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Science **343**, 788 (2014);
DOI: 10.1126/science.1243529

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A Common Cellular Basis for Muscle Regeneration in Arthropods and Vertebrates

Nikolaos Konstantinides[1,2,3]* and Michalis Averof[1,3,4]*

Many animals are able to regenerate amputated or damaged body parts, but it is unclear whether different taxa rely on similar strategies. Planarians and vertebrates use different strategies, based on pluripotent versus committed progenitor cells, respectively, to replace missing tissues. In most animals, however, we lack the experimental tools needed to determine the origin of regenerated tissues. Here, we present a genetically tractable model for limb regeneration, the crustacean *Parhyale hawaiensis*. We demonstrate that regeneration in *Parhyale* involves lineage-committed progenitors, as in vertebrates. We discover *Pax3/7*-expressing muscle satellite cells, previously identified only in chordates, and show that these cells are a source of regenerating muscle in *Parhyale*. These similarities point to a common cellular basis of regeneration, dating back to the common ancestors of bilaterians.

Regeneration relies on specific populations of progenitor cells, which serve as the source of new cells in the regenerated tissues. Progenitors may be undifferentiated stem cells or differentiated cells that have the capacity to dedifferentiate, proliferate, and redifferentiate to produce new functionally specialized cells (*1*). Their identity and degree of commitment are relevant for addressing fundamental questions in regenerative biology, such as the role of cellular memory and plasticity during regeneration.

Although the capacity to regenerate is widespread in animals, the evolutionary origins of regeneration remain unexplored. Among animals with extensive regenerative abilities, progenitor cells have been identified only in planarians, vertebrates, and cnidarians. These animals use different strategies to replace missing tissues. In planarians, all tissues regenerate from a common pool of pluripotent progenitor cells (*2*, *3*), whereas in vertebrates, different cell types arise from distinct progenitors (*4*–*7*). Cnidarians use progenitor cells that are specialized to different degrees in different species (*8*–*10*). In most animal phyla, we lack the tools to rigorously identify these progenitor cells and to map their lineage commitments. Thus, we do not know if similar types of progenitors exist across diverse phyla, whether there are shared regenerative strategies, and which of these strategies is most ancient.

Here, we establish the crustacean *Parhyale hawaiensis* as a genetically tractable model for limb regeneration. *Parhyale* can fully regenerate all amputated appendages throughout their lifetime (Fig. 1A). Regeneration restores all of the cell types that can be observed in adult limbs, including epidermis, neurons, and muscles. Several of these cell types can be visualized with the use of gene trap lines and reporter constructs (fig. S1). The speed of regeneration varies among individuals and correlates with the frequency of moulting (fig. S2). Young adults typically need 5 to 8 days to regenerate a patterned thoracic limb, such as the one shown in Fig. 1B.

We used cellular, morphological, and genetic markers to define a timeline for limb regeneration in *Parhyale* young adults (Fig. 1C). Wound closure takes place within a day of amputation, as seen by the development of a melanized scab at the wound surface (Fig. 1, D to D″). This is followed by the formation of a blastema, consisting of proliferating cells underlying the wound, which can be visualized by EdU incorporation 2 to 3 days after amputation (Fig. 1, E to E″, and fig. S3). Approximately 4 to 6 days after amputation, the distal tip of the newly regenerated appendage becomes apparent, visualized with the *Distal*^{DsRed} gene trap (*11*) (Fig. 1, F to F″, and movie S1). During the following days, the regenerating limb grows in size, acquiring its characteristic pattern of limb segments. The axis of the limb is folded (often S-shaped) to accommodate the growing appendage in the limited space available within the exoskeleton of the amputated limb (Fig. 1B). The fully regenerated limb is revealed during the next moult. Muscles, visualized with the *PhMS-DsRed* reporter (*12*), regenerate after the epidermis, within a week from moulting (fig. S1B′).

