Built for Jumping: The Design of the Frog Muscular System

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Frogs must generate a high level of mechanical power when they jump. The muscular system of frogs that jump is presumably designed to deliver these high powers. The length changes and activation pattern that muscles undergo during jumping were measured, and isolated muscle bundles were driven through this in vivo pattern. During jumping, muscles generated maximum power. Specifically, the muscle fibers (i) operated at optimal sarcomere lengths, (ii) operated at optimal shortening velocities, and (iii) were maximally activated during power generation. Thus, many different parameters must have evolved in concert to produce a system capable of this explosive jumping movement.

Over the past four decades much has been learned about the mechanism of muscle contraction (*1*, *2*) and about the large variation in the kinetics of contraction (*3*). In contrast, we know little about how muscle is used during locomotion. An understanding of how muscle actually functions in vivo may explain why contraction kinetics vary so greatly. We chose to study the frog to explore muscle function during locomotion because most of our understanding of the mechanism of muscle contraction is based on frog muscle (*1*, *2*, *4*). In addition, frogs recruit all of their extensor muscle fibers during jumping (*5*), so isolated muscle experiments can be related directly to muscle function during locomotion. Finally, the jump of the frog is a fundamentally different mode of locomotion ("single shot" explosive movement) than the cyclical movements examined in fish (*6*) and scallops (*7*) and thus provides the opportunity to examine a muscular system evolving under different constraints.

The jumping of the frog is one of the most thoroughly studied locomotory movements (*5*, *8*). During a maximal jump, a frog accelerates from a stationary crouched position to high vertical and forward velocities in under 100 ms (*5*, *8*). To produce these rapid increases in potential energy and kinetic energy, the muscles of the frog must generate a high level of mechanical power. Ultimately, the distance a frog travels is directly proportional to power production (*5*).

For the muscles of the frog to generate their maximum mechanical power, they must (i) operate over the plateau of the sarcomere length (SL)–tension curve where maximum force is generated, (ii) operate at the appropriate V/V_{max} (where V is shortening velocity during jumping and V_{max} is the maximum velocity of shortening) where maximum power is generated, and (iii) be maximally activated (*9*).

To test whether the muscular system is designed to achieve these conditions, we first measured the SL changes, V, and the activation pattern of the semimembranosus muscle (SM) during maximal jumps in the frog, *Rana pipiens*. This was accomplished by a combination of high-speed motion pictures, anatomical analysis, and electromyography (Fig. 1).

Frogs were filmed from above and from the side at 200 frames per second during maximal jumps at 25°C. Electromyograms (EMGs) were recorded with bipolar electrodes (*6*, *10*) made of 50-μm nickel wire and were electronically synchronized to the motion picture film to within ±0.5 ms (*6*).

The three-dimensional joint angle of the hip and knee were determined from the films by analysis with a computer-aided design (CAD) program. We determined muscle-tendon length changes by cutting either the proximal or distal end of the muscle (which was made inextensible by fixation with formalin) and driving the hip or knee joints, respectively, through their in vivo three-dimensional angle changes [on the basis of (*11*)] in a custom-made jig (*12*).

The SL excursion during the jump was calculated from the SL value measured in the crouched position and the percent length change of the muscle determined above. We determined the SL in the crouched position by rapidly freezing resting or active muscle at its in vivo muscle length and measuring SL in frozen sections (*13*).

The SM is principally a hip extensor muscle, with an average moment arm around the hip of 4.4 mm and a flexor moment about the knee of 0.08 mm. During six maximal jumps (distance = 0.67 ± 0.03 m, mean ± SE), the SM shortened 7.51 ± 0.17 mm (Fig. 1B) from an initial length of 33.6 ± 0.35 mm. This corresponds to SL shortening from 2.34 μm (± 0.013, n = 161, from four frogs) in the crouched position to 1.82 ± 0.01 μm at the point of takeoff (Fig. 2A) (*14*). Shortening started 26 ± 3.4 ms after the start of the EMG burst, and after an additional ~5 ms, the muscle attained a constant velocity of 3.43 ± 0.10 muscle lengths per second (ML/s; ML defined at 2.34 μm; Fig. 1, A and B). The constancy of muscle shortening velocity (mdθ/dt) reflects that during jumping both the angular velocity of the hip (dθ/dt) and the moment around the hip (m) were nearly constant.

