Establishing genetic transformation for comparative developmental studies in the crustacean *Parhyale hawaiensis*

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The amphipod crustacean *Parhyale hawaiensis* has been put forward as an attractive organism for evolutionary developmental comparisons, and considerable effort is being invested in isolating developmental genes and studying their expression patterns in this species. The scope of these studies could be significantly expanded by establishing means for genetic manipulation that would enable direct studies of gene functions to be carried out in this species. Here, we report the use of the *Minos* transposable element for the genetic transformation of *P. hawaiensis*. Transformed amphipods can be obtained from ≈30% of surviving individuals injected with both a *Minos* element carrying the *3xP3-DsRed* fluorescent marker and with mRNA encoding the *Minos* transposase. Integral copies of the transposon are inserted into the host genome and are stably inherited through successive generations. We have used reporter constructs to identify a muscle-specific regulatory element from *Parhyale*, demonstrating that this transformation vector can be used to test the activity of *cis*-regulatory elements in this species. The relatively high efficiency of this transgenic methodology opens new opportunities for the direct study of *cis*-regulatory elements and gene functions in *Parhyale*, allowing functional studies to be carried out beyond previously established model systems in insects.

arthropods | evolution | functional studies | transgenesis

Developmental biology relies on the use of model organisms, such as *Drosophila*, *Caenorhabditis*, or the mouse, to elucidate the mechanisms that control patterning and growth. Comparative studies are needed to extend this knowledge to other organisms, to uncover the extent to which these processes are conserved among different species, and to understand how these mechanisms change during evolution. Thus far, comparative developmental studies have relied primarily on the isolation of homologous genes, and comparisons of their sequences and expression patterns among different species. This approach has yielded valuable information concerning the possible roles played by gene duplication, changes in gene expression, and the evolution of protein sequences during evolution (e.g., refs. 1–4). However, the scope of this research has been limited by our inability to assess gene functions directly in nonmodel species. This limitation has precluded the characterization of new gene functions in nonmodel species and has biased comparative studies toward "candidate genes" that are well studied (in model organisms) and have relatively conserved functions.

To overcome these limitations, it is necessary to develop techniques for genetic manipulation that will allow functional studies in organisms that have not been genetically tractable in the past. Here, we focus on the amphipod crustacean *Parhyale hawaiensis*, an organism that has attracted attention for its potential to serve as a "model" crustacean in comparative developmental studies. *Parhyale* has a set of attributes that make it a very promising system for genetic-developmental research: it is easy to culture in large numbers in the laboratory, it has a relatively rapid lifecycle (≈2 months' generation time), and its transparent embryos are accessible at all stages, offering possibilities for genetic and developmental manipulations (5–7). Considerable effort has already been invested in describing embryonic development in this species, a number of tools and methods have been established for isolating developmental genes and studying their expression patterns (cDNA libraries, protocols for *in situ* hybridization, and immunohistochemistry), and an EST screen has been undertaken as a first step toward genomic-scale research (N. Patel, personal communication). Thus, establishing transgenesis in *Parhyale* would make this organism a very attractive system for comparative developmental research.

Establishing an efficient transformation methodology involves selecting and putting together a number of essential components, which include the following: (*i*) The transformation vector: Transposable elements that have the ability to transpose in a wide range of species are the most promising vectors for transforming new species of interest (8, 9). We have focused on the *Minos* transposable element (10), a member of the mariner/Tc1 family, which has been shown to be active in a wide range of animals and is able to carry relatively large insert sizes (11–17). (*ii*) A source of transposase: The generation of stable transformants requires a transient source of active transposase. Transposase can be provided in the form of a helper plasmid (expressing the transposase gene under a promoter that is active in the species of interest) or in the form of *in vitro*-produced transposase mRNA (18). (*iii*) A transformation marker: The identification of transgenic individuals, among a large number of untransformed animals, relies on the use of a dominant marker carried in the transposable element vector. Fluorescent proteins are widely used as transformation markers, because they can be scored easily and reliably in live animals (19). (*iv*) Active cis-regulatory elements: Regulatory elements that are active in the species of interest are required to express transformation markers and the transposase (when using a helper plasmid). An artificial *Pax6*-responsive promoter, named *3xP3* (20), has been shown to be active in a variety of animals (19, 21, 22). (*v*) An *in vivo* delivery system: Transformation requires efficient delivery of these components into the germ line of the targeted organism to generate individuals with transformed germ cells. The ability to culture and breed these animals to obtain transformed progeny is also essential. Microinjection of early embryos appears to be an effective way to target the germ line of *Parhyale* (5).

