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N-terminal short fragment of *TUP1* confers resistance to 5-bromodeoxyuridine in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

Small molecules that exhibit biological activity have contributed to the understanding of the molecular mechanisms of various biological phenomena. 5-Bromodeoxyuridine (BrdU) is a thymidine analogue that modulates various biological phenomena such as cellular differentiation and cellular senescence in cultured mammalian cells. Although BrdU is thought to function through changing chromatin structure and gene expression, its precise molecular mechanisms are not understood. To study the molecular mechanism for the action of BrdU, we have employed the yeast *Saccharomyces cerevisiae* as a model system, and screened multi-copy suppressor genes that confer resistance to BrdU. Our genetic screen has revealed that expression of the N-terminal short fragment of *TUP1*, and also disruption of *HDA1* or *HOS1*, histone deacetylases that interact with *TUP1*, conferred resistance to BrdU. These results suggest the implication of the chromatin proteins in the function of BrdU, and would provide novel clues to answer the old question of how BrdU modulates various biological phenomena.

1. Introduction

5-Bromodeoxyuridine (BrdU) is a thymidine analogue that is widely used to measure DNA synthesis immunochemically in living cells. Besides, in the 1970s, it was frequently used as a reagent that regulates cellular differentiation in various mammalian cell types together with cAMP and butyrate [1]. The molecular targets for cAMP and butyrate have successfully been identified as cAMP-dependent protein kinases and histone deacetylases, respectively, and identification of their targets has greatly contributed to the understanding of the molecular mechanisms for cell signaling and gene expression. On the other hand, however, a molecular target for BrdU has been left unidentified.

In the previous study, we have shown that BrdU induces a senescence-like phenomenon in cultured mammalian cells [2]. To gain an insight into molecular mechanisms for BrdU-induced cellular senescence, we have extensively characterized genes that respond to BrdU in human cells, and have found that they are located on particular regions of human chromosomes, forming clusters on or nearby Giemsa-dark bands of human chromosomes [3–6]. We also found that AT-tract minor groove binders, such as distamycin A, netropsin, Hoechst 33258, and the AT-hook protein HMG-I, potentiate the effects of BrdU in human cells [4,7]. The earlier studies by others indicate that BrdU decondenses particular regions of chromosomes [8], and changes the interaction between 5-bromouracil-substituted DNA and DNA-binding proteins, such as histones [9], non-histone proteins [10], a *lac* repressor [11], and chromosomal proteins [12]. In addition, we have recently shown that BrdU disrupts nucleosome positioning and changes gene expression through certain types of AT-rich sequence in the yeast *Saccharomyces cerevisiae* [13,14].

One effective strategy to identify a molecular target of BrdU would be the isolation and characterization of the mutants that are irresponsive to BrdU. Then, BrdU-resistant mutants have been isolated from cultured mammalian cells to identify their responsible genes. Although the majority of the mutants isolated with a standard method are defective in up-take of thymidine/BrdU into their genomes [15,16], Davidson and Kaufman have successfully isolated BrdU-resistant mutants with normal up-take of thymidine/BrdU from Syrian hamster melanoma line RPMI 3460 [17]. We also isolated and characterized BrdU-resistant mutants from mouse thymidine-auxotrophic FM3A cells (unpublished results), but their causative mutations are yet to be identified.

To identify a molecular target of BrdU, we have employed a genetic approach with a thymidine-auxotrophic strain of the yeast *S. cerevisiae* [18], because genetic system in yeast has been proven to be useful in identifying responsible genes in various biological phenomena. In the previous study, we reported the identification of several multi-copy suppressor genes that confer resistance to BrdU [19,20]. In this study, we further extended our genetic screen, and found a novel suppressor gene, N-terminal *TUP1*. Tup1p forms a protein complex with Ssn6p/Cyc8p, and represses the expression

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of a variety of genes through interaction with histone deacetylases such as Hda1p, Rpd3p, Hos1p, and Hos2p [21–26]. We also found that disruption of *HDA1* or *HOS1* caused resistance to BrdU as well, and further, N-terminal *TUP1* and disruption of *HDA1* worked in the same genetic pathway. These findings are in good agreement with the view that BrdU modulates various biological phenomena through changing chromatin structure and gene expression. Characterization of these genes would help understanding the molecular mechanisms for various biological phenomena induced by BrdU.

2. Materials and methods

2.1. Yeast

The thymidine-auxotrophic (thy$^-$) strain YKH1 in *S. cerevisiae* was used throughout this study [18]. The genotype of YKH1 is as follows: *MAT*a *lys2 trp1 his3 cdc21*/*tmp1*::*LEU2* P_{ADH1}-*TK*. Yeast cells were transformed with plasmids according to a standard protocol in which lithium acetate and polyethylene glycol 4000 are used. Yeast transformants were selected on SD plates supplemented with thymidine without an appropriate amino acid(s).

2.2. Screening of multi-copy suppressor genes

Genomic DNA of NNY11 [18] from which YKH1 was derived was partially digested with *Sau*3AI, and cloned into a multi-copy type vector, pYBT2, which contains a 2-μm origin of replication and a *TRP1* selection marker. YKH1 cells were transformed with this genomic DNA library, and BrdU-resistant clones were selected by culturing them on SD plates supplemented with 1.8 mM of thymidine and 1.2 mM of BrdU without tryptophan. Plasmids were recovered from these BrdU-resistant clones by transformation of *Escherichia coli* with DNAs of these yeast clones. Isolated plasmids were re-introduced into YKH1 cells to confirm the sensitivity to BrdU, and were partially sequenced [19,20].

