Prohibitin1 acts as a neural crest specifier in *Xenopus* development by repressing the transcription factor E2F1

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**SUMMARY**

Prohibitin1 (phb1), which was initially described as an inhibitor of cell proliferation, is a highly conserved protein found in multiple cellular compartments. In the nucleus it interacts with the transcriptional regulators Rb and E2F1 and controls cell proliferation and apoptosis. Here we unravel an unexpected novel function for phb1 in *Xenopus* cranial neural crest (CNC) development. Xphb1 is maternally expressed; zygotically expressed neurula stage transcripts accumulate in the CNC and the neural tube. Knockdown of Xphb1 by antisense morpholino injection results in the loss of foxD3, snail2 and twist expression, whereas expression of c-myc, AP-2 and snail1 remains unaffected. Xphb2, its closest relative, cannot substitute for Xphb1, underlining the specificity of Xphb1 function. Epistatic analyses place Xphb1 downstream of c-myc and upstream of foxD3, snail2 and twist. To elucidate which subdomain in Xphb1 is required for neural crest gene regulation we generated deletion mutants revealed a crucial mitochondrial function for prohibitins in mitochondria (Mishra et al., 2006). Studies in *C. elegans* have revealed a crucial mitochondrial function for prohibitins in from the neural fold. In addition, snail2, sox10 and twist are activated, which serves to maintain CNC pluripotency and survival but also to regulate the expression of genes required for cell migration and differentiation (Meulemans and Bronner-Fraser, 2004; Sauka-Spengler and Bronner-Fraser, 2005; Steventon et al., 2005). This view of hierarchy in neural crest gene activation is most likely an oversimplification because there is mounting evidence of a cross-regulation between snail1, snail2 and twist in the induction of neural crest at the gastrula stage (Carl et al., 1999; Zhang et al., 2006; Zhang and Klymkowsky, 2009). Although we have to reconsider the regulatory network at the level of neural crest specification, the upstream requirement of the neural plate border specifiers appears correct. In addition to their role in the CNC, neural plate border specifiers such as pax3 and zic1 collaborate to stimulate neural crest formation (Hong and Saint-Jeannet, 2007). In addition, gbx2 collaborates with zic1 in activating neural crest genes and inhibiting the placodal fate (Li et al., 2009).

Not all homologs of neural crest specifier genes that are upregulated in human tumors share common functions with their orthologs in neural crest cells. snail1 and snail2 behave similarly by promoting EMT and blocking apoptosis. The proto-oncogene c-myc, however, operates differently. When overexpressed or mutated, c-myc promotes tumor growth by facilitating cell cycle entry (Herold et al., 2009; Morrish et al., 2009) and sensitizes cells for apoptosis (Hoffman and Liebermann, 2008). In neural crest development, c-myc neither influences cell proliferation nor cell death; rather, it is required for snail1, snail2 and twist expression (Bellmeyer et al., 2003).

The human prohibitin homologs, PHB (PHB1) and PHB2, are tumor suppressors that share 53% amino acid sequence identity; these proteins are localized to the plasma membrane, nucleus and mitochondria (Mishra et al., 2006). Studies in *C. elegans* have

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**INTRODUCTION**

The cranial neural crest (CNC) shapes the vertebrate face. These cells possess two exceptional properties: pluripotency and motility. CNC cells differentiate to melanocytes, peripheral neurons, glia, cartilage, bone and the inflow tracts of the heart, based on an orchestrated gene regulatory network and their migratory trajectories (Sauka-Spengler and Bronner-Fraser, 2008). CNC cells share important characteristics with carcinoma cells in undergoing epithelial-mesenchymal transition (EMT) and tissue invasion (Acloque et al., 2009). They express genes involved in tumor formation, such as c-myc, snail1 and snail2 (slug). They also upregulate mesenchymal cadherins and metalloproteinasises, which are enriched during metastasis (Kuriyama and Mayor, 2008). Therefore, deciphering the regulatory network of CNC development would not only help to understand congenital defects of craniofacial development but also provide insight into gene functions in tumor formation and progression.

