Differential Sensitivity of v-Myb and c-Myb to Wnt-1-induced Protein Degradation

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1The abbreviations used are: AMV, avian myeloblastosis virus; CBP, cAMP responsive element binding protein (CREB) binding protein; c-Myb, c-myb proto-oncogene product; DBD, DNA-binding domain; HIPK2, Homeodomain-interacting protein kinase 2; NLK, Nemo-like kinase; NRD, negative regulatory domain; TAK1, TGF-β-
activated kinase 1; v-Myb, viral myb gene product.
ABSTRACT

Recently we have shown that the c-myb proto-oncogene product (c-Myb) is degraded in response to Wnt-1 signaling via the pathway involving TAK1 (TGF-β-activated kinase), HIPK2 (Homeodomain-interacting protein kinase 2), and NLK (Nemo-like kinase). NLK and HIPK2 bind directly to c-Myb, which results in the phosphorylation of c-Myb at multiple sites, followed by its ubiquitination and proteasome-dependent degradation. The v-myb gene carried by avian myeloblastosis virus (AMV) has a transforming capacity, but the c-myb proto-oncogene does not. Here, we report that two characteristics of v-Myb make it relatively resistant to Wnt-1-induced protein degradation. First, HIPK2 binds with a lower affinity to the DNA-binding domain (DBD) of v-Myb than to that of c-Myb. The mutations of three hydrophobic amino acids on the surface of the DBD in v-Myb decrease the affinity to HIPK2. Secondly, a loss of multiple NLK phosphorylation sites by truncation of the C-terminal region of c-Myb increases its stability. Among fifteen putative NLK phosphorylation sites in mouse c-Myb, the phosphorylation sites in the C-terminal region are more critical than other sites for Wnt-1 induced protein degradation. The relative resistance of v-Myb to Wnt-1-induced degradation may explain, at least in part, the differential transforming capacity of v-Myb versus c-Myb.
INTRODUCTION

The \textit{v-myb} oncogene, which is carried by the avian myeloblastosis virus (AMV), and causes acute myeloblastic leukemia, is derived from the \textit{c-myb} proto-oncogene (1). Studies of \textit{c-myb}-deficient mice showed that \textit{c-myb} is essential for the proliferation of immature hematopoietic cells and early T cell development (2, 3). The \textit{c-myb} gene product (c-Myb) is a transcriptional activator that recognizes a specific DNA sequence (4-7). Multiple c-Myb target genes, including \textit{c-myc}, have been identified, and they are involved in cell cycle control, lineage commitment during differentiation, and blockage of apoptosis (8-12). Three functional domains were identified in c-Myb that are responsible for DNA binding, transcriptional activation, and negative regulation (5). The DNA-binding domain (DBD) in the N-terminal region of c-Myb consists of three imperfect tandem repeats of 51-52 amino acids, each containing a helix-turn-helix variation motif (13). The transcriptional coactivator cAMP responsive element binding protein (CREB) binding protein (CBP) binds to the transcriptional activation domain to mediate trans-activation (14).

The \textit{v-myb} gene product (v-Myb) encoded by AMV is an amino (N)- and carboxy (C)-terminally truncated version of chicken c-Myb (1) and has a strong transformation activity in hematopoietic cells, whereas c-Myb has not. This difference is at least partly due to a deletion of the negative regulatory domain (NRD) located in the C-terminal portion of the molecule (15-17). The NRD appears to contain multiple
subdomains, and the deletion of any of these increases both the trans-activation and transformation capacity of c-Myb (18, 19). The v-Myb encoded by AMV lacks the C-proximal region of the NRD. Mutations of the leucine-rich region in the NRD are sufficient for oncogenic activation of c-myb. Although the mechanism by which c-Myb is regulated by its NRD has not been completely clarified, several possibilities have been suggested. First, deletion or mutations of the NRD reduces the affinity for corepressors, leading to increased c-Myb activity (20, 21). Two corepressors, BS69 and TIF1β, bind directly to the NRD and negatively regulate c-Myb-mediated trans-activation. Since other multiple corepressors, including Ski, N-CoR, and mSin3A, also bind to the DBD together with TIF1β, a deletion of NRD also reduces interactions with these corepressors. Further, three point mutations in the DBD of v-Myb reduce the affinity for these corepressors. Second, the C-truncated form of c-Myb is more resistant to proteasome-dependent protein degradation compared to c-Myb (22). However, the mechanism by which c-Myb is stabilized by its C-terminal truncation remains unknown.

