BRIEF REPORT

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The localization of histone H3K27me3 demethylase Jmjd3 is dynamically regulated

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Abbreviations: PTMs, posttranslational modifications; H3K27me3, Histone 3 lysine 27 trimethylation; Jmjd3, Jumonji-C domain containing protein 3; JmjC domain, Jumonji C domain; cNLS, classical nuclear localization signal; NES, nuclear export signal

Jmjd3 is required for cellular differentiation and senescence, and inhibits the induction of pluripotent stem cells by demethylating histone 3 lysine 27 trimethylation (H3K27me3). Although recent studies reveal crucial biological roles for Jmjd3, it is unclear how its demethylase activity is controlled. Here, we show that nuclear localization of Jmjd3 is required for effective demethylation of H3K27me3. Our subcellular localization analysis of Jmjd3 shows that the N-terminal region of the protein is responsible for its nuclear placement, whereas the C-terminal region harboring the catalytic Jumonji C (JmjC) domain cannot situate into the nucleus. We identify two classical nuclear localization signals (cNLSs) in the N-terminal domain of Jmjd3. Forced nuclear emplacement of the catalytic domain of Jmjd3 by fusion with a heterologous cNLS significantly enhances its H3K27me3 demethylation activity. A dynamic nucleocytoplasmic shuttling of endogenous Jmjd3 occurs in mouse embryonic fibroblasts. Jmjd3 is localized both into the cytoplasm and the nucleus, and its nuclear export is dependent on Exportin-1, as treatment with leptomycin B triggers nuclear accumulation of Jmjd3. These results suggest that the subcellular localization of Jmjd3 is dynamically regulated and has pivotal roles for H3K27me3 status.

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Introduction

Posttranslational modifications (PTMs) of histones are indispensable for several genomic functions, such as maintenance of genome stability and regulation of gene expression.[1,2] Mediated by the Polycomb Repressive Complex (PRC) complex 2, histone 3 lysine 27 trimethylation (H3K27me3) is one of the associative repressive PTMs and results in the formation of facultative heterochromatin.[3] H3K27me3 PTM is exemplified in female X-chromosome inactivation (XCI), a crucial epigenetic process that balances the X-linked dose disparity between XX females and XY males by transcriptionally silencing one of the two female X chromosomes. H3K27me3 decorates the entire silenced X chromosome.[4] During epigenetic reprogramming of female somatic cells into induced pluripotent stem cells (iPSCs), the inactivated X-chromosome is reactivated, as becomes evident by the erasure of the H3K27me3 PTM along this X.[5] The H3K27me3 mark is removed by histone demethylases after a variety of stimuli, such as induction of cellular senescence and differentiation to activate the expression of target genes. Jmjd3 (also known as Kdm6b) is a specific demethylase for H3K27me3 and contains a Jumonji C (JmjC) catalytic domain, which is broadly conserved among the histone demethylase protein family.[6-11] Several studies revealed that Jmjd3 has crucial roles in a variety of biological processes. Jmjd3 is required for the polarization of macrophages into mature M2-macrophages.[12,13] The overexpression of Jmjd3 in neural stem cells induces the expression of neurogenic genes, while the depletion of Jmjd3 causes a defect in the differentiation of ESCs to the neural lineage.[14,15] Jmjd3 is induced by oncogenic Ras signaling and arrests cell proliferation by increasing the expression levels of *Ink4a/Arf*, which inhibits cyclin-dependent kinases 4 and 6 (Cdk4/6) and stabilizes p53.[16,17] Interestingly, a recent study showed that Jmjd3 decreases the efficiency of the generation of iPSCs cells via *Ink4a/Arf*-dependent and -independent mechanisms.[18] These studies suggest that Jmjd3 functions as an epigenetic barrier for preventing tumorigenesis and ectopic transdifferentiation. Therefore, understanding the molecular controls that regulate Jmjd3 demethylase activity is crucial for the finding of novel treatments of cancer and the improvement of cellular engineering. However, the regulating mechanisms of Jmjd3 activity are fully unknown. This prompted us to elucidate the regulation of the nuclear localization of Jmjd3.

Nuclear import of macromolecules occurs via nuclear pore complexes (NPCs); in addition, a classical nuclear localization signal (cNLS), consisting of a basic amino-acids cluster, is required in many cases.[19] Importin-α and Importin-β mediate nuclear localization of cNLS-containing proteins.[20,21] Importin-α recognizes a target cNLS and binds to Importin-β, subsequently

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forming a ternary complex in the cytoplasm. Importin-β confers the ability to cross the NPCs by interacting with the proteins comprising the pore complex, the nucleoporins. Nuclear import and nuclear export are both crucial for the regulation of the subcellular localization of macromolecules. Exportin-1, also known as Crm-1, is one of the best-characterized nuclear export factors recognizing leucine- or isoleucine-rich nuclear export signals (NESs) on target proteins.[22-24] Here, we report the first identification of functional NLSs of Jmjd3 and the dynamics of subcellular localization of Jmjd3 protein in mouse embryonic fibroblasts via its Exportin-1 mediated nuclear export.

