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A SET-domain-independent role of WRAD complex in cell-cycle regulatory function of mixed lineage leukemia

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ABSTRACT

MLL, the trithorax ortholog, is a well-characterized histone 3 lysine 4 methyltransferase that is crucial for proper regulation of the *Hox* genes during embryonic development. Chromosomal translocations, disrupting the *Mll* gene, lead to aggressive leukemia with poor prognosis. However, the functions of MLL in cellular processes like cell-cycle regulation are not well studied. Here we show that the MLL has a regulatory role during multiple phases of the cell cycle. RNAi-mediated knockdown reveals that MLL regulates S-phase progression and, proper segregation and cytokinesis during M phase. Using deletions and mutations, we narrow the cell-cycle regulatory role to the C subunit of MLL. Our analysis reveals that the transactivation domain and not the SET domain is important for the S-phase function of MLL. Surprisingly, disruption of MLL–WRAD interaction is sufficient to disrupt proper mitotic progression. These mitotic functions of WRAD are independent of SET domain of MLL and, therefore, define a new role of WRAD in subset of MLL functions. Finally, we address the overlapping and unique roles of the different SET family members in the cell cycle.

INTRODUCTION

Mixed lineage leukemia (MLL or MLL1) protein, a human ortholog of *Drosophila* trithorax, was first identified for its involvement in chromosomal translocations associated with acute leukemia in infants and adults (1). Subsequent studies revealed its critical role in proper regulation of the homeobox-containing (*Hox*) genes during embryonic development (2). MLL also plays a vital role in regulating hematopoietic stem cell self-renewal and progenitor cell expansion (2,3). *Mll* encodes a 3969-aa nuclear protein that gets proteolytically processed into two subunits, MLL_N and MLL_C (4). These subunits self-associate through PHD1, PHD4 and FYRN domains present in MLL_N, and FYRC domain present in MLL_C subunit, to confer stability to each other (4,5). MLL_N contains several motifs involved in DNA binding (AT hooks, CXXC domain) and chromatin recognition (plant homeodomain fingers, bromo domain) and is thought to be responsible for targeting the MLL complex to DNA (6). By contrast, MLL_C is the transcriptional effector that possesses a transactivation domain (TAD) and a conserved Su(var)3–9, Enhancer-of-zeste, Trithorax (SET) domain that specifically methylates lysine 4 of histone H3, an epigenetic mark associated with active transcription.

Methylation of H3K4 is an important regulatory pathway that is highly conserved from yeast to mammals (7). However, in contrast to yeast, which has only one H3K4 histone methyltransferase (HMT)—Set1 (COMPASS)—there are at least six H3K4 HMTs in mammalian cells, namely, MLL1 to MLL4 (KMT2A to KMT2D) and Set1A and Set1B (KMT2F and KMT2G). While yeast Set1 is capable of mono, di and tri methylating H3K4, mammalian HMTs have variable intrinsic capability to methylate H3K4 leading to distinct cellular roles (8–11). However, despite the recent advances in understanding the unique functions of SET1 family, our knowledge of their role in biological processes like cell proliferation is extremely limited.

The SET1 family is active only in the context of a multisubunit complex, sharing four common highly conserved components, namely, WDR5, RbBP5, Ash2L and Dpy30 (referred to as WRAD) that are related to yeast Set1 COMPASS complex. Additional complex-specific subunits are also needed in different functional context (12). Independent studies have shown that a minimal four-component complex, including WDR5, RbBP5 and Ash2L along with the SET-domain subunit can reconstitute most of the H3K4-specific HMT activity of the MLL core complex, while Dpy30 is required to increase the enzymatic activity of the above complex (13–15). *In vitro* experiments show that in absence of WRAD complex, MLL is a weak monomethyltransferase. However, in the presence of WDR5–RbBP5–Ash2L (W–R–A), MLL displays di- and weak tri-methyltransferase activity, further highlighting the

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importance of WRAD in the enzymatic activity of SET domain of MLL (13). Recent studies demonstrate that W–R–A form a stable subcomplex that is capable of interacting with the other members of the SET1 family as well (14,16). The WD40 repeat-containing protein—WDR5—is critical for these interactions, as it bridges the interactions between the catalytic SET domain and RbBP5, thereby maintaining the structural integrity of the complex (14,16). Thus, so far, the only known function of WRAD in relation to SET1 family is its effect on the enzymatic activity/stability of SET complexes. Here, we report a novel non-SET-domain function of WRAD with MLL in cell-cycle regulation.

MLL is known to associate with most transcriptionally active genes (17,18). It is firmly believed that maintenance of the transcriptional status of target genes by MLL is achieved through chromatin modifications. Supporting this hypothesis, MLL has been shown to directly bind to the promoter regions of a subset of *Hox* genes. At these promoters, MLL recruits large multiprotein complex capable of depositing methylation and acetylation marks associated with active transcription (19,20). *Hox* gene expression is initiated normally in *Mll*-knockout mice, but is not sustained past embryonic day 10.5 leading to embryonic lethality (2). However, the MLL-associated H3K4 methyltransferase activity is not required *in vivo*, as mice with MLL SET domain homozygous deletion are born with a relatively mild phenotype (21). Strikingly, MLL-deficient cells show no global changes in H3K4 methylation and only a small subset of genes exhibit loss of H3K4 methylation upon loss of MLL (9,18,20). Nonetheless, expression of a large number of genes is affected upon loss of MLL (9), indicating that MLL may regulate transcription independent of its SET domain.

Although the role of MLL (i) in maintaining the expression of *Hox* genes and (ii) in MLL pathology has been relatively well understood, the participation of MLL in many other important cellular processes remains elusive. Recent reports show that MLL proteins are involved in regulation of the cell cycle (17,22–24). MLL regulates S-phase checkpoint by methylating H3K4 at late replication origins and prevents them from re-firing (22). MLL also interacts with several E2F proteins directly or indirectly, and brings about the transcriptional activation of E2F-dependent genes during G1- to S-phase transition (23–25). In direct contrast to this function, MLL regulates the expression of cyclin-dependent kinase inhibitor genes (17). All of the above-mentioned functions have been attributed to the histone methyltransferase activity of MLL. The expression of MLL is itself tightly regulated during the cell cycle with maximal expression at G1/S and M phases (26). While these reports provide valuable insight into the regulatory role of MLL, how MLL exerts its control during the cell cycle is far from known. In order to further analyze the functions of MLL during the cell cycle, we knocked down MLL by RNAi in normal and transformed cells. Our studies reveal that MLL not only regulates cell proliferation and S-phase progression, but also participates in segregation and cytokinesis during cell division. Importantly, MLL utilizes two different activities, i.e. the transcriptional activity from transactivation domain to regulate S phase and protein–protein interactions from 'Win' motif to regulate M phase. Both these activities are independent of its more classical regulation via SET domain. We also characterize the role of other SET1 family members in cell-cycle progression.

MATERIALS AND METHODS

Cloning and site-directed mutagenesis

Flag epitope-tagged complementary DNA (cDNA) expression constructs encoding WDR5, full-length MLL, MLL_N, MLL_C and MLL SET domain deletion (MLLΔSET, aa3829–3969) were generated by polymerase chain reaction (PCR) amplification using high-fidelity polymerase (Agilent) and cloned in Xho I linearized pBabe-Flag vector (WDR5 and MLL cDNAs were a generous gift from R. Roeder). MLL ΔSET ΔWin R3765A , MLL_C ΔTAD (aa2847–2855) and MLL_C ΔFYRC (aa3666–3747) mutations were generated by PCR-mediated mutagenesis. Similarly, pBabe WDR5 was subjected to sequential PCR-mediated mutagenesis to generate either siRNA-resistant WDR5 (resistant to siRNA #2, also see Supplementary Data) followed by F133L, F263A, Q289A and Y191F point mutations or vice versa [WDR5 F263A and WDR5 Q289A were generously provided by J.F. Couture, (27,28)]. Every insert and mutation was verified by sequencing the entire cDNA construct.

Cell culture, transfection and stable cell-line generation

U2OS, IMR-90tert and MCF7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, L-glutamine and penicillin/streptomycin. Cells stably expressing Flag-tagged WDR5, MLL and all the mutants of WDR5 and MLL were obtained by transducing U2OS cells with retroviral particles generated in the Phoenix amphotropic virus packaging line (29). The only exception was Flag-MLL_CΔFYRC and Flag-MLL_CΔTAD, which were in pcDNA backbone and were transfected using Lipofectamine-2000 (Invitrogen). The transduced and transfected cells were selected using 4 μg/ml and maintained using 2 μg/ml Puromycin (Invitrogen). Drug-resistant colonies were tested for recombinant gene expression either by immunoblot (WDR5) or immunofluorescence (MLL).

siRNA transfections

siRNA transfections were performed as described (30). Cells were harvested 72 or 96 h after first transfection and either lysed in sodium dodecylsulphate Laemmli buffer, subjected to Western blotting, RNA extraction, or fixed for immunofluorescence studies. Oligonucleotide sequences are provided in Supplementary data.