This report shows stable transformation in a noninsect arthropod species. We establish the use of a *Minos* transposable element carrying a *3xP3-DsRed* fluorescent marker as an efficient vector for transformation in *Parhyale*. We show that

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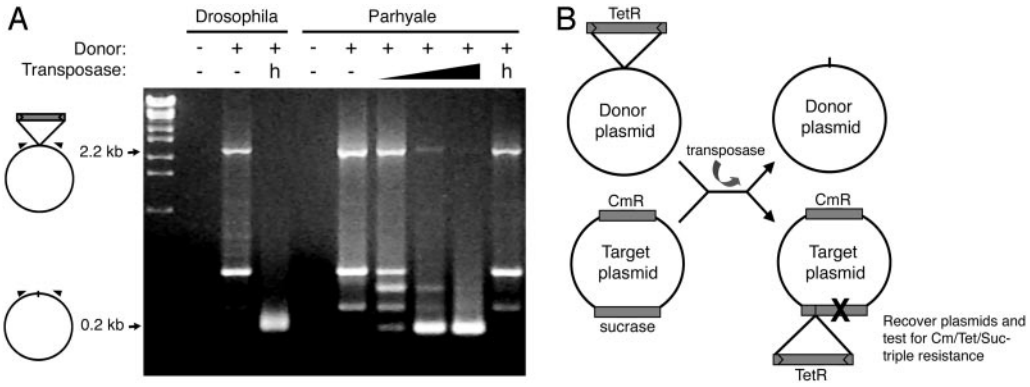

Fig. 1. Interplasmid assays for *Minos* excision and transposition in *Parhyale*. (*A*) Results of excision assay. Each lane represents the results of a single experiment, where a donor plasmid (carrying the *Minos* element) and a source of transposase have been injected into *Drosophila* or *Parhyale* embryos; transposase was provided in the form of a helper plasmid (h) or increasing amounts of transposase mRNA. PCR reactions were carried out by using primers flanking the *Minos* element in the donor plasmid. The 2.2-kb band results from amplification of the unexcised *Minos* element in the donor plasmid, whereas the 0.2-kb band results from amplification after *Minos* excision. Additional bands of intermediate size appear exclusively in lanes that contain the 2.2-kb band; these bands are probably due to the presence of the *Minos* long-inverted repeats in this band. (*B*) Illustration of the transposition assay. A donor plasmid with a *Minos* element carrying the tetracycline resistance gene (TetR), a target plasmid carrying the chloramphenicol resistance (CmR) and sucrase genes, and a source of transposase are coinjected into early *Parhyale* embryos. The plasmids are then recovered and screened for the presence of target plasmids carrying a *Minos* element inserted into the sucrase gene. The screen is carried out by triple selection for chloramphenicol resistance, tetracycline resistance, and tolerance to sucrose. The frequencies of *Minos* interplasmid transposition are given in Table 1.

this element can be used to test the activity of cis-regulatory elements and to generate new cell/tissue-specific markers in this organism.

Materials and Methods

***Parhyale* Rearing and Microinjection.** A culture of *P. hawaiensis* was initiated from a small number of individuals kindly provided by William Browne and Nipam Patel (6) and maintained as described in refs. 6 and 7. Embryos were collected and kept as described in ref. 7.

Microinjections were carried out by using a Narishige IM-300 microinjector with customized needles prepared from borosilicate glass capillaries (Harvard Apparatus GC100F-10) on a Sutter Instruments (Novato, CA) P-87 puller and a Narishige (Tokyo) EG-40 beveller. The small diameter (≈1–2 μm) and sharpness of the needle tip were critical for the survival rates of the injected amphipods. Embryos were processed a few at a time to avoid desiccation: one to four embryos were placed in a trough of 2% agarose (in artificial seawater) under a film of artificial seawater, injected under a compound microscope by using a Leitz M or a Narishige MO-108 micromanipulator, and then immediately transferred to a Petri dish with artificial seawater. All injected mixes were prepared in water containing 0.05% of the inert dye phenol red (Sigma).