Results

Basic amino acid clusters within the N-terminus of Jmjd3 functions as nuclear localization signals

We cloned various Flag-tagged constructs of Jmjd3 (full-length [Jmjd3-FL], amino acids [aa] 1–1641; N-terminal [Jmjd3-N], aa 1–709; and C-terminal [Jmjd3-C], aa 672–1641 [**Fig. 1A**]) and transfected these constructs into human embryonic kidney (HEK) cells, followed by staining with anti-Flag antibodies. The expression of recombinant proteins was confirmed by western blot analysis (**Fig. 1B**). We were surprised to see that the localization of Jmjd3-C (the catalytic JmjC domain-containing protein) was restricted to the cytoplasm, while the Jmjd3-FL and Jmjd3-N constructs were predominantly localized into the nucleus (**Fig. 1C and D**). These results indicate that the N-terminal region is responsible for the nuclear localization of Jmjd3. Consistent with this, we identified two putative NLSs (a bipartite and a monopartite cNLS) within the N-terminal region of Jmjd3, and designated these NLS1 and NLS2, respectively (**Fig. 2A**). To test whether these sequences function as NLSs, we constructed single NLS1 or NLS2 Jmjd3-N mutants by substituting lysine 198 and arginine 199, or lysine 228 and arginine 229 residues with alanine, respectively. Wild type (WT) and NLS mutants (NLS-Mut) of Jmjd3-N were expressed into HEK cells and their localization was observed by anti-Flag immunostaining. As shown in **Figure 2B**, the NLS1 mutation significantly perturbs the nuclear placement of Jmjd3-N. In contrast, the NLS2 mutation has no significant effect for nuclear accumulation. To test whether NLS1 and NLS2 function redundantly, we also constructed a double Jmjd3 mutant for both NLS1 and NLS2 (NLS1+2 Mut). Interestingly, Jmjd3 NLS1+2 Mut did not accumulate in the nucleus (**Fig. 2B and C**), indicating that both NLSs (NLS1 and NLS2) functionally cooperate for the nuclear localization of Jmjd3. Taken together, NLS1 is sufficient for the nuclear localization of Jmjd3 and NLS2, albeit weaker, is also a functional NLS.

Forced nuclear localization of Jmjd3 enhances demethylation of H3K27me3

Next, we asked whether the localization of Jmjd3 would affect H3K27me3 demethylation efficiency. To target Jmjd3-C into the nucleus, we cloned Jmjd3-C with a cNLS derived from the Simian virus 40 (SV40) large T antigen (Jmjd3-C+SV40NLS), and transfected this construct into HEK cells. Comparable expression levels of Jmjd3-C and Jmjd3-C+SV40NLS were confirmed by western blot (**Fig. 3A**). As shown in **Figure 3B**, Jmjd3-C+SV40NLS was predominantly localized into the nucleus and more effectively demethylated H3K27me3 (arrows) than Jmjd3-C (arrowheads) (**Fig. 3B and C**; **Fig. S1B**). On average, the signal intensity of H3K27me3 of Jmjd3-C+SV40NLS transfectants was reduced about 30% as compared with control cells (**Fig. 3C**; **Fig. S1B**). In contrast, Jmjd3-C transfectants did not significantly alter their H3K27me3 levels (**Fig. S1A**). Individually, 18% of Jmjd3-C+SV40NLS-expressing cells and only 4% of Jmjd3-C-expressing cells showed a reduction in H3K27me3 signal of more that 50% of that of control cells (average of two independent experiments). We queried whether nuclear accumulation is also required for the demethylase activity of the other H3K27-specific demethylase, UTX (also known as Kdm6a).[7,8,10] The JmjC domain harboring the C-terminus of human UTX (aa 662–1402; shown as UTX-C) was cloned and transfected into HEK cells. As shown in **Figure 3E**, UTX-C shows exclusive cytoplasmic localization. In accordance with these results, we cannot observe a predicted cNLS within this fragment. We constructed an SV40NLS fusion for UTX-C and transfected into HEK cells. Similar to what was observed with Jmjd3, UTX-C+SV40NLS localized to the nucleus in all transfectants, resulting in increased demethylase activity (arrows) compared with both the control and UTX-C (**Fig. 3D–F**; **Fig. S2**). Collectively, these results indicate that nuclear accumulation of both Jmjd3 and UTX is required for effective demethylation of H3K27me3.

Dynamic regulation of endogenous Jmjd3 localization

To confirm the physiological relevance of the subcellular localization of Jmjd3, we investigated the localization of endogenous Jmjd3 in mouse embryonic fibroblasts (MEFs). As shown in **Figure 3G**, Jmjd3 shows heterogeneous distribution among cells. In some cells, Jmjd3 is predominantly localized into the nucleus (indicated as arrow in **Fig. 3G**) although uniform distribution is also observed in other individual cells (arrow head in **Fig. 3G**), suggesting that the subcellular localization of the endogenous Jmjd3 protein is dynamically regulated.

Exportin-1 mediates the nuclear export of Jmjd3

To further test the dynamics of Jmjd3 localization, we treated MEFs with mitomycin C (MMC), a small chemical that induces DNA double strand breaks and replicative senescence. Following MMC treatment we observed that the placement of Jmjd3 is altered with a reduction in the ratio of cells showing a nuclear accumulation. As shown in **Figure 4A and B**, only 4% of MMC-treated cells show nuclear enrichment of Jmjd3 (MMC+ETOH panel in **Fig. 4A and B**), which is nearly 4 times lower (17%) than in control cells (Con+EtOH panel in **Fig. 4A and B**). These results suggest that the mechanism(s) regulating the subcellular localization of Jmjd3 is mediated in response to extracellular signals or exogenous stresses.