2.3. Up-take of BrdU into genome DNA

Genomic DNA was prepared from yeast cells cultured in medium containing 1.8 mM of thymidine and 1.2 mM of BrdU. DNA was digested with *Hinf*I, run on an agarose gel, and transferred onto a nitrocellulose filter after being stained with ethidium bromide. BrdU in DNA was probed with a mouse monoclonal antibody (Sigma), and detected with a chemiluminescence detection kit according to the supplier's instruction (ECL, Amersham Bioscience). The signal was quantified with an image analyzer (FLA-5000, Fuji Film).

2.4. Assay of sensitivity to BrdU

Ten-fold serially diluted culture of yeast was spotted on the dThd plates supplemented with 3 mM of thymidine and on the BrdU plates supplemented with 0.9–1.2 mM of BrdU and 2.1–1.8 mM of thymidine. These plates were incubated at 30 °C for 5–7 days.

2.5. Construction of plasmids

The 1.7-kb *Bam*HI, the 1.2-kb *Bam*HI-*Nde*I, and the 0.5-kb *Bam*HI-*Nde*I fragments were cut out from pYBT442 and cloned into pYBT2 to construct the plasmids pYBT444, pYBT445 and pYBT446, respectively. Digestion of pYBT442 with *Bam*HI and subsequent self-ligation yielded pYBT443. pYBT447, pYBT448, and pYBT449 which carry full-length *TUP1*, N-terminal 90 amino acid residues of *TUP1*, and N-terminal 70 amino acid residues of *TUP1*, respectively, were made by cloning the sequences that were amplified by PCR from genomic DNA with the following primers into pYBT2: 5′-AGACATAAACATATGTATATAAGAACAAC-3′ and 5′-ACCCGGGTGT TCTGTTCTTAATTTGGCGC-3′ for pYBT447; 5′-AGACATAAACA TATG TATATAAGAACAAC-3′ and 5′-AGAATTCCTATTGATGGTCTCTTTGCT CC-3′ for pYBT448; 5′-AGACATAAACATATGTATATAAGAAC AAC-3′ and 5′-AGAATTCCTAGTCCTTCATTTTCCTGTGA-3′ for pYBT449. To construct the targeting vectors to disrupt *TUP1* and *RPD3*, the *TUP1* and *RPD3* sequences were amplified from yeast genomic DNA by PCR with the primers: 5′-CCTTCTTCACTCTTGACGCC-3′ and 5′-CAATATTCAGAAACACAGGAAAAGG-3′ for *TUP1*; 5′-GGTTG TCGTAAAAATGGAAGAC-3′ and 5′-GGGACGAGACGTTTAGATAG-3′ for *RPD3*. These sequences were cloned into the pGEM-T Easy vector (Promega), following which the sequence of the *HIS3* gene was inserted at the site of *Nsp*V or *Eco*RI/*Eco*RV to make knock-out constructs for *TUP1* or *RPD3*, respectively.

2.6. Disruption of yeast genes

To isolate *tup1*Δ and *rpd3*Δ, the targeting plasmids described above were linearized with *Not*I. To isolate other disruptants, a PCR-based gene deletion technique was employed [27]. In brief, knock-out constructs were made by amplification of the *HIS3* sequence on pFA6a-MN-His3MX6 [28] by PCR with the following primers: 5′-CGACTGGAAAAAAAAAATTAGGAAAAATGAATCCGGG CGGTGAACAAACAATAATGGGTTTAGCTTGCCTCGTCC-3′ and 5′-ACA CAACATTTCTCGTTGATTATAAATTAGTAGATTAATTTTTTGAATGCGA ATTCGAGCTCGTTTAAAC-3′ for *SSN6*/*CYC8*; 5′-ATATTGAGAAAGG-GAAAGTTGAGCACTGTAATACGCCGAACAGATTAAGCGTTTAGCTTGC CTCGTCC-3′ and 5′-CATAAGGCATGAAGGTTGCCGAAAA AAAATTAT-TAATGGCCAGTTTTTCCGAATTCGAGCTCGTTTAAAC-3′ for *HDA1*; 5′-ATGTACTGTAATATGAATTAATAAACACCTGTCCATTTTAGAAAAACGC TGTTTAGCTTGCCTCGTCC-3′ and 5′-TGCGGCGTTCGCATTATTAATTT GTATTCAAACGACTAATTAAAACTATCGAATTCGAGCTCGTTTAAAC-3′ for *HOS1*; 5′-ATTACTCAAGTACGTTAAAATCAGGTATC AAGTGAA-TAACAACACGCAACGTTTAGCTTGCCTCGTCC-3′ and 5′-CGGGAGAT-TAACCGAATAGCAAACTCTTAAACTATGAAAAGGCAATCAATGAATTC-GAGCTCGTTTAAAC-3′ for *HOS2*. YKH1 cells were transformed with these constructs and were selected on SD medium supplemented with thymidine without histidine to isolate disruptants.