Interplasmid Assays. Excision and transposition assays were carried out by using the donor plasmid pMiLRTetR(L), the target plasmid pBC/SacRB and the helper plasmid pHSS6hsILMi20 (14) injected at 150, 300, and 280 ng/μl, respectively. Capped mRNA encoding the *Minos* transposase was synthesized *in vitro* from the template plasmid pBlueSKMimRNA (17) and injected at 75, 150, or 300 ng/μl. The injections were carried out in 1- to 16-cell embryos, as described above. Purification of nucleic acids, PCR reactions, and recovery of transposition products were done as described in ref. 14.

***Parhyale* Transformation.** The donor plasmid pMi{3xP3-DsRed}, carrying the *Minos{3xP3-DsRed}* transposon, is a derivative of pMi{3xP3-EGFP} (17). The DsRedT1 coding sequence (23) was excised as an NcoI/NotI fragment from plasmid pSPDsRedT1 (5) and cloned into NcoI/NotI (partial) cut pMi{3xP3-EGFP}, replacing the EGFP coding sequence with that of DsRedT1. *Minos* transposase mRNA was prepared from the pBlueSKMimRNA plasmid as described in ref. 17.

Microinjections were carried out in one- to four-cell-stage embryos, targeting the blastomeres known to give rise to the germ line (5). The donor plasmid pMi{3xP3-DsRed} and *Minos* transposase mRNA were injected at 500 and 300 ng/μl, respectively. The injected individuals (G_0s) were then raised to adulthood and crossed with wild-type *Parhyale* of the opposite sex. The progeny of these crosses (G_1s) were scored as late embryos for DsRed expression under a Leica MZ FLIII fluorescence stereoscope equipped with a DsRed filter set (Chroma 41002c) or under a Leica DMIRB fluorescence inverted microscope by using the TRITC filter set.

Genomic DNA was prepared from pools of late embryos or single adults by using standard *Drosophila* DNA isolation protocols adjusted for smaller samples (24). Approximately 2 μg of genomic DNA were digested with SacI, size-separated by agarose gel electrophoresis, and blotted onto PROTRAN nitrocellulose membranes (Schleicher & Schuell). Radiolabeled DsRedT1 probe and hybridizations were carried out by using standard techniques in ref. 25 and the signal was visualized by using a PhosphorImager (Molecular Dynamics).

Cloning and Testing of *Parhyale cis*-Regulatory Element. A short fragment close to the amino terminus of an endogenous *hsp70* gene was amplified from *Parhyale* genomic DNA by using the degenerate primers Hsp70F1 (5′-ACIACITAYTCITGYGTIGG-3′) and Hsp70R1 (5′-AAIGGCCARTGYTTCAT-3′). Based on the sequence of this fragment, specific primers

Table 1. Frequency of *Minos* interplasmid transposition

Transposase mRNA, ng/μl	Disrupted targets, %
0	0
75	0.006
150	0.04
300	0.4

Transposition rates were quantified as the proportion of triple-resistant TetR/CmR/suc colonies (disrupted target plasmids) over the total number of CmR colonies (number of target plasmids screened).

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Table 2. Summary of *Parhyale* transformation experiments

Transposon name	Transposon length, kb	Concentration of donor plasmid, ng/μl	Concentration of transposase mRNA, ng/μl	Injected G_0 embryos	Surviving and fertile G_0s	G_0s with transformed progeny*	Transformation efficiency,† %	No. of insertions‡
Mi{3×P3-DsRed}	2.0	500	300	≈250	16	4	25	>10
Mi{3×P3-DsRed; reporter constr. 1}§	4.5	300	100	≈50	22	6	27	n/a
Mi{3×P3-DsRed; PhMS-DsRed}	5.3	300	100	≈200	10	3	30	1–5
Mi{3×P3-DsRed; reporter constr.2}§	5.7	300	100	≈80	7	2	29	n/a

n/a, data not available.
*Transformed progeny identified based on *3×P3-DsRed* expression.
†Transformation efficiency is percentage of fertile G_0s producing transformed G_1 progeny.
‡Number of *Minos* insertions determined by Southern analysis.
§Other reporter constructs (not mentioned in the text).