To address whether pluripotent or lineally restricted progenitor cells give rise to the new tissues of regenerated limbs, we marked individual cell lineages in early embryos and followed their regenerative contributions in adults. *Parhyale* embryos have a stereotypic early cell lineage: At the eight-cell stage, three blastomeres (El, Er, and Ep) are fated to produce the ectoderm, three (ml, mr, and Mav) give rise to mesoderm, one (en) produces cells that localize in the gut, and one (g) gives rise to the germ line (*13*) (Fig. 2A). We stably marked each of these blastomere lineages by injecting early embryos with a *Minos* transposon carrying a fluorescence marker [enhanced green fluorescent protein (EGFP) or DsRed] driven by the *PhHS* promoter, which is activated in all cell types after heat shock (*14*). Integration of the transposon in the genome of individual blastomeres produced mosaic animals in which the marked lineages could be identified (Fig. 2, A to C). By injecting ~4000 early embryos and screening the survivors as late embryos and juveniles, we identified 79 individuals in which specific cell lineages were marked. These individuals were raised to adulthood, subjected to limb amputations, and allowed to regenerate. The contribution of each marked lineage was then assessed on the regenerated limbs (Fig. 2, B and C, and supplementary materials and methods). Results are summarized in Fig. 2D.

We consistently observed that the descendants of blastomeres El, Er, and Ep gave rise to regenerated ectodermal cell types, namely epidermis and neurons, but never to mesodermal cells, such as muscle (Fig. 2B). Conversely, the descendants of blastomeres ml, mr, and Mav gave rise to regenerated muscle, but not to epidermis or neurons (Fig. 2C). We did not observe any contribution from the en and g lineages to regenerated appendages. We found no blastomere lineages contributing to both ectodermal and mesodermal cell types, implying an absence of pluripotent progenitors and of transdifferentiation across ectoderm and mesoderm. These results demonstrate that, in *Parhyale*, regenerative progenitor cells have a developmental potential that is restricted with respect to germ layers. These progenitors may be stem cells or differentiated cells that have retained the capacity to proliferate.

We also found that marked cell lineages only contribute to regenerating limbs locally, within the body region that was originally populated by each lineage: El and ml contribute to regeneration of appendages on the left side of the body, Er and mr to appendages in the right, Ep to posterior thoracic appendages, and Mav to regenerated antennae (Fig. 2D). The fact that no lineage contributed to all limbs suggests that there is no central pool of progenitor cells for the whole body—the progenitor cells reside locally.

Our experiments show that *Parhyale* limb regeneration involves at least two distinct types of progenitor cells: ectodermal and mesodermal. These progenitors derive from distinct cell lineages, have predetermined (lineally restricted) regenerative capacities, and are present near the regenerating tissue. This is highly reminiscent of vertebrates—in which distinct progenitors give rise to different ectodermal and

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mesodermal cells during limb, fin, and tail regeneration (*4*–*6*)—and differs from the strategy used by planarians involving pluripotent stem cells (*2*, *3*).

While studying the *PhMS-DsRed* reporter line, where DsRed is expressed in muscles (*12*), we noticed another DsRed-expressing cell type that is tightly associated with muscles within each limb (Fig. 3A). These compact cells reminded us of the satellite cells, which can serve as muscle progenitors during muscle maintenance, growth, and regeneration in vertebrates (*4*, *15*–*19*). Satellite cells are characterized by expression of Pax3/7 family transcription factors (*19*, *20*). Using two antibodies that recognize Pax3/7 proteins in a wide range of animals (*21*) and in situ hybridization, we saw that the muscle-associated cells of *Parhyale* also express Pax3/7 (Fig. 3, A to C, and fig. S6). The expression level is variable but is consistently above background in these cells (fig. S7). By genetically marking mesodermal cell lineages (as described earlier), we could demonstrate that these Pax3/7-positive cells have a mesodermal origin (Fig. 3D). Based on these characteristics, we refer to these mesodermal Pax3/7-positive cells as satellite-like cells (SLCs).

