A conserved histone deacetylase with a role in the regulation of cytokinesis in *Schizosaccharomyces pombe*

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**Abstract**

**Background:** In *Schizosaccharomyces pombe* the SET domain protein, Set3p - together with its interacting partners, Snt1p, and Hif2p - form a complex that aids in preventing cell division failure upon mild cytokinetic stress. Intriguingly, the human orthologs of these proteins (MLL5, NCOR2, and TBL1X) are also important for the faithful completion of cytokinesis in tissue culture cells. Since MLL5, NCOR2, and TBL1X form a complex with the histone deacetylase, HDAC3, we sought to determine if an orthologous counterpart played a regulatory role in fission yeast cytokinesis.

**Results:** In this report we identify the *hos2* gene as the fission yeast HDAC3 ortholog. We show that Hos2p physically interacts with Set3p, Snt1p, and Hif2p, and that *hos2*Δ mutants are indeed compromised in their ability to reliably complete cell division in the presence of mild cytokinetic stresses. Furthermore, we demonstrate that over-expression of *hos2* causes severe morphological and cytokinetic defects. Lastly, through recombinase mediated cassette exchange, we show that expression of human HDAC3 complements the cytokinetic defects exhibited by *hos2*Δ cells.

**Conclusions:** These data support a model in which Hos2p functions as an essential component of the Set3p-Snt1p-Hif2p complex with respect to the regulation of cytokinesis. The ability of human HDAC3 to complement the cytokinesis defects associated with the deletion of the *hos2* gene suggests that further analysis of this system could provide insight into the role of HDAC3 in both the regulation of cell division, as well as other biological processes influenced by HDAC3 deacetylation.

**Keywords:** Fission yeast, Cytokinesis, Cell division, Histone deacetylase
the cytoplasm and the extended activation of the SIN. In the absence of either Clp1p or Rad24p, cells are unable to maintain SIN signalling leading to cytokinesis failure and the generation of inviable, multinucleate cells [13,15,18].

A useful strategy in both identifying these regulators, and in defining their roles, has involved the treatment of fission yeast cells with the actin depolymerising drug, Latrunculin A (LatA) [11-15]. At the concentrations used (20–50 times less than that needed to completely depolymerize the actin cytoskeleton) such treatment impedes constriction of the actomyosin ring and is lethal to both clp1Δ and rad24Δ mutants (due to their inability to prolong the cytokinesis competent state). Wild-type cells in contrast, are able to complete cell division under these conditions, albeit at rates slower than in untreated cells.

Interestingly, a recent genome-wide genetic screen based on the isolation of deletion mutants hyper-sensitive to LatA, identified set3, hif2, and snt1 and showed that their respective gene-products form a nuclear-localized complex required for the dependable execution of cytokinesis. Further analysis demonstrated that set3Δ mutants were unable to properly modulate the expression of stress response genes, suggesting a role for the Set3p complex in effecting changes in gene expression required to counter the effects of LatA induced stress [19].

Intriguingly, the set3, snt1, and hif2 genes are orthologous to human MLL5, NCOR2 and TBL1X, which together encode components of a histone deacetylase complex. Remarkably, knockdown of either of these genes in human HeLa cells results in increased rates of cytokinesis failure [20]. Since NCOR2 and TBL1X physically associate with the type I histone de-acetylase, HDAC3 - a highly conserved histone deacetylase with orthologs from Dictyostelium to multicellular mammals - we sought to determine if an orthologous counterpart played a regulatory role in fission yeast cytokinesis [20-22].

Here we identify the hos2 gene as the fission yeast HDAC3 ortholog. Hos2p, also known as Hda1p, is a non-essential histone de-acetylase known to affect H4K16 acetylation (primarily in the 5’ end of genes) as well as gene silencing and sporulation efficiency [23-25]. In this report we show that Hos2p exists in a complex with Set3p, Snt1p, and Hif2p, and that hos2Δ mutants are also compromised in their ability to complete cytokinesis in the presence of low doses of LatA. Furthermore, a role in the regulation of cell division is supported by the severe morphological and cytokinetic defects observed upon hos2 over-expression.

Lastly, we provide strong support for the conservation of HDAC3 function by demonstrating the ability of human HDAC3 to complement the cytokinetic defects exhibited by hos2Δ cells.

Results

Hos2p is required for the successful completion of cytokinesis in response to perturbation of the cell division machinery

[20-22] To determine if an ortholog of HDAC3 existed in S. pombe, and if it too played a role in the regulation of cytokinesis, a BLAST search using human HDAC3 as query was performed. This analysis revealed strong conservation of amino acid sequence between HDAC3 and fission yeast Hos2p (not be confused with the DASH complex subunit, Dad2p, which is also sometimes referred to using the gene name, hos2). The proteins share 51% identity (63% similarity), are of similar length (427 and 437 aa, respectively), and possess a single histone deacetylase domain (PFAM00850) that comprises almost the entire length of the protein (Additional File 1).

To determine if Hos2p played a role in cytokinesis, the hos2 gene deletion mutant was purchased from the commercial supplier, Bioneer. After confirmation of the deletion via colony PCR, wild-type and hos2Δ strains were grown to mid-log phase and serial dilutions plated onto YES media containing either 0.5 μM LatA or DMSO (solvent control).