Recently we have shown that c-Myb is phosphorylated and degraded via the Wnt-1 signaling pathway involving TAK1 (TGF-β-activated kinase), HIPK2 (Homeodomain-interacting protein kinase 2), and NLK (Nemo-like kinase) (23). NLK and HIPK2 bind directly to c-Myb, resulting in its phosphorylation at multiple sites, followed by its ubiquitination and proteasome-dependent degradation. Since Wnt
signaling controls differentiation or apoptosis in many cell types, including hematopoietic cells (24, 25), the Wnt-induced c-Myb degradation may play some role in the proliferation and differentiation of hematopoietic cells. Here, we report that v-Myb is relatively resistant to Wnt-1-induced protein degradation.

MATERIALS AND METHODS

**Plasmids** --- The chicken cytoplasmic β-actin promoter was used to express various forms of Myb in CV-1 cells. N- and C-terminally truncated forms of Myb (NT2-V, V-CTV, and NT2-CTV) with FLAG tag at their C-termini were expressed from the chicken cytoplasmic β-actin promoter. The pact-v-Myb-FLAG plasmid to express v-Myb-FLAG, in which the FLAG tag is linked to the C-terminus of v-Myb, was constructed. Plasmids to express various components of the Wnt-1-TAK1-HIPK2-NLK pathway have been described previously (23).

**Western Blotting** --- CV-1 cells were transfected with a mixture of the c-Myb expression plasmid (6 µg), various amounts of the plasmids that express components of the Wnt-1-TAK1-HIPK2-NLK pathway (amounts shown in Figure legends), and the internal control plasmid pact-β-gal (0.3 µg). Total plasmid amounts were adjusted to 8.3 µg by adding empty plasmid. Forty-hours after transfection, cells were lysed in SDS sample buffer with mild sonication and subjected to SDS-PAGE followed by Western blotting and detection by ECL (Amersham Biosciences, Piscataway, NJ).
detect c-Myb, the anti-c-Myb monoclonal antibody 1-1, or the rabbit polyclonal antibody, which was raised against the GST-CT5 fusion protein, were used. To detect the N- and C-terminally truncated forms of Myb including v-Myb, the anti-FLAG antibody (M2, Sigma, St. Louis, MO) was used. The transfection efficiency was determined by measuring β-galactosidase activity in aliquots of cells and the amounts of lysate used for Western blotting were normalized based on the β-galactosidase activity.

**CAT Reporter Assays** --- Using the CaPO₄ method, CV-1 cells (4 x 10⁵ cells per 100 mm dish) were transfected with a mixture of the pc-myc-CAT reporter containing the human c-myc promoter (8) (4 µg), plasmids expressing various forms of Myb (2 µg), plasmids expressing various components of the Wnt-1-TAK1-HIPK2-NLK pathway (amounts shown in Figure legends), and the internal control plasmid pact-β-gal (0.3 µg). Forty hours after transfection, CAT assays were performed.

**GST Pull-down Assays and Yeast Two-hybrid Assay** --- GST pull-down assays were performed as described (23). To increase the solubility of GST fusion proteins expressed in bacteria, the thioredoxin coexpression system (26) was used. The binding buffer consisted of 20 mM HEPES (pH 7.5), 1 mM DTT, 0.1% NP-40, and 150 mM (for the interaction between Myb and HIPK2). Yeast two-hybrid assays were performed as described (14).
RESULTS

v-Myb Is Relatively Resistant to Wnt-1-induced Protein Degradation --- As we reported recently (23), coexpression of Wnt-1 and mouse c-Myb led to the degradation of c-Myb (Fig. 1A, upper left). In contrast, v-Myb encoded by AMV was not degraded by Wnt-1 coexpression (Fig. 1A, lower left). AMV v-Myb contains an 11 amino acid fragment derived from the Gag gene of AMV at its N-terminus. We confirmed that this small Gag protein fragment does not affect Wnt-1-regulated protein stability (data not shown). In addition, species differences cannot explain the differential degradation, because both mouse and chicken c-Myb were degraded by coexpression with NLK (Fig. 1A, upper right). Although v-Myb degradation by Wnt-1 was negligible, overexpression of NLK induced a partial degradation of v-Myb (Fig. 1A, lower right). In the case of Wnt-1 expression, the signal from overexpressed Wnt-1 may be mediated by endogenous NLK, whereas in the case of overexpression of NLK, the kinase may interact directly with Myb to induce its degradation.