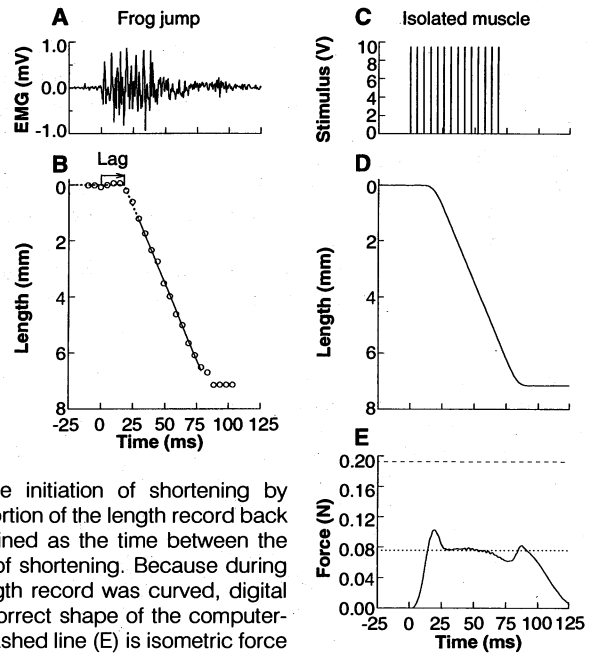

Fig. 1. Frog muscle function during jumping. (**A**) and (**B**) show the stimulation and length change patterns, respectively, that the SM undergoes during a maximal jump. (**C** and **D**) An isolated muscle bundle driven through the in vivo stimulation and length change patterns, and (**E**) the resulting force production of the muscle. The isolated muscle bundles were stimulated at either 200 or 120 pulses per second (pps), and there was no significant effect of frequency over this range. The stimulation duration was determined from the EMG (*29*). The phase of the stimulus with respect to the length change was determined in the following manner. We determined the initiation of shortening by extrapolating the constant velocity portion of the length record back to zero length (B). The lag was defined as the time between the onset of the EMG and the initiation of shortening. Because during jumping the early portion of the length record was curved, digital smoothing was used to obtain the correct shape of the computer-generated length change (D). The dashed line (E) is isometric force and the dotted line is the steady-state force generated by the same muscle at the same V during a force-velocity experiment (Fig. 2). The jump shown in (A) and (B) is the longest measured (distance = 0.8 m, V = 3.78 ML/s), and this was reproduced in (C) to (E).

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To determine whether the SM generated maximum power during these in vivo conditions, we used a computer-controlled servo and stimulator system (6) to perform isolated muscle mechanics experiments. Several experiments, in which tension and active SL (laser diffraction) were measured during "fixed-end" tetani, showed that the standard frog SL-tension curve (2) fit the SM data, so the standard curve was used to represent the SL-tension curve of the SM (Fig. 2A). During a jump, the SM operates mostly over the plateau where maximum force is generated (Fig. 2A). This suggests that during evolution the muscle origin, insertion, and angle of pennation have been adjusted to give the appropriate gear ratio [(change in body movement)/(change in SL)] so that fibers shorten mainly over the plateau during jumping. Although the SM shortened somewhat beyond the plateau (15), it never produced less than 90% of the force generation at optimal SL.