For growth curve generation, 3.8×10^4 cells were transfected in duplicate plates with control, MLL#1 or MLL#2 siRNA on day 0, harvested, stained with trypan blue and counted every 24 h for 4 days. Results were averaged and plotted.

RNA isolation and quantitative real-time polymerase chain reaction

RNA was extracted either by Trizol extraction method (Life Technologies) or Direct-zol RNA Miniprep kit (Zymo Research). Two micrograms of the total RNA was used to prepare cDNA using Superscript III reverse transcriptase (Invitrogen) and cDNA was used for quantitative real-time polymerase chain reaction (RT-qPCR) using QuantiTect SYBR green PCR kit (Qiagen). Each sample was run in triplicate. The amplification was performed and detected using 7500 Real Time PCR system (Applied Biosystems). Transcripts were normalized to the housekeeping gene GAPDH by using $-\Delta\Delta$CT method (31) and percentage expression relative to untransfected sample is shown. Primer sequences are provided in Supplementary data.

Immunoblots

Immunoblot analysis was performed and proteins were detected either with Licor-Biosciences imaging system as described previously (23) or by chemiluminescence method (Amersham ECL Plus-RPN2132) using anti-mouse immunoglobulin G (IgG) (Sigma-Aldrich-A9044) and anti-rabbit IgG (Sigma-Aldrich-A0545).

Immunofluorescence

For immunofluorescence, cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.2% Triton X-100 in phosphate buffered saline for 5 min and then blocked with 1% bovine serum albumin at room temperature (RT) for 1 h. For bromodeoxyuridine (BrdU) staining, cells were fixed with 4% paraformaldehyde at RT for 15 min, and permeabilized with 0.2% Triton X-100 at RT for 15 min. Permeabilization was followed by denaturation using 2-M Hydrochloric acid treatment and neutralization by 1-M borate buffer.

The cells were subsequently incubated with primary antibodies (see Supplementary data) at RT for 3 h. After washing, the cells were incubated with Alexa 488 (1:1000)- or Alexa 633 (1:500)-conjugated anti-rabbit or anti-mouse antibodies (Invitrogen-Molecular Probes) at RT for 1 h. The nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and cells were mounted using VECTASHIELD Mounting Medium (Vector Laboratories-H1200). The fluorescent images were captured on ZIESS LSM 510 META inverted confocal microscope and analyzed with LSM software. Minimum 200 cells were counted from at least two independent experiments in each case to calculate the percentage of defective cells.

RESULTS

Loss of MLL results in growth arrest

In order to decipher the cell proliferation functions of MLL, we made use of RNAi technology. To avoid off-target effects, we selected two different siRNAs against the human MLL messenger RNA (mRNA), and as control, used siRNA against firefly luciferase mRNA not present in human cells (30). We monitored the knockdown efficiency of our siRNAs on the MLL mRNA and protein levels by RT-qPCR and Western blot analysis, respectively. As shown in Figure 1A, 72 h after siRNA treatment, MLL mRNA levels were reduced by 70% in the two MLL-specific siRNAs used. Similarly, MLL protein levels were considerably reduced in

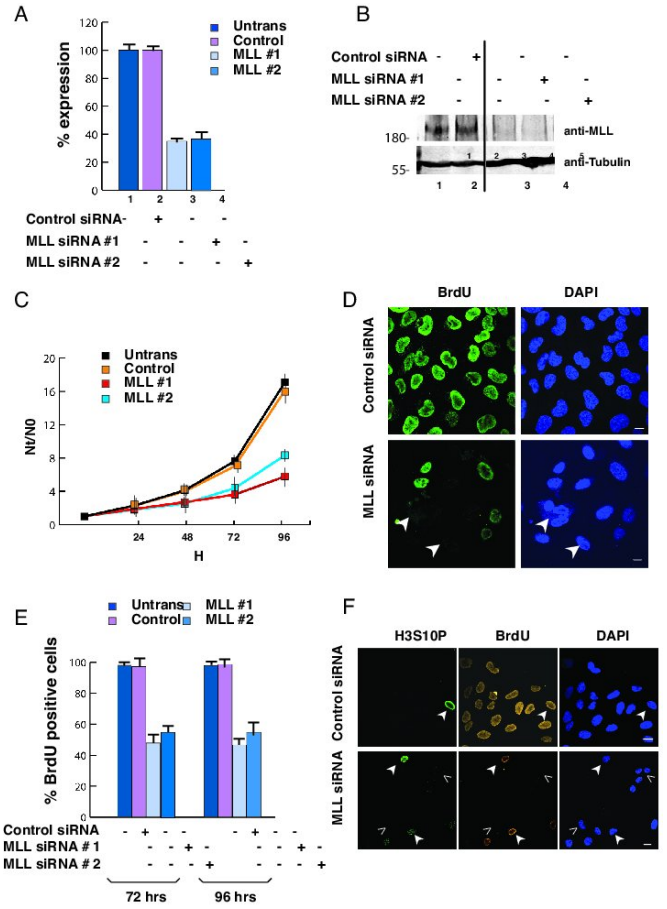

Figure 1. MLL loss-of-function leads to growth arrest. (**A**) RT-qPCR was carried out to analyze mRNA transcript levels of MLL in cells transfected with two different MLL siRNAs (siRNA #1 or siRNA #2). Untransfected and luciferase siRNA-transfected cells were used as control. Transcripts were normalized to the housekeeping gene GAPDH by using $-\Delta\Delta$CT method and percentage expression relative to untransfected sample is shown. (**B**) Immunoblot analysis of MLL knockdown was done using MLL siRNA #1 or siRNA #2. Untransfected and luciferase siRNA-transfected cells were used as control. The blot was probed with anti-MLL and anti-tubulin antibody. 180 and 55: molecular weight markers. (**C**) Growth curves of untransfected (black), control siRNA-transfected (orange) or MLL siRNA [siRNA #1(red) or #2 (blue)]-transfected U2OS cells were generated by plotting the total number of live cells (N_t) divided by number of cells seeded on day 0 (N_0). The cells were harvested at 24, 48, 72 and 96 h from duplicate experiments after siRNA treatment, stained with trypan blue, counted, and averaged results are shown. (**D**) Immunofluorescence analysis of BrdU incorporation in control or MLL siRNA-transfected cells was done by staining cells with anti-BrdU antibody and DAPI. Arrowheads point to BrdU-negative cells. Scale: 5 μm. (**E**) Quantification of BrdU-positive cells was done in untransfected, control siRNA, MLL siRNA #1- or MLL siRNA #2-transfected cells 72 or 96 h after treatment. (A, C, E) Data are represented as mean ± SD. (**F**) BrdU incorporation and cell-cycle phase analysis in control or MLL siRNA-transfected cells was done by staining cells with anti-BrdU or anti-H3S10P antibody and DAPI. Closed arrowheads point to H3S10P and BrdU-positive cells. Open arrowheads point to BrdU-negative cells. Scale: 5 μm.

the two MLL siRNAs used, but remained unaffected in the control siRNA-treated cells (Figure 1B).

Previously, it has been reported that impaired MLL activity affects cell proliferation in mammalian cells (24,26,32). To examine the effect on cell proliferation, we compared the growth rates of untreated U2OS cells with those treated with MLL or control siRNA. Consistent with previous observations (26,32,33), MLL siRNA-treated cells displayed a decrease in cell growth 48 h after siRNA treatment as opposed to control siRNA-treated cells (Figure 1C). By 72 h, the growth retardation was pronouncedly high in MLL-depleted cells. We also noticed considerable cell death in MLL siRNA-treated samples. This observation is consistent with those reported by Mandal *et al.* (32). Even though we used trypan blue dye exclusion method to score live cells in these samples, in order to ensure that the growth inhibition was true and not due to cell death observed, we determined the proliferative status of individual MLL-depleted cells. For this, we assayed the cells for S-phase entry using long-term BrdU incorporation by indirect immunofluorescence staining (IFS) followed by microscopy. As shown in Figure 1D, when stained with BrdU antiserum and DAPI to mark the nuclei, all control siRNA-treated cells showed BrdU incorporation, whereas a large number of MLL siRNA-treated cells were unable to incorporate BrdU (see arrowheads). When quantified, 72 h after siRNA treatment, about 50% of MLL siRNA-treated cells were deficient in incorporating BrdU. We repeated the assay 96 h after siRNA treatment, but found no further increase in BrdU-negative cells (Figure 1E), indicating that the cells were stably arrested upon loss of MLL. Our results show that MLL is required for mammalian cells to proliferate.