As reported recently (23), the c-Myb-induced trans-activation of the c-myc promoter was inhibited by c-Myb coexpression with either Wnt-1 or NLK, because overexpression of either component of the Wnt-1-NLK pathway induces the degradation of c-Myb (Fig. 1B, left). In contrast, the activation of c-myc promoter by the AMV-encoded v-Myb, was not inhibited by Wnt-1, rat Frizzled 1 (R-Fz1), or TAK1+TAB1 (Fig. 1B, right). Although coexpression of HIPK2 or NLK partially
inhibited v-Myb-dependent trans-activation of the c-myc promoter, the degree of inhibition was much weaker than seen with c-Myb.

Partial inhibition of v-Myb activity by HIPK2 or NLK could be due to partial degradation of v-Myb, or mechanisms other than protein degradation may contribute to this inhibition. The kinase activity of NLK is needed to induce the degradation of c-Myb (23), but we observed that overexpression of kinase-negative NLK also partly inhibited c-Myb activity (data not shown). We have observed that binding of in vitro-translated c-Myb to a GST-CBP resin was inhibited by the kinase-negative NLK (Fig. 2). Similar results were also obtained by using v-Myb (Fig. 2). This suggests that overexpressed NLK blocks the interaction between CBP and c-Myb or v-Myb by directly binding to c-Myb or v-Myb, which leads to inhibit trans-activation.

**Truncation of c-Myb Weakens Wnt-1-induced Negative Regulation** --- In addition to the N- and C-terminal truncation, AMV v-Myb is different from mouse c-Myb at multiple residues due to species specific differences as well as mutations which have occurred during viral replication. To determine which regions of v-Myb are responsible for resistance to Wnt1-induced protein degradation, we generated two chimeric proteins between of c-Myb and v-Myb (Fig. 3A). NT2-V was generated by replacement of the N-terminal half of v-Myb, which contains the DBD, with the corresponding fragment of mouse c-Myb. V-CTV was made by replacement of the C-
terminal half of v-Myb, which contains the transcriptional activation domain and a part of the NRD, with the corresponding fragment of mouse c-Myb. Both chimeric proteins were only partially degraded by coexpression of Wnt-1 (Fig. 3B). In addition, NT2-CTV, which was generated by truncation of both N- and C-terminal regions of c-Myb, was also apparently resistant to Wnt-1-induced degradation (Fig. 3B). Consistent with these results, Wnt-1 did not inhibit trans-activation of the c-myc promoter mediated by NT2-V, V-CTV, or NT2-CTV (Fig. 3C). Thus, truncation of either the N- or C-terminal region of c-Myb confers resistance to Wnt-1-induced protein degradation.

**Point Mutations in the DBD of v-Myb Weaken Wnt-1-induced Negative Regulation**

In addition to the truncation, we also found that point mutations in v-Myb contribute to the relative resistance to Wnt-1-induced degradation. Three hydrophobic amino acids on the surface of repeat 2 in the DBD of c-Myb, which are changed to non-hydrophobic amino acids in v-Myb (Fig. 4A) (27, 28), are critical for interactions with the transcription factor C/EBPβ on the target promoter (29) and with corepressors (21). Therefore, we investigated whether the change of these three amino acids has any effect on Wnt-1-induced protein degradation. The c-Myb-3M mutant, in which these three amino acids were mutated to non-hydrophobic amino acids as in v-Myb, were relatively resistant to Wnt-1-induced protein degradation (Fig. 4B). To further confirm the role of these three point mutations, we examined the effect of
increasing amounts of HIPK2 on Myb-induced trans-activation of the c-myc promoter (Fig. 4C). Consistent with the results shown in Figure 1B, overexpression of HIPK2 almost completely inhibited the c-Myb-dependent activation of the c-myc promoter, whereas HIPK2 only partially inhibited (about 40%) the v-Myb-induced activation. HIPK2 also only partially inhibited the c-Myb-3M-dependent activation of the c-myc promoter, similar to the response of v-Myb.