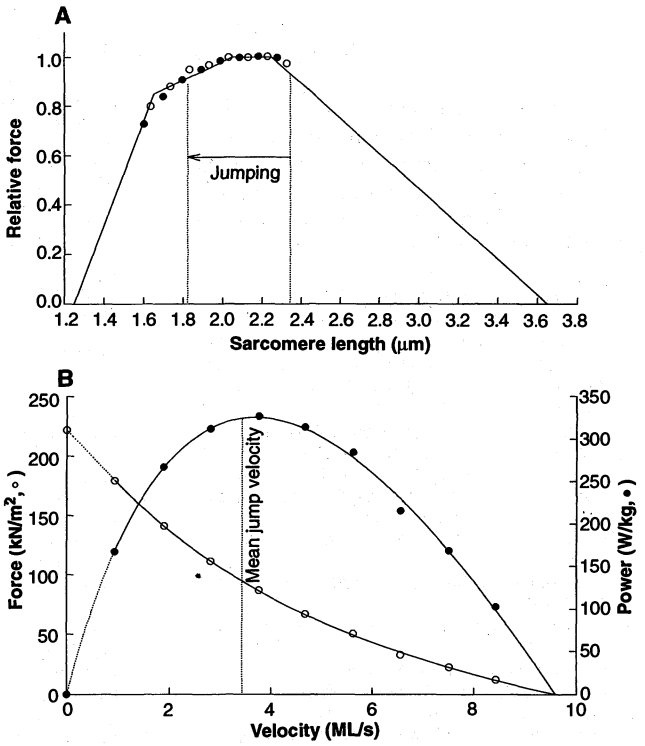

Fig. 2. Where does the SM muscle operate on its SL-tension and force-velocity curves? (**A**) Results from fixed-end tetani of two preparations (open and solid symbols) show that the data are well fit by the classic SL-tension curve (*2*). The SL-tension relation was not studied at SL>2.35 μm, because frogs do not use these SLs during jumping and because of well-known experimental problems associated with fixed-end contractions at long SLs (*2*). Muscle shortening during jumping is shown by the arrow. (**B**) A typical force-velocity and power-velocity curve. The power curve was calculated from the force-velocity fit. At the *V* used during jumping, the muscle operates over the portion of the power curve where at least 99% of maximum power is generated. Force is shown by open symbols and power is shown by closed symbols. At loads above 0.8 of isometric force (*19*), cubic splines (dotted lines) were used to extrapolate the force-velocity curve to isometric force and the power-velocity curve to zero power, respectively.

Isovelocity contractions (*16*) were used to determine the force-velocity characteristics of the muscle. A force-velocity and power-velocity curve from one preparation are shown in Fig. 2B. Frog muscle at 25°C can shorten faster and generate greater power than muscles from most other animals (*17, 18*). On average (n = 6), V_{max} was 10.35 ± 0.20 ML/s (*19*) and maximum power was 371 ± 7 W per kilogram of muscle mass. The V at which maximum power was generated was 3.44 ± 0.13 ML/s, which matches closely the average V during jumping (3.43 ML/s). This occurs at a V/V_{max} = 0.33 (Fig. 2B). Although histochemistry (*20*) shows that the SM contains the two fastest twitch amphibian fibers (amphibian type 1 and type 2), mechanics experiments (*18, 21*) show relatively little difference (~20%) in V_{max} between these fiber types. This suggests that V_{max} of each fiber type has been adjusted during evolution such that during jumping, the muscle operates at the appropriate V/V_{max} for maximal power generation.

Although the SM shortens at the correct SLs and V/V_{max} for maximum power generation, the question arises whether the muscle is stimulated for a sufficient time before shortening to be maximally activated during jumping. For the jump of the frog, we considered activation to be maximal if the muscle generated the same force (and power) under in vivo conditions as it did at the corresponding V during the force-velocity experiments (where maximal activation was achieved by stimulating the SM for 60 ms before shortening).

Force generation while the muscle underwent its in vivo length change and stimulation pattern rose to about 60% of isometric force and then fell to an average constant level (41 ± 2%), corresponding to the V at which the muscle is shortening (Fig. 1, C to E). Despite the short lag between the EMG and muscle shortening (18 ms), the force the muscle generated under the in vivo conditions was greater than or equal to the force generated at that V on the force-velocity curve, suggesting that the muscle was maximally activated.