Interestingly, the hos2Δ strain demonstrated a substantial decrease in viability when grown in the presence of LatA. In contrast, while the rate of growth of wild-type cells decreased in LatA media, viability was not affected (note the formation of small colonies even at the lowest dilution) (Figure 1A).

To determine if the sensitivity to LatA was related to defects in cytokinesis, both wild-type and hos2Δ strains were grown in liquid YES media and then treated with either 0.5 μM LatA or DMSO for 5 hours at 30°C. Cells were then fixed and stained with DAPI and analine blue to visualize nuclei and cell wall/septal material, respectively. No obvious morphological or cytokinesis phenotypes were observed in hos2Δ cells under normal growth conditions. However, in LatA media, hos2Δ mutants were severely impaired in their ability to complete cell division and accumulated a large proportion of tetra-nucleate cells with fragmented septa. In contrast, the majority of wild-type cells were bi-nucleate and formed functional, albeit thickened and sometimes malformed septa (Figure 1B).

To quantitate the data, cells were classified into four different phenotypic categories: i) uni-nucleate cells, ii) bi-nucleate cells with a functional septum (i.e. the septum completely bisects the cell), iii) bi-nucleate cells with a fragmented septum (i.e. the septum is non-functional and does not completely bisect the cell), and iv) tetra-nucleate cells. This analysis revealed that while over 40% of hos2Δ cells were tetra-nucleate, only 6% of wild-type cells...
Figure 1 hos2Δ and hos2-Y321H mutants are hyper-sensitive to LatA treatment. (A) Ten-fold serial dilutions of logarithmically growing cells of the indicated genotype were plated onto YES plates containing 0.5 μM LatA or DMSO (solvent control) at 30°C for 3 d. (B) Cells of the indicated genotype were grown to mid-log phase at 30°C and then treated with 0.5 μM LatA for 5 h before being fixed and stained with DAPI (nuclei) and aniline blue (cell wall/septa). Bar, 10 μm. (C) Quantitation of phenotypes of cells treated as in B. Between 200 and 500 cells were counted for each genotypic class.
showed a similar phenotype. Moreover, while 72% of wild-type cells were either mono-nucleate, or bi-nucleate (with a functional septum), only 31% of hos2Δ cells were similarly distributed (Figure 1C). The phenotypes observed in hos2Δ mutants upon LatA treatment are unlikely to be due to defects in SIN activity since two independent markers of active SIN signalling - Cdc7p localization to a single SPB, and increased export of Clp1p from the nucleolus to the cytoplasm [15] - were normal in hos2Δ mutants upon exposure to LatA (Additional File 2).

An important role for Hos2p in responding to LatA treatment was also supported by synthetic genetic interactions between the hos2Δ and clp1Δ mutations. Since Clp1p is required for the function of the cytokinesis checkpoint, weak cytokinesis mutants often display stronger phenotypes in clp1Δ backgrounds [15]. To test if this were true in the case of hos2Δ mutants, clp1Δ, hos2Δ and clp1Δ hos2Δ mutants were examined after treatment with both 0.1 μM and 0.5 μM LatA. Interestingly, while hos2Δ and clp1Δ single mutants were viable at 0.1 μM LatA, double mutants displayed severe cytokinesis defects at this concentration (Additional File 3).

Lastly, we also noted that the presence of the hos2Δ mutation was capable of lowering the restrictive temperature of the ts cdc15-140 mutation by ~2°C (cdc15 encodes an F-BAR protein required for contractile ring assembly; [26]) (Additional File 4). Interestingly, this decrease in the restrictive temperature of the cdc15-140 mutation is similar to that caused by the presence of the set3Δ, snt1Δ, and hif2Δ gene deletions in cdc15-140 backgrounds. These data further support a common function for the hos2, set3, snt1, and hif2 genes [19].

Once having established that the Hos2p protein was indeed involved in the regulation of cytokinesis, we explored the possibility that the protein’s deacetylase activity was related to its function. To this end we created a mutant strain expressing a form of Hos2p in which the catalytically active tyrosine residue in the catalytic pocket was replaced with a catalytically inactive histidine residue (Y321H). Interestingly, the cytokinesis phenotypes of this mutant were more severe than those displayed by hos2Δ cells. Cells bearing the Y321H mutation were almost completely inviable in the presence of LatA and furthermore, over 60% were tetra-nucleate after 5 hours growth in liquid media containing 0.5 μM LatA (Figure 1A-C).

To more closely examine the effects of LatA on cytokinesis we created a hos2Δ strain expressing a marker of the actomyosin ring, Rlc1-GFP [27]. Using the CellAsics ONIX Microfluidic Perfusion Platform, we were able to monitor the constriction of the actomyosin ring while perfusing liquid YES media containing either 0.5 μM LatA or DMSO as a solvent control. In DMSO media, wild-type cells were able to fully constrict the ring in approximately ~25 minutes. Similarly, rings in hos2Δ mutants grown in DMSO media displayed comparable kinetics and were also able to fully constrict in ~25 minutes (Figure 2, top two rows; Additional Files 5 and 6). However, when grown in LatA media, hos2Δ cells displayed dramatic differences in phenotype compared to wild type. The majority of wild-type cells were able to form and constrict the ring in the presence of LatA, albeit over a much longer time frame (~90 minutes) than DMSO controls (7 out of 9 cells). The remainder (2 out of 9 cells) were able to maintain the integrity of the ring over this time frame, but were not able to fully constrict the ring over the 90 minute time-lapse. In contrast, hos2Δ cells could not preserve the physical integrity of the ring. In LatA treated hos2Δ mutants, Rlc1-GFP signal did not constrict and instead appeared to fragment (8 out of 8 cells) within 10–15 minutes (Figure 2, bottom two rows; Additional Files 7 and 8).

