Published in final edited form as:

Nature. 2013 May 9; 497(7448): 196–197. doi:10.1038/nature12097.

Death brings new life to muscle

Shannon F. Yu and Mary K. Baylies

Program in Developmental Biology, Sloan-Kettering Institute, New York, New York 10065, USA.

Abstract

Fusion of muscle cells called myoblasts underlies the generation and maintenance of skeletal muscle throughout an animal's life. Emerging data indicate that cell death acts as a signal to enhance these processes in mammals.

The multinucleated myofibres that comprise skeletal muscle are generated from the iterative fusion of mononucleated myoblasts. During muscle development, myoblast fusion is mediated by an evolutionarily conserved signalling pathway that alters the actin cytoskeleton^{1,2} — a network of filaments that controls cellular movement and organization. A second, but related, pathway that regulates the actin cytoskeleton drives phagocytosis, the process by which phagocytic cells engulf dead cells³. On page 263 of this issue, Hochreiter-Hufford *et al.*⁴ show that the phagocytic pathway also contributes to myoblast fusion. Specifically, they find that the presence of cells dying by the process of apoptosis, and a receptor that recognizes these dying cells, potentiate myoblast fusion during muscle development, regeneration and repair*.

The phospholipid phosphatidylserine is normally present in the inner leaflet of the cell membrane, but serves as an 'eat me' signal when exposed on the outer leaflet in apoptotic cells (Fig. 1). On phagocytic cells, BAI1, the seven-pass transmembrane receptor for phosphatidylserine, signals through the conserved ELMO/Dock180/Rac1 signalling pathway to regulate the downstream actin cytoskeleton network and promote engulfment of dying cells⁵.

In the fruitfly *Drosophila*, four transmembrane receptors mediate myoblast adhesion, which subsequently activates the ELMO/Myoblast city/Rac signalling module. (Myoblast city is the *Drosophila* version of Dock180.) At the site of myoblast–myoblast contact, this module remodels the actin cytoskeleton to drive fusion. The ELMO/Dock180/Rac1 module is conserved in zebrafish and mammals. In mammals, however, numerous receptors have been found that mediate myoblast–myoblast contact and adhesion between myoblasts and the developing myofibres called myotubes, with related and novel proteins continuing to be identified^{1,2}. It remains unclear how the different receptors contribute to and coordinate the fusion process.

Previous studies^{6–8} have linked exposure of phosphatidylserine on myoblasts to the enhancement of fusion, yet the receptor mediating this signal has not been identified. Hochreiter-Hufford *et al.* asked whether BAI1 might transduce the phosphatidylserine signal during mammalian myoblast fusion. They find that the levels of this receptor increase during fusion *in vitro*, and that it is expressed in actively fusing myotubes *in vivo*. Moreover, increasing the expression of BAI1 enhanced fusion in an ELMO/Dock180/Rac1-dependent manner *in vitro*, indicating that the signalling pathway downstream of this receptor is intact (Fig. 1).

The authors also report that BAI1 seems to be required for muscle development and repair *in vivo*. The muscles of mice lacking the *Bai1* gene were fully formed but were smaller than those of control animals. And after injury, the regenerating muscles of the mutant mice remained smaller and contained fewer centrally localized nuclei; centrally positioned nuclei are a hallmark of muscle repair⁹. That muscles can still undergo some fusion in the absence of BAI1 suggests that the BAI1-related proteins BAI2 and/or BAI3, which are also expressed in muscle, may compensate for its loss.

Contact between apoptotic cells and myotubes has been documented^{4,7} during normal muscle development *in vivo*. Hochreiter-Hufford and co-workers show that phosphatidylserine externalization, together with the activity of the caspase enzymes that mediate apoptosis, are necessary to enhance fusion. In agreement with previous work¹⁰, they find that inhibiting caspase activity using a pan-caspase inhibitor blocks fusion *in vitro*. Strikingly, the researchers could overcome this inhibition by adding apoptotic myoblasts, or even apoptotic thymocytes — unrelated cells belonging to the blood lineage. Thus, various types of apoptotic cell can provide a cue to healthy muscle cells to promote fusion.