PhHsR2 (5′-TGTCGTCCTGTTGCCCTGGTC-3′) and PhHsR3 (5′-ACCTTGCCATGCTGGAACACC-3′) were designed, and a fragment containing ≈2.5 kb of the gene's upstream sequence was recovered by PCR on *Parhyale* genomic DNA, where primer PhHsR2 had hybridized spuriously in the upstream sequence. The sequence upstream of the ATG start codon was placed in front of a *DsRedT1/SV40polyA* reporter cassette, and the whole transcription unit was incorporated into the *Minos{3xP3-DsRed}* transposon. Cloning details are available on request. The resulting plasmid pMi{3xP3-DsRed; PhMS-DsRed} and *Minos* transposase mRNA were coinjected into one- and two-cell stage embryos at 300 and 100 ng/μl, respectively.

Results

Interplasmid Assays Demonstrate *Minos* Activity in *Parhyale*. Establishing transformation in a new organism requires often laborious and time-consuming experiments to test the effectiveness of transposable element vectors and sources of transposase in the organism of interest. Plasmid-based assays have been devised to facilitate and speed up this process. We have used two rapid and sensitive assays, the excision and transposition assays (14), to test the activity of the *Minos* transposable element in the cellular environment of *Parhyale*.

The excision assay tests the ability of an element to excise from a plasmid in the relevant cellular environment, when provided with a suitable source of transposase. This assay involves injecting a "donor" plasmid carrying the transposable element, with or without a source of transposase, into early *Parhyale* embryos, incubating these embryos for 24 h, extracting DNA, and carrying out PCR reactions to determine whether the element has excised from the donor plasmid (by using primers flanking the transposon on the donor plasmid). Injections with *Minos* transposase mRNA supported excision of the *Minos* element from the donor plasmid in a dose-dependent manner, with higher concentrations of injected mRNA resulting in increased excision, as monitored by the conversion of the 2.2-kb PCR band into the diagnostic 0.2-kb band (Fig. 1*A*). In contrast, the diagnostic band was not detected in the absence of a transposase source, showing that *Minos* excision is transposase-dependent and suggesting that there is no endogenous source of transposase in *Parhyale* embryos that could cross-mobilize *Minos* (Fig. 1*A*). Injections with a helper plasmid commonly used in insects (11, 14, 17) also showed no evidence of excision (Fig. 1*A*), suggesting that the *Drosophila hsp70* promoter used in this plasmid to express the transposase is probably inactive in *Parhyale*.

The transposition assay tests the ability of an element to be mobilized from its original site in the donor plasmid to a new site