Consistent with a possible involvement of SLCs in regeneration, Pax3/7-positive cells proliferate in the amputated limb stump and contribute to the regeneration blastema 2 to 3 days after amputation (Fig. 3E). To directly test whether SLCs can act as muscle precursors during regeneration, we transplanted isolated SLCs from the limbs of *PhMS-EGFP* transgenic animals into the amputated limbs of wild-type animals and examined the contribution of those EGFP-marked cells after regeneration (Fig. 4A and supplementary materials). The EGFP-marked cells expressed Pax3/7 (fig. S7) and were free of muscle cells. Out of 72 limbs that received marked SLCs, 12 contained one or a few EGFP-expressing muscle fibers after regeneration (Fig. 4, B to D). To test whether muscle fibers could derive from unlabeled cells that were inadvertently transplanted together with the EGFP-marked SLCs, we also transplanted unlabeled (non–EGFP-expressing) cells from *PhMS-EGFP* donors to wild-type recipients; no EGFP-expressing muscles were observed after regeneration in 34 limbs. These results demonstrate that SLCs are capable of functioning as progenitor cells for regenerated muscle. Our experiments do not exclude the involvement of other types of muscle progenitors, in addition to SLCs.

Our study has revealed a number of key similarities between arthropod and vertebrate

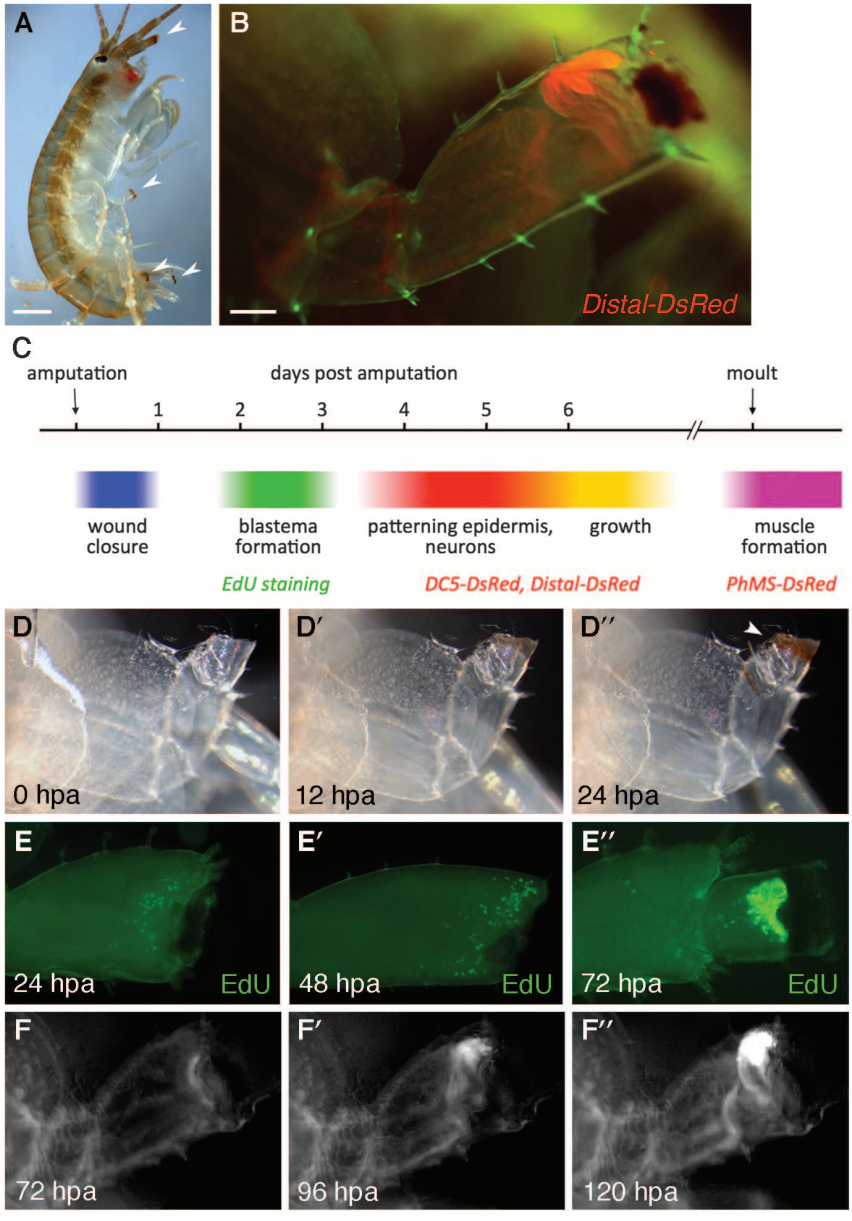
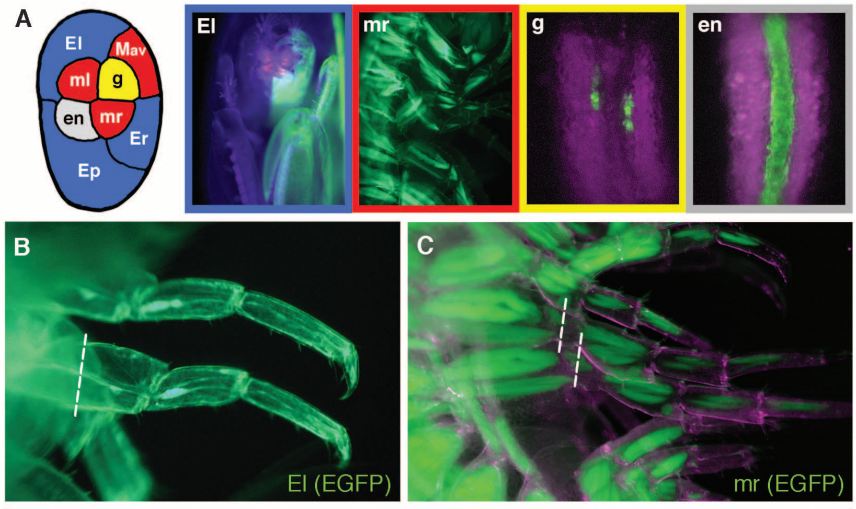


