Supplemental experimental procedures

Expression Plasmids

pbphi-multi cloning site

A vector was created for maternal expression of a tdTomato-PCP fusion protein, which permits detection of reporter genes containing PP7 RNA stem loops. The first was to modify the pbphi-yellow reporter plasmid used in a previous study (Lagha et al., 2013). Two DNA fragments (5′-TCG AGG GGG GAA GCT TGG CGG ATC AGG CTC GGG ATC ATC GGC TAG CGG GGG GGA TCC GGG GGT-3′) and (5′-TCG AGG GGG GAA GCT TGG CGG ATC AGG CTC GGG ATC ATC GGC TAG CGG GGG GGA TCC GGG GGT-3′) were annealed and inserted into the pbphi-yellow expression vector using Xho-XbaI restriction sites.

pbphi-nanos promoter

A DNA fragment containing the *nanos* promoter was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CGC TTC GAC CGT TTT AAC CTC 3′) and (5′-CCC CCC TCG AGG GCG AAA ATC CGG GTC GAA A-3′) and digested with NotI and XhoI. The resulting fragment was inserted between the NotI and XhoI sites in the pbphi-multi cloning site.

pbphi-nanos promoter-αTubulin 3′UTR

A DNA fragment containing the *αTubulin* 3′UTR was amplified from genomic DNA using primers (5′-GGG GGG GAT CCG CGT CAC GCC ACT TCA ACG C-3′) and (5′-CCC CCT CTA GAG AGC TTC GCA TGG TTT TGC C-3′) and digested with BamHI and XbaI. The resulting fragment was inserted between the BamHI and XbaI sites in the pbphi-nanos promoter.

pbphi-nanos>PCP-αTubulin 3′UTR

A DNA fragment containing the coding sequence of the PP7 coat protein was amplified from pURA-MET28 PCP-2x-yeGFP (Larson et al., 2011) using primers (5′-GGG GGG CTA GCA TGT CCA AAA CCA TCG TTC T-3′) and (5′-CCC CCG GAT CCC TAA CGG CCC AGC GGC ACA AGG T-3′) and digested with NheI and

BamHI. The resulting fragment was inserted between the NheI and BamHI sites in the pbphi-nanos promoter-αTubulin 3´UTR.

pbphi-nanos>tdTomato-PCP-αTubulin 3´UTR

A DNA fragment containing *tdTomato* was amplified from tdTomato-Moesin (Hannibal et al., 2012) using primers (5´-GGG GGC TCG AGA TGG TGA GCA AGG GCG AGG A-3´) and (5´-CCC CCA AGC TTC TTG TAC AGC TCG TCC ATG C-3´) and digested with XhoI and HindIII. The resulting fragment was inserted between the XhoI and HindIII sites of the pbphi-nanos>PCP-αTubulin 3´UTR expression vector.

pbphi-nanos>SV40NLS-tdTomato-PCP-αTubulin 3´UTR

Two DNA fragment (5´-GGG GGC TCG AGA TGC CTA AGA AAA AGA GGA AGG TTG GAT CAG GCT CGG GAT CAC TCG AGG GGG G-3´) and (5´-CCC CCC TCG AGT GAT CCC GAG CCT GAT CCA ACC TTC CTC TTT TTC TTA GGC ATC TCG AGC CCC C-3´) were annealed and then digested with XhoI. The resulting fragment was inserted into the unique XhoI site of the pbphi-nanos>tdTomato-PCP-αTubulin 3´UTR expression vector.

pBlueScript-24xPP7

Reporter genes containing MS2 and PP7 RNA stem loop sequences were made as follows. The first was to create 24 copies of PP7 stem loops. A synthesized DNA oligonucleotide (5´-CCC CCG GAT CCT ACG GTA CTT ATT GCC AAG AAA GCA CGA GAC GAT ATG GCG TCC GTG CCT CCA GGT CGA ATC TTC AAA CGA CGA GAG GAT ATG GCC TCC GTC GCT CCA GTA TTC CAG GGT TCA TCA GAT CTG GGG G-3´) was digested with BamHI and BglII, and inserted into the pBlueScript SK vector (pBlueScript-2xPP7). A DNA fragment containing 2x PP7 was purified from pBlueScript-2xPP7 using BamHI and NotI, and cloned into pBlueScript-2xPP7 using BglII and NotI restriction sites (pBlueScript-4xPP7). By repeating this procedure, a series of vectors containing different copy numbers of the PP7 stem loop were created (pBlueScript-8xPP7, pBlueScript-16xPP7 and pBlueScript-24xPP7).

pbphi-snaPr-yellow-1

A DNA fragment containing *sna* promoter was amplified from genomic DNA using primers (5′-GGG GGC TCG AGG ACA GCG GCG TCG GCA GAG G-3′) and (5′-CCC CCG GAT CCT GGT TGC GTT CTC AAC GAG A-3′) and digested with XhoI and BamHI. The resulting fragment was inserted between the XhoI and BamHI sites in pbphi-yellow.