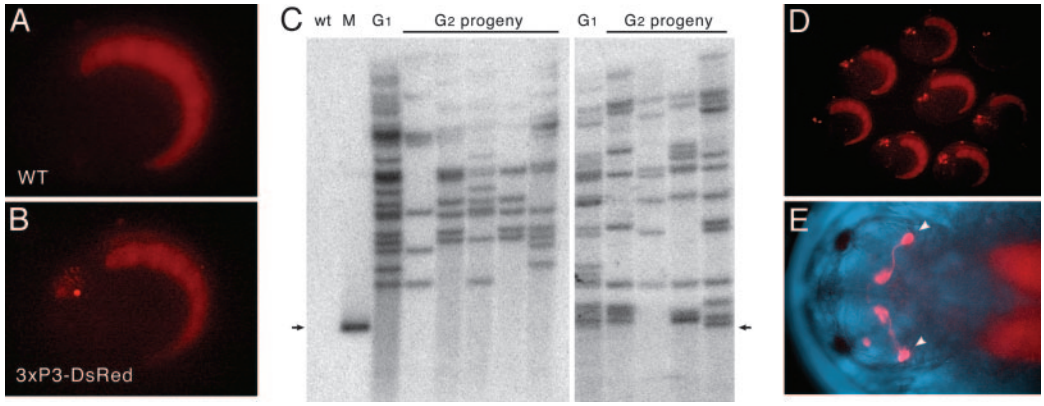

Fig. 2. Transgenesis in *Parhyale* by using the *Minos{3xP3-DsRed}* element. (*A* and *B*) Lateral views of late *Parhyale* embryos, visualized on a fluorescence microscope with filter settings for DsRed. (*A*) Normal, untransformed individuals have a dorsal crescent of diffuse fluorescence, which is due to autofluorescence of the yolk. (*B*) Individuals transformed with the *Minos{3xP3-DsRed}* element exhibit an additional spot of strong fluorescence in the head. (*C*) Genomic Southern blots from single *Parhyale* individuals, probed for the presence of *Minos{3xP3-DsRed}* insertions. Each band corresponds to a different *Minos* insertion. The first lane (wt) contains genomic DNA from untransformed individuals. The second lane (M) contains digested *Minos{3xP3-DsRed}* plasmid DNA; the 1.6-kb band represents the minimum band size expected for integral *Minos* insertions (marked by arrows). The other lanes show individual transformed G_1s and a number of their G_2 progeny. (*D*) Progeny of a single G_0 parent carrying multiple *Minos* insertions; all embryos are positive for *3xP3*-driven fluorescence. (*E*) Localization of *3xP3-DsRed* fluorescence in a strongly expressing transgenic embryo. The bilateral fluorescent spots (white arrowheads) lie posterior to the compound eyes (dark spots) and appear to extend projections toward the brain. The image shows a dorsal view of a late embryo, with merged brightfield and fluorescence (red) channels.

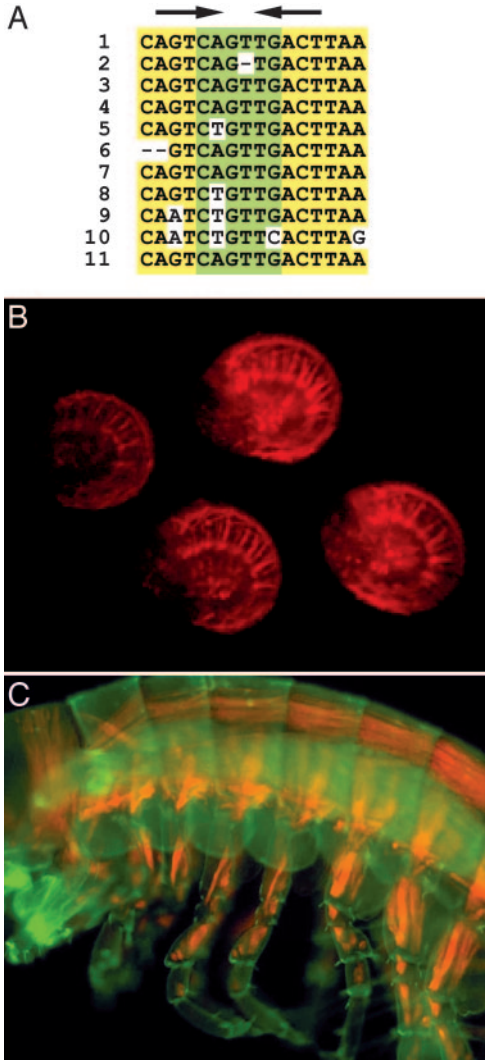

Fig. 3. Identification of a *Parhyale* muscle-specific cis-regulatory element. (*A*) Alignment between the 11 tandem repeats contained in the *PhMS* fragment. Conservation within the putative b-HLH binding site (CAGTTG) is highlighted in green, and conservation in other parts is highlighted in yellow. Arrows indicate inverted repeat structure. (*B*) Population of late embryos transformed with the *PhMS-DsRed* construct, showing fluorescence in muscles. (*C*) Juvenile transformed with the *PhMS-DsRed* construct, showing muscle-specific fluorescence in red. Fluorescence in the green channel is due to autofluorescence of the cuticle.

