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Muscle nuclei remember to cheat death

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Getting buff is hard work and invariably involves lots of repetitive and exhausting resistance exercise. Unfortunately, stop the workouts and those hard fought gains can be lost easily. The only heartening part is that getting back into shape is far easier the second time around, a phenomenon known as 'muscle memory' (Staron *et al.* 1991). For many years the prevailing view was that changes within the central nervous system were responsible for this capability. This view is evolving, and recent data suggest that adaptive mechanisms within the muscle itself are responsible for much of this memory.

The muscle fibre is one of the few syncytial cells within the body and can contain many hundreds of nuclei. This makes sense when one appreciates that individual muscle fibres can be enormous, achieving lengths of up to ∼600 mm (23 in) (for example in the sartorius muscle) and volumes that are five orders of magnitude greater than a typical mononucleated cell (Bruusgaard *et al.* 2003). Muscle is also a very plastic tissue that can add or lose contractile proteins when it undergoes hypertrophy or atrophy, respectively. Many studies have proposed that the number of nuclei change in parallel with muscle mass to ensure that the cytoplasmic-to-nuclear volume is maintained, a phenomenon known as the 'myonuclear domain hypothesis'. Analysing these data is complicated by the fact that muscle is a heterogeneous tissue composed of many different cell types, and determining that a given nucleus has been added to, or lost from, the myoplasm is imprecise. To help overcome this technical challenge the Gundersen lab built upon the pioneering approaches of Jeff Lichtman (Lichtman et al. 1987), and devised techniques for monitoring nuclei over time within individual living muscles fibres *in vivo* (Bruusgaard et al. 2003, 2008). In these experiments the extensor digitorum longus (EDL) or soleus muscles of anaesthetized mice were exposed under a microscope and individual fibres were injected with fluorescent dyes that differentially labelled the cytoplasm and the nuclei. The incision was then sutured and the mice manipulated in ways that would induce skeletal muscle atrophy or hypertrophy, such as denervation and overload, respectively. The same muscle fibre could then be observed over a span of days or weeks to determine the number and location of nuclei within a uniquely identified cell. Using this approach they demonstrated unambiguously that muscle nuclei are virtually never lost during atrophy. The massive increase in apoptotic nuclei that accompanies atrophy reflects the fate of non-muscle stromal and satellite cells, not the myonuclei. This makes teleological sense given that all of the nuclei within a muscle fibre share a common cytoplasm. Under these circumstances, it is not clear how some nuclei would be protected while their neighbours would be completely degraded during apoptosis. The persistence of myonuclei in atrophic muscle not only challenged well-accepted dogma, it also suggested a new epigenetic mechanism for muscle memory.

In a new study described in this issue of *The Journal of Physiology*, Egner *et al.* (2013) extend this analysis to inquire about the fate of new nuclei that are acquired during hypertrophy following exercise and/or anabolic steroid treatment. Female mice received subcutaneous testosterone propionate or placebo pellets, then 2 weeks later their EDL and soleus muscles were excised and examined. In a subset of animals, these muscles were also forced to perform extra work by truncating their primary synergistic muscles. Testosterone and exercise independently led to hypertrophy and the anticipated increases in muscle cross-sectional area (CSA) and myonuclear number. In animals that received both treatments, these effects were additive and the muscles displayed an ∼90% increase in the number of myonuclei. Testosterone levels and muscle CSA both declined rapidly when the hormone pellets were removed. However, consistent with earlier reports, this atrophy was not accompanied by a concomitant loss of myonuclei.

These results raise an obvious question: are these 'surplus' nuclei beneficial? To test this hypothesis, animals were pre-treated with either steroid or placebo for 2 weeks, deprived of hormone for an additional 3 weeks, and then the muscles were forced to work. Prior steroid exposure conferred a distinct advantage to the muscles, with a 44% increase in CSA rather than the 17% increase observed in controls. These benefits were not transient. When the muscles were induced to hypertrophy 3 months after steroid treatments had ended (the mouse equivalent of 10 years of human lifespan), the steroid-treated group displayed a 31% increase in CSA compared to a modest 6% in placebo controls.

Taken together, these data suggest that: (1) once you acquire a myonucleus, it is essentially permanent; and (2) more nuclei translate into greater capacity for regrowth, which presumably translates into enhanced muscle strength and/or speed. These observations have significant public health implications, especially with regards to resistance exercise in the young and the potential to reduce the risk of sarcopenia during ageing. There are also potential ramifications for the sporting community. All athletes acquire their myonuclei through persistent hard work, but some individuals can unfairly supplement their pool by cheating with anabolic steroid use. Since the benefits of steroid administration may persist well after the circulating hormone levels have returned to baseline, it may be very hard to catch violators. Obtaining muscle biopsies from athletes to determine if they have ill-gotten nuclei is invasive, unethical (akin to assault), and likely to be ambiguous. This study reveals a potential new hurdle in the arms race between athletes and ethics, and offers the demoralizing realization that perhaps some cheaters may prosper.

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