3. Results

3.1. Isolation of a multi-copy suppressor that confers resistance to BrdU

In the previous study, we have established a thymidine-auxotrophic strain, YKH1, to examine the effect of BrdU on the growth of the yeast *S. cerevisiae* [18]. YKH1 cells grow in medium supplemented with thymidine, but not in medium supplemented with increased concentrations of BrdU [18]. To isolate multi-copy suppressors for BrdU-induced growth defect, we transformed YKH1 cells with a genomic DNA library constructed on a multi-copy type vector, pYBT2, as previously described [19,20]. We isolated yeast clones that grew on selective plates that contained BrdU, and recovered plasmids from them. In this study, we focused one plasmid designated as pYBT442, which contains an approximately 4.0-kb insert DNA that carries two intact and two partial gene sequences on yeast chromosome III (Fig. 1A). To identify the gene responsible for resistance to BrdU on pYBT442, we made several deletion constructs (Fig. 1A). Transformation of YKH1 cells with these plasmid constructs revealed that expression of N-terminal *TUP1* led to resistance to BrdU (Fig. 1A and B).

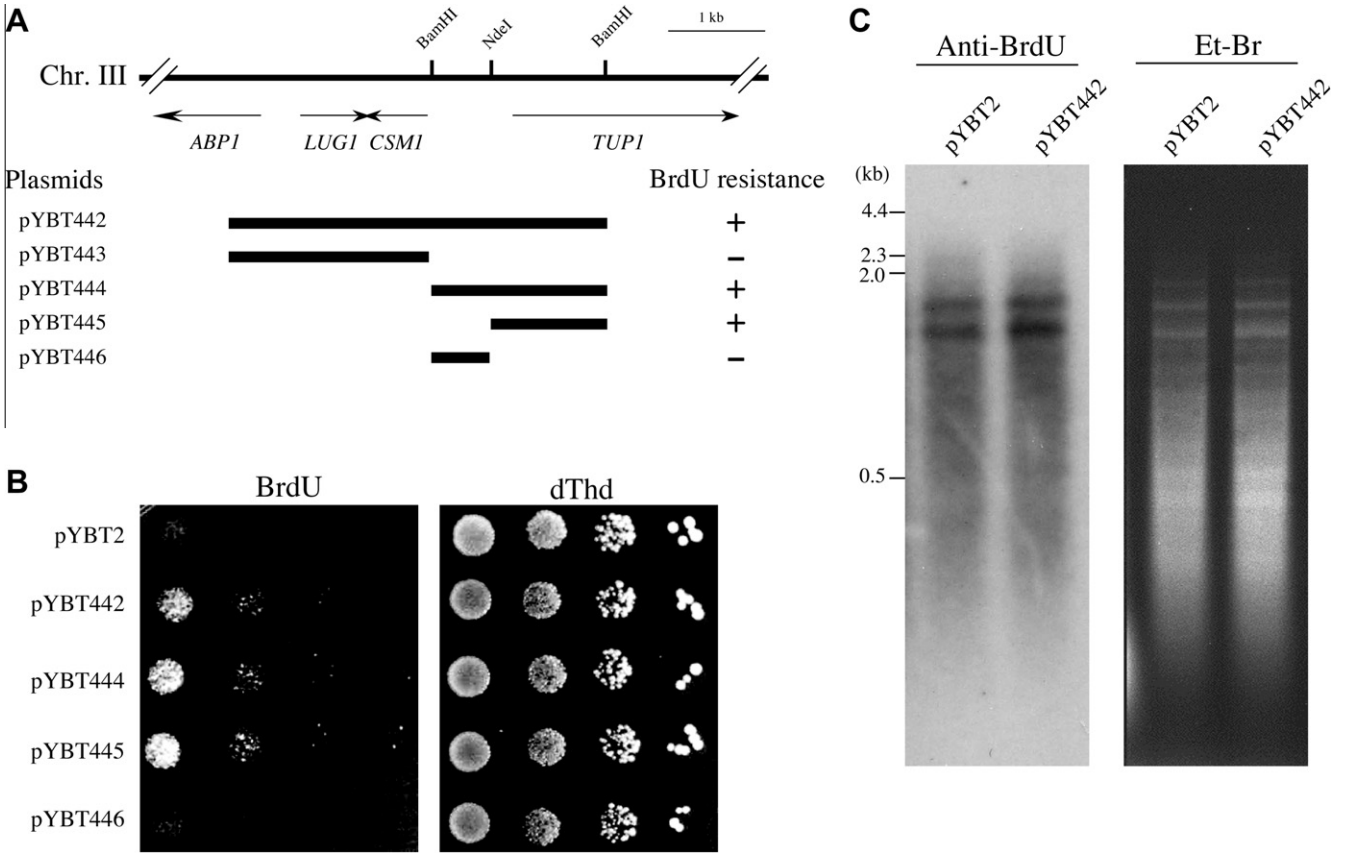

Fig. 1. Identification of a multi-copy suppressor gene. (A) The DNA regions cloned in pYBT442 and in its derivatives are shown as black bars. Genes on chromosome III are indicated by arrows with direction of gene expression. (B) Ten-fold serially diluted culture of yeast cells was spotted and cultured on the dThd plates supplemented with 3 mM of thymidine, and on the BrdU plates supplemented with 1.2 mM of BrdU and 1.8 mM of thymidine. (C) DNA harvested from yeast cells cultured with BrdU was stained with Et–Br (right), and blotted onto a nitrocellulose filter. BrdU in DNA was detected with an antibody against BrdU (left).