Hochreiter-Hufford *et al.* did not observe fusion between apoptotic myoblasts and myotubes, whereas earlier work^{7,8} indicated that even non-apoptotic myoblasts show a feature of apoptosis, transiently externalizing phosphatidylserine before fusion. It could be that different cell populations contribute to fusion: fusion-capable myoblasts transiently expose phosphatidylserine without undergoing apoptosis; and dying myoblasts (and other cell types) enhance the fusion of healthy myoblasts without participating in fusion themselves. Indeed, as noted earlier, apoptotic mouse thymocytes, a cell type that does not fuse, could restore fusion in caspase-inhibited myoblasts. It is not clear how phosphatidylserine is presented by apoptotic cells and non-apoptotic cells so as to allow fusion of the latter but not of the former. Additional phosphatidylserine-binding proteins are required for myoblast fusion *in vitro*¹¹, suggesting that phosphatidylserine may affect parallel, yet different, pathways during myoblast fusion.

If the apoptotic cells are not fusing with myotubes, how is the phosphatidylserine–BAI1 signal integrated with the other elements of the fusion machinery? It is likely that, in this case, BAI1 is not participating in the regulation of actin necessary for cell fusion, despite the fact that its influence on fusion depends on ELMO/Dock180/Rac1 signalling within the myotube. Because signalling by BAI1 can restore fusion capacity in caspase-inhibited myoblasts, which have decreased expression of several regulators of muscle-gene transcription¹⁰, this receptor may target genes essential for myoblast differentiation.

Transient externalization of phosphatidylserine has been observed in other cell-fusing systems — for instance, in trophoblast cells during placental development¹². So the exposure of this phospholipid may be part of a conserved fusion mechanism. But is phosphatidylserine–BAI1 signalling common to all fusing systems? Moreover, apoptotic epithelial cells have previously been linked to compensatory proliferation and regeneration in a variety of organisms — from *Hydra* and planarians to *Drosophila* and mice¹³. It would be interesting to know whether phosphatidylserine–BAI1 signalling also induces proliferation and differentiation of satellite cells, the resident stem cells of skeletal muscle.

The present work provides a new dimension to the fields of myoblast fusion, muscle differentiation and muscle regeneration. A better understanding of the link between apoptosis, BAI1 signalling and fusion could lead to interventions that aid muscle recovery, be it after intense physical training or under conditions of disease-associated muscle wasting, prolonged bed rest or in the elderly.

References

1. Rochlin K, Yu S, Roy S, Baylies MK. Dev. Biol. 2010; 341:66–83. [PubMed: 19932206]
2. Abmayr SM, Pavlath GK. Development. 2012; 139:641–656. [PubMed: 22274696]
3. Gumienny TL, et al. Cell. 2001; 107:27–41. [PubMed: 11595183]
4. Hochreiter-Hufford AE, et al. Nature. 2013; 497:263–267. [PubMed: 23615608]
5. Park D, et al. Nature. 2007; 450:430–434. [PubMed: 17960134]
6. Sessions A, Horwitz AF. FEBS Lett. 1981; 134:75–78. [PubMed: 9222328]
7. van den Eijnde SM, et al. J. Cell Sci. 2001; 114:3631–3642. [PubMed: 11707515]
8. Jeong J, Conboy IM. Biochem. Biophys. Res. Commun. 2011; 414:9–13. [PubMed: 21910971]
9. Chargé SBP, Rudnicki MA. Physiol. Rev. 2004; 84:209–238. [PubMed: 14715915]
10. Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA. Proc. Natl Acad. Sci. USA. 2002; 99:11025–11030. [PubMed: 12177420]
11. Leikina E, et al. J. Cell Biol. 2013; 200:109–123. [PubMed: 23277424]
12. Gauster M, Moser G, Orendi M, Huppertz B. Placenta. 2009; 30:S49–S54. [PubMed: 19027159]
13. Bergmann A, Steller H. Sci. Signal. 2010; 3:re8. [PubMed: 20978240]

Figure 1. Helping hand from a dying cell

Exposure of phosphatidylserine on the outer leaflet of the cell membrane, rather than its normal location in the inner leaflet, marks apoptotic cells for engulfment by phagocytic cells (not shown). During phagocytosis, phosphatidylserine is recognized by the membrane receptor BAI1, which normally signals through the ELMO/Dock180/Rac1 pathway to drive actin cytoskeleton rearrangements and engulfment of apoptotic cells³. In muscle cells, however, this pathway does not lead to engulfment of apoptotic cells. But Hochreiter-Hufford *et al.*⁴ report that phosphatidylserine–BAI1 signalling also enhances myoblast fusion — with healthy myoblasts and with the multinucleated myotubes — in an ELMO/

Dock180/Rac1-dependent manner. The precise mechanism by which this occurs remains unclear, but it does not require fusion or engulfment of the apoptotic cell.