For a more clear determination of the time course of activation, the muscle was driven through the in vivo length changes, with the first stimulus preceding shortening by different times (Fig. 3, A and B). When the first stimulus preceded shortening by less than 18 ms, the muscle generated less force for much of the shortening phase of

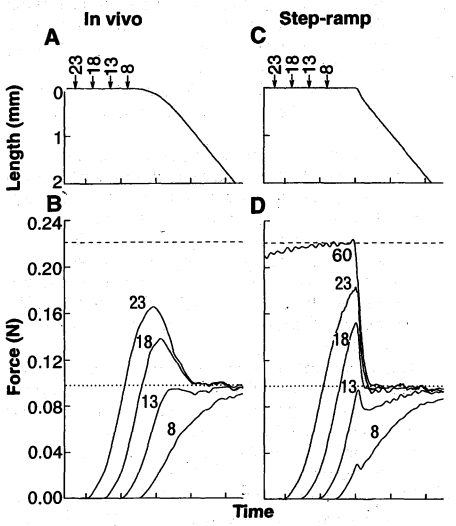

Fig. 3. The SM muscle is maximally activated for the entire shortening phase of jumping. To determine the time course of activation, we compared the force generated by the muscle shortening at the V of the fastest jump (3.78 ML/s) in contractions where the initial stimulus preceded shortening by various amounts with the steady-state force generated while shortening at the same V during the force-velocity curve measurements (in which the muscle is known to be maximally activated). (**A** and **B**) The muscle underwent the in vivo length changes (A), with the first stimulus preceding shortening by various times (shown by arrows, times in milliseconds). (B) Force record of the muscle given these lags; the dotted line is the force level from the force-velocity curve. (**C** and **D**) The muscle undergoing the step-ramp protocol, with different lags. In this case, the step imposed on the muscle was adjusted for the various lags so as to bring the force quickly down to the constant level associated with the V. In the case of 8 and 13 ms, no step was imposed, as the force at the time of the step was below the force-velocity level. (C) Length change and (D) force records of SM undergoing step-ramp protocol; dashed line in (B) and (D) is isometric force. The dotted line in (D) represents force from the force-velocity curve. Each division on the x axes represents 10 ms.

the jump, which signifies a lack of complete activation. When the stimulus preceded shortening by at least 18 ms (the shortest lag measured during a jump), the force the muscle generated was greater than or equal to the corresponding force from the force-velocity curve, suggesting the muscle was maximally activated. Because the force early in the shortening phase was also effected by the low V and a stretched series elastic component, we drove the muscle through the step-ramp protocol (*16*) [which rapidly (~2 ms) unloads the series elastic component and attains the correct V; Fig. 3, C and D] to determine how early in shortening the muscle becomes maximally activated. The activation time course was similar to that in Fig. 3B, suggesting that the smallest lag observed during jumping (18 ms) is sufficient for the fibers to be maximally activated throughout the shortening phase. This also shows that although the rate of activation processes may vary slightly in amphibian type 1 and type 2 fibers (*22*), activation processes are sufficiently fast in both fiber types to ensure maximal activation during jumping.

Comparison of isolated muscle and whole animal power production further supports the conclusion that fibers were maximally activated and shows that most of the extensor muscles in the frog hindlimb probably behaved similarly to the SM during jumping. Peak power generated by the whole frog during jumping was determined from the films by measuring the change in the position of the center of mass of seven segments of the frog with time (*23*) and was 67.2 ± 5.4 W per kilogram of body mass. Because the maximum power generated by the SM was 371 W per kilogram of muscle mass and only about 17% of the body mass (*24*) can be involved in powering jumping [that is, maximum possible whole animal power = 371 W/(kilogram of muscle) × 0.17 (kilogram of muscle/kilogram of body mass) = 63 W/(kilogram of body mass)], all the muscle fibers from each extensor muscle are probably recruited and stimulated at a sufficiently high frequency (*25*) and shorten over the appropriate SLs and V/V_{max} for maximum power generation during jumping. In addition, preliminary results for the cruralis muscle, a large knee extensor, indicate that it is activated and shortens much like the SM (*26*).