in a "target" plasmid. This assay involves coinjecting the donor and target plasmids, with or without a source of transposase, into early embryos of *Parhyale*, incubating these embryos for 24 h, extracting DNA, transforming bacteria with the recovered plasmids, and screening these bacterial clones for the presence of target plasmids containing the transposed *Minos* element. The screen is carried out by positive selection for the chloramphenicol-resistance (*CmR*) and tetracycline-resistance (*TetR*) marker genes carried by the target plasmid and the *Minos* transposon, respectively; further specificity is achieved by selecting for the insertional inactivation of the sucrase gene in the target plasmid (Fig. 1*B*). Using this assay, we detected interplasmid transposition of the *Minos* element exclusively in the presence of transposase mRNA (Table 1). The proportion of target plasmids hit by the transposon depended on the concentration of the injected mRNA, with a doubling dose of mRNA causing ≈10 times higher frequencies of insertion (over a range of 75–300 ng/μl of transposase mRNA; Table 1). Multiple independent transposition events were documented by restriction digests of purified plasmids from triple resistant colonies (data not shown).

Taken together, these results indicate that the *Minos* element can be efficiently mobilized in early *Parhyale* embryos, in a controlled transposase-dependent fashion, by using *in vitro* synthesized transposase mRNA.

Transformation of *Parhyale* by Using *Minos{3xP3-DsRed}*. Establishing transgenesis also requires a marker gene for identifying transformed individuals. We combined the coding sequence of the DsRedT1 fluorescent protein (23) with the artificial 3xP3 promoter (20) and introduced this marker gene into the *Minos* vector to generate *Minos{3xP3-DsRed}*. We used DsRedT1 because, compared with EGFP, it shows a stronger signal and lower background/autofluorescence in *Parhyale* embryos (data not shown; also see ref. 5). The artificial *Pax6*-responsive *3xP3* promoter was selected for its potential to drive expression in a wide range of animals (19, 21, 22).

We microinjected early *Parhyale* embryos with a plasmid carrying the *Minos{3xP3-DsRed}* transposon together with *Minos* transposase mRNA. The individuals raised from these injected embryos (G_0s) were crossed, and their progeny (G_1s) were screened for DsRed fluorescence. Among the surviving G_0s, 25% gave rise to progeny that had a pair of bilaterally symmetric spots of red fluorescence in the head region (Table 2 and Fig. 2 *A* and *B*). This fluorescence was detected in several different rounds of injections but was never observed in wild-type *Parhyale* embryos, suggesting that it is associated with the expression of the *3xP3-DsRed* marker.

Integration of the *Minos* element into the genome of fluorescent G_1 progeny was confirmed by PCR with *Minos*-specific primers (data not shown) and by Southern blots. Genomic DNA was digested with SacI and probed with the DsRedT1 coding sequence, resulting in a single band per *Minos* insertion. The insertions detected by Southern blots varied in length, depending on the flanking genomic sequence, and all of them were longer than 1.6 kb, the minimum size expected for integral copies of the *Mi{3xP3-DsRed}* element (Fig. 2*C*). Surprisingly, Southern blots carried out on pools of transgenic G_1 siblings revealed that their G_0 parents carried tens of *Minos* insertions in their germ lines (data not shown). This finding was consistent with the observation that all these G_0s gave rise exclusively to fluorescent G_1 progeny (Fig. 2*D*), indicating that each G_1 had inherited a subset of these insertions.

To analyze the segregation and stability of these *Minos* insertions from one generation to the next, we crossed transgenic G_1 amphipods to wild-type animals to obtain transgenic G_2 progeny. Each G_1 parent and individual G_2 progeny were then subjected to Southern analysis and were compared with each other for the number and size of their insertions. In all cases studied, the G_2 progeny had inherited different subsets of the *Minos* insertions found in their parent (Fig. 2*C*). Most importantly, no new bands were ever detected in the G_2 progeny, demonstrating the stability of *Minos* insertions in the amphipod genome (Fig. 2*C*). These observations are consistent with the results of the interplasmid assays (Fig. 1 and Table 1), indicating that *Minos* mobility in *Parhyale* strictly depends on an exogenously supplied source of transposase. The stability of *Minos* insertions is also reflected in our ability to maintain individual transgenic lines for several generations.