Cell arrest in G1 phase upon loss of MLL

The considerable cell death in MLL siRNA-treated samples made it difficult to ascertain the specific cell-cycle phase that MLL-depleted cells arrested in, by flow cytometry. Therefore, we again made use of IFS to determine the cell-cycle phase of individual MLL-depleted cells. We used G2/M-specific histone 3 serine 10 phosphorylation (H3S10P) staining to distinguish G1 cells from G2/M cells. As shown in control cells, all control siRNA-treated cells stained for BrdU and showed either H3S10P-negative G1 cells or H3S10P-positive G2/M cells (Figure 1F). In MLL siRNA-treated cells, 50% of cells did not incorporate BrdU, indicating that they were unable to pass through S phase in past 24 h. Even though we saw an overall increase in H3S10P-positive cells compared to control cells (18.19 ± 0.69% in test versus 7.35 ± 0.64% in control), out of BrdU-negative cells, the overwhelming population was H3S10P-negative, indicating that the MLL-depleted cells arrested in G1 phase. Taken together with BrdU incorporation deficiency, our results suggest that MLL-depleted cells are unable to progress through S phase.

Loss of MLL gives rise to M-phase defects

When examined 72 h after siRNA treatment, the MLL siRNA-treated cells displayed high number of binucleated cells. The MLL siRNA treatment resulted in fewer numbers of cells with elongated shapes and binucleated cells (see

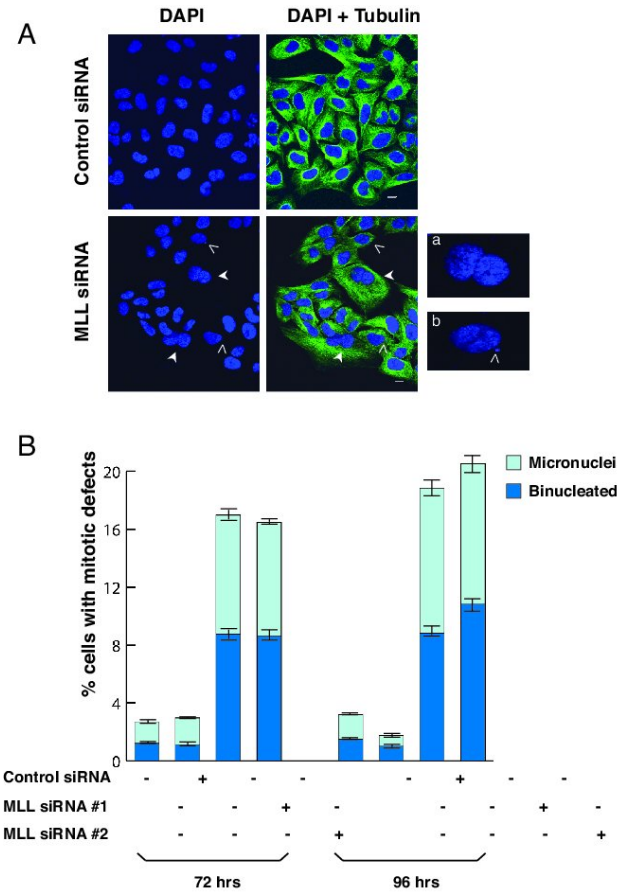

Figure 2. MLL depletion leads to mitotic defects. (**A**) Immunofluorescence analysis showing mitotic defects (binucleation and micronuclei) upon MLL depletion in U2OS cells. The cells were stained with DAPI and anti-tubulin antibody. Closed arrowheads and panel *a* show binucleated cells; open arrowheads and panel *b* show cells with micronuclei. Scale: 5 μm. (**B**) The percentage of the U2OS cells displaying mitotic defects was quantified in untransfected, control siRNA, MLL siRNA #1- or MLL siRNA #2-transfected cells 72 or 96 h after treatment. Data are represented as mean ± SD. Significant *P*-values (<0.01) were obtained with Student's *t*-test.

closed arrowhead in Figure 2A and panel *a*) as opposed to control siRNA-treated U2OS cells, which were clustered and displayed a compact shape. These observations indicated that, along with cell growth, the loss of MLL function could lead to a binucleation defect, probably arising from defective cytokinesis. We also noticed the presence of micronuclei in MLL-depleted cells. Micronuclei are recognized as small distinct bodies of chromatin in the cytoplasm of interphase mammalian cells and considered as marker of chromosome loss during mitotic segregation (see open arrowhead in Figure 2A and panel *b*). As both these phenotypes i.e. cells with micronuclei or binucleation, arise due to defects in M phase, we will refer to these segregation and cytokinesis defective cells together as cells with mitotic defects hence on.

In order to quantify the cells with mitotic defects, we stained the siRNA-treated cells with α-tubulin antiserum to mark the cell border, and DAPI to identify the nuclei. U2OS cells displayed 2–4% defective cells even before any siRNA

treatment, and control siRNA treatment did not exacerbate the count. In contrast, MLL siRNA treatment resulted in significantly higher number of cells displaying mitotic defects (Figure 2B). Interestingly, unlike the BrdU incorporation assay, this assay showed a 4–5% increase in number of defective cells when harvested at 96 h (Figure 2B; compare 72- and 96-h MLL siRNA-treated samples). This increase in number of cells with mitotic defects indicates that the cells, which continue to proliferate in absence of MLL, may do so with defective mitosis and cytokinesis. Taken together, our results indicate that MLL may regulate multiple steps in mitosis and depletion of MLL results in segregation and cytokinesis defects.

Loss of MLL induces cell-cycle defects in MCF7 and IMR-90tert cells

To determine whether the cell-cycle functions of MLL are limited to U2OS cells, we assayed for loss-of-function of MLL in p53- and pRb-positive MCF7 breast carcinoma cells and non-tumor human diploid embryonic lung fibroblasts IMR-90tert cells. As shown in Figure 3, a large number of both the MCF7 (Figure 3A) and IMR-90tert (Figure 3B) cells were unable to incorporate BrdU in MLL siRNA-treated cells, whereas the control siRNA-treated cells exhibited no problems in BrdU uptake. As in U2OS cells, the MLL knockdown resulted in significant number of cells with mitotic defects in both the cell lines, where cells with micronuclei and binucleation were evident (Figure 3C and D). Our results indicate that MLL is a broad regulator of cell cycle and its functions are not confined to a particular cell type.

Depletion of WRAD components causes S- and M-phase progression defects

MLL along with WRAD exists as a core complex (Figure 4A). The WRAD components are essential for the catalytic activity of MLL HMT complex (34). As MLL is a transcriptional co-activator and acts by its HMT activity, we reasoned that the activity of WRAD will also be essential for the cell proliferation functions of MLL complex. Therefore, we used two different siRNAs to deplete each WRAD component individually and assayed these cells for BrdU incorporation. These proteins have been reported to be highly abundant in the cell, but RNAi treatment could successfully knock down all four components (35; Supplementary Figure S1). As shown in Figure 4B, knockdown of WRAD components resulted in cells deficient in BrdU incorporation, although to different degrees. Loss of WDR5 and RbBP5 resulted in about 50% cells unable to incorporate BrdU, whereas loss of Ash2L and Dpy30 about 30%. However, the cells treated with Ash2L and Dpy30 siRNA eventually displayed 50% BrdU negative cells, 96 h after siRNA treatment, suggesting that either the loss of Ash2L and Dpy30 may have delayed effect on cell proliferation or the Ash2L and DPy30 siRNA treatment may take longer to be effective (due to protein stability). In support of latter, it has previously been reported that Ash2L protein is very stable in transformed cell lines (36).

