Intracellular Ca\(^{2+}\) dynamics regulate various cellular functions. As the classic study by Ebashi reported,\(^1\) skeletal muscle contraction is regulated by cytoplasmic Ca\(^{2+}\) released from the sarcoplasmic reticulum, which in turn activates troponin C. Since that discovery, Ca\(^{2+}\) action has drawn much attention in basic biomedical research because cytosolic Ca\(^{2+}\) can activate Ca\(^{2+}\)-dependent proteins, and thereby modulate cellular activities and functions. However, recent studies have highlighted the significance of Ca\(^{2+}\) removal from the endoplasmic reticulum (ER).

Increased cytosolic Ca\(^{2+}\) is caused by either Ca\(^{2+}\) release from calcium stores (e.g., ER) or Ca\(^{2+}\) uptake from the extracellular space. When ER Ca\(^{2+}\) is released into the cytosol via ER Ca\(^{2+}\) channels, Ca\(^{2+}\) depletion occurs simultaneously in the ER. ER Ca\(^{2+}\) depletion is potentially harmful to cells, as it interferes with proper folding of proteins that are synthesized on the rough ER, because of dysfunction of ER Ca\(^{2+}\)-dependent molecular chaperones (e.g., calreticulin and calnexin). ER stress is a cellular condition in which misfolded proteins accumulate in the ER.\(^2\) In addition to ER Ca\(^{2+}\) depletion, various other factors (e.g., oxidative stress, glucose shortage, or heavy burden of protein synthesis) cause ER stress.\(^3\) We have recently found that ER Ca\(^{2+}\) depletion-dependent ER stress plays a physiological role in skeletal muscle differentiation.

We previously demonstrated that ER stress occurs in differentiating myoblasts (muscle precursor cells).\(^4\) During skeletal muscle development, myoblasts fuse to form multinucleated cells known as myofibers. Prior to cell fusion, activation of activating transcription factor-6 (ATF6) and caspase-12, an ER stress sensor and the ER stress-specific proapoptotic protease, respectively, is detected. In differentiating myoblasts, ER stress induces many proteins including CCAAT/enhancer-binding protein homologous protein (CHOP), a stress-response transcription factor. Caspase-12 activation results in apoptosis in a subpopulation of differentiating myoblasts. Blocking ER stress signaling inhibits synthesis of muscle-specific proteins, cell fusion, and apoptosis, demonstrating the importance of ER stress in myogenesis. Nevertheless, the cause of ER stress was not identified.

Our recent study provided evidence that Ca\(^{2+}\) release from the ER, mainly through inositol 1, 4, 5-triphosphate receptors (IP\(_3\)R) and ryanodine receptors (RyR), is the cause of ER stress in differentiating myoblasts\(^5\) (Fig. 1). Treatment of myoblasts with a combination of specific inhibitors for these Ca\(^{2+}\) channels (2-aminoethoxydiphenyl borate and dantrolene, respectively) suppressed CHOP induction, and more importantly, myoblast differentiation. With inhibition of both Ca\(^{2+}\) channels, myoblasts did not express myosin, and myoblast fusion did not occur.\(^5\) These observations suggest that ER Ca\(^{2+}\) depletion is a prerequisite for myoblast fusion.

In general, ER loss of Ca\(^{2+}\) is closely associated with Ca\(^{2+}\) uptake from the extracellular space. In response to Ca\(^{2+}\) loss, the ER Ca\(^{2+}\) sensor stromal interaction molecule 1 (STIM1) oligomerizes and translocates to sites close to the plasma membrane, where it interacts and activates Ca\(^{2+}\) channels of the Orai family proteins.\(^6\) Ca\(^{2+}\) influx across the plasma membrane helps refill the ER via sarcoplasmic/endoplasmic reticulum-specific Ca\(^{2+}\) ATPase (SERCA). A series of these events is called store-operated Ca\(^{2+}\) entry (SOCE).\(^7\) Consistent with the presence of such an “automatic” mechanism, STIM1
oligomerization occurs in differentiating myoblasts, prior to or upon CHOP induction. This supports the idea that ER Ca\(^{2+}\) release is the cause of ER stress during myogenesis.

As well as stress responses that include ATF6 activation and CHOP induction, ER Ca\(^{2+}\) depletion induces a specific change in ER morphology. We found that ER-derived globular structures, termed stress-activated response to calcium depletion (SARC) bodies, are present prior to myoblast fusion both in vitro and in vivo. Although SARC bodies are derived from rough ER, they consist of pseudoconcentric cisternae with smooth surfaces and narrow lumens. These SARC bodies appear after STIM1 oligomerization, and eventually disappear during the repeated fusion of myoblasts. They can also be induced in growing myoblasts by SERCA inhibitors, which are typical ER stressors. Other ER stress inducers that act through different mechanisms (e.g., an inhibitor of N-linked glycosylation in the ER) do not induce SARC bodies, suggesting that among causes of ER stress, SARC body formation is specific to ER Ca\(^{2+}\) depletion.

These new findings highlight the importance of ER Ca\(^{2+}\) depletion in transmitting differentiation signals in a cell-autonomous manner, and acting as a regulatory mechanism for differentiation. Ca\(^{2+}\) release from the ER may also contribute, at least partially, to cytosolic Ca\(^{2+}\) signaling. It has been suggested that increased cytosolic Ca\(^{2+}\) prior to myoblast fusion induces calcineurin, which in turn activates myocyte enhancer factor 2A (MEF2) and myogenic differentiation 1 (MyoD), transcription factors that are essential for muscle differentiation.

ER Ca\(^{2+}\) depletion is compensated by SOCE almost instantaneously, although appears to be maintained sufficiently to cause ER stress and SARC body formation. Thus, it is possible that SOCE and/or Ca\(^{2+}\) uptake through SERCA may become transiently hypofunctional. Identifying the mechanism of ER Ca\(^{2+}\) depletion during myoblast differentiation may contribute to developing means of regulating cellular Ca\(^{2+}\) dynamics.

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