3.2. Up-take of BrdU was not influenced by pYBT442

We next examined whether incorporation of BrdU into genome DNA was influenced by the expression of N-terminal *TUP1*. For this, we harvested DNA from yeast cells cultured with BrdU, and blotted it onto a nitrocellulose filter. Detection of BrdU in DNA with an antibody against BrdU indicated that expression of N-terminal *TUP1* did not change the up-take of BrdU into genome DNA (Fig. 1C). Then, N-terminal *TUP1* caused resistance to BrdU without changing the incorporation of BrdU into genome DNA, unlike the case for *HAM1* [19].

3.3. N-terminal 90 amino acid resides of Tup1p conferred resistance to BrdU

Sequencing of pYBT442 revealed that it contains the N-terminal 754-bp sequences of *TUP1*, but unexpectedly, a nonsense mutation (CAG–TAG) was found at the position of 352 in *TUP1* on pYBT442 (Fig. 2A). Then, pYBT442 expresses the N-terminal 117 amino acid residues of Tup1p, designated as Tup1p^{N117} hereafter. This mutation seems to have arisen during preparation of the genomic DNA library, because it is not found in the genome of the parental YKH1 cells (data not shown). To further determine the amino acid residues required for resistance to BrdU, we constructed the plasmids pYBT448 and pYBT449 which express N-terminal 90 amino acid residues and N-terminal 70 amino acid residues of Tup1p, respectively, (Fig. 2A). Transformation of YKH1 cells with these plasmids revealed that the N-terminal 90 amino acid residues of Tup1p were required for resistance to BrdU (Fig. 2B). These amino acid residues of Tup1p are reported to be involved in tetramerization of Tup1p and in interaction with Ssn6p, but not in transcriptional repression [29–31].

3.4. Overexpression or deletion of TUP1 did not confer resistance to BrdU

Since our genetic screen failed to identify clones that contain *TUP1* except for pYBT442, we examined whether wild-type *TUP1*, as well as *TUP1*^{N117}, regulates the sensitivity to BrdU. Interestingly, overexpression or deletion of wild-type *TUP1* did not cause significant changes in the sensitivity to BrdU (Figs. 2A, B and 3A). This finding indicates that wild-type *TUP1* was not directly involved in the regulation of the sensitivity to BrdU, and thus suggests that *TUP1*^{N117} conferred resistance to BrdU possibly through interaction with proteins other than *TUP1*. This view is supported by the finding that expression of *TUP1*^{N117} led to resistance to BrdU even in *tup1*Δ cells (Fig. 3A).

3.5. TUP1^{N117} functions in an SSN6-dependent manner

We next examined the implication of *SSN6*, which forms a co-repressor complex with *TUP1*, in the resistance to BrdU by *TUP1*^{N117}. Disruption of *SSN6* resulted in an increased sensitivity to BrdU, and importantly, *TUP1*^{N117} did not change the sensitivity to BrdU in *ssn6*Δ cells (Fig. 3B). This result suggests that *TUP1*^{N117} required *SSN6* for resistance to BrdU.