CNC is induced at the neuroectoderm/ectoderm border under the influence of defined levels of BMP, canonical Wnt, retinoic acid (RA), FGF and notch signaling (Basch and Bronner-Fraser, 2006). These signals activate the neural plate border specifiers pax3, zic1 and dlx, which in turn activate expression of the more restricted, localized CNC specifiers, including c-myc, foxD3, AP-2, snail1 and sox9. The latter are thought to initiate CNC fate specification by controlling CNC proliferation and apoptosis, EMT and emigration

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development and lifespan control (Artal-Sanz and Tavernarakis, 2009; Artal-Sanz et al., 2003); in mitochondria, the prohibitins localize predominantly to the inner membrane where they form a multimeric complex with a chaperone-like function (Osman et al., 2009). Nuclear prohibitins inhibit cell proliferation and suppress apoptosis (Fusaro et al., 2002; Wang et al., 1999a). Here, we report that Xenopus prohibitin1 (Xphb1) acts downstream of c-myc in neural crest development to control snail2, foxD3 and twist expression. Whereas PHB1/PHB1 and PHB2/PHB2 appear functionally synonymous in C. elegans and human cell lines, this is not the case in Xenopus. The role of Phb1 in neural crest development is not associated with cell proliferation or apoptosis but involves its ability to bind to and repress the activity of the transcription factor E2F1.

MATERIALS AND METHODS

Xenopus embryos, micromanipulation and lineage tracing

Embryos were obtained by in vitro fertilization and staged according to Nieuwkoop and Faber (Nieuwkoop and Faber, 1967). mRNA for injection experiments was synthesized in vitro using the mMessage mMachine Kit (Ambion, Norwalk, CT, USA). Xphb1 (phb1 MO, 5′-ATCCCTGTT-CTTCCACACGCTAAT-3′) and c-myc [c-myc MO, as previously described (Bellmeyer et al., 2003)] morpholino antisense oligonucleotides were purchased from Gene Tools (Philomath, OR, USA). As a control we used the 3′carboxyfluorescein-tagged standard control morpholino oligonucleotide (co MO) designed by Gene Tools.

Unless indicated otherwise, all injections were performed into the animal hemisphere of one blastomere of 2-cell stage embryos as follows: 700 pg RNA, 16 ng phb1 MO, 11 ng c-myc MO. Embryos were co-injected with lacZ (β-gal) DNA (50 pg) to identify the manipulated side. Embryos were fixed in MEMFA at stage 18 (unless otherwise noted) and successively processed for β-gal staining (Sive et al., 2000) using X-Gal (Applichem, Darmstadt, Germany) as substrate. In the rescue experiment, in which Hphb1 mRNA was co-injected, dextran-FITC (4 pg; Molecular Probes, Eugene, OR, USA) was used as a lineage tracer.

Constructs

Xphb1 and Xphb2 were amplified from neurola stage embryos. Xphb1 was fused terminally with six copies of the c-myc epitope in the pCS2+ vector (pCS2+-myc). All Xphb1 mutants, Xphb1AN-term (amino acids 116-275), Xphb1ΔRaf-1 (amino acids 1-243) and Xphb1AC-term (amino acids 1-188), were subcloned into pCS2+-myc. Primers are listed in Table S1 in the supplementary material. The mutant Hphb1Δ185-214, a kind gift of S. Chellappan (Columbia University, New York, USA), was subcloned into the pCS2+-vector and the Hphb1 (pcDNA3-Phb) plasmid was kindly provided by T. Rudel (Julius-Maximilians-Universität, Würzburg, Germany).

Whole-mount analysis

In situ hybridization was performed as described (Gawantka et al., 1995). Antisense DIG-labeled probes were synthesized with the DIG RNA Labeling Kit (Roche, Basel, Switzerland) using template cDNA encoding Xphb1, twist (Hopwood et al., 1989), snail2 (Mayor et al., 1995), snail1 (Essex et al., 1993), foxD3 (Sasai et al., 2001), c-myc (Bellmeyer et al., 2003) AP-2 (Luo et al., 2003), Bbra (Smith et al., 1991), chordin (Sasai et al., 1995), sox2 (Kishi et al., 2000), cytokeratin Xk81A1 (Jonas et al., 1985), pax3 (Bang et al., 1997), zic3 (Nakata et al., 1997) and meis3 (Salzberg et al., 1999). Images were captured as described previously (Etard et al., 2005). The probe for cytokeratin Xk81A1 was kindly provided by R. Mayor (UCL, London, UK). The results of at least three independent experiments were averaged, and statistical significance was calculated using Student’s t-test. Proliferation was estimated at stage 18 by TUNEL staining. Western blots were carried out as described (Unterseher et al., 2004). Lysates corresponding to half an embryo were separated by SDS-PAGE. Rabbit polyclonal anti-prohibitin antibody was obtained from Abcam (1:4000; Cambridge, UK) and alkaline phosphatase-conjugated secondary antibody from Dianova (1:3000). β-catenin (a kind gift of Ralph Rupp, München, Germany), with Coomassie Brilliant Blue (Serva, Heidelberg, Germany) staining, was used as a loading control.