pbphi-snaPr-yellow-2

A DNA fragment containing the 100 bp sequence downstream of *αTubulin* gene was amplified from genomic DNA using primers (5′-GGG GGG CTA GCA AGC TTT CCG AAT TTA ACT ACG AGT C-3′) and (5′-CCC CCT CTA GAG CCT GCA CAA AGT ACA TAC A-3′) and digested with NheI and XbaI. The resulting fragment was inserted into the unique XbaI site in pbPhi-snaPr-yellow-1. Subsequently, a DNA fragment containing the 100 bp sequence downstream of *αTubulin* gene was amplified from genomic DNA using primers (5′-GGG GGA AGC TTT CCG AAT TTA ACT ACG AGT C-3′) and (5′-CCC CCT CTA GAG CTA GCG CCT GCA CAA AGT ACA TAC A-3′) and digested with HindIII and XbaI. The resulting fragment was inserted into between the HindIII and XbaI sites.

pbphi-snaPr-yellow-3

A DNA fragment containing the *sna* promoter was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CTC TAG AGA CAG CGG CGT CGG CAG AGG-3′) and (5′-CCC CCG GAT CCT GGT TGC GTT CTC AAC GAG A-3′) and digested with NotI and BamHI. The resulting fragment was inserted between the NotI and BamHI sites in pbphi-yellow.

pbphi-snaPr-MS2-yellow

A DNA fragment containing 24x MS2 stem loops was purified from pCR4-24xMS2SL-stable (Bertrand et al., 1998) by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BamHI site in the pbphi-snaPr-yellow-2 reporter vector. Subsequently, a DNA fragment containing a partial sequence from *lacZ* was amplified using primers (5′-GGG GGA CTA GTC GCT GGG GAA TGA ATC AGG C-3′) and (5′-CCC CCA CTA GTT CCA GCG GTG

CAC GGG TGA A-3′) and digested with SpeI. The resulting fragment was inserted into the unique SpeI site of the plasmid.

pbphi-evePr-MS2-yellow

A series of reporter genes containing different core promoter sequences were created as follows. First, a DNA fragment containing the *eve* promoter was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CTT TGC CTG CAG AGC GCA GCG-3′) and (5′-CCC CCG GAT CCA ACG AAG GCA GTT AGT TGT T-3′) and digested with NotI and BamHI. The resulting fragment was inserted between the NotI and BamHI sites in pbphi-snaPr-yellow-2. Subsequently, a DNA fragment containing 24x MS2 stem loops was inserted into the unique BamHI site.

pbphi-sogPr-MS2-yellow

A DNA fragment containing the *sog* promoter was amplified from genomic DNA using primers (5′-GGG GGC TCG AGA AGC TTG CCG TTG CAT GTT GCC GCC G-3′) and (5′-CCC CCG GAT CCG CCG AAC CCA AAA AGC CGA A-3′) and digested with XhoI and BamHI. The resultant fragment was inserted between the XhoI and BamHI sites in pbphi-snaPr-yellow-2. Subsequently, a DNA fragment containing 24x MS2 stem loops was inserted into the unique BamHI site.

pbphi-snaPr-PP7-yellow

A DNA fragment containing 24x PP7 stem loops was purified from pBlueScript-24xPP7 by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BamHI site of the pbphi-snaPr-yellow-3 reporter vector. Subsequently, a DNA fragment containing a partial sequence from *lacZ* was amplified using primers (5′-GGG GGA CTA GTC GGT CAA TCC GCC GTT TGT T-3′) and (5′-CCC CCA CTA GTG TTA CGC GTT CGC TCA TCG C-3′) and digested with SpeI. The resulting fragment was inserted into the unique SpeI site of the plasmid.

pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr

Plasmid DNAs containing linked *MS2-yellow* and *PP7-yellow* reporter genes were created as follows. Towards this end, a DNA fragment containing snaPr-PP7-yellow was purified from pbphi-snaPr-PP7-yellow by digesting with XbaI. The resulting fragment was inserted into the unique XbaI site in pbphi-snaPr-MS2-yellow.

Subsequently, a DNA fragment containing the *sna* shadow enhancer was amplified from genomic DNA using primers (5′-GGG GGA AGC TTG CAT TGA GGT GTT TTG TTG G-3′) and (5′-CCC CCG CTA GCT AAA TTC CGA TTT TTC TTG TCT GGG-3′) and digested with HindIII and NheI. The resulting fragment was inserted between the HindIII and NheI sites of the plasmid.

pbphi-snaPr-MS2-yellow-sna primary-yellow-PP7-snaPr

A series of linked *MS2-yellow* and *PP7-yellow* reporter genes containing different shared enhancers were created as follows. First, a DNA fragment containing the *sna* proximal primary enhancer was amplified from genomic DNA using primers (5′-GGG GGA GCT TCC CGC CGA AGG ATT CCG AGA-3′) and (5′-CCC CCA CTA GTG CCA TTT GGT GGT GGT TCT TCT TC-3′) and digested with HindIII and SpeI. The resulting fragment was inserted between the HindIII and NheI sites of pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr.