Fig. 1. Limb regeneration in *Parhyale*. (**A**) Adult *Parhyale* with amputated antenna, thoracic leg, and uropods (arrowheads). Scale bar, 1 mm. (**B**) Regenerating thoracic leg visualized by the *Distal*^{DsRed} gene trap (*11*) (red), within the cuticle of the amputated limb (green autofluorescence). Scale bar, 100 μm. (**C**) Timeline of *Parhyale* leg regeneration, representing the average speed of regeneration in ~6-month-old adults. (**D** to **F″**) Different phases of leg regeneration are characterized by melanized scab formation at the wound surface (arrowhead) (D, D′, D″); proliferating cells in the blastema, labeled by EdU (green) (E, E′, E″); and morphogenesis of the leg visualized by the *Distal*^{DsRed} gene trap (F, F′, F″) (time-lapse shown in movie S1). hpa, hours postamputation.



D

Marked lineages	Regenerated tissues	Ectoderm Left	Ectoderm Right	Ectoderm Posterior	Mesoderm Left	Mesoderm Right	Mesoderm Anterior
	El (n=19)	26/27	0/27	0/7	0/20	0/19	0/23
	Er (n=18)	0/25	21/21	0/10	0/19	0/18	0/21
	Ep (n=11)	0/18	0/21	10/10	0/14	0/20	0/21
	ml (n=12)	0/6	0/9	0/8	10/11	0/11	0/10
	mr (n=10)	0/13	0/7	0/9	0/14	11/11	0/8
	Mav (n=6)	0/7	0/6	0/8	0/11	0/10	12/12
	en (n=13)	0/21	0/38	0/22	0/25	0/25	0/34
	g (n=8)	0/17	0/21	0/16	0/18	0/14	0/21

Fig. 2. Contribution of marked cell lineages to regenerated tissues. (**A**) At the eight-cell stage, the *Parhyale* embryo has blastomeres contributing specifically to the ectoderm (blue), mesoderm (red), gut (gray), and germ line (yellow) (*13*). Images show EGFP-marked lineages in the ectoderm (El, ventral view), mesoderm (mr, lateral view), germ line (g, dorsal view), and gut (en, dorsal view). (**B** and **C**) EGFP-marked mosaics showing the contributions of the El and mr lineages during regeneration. After amputation, the El lineage contributed to epidermis and neurons (B), whereas the mr lineage contributed to muscles (C). Amputation planes are marked by dashed lines; limbs are visualized by reflected light in (C) (magenta). (**D**) Summary of lineage contributions to regenerated limb tissues (number of marked limbs/number of limbs tested). n, number of animals in which the particular blastomere lineage was labeled.