RESULTS

Xenopus prohibitin1 (Xphb1) is expressed in CNC and is required for twist expression

In a search for genes expressed in migrating cells of the early Xenopus embryo we identified prohibitin1 (Xphb1) in neural crest cells by whole-mount in situ hybridization (ISH) (Fig. 1). At blastula stage, Xphb1 transcripts localized in the animal hemisphere, accumulated in the posterior dorsal area at late gastrula and concentrated in CNC and neural tube at neurula stage (Fig. 1A-C). When gastrula embryos were cut into two halves (semi-sections), Xphb1 was detected in the mesendoderm at stage 10 (Fig. 1D,d) and in the posterior neuroectoderm and mesoderm at stage 11.5 (Fig. 1E,e). The sense Xphb1 probe yielded no signal (Fig. 1d’,e’). Xphb1 expression in CNC was maintained during their migration, and additional expression areas were found in the eye, the otic vesicle and the brain (Fig. 1F,G). Double ISH for Xphb1 and twist revealed partial overlap of the expression domains at stage 18 (Fig. 1H-H’). Xphb2, which shares 53% amino acid identity with Xphb1, was not detected in the neural crest (data not
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Fig. 1. Xphb1 is expressed in the presumptive cranial neural crest (CNC) region. (A–H) In situ hybridization (ISH) for Xphb1 (A–H) and Xphb1 plus twist (H’). (A) At blastula (stage 9) Xphb1 is expressed in the dorsal animal ectoderm (arrowheads; animal pole is towards the top). (B) At late gastrula stage (stage 11.5) Xphb1 transcripts are found in the posterior dorsal area (arrowhead). (C) At neurula stage (dorsal view) Xphb1 is detected in migrating neural crest (arrowhead) and in the neural tube. (D,E) Semi-sections of stage 10 (D) and stage 11.5 (E) gastrula stage Xenopus embryos (animal pole towards the top). Insets demonstrate Xphb1 expression in the mesendoderm (d) and in the posterior neuroectoderm including mesoderm (e). No Xphb1 mRNA is detectable in comparable regions of sense control embryos (d/e). (F,G) From neurulation onwards (F, stage 23; G, stage 28; lateral view) Xphb1 expression is restricted to the neural crest territory (arrowheads), the eye and brain. (H,H’) Double ISH at stage 18 shows that Xphb1 (H, purple) is partially co-expressed with twist (blue) in the neural crest (H’). Xphb1 (purple arrowhead) and twist (blue arrowhead) are also expressed in non-overlapping regions. Scale bars: 400 μm. (I) RT-PCR analysis of Xphb1 expression at the indicated stages. Ornithine decarboxylase (ODC) was used as an internal control. Additional controls were performed without reverse transcriptase (–RT) and without cDNA (H2O).

Fig. 2. Xphb1 is required for CNC formation. (A) The phb1 MO, showing its binding site in Xphb1. Nucleotides that are identical between Xphb1 and human PHB1 (Hphb1) or Xphb2 are indicated (asterisks). A standard control morpholino (co MO) tagged with 3’ carboxyfluorescein was used as control. (B) In TnT in vitro translation, phb1 MO specifically blocks the translation of Xphb1 (lane 2). The phb1 MO had no effect on the translation of Hphb1 (lane 3) or Xphb2 (lane 6). (C) Injection (*) of phb1 MO, but not of co MO, efficiently inhibited twist expression. This was rescued by co-injection of Hphb1 mRNA or Xphb1 mRNA lacking the morpholino binding site, whereas Xphb2 mRNA showed no effect. β-gal was used as a lineage tracer (light blue). The co MO-injected side appears fluorescently labeled. Beneath is shown the percentage of Xenopus embryos with reduced twist expression. n, number of embryos analyzed. **, P<0.005. Error bars indicate standard error.
The accumulation of Xphb1 in CNC prompted us to examine whether depletion of Xphb1 affects neural crest development. We designed an antisense morpholino (phb1 MO) that specifically blocks Xphb1 translation while Xphb2 synthesis remains unaltered (Fig. 2A,B). Single-sided injections of phb1 MO at the 2-cell stage led to the loss of twist expression in the CNC (Fig. 2C). Co-injection of lacZ DNA served to identify the injected side. Neither lacZ DNA alone nor control morpholino (co MO) injection affected twist expression, pointing to a specific phb1 MO effect. This was confirmed by rescue experiments. Both Xphb1 mRNA that lacks the morpholino binding site and mRNA encoding the human homolog PHB1 (Hphb1), which shows 90% amino acid identity to Xphb1, rescued the phb1 MO phenotype. Importantly, Xphb2 mRNA injection did not rescue the depletion of Xphb1 (Fig. 2C).