Not only is nuclear import important, but nuclear export also contributes to the regulation of the nuclear localization of macromolecules. Exportin-1 is a well-known nuclear export factor that recognizes clusters of hydrophobic amino acids such as leucine and isoleucine.[22-24] To test whether Jmjd3 is exported from

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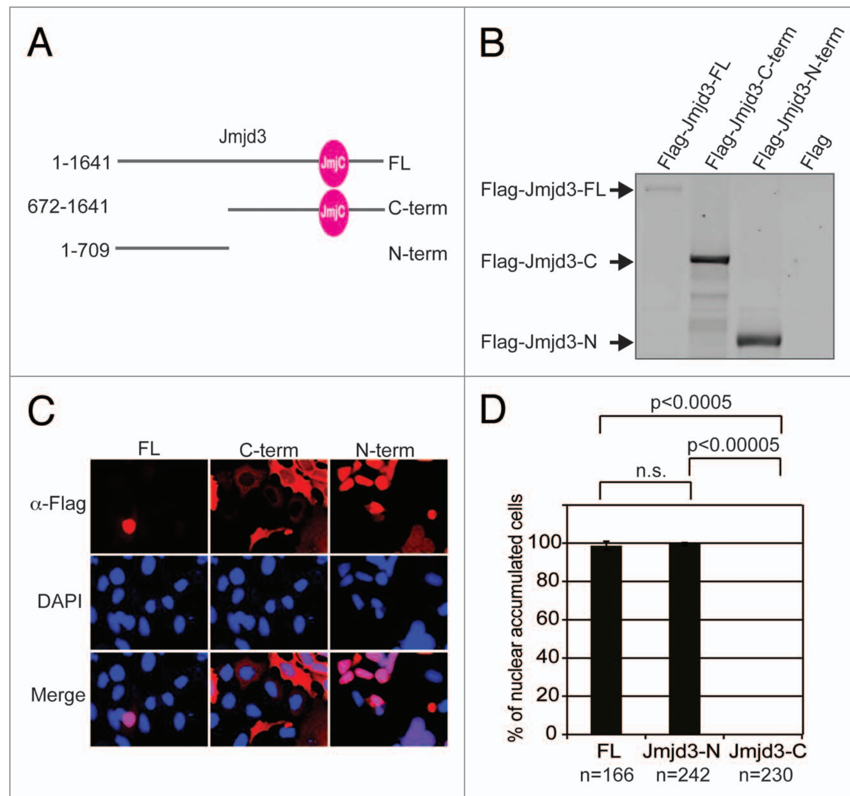

Figure 1. The N-terminal region is required for nuclear localization of Jmjd3 (**A**) Schematic presentation of Jmjd3 domain structure, including the catalytic JmjC domain. HEK cells were transfected with expression vectors for Flag-tagged full-length (FL), C-terminal domain (C-term), and N-terminal domain (N-term) of Jmjd3. (**B**) The expression of Jmjd3 full-length and deletion mutants was confirmed by western blot using anti-Flag antibodies. (**C**) The subcellular localization of these proteins was observed with immunofluorescence with anti-Flag antibodies (red). Cell nuclei were stained with DAPI (blue). (**D**) Quantification of the transfected cells showing the nuclear accumulation of Jmjd3 recombinant proteins. The values are shown as the mean of three independent experiments. n = total numbers of counted cells. Error bars equal one standard deviation from the mean. Statistics were performed using the Student's *t* test analysis.

the nucleus by Exportin-1, we treated MEFs with leptomycin B (LMB), a specific chemical inhibitor for Exportin-1-dependent nuclear export. LMB treatment alone resulted in an approximate 3-fold increase (47%) in the ratio of Jmjd3 nuclear accumulation (**Fig. 4A and B**, see the Con+LMB panel). Furthermore, cells treated with MMC followed by LMB (MMC+LMB panel) show an increased ratio of the nuclear accumulation of Jmjd3 as compared with cells treated with MMC alone (**Fig. 4A and B**, MMC+LMB panel). These data suggest that Exportin-1-mediated nuclear export is the major consequence of MMC-induced alteration of Jmjd3 localization, rather than the inhibition of nuclear import.

To evaluate the correlation between nuclear Jmjd3 amount and the level of H3K27me3, we next performed co-immunostaining of MEFs with anti-Jmjd3 and anti-H3K27me3 antibodies. As shown in **Figure 4C** (see left panels, Jmjd3 hi), some of the MEFs showed a negative correlation between the nuclear signals of Jmjd3 vs. H3K27me3 signals. In contrast, we also observed a positive correlation between nuclear Jmjd3 and H3K27me3 signals in a subset of these cells (**Fig. 4C**, see right panels, Jmjd3 lo). The nuclear signals of Jmjd3 and H3K27me3 were plotted from individual cells. We cannot conclude that nuclear Jmjd3 protein negatively correlates with H3K27me3 levels (**Fig. 4D**; **Fig. S3**). This may be due to the effects of other H3K27me3 regulators, such as the polycomb repressive complex 2 (PRC2) and the other H3K27 demethylase, UTX. Based on these findings, we propose a model in which Jmjd3 nuclear import and export (**Fig. 5**) are dynamically regulated.