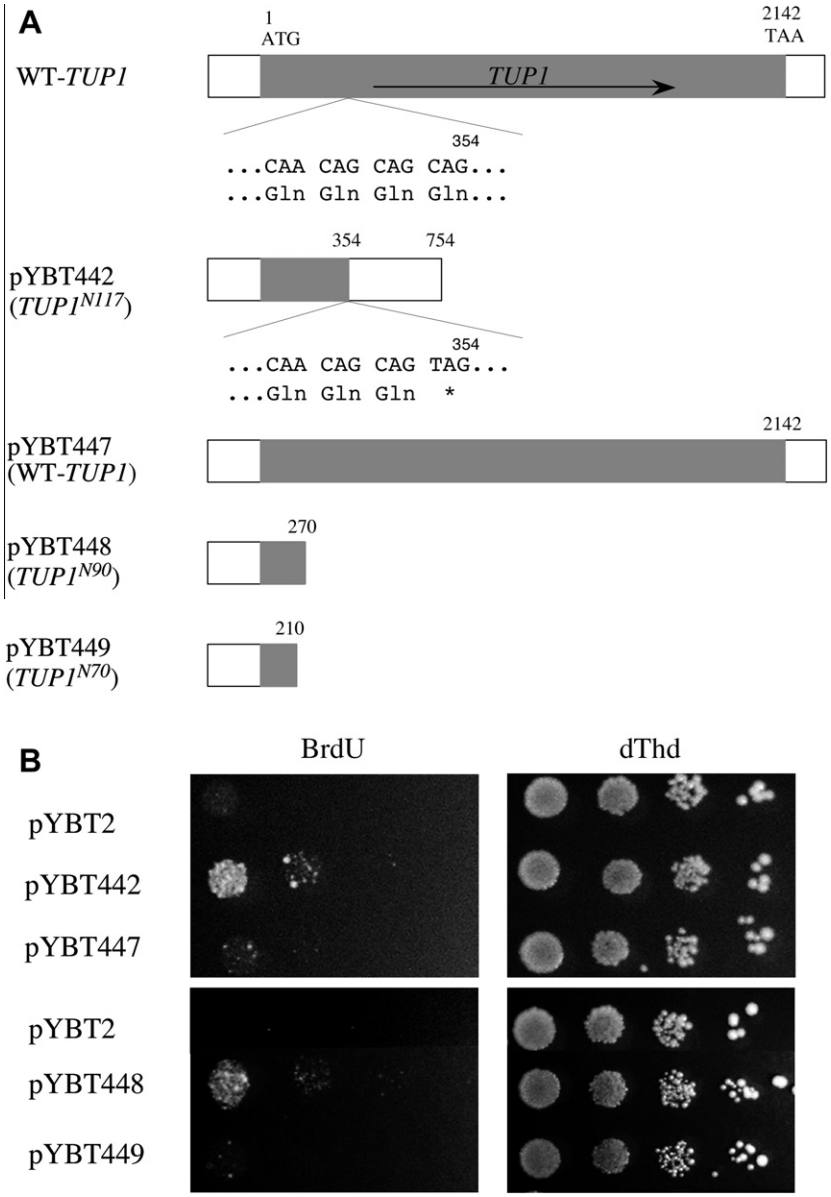

Fig. 2. Determination of the amino acid residues required for resistance to BrdU. (A) The DNA regions of *TUP1* cloned in the plasmid constructs are shown. A nonsense mutation observed in pYBT442 is shown together with its wild-type sequence. (B) Ten-fold serially diluted culture of yeast cells was spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (1.2 mM BrdU, 1.8 mM thymidine).

3.6. *TUP1*^{N117} on a low-copy number plasmid conferred resistance to BrdU

We examined the expression level of Tup1p^{N117} required for resistance to BrdU. Expression of *TUP1*^{N117} from a YCp, low-copy number vector led to resistance to BrdU as effectively as that from a YEp, high-copy number vector (data not shown). Then, a high level expression of Tup1p^{N117} was not necessary for resistance to BrdU, though it was originally isolated as a multi-copy suppressor gene.

3.7. Disruption of histone deacetylases conferred resistance to BrdU

Tup1p forms a protein complex with Ssn6p, and represses the expression of a variety of genes through interaction with histone deacetylases [21–26]. To date, the Tup1p-Ssn6p complex has been shown to interact with the histone deacetylases, Hda1p, Rpd3p, Hos1p, and Hos2p [23–25]. Since BrdU is thought to modulate various biological processes through changing chromatin structure and gene expression, we examined whether the above histone deacetylases play a role in the regulation of the sensitivity to BrdU. For this, we constructed mutants deficient in these histone deacetylases, and examined their sensitivity to BrdU. Interestingly, *hda1*Δ and *hos1*Δ cells showed resistance to BrdU (Fig. 4A), though some clonal variation in resistance to BrdU was observed in these mutants. The majority of *hda1*Δ and *hos1*Δ clones showed the resistance to BrdU shown in Fig. 4A; however, a few clones showed weaker or higher resistance to BrdU (data not shown). We also found that expression of *TUP1*^{N117} did not further enhance the resistance to BrdU of *hda1*Δ cells (Fig. 4B), which result suggests that *TUP1*^{N117} and disruption of *HDA1* worked in the same genetic pathway. Since expression of *TUP1*^{N117} enhanced the resistance to BrdU of *hos1*Δ cells (Fig. 4C), Tup1p^{N117} might cause resistance to BrdU by inhibiting the function of Hda1p through direct binding to it [24].

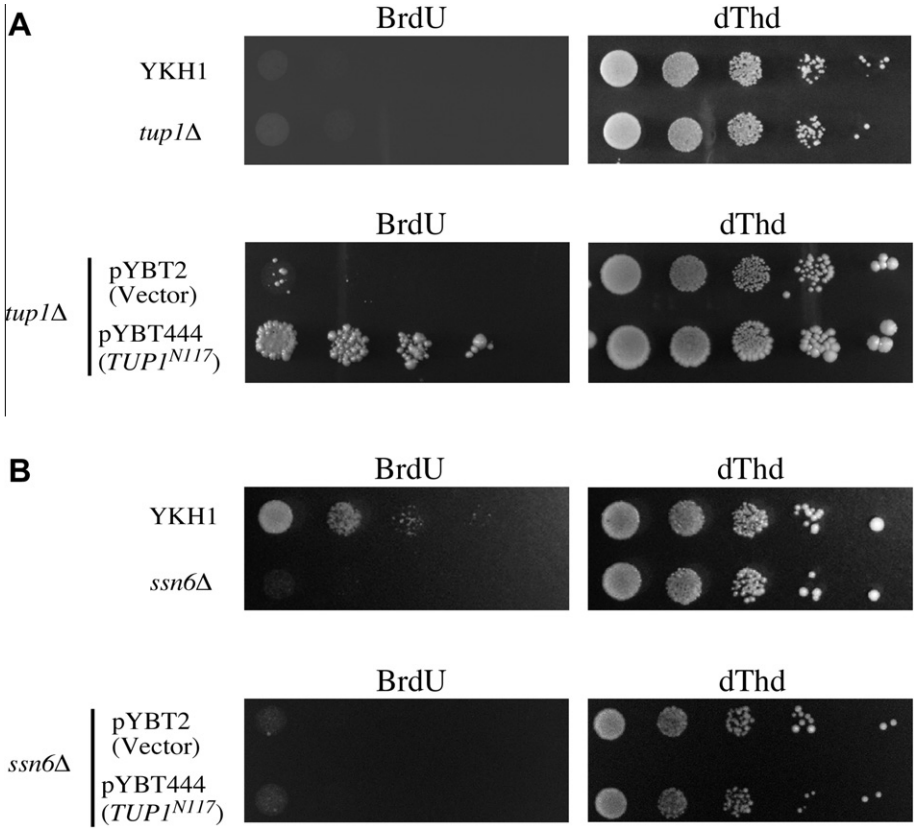

Fig. 3. Effect of deletion of *TUP1* on resistance to BrdU. (A) Ten-fold serially diluted cultures of YKH1 cells and *tup1*Δ cells, and *tup1*Δ cells harboring pYBT2 or pYBT444 were spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (1.2 mM BrdU, 1.8 mM thymidine). (B) Ten-fold serially diluted cultures of YKH1 cells and *ssn6*Δ cells, and *ssn6*Δ cells harboring pYBT2 or pYBT444 were spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (0.9 mM BrdU, 2.1 mM thymidine).