We next assayed the WRAD siRNA-treated samples for mitotic defects. Again like MLL, WRAD siRNA-treated

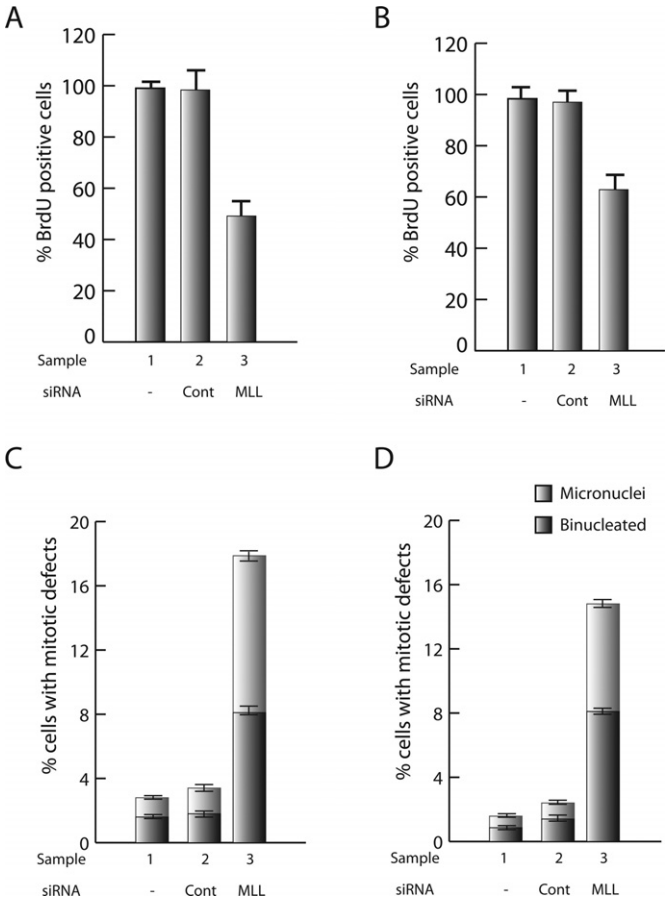

Figure 3. MLL RNAi gives rise to cell proliferation and mitotic defects in MCF7 and IMR-90tert cells. (**A**–**B**). BrdU labeling was done in MCF7 (A) and IMR-90tert (B) cells following MLL depletion by siRNA. The untransfected (−), control siRNA (cont) and MLL siRNA#1(MLL)-transfected cells were stained with anti-BrdU 72 h after treatment. Data are represented as mean ± SD. (**C**–**D**). Percentage of cells displaying mitotic defects (binucleation and micronuclei) in MCF7 (C) and IMR-90tert (D) cells upon MLL siRNA treatment. Untransfected, control siRNA and MLL siRNA #1-transfected cells were stained with DAPI and anti-tubulin antibody 72 h after siRNA treatment. Data are represented as mean ± SD. Significant *P*-values (<0.006) were obtained with Student's *t*-test.

cells displayed both micronuclei formation and binucleation, although with some variation in number of defective cells. However, overall cell count with mitotic defects was similar to that of MLL knockdown indicating that WRAD participated in the same pathway as MLL to regulate mitosis.

In order to examine the long-term phenotype of MLL complex depletion, the percentages of cells with mitotic defects were determined for 120 h consecutively in control siRNA-treated U2OS and WDR5 siRNA-treated cells (Supplementary Figure S2). The number of cells with mitotic defects in WDR5 siRNA-treated samples increased steadily till 96 h and then abruptly decreased to level of control siRNA-treated samples, probably, because the silencing effects of WDR5 siRNA were not sustained after 96 h. Our results indicate that loss of components of MLL core complex results in accumulation of cell population undergoing error-prone mitosis and cytokinesis. Taken together,

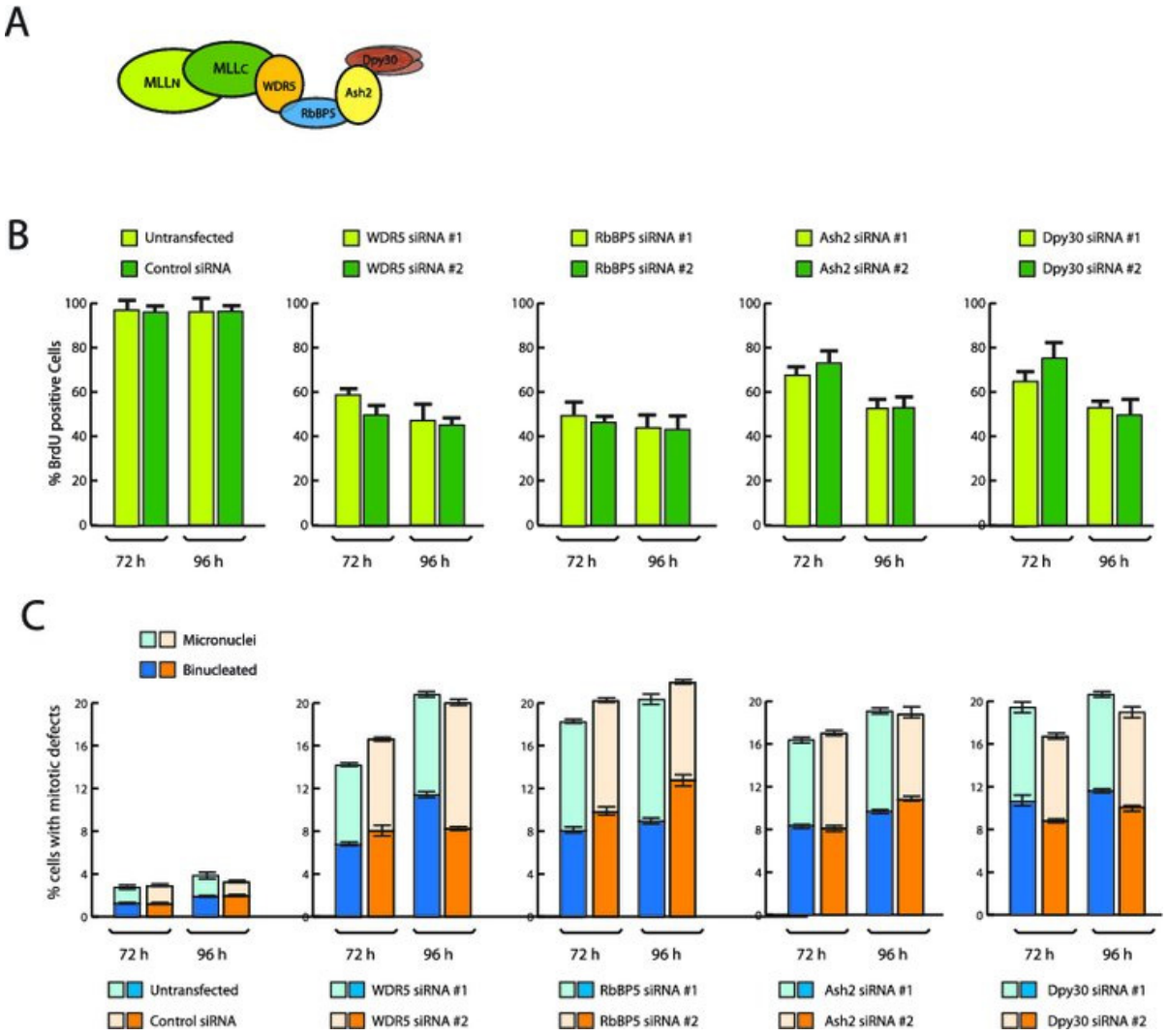

Figure 4. RNAi of WRAD complex leads to cell proliferation and mitotic defects. (**A**) The model of functional MLL HMT core complex. WRAD interacts with MLL_C subunit. WDR5 forms a bridge by interacting with MLL_C subunit on one side and RbBP5 on other (27,34). (**B**) BrdU incorporation assay was performed in U2OS cells following depletion of each component of WRAD complex by using two different siRNAs for each protein. The untransfected, control siRNA, WDR5 siRNA (#1 or #2), RbBP5 siRNA (#1 or #2), Ash2L siRNA (#1 or #2) and Dpy30 siRNA (#1 or #2)-transfected cells were subjected to long-term BrdU labeling. The cells were harvested at 72 and 96 h after siRNA treatment and stained with DAPI and anti-BrdU antibody. Data are represented as mean ± SD. (**C**) Percentage of cells displaying binucleation (dark blue and orange) and micronuclei (light blue and apricot) upon siRNA treatment. The cells were transfected with siRNA as indicated and stained with DAPI and anti-tubulin 72 or 96 h after siRNA transfection. Data are represented as mean ± SD. Significant *P*-values (<0.01) were obtained with Student's *t*-test.

our observations here point to a role of MLL and components of MLL core complex—WRAD—in cell-cycle regulatory function in S- and M-phase progression.