Over-expression of Hos2p results in severe morphological and/or cytokinetic defects

To explore whether Hos2p played a dosage dependent role in cytokinesis we decided to test the effects of hos2 over-expression using the pREP series of thiamine repressible expression vectors [28,29]. To this end, full length hos2 was cloned downstream of the nmt1/41/81 promoters present within the pREP1/41/81 plasmids and the constructs were transformed into wild-type strain, JK484 (Table 1). Full strength expression is obtained from the nmt1 promoter, while nmt41 and nmt81 promoters contain site mutations that decrease expression to intermediate and low levels, respectively [28].

In the presence of thiamine (repressed) cells expressing hos2 from either nmt41, or nmt81 promoters (as well as an empty vector control) displayed normal growth whereas cells expressing hos2 from the nmt1 promoter showed slight growth inhibition (most likely due to the fact that expression from nmt1 is somewhat “leaky”) [28,29]. On the other hand, when grown in the absence of thiamine (de-repressed) cells expressing hos2 displayed an inhibition of growth ranging from severe (in the case of the nmt1 promoter) to intermediate (in the case of the nmt41 promoter) to mild (in the case of the nmt81 promoter) (Figure 3A, B).

Interestingly, expression of hos2 from the nmt1 promoter also led to a series of unusual and pleiotropic phenotypes related to morphogenesis and/or cytokinesis. While the majority of cells appeared normal, others displayed phenotypes ranging from i) slight morphological abnormalities such as a rounded, de-polarized appearance, ii) misplaced, but otherwise normal septa, iii)
abnormally excessive and mis-localized septal deposition, iv) multiple septa v) highly elongated cells containing multiple aberrant deposits of septal material and vi) highly unusual branched and elongated cells (Figure 3C; Table 2). This broad range of phenotypes further supports a model where Hos2p plays a role in the regulation of cytokinesis and/or morphogenesis.

**Hos2p is nucleo-cytoplasmic and physically interacts with Set3p, Snt1p, and Hif2p**

We have previously shown that the Set3p, Snt1p, and Hif2p form a nuclear-localized complex. If indeed a member of this complex, Hos2p would be expected to localize (at least in part) to the nucleus. To test this prediction we created a strain expressing a C-terminal Hos2-GFP fusion protein under control of the native hos2 promoter. Hos2p localized to both the cytoplasm and to the nucleus as judged by co-staining with the nuclear dye, DAPI (Figure 4A). Furthermore, we determined that the intracellular distribution of Hos2p was not affected by LatA treatment (data not shown), nor did its localization change as a function of cell cycle position (Figure 4A). The localization of the Scw1p protein (enriched in the cytoplasm relative to the nucleus) was used as a control to ensure the validity of the observed nuclear signal (Figure 4A, bottom panels) [30].

To determine if Hos2p was able to physically interact with members of the Set3p complex, in-vivo co-immunoprecipitation experiments using Myc or HA epitope tagged alleles were performed. Significantly, Hos2-Myc fusion proteins could be detected in anti-HA immunoprecipitates of Snt1-HA Hos2-Myc, Hif2-HA Hos2-Myc, and Set3-HA Hos2-MyC extracts, but not in extracts expressing only Hos2-Myc (Figure 4B). These results are consistent with high throughput proteomics experiments aimed at defining fission yeast protein complexes related to histone modification [31]. Taken together, these data suggest that Hos2p molecules can indeed exists within a complex with Snt1p, Hif2p, and/or Set3p.

**Expression of human HDAC3 complements the cytokinesis defects associated with the hos2 gene deletion**

To further explore the possibility of functional conservation between human HDAC3 and fission yeast Hos2p, we utilized the technique of recombinase mediated cassette exchange to replace the endogenous Hos2p open
reading frame in fission yeast with the human HDAC3 gene (Additional File 9) [32]. This approach first required the creation of a “base strain” in which the hos2 gene was replaced with a deletion cassette composed of the ura4 selectable marker flanked by the Cre recombinase recognitions sites, loxP and loxM3. As expected, the hos2Δ “base strain” created in this manner was indistinguishable from the Bioneer gene deletion mutant in the ura4-D18 strain (base strain) for maintaining genomic integrity. While the absence of cytokinesis is tolerated under certain specialized circumstances (e.g. the development of Drosophila embryos) it is normally essential for cellular proliferation, differentiation and for maintaining control over ploidy [1,2,33]. Thus, knowledge of the regulatory networks governing cytokinesis is an important component of our basic understanding of eukaryotic cell biology. Moreover, aspects of this knowledge related to cytokinesis failure may also be relevant to our understanding of the mechanisms important for maintaining genomic integrity.