4. Discussion

BrdU is an interesting reagent that affects various biological processes such as cellular differentiation and cellular senescence in cultured mammalian cells. To reveal the molecular mechanism for the action of BrdU, many mutants with altered sensitivity to BrdU have been isolated from cultured mammalian cells. For example, BrdU-resistant mutants of Syrian hamster melanoma line RPMI 3460 grow even when more than 90% of thymine is substituted with 5-bromouracil in their genomes [17]. We have also isolated BrdU-resistant mutants from mouse thymidine-auxotrophic FM3A cells (unpublished results), but their responsible genes are yet to be identified. Then, we have established an experimental system with the yeast *S. cerevisiae* in which the growth of yeast cells depends on extracellular thymidine/BrdU [18]. Since genetic research in yeast is easier than that in mammalian cells, it should facilitate the understanding of the molecular mechanism for the action of BrdU in mammalian cells.

Our genetic screen has revealed that expression of the N-terminal 90 amino acid residues of Tup1p conferred resistance to BrdU in yeast. *TUP1* was originally identified as a gene responsible for altered up-take of deoxythymidine-5′-monophosphate (*TUP1*: deoxy*T*hymidine monophosphate *Up*-take 1), and subsequent studies indicate that *TUP1* acts as a general transcriptional repressor with *SSN6* [22,32]. Then, the altered up-take of deoxythymidine monophosphate is presumably caused by inappropriate gene expression by a defect in *TUP1*. However, it seems to be unlikely that $TUP1^{N90}$ conferred resistance to BrdU by affecting the metabolism of thymidine/thymine because *TUP1* is not intrinsically involved in the regulation of the sensitivity to BrdU. This was virtually confirmed by the finding that $TUP1^{N117}$ did not change the substitution ratio of thymidine with BrdU in genome DNA (Fig. 1C), which is a key determinant of the sensitivity to BrdU in cells [18].

Since the N-terminal 90 amino acid residues of Tup1p are involved in tetramerization of Tup1p and interaction with Ssn6p, [29–31], we first hypothesized that $Tup1p^{N117}$ functions dominant-negatively over wild-type Tup1p or over the Tup1p–Ssn6p complex. However, the finding that deletion of *TUP1* did not lead to resistance to BrdU excluded the above possibility, and indeed, $TUP1^{N117}$ led to resistance to BrdU even in the absence of wild-type *TUP1* (Fig. 3). Hence, it is the most likely that $Tup1p^{N117}$ influenced the sensitivity to BrdU through interaction with proteins other than Tup1p. The Tup1p–Ssn6p complex interacts with several histone deacetylases such as Hda1p, Hos1p, Hos2p, and Rpd3p, to repress gene expression [23–26]. We then investigated the implication of these histone deacetylases in resistance to BrdU, and found that disruption of *HDA1* or *HOS1* led to resistance to BrdU. Further, we also found that $TUP1^{N117}$ and disruption of *HDA1* worked in the same genetic pathway (Fig. 4). Thus, $TUP1^{N117}$ might cause resistance to BrdU by inhibiting the function of *HDA1* through direct binding to it. Interestingly, we have previously shown that another type of histone deacetylase, *SIR2*, is involved in the regulation of the sensitivity to BrdU [20]. These findings suggest that histone acetylation/deacetylation at specific chromosome loci may play a role in the function of BrdU.

Histone deacetylases regulate gene expression through modification of histones, which is frequently associated with changes in