MLL_C subunit is required for S- and M-phase progression

To identify the regions of MLL required to promote S- and M-phase progression, a set of recombinant Flag epitope-tagged MLL protein deletions or mutations were stably expressed in U2OS cells. Figure 5A shows the structure of these recombinant MLL proteins: (i) F-MLL representing recombinant full-length precursor protein; (ii) F-MLL ΔSET lacking the SET domain; (iii) F-MLL ΔSETΔWin lacking the SET domain and point mutation in Win motif (R3765A) (iv) F-MLL_N representing the N subunit; (v) F-MLL_C representing the C subunit; (vi) F-MLL_C ΔFYRC lacking the FYRC region and (vii) F-MLL_C ΔTAD lacking the transactivation domain. Two independent clones were used and expressing clones were identified by IFS using Flag antibody (Figure 5B).

Out of our two MLL-specific siRNAs, siRNA #1 is directed against the coding sequence, whereas siRNA #2 binds the 3′ UTR of MLL precursor mRNA. As our recombinant MLL constructs lacked the 3′UTR sequence, we could use siRNA #2 to specifically deplete endogenous MLL, but not recombinant MLL in these stable cell lines. Each cell line was treated with control and MLL siRNA (#2) and analyzed for S-phase progression. Figure 5C shows that while wild-type cells showed deficiency in incorporating BrdU upon MLL siRNA treatment, cells stably expressing F-MLL were able to incorporate BrdU just

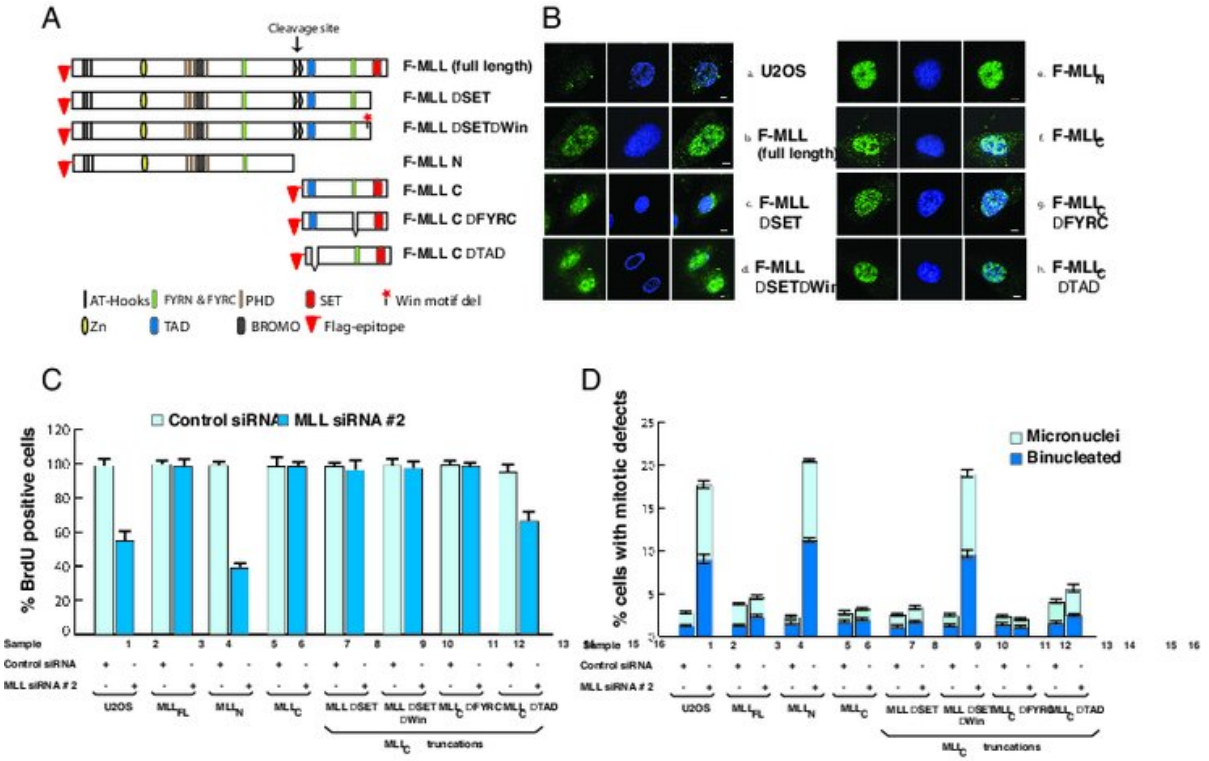

Figure 5. MLL_C subunit rescues cell proliferation and mitotic defects in MLL-depleted cells. (**A**) The figure shows schematic representation of MLL full-length protein with various domains. Recombinant full-length and mutant MLL proteins were expressed with Flag-epitope-tag (F) fused at their N terminal. (**B**) The expression of ectopic MLL full-length and mutant proteins was checked by immunofluorescence. The U2OS cells were fixed and immunostained with anti-Flag serum to detect expressed Flag-tagged recombinant MLL (full-length) and mutant constructs in (a) wild-type cells (as control), and, cells expressing (b) F-MLL representing recombinant full-length precursor protein; (c) F-MLL ΔSET lacking the SET domain; (d) F-MLL ΔSETΔWin lacking the SET domain and point mutation in Win motif (R3765A); (e) F-MLL_N representing the N subunit; (f) F-MLL_C representing the C subunit; (g) F-MLL_C ΔFYRC lacking the FYRC region and (h) F-MLL_C ΔTAD lacking the TAD. Scale: 5 μm. BrdU staining (**C**) and mitotic defects (**D**) quantifications were done in U2OS cells and stable cell lines expressing full-length or mutant MLL protein following treatment with control siRNA or MLL siRNA #2 for 72 h. Data are represented as mean ± SD. Significant *P*-values (<0.01) were obtained with Student's *t*-test (D).

like control siRNA-treated cells (see Figure 5C, samples 2 versus 4), indicating that the S-phase progression defect induced by MLL siRNA treatment was specific to MLL protein depletion and could be rescued by reconstituting the MLL expression. Out of the two MLL subunits, expression of the MLL_C subunit alone, but not the MLL_N subunit, was adequate to restore the ability of cells to incorporate BrdU just like control siRNA-treated cells (Figure 5C, sample 8 and 6). Further analyzing for requirement of the different domains present in MLL_C, and consistent with the non-requirement of MLL_N subunit, the absence of FYRC region did not affect the ability of MLL_C subunit to rescue BrdU incorporation in MLL siRNA-treated cells (Figure 5C, sample 14). Surprisingly, deletion of SET domain also did not compromise the MLL_C activity to rescue S-phase progression (Figure 5C, sample 10). However, deletion of nine amino acid transactivation domain reduced the ability of cells to incorporate BrdU (Figure 5C, sample 16). Our results indicate that the transcriptional activity of TAD domain of MLL and not the methyltransferase activity of SET domain is required for passage of cells into S phase.

To determine the region of MLL involved in mitosis and cytokinesis, we quantified cells displaying mitotic defects. Expression of recombinant MLL protein deletions or mutations did not induce obvious mitotic defects (Figure 5D, compare sample 1 with samples 3, 5, 7, 9, 11, 13 and 15). The reconstitution of the full-length MLL expression displayed marked reduction in number of cells with mitotic defects indicating that, indeed absence of MLL was responsible for causing errors in cell division (Figure 5D, sample 2 versus 4). Similar to full-length protein, expression of MLL_C subunit was sufficient to reduce the number of cells with mitotic defects (Figure 5D, sample 8). In contrast, expression of MLL_N subunit had no effect on the number of cells displaying mitotic defects, indicating that MLL_N subunit cannot independently rescue the segregation and cytokinesis defects induced upon MLL knockdown.

We next analyzed the cells for mitotic defects in the different domain deletions of MLL_C subunit. Similar to BrdU incorporation, FYRC and SET domain deletions did not affect the ability of MLL_C subunit to rescue mitotic defects in MLL siRNA-treated cells (Figure 5D, samples 10 and 14). To our surprise, however, deletion of even transcriptional activation domain did not give rise to mitotic defects (Figure 5D, sample 16). These observations imply that neither the transcriptional activity nor the methyltransferase activity of MLL plays any part in MLL's role in mitotic progression.