A relationship between cytokinesis failure and genome integrity (and thus tumourigenesis) was first proposed by Theodor Boveri in 1914 [34]. In his classic manuscript “Concerning the origin of malignant tumours” Boveri hypothesized that tetraploid intermediates derived from cytokinetic failure might undergo chaotic multipolar mitoses leading to numerical and/or structural chromosomal defects. Recent experimental evidence (reviewed in [33,35,36]) provides strong support

| Table 1 Strains used in this study |
|-----------------------------------|----------------|---------------|
| **Strain Name**                  | **Relevant Genotype** | **Source**    |
| JKR                             | clp1::ura4 ura4-D18 h | JK Collection |
| JK468                           | hos2::KanMX4 ura4-D18 leu-32 ade6-216 | Bioneer       |
| JK484                           | ura4::D18 leu-32 ade6-216 his3-D1 | JK Collection |
| JK561                           | hos2-GFP::ura4 ura4-D18 leu-32 ade6-210 his3-D1 | This Study |
| JKD68                           | hos2::Y321H::ura4 ura4-D18 | This Study |
| JKD69                           | rlc1GFP::ura4 hos2::KanMX4 ura4-D18 | This Study |
| JKD73                           | hos2::ura4+ ura4-D18 leu-32 ade6-216 his3-D1 (base strain) | This Study |
| JKD44                           | hos2::osa26 ura4-D18 leu-32 ade6-216 his3-D1 | This Study |
| JKD45                           | hos2::HDAC3Hs ura4-D18 leu-32 ade6-216 6his3-D1 | This Study |
| JKD79                           | hos2::KanMX4 clp1::ura4 ura4-D18 | This Study |
| JK761                           | hos2::KanMX4 cdc15-140 ade6-21x leu-1-32 | JK Collection |
| JKD76                           | cdc7GFP::ura4 hos2::ura4 ura4-D18 | This Study |
| JKD78                           | rlc1GFP::KanMX4 hos2::ura4 ura4-D18 | This Study |
| MBY154                          | cdc7s15-140 ade6-21x leu-1-32 h+ | JK Collection |
| MBY624                          | rlc1GFP::ura4 ura4-D18 h+ | JK Collection |
| MBY978                          | clp1GFP::KanMX4 ura4-D18 leu-1-32 ade6-216 h+ | JK Collection |
| MBY2415                         | cdc7GFP::ura4 ura4-D18 ade6-21x leu-1-32 ura4::D18 his3-D1 h+ | JK Collection |
| SCG5                            | hos2::myc::ura4 ura4-D18 leu-32 ade6-210 his3-D1 | This Study |
| SCG10                           | set3-HA::ura4 hos2::myc::ura4 ura4-D18 | This Study |
| SCG11                           | snr1-HA::ura4 hos2::myc::ura4 ura4-D18 | This Study |
| SCG14                           | his2-HA::ura4 hos2::myc::ura4 ura4-D18 | This Study |

In this way the hos2::HDAC3Hs (Hs, Homo sapiens) strain - in which the human HDAC3 gene is under control of the S. pombe hos2 promoter - was created. In addition a control strain, in which the S. pombe hos2 gene was re-engineered back into the base strain, hos2::hos2Sp (Sp, Schizosaccharomyces pombe) was used as a control.

Remarkably, expression of human HDAC3 in S. pombe was able to both restore viability and substantially complement the cytokinesis phenotypes characteristic of the base strain, albeit not to the same extent as the re-introduced hos2 gene (Figure 5A-C). While the base strain displayed over 50% tetra-nucleate cells after 5 hours, the hos2::hos2Sp strain displayed only 5% tetra-nucleate cells and the hos2::HDAC3Hs strain only 18% tetra-nucleate cells. Thus, expression of the human HDAC3 is indeed capable of at least partially complementing the loss of the S. pombe hos2 gene.

Lastly, to provide further evidence of conserved function, we proceeded to over-express human HDAC3 under the control of the nmt1 promoter using the pREP1 plasmid. Similar to the over-expression of hos2, over-expression of HDAC3 resulted in a decrease in growth rate in the absence of thiamine (de-repressed) and similar morphological and cytokinesis phenotypes (compare Figure 3B,C with Figure 6B,C). Just as with the case of the hos2 gene, over-expression of HDAC3 resulted in a similar range of pleiotropic phenotypes including i) cells with a rounded, de-polarized appearance, ii) misplaced septa, iii) abnormally excessive and mis-localized septal deposition, iv) multiple septa v) highly elongated cells containing multiple aberrant deposits of septal material and vi) elongated and branched cells (Figure 6B,C; Table 2). Taking all data together, these results suggest that Hos2p and human HDAC3 function in a similar manner and modulate similar biological processes in S. pombe.

**Discussion**

While the absence of cytokinesis is tolerated under certain specialized circumstances (e.g. the development of Drosophila embryos) it is normally essential for cellular proliferation, differentiation and for maintaining control over ploidy [1,2,33]. Thus, knowledge of the regulatory networks governing cytokinesis is an important component of our basic understanding of eukaryotic cell biology. Moreover, aspects of this knowledge related to cytokinesis failure may also be relevant to our understanding of the mechanisms important for maintaining genomic integrity.