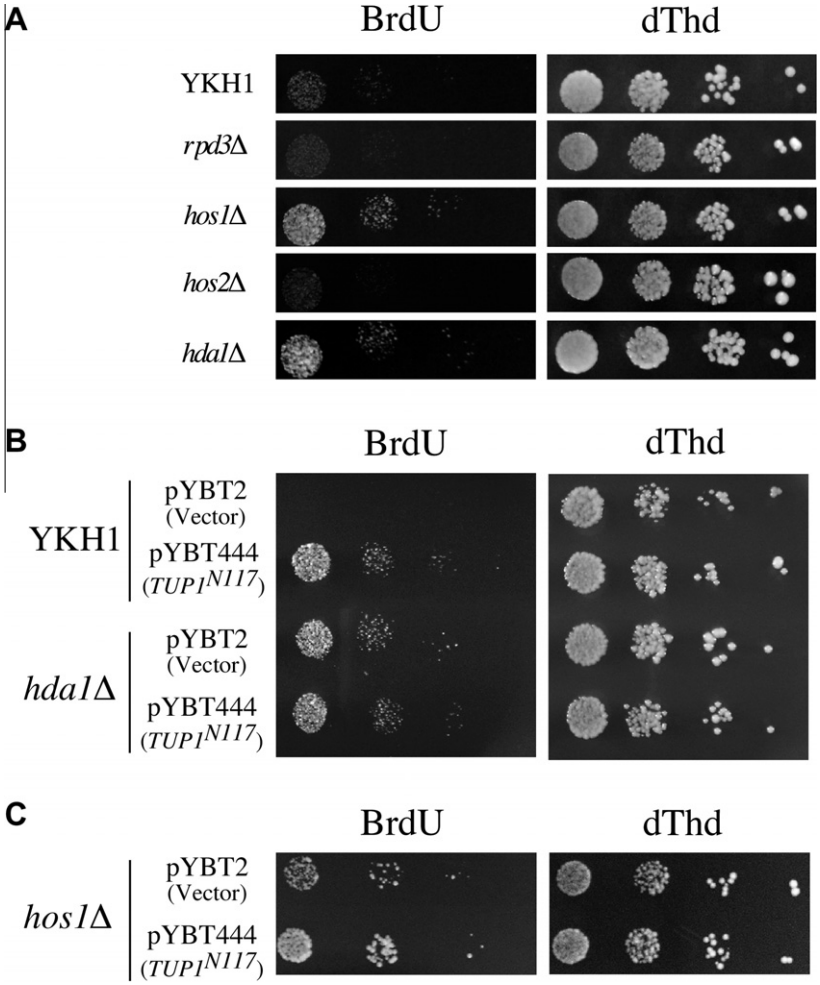

Fig. 4. Effect of deletion of histone deacetylases on the sensitivity to BrdU. (A) Ten-fold serially diluted cultures of mutants in histone deacetylases were spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (1.2 mM BrdU, 1.8 mM thymidine). (B) Ten-fold serially diluted cultures of YKH1 cells or *hda1*Δ cells harboring pYBT2 or pYBT444 were spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (1.2 mM BrdU, 1.8 mM thymidine). (C) Ten-fold serially diluted cultures of *hos1*Δ cells harboring pYBT2 or pYBT444 were spotted and cultured on the dThd plates (3 mM thymidine) and on the BrdU plates (1.2 mM BrdU, 1.8 mM thymidine).

nucleosome/chromatin structure. On the other hand, BrdU is incorporated into DNA in the place of thymidine, and thus is predicted to function through AT-rich sequences. Indeed, substitution of thymine with 5-bromouracil changes DNA structure in vitro: the structure of DNA containing AT-rich sequences is changed into a possible "A-form-like structure" [4,13]. This change in DNA structure causes altered nucleosome positioning, which is followed by altered gene expression in *S. cerevisiae* [13,14]. These findings suggest the possibility that BrdU regulates nucleosome/chromatin structure and gene expression through changing the interaction between 5-bromouracil-substituted DNA and histones, while histone deacetylases do through modification of histones.

In summary, we have identified several chromatin proteins that regulate the sensitivity to BrdU. This finding is in agreement with the view that BrdU exhibits biological activities through changing chromatin structure and gene expression. Further functional characterization of these genes would provide clues to answer the old question of how BrdU modulates various biological phenomena.

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References

[1] F.H. Wilt, M. Anderson, The action of 5-bromodeoxyuridine on differentiation, Dev. Biol. 28 (1972) 443–447.
[2] E. Michishita, K. Nakabayashi, T. Suzuki, S.C. Kaul, H. Ogino, M. Fujii, Y. Mitsui, D. Ayusawa, 5-Bromodeoxyuridine induces senescence-like phenomena in mammalian cells regardless of cell type or species, J. Biochem. (Tokyo) 126 (1999) 1052–1059.
[3] T. Suzuki, S. Minagawa, E. Michishita, H. Ogino, M. Fujii, Y. Mitsui, D. Ayusawa, Induction of senescence-associated genes by 5-bromodeoxyuridine in HeLa cells, Exp. Gerontol. 36 (2001) 465–474.
[4] T. Suzuki, E. Michishita, H. Ogino, M. Fujii, D. Ayusawa, Synergistic induction of the senescence-associated genes by 5-bromodeoxyuridine and AT-binding ligands in HeLa cells, Exp. Cell Res. 276 (2002) 174–184.
[5] S. Minagawa, K. Nakabayashi, M. Fujii, S.W. Scherer, D. Ayusawa, Functional and chromosomal clustering of genes responsive to 5-bromodeoxyuridine in human cells, Exp. Gerontol. 39 (2004) 1069–1078.
[6] S. Minagawa, K. Nakabayashi, M. Fujii, S.W. Scherer, D. Ayusawa, Early BrdU-responsive genes constitute a novel class of senescence-associated genes in human cells, Exp. Cell Res. 304 (2005) 552–558.
[7] W. Satou, T. Suzuki, T. Noguchi, H. Ogino, M. Fujii, D. Ayusawa, AT-hook proteins stimulate induction of senescence markers triggered by 5-bromodeoxyuridine in mammalian cells, Exp. Gerontol. 39 (2004) 173–179.
[8] A.F. Zakharov, L.I. Baranovskaya, A.I. Ibraimov, V.A. Benjusch, V.S. Demintseva, N.G. Oblapenko, Differential spiralization along mammalian mitotic chromosomes. II. 5-bromodeoxyuridine 5-bromodeoxycytidine-revealed differentiation in human chromosomes, Chromosoma 44 (1974) 343–359.
[9] S. Lin, D. Lin, A.D. Riggs, Histones bind more tightly to bromodeoxyuridine-substituted DNA than to normal DNA, Nucleic Acids Res. 3 (1976) 2183–2191.
[10] T.M. Fasy, B.R. Cullen, D. Luk, M.D. Bick, Studies on the enhanced interaction of halodeoxyuridine-substituted DNAs with H1 histones and other polypeptides, J. Biol. Chem. 255 (1980) 1380–1387.