The fluorescence associated with the expression of the *3xP3-DsRed* marker persists through most of *Parhyale* development, from late embryos to adults, and can serve as a reliable marker at any of these stages. It is interesting to note, however, that unlike *3xP3*-driven expression in many insects (19), the DsRed-expressing cells in *Parhyale* do not colocalize with the photoreceptors of the developing compound eyes (Fig. 2*E*). In strongly

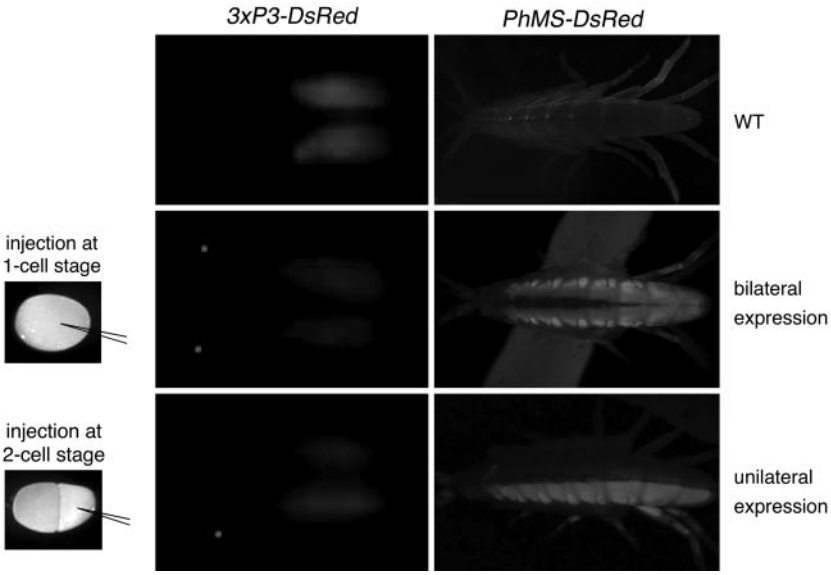

Fig. 4. Early transgene expression in injected G_0 amphipods. Fluorescence in G_0 individuals injected with *Minos* elements carrying the *3xP3-DsRed* construct (shown as late embryos) or the *PhMS-DsRed* construct (shown as adults). Fluorescence is compared among uninjected individuals (WT) and individuals injected as one-cell stage or two-cell stage embryos. Injection of a single blastomere at the two-cell stage gives rise to unilateral fluorescent patterns; this pattern is consistent with lineage tracing experiments showing that each of these blastomeres gives rise to the left or the right half of the ectoderm and mesoderm (5). In contrast, injections at the one-cell stage can give rise to individuals with bilateral fluorescence (as shown here) and to individuals with unilateral fluorescent patterns (presumably due to later integration events). All images are dorsal views.

expressing lines, it is possible to see these cells sending cellular projections toward the brain (Fig. 2*E*), suggesting that they might correspond to yet uncharacterized sensory/photoreceptive cells.

Identification of *Parhyale* cis-Regulatory Elements by Using Reporter Constructs. Transgenesis could be applied to test the activity of putative cis-regulatory sequences in *Parhyale*, provided that the transformation vector is capable of carrying a reporter construct in addition to the *3xP3-DsRed* marker gene. To test the feasibility of such experiments, we cloned genomic fragments that lie upstream of *Parhyale* hsp70 family genes and assessed their ability to drive the expression of DsRed. The reporter constructs we made did not contain any heterologous core promoter sequences but relied entirely on the activity of the regulatory sequences upstream of the putative translation start codon in these fragments. These constructs were then incorporated in the *Minos*{*3xP3-DsRed*} vector and injected with transposase mRNA into early *Parhyale* embryos (Table 2).

With one of these genomic fragments, we obtained a consistent pattern of strong *DsRed* fluorescence in muscles, in addition to the fluorescence attributed to the *3xP3-DsRed* marker (in three of three independent transgenic lines). This fragment, named *PhMS* (for *P. hawaiensis* muscle specific), was able to drive expression in all muscles, from ≈70% of embryogenesis (when the muscle fibers are forming) to adulthood (Fig. 3 *B* and *C*). Examining the DNA sequence of *PhMS* revealed an array of 11 almost-perfect tandem repeats of a 16-bp sequence (Fig. 3*A*). These repeats contain the core sequence CAGTTG, which can serve as a binding site for myogenic b-HLH transcription factors (26). The presence of these binding sites is consistent with the specific activation of this element in muscles. This muscle-specific cis-regulatory element could be used to drive the expression of a second transformation marker in *Parhyale*, in experimental settings requiring multiple transgenes.