A relationship between cytokinesis failure and genome integrity (and thus tumourigenesis) was first proposed by Theodor Boveri in 1914 [34]. In his classic manuscript “Concerning the origin of malignant tumours” Boveri hypothesized that tetraploid intermediates derived from cytokinetic failure might undergo chaotic multipolar mitoses leading to numerical and/or structural chromosomal defects. Recent experimental evidence (reviewed in [33,35,36]) provides strong support
Over-expression of Hos2p results in growth inhibition and in phenotypic defects related to morphogenesis and/or cytokinesis. (A) Strains expressing hos2 from the nmt1/41/81 series of promoters were streaked to EMM media in the presence (repressed) or absence (de-repressed) of thiamine and incubated at 30°C for two days. (B) Colony morphology of strains treated as in A. Bar, 50 μm (C) Representative examples of the pleiotropic cellular morphology exhibited by cells expressing hos2 from the nmt1 promoter; (i) cells with a rounded, de-polarized appearance, (ii) cells with misplaced septa, (iii) cells with abnormally excessive and mis-localized septal deposition, (iv) cells with multiple septa (v) highly elongated cells containing multiple aberrant deposits of septal material, (vi) elongated and branched cells. Bar, 10 μm.
for Boveri’s assertions; most notably the observation that tetraploid mouse mammary epithelial cells (generated by the inhibition of cytokinesis) display increased rates of aneuploidy and give rise to malignant tumours when transplanted into nude mice [37].

Interestingly, a recent genome-wide RNAi screen, aimed at discovering gene-products important for the fidelity of cell division in HeLa cells, identified physical interactors of HDAC3 as being important for cytokinesis [20]. In this work Kittler et al., showed that knockdown of MLL5, NCoR2, or TBLIX (genes encoding putative chromatin-binding proteins implicated in transcriptional regulation) resulted in increased rates of cytokinesis failure and the generation of tetraploid intermediates [20]. Remarkably, these genes are orthologous to three fission yeast genes (set3, snt1, and hif2) recently identified as playing a role in the reliable execution of cytokinesis and which physically interact with the S. pombe HDAC3 ortholog, Hosp2p (Figure 4B). These results not only further validate the use of S. pombe as a eukaryotic model, but also raise the intriguing question of whether these orthologous complexes represent an evolutionarily conserved module important for the faithful execution of cell division.

In this report we identify the fission yeast ortholog of HDAC3 and provide further evidence of cross-species conservation. First, the expression of human HDAC3 clearly complements the cytokinesis defects displayed by hos2Δ mutants (Figure 5). Second, over-expression of Hos2p or HDAC3 both result in phenotypes related to

Table 2 Quantitation of phenotypic abnormalities upon hos2 or HDAC3 over-expression from the nmt1 promoter in the absence of thiamine

| Morphological Abnormality       | pREP1 | pREP1-hos2 | pREP1-HDAC3 |
|---------------------------------|-------|------------|-------------|
| Normal                          | >99%  | 52%        | 54%         |
| Rounded/Depolarized             | <1%   | 26.5%      | 29%         |
| Misplaced septa                 | <1%   | 9%         | 5%          |
| Excessive/Misplaced septal      | 0     | 6%         | 6%          |
| deposits                        |       |            |             |
| Multiple septa                  | 0     | 2%         | 2%          |
| Elongated with multiple aberrant deposits of septal material | 0     | 3%         | 2%          |
| Elongated and Branched          | 0     | 1.5%       | 1.5%        |

Figure 4 Hos2p localizes to both the cytoplasm and the nucleus and physically interacts with Set3p, Snt1p, and Hif2p. (A) Cells expressing the Hos2-GFP fusion protein were grown to mid-log phase at 30°C in YES media, fixed, and then stained with DAPI and observed using the DAPI and GFP filter sets. Bar, 5 μm. (B) Cells expressing the indicated fusion proteins were grown to mid-log phase in YES, lysed under native conditions, and subjected to anti-HA immunoprecipitations. Both total lysates and immunoprecipitates were resolved by SDS-PAGE and immunoblotted with antibodies specific for the Myc epitope.
morphogenesis and/or cytokinesis (Figure 6). With respect to functional conservation, it is also important to note the similarity in localization between Hos2p and HDAC3. HDAC3 is the only member of the class I HDACs to localize to the cytoplasm, as well as the nucleus, owing to the presence of both nuclear import and export signals [38]. Thus, our observation that Hos2p localizes to both of these intracellular compartments represents another level at which Hos2p and HDAC3 share similarities (Figure 4A). Furthermore, the cytoplasmic localization observed for Hos2p is consistent with immunofluorescence data demonstrating that HA epitope tagged Hos2p is predominantly cytoplasmic as well as with global GFP-fusion based localisation studies showing both nuclear and cytoplasmic localization [39,40].