WRAD is required for a non-SET-domain function of MLL

Our results indicate that the SET domain of MLL_C is not required for either S- or M-phase progression as the cells expressing F-MLL ΔSET behaved similar to cells expressing F-MLL construct (Figure 5C and D, see samples 4 and 10). In this light, our observations with knockdown of WRAD components are perplexing as it is well documented that WRAD is required for the catalytic activity of SET-domain-mediated HMT activity. Here, for ease of discussion, we would like to refer to the SET-domain-mediated activity as methyltransferase activity and TAD-domain-mediated activity as transcriptional activity. We reasoned that WRAD might be involved in the non-SET-domain-mediated transcriptional activity of MLL. To test this hypothesis, we made a mutation in the WDR5 interacting (Win) motif of MLL ΔSET construct by changing arginine 3765 to alanine. Recent reports have characterized this motif as being sufficient for interaction with WDR5 and point mutation of Arg3765 in this motif abolishes the binding of the whole WRAD complex to MLL (37,38). If WRAD interaction is required for the transcriptional activity of MLL, abolishing the MLL-WDR5 interaction should affect MLL's cell proliferation functions. Contrary to our hypothesis, cells expressing F-MLL ΔSETΔWin readily incorporated BrdU upon MLL siRNA treatment (Figure 5C, samples 11 and 12), indicating that WRAD does not influence the S-phase progression functions of MLL TAD domain. However, cells expressing F-MLL ΔSETΔWin did display strikingly high number of cells with mitotic defects, indicating that WRAD is required for M-phase functions of MLL (Figure 5D, samples 11 and 12). Taken together, our results show that WRAD is required for a specific subset of MLL non-SET-domain functions.

Mutation in WDR5 confirms MLL-specific cell-cycle function of WRAD

Recent reports predicted the existence of WRAD subcomplexes lacking the HMT subunits (13,39). Additionally, WDR5 and Dpy30 have been found associated with other chromatin regulatory complexes (34,35). In order to further clarify the role of WRAD complex in MLL cell proliferation functions observed here, we studied these interactions with specific WDR5 point mutations. As shown in Figure 4A, WDR5 forms a bridge between MLL and RbBP5, interacting directly with MLL via the 'Win' motif on one side and with RbBP5 on the opposite side (27,37,38). Strikingly, the Win motif sequence shares sequence homology with the histone H3 N terminus. Mutations in WDR5, that disrupts the interactions with histone H3, particularly substitutions of Phe133 and Phe263, also disrupt its binding with MLL, and the H3K4 HMT activity. In contrast, substitution of Tyr191 that impairs the H3 binding of WDR5 and HMT activity, does not affect the interactions of WDR5 with MLL and the core complex (14,37). Similarly, mutations in the RbBP5 binding residues of WDR5 (namely, Asn 225, Leu240 and Gln 289) impair the RbBP5 binding and the HMT activity of the core complex (27).

Unlike MLL, both siRNA oligonucleotides targeted coding sequence in WDR5. Therefore, to prevent the destruction of the recombinant WDR5 mRNAs, we introduced

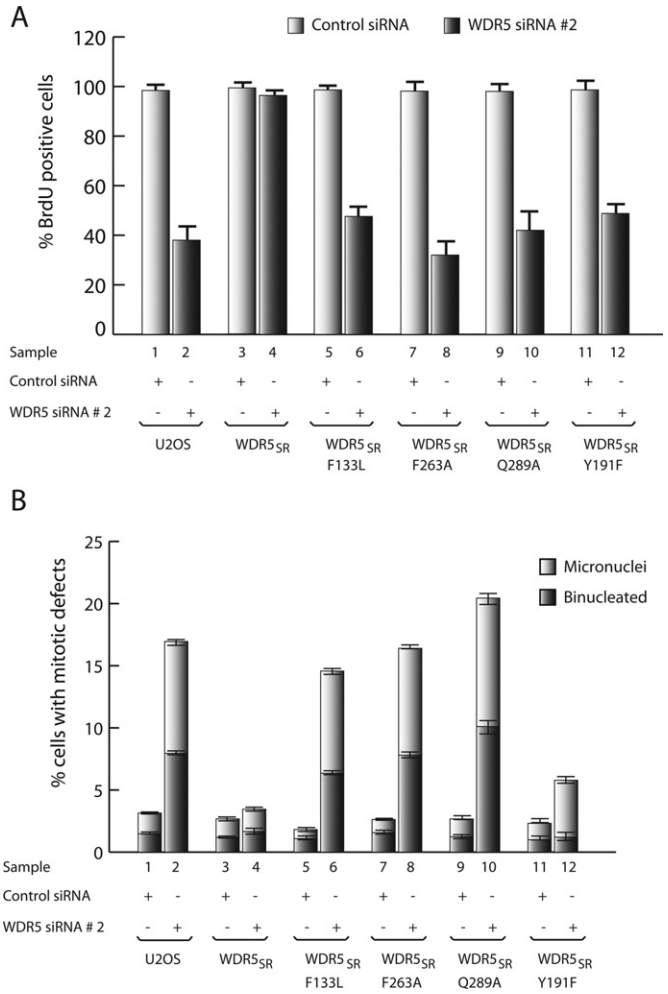

Figure 6. Mutational analysis of WDR5 protein. (**A** and **B**) U2OS cells and cells stably expressing siRNA-resistant full-length or point mutants of WDR5 protein were treated with WDR5 siRNA #2 for 72 h and scored for BrdU incorporation (A) and mitotic defects (B) as described earlier. Data are represented as mean ± SD. Significant *P*-values (<0.004) were obtained with Student's *t*-test (B).

six silent mutations into the *WDR5* sequence corresponding to the siRNA #2, creating siRNA-resistant WDR5 ($WDR5_{SR}$) proteins (40; see Supplementary methods). We also Flag epitope-tagged this recombinant $WDR5_{SR}$, thus creating F-$WDR5_{SR}$. When treated with siRNA #2 and analyzed by immunoblot as shown in Supplementary Figure S3, the endogenous WDR5 disappeared, but not the recombinant $WDR5_{SR}$ protein. As expected, the silent mutations did not affect the ability of $WDR5_{SR}$ to rescue the cell proliferation defects acquired upon WDR5 siRNA treatment (Figure 6A, compare samples 2 and 4). In order to analyze the WDR5 functions, we created F133L, F263A, Q289A and Y191F mutations individually in $WDR5_{SR}$ and verified the expression of all four constructs upon siRNA treatment (Supplementary Figure S3). In our assay, $WDR5_{SR}$ F133L and $WDR5_{SR}$ F263A mutants could not rescue the S-phase progression (Figure 6A). Similarly, $WDR5_{SR}$ Q289A mutation, which disrupted RbBP5 binding, also proved inefficient in S-phase rescue. To our surprise, the Y191F mu-

tant sample also exhibited cells, which were unable to uptake BrdU, indicating that interaction of WDR5 with MLL was not sufficient for cells to progress to S phase.

We next checked these WDR5 mutants for rescuing mitotic defects in WDR5 siRNA-treated cells. None of the three mutants, namely, F133L, F263A and Q289A, were able to rescue the defective phenotypes in mitosis, indicating that WDR5 and RbBP5 interacted with MLL to ensure uneventful mitotic progression. In contrast to BrdU incorporation, however, Y191F mutation did not result in any binucleation and very little micronuclei formation (Figure 6B sample 12), suggesting that interaction of WDR5 with MLL, but not H3, was enough for proper M-phase progression. Taken together, our results show that the interactions of WRAD—HMT core complex—with MLL are essential for M-phase but not S-phase progression.

The functions of other SET1 family members in cell-cycle regulation

Our results so far show that loss of both MLL and WRAD results in S- and M-phase progression defects, but, while WRAD may have a role in the M-phase functions of MLL, their involvement in S-phase functions of MLL is not clear. Further, mutations in WDR5 show that Y191F, which is capable of interacting with MLL, rescues M- but not S-phase progression defect induced by loss of WDR5. WRAD components complex with other SET1 family members as well (16). Moreover, SET1 family members have overlapping as well as unique functions (12). In order to clarify if other SET members participated or duplicated the functions of MLL in cell-cycle progression and, therefore, could explain the results with WRAD and Y191F WDR5 mutant, we undertook further experiments.