Three Point Mutations in the DBD of v-Myb Decreases the Affinity for HIPK2 --

To further analyze the mechanism by which the three point mutations in the DBD of v-Myb affect Wnt-1-induced protein degradation, we examined the effect of these point mutations on the affinity of HIPK2 and NLK for Myb. Both HIPK2 and NLK kinases bind directly to the DBD of c-Myb (23). An in vitro-translated c-Myb fragment (R23) which contains only repeats 2 and 3 of the DBD efficiently bound to the GST-HIPK2 fusion proteins (Fig. 5A, middle panel). In contrast, the in vitro-translated R23 mutant fragment (R23-3M), which contained the three point mutations, failed to bind to GST-HIPK2. Both wild-type and mutant R23 fragments efficiently bound to the GST-NLK fusion proteins (Fig. 5A, right panel). To further confirm this, we used a yeast two-hybrid assay. LexA DBD fusion proteins containing the wild-type or mutated R23 were coexpressed with the VP16-HIPK2 fusion proteins in yeast cells harboring the lacZ reporter containing LexA-binding sites. The wild-type R23 fusion
gave rise to higher levels of lacZ than the mutant R23 fusion (Fig. 5B). The expression levels of LexA-wild-type R23 was about half that of the mutant R23 fusion (Fig. 5C, left). Therefore, the affinity of one molecule of mutant R23 with HIPK2 was estimated to be about one fourth that of wild-type R23 (Fig. 5C, right). Thus, the three point mutations in the DBD of v-Myb decrease the affinity for HIPK2.

The C-proximal NLK Phosphorylation Sites of c-Myb Are Critical for NLK-induced Protein Degradation --- The results of Figure 3 suggested that a truncation of the N- or C-terminal region of c-Myb confers resistance to Wnt-1-induced protein degradation. The C-terminal half of c-Myb contains the multiple NLK phosphorylation sites, whereas the N-terminal region does not, suggesting that the removal of multiple NLK phosphorylation sites by a truncation of the C-terminal region may be responsible for the reduced sensitivity to Wnt-1 induced protein degradation. Mouse c-Myb contains fifteen putative NLK phosphorylation sites, and replacement of all fifteen of these Ser or Thr residues by Ala (15A mutant) completely blocked Wnt-1-induced protein degradation (23). To examine the role of NLK phosphorylation sites in the C-terminal region, we introduced Ala mutations in these sites (Fig. 6A). Ala mutations at the C-terminal two sites alone (C2A) did not confer complete resistance to Wnt-1-induced protein degradation, although this mutant was slightly resistant compared to wild-type c-Myb. However, mutants in which three or nine sites in the C-terminal
region were replaced by Ala (C3A and C9A) were resistant to Wnt-1 and NLK-induced protein degradation (Fig. 6AB).

There are 12 Ser/Thr residues that are conserved between chicken and mouse c-Myb, but three residues (208T, 227S, and 233S) are changed in chicken c-Myb (Fig. 6A). We speculated that loss of these three NLK phosphorylation sites in chicken c-Myb may affect its sensitivity to Wnt-1-induced protein degradation. To investigate this, we generated a mouse c-Myb mutant in which the three residues at 208, 227, and 233 were replaced by Ala (N3A). However, N3A was degraded by Wnt-1 and NLK (Fig. 6B). This is consistent with the results that chicken and mouse c-Myb have similar sensitivities to Wnt-1-induced protein degradation (Fig. 1A). Introducing Ala mutations into the C-terminal three or nine sites of the N3A mutant (N3A+C3A or N3A+C9A) conferred resistance to Wnt-1 and NLK-induced protein degradation.

We also examined the role of the N-proximal NLK phosphorylation sites. Introducing Ala mutations into the N-terminal five or seven sites (N5A and N7A) changed the sensitivity to Wnt-1-induced protein degradation, but not to the NLK-induced protein degradation (Fig. 6B). Thus, the NLK phosphorylation sites in the C-terminal region are more important for Wnt-1-induced protein degradation than the N-proximal phosphorylation sites.

DISCUSSION
We have demonstrated that AMV-encoded v-Myb is relatively resistant to Wnt-1-induced protein degradation. Wnt signaling controls the differentiation or apoptosis of many cell types, including hematopoietic cells (24, 25), and it was recently shown that Wnt5a functions as a tumor suppressor in hematopoietic tissue (30). Therefore, it is possible that resistance of v-Myb to Wnt-mediated protein degradation partially contributes to leukemogenesis.