These experiments demonstrate that the use of reporter constructs to study cis-regulatory elements is now an available experimental option in *Parhyale*. In several applications involving transgenesis, however, the ability to work with single transgene insertions is important (e.g., to avoid unwanted position or dosage effects). In an effort to decrease the number of *Minos* insertions present in each transformed amphipod, we carried out all of the injections with reporter constructs by using lower concentrations of both the donor plasmid and the transposase mRNA (Table 2). Southern blots of three different pools of transgenic G_1 siblings carrying the *3xP3-DsRed* and *PhMS-DsRed* transgenes, showed that they contained significantly fewer *Minos* insertions (Table 2). Two of the G_0 parents transmitted a single insertion to a proportion of their G_1 progeny, whereas one transmitted approximately five insertions, giving rise exclusively to transformed progeny. Importantly, the transformation efficiency in these experiments was as high as with the smaller transposon (Table 2), demonstrating that *Minos* is able to carry relatively large insert sizes without a significant change in transformation rates.

Early Transgene Expression in the Injected G_0 Amphipods. Raising a large number of injected individuals, mating them, and screening their progeny are among the most time-consuming steps in the process of generating transgenic amphipods, requiring ≈2–3 months from injection to obtaining transformed G_1 embryos or juveniles. During the course of these experiments, however, we noticed that it is possible to have an early indication as to the success of a transformation experiment and to preselect individuals that are likely to produce transformed progeny by looking for the expression of the transgenes in the injected G_0 individuals themselves. By screening late G_0 embryos, we noticed that ≈33% of those injected at the one-cell stage and ≈24% of those injected at the two-cell stage showed fluorescence in the characteristic *3xP3* or reporter patterns described earlier. The majority of these individuals that survived produced transformed progeny, indicating that fluorescence observed in G_0s is a good predictor of germ-line transformation.

Fluorescent G_0 individuals are likely to be mosaically transformed, depending on when and where in the embryo transposition has taken place. We observed that some of the embryos

injected at the two-cell stage, when each blastomere is fated to give rise only to the left or to the right half of the ectoderm and mesoderm (5), exhibited unilateral expression of the transgenes (Fig. 4). In contrast, some of the embryos injected at the one-cell stage showed bilateral fluorescence (Fig. 4).

The prescreening of G_0s for fluorescence just a few days after the injections could prove to be an important feature of transformation experiments in *Parhyale*, because it allows us to have an early warning as to the potential success or failure of an experiment or a first glimpse on the activity of a reporter construct months before we are able to obtain the definitive results of the experiment. This advantage is unique to *Parhyale* among arthropods where genetic transformation has been achieved and is probably due to our ability to obtain integration events in very early stages, giving rise to a very low degree of mosaicism in this organism.

Future Prospects. Our experiments demonstrate that transgenic lines can be obtained reliably and efficiently in *Parhyale* by using the *Minos* transposable element as a vector. The high transformation frequencies obtained, where ≈30% of surviving G_0s produce transformed progeny, now allow direct functional studies to be carried out in this organism. We have shown that *Minos*-mediated transgenesis can be used to test the activity of cis-regulatory elements in *Parhyale*, using reporter constructs, and we are confident that the isolation of new tissue-specific or inducible cis-regulatory elements will allow us to carry out gain-of-function experiments by gene misexpression in the future. Combined with loss-of-function studies by using RNA interference (which have recently been achieved in crustaceans; ref. 27), these approaches now provide an opportunity to assess gene functions in crustaceans directly without the need to extrapolate from experiments carried out in *Drosophila*. These advances are particularly valuable for studying functions that have changed during evolution and need to be examined in the context of individual species of interest.

The relatively high efficiency of this transgenic methodology could also allow genetic screens to be carried out in *Parhyale*, providing the opportunity to identify and study genes on the basis of their expression patterns and functions in this organism.

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