Based on the shared and unique partners of the protein complexes they occur in, previous reports have classified MLL family members broadly into three categories, MLL (or MLL1)/MLL2, MLL3/MLL4 and SET1A/ SET1B (12). MLL and MLL2 more closely resemble the *Drosophila* Trithorax and can complex with Menin, a tumor suppressor protein (41). The other two, MLL3 and MLL4, occur complexed with Pax transactivation domain-interacting protein (PTIP), the nuclear receptor coactivator Ncoa6 and the H3K27 demethylase UTX (42,43). Human Set1A and Set1B most closely resemble the yeast Set1/COMPASS. Wdr82 is a unique component of Set1A/Set1B complex and does not interact with the MLL complexes (10). In order to decipher the functions of the SET1 family members, we used two different siRNAs to deplete Set1A, from the SET1A/SET1B group; and MLL3 from the MLL3/MLL4 group. Even though we have studied the effects of MLL from MLL/MLL2, we still choose MLL2 to confirm our findings. We also used previously published siRNA sequences to knock down Menin, PTIP and Wdr82 (10,41,44).

We assayed the Set1A, MLL2 and MLL3 siRNA-treated samples for BrdU incorporation (Figure 7A). Despite the different level of knockdown observed in the siRNA-treated samples (Supplementary Figure S4), loss of Set1A, MLL2 and MLL3 resulted in pronounced and almost similar loss in cell proliferation as observed by failure of BrdU uptake by siRNA-treated cells. In agreement with these results, RNA interference of the unique components of different MLL complexes, Wdr82, Menin and PTIP, also showed reduction in BrdU incorporation (Figure 7B). Our results indicate that all MLLs may have a redundant function in promoting S-phase progression. In contrast, when assayed for mitotic defects, only samples treated with Set1A siRNA displayed obvious phenotype, and not MLL2 or MLL3 siRNA-treated samples (Figure 7C). In fact, the counts for cells with mitotic defects were higher than those obtained for MLL or WRAD [compare Figure 7 (samples 2 and 3) with Figures 2B and 4C]. Similarly, knockdown of Set1A protein complex component WDR82 displayed considerable number of cells with mitotic defects, but not Menin or PTIP (Figure 7D). Together, our results suggest that while all MLL complexes play a role in regulating S-phase progression, only MLL and SET1A are the major protein complexes responsible for facilitating M-phase progression.

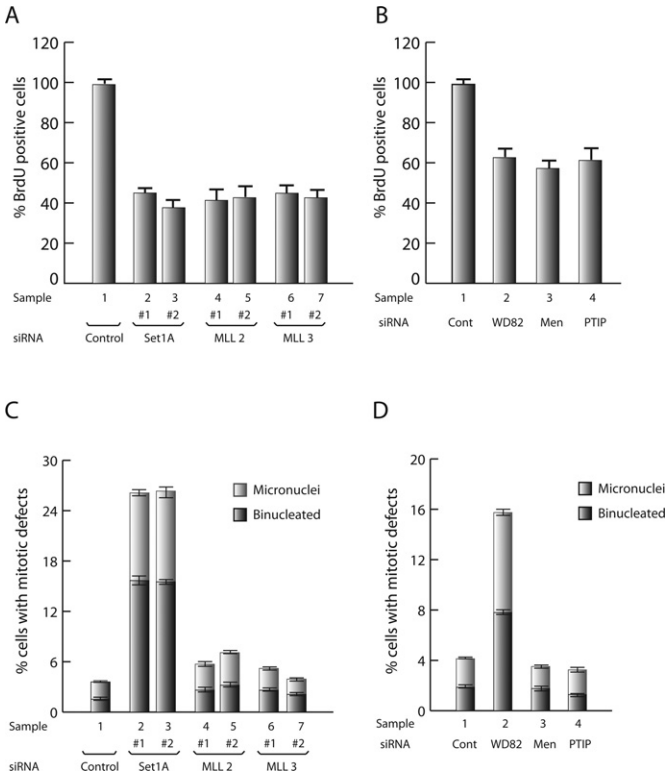

Figure 7. SET1 family regulates cell growth and mitosis. (**A**–**D**) Different members of SET1 family were knocked down using siRNA and BrdU incorporation assay (A-B) and mitotic defects analyses (C-D) were done in U2OS cells as indicated. (A,C) #1 and #2 denote two different siRNAs used. Cont, control; Men, Menin; Wd82, WDR82. Significant *P*-values (<0.01) were obtained with Student's *t*-test (C-D).

DISCUSSION

Here we have made use of RNAi to define the cell proliferation functions of MLL. Our results show that MLL regulates two different phases of cell cycle, namely, the S-phase and the M-phase progression. In M phase, MLL is critical not only for proper chromosome segregation, but also cytokinesis, as seen by appearance of micronuclei and

binucleated cells. These defects were specific to MLL depletion as expression of recombinant MLL could rescue both S-phase and the M-phase progression defects. All these activities of MLL are observed in various transformed as well as untransformed cell types indicating that functions of MLL encompass over a broad range. Our results also highlight the role of other MLL family members and the MLL core complex components—WRAD—in the progression of these phases.

MLL regulates S- and M-phase progression by two different activities

We show that loss of MLL results in growth arrest and MLL-depleted cells are unable to incorporate BrdU. We further analyzed the phase of arrested cells by staining with G2/M-specific H3S10 P antisera. Our results imply that majority of cells are arrested in G1 phase. Upon reconstitution of MLL expression by recombinant proteins, we show that the BrdU incorporation defect can be rescued by MLL_C subunit. We used specific deletions of TAD, FYRC and SET domain in MLL_C subunit to show that the S-phase progression is dependent on the transcriptional activity of TAD domain.

Previous studies including ours have indicated that MLL participates in the progression of S phase (22–24,26,30). MLL complexes with Host cell factor-1 (HCF-1) to interact with E2F1 and activate S-phase genes (23). MLL and MLL2 also interact directly with other E2Fs to affect passage to S phase (24). Moreover, ChIP analysis revealed that E2F-binding sequences were enriched in MLL-occupied regions in interphase cells (45). All these studies implied that the methyltransferase activity of MLL was responsible for S-phase progression. Our mutational analysis conclusively shows that it is the transactivation domain, which is the effector of MLL activity in S phase. In support of our observations, while H3K4 trimethylation was affected only on a small number of MLL-occupied genes in MLL null mouse embryonic fibroblasts (MEFs), the transcription of about 4700 genes was affected upon loss of MLL, indicating that the transcriptional activity and not the methyltransferase activity may be the major activity of MLL (9).

Our analysis of MLL siRNA-treated cells also revealed cells displaying micronuclei and binucleation. Both phenotypes are outcomes of an error-prone mitosis and lead to aneuploidy. The micronuclei also arise due to an erroneous DNA replication, but it is unlikely here. First, we do see an increase in number of mitotic cells in MLL-siRNA-treated samples. Second, others have suggested a role of MLL in mitosis where accumulation of mitotic cells and delay in mitotic progression was observed upon MLL knockdown (26,45). How does MLL regulate mitosis? MLL has been shown to bind DNA during mitosis where it is proposed to facilitate the inheritance of active gene transcription state during cell division by mitotic bookmarking (45). Curiously stable H3K4 methylation marks are present on the same chromatin. It has been suggested that MLL may be involved in non-enzymatic functions during mitosis and our mutational analysis supports this hypothesis. In our assays, the deletion of either TAD or SET domain did not induce mitotic defects indicating that these domains are not essential for function of MLL in mitosis. However, a point mutation in MLL 'Win' motif was sufficient to cause these defects to appear indicating that physical protein–protein interaction of MLL with WRAD is playing a role in mitosis. This result is supported by the analyses from corresponding mutation in WDR5, which bridges the interaction between MLL and remaining components of WRAD (see below). It has also been suggested that one role of MLL is to tether WRAD to its active site in mitosis and then 'hand it off' to Set1 complexes in G1 when they re-associate with DNA. However, this kind of passive function should not induce any defects in mitosis itself, but in reactivation of genes in G1 phase. It is also possible that MLL serves non-chromatin functions in mitosis. Consistent with the latter hypothesis, yeast Set1 has been shown to methylate a non-histone mitotic protein—Dam1—using the intact COMPASS machinery (46). Recently, MLL has been shown to self-methylate itself and Ash2L proving that such non-histone targets exist in higher organisms as well (47).

Role of WRAD in a SET-domain-independent function of MLL

Our studies revealed the requirement of common HMT core complex components in S- and M-phase progression. RNAi of all four proteins, namely, WDR5, RbBP5, Ash2L and Dpy30, resulted in cells deficient in BrdU incorporation. These findings are consistent with previous observations where different components of WRAD have been implicated in cell proliferation. For example, knockdown of Ash2L in different cell lines inhibited cell proliferation and BrdU incorporation (36). Similarly, loss of Dpy30 displayed acute proliferation defect and gene expression analysis indicated that genes involved in proliferation and cell cycle were affected (48). However, the observations made here do not seem to be replicated in embryonic stem cells (ESCs) as depletion of WDR5, RbBP5 or Dpy-30 does not show any remarkable change in cell proliferation or affect self-renewal capacity of ESCs (3,49). We also observed a previously unreported function of WRAD in mitotic progression. Like MLL, knockdown of WRAD components gave rise to cells displaying micronuclei or binucleation, indicating their role in multiple stages of mitosis.