Discussion

In this study, we show that Jmjd3 has two functional NLSs in its N-terminal region. These NLSs are typically bi-partite and mono-partite cNLSs, most often recognized by Importin-α. This suggests that the Importin-α/β pathway mediates the nuclear localization of Jmjd3. Our data also reveal that nuclear accumulation of Jmjd3 is indispensable for effective demethylation of H3K27me3. Therefore, nuclear transport of Jmjd3 is a critical step for erasing this PTM and regulating its downstream events, such as the induction of gene expression. Nuclear import by the Importin-α/β pathway is regulated by multiple mechanisms that operate in a context-dependent manner. It is well known that several PTMs of cargo molecules affect their interaction with Importin-α. In addition, cellular stresses, such as H_2O_2 treatment and heat shock, induce the nuclear accumulation of

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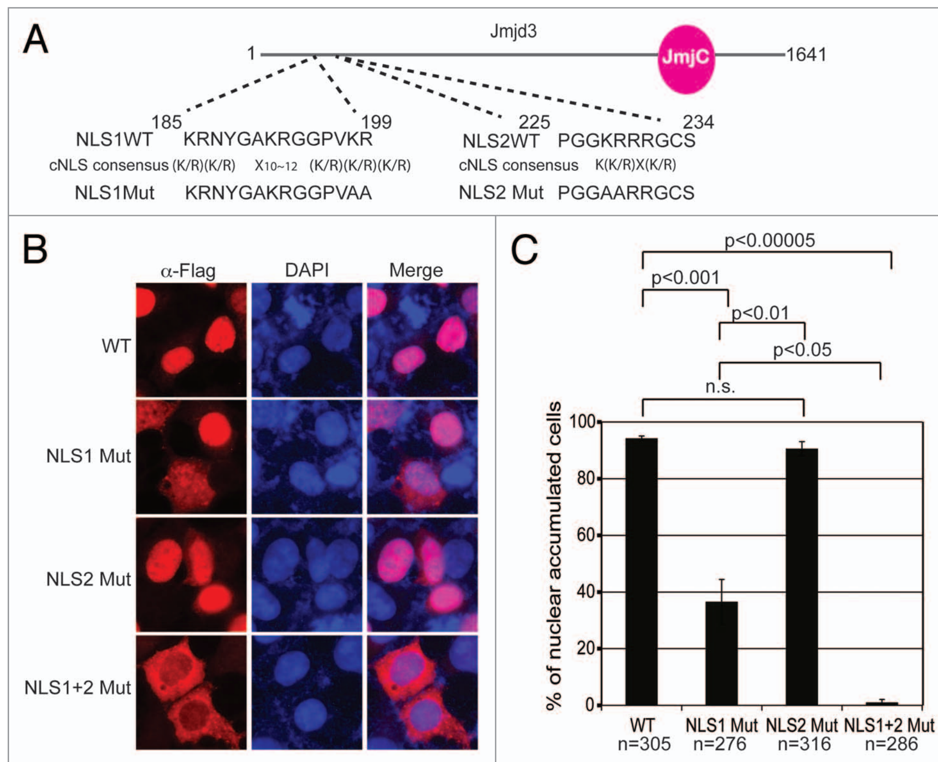

Figure 2. N-terminal cNLSs are responsible for nuclear localization of Jmjd3. (**A**) Predicted cNLSs within the N-terminal region of Jmjd3. NLS1 and/or NLS2 were mutated by site directed mutagenesis. (**B**) Localization of NLS mutants of Jmjd3. Wild type (WT), mutants of NLS1 (NLS1 Mut), NLS2 (NLS2 Mut), and both NLS1 and NLS2 (NLS1+2 Mut) were transfected into HEK cells. The localization of each protein was observed by anti-Flag antibodies (red). Cell nuclei were visualized with DAPI (blue). (**C**) Quantification of transfected cells showing nuclear accumulation of Jmjd3 recombinant proteins. Values are shown as mean of three independent experiments. n = total numbers of counted cells. Error bars represent one standard deviation from the mean. Statistics were performed using the Student's *t* test analysis.

Importin-α and perturb the nuclear transport via Importin-α/β.[25] Our data show that nuclear export is also critical for regulating Jmjd3 localization. Nuclear accumulation of Jmjd3 with LMB treatment suggests that Jmjd3 is exported from the nucleus by Exportin-1 and this nuclear export is enhanced by MMC treatment. Taken together, these results demonstrate that the subcellular localization of Jmjd3 is tightly regulated by a balance between nuclear import and nuclear export, although it is not yet clear how extracellular signals, such as MMC treatment, can modulate its dynamic localization. It has been shown that MMC treatment results in the increased expression of *p21*, an inhibitor of cyclin-dependent kinases Cdk2 and Cdk4.[26,27] It is possible that these Cdks enhance nuclear import of Jmjd3 and/or inhibit its nuclear export, not only after MMC treatment but also in a cell cycle-dependent manner. As shown in **Figure 4C and D**, we saw no clear negative correlation between the amount of nuclear Jmjd3 and H3K27me3 levels, presumably due to the fluctuation of other H3K27me3 regulators (PRC2 or UTX) in the individual cells. In addition, H3K27me3 levels at specific gene loci may be significantly reduced in Jmjd3-accumulating cells. Further research is required to understand the effects of the regulation of Jmjd3 subcellular localization on epigenetic homeostasis and reprogramming.

Material and Methods

Cell culture and transfection

Human embryonic kidney (HEK) cells and primary mouse embryonic fibroblasts (MEFs) derived from embryonic day 13.5 embryos were maintained in DMEM supplemented with 10% Fetal Bovine Serum, 2 mM glutamine, and penicillin/streptomycin. Transfection of all the expression vectors was performed using Lipofectamine 2000 (Life Technologies) per manufacture's recommendation. The transfectants were subjected to western blot or immunostaining 24 h after transfection. 5×10^4 MEFs were plated on a 12-well tissue culture dish with coverslips and cultured overnight. MEFs were then treated with 10 μg/ml of mitomycin C (MMC) (Sigma-Aldrich) or mock-treated (Control) for 2 h. The cells were washed twice with phosphate buffered saline (PBS) and cultured for 24 h in normal media conditions as described above. Either leptomycin B (LMB) (Sigma-Aldrich) or vehicle (ethanol) was added at 10 nM and the cells were cultured an additional 2 h.