We also provide genetic evidence supporting a catalytic role for Hos2p through the analysis of strains bearing the Y321H mutation. The mutated tyrosine residue catalyzes stabilization of the transition state between acetyl-lysine and lysine thereby allowing for catalysis of lysine deacetylation; this is not chemically possible through a histidine residue [41]. As expected if Hos2p
Similarly to the over-expression of Hos2p, over-expression of human HDAC3 results in growth inhibition and in phenotypic defects related to morphogenesis and/or cytokinesis. (A) Strains expressing hos2 or HDAC3 from the nmt1 promoter were streaked to EMM media in the presence (repressed) or absence (de-repressed) of thiamine and incubated at 30°C for two days. (B) Colony morphology of strains treated as in A. Bar, 50 μm (C). Representative examples of the pleiotropic cellular morphology exhibited by cells expressing HDAC3 from the nmt1 promoter: i) cells with a rounded, de-polarized appearance, ii) cells with misplaced septa, iii) cells with abnormally excessive and mis-localized septal deposition, iv) cells with multiple septa v) highly elongated cells containing multiple aberrant deposits of septal material, vi) elongated and branched cells. Bar, 10 μm.
played a catalytic role, hos2-Y321H mutants displayed phenotypes similar to those exhibited by hos2Δ strains. In fact, the severity of the cytokinesis defects was greater in the site-mutant compared to the gene deletion (Figure 1). We speculate that the increase in severity of the phenotype may be related to the presence of the mutant protein interfering with other components of the pathway in a dominant negative fashion.

While targeted deacetylation is likely an important aspect of Hos2p function, the physiological substrates of the Hos2p-Set3p-Snt1p-Hif2p complex remain unknown. One possibility is that cytokinetic failure is related to transcriptional defects stemming from the abnormal acetylation of histones. A role in transcription is supported by the observation that set3Δ mutants are compromised in their ability to alter the expression of stress response genes upon LatA treatment. Thus, cell division failure may be a manifestation of the inability of the mutants to properly counter the effects of LatA induced stress leading to the direct and/or indirect effects on the function of the cytokinetic machinery. When considering such models it should also be noted that the substrate specificity of HDACs is not restricted to histones. In fact, many non-histone substrates, including transcription factors, signalling molecules, and nuclear import factors have been identified as targets [42]. Thus, the possibility exists that the complex may function through both the modulation of transcription and/or the post modification of non-histone targets.

Finally, while highly speculative, it is interesting to note that the liver specific deletion of HDAC3 results in hepatocellular carcinoma in mice [43]. In this work, 20 out of 20 hos2Δ mutants displayed low grade hepatocellular carcinoma at a mean age of 10.2 months. Although a role for cytokinesis failure in this phenotype has not been examined, it is interesting to speculate as to whether it played any role in tumor development in this context. Regardless, the demonstration of functional conservation between HDAC3 and Hos2p suggests that further analysis of these proteins in fission yeast might translate into a theoretical framework for understanding the role of HDAC3 in both the regulation of cytokinesis as well as other biological processes influenced by HDAC3 deacetylation.

**Conclusions**

In this work we demonstrate a role for the histone deacetylase, Hos2p, in promoting the faithful and dependable execution of cytokinesis in fission yeast. Analysis of catalytically inactive hos2-Y321H mutants suggests that its role in this process is mediated through its deacetylase activity. In addition, co-immunoprecipitation experiments indicate that Hos2p regulates cytokinesis as part of a complex with Set3p, Snt1p, and Hif2p (all of which play a documented role in preventing cell division failure). Lastly, the ability of human HDAC3 to complement the cytokinesis defects exhibited by hos2Δ mutants suggests that continued analysis of this system could translate into a theoretical framework for understanding how HDAC3 functions in more developmentally complex organisms.

**Methods**

**Yeast methods**

All Schizosaccharomyces pombe strains used in this study are listed in Table 1. Strains were derived from the Karagiannis lab collection, constructed during the course of this work, or purchased from Bioneer Corporation (Alameda, CA). *S. pombe* cells were cultured in YES or in Edinburgh Minimal Media (EMM) supplemented with adenine, histidine, leucine, and/or uracil. Liquid cultures were grown with shaking (200 rpm) at 30°C [44]. In experiments involving Latrunculin treatment, *S. pombe* cells were grown to mid log phase (O.D. 0.2) and treated with 0.1-0.5 μM of Latrunculin A (Enzo Life Sciences International, Plymouth Meeting, Pennsylvania) dissolved in DMSO. Cells were grown at 30°C with shaking at 200 rpm for 3–6 hrs, before being fixed with ethanol and stored in PBS pH 7.4. All experiments were repeated a minimum of three times. Plasmid vectors were transformed into *S. pombe* using the lithium acetate protocol according to Forsburg and Rhind [44].

**Fluorescence microscopy**

To observe nuclei and cell wall/septa material, cells were prepared as described above (see Yeast Methods) and mixed with 0.02 μg/μL 4′,6-diamidino-2-phenylindole (DAPI) and 1 μg/μL aniline blue. In colocalization experiments, *S. pombe* cells expressing the Hos2-GFP fusion were fixed with ethanol and stored in PBS pH 7.4. To observe nuclei and cell wall/septa material, cells were mixed with DAPI and 1 μg/μL aniline blue prior to observation with the DAPI and GFP filter sets. In time-lapse experiments log-phase cells expressing the Rlc1-GFP fusion were imaged live using the CellAsics ONIX™ Microfluidic Perfusion Platform while perfusing liquid YES growth medium containing 0.5 μM LatA or DMSO as a solvent control. All Images were acquired using a Leica DMI6000B inverted microscope equipped with a 100X Plan Apochromat 1.4 NA Oil objective, a Photometrics QuantEM:512SC EMCCD camera, in conjunction with a BDCARVII spinning disk confocal imager (Z-resolution of 0.5 μm) driven by Metamorph software.