pbphi-snaPr-MS2-yellow-rho NEE-yellow-PP7-snaPr

A DNA fragment containing the *rho* NEE was amplified from genomic DNA using primers (5′-GGG GGA AGC TTC CTC AGG TCG AGT TCC TCC ATC CTC-3′) and (5′-CCC CCG CTA GCT TCC TCT GCT CAA AAT CAA AAT GAT-3′) and digested with HindIII and NheI. The resulting fragment was inserted between the HindIII and NheI sites of pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr.

pbphi-snaPr-MS2-yellow-Kr CD2-yellow-PP7-snaPr

A DNA fragment containing the *Kr* CD2 enhancer was amplified from genomic DNA using primers (5′-GGG GGA AGC TTG TAA GTT CCC ATA TTT CGG A-3′) and (5′-CCC CCG CTA GCT GGG TAC TTC GCT GAG TTG A-3′) and digested with HindIII and NheI. The resulting fragment was inserted between the HindIII and NheI sites of pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr.

pbphi-snaPr-MS2-yellow-iab5-yellow-PP7-snaPr

A DNA fragment containing the IAB5 enhancer was amplified from genomic DNA using primers (5′-GGG GGA AGC TTG TCG ACT ACT ACG CCG ATT CTG CTG G-3′) and (5′-CCC CCG CTA GCG TCG ACG GAG GCG GCA AAT GCA CAA A-3′) and digested with HindIII and NheI. The resulting fragment was inserted

between the HindIII and NheI sites of pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr.

pbphi-snaPr-MS2-yellow-sna shadow- gypsy insulator-yellow-PP7-snaPr

To selectively inhibit enhancer interactions with the *PP7-yellow* reporter gene, a DNA fragment containing the *gypsy* insulator was amplified from genomic DNA using primers (5′-GGG GGG CTA GCC TGG CCA CGT AAT AAG TGT G-3′) and (5′-CCC CCG CTA GCG TTG TTG GTT GGC ACA CCA C-3′) and digested with NheI. The resulting fragment was inserted into the unique NheI site of pbphi-snaPr-MS2-yellow-sna shadow-yellow-PP7-snaPr.

pbphi-sna shadow-linker-snaPr-PP7-yellow

Plasmid containing linked *MS2-yellow* and *PP7-yellow* reporter genes in asymmetric configuration was created as follows. First, a DNA fragment containing the shadow enhancer was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CAA GCT TGC ATT GAG GTG TTT TGT TGG-3′) and (5′-CCC CCG CGG CCG CGC TAG CTA AAT TCC GAT TTT TCT TGT-3′) and digested with NotI. The resulting fragment was inserted into the NotI site in pbphi-snaPr-PP7-yellow. Subsequently, a 1 kb linker sequence was amplified from p*galK* (Warming et al., 2005) using primers (5′-GGG GGG CTA GCC TGC CCT GCG CGA TTG ATT A-3′) and (5′-CCC CCG CTA GCT CAG CAC TGT CCT GCT CCT T-3′) and digested with NheI. The resulting fragment was inserted into the NheI site of the PP7 reporter vector.

pbphi-linker-snaPr-MS2-yellow

A DNA fragment containing the *sna* promoter was amplified from genomic DNA using primers (5′-GGG GGC TCG AGA AGC TTA GAT CTG ACA GCG GCG TCG GCA GAG G-3′) and (5′-CCC CCG GAT CCT GGT TGC GTT CTC AAC GAG A-3′) and digested with XhoI and BamHI. The resulting fragment was inserted between the XhoI and BamHI sites of pbphi-snaPr-MS2-yellow. Subsequently, a 1 kb linker sequence was amplified from p*galK* (Warming et al., 2005) using primers (5′-GGG GGA GAT CTC TGC CCT GCG CGA TTG ATT A-3′) and (5′-CCC CCA GAT CTT CAG CAC TGT CCT GCT CCT T-3′) and digested with BglII. The resulting fragment was inserted into the unique BglII site of the MS2 reporter vector.

pbphi-snaPr-MS2-yellow-sna shadow-linker-snaPr-PP7-yellow

A DNA fragment containing linker-snaPr-MS2-yellow was purified from pbphi-linker-snaPr-MS2-yellow by digesting with HindIII. The resultant fragment was inserted into the unique HindIII site in pbphi-sna shadow-linker-snaPr-PP7-yellow.

Supplemental references

Hannibal, R. L., Price, A. L., Parchem, R. J., and Patel, N. H. (2012). Analysis of *snail* genes in the crustacean Parhyale hawaiensis: insight into *snail* gene family evolution. Dev Genes Evol *222*, 139-151.

Warming, S., Costantino, N., Court, D. L., Jenkins, N. A., and Copeland, N. G. (2005). Simple and highly efficient BAC recombineering using *galK* selection. Nucleic Acids Res *33*, e36.