Plasmids and antibodies

Full-length and deletion mutants of Jmjd3 and UTX were amplified by PCR using as templates the constructs deposited to Addgene by Dr. Kai Ge's laboratory: pCS2-Jmjd3 (plasmid #17440) and pCS2-UTX (plasmid #17438).[10] Amplified fragments were cloned into pcDNA3.1 (Life Technologies)

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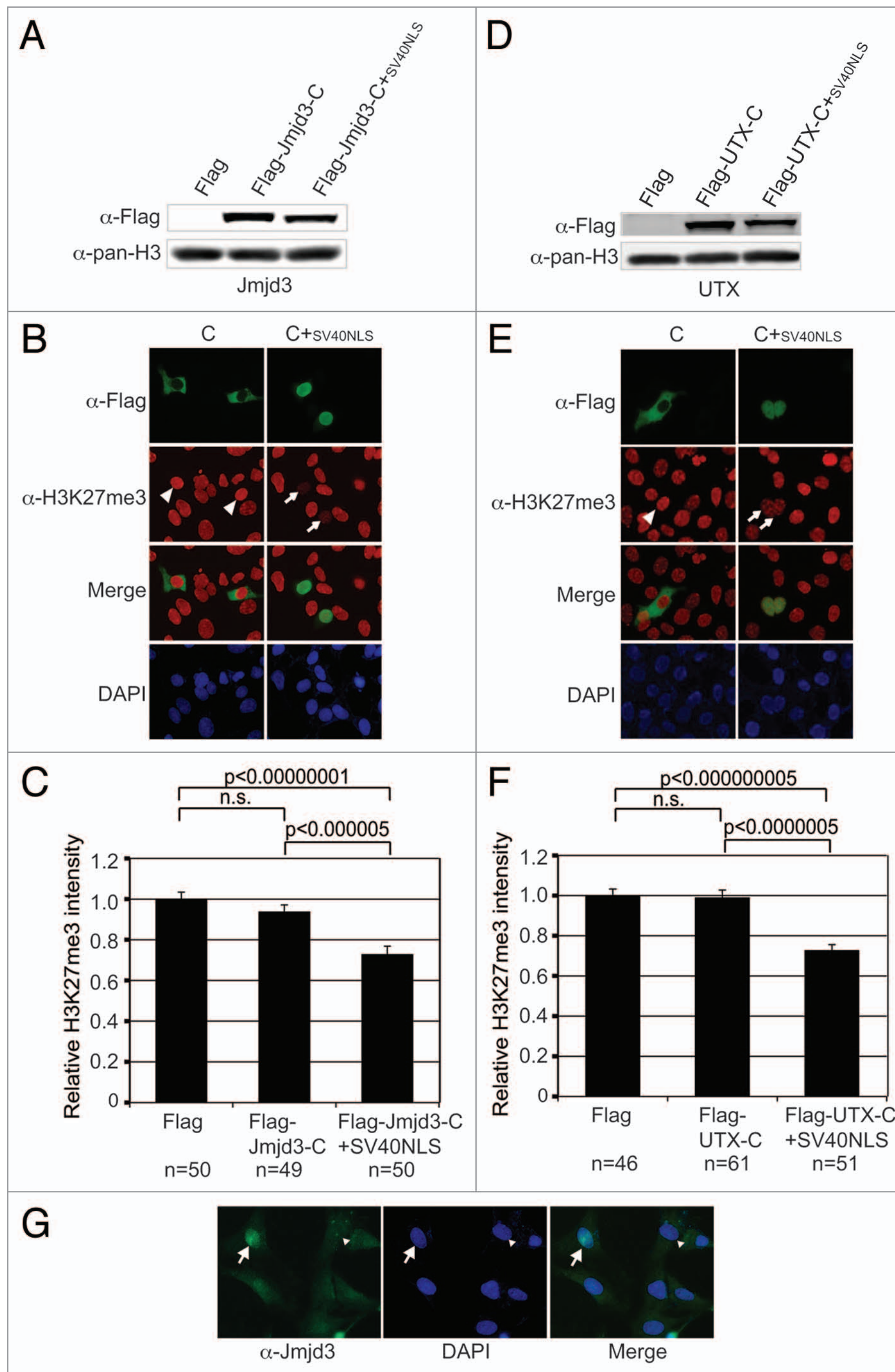

Figure 3. Nuclear accumulation of Jmjd3 is required for effective demethylation of H3K27me3. SV40NLS was fused with C-terminal domain of Jmjd3 (Jmjd3-C+SV40NLS). HEK cells were transfected with Flag-tagged Jmjd3-C+SV40NLS or Jmjd3-C. (**A**) Anti-Flag western blot confirms the expression of these proteins. Pan-histone H3 levels were used a protein loading control. (**B**) Localization of Jmjd3-C or Jmjd3-C+SV40NLS and level of H3K27me3 were determined by immunofluorescence using anti-Flag antibodies (green) and anti-H3K27me3 antibody (red). Cell nuclei were stained with DAPI (blue). Arrowheads show nuclear H3K27me3 levels in the Jmjd3-C transfectants, whereas arrows show H3K27me3 levels in the Jmjd3-C+SV40NLS transfectants. (**C**) The signal intensity of H3K27me3 in Jmjd3-C or Jmjd3-C+SV40NLS transfected cells was quantified. Values represent the mean of individual transfectants. n = total numbers of counted cells. The error bars represent one standard error. Statistics were performed using the Student's *t* test analysis. (**D**) Expression of Flag-UTX-C and Flag-UTX-C+SV40NLS was confirmed by anti-Flag western blot. Pan-histone H3 levels were used as a protein loading control. (**E**) Localization of UTX-C or UTX-C+SV40NLS and level of H3K27me3 were determined by immunofluorescence using anti-Flag antibodies (green) and anti-H3K27me3 antibodies (red). Cell nuclei were stained with DAPI (blue). Arrowheads show nuclear H3K27me3 levels in the UTX-C transfectants, whereas arrows show H3K27me3 levels in the UTX-C+SV40NLS transfectants. (**F**) The signal intensity of H3K27me3 in UTX-C or UTX-C+SV40NLS transfected cells was quantified. The values represent the mean of the individual transfectants. n = total number of counted cells. Error bars represent one standard error from mean. Statistics were performed using the Student's *t* test analysis. (**G**) Heterogeneous subcellular localization of Jmjd3 in MEFs. MEFs were stained with anti-Jmjd3 antibodies (green) and DAPI (blue). Arrow and arrowhead show cells in which Jmjd3 accumulated in the nucleus and was distributed between cytoplasm and nucleus, respectively.