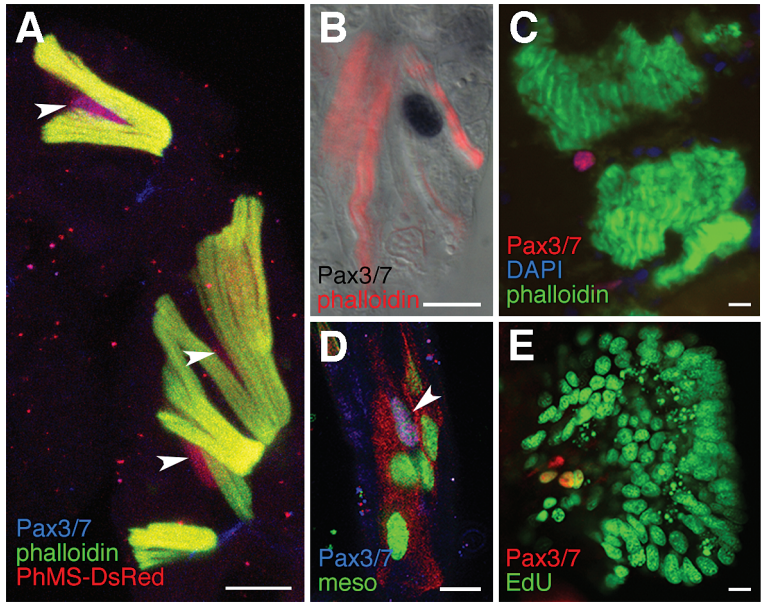


Fig. 3. Satellite-like cells (SLCs) in *Parhyale*. (**A**) SLCs (arrowheads) in the ischium and merus of a thoracic limb, from a late embryo carrying the *PhMS-DsRed* reporter, stained with antibodies for DsRed (red) and Pax3/7 (blue) and with phalloidin to mark muscles (green). The *PhMS* regulatory sequence carries putative MyoD binding sites (*12*). (**B**) SLC in *Parhyale* limb stained with an antibody for Pax3/7 (black) and phalloidin (red). SLC nuclei have a diameter of 5 to 10 μm and occupy most of the cell's volume. (**C**) SLC in an adult *Parhyale* limb sectioned transversely and stained with an antibody for Pax3/7 (red), 4′,6-diamidino-2-phenylindole (DAPI) (blue), and phalloidin (green). SLC nuclei are associated with muscle fibers in late embryos, juveniles, and adults. (**D**) SLCs have a mesodermal origin, seen by the colocalization of Pax3/7-positive nuclei (blue) with a marker for mesoderm (arrowhead) (seven out of seven SLCs scored in late embryos). Mesodermal cells were clonally marked with nuclear-localized EGFP (green) and membrane-localized tdTomato (red). (**E**) Pax3/7-expressing cells (red) contribute to the regeneration blastema (EdU, green). The amputation plane was at the edge of the blastema, on the right. Four Pax3/7-positive cells are seen in the proximal part of the blastema, three of which are positive for EdU (on average, 2.5 Pax3/7-positive cells per blastema, scored on 12 blastemas). Individual fluorescence channels for these images are shown in fig. S4. Scale bars, 10 μm.

limb regeneration. First, regenerated ectodermal and mesodermal cells derive from distinct progenitor cells, rather than a common pool of pluripotent progenitors. Second, the progenitors are present locally in the amputated limb stump. Third, muscle regeneration involves a similar progenitor cell type, the satellite-like cells, which are tightly associated with muscles before regeneration and contribute to newly formed muscle fibers. We suggest that these similarities reflect cell types and repair strategies that evolved in the common ancestors of protostomes and deuterostomes in Precambrian times.