Mutation of the three hydrophobic amino acids on the surface of repeat 2 of v-Myb reduces the affinity for HIPK2. These three hydrophobic amino acids in c-Myb are involved in interaction not only with HIPK2 but also with other proteins, including corepressor Ski and C/EBPβ (21, 29). HIPK2 and Ski negatively regulate the c-Myb-dependent trans-activation of the c-myc promoter by inducing protein degradation and by recruiting the HDAC complex, respectively (23, 21). These data indicate that v-Myb can escape both of these types of negative regulation because it possesses these point mutations. c-Myb and C/EBPβ, through its interaction with the three hydrophobic amino acids in repeat 2 of the DBD of c-Myb, synergistically activates transcription of mim-1, which encodes one of the hematopoietic differentiation marker (31, 29). v-Myb cannot activate the mim-1 gene due to mutation of these amino acids, suggesting that the expression of differentiation markers, but not proliferation control genes, may be inhibited by these point mutations. Thus, these mutations in v-Myb block expression of the mim-1 differentiation marker and the negative regulation of by HIPK2 and Ski,
suggesting that these mutations may contribute to v-Myb-induced transformation by multiple mechanisms.

In addition to the point mutations in the DBD, a deletion of the C-terminal region of c-Myb which contains nine NLK phosphorylation sites also contributes to the resistance of v-Myb to Wnt-1-induced protein degradation, since the c-Myb mutant in which these nine sites were mutated to Ala was not degraded by Wnt-1. The importance of the number of phosphorylated sites for substrate recognition by ubiquitin E3 ligase was shown by the report that elimination of the Cdk inhibitor Sic1 by the SCF\textit{Cdc4} ubiquitin ligase at the onset of S phase requires phosphorylation of Sic1 on at least six of its nine Cdc4-phosphorylation sites (32). Identification of the specific E3 ligase which recognizes the NLK-phosphorylated c-Myb will further contribute to understanding the mechanism of v-Myb-induced leukemogenesis.

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**FIGURES LEGENDS**

Fig. 1. *v-Myb is relatively resistant to Wnt-1-induced protein degradation.*  

A, Differential sensitivity of v-Myb and c-Myb to Wnt-1-induced protein degradation. CV-1 cells were transfected with a plasmid to express mouse or chicken c-Myb, or AMV v-Myb, and an internal control plasmid pact-β-gal with (+ or ++) or without (-) a Wnt-1 (+, 0.5 µg; ++, 2.0 µg) or NLK (+, 0.3 µg) expression plasmid. Total cell lysates were prepared and analyzed by Western blotting using anti-Myb or anti-FLAG antibodies. The relative density of each band is shown below by a bar graph. Values shown are the averages ± standard deviations of three independent experiments.  

B, Wnt-1 cannot negatively regulate the v-Myb-dependent activation of the c-myc promoter. CV-1 cells were transfected with a mixture of the pc-myc-CAT reporter and either the c-Myb (left) or v-Myb (right) expression plasmid or the control plasmid with (+, 0.5 µg; ++, 2.0 µg) or without (-) a plasmid to express Wnt-1, Fz1, TAK1+TAB1, or NLK. CAT activity was then measured. The degree of activation by c-Myb and v-Myb in the absence of overexpression of any component of the Wnt-1-NLK pathway was 10.4 ± 1.4 and 17.1 ± 1.9 fold, respectively. The relative degree of *trans*-activation by Myb in the presence of the component of the Wnt-NLK pathway compared to that in the absence of the component is indicated. The averages of three experiments are shown with standard deviations.
FIG. 2. **NLK blocks the interaction between c-Myb and CBP.** *(Left)* The binding of *in vitro*-translated c-Myb and v-Myb to GST-CBP in the presence of increasing amounts of either *in vitro*-translated NLK-K155M (kinase-negative mutant) or control lysates was examined. *(Right)* The amount of c-Myb and v-Myb bound to GST-CBP in the presence of NLK-K155M or control lysates is indicated by a bar graph.

FIG. 3. **Truncation of c-Myb weakens Wnt-1-induced negative regulation.** *A,* The functional domains in c-Myb are shown at the top. Chimeric Myb constructs are shown schematically below. The portion derived from AMV v-Myb is indicated by a shaded box, while that from mouse c-Myb is shown by an open box. The point mutations in v-Myb are indicated by dots. *B,* Truncation of c-Myb confers resistance to Wnt-1-induced protein degradation. CV-1 cells were transfected with a plasmid to express the indicated Myb and the internal control plasmid pact-β-gal with (+, 2.0 µg) or without (-) the Wnt-1 expression plasmid. Total cell lysates were prepared and analyzed by Western blotting using anti-Myb or anti-FLAG antibodies. The relative density of each band is shown by a bar graph on the *right.* Values shown are the averages ± standard deviations of at least three independent experiments. *C,* Wnt-1 cannot inhibit *trans-*activation by the truncated form of c-Myb. CV-1 cells were transfected with a mixture of the c-*myc*-CAT reporter and a plasmid to express the indicated form of Myb, or the control plasmid with (+, 0.5 µg; ++, 2.0 µg) or without (-) the Wnt-1 expression
plasmid. CAT activity was then measured. The degree of activation by NT2-V, V-CTV, and NT2-CTV in the absence of Wnt-1 overexpression was 13.9 ± 1.2, 8.0 ± 0.8, and 4.7 ± 0.4 fold, respectively. The relative degree of trans-activation by each form of Myb in the presence of Wnt-1 compared to that in the absence of Wnt-1 is indicated. The averages of three experiments are shown with standard deviations.