[11] S.Y. Lin, A.D. Riggs, The binding of lac repressor and the catabolite gene activator protein to halogen-substituted analogues of poly[d(A-T)], Biochim. Biophys. Acta 432 (1976) 185–191.
[12] M.D. Bick, E.A. Devine, Interaction of chromosomal proteins with BrdU substituted DNA as determined by chromatin-DNA competition, Nucleic Acids Res. 4 (1977) 3687–3700.
[13] K. Miki, M. Shimizu, M. Fujii, M.N. Hossain, D. Ayusawa, 5-Bromouracil disrupts nucleosome positioning by inducing A-form-like DNA conformation in yeast cells, Biochem. Biophys. Res. Commun. 368 (2008) 662–669.
[14] K. Miki, M. Shimizu, M. Fujii, S. Takayama, M.N. Hossain, D. Ayusawa, 5-bromodeoxyuridine induces transcription of repressed genes with disruption of nucleosome positioning, FEBS J. 277 (2010) 4539–4548.
[15] D.R. Dubbs, S. Kit, Effect of halogenated pyrimidines and thymidine on growth of l-cells and a subline lacking thymidine kinase, Exp. Cell Res. 33 (1964) 19–28.
[16] T. Hori, D. Ayusawa, K. Shimizu, H. Koyama, T. Seno, Chromosome breakage induced by thymidylate stress in thymidylate synthase-negative mutants of mouse FM3A cells, Cancer Res. 44 (1984) 703–709.
[17] E.R. Kaufman, R.L. Davidson, Novel phenotypes arising from selection of hamster melanoma cells for resistance to BUdR, Exp. Cell Res. 107 (1977) 15–24.
[18] M. Fujii, H. Ito, T. Hasegawa, T. Suzuki, N. Adachi, D. Ayusawa, 5-Bromo-2′-deoxyuridine efficiently suppresses division potential of the yeast *Saccharomyces cerevisiae*, Biosci. Biotechnol. Biochem. 66 (2002) 906–909.
[19] S. Takayama, M. Fujii, A. Kurosawa, N. Adachi, D. Ayusawa, Overexpression of HAM1 gene detoxifies 5-bromodeoxyuridine in the yeast *Saccharomyces cerevisiae*, Curr. Genet. 52 (2007) 203–211.
[20] M. Fujii, K. Miki, S. Takayama, D. Ayusawa, Identification of genes that affect sensitivity to 5-bromodeoxyuridine in the yeast *Saccharomyces cerevisiae*, Mol. Genet. Genomics 283 (2010) 461–468.
[21] F.E. Williams, U. Varanasi, R.J. Trumbly, The CYC8 and *TUP1* proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex, Mol. Cell Biol. 11 (1991) 3307–3316.
[22] C.A. Keleher, M.J. Redd, J. Schultz, M. Carlson, A.D. Johnson, Ssn6-*TUP1* is a general repressor of transcription in yeast, Cell 68 (1992) 709–719.
[23] A.D. Watson, D.G. Edmondson, J.R. Bone, Y. Mukai, Y. Yu, W. Du, D.J. Stillman, S.Y. Roth, Ssn6-*TUP1* interacts with class I histone deacetylases required for repression, Genes Dev. 14 (2000) 2737–2744.
[24] J. Wu, N. Suka, M. Carlson, M. Grunstein, *TUP1* utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast, Mol. Cell. 7 (2001) 117–126.
[25] J.K. Davie, D.G. Edmondson, C.B. Coco, S.Y. Dent, *TUP1*-Ssn6 interacts with multiple class I histone deacetylases in vivo, J. Biol. Chem. 278 (2003) 50158–50162.
[26] D. Robyr, Y. Suka, I. Xenarios, S.K. Kurdistani, A. Wang, N. Suka, M. Grunstein, Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases, Cell 109 (2002) 437–446.
[27] M.S. Longtine, A. McKenzie 3rd, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, J.R. Pringle, Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.
[28] M. Schmid, T. Durussel, U.K. Laemmli, ChIC and ChEC; genomic mapping of chromatin proteins, Mol. Cell. 16 (2004) 147–157.
[29] U.S. Varanasi, M. Klis, P.B. Mikesell, R.J. Trumbly, The Cyc8 (Ssn6)-*TUP1* corepressor complex is composed of one Cyc8 and four *TUP1* subunits, Mol. Cell. Biol. 16 (1996) 6707–6714.
[30] C. Jabet, E.R. Sprague, A.P. VanDemark, C. Wolberger, Characterization of the N-terminal domain of the yeast transcriptional repressor *TUP1*. Proposal for an association model of the repressor complex *TUP1* x Ssn6, J. Biol. Chem. 275 (2000) 9011–9018.
[31] D. Tzamarias, K. Struhl, Functional dissection of the yeast Cyc8-*TUP1* transcriptional co-repressor complex, Nature 369 (1994) 758–761.
[32] R.B. Wickner, Mutants of *Saccharomyces cerevisiae* that incorporate deoxythymidine-5′-monophosphate into deoxyribonucleic acid in vivo, J. Bacteriol. 117 (1974) 252–260.