**Cloning methods**

*S. pombe* strains expressing carboxy-terminal epitope tagged fusion proteins were constructed using a PCR based cloning strategy. To create the Hos2-GFP and Hos2-Myc expressing strains a C-terminal fragment of the hos2 gene was PCR amplified using High-Fidelity PCR Enzyme Mix (Fermentas Life Sciences) from *S.
pombe genomic DNA with the forward primer 5′-GGG GGG GGA ATT TCT GAA CGA ATT TTT CGC ACC AGA T-3′ and reverse primer 5′-GGG GGC CCG GGG CCT CGA ACG CGA ACA TC-3′ and cloned into frame into the EcoRI and Smal sites of the pJK210-GFP and pJK210-Myc vectors respectively. Molecular cloning of the desired C-terminal fragments was confirmed by restriction digestion and DNA sequencing. Plasmid clones containing the desired C-terminal fragment were transformed into S. pombe strain MBY1343 (ura4-D18). Ura4⁺ integrants were selected for by growth on EMM lacking uracil and subjected to colony PCR to identify clones in which the construct had integrated into the genome via homologous recombination. HA epitope-tagged versions of Set3p, Hif2p, and Snt1p were created as described earlier.

Thiamine repressible hos2 expression plasmids were created by PCR amplifying the hos2 gene (forward primer: 5′-GGG GGA GGA TTA ATA TGG CGA CAT CCA CAC-3′; reverse primer: 5′-GGG GGG GAT CCT CAG CCT CGA AGC CGA AC-3′) from wild-type genomic DNA and cloning into the Ndel and BamHI sites of pREP1/41. Plasmids were then transformed into strain JK484 (Table 1) using the LiAc method and Leu⁺ transformants selected in EMM media, and then cultured for 24 hrs. Cells were then fixed with ethanol, stained with DAPI and analine blue, and imaged using a Leica DMI6000B microscope driven by Metamorph software using the DAPI filter set.

To determine the effects of HDAC3 over-expression, the full-length human HDAC3 cDNA, was PCR amplified (forward primer: 5′-GGG GGA TTA ATA TGG CGA CAT CCA CAC-3′; reverse primer: 5′-CGA TCG GAT CCT TAA ATC TCC ACA TCG TT-3′) from the pOTB7 plasmid (Open Biosystems) and cloned into the Ndel and BamHI sites of the pREP series of plasmids. The plasmids were then transformed into JK484 (Table 1) and the effects of over-expression examined as described above.

To create the hos2-Y321H mutant, an N-terminal fragment of the hos2 gene was PCR amplified with a reverse primer incorporating the Y321H mutation (forward primer #1: 5′-GGG GGG GAT CCT GAC GTG GTG AGG CTA GTG GAT TC-3′; reverse primer #1: 5′-TTT CTA AGA GTA TGA CCA CCA CCT-3′). Similarly, a C-terminal fragment of the hos2 gene was PCR amplified with a forward primer incorporating the Y321H mutation (forward primer #2: 5′-GAG GTG GTG GTG ATC AAT CTA TAA ATT GAA A-3′; reverse primer #2: 5′-GGG GGC CCG GGG CCT CGA ACG CGA ACA TC-3′). A full-length product containing the desired mutations was then PCR amplified using forward primer #1, reverse primer #2, and the products of the first two reactions as template. The full-length product was then cloned into the EcoRI and Smal sites of the pJK210 vector and the construct transformed into JK484 (Table 1) to obtain integrants. Ura⁺ transformants were then isolated and subjected to colony PCR to identify clones in which the construct had integrated into the genome via homologous integration.

Biochemical and immunological methods

Cells of the indicated genotype were grown to the mid-log phase at 30°C, collected by centrifugation, and resuspended in STOP buffer (10 mM Tris–HCl pH 8.0, 150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM Na₃VO₄, 50 mM B-glycerophosphate, 15 mM p-nitrophenyl phosphate, ¼ Tablet Sigma Protease Inhibitors). Immunoprecipitations were performed using Protein G Dynabeads® (Invitrogen) according to the manufacturer’s protocol. Briefly, anti-HA antibodies (HA.11; Sigma) were incubated with Protein G Dynabeads in extraction buffer. Cell extracts were then added to the antibody-bound bead slurry. After incubation and repeated washing with extraction buffer, the bound proteins were eluted by incubation at 96°C for 5 minutes. The eluted proteins were then subjected to SDS-PAGE, transferred to PVDF membranes and probed with anti-Myc antibodies (9E10; Sigma).