Phb1 is known from mammalian cell culture studies to control cell proliferation and apoptosis (Joshi et al., 2003; McClung et al., 1989; Wang et al., 1999a; Wang et al., 1999b). To address whether loss of the twist signal is caused by reduced cell proliferation or increased cell death we performed phospho-histone H3 immunostainings and TUNEL assays. As shown in Fig. 3, phb1 MO injections influenced neither cell proliferation nor apoptosis. This suggested that Xphb1 might be required in neural crest gene activation, rather than in controlling CNC proliferation or cell death.

Neural crest induction takes place during gastrulation and depends on signals secreted from the dorsolateral mesoderm (Steventon et al., 2009). To exclude the possibility that Xphb1 knockdown affects mesoderm formation and consequently neural crest induction, we examined the expression patterns of Xbra and chordin by ISH. Both genes were expressed normally upon Xphb1 depletion (see Fig. S2 in the supplementary material).

**Xphb1 operates downstream of c-myc in regulating snail2, foxD3 and twist**

Next, we aimed to better allocate Xphb1 into the regulatory network of neural crest genes by expanding ISH studies of marker genes and by performing epistatic analyses. As shown in Fig. 4, depletion of Xphb1 resulted in loss of snail2 and foxD3, in addition to twist, but did not alter c-myc, snail1 or AP-2 expression. Reduction of twist, snail2 and foxD3 expression was observed in nearly 80% of the embryos (see Fig. S3A,B in the supplementary material). We further confirmed these findings by real-time PCR. twist mRNA was reduced by 50% and snail2 by 40% in phb1 MO-injected embryos; these effects were rescued by Hphb1 mRNA injection (see Fig. S4A in the supplementary material). No changes in the amount of c-myc or snail1 transcripts were found in Xphb1 morphants; a small decrease in AP-2 mRNA was detected by real-
time PCR, but not by ISH (see Fig. S4B in the supplementary material; compare with Fig. 4).

To examine whether c-myc acts upstream of Xphb1, embryos were injected with antisense c-myc morpholino (c-myc MO). There was a decrease in Xphb1 CNC expression in 90% of the injected embryos, as monitored by ISH (Fig. 5A). Real-time PCR showed a reduction in relative Xphb1 expression (normalized to the level of ODC expression). **, P<0.005. (C) c-myc MO-mediated depletion of twist, snail2 and foxD3 was rescued by co-injection of Xphb1 mRNA (twist, **, P<0.005; snail2, *, P<0.05; foxD3, **, P<0.005), whereas Xphb2 had no effect. n, number of embryos analyzed. Error bars indicate standard error.

To separate Xphb1 clearly from the group of neural plate border specifiers we investigated whether Xphb1 knockdown alters the expression of pax3, zic3 and meis3. The expression patterns of these neural plate border specifiers were unchanged upon Xphb1 depletion (Fig. 7A-C). In line with these results, we did not observe a broadening or reduction of the epidermal or neural tissue, as examined by ISH for Xk81A1 (an epidermal marker) and sox2 (a neural marker) (Fig. 7D,E). Double ISH for sox2 and snail1 or snail2 further confirmed that the ratio between neural plate and CNC areas remains stable in the absence of Xphb1 (Fig. 7F,G).