**FIG. 4. Point mutations in the DBD of v-Myb weaken Wnt-1-induced negative regulation.** A. The functional domains in c-Myb are shown at the top. Myb constructs used are shown schematically below. The point mutations in v-Myb are indicated by dots. B, Point mutations in v-Myb weaken Wnt-1-induced protein degradation. Experiments similar to those described in Fig. 3B were performed using plasmids expressing wild-type and the 3M mutant c-Myb. The relative density of each band is shown below. Values shown are the averages ± standard deviations of at least three independent experiments. C, Negative regulation of various forms of Myb by HIPK2. Experiments similar to those described in Fig. 3C were performed using the plasmid expressing various forms of Myb with increasing amounts of the HIPK2 expression plasmid. The degree of activation by v-Myb, c-Myb, and c-Myb-3M in the absence of HIPK2 overexpression was 18.9 ± 1.9, 4.7 ± 0.5, and 11.9 ± 1.8, fold, respectively. The relative degree of trans-activation by each form of Myb in the presence of HIPK2 compared to that in the absence of HIPK2 is indicated. The averages of three
FIG. 5. **Three point mutations in the DBD of v-Myb decreases the affinity for HIPK2.**

A, GST pull down assays. *(Upper)* The protein fragments containing repeats 2 and 3 are shown. Three point mutations in v-Myb R2 are indicated by circles. *(Lower left)* The GST-HIPK2ID, which contains the c-Myb-interacting region of HIPK2, and GST-NLK fusion proteins used in the binding assays were analyzed by SDS-PAGE followed by Coomassie blue staining. The binding of *in vitro*-translated R23 and R23-3M to GST-HIPK2ID *(Lower middle)* or GST-NLK *(Lower right)* is shown. The input lanes were loaded with 10% of the amount used for the binding assays. B, Interaction measured by yeast two hybrid assays. Three independent transformants harboring the plasmids indicated at *left* were isolated and grown, and their β-galactosidase activities were measured. Values shown are the averages ± standard deviations of duplicate assays from three independent transformants. C, Degree of interaction normalized to the amounts of protein expressed. *(Left)* To compare the expression levels of two bait proteins, whole cell lysates were prepared from the transformants expressing LexA-R23 or LexA-R23-3M and used for Western blotting with anti-LexA antibody. To precisely compare the protein levels, decreasing amounts of proteins were used for Western blotting. *(Right)* The relative degree of interaction between HIPK2 and R23 or R23-3M was calculated by subtracting the basal β-galactosidase activity from the
activity obtained in (B), and then normalized by expression level of the proteins. Similar results were obtained using two other independent transformants (data not shown).

**FIG. 6.** The C-proximal NLK phosphorylation sites of c-Myb are critical for NLK-induced protein degradation. 

**A,** Domain structure of c-Myb and the various Myb mutants used. The Ser and Thr residues linked to Pro are shown by vertical arrows. The residues that were mutated to Ala are indicated by closed circles. The results of the c-Myb degradation assays (B) are summarized on the right. The relative sensitivities of the various forms of c-Myb to Wnt-1 or NLK-induced protein degradation are designated +, ±, and - to indicate degradation of greater than 90%, 55-65%, and less than 50% of the control levels, respectively. 

**B,** Effect of c-Myb mutations on NLK-induced c-Myb degradation. CV-1 cells were transfected with a plasmid that expresses a particular form of c-Myb and the pact-β-gal plasmid together with a plasmid that expresses Wnt-1 or NLK. Whole cell lysates were prepared and analyzed by Western blotting using an anti-c-Myb antibody. The relative density of each band is shown below the panels. Values shown are the averages ± standard deviations of at least three independent experiments.
Figure 1. Kanei-Ishii, C. et al.
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Differential sensitivity of v-Myb and c-Myb to Wnt-1-induced protein degradation
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