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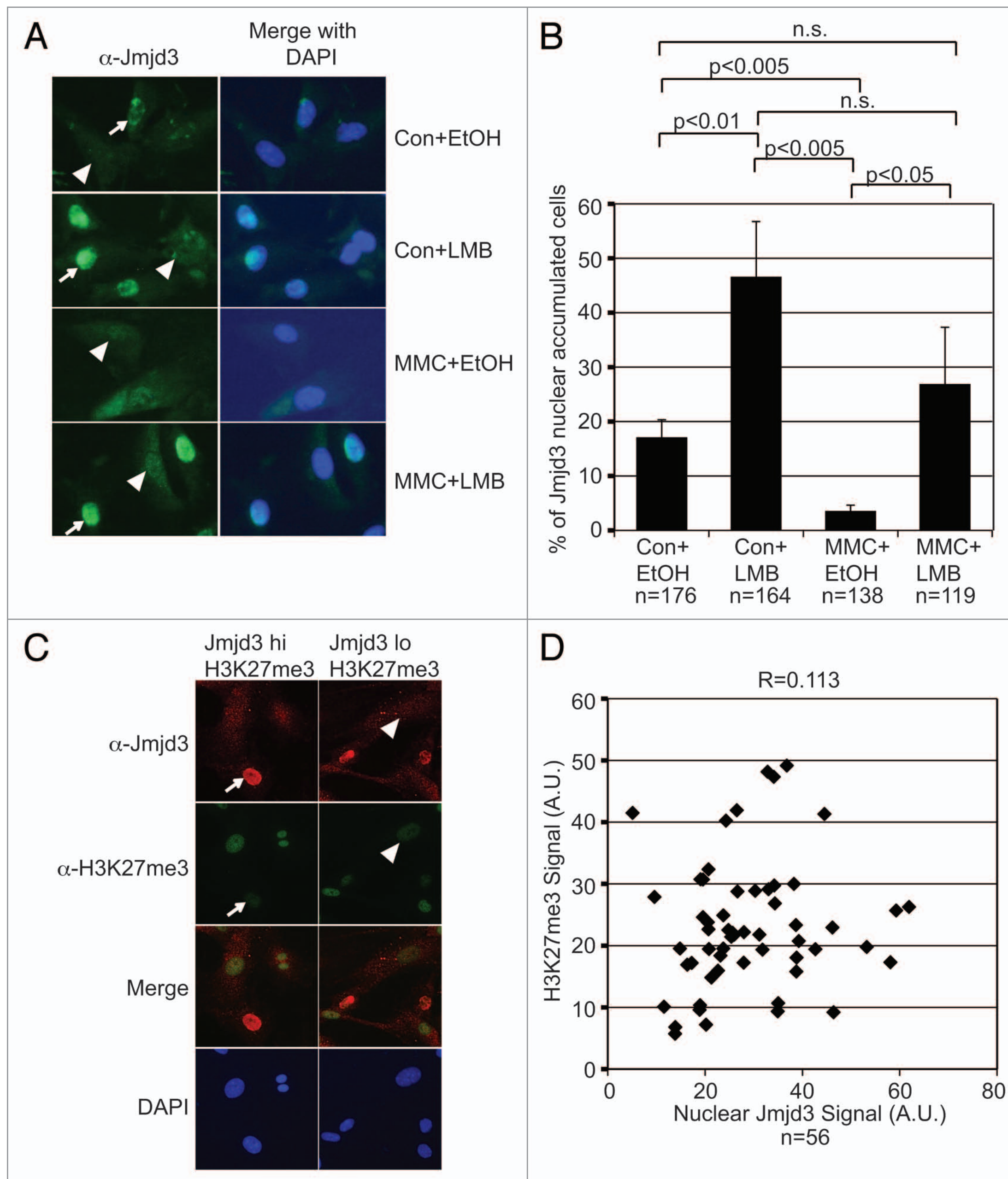