**Xphb1 specifies neural crest by repressing E2F1**

Next, we aimed to determine whether a specific domain in Xphb1 is required to regulate snail2, foxD3 and twist. Prohibitin1 consists of a transmembrane domain (TM) required for mitochondrial localization, as well as binding sites for the nuclear transcription factors Rb and E2F1 (Mishra et al., 2006; Wang et al., 1999a; Wang et al., 1999b) (Fig. 8A). Human PHB1 has also been reported to interact with the ring-finger protein 2 (RNF2) (Choi et al., 2008) and to bind RAF1 (Rajalingam et al., 2005; Wang et al., 1999b). Within the Raf-1 binding site, a nuclear export signal (NES) sequence is located (Mishra et al., 2006). We deleted the N-
terminus containing the TM domain and the Rb binding site (Xphb1ΔN-term) and generated two different C-terminal truncations: Xphb1ΔRaf-1, which lacks the Raf-1 binding site and the NES motif; and Xphb1ΔC-term, which lacks the Raf-1 binding site, the NES motif and the E2F1-binding sequence (Fig. 8A). All deletion mutants could be detected by their myc tag in embryo lysates (Fig. 8B). When these mutants were analyzed for their ability to rescue the Xphb1 morphant phenotype, we found that Xphb1ΔC-term was insufficient to restore twist expression (Fig. 8C). Injections of mRNAs encoding the deletion mutants Xphb1ΔN-term and Xphb1ΔRaf-1, however, recovered twist expression in 60% and 50% of injected embryos, respectively (Fig. 8C). These findings point to a crucial role of the E2F1 binding site for Xphb1 function in CNC. This was confirmed when the rescue ability of human prohibitin 1 deleted in the E2F1 binding site was examined, as Hphb1Δ185-214 was unable to reconstitute twist expression in Xphb1 morphants (Fig. 8C).

These results prompted us to investigate the functional relationship between Xphb1 and E2F1. We injected a Gal4-DBD-E2F1 fusion construct (Choi et al., 2008) into embryos and measured the response of a pFR-luciferase trans-reporter plasmid at different concentrations of co-injected Xphb1 mRNA. As shown in Fig. 9A, the luciferase activity dropped by nearly 50%, indicating a repressive effect of Xphb1 on E2F1.

We then asked whether expression of E2F1 influences twist expression. Single-sided injections of human E2F1 DNA led to downregulation of twist expression, which was restored when Xphb1 mRNA was co-injected (Fig. 9B). This was also observed for snail2 and foxD3 (data not shown). We also tested the influence of E2F1 in Xphb1-depleted embryos. When phb1 MO was injected at low doses, an additive effect was observed in the presence of low concentrations of E2F1 (Fig. 9C). These results indicate that Xphb1 serves to repress the transcription factor E2F1 in order to promote twist, snail2 and foxD3 expression.

**DISCUSSION**

CNC development requires a complex signaling network, which includes key regulators that are often found to be dysregulated in tumors. In this study we identify Xphb1, the *Xenopus* homolog of the tumor suppressor gene prohibitin 1, as a novel neural crest specifier operating downstream of c-myc in regulating snail2, foxD3 and twist. This distinguishes Xphb1 from the neural plate border specifiers and places it among the CNC specifiers (Fig. 10).

Importantly, Xphb1 is not required for the activation of snail1 or AP-2 through c-myc. Bellmeyer et al. have demonstrated that c-myc expression marks CNC already at mid-gastrula (stage 11) and induces the CNC genes snail2, snail1, sox9, foxD3 and twist (Bellmeyer et al., 2003). Therefore, the branching in Xphb1-dependent and -independent c-myc targets observed here is surprising and has not been reported previously. Depletion of the CNC specifiers sox10 and sox9, for example, results in loss of twist, foxD3, snail2 and snail1 expression (Honore et al., 2003; Lee et al., 2004; Spokony et al., 2002). More recently, a disjunctive regulation of snail1 and snail2 has been demonstrated in RhoV-depleted embryos. RhoV is transiently expressed in CNC between stages 12 and 21. It is essential for the expression of snail2, sox9, sox10, twist and foxD3, but not for snail1 (Guemar et al., 2007). The function of this small GTPase, however, is believed to be in the context of cell rearrangements rather than nuclear activity.

Disturbances in neural fold formation may lead to shifts between neural plate and neural crest territories, thereby indirectly influencing gene expression (Guemar et al., 2007). In contrast to RhoV, overexpression of Xphb1 did not enlarge the CNC area and its depletion did not alter the border between neural plate and epidermis (Fig. 7). We conclude that Xphb1 is unable to change the cell fate from neural or epidermal progenitors to CNC. Furthermore, we could allocate Xphb1 a nuclear activity because deletion of its binding site for the transcription factor E2F1 abolished its rescue ability in morphants (Fig. 8). Neither depletion of the TM domain and the Rb binding site, nor of the Raf-1 binding domain with the NES motif, severely affected the rescue capacity, pointing to a pivotal role...
for the interaction with E2F. Intriguingly, E2F overexpression led to a decrease in twist, snail2 and foxD3 expression, which was recovered by overexpression of Xphb1. Depletion of Xphb1, conversely, promoted a reduction in twist expression through E2F1. These findings, and the decrease in Gal4-DBD-E2F1 activity in presence of prohibitin in the pFR-luciferase reporter, suggest that Xphb1 is required to repress E2F1 function in CNC development.

