tumors with *chfr* losses.

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