References and Notes

1. E. M. Tanaka, P. W. Reddien, *Dev. Cell* **21**, 172–185 (2011).
2. P. W. Reddien, A. Sánchez Alvarado, *Annu. Rev. Cell Dev. Biol.* **20**, 725–757 (2004).
3. D. E. Wagner, I. E. Wang, P. W. Reddien, *Science* **332**, 811–816 (2011).
4. C. Gargioli, J. M. Slack, *Development* **131**, 2669–2679 (2004).
5. M. Kragl *et al.*, *Nature* **460**, 60–65 (2009).
6. S. Tu, S. L. Johnson, *Dev. Cell* **20**, 725–732 (2011).
7. Y. Rinkevich, P. Lindau, H. Ueno, M. T. Longaker, I. L. Weissman, *Nature* **476**, 409–413 (2011).
8. H. R. Bode, *J. Cell Sci.* **109**, 1155–1164 (1996).
9. W. A. Müller, R. Teo, U. Frank, *Dev. Biol.* **275**, 215–224 (2004).
10. T. C. G. Bosch, *Bioessays* **31**, 478–486 (2009).
11. Z. Kontarakis *et al.*, *Development* **138**, 2625–2630 (2011).

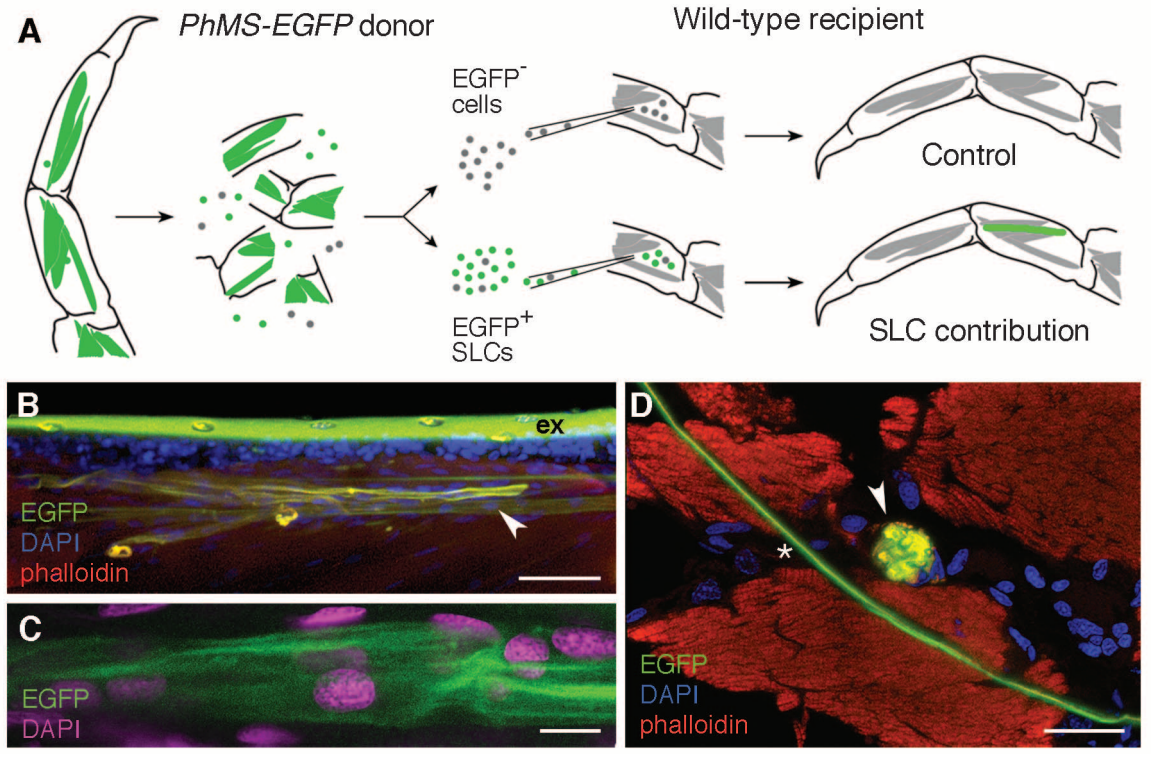


Fig. 4. Transplanted SLCs contribute to muscle regeneration. (**A**) Schematic representation of the transplantation experiment designed to test the contribution of SLCs to regenerated muscle. EGFP-marked SLCs were taken from the limbs of transgenic donors carrying the *PhMS-EGFP* construct (*12*) and transplanted into freshly amputated limbs of nontransgenic recipients. In control experiments, non–EGFP-expressing cells were transplanted using the same donor and recipient lines. (**B**) SLC-derived muscle fibers were detected by EGFP fluorescence (arrowhead, green) in recipient limbs also stained with phalloidin (red) and DAPI (blue). ex, autofluorescence of the chitinous exoskeleton. Scale bar, 50 μm. (**C**) Higher-magnification view of EGFP-expressing muscle. Scale bar, 10 μm. (**D**) Transverse section of a regenerated recipient limb, showing an EGFP-expressing muscle fiber (arrowhead), stained with an antibody for EGFP (green) and phalloidin to mark muscles (red). The asterisk marks autofluorescence from a slice of cuticle that was displaced during sectioning. Scale bar, 20 μm.

12. A. Pavlopoulos, M. Averof, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7888–7893 (2005).
13. M. Gerberding, W. E. Browne, N. H. Patel, *Development* **129**, 5789–5801 (2002).
14. A. Pavlopoulos *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13897–13902 (2009).
15. J. I. Morrison, P. Borg, A. Simon, *FASEB J.* **24**, 750–756 (2010).
16. A. Sacco, R. Doyonnas, P. Kraft, S. Vitorovic, H. M. Blau, *Nature* **456**, 502–506 (2008).
17. C. Lepper, T. A. Partridge, C. M. Fan, *Development* **138**, 3639–3646 (2011).
18. R. Sambasivan *et al.*, *Development* **138**, 3647–3656 (2011).
19. Y. X. Wang, M. A. Rudnicki, *Nat. Rev. Mol. Cell Biol.* **13**, 127–133 (2012).