In Xenopus, an E2F ortholog is maternally expressed. E2F mRNA specifically localizes during neurula stage in brain, neural tube and neural crest (Suzuki and Hemmati-Brivanlou, 2000) and E2F seems to be more widely used in regulating transcription than Xphb1. The latter is supported by our findings that E2F1 also reduces c-myc, snail1 and AP-2 expression and that this is not rescued by Xphb1 (data not shown). Expression of dominant-negative E2F constructs results in disturbances of ventral and posterior cell fates (Suzuki and Hemmati-Brivanlou, 2000) and in the inhibition of cell cycle progression from mid-blastula stage onwards (Tanaka et al., 2003). Based on our results from phospho-histone H3 immunostaining and TUNEL assay, we can exclude the possibility that Xphb1, together with E2F1, balances cell proliferation in CNC development (Fig. 3).

In mammalian cells, multiple mechanisms of prohibitin-mediated repression of E2F1 function have been reported, some of which are Rb independent. Importantly, all of these mechanisms involve chromatin remodeling. Prohibitin1 is able to recruit histone deacetylase 1 (HDAC1) and the co-repressor N-CoR into the E2F1 transcription complex, thereby abolishing E2F1 function (Wang et al., 2002a). Prohibitin1 has also been shown to recruit Bg-1/Brm, which are part of the SWI/SNF chromatin remodeling complex (Wang et al., 2002b). Choi et al. reported that prohibitin1 binds RNF2 and recruits it to E2F1-response promoters (Choi et al., 2008). RNF2 belongs to the polycomb family. The presence of RNF2 in polycomb complexes correlates with histone H2A ubiquitylation (Wang et al., 2004). Therefore, it seems most likely that Xphb1 promotes chromatin rearrangements that are essential for the activation of those CNC specifier genes that contain E2F1-response elements in their promoters. XBrg1, the catalytic subunit of the SWI/SNF complex, is contributed maternally in Xenopus embryos, but transcripts accumulate in the presumptive CNS, neural crest and in the otic vesicle (Seo et al., 2005). Loss-of-function studies revealed a requirement for XBrg1 in neurogenesis. Importantly, single-sided antisense XBrg1 morpholino injections yield a lateral

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**Fig. 8.** The E2F1-binding domain is required for CNC formation. (A) Model of prohibitin1 protein domains (according to the human protein structure). Beneath are shown the various Xphb1 mutants (Xphb1ΔN-term, Xphb1ΔRaf-1, Xphb1ΔC-term) and a human PHB1 mutant that lacks the E2F1 binding site (Hphb1Δ185-214). Deletions are indicated by the thin lines. TM, transmembrane domain; NES, nuclear export signal. (B) In western blots, Xphb1 mutants were detected by anti-myc (9E10) antibody. Coomassie Brilliant Blue (CBB) staining was used to reveal equal loading. (C) Xphb1 mutants and Hphb1Δ185-214 co-injected with phb1 MO (*) and analyzed by twist ISH showed that both of the mutants that lack the E2F1 binding site were unable to rescue twist expression. n, number of embryos analyzed. **, P<0.005; *, P<0.05. Error bars indicate standard error.
broadening of the sox2 domain (Seo et al., 2005), which might also affect the CNC domain. The homologs of RNF2 and HDAC1 in Xenopus have not been cloned. However, two other members of the HDAC complex, xSin3 and xRBD3, have been reported to co-immunoprecipitate with FoxN3, a protein required in craniofacial and eye development (Schuff et al., 2007).

Taking our results together with those of previous studies, we modified the regulatory gene network of CNC development by adding Xphb1 to the group of CNC specifiers (Fig. 10). We further demonstrate that Xphb1 separates c-myc target genes into those that are prohibitin1/E2F-dependent and -independent. As prohibitin1 is able to recruit a broad set of chromatin modifiers to the E2F transcription complexes, future studies of epigenetic events might help to elucidate the molecular mechanism of Xphb1 in neural crest specification.

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The authors declare no competing financial interests.

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