Figure 4. Dynamic regulation of subcellular localization of endogenous Jmjd3. MEFs were treated with 10 μg/ml of mitomycin C (MMC) or mock control for 2 h. Twenty-four hours after MMC treatment, leptomycin B (LMB) or equivalent volume of ethanol (EtOH) vehicle was added to the culture medium and the cells were cultured an additional 2 h. (**A**) Cells were then subjected to immunofluorescence using anti-Jmjd3 antibodies (green) and nuclei were stained with DAPI (blue). Arrows show the cells where Jmjd3 is accumulated in the nucleus. The arrowhead shows a representative cell where Jmjd3 is not accumulated in the nucleus. (**B**) Percentages of cells in which Jmjd3 is accumulated in the nucleus . The values are shown as the mean of three independent experiments. n = the total number of counted cells for each experimental condition. Error bars represent one standard deviation. Statistics were performed using the Student's *t* test analysis. (**C**) Co-immunostaining of Jmjd3 and H3K27me3 in MEFs. Non-treated MEFs were stained with anti-Jmjd3 antibodies (red), anti-H3K27me3 antibodies (green), and DAPI (blue). Left panels Jmjd3 hi shows the negative correlation between Jmjd3 and H3K27me3 signals (indicated with an arrow); Right panels Jmjd3 lo show the positive correlation between Jmjd3 and H3K27me3 signals (indicated with an arrowhead). (**D**) Plotted signals of nuclear Jmjd3 and H3K27me3 in individual cells. n= the total numbers of counted cells; A.U., arbitrary unit of fluorescence; R, correlation coefficient.

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containing an N-terminal triple Flag-tag (a gift from Ramnik Xavier). To mutate NLS1 (lysine 198 and arginine 199) and/or NLS2 (lysine 228 and arginine 229) into alanine, site-directed mutagenesis was performed using Quick-change site directed mutagenesis kit (Agilent). The sequence for NLS of SV40 large T antigen was inserted by using annealed oligonucleotide. The sequences of the oligonucleotides are shown in **Table S1**. Anti-Flag M2 mouse (Sigma-Aldrich), anti-Jmjd3 rabbit (GeneTex),

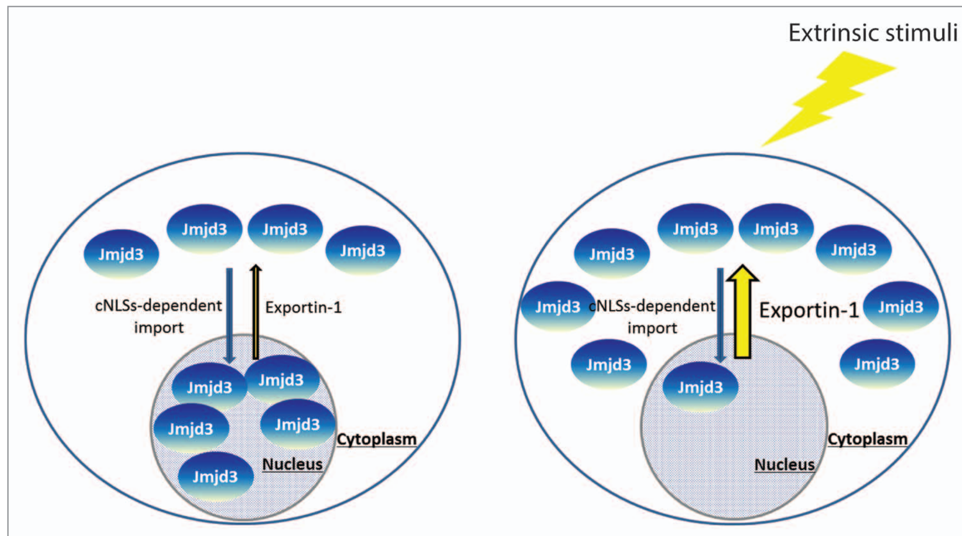

Figure 5. Dynamic regulation of subcellular localization of Jmjd3. A model for the regulation of nuclear import and nuclear export of Jmjd3. Left: in non-stimulated cells, Jmjd3 is localized to both cytoplasm and nucleus. Nuclear export and import of Jmjd3 is mediated by Exportin-1 and presumably mediated by Importin-α/β, respectively. Right: Exportin-1 mediated nuclear export of Jmjd3 is enhanced by extrinsic stimuli such as MMC treatment. This results in a reduction of nuclear Jmjd3 protein.

anti-pan-H3 rabbit (Abcam), and anti-trimethylated-H3K27 rabbit antibodies (Millipore) were used for western blot and immunostaining. For co-immunostaining with Jmjd3 and H3K27me3, anti-H3K27me3 mouse antibodies (Active Motif) were used together with anti-Jmjd3 antibodies.

Immunofluorescence

The cells were fixed with 3.7% formaldehyde for 15 min at R.T. After permeabilization with 0.5% Triton X-100, the cells were blocked with 3% skim milk, and then incubated with primary antibody. Alexa-488 or Alexa-546 labeled antibodies (Life Technologies) were used as secondary antibodies. All the images were captured using an ECLIPSE 80i microscope with a DXm1200F CCD camera and AT-1 software (Nikon). The acquired images were analyzed with Image J (NIH, http://imagej.nih.gov/ik/).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
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References