20. I. M. L. Somorjai, R. L. Somorjai, J. Garcia-Fernàndez, H. Escrivà, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 517–522 (2012).
21. G. K. Davis, J. A. D'Alessio, N. H. Patel, *Dev. Biol.* **285**, 169–184 (2005).

Acknowledgments: We thank A. Pavlopoulos, Z. Kontarakis, E. Kabrani, N. Patel, and M. Grillo for sharing reagents and unpublished information and Z. Kontarakis, K. Echeverri, E. Houliston, M. Telford, V. Laudet, F. Alwes, and two anonymous referees for suggesting improvements. Our research was supported by the Heraklitos II program of the Ministry of Education (Greece) and the European Social Fund, and by grant ANR-12-CHEX-0001-01 of the Agence Nationale de la Recherche (France).

Supplementary Materials

www.sciencemag.org/content/343/6172/788/suppl/DC1
Materials and Methods
Figs. S1 to S7
References (*22*–*24*)
Movie S1

22 July 2013; accepted 20 December 2013
Published online 2 January 2014;
10.1126/science.1243529

Somites Without a Clock

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The formation of body segments (somites) in vertebrate embryos is accompanied by molecular oscillations (segmentation clock). Interaction of this oscillator with a wave traveling along the body axis (the clock-and-wavefront model) is generally believed to control somite number, size, and axial identity. Here we show that a clock-and-wavefront mechanism is unnecessary for somite formation. Non-somite mesoderm treated with Noggin generates many somites that form simultaneously, without cyclic expression of Notch-pathway genes, yet have normal size, shape, and fate. These somites have axial identity: The Hox code is fixed independently of somite fate. However, these somites are not subdivided into rostral and caudal halves, which is necessary for neural segmentation. We propose that somites are self-organizing structures whose size and shape is controlled by local cell-cell interactions.

The mesoderm of the embryo, from which the cardiovascular and musculoskeletal systems arise, derives from the primitive streak (PS) during gastrulation. A high level of bone morphogenetic protein (BMP) at the posterior PS generates ventral mesoderm (blood vessels, lateral and extraembryonic mesoderm), whereas lower levels near the anterior tip generate paraxial mesoderm, from which somites (future striated muscle and axial skeleton) develop (*1*). Somites are epithelial spheres that form sequentially from head to tail on either side of the spinal cord. The combination of a molecular clock (cell-autonomous Notch and Wnt oscillations) and a wave traveling the length of the paraxial mesoderm (*2*, *3*) is thought to regulate the number, size, timing of formation, and

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