To ensure that the effects observed on cell proliferation are due to the interaction of WDR5 with MLL, and not due to WDR5's interaction with other chromatin-modifying complexes (34,35), we made point mutations in residues of WDR5 that are known to interact with MLL (F133L and F263A) and RbBP5 (Q289A) in the MLL core complex. All the three WDR5 mutants were unable to rescue S- as well as M-phase progression. Further, in order to distinguish between interaction of WDR5 with MLL or H3, we made use of Y191F substitution in WDR5. WDR5 Y191F is capable of interacting with MLL, but not H3 (14,37). We expected that it would rescue both S- and M-phase progression. However, interaction of WDR5 Y191F with MLL was enough to largely rescue binucleation and micronuclei formation, but not S-phase progression. Here, it should be stated that WDR5 has been shown to bind the Win motif of other SET1 family members, mostly using same residues as those involved in interaction with MLL, though with

different equilibrium dissociation constant (16,50). While the residues of WDR5 that interact with MLL are well studied, the interactions of WDR5 with other MLL family members are not so well defined (16,37). The crystal structure suggests that Y191 has a role in WDR5's interactions with other MLL members (16,50). However, this is yet to be shown experimentally. Therefore, we cannot rule out the possibility that WDR5 acts with other SET1 family members (but not MLL) to regulate S-phase progression. WRAD has also been reported to have an intrinsic methyltransferase activity raising the possibility that it may not need any SET-domain protein to carry out its S-phase functions (13,39).

Till date the primary function of WRAD reported in relation to H3K4 HMTs, has been to participate in the enzymatic activity of SET-domain-dependent methylation of H3K4. Besides this, WRAD components have also been implicated in recruitment and stabilization of SET1 family complexes to their target sites on the chromatin in context of H3K4 methylation (34). Our studies reveal a novel function of WRAD, which is independent of the SET domain of MLL. Here protein–protein interactions of WDR5 with MLL, and by correlation other WRAD components, are essential and sufficient for mitotic progression. Why does MLL need WRAD for mitotic function? WRAD components, between themselves, contain many diverse domains e.g. WD40 repeats in WDR5 and RbBP5 for protein–protein interaction, and winged helix in Ash2L for DNA binding. Additionally, WDR5 can interact with chromatin modifications and long intergenic noncoding RNA (34,51,52). Our analyses show that MLL_C, which does not have many domains like MLL_N, is capable of rescuing M-phase progression. Therefore, it is likely that WRAD increases the interaction capability to MLL_C to carry out its mitotic functions.

Role of unique SET1 complex-associated members in cell cycle

As mentioned before, each of the SET1 complexes is also associated with unique members, which may further increase their functional diversity. We used the strategy employed by Wang *et al*. (9) and targeted Menin, PTIP or WDR82 mRNA to substantiate our RNAi experiments with MLL/MLL2, MLL3 and Set1A complexes, respectively. Pleasingly, our observations with the unique members were similar to those observed with their corresponding SET-domain protein (with the exception of Menin).

Consistent with our results in Set1A-knockdown samples, Wdr82-depleted samples showed defects in S- and M-phase progression. WDR82 is implicated in affecting the stability of the entire Set1A complex (10). Wdr82 also interacts with a number of proteins involved in mitosis like PP1 phosphatase complex and, Bod1 and Bod1L1, protein involved in chromosome segregation (35,53). Therefore, consistent with our findings, it is likely that WDR82 has a crucial role in mitosis either through a stable Set1A complex or by bringing in new partners to the Set1A complex. In contrast, similar to MLL3 knockdown, PTIP knockdown exhibited cells unable to incorporate BrdU but displayed no obvious mitotic defects. PTIP has been implicated in cell proliferation as MEFs from *PTIP* null mouse fail to proliferate in culture (54). PTIP mutants are also unable to progress through mitosis due to failure of chromosomes to condense early on, but this has been attributed to its role in DNA damage post-DNA replication and not segregation and cytokinesis processes being discussed here (54).

Menin has an essential role in the HMT activities of MLL (41). Our results show that Menin knockdown results in cell-cycle arrest, but not mitotic defects as seen with MLL knockdown. This suggests that while Menin is an essential component of the MLL HMT activity, it may not play a role in all the functions of MLL. Indeed, only about 20% of MLL complexes are found to contain Menin (35). Another member of HMT complexes—HCF-1—has been shown to regulate both G1- to S-phase transition and mitotic progression, particularly cytokinesis (55). HCF-1 has been identified associated with about 50% of MLL and Set1 complexes—both complexes, which regulate mitotic progression in our assays—but not MLL3/4 complexes. However, both HCF-1 and Menin associate with MLL_N subunit (41) and our results show that MLL_C is essential in mitotic functions of MLL. This fact, and our results with Menin, therefore, make it difficult to reconcile how they may contribute to MLL's functions in mitosis. However, it needs to be stated that formation of micronuclei and binucleation are results of aberrant segregation and cytokinesis, processes that occur late in mitosis. It is likely that MLL and other proteins may have a function early on in mitosis, but here, as we are only scoring for appearance of micronuclei or binucleation, we are unable to detect such early mitotic functions. Therefore, a more thorough analysis of MLL and its interacting partners is required to understand the full repertoire of the functions of these proteins in mitosis.

Common and unique roles of SET1 family members

There are six H3K4 HMTs in mammals. Although they have a common SET domain, they perform overlapping and unique functions (12). This is highlighted by the experiments in mice where deletion of many of these proteins, like MLL, MLL2 or MLL4, results in embryonic lethality (2,56–58). Nonetheless, here we show that all HMT complexes participate in cell proliferation. Our observations are consistent with previous studies where many of these proteins have been shown to give rise to growth arrest (57,59–62). Although we only show a G1-phase arrest for MLL-knockdown samples, we speculate that it will be the same phase for other members also. Our speculations are supported by the observations that many MLLs have been reported to interact with E2Fs, transcription factors responsible for G1- to S-phase transition (23,24). However, different MLLs may affect same or different genes sets required for cell proliferation. Our results also suggest that different MLLs may utilize WRAD differently to regulate cell proliferation.

We also analyzed the role of different MLLs in mitotic progression. Curiously, only MLL and Set1A knockdown resulted in cells displaying micronuclei and binucleation. Our contrasting observations with MLL and MLL2 in mitosis are curious but not unexpected. Although the two proteins have similar domain organization, the *MLL* and

MLL2 null mice are embryonic lethal, indicating that one cannot replace all functions of other protein (2,57,58). In our assay, MLL2 did not show any mitotic defects indicating that regulation of mitosis may be one of the functions where their roles do not overlap. In direct contrast, MLL and Set1A are dissimilar in structure and function. Structurally, Set1A has few structured conserved domains and these are similar to those present in yeast Set1 protein. In contrast, MLL has a number of domains, which can target it to DNA, interact with histone modifications and mediate protein–protein interactions. Because of its structure, MLL is thought to be closer to *Drosophila* trithorax. By virtue of their SET domain, both enzymes in association with WRAD can di and trimethylate histones. However, while MLL is thought to be responsible for targeting *Hox* genes in particular, Set1A is the primary methyltransferase in human cells. MLL has already been implicated in regulating mitosis. Indirect evidence for role of Set1 in mitosis has been mounting: (i) in yeast, Set1 has been shown to methylate a kinetochore protein Dam1; (ii) greater proportion of cells are in G2 phase in yeast *set1* mutants and finally (iii) mammalian Set1A complex has been shown to interact with Bod1 and Bod1L1, proteins that are important for proper chromosome segregation during mitosis (35,46,62). Despite the differences outlined above, our results show that both proteins participate in the same pathway to regulate mitosis, indicating that either both proteins can functionally compensate for any one of the processes outlined above or their common function is yet to be discovered.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Author contributions: A.A. performed all experiments except the ones mentioned below. S.N.V. performed experiments presented in SF 1B and made all constructs and mutants used in Figures 5 and 6. S.T. performed experiments presented in 1C, SF 3A and made all stable cell lines used in Figures 5 and 6. S.T. and A.A. designed and analyzed the experiments and wrote the manuscript. A.A. is working towards obtaining a PhD degree from Manipal University.

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