1. Masumoto H, Hawke D, Kobayashi R, Verreault A. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. Nature 2005; 436:294-8; PMID:16015338; http://dx.doi.org/10.1038/nature03714
2. Turner BM. Cellular memory and the histone code. Cell 2002; 111:285-91; PMID:12419240; http://dx.doi.org/10.1016/S0092-8674(02)01080-2
3. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature 2011; 469:343-9; PMID:21248841; http://dx.doi.org/10.1038/nature09784
4. Lee JT, Bartolomei MS. X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell 2013; 152:1308-23; PMID:23498939; http://dx.doi.org/10.1016/j.cell.2013.02.016
5. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 2007; 1:55-70; PMID:18371336; http://dx.doi.org/10.1016/j.stem.2007.05.014
6. De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell 2007; 130:1083-94; PMID:17825402; http://dx.doi.org/10.1016/j.cell.2007.08.019
7. Lan F, Bayliss PE, Rinn JL, Whetstine JR, Wang JK, Chen S, Iwase S, Alpatov R, Issaeva I, Canaani E, et al. A histone H3 lysine 27 demethylase regulates animal posterior development. Nature 2007; 449:689-94; PMID:17851529; http://dx.doi.org/10.1038/nature06192
8. Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 2007; 449:731-4; PMID:17713478; http://dx.doi.org/10.1038/nature06145
9. Xiang Y, Zhu Z, Han G, Lin H, Xu L, Chen CD. JMJD3 is a histone H3K27 demethylase. Cell Res 2007; 17:850-7; PMID:17923864; http://dx.doi.org/10.1038/cr.2007.83
10. Hong S, Cho YW, Yu LR, Yu H, Veenstra TD, Ge K. Identification of JmjC domain-containing UTX and JMJD3 as histone H3 lysine 27 demethylases. Proc Natl Acad Sci U S A 2007; 104:18439-44; PMID:18003914; http://dx.doi.org/10.1073/pnas.0707292104
11. Shi Y, Whetstine JR. Dynamic regulation of histone lysine methylation by demethylases. Mol Cell 2007; 25:1-14; PMID:17218267; http://dx.doi.org/10.1016/j.molcel.2006.12.010
12. Ishii M, Wen H, Corsa CA, Liu T, Coelho AL, Allen RM, Carson WF 4th, Cavassani KA, Li X, Lukacs NW, et al. Epigenetic regulation of the alternatively activated macrophage phenotype. Blood 2009; 114:3244-54; PMID:19567879; http://dx.doi.org/10.1182/blood-2009-04-217620
13. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nat Immunol 2010; 11:936-44; PMID:20729857; http://dx.doi.org/10.1038/ni.1920

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14. Jepsen K, Solum D, Zhou T, McEvilly RJ, Kim HJ, Glass CK, Hermanson O, Rosenfeld MG. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature 2007; 450:415-9; PMID:17928865; http://dx.doi.org/10.1038/nature06270
15. Burgold T, Spreafico F, De Santa F, Totaro MG, Prosperini E, Natoli G, Testa G. The histone H3 lysine 27-specific demethylase Jmjd3 is required for neural commitment. PLoS One 2008; 3:e3034; PMID:18716661; http://dx.doi.org/10.1371/journal.pone.0003034
16. Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J, Helin K. The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. Genes Dev 2009; 23:1171-6; PMID:19451217; http://dx.doi.org/10.1101/gad.510809
17. Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenführ M, Maertens G, Banck M, Zhou MM, Walsh MJ, et al. Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. Genes Dev 2009; 23:1177-82; PMID:19451218; http://dx.doi.org/10.1101/gad.511109
18. Zhao W, Li Q, Ayers S, Gu Y, Shi Z, Zhu Q, Chen Y, Wang HY, Wang RF. Jmjd3 inhibits reprogramming by upregulating expression of INK4a/Arf and targeting PHF20 for ubiquitination. Cell 2013; 152:1037-50; PMID:23452852; http://dx.doi.org/10.1016/j.cell.2013.02.006
19. Lange A, Mills RE, Lange CJ, Stewart M, Devine SE, Corbett AH. Classical nuclear localization signals: definition, function, and interaction with importin alpha. J Biol Chem 2007; 282:5101-5; PMID:17170104; http://dx.doi.org/10.1074/jbc.R600026200
20. Görlich D, Prehn S, Laskey RA, Hartmann E. Isolation of a protein that is essential for the first step of nuclear protein import. Cell 1994; 79:767-78; PMID:8001116; http://dx.doi.org/10.1016/0092-8674(94)90067-1
21. Imamoto N, Shimamoto T, Kose S, Takao T, Tachibana T, Matsubae M, Sekimoto T, Shimonishi Y, Yoneda Y. The nuclear pore-targeting complex binds to nuclear pores after association with a karyophile. FEBS Lett 1995; 368:415-9; PMID:7635189; http://dx.doi.org/10.1016/0014-5793(95)00699-A
22. Fornerod M, Ohno M, Yoshida M, Mattaj IW. CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 1997; 90:1051-60; PMID:9323133; http://dx.doi.org/10.1016/S0092-8674(00)80371-2
23. Ossareh-Nazari B, Bachelerie F, Dargemont C. Evidence for a role of CRM1 in signal-mediated nuclear protein export. Science 1997; 278:141-4; PMID:9311922; http://dx.doi.org/10.1126/science.278.5335.141
24. Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, Yanagida M, Nishida E. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. Nature 1997; 390:308-11; PMID:9384386; http://dx.doi.org/10.1038/36894
25. Miyamoto Y, Saiwaki T, Yamashita J, Yasuda Y, Kotera I, Shibata S, Shigeta M, Hiraoka Y, Haraguchi T, Yoneda Y. Cellular stresses induce the nuclear accumulation of importin alpha and cause a conventional nuclear import block. J Cell Biol 2004; 165:617-23; PMID:15184398; http://dx.doi.org/10.1083/jcb.200312008
26. Ohtsuka T, Jensen MR, Kim HG, Kim KT, Lee SW. The negative role of cyclin G in ATM-dependent p53 activation. Oncogene 2004; 23:5405-8; PMID:15077171; http://dx.doi.org/10.1038/sj.onc.1207693
27. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 1993; 75:805-16; PMID:8242751; http://dx.doi.org/10.1016/0092-8674(93)90499-G

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