Recombinase mediated cassette exchange

The hos2 “base” strain was created by deleting the hos2 coding sequence with the loxP-ura4⁺-loxM3 deletion cassette amplified from the pAW1 plasmid (forward primer: 5′-GGT TCT TAA TTT TAT TTA TCC TTC TCT TGT TCT TTC TTT AGA AAA GAT ATT TTC ATT TAA TTT GTC TCG CGG TTT TTT TTA GTA TAG TCT GTA CCG CAT GGG GTT AAT TAA-3′; reverse primer: 5′-TAT AGG GTC AAT TAT TAA TAT TTA CAA TGT CTA AAT AATA GAT TGA TGT TTT TAC GGA TAT CAA ATA AAA AGT CGA AAA TTT AAT ATT AGA ATT CGA GCT CTG TTT AAC-3′). Deletion cassettes were transformed into JK484 using the lithium acetate method to obtain integrants. Ura⁺ transformants were then isolated and subjected to colony PCR to identify clones in which the construct had integrated into the genome via homologous integration.
The hos2 “exchange” cassette was constructed by PCR amplifying the hos2 gene from the pREP1-hos2 plasmid (forward primer: 5’-GTC CTG AGA CAT CCA CAC-3’; reverse primer: 5’-GGC GGC AGC TCT CAG CCT CGA ACG CGA AC-3’). The amplicon was then cloned into the XhoI and SacI sites of the pA/W8X plasmid. The HDAC3 exchange cassette was constructed by PCR amplifying the HDAC3 cDNA gene from the pOTB7 plasmid (Open Biosystems) (forward primer: 5’-GGC GG C TCG AGA TGG CCA AGA CCG TGG C-3’; reverse primer: 5’-GGG GCC AGC TCT CAG CCT CGA ACG CGA AC-3’). The amplicon was then cloned into the XhoI and SacI sites of the pA/W8X plasmid. Exchange of the ura4+ gene with either hos2 or HDAC3 was achieved according to the protocol of Watson et al. [32].

Additional files

**Additional file 1:** Hos2p shares significant sequence similarity to human HDAC3. (A) Phylogenetic relationship of Hos2p orthologs from yeast to humans. (B) Domain structure of fission yeast Hos2p, and human HDAC3. Red diamond indicates conserved Y321 residue necessary for catalysis. (C) ClusterAIF alignment of fission yeast Hos2p, and human HDAC3. Stars indicate amino acid identity and “.” indicates amino acid similarity. Red arrow indicates conserved Y321 residue necessary for catalysis.

**Additional file 2:** Cldp1 and Cdc7p localization upon LatA treatment in hos2Δ backgrounds. (A) Wild-type and hos2Δ strains expressing Clp1-GFP were grown to mid-log phase at 30°C and then treated with 0.5 μM LatA for 3 h before fluorescence imaging. Arrows indicate cells in which Clp1p is enriched in the cytoplasm indicating active SIN signaling. Bar, 10 μm. (B) Wild-type and hos2Δ strains expressing Cdc7p-GFP were grown to mid-log phase at 30°C and then treated with 0.5 μM LatA for 3 h before fluorescence imaging. Arrows indicate cells in which Cdc7p localizes asymetrically to one of two available SPBs indicating active SIN signaling. Bar, 10 μm.

**Additional file 3:** cldp1Δ hos2Δ double mutants display increased sensitivity to LatA relative to both cldp1Δ and hos2Δ single mutants. Cells of the indicated genotype were grown to mid-log phase at 30°C and then treated with 0.1 or 0.5 μM LatA for 5 h before being fixed and stained with DAPI (nuclei) and aniline blue (cell wall/septa). Bar, 10 μm.

**Additional file 4:** the hos2 gene deletion reduces the restrictive temperature of the cdc15-140 mutagenesis. Cells of the indicated genotype were streaked to YES plates and incubated overnight at 25°C, 31°C, or 36°C. Bar, 50 μm.

**Additional file 5:** Time-lapse movie of Rlc1-GFP dynamics in wild-type cells treated with DMSO.

**Additional file 6:** Time-lapse movie of Rlc1-GFP dynamics in hos2Δ cells treated with DMSO.

**Additional file 7:** Time-lapse movie of Rlc1-GFP dynamics in wild-type cells treated with 0.5 μM LatA.

**Additional file 8:** Time-lapse movie of Rlc1-GFP dynamics in hos2Δ cells treated with 0.5 μM LatA.

**Additional file 9:** Schematic representation of recombinase-mediated cassette exchange. A “base-strain” is first created in which the hos2 open reading frame is replaced with a deletion cassette composed of the ura4+ selectable marker flanked by the Cre recombinase recognition sites, loxP and loxM3. Next, the “exchange” plasmid (pAW8X-HDAC3) containing the Cre recombinase gene under control of the nmt41 promoter is transformed into the base strain.

**Expression of the recombinase permits exchange of the ura4+ cassette with the HDAC3 sequence.**

**Abbreviations**

HDAC: Histone Deacetylase; SIN: Septation Initiation Network; YES: Yeast Extract Supplements; EMWA: Edinburgh Minimal Media; LαA: Lauruncin A; DMSO: Dimethyl sulfoxide; LatA: 4,6-diamidino-2-phenylindole; PBS: Phosphate Buffered Saline.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JK designed the experiments and drafted the manuscript. CG, JH, SR, and JK performed the experiments and analysed the data. All authors read and approved the final manuscript.

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**Acknowledgements**

This work was supported by the National Sciences and Engineering Research Council of Canada, the University of Western Ontario Academic Development Fund, and the Canada Foundation for Innovation. We would also like to thank members of the UWO Biology and Biochemistry Departments for helpful discussions and/or critical reading of the manuscript.

**Received:** 22 December 2011 **Accepted:** 20 April 2012 **Published:** 4 May 2012

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