Thus, the muscular system of frogs that jump is designed so that the three necessary conditions for maximum power generation are met. In agreement with fish studies (*27*), the values of a number of underlying parameters have evolved to meet the first two conditions. Specifically, the fiber gear ratio and myofilament lengths are set so that muscles work mainly over the plateau of the SL-tension curve, and the V_{max} and the gear ratio are set so that extensors operate at the appropriate V/V_{max} for maximum mechanical power generation. In addition, the present results show that during one-shot frog jumps there is a third design goal met; various processes involved in muscle activation [calcium release, binding to troponin, crossbridge transitions (*28*)] are set to be rapid enough to enable the muscle to be maximally activated by the beginning of the shortening phase of the jump. Finally, muscle force in the frog remains constant during shortening, permitting maximum power to be generated throughout a jump. By contrast, muscle force in animals using cyclical movements, such as fish swimming, declines during shortening (because of shortening deactivation and early cessation of stimulation) so that the muscle can be subsequently relengthened without resistance (*6*).

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9. From a molecular viewpoint, all of the crossbridges must be engaged in active cycling and the filaments must be moving past one another at the appropriate speed for maximum power generation.
10. Frogs were anesthetized with MS-222 (1 mg/ml) for the implantation procedure. The short electrodes were fed subcutaneously to a connecter sutured to the frog's back.
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12. The displacement of the cut end along the line of action of the femur is equal to the muscle-tendon length change about a given joint (dL). The moment (m) was calculated as the quotient of $dL/d\theta$ where $d\theta$ is the joint angle change. The total muscle-tendon length change was taken as the sum of the dLs around the hip and knee. We calculated the muscle length change assuming the length change in the connective tissue to be negligible (*14*).
13. The in vivo length was set by killing the frog and fastening the frog hindlimb in the crouched position. The muscle was frozen by plunging the hindlimb in liquid N_2–cooled isopentane either during stimulation of the motor nerve (active) or in a relaxed state.
14. The total length change of the muscle-tendon complex was assumed to occur in the muscle, on the basis of the following reasoning. The SL measured in the active case was 2.21 μm (±0.014, n = 184, from three frogs) compared with 2.34 μm in the passive case, thus representing an ~5% decrease in SL during maximum isometric tension. Because an ~1.5% length change is known to be associated with the intrinsic series elasticity of the fibers (*16*), the maximum that could be associated with the connective tissue under isometric conditions is 3.5%. During shortening, however, the muscle generated only ~40% of isometric force, setting an upper limit of 1.4% (that is, 40% × 3.5%) for the length change associated with connective tissue during jumping. This value seems reasonable because the SM has only a short (~2 mm), stout tendon at the knee and a direct attachment of the fibers at the pelvis. This term was ignored because it has no effect on V and little effect on SL (0.03 μm).
15. During jumps, force and power fell rapidly before takeoff, so that very little power was generated at the shorter SLs.
16. In this method, the muscle was given a high-velocity length step to unload the series elastic component and thereafter shortened at constant velocity [F. J. Julian, L. C. Rome, D. G. Stephenson, S. Striz, *J. Physiol.* **370**, 181 (1986)].
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19. Over the loads of 0.05 to 0.8 of isometric tension, the force-velocity curves were well fit by the Hill curve (Fig. 2B) when the curve was not constrained to pass through isometric tension. Typical r^2 values for these curves were 0.99. At low loads (0.01 to 0.03 of isometric tension), velocity exceeded the Hill curve, and including these points raised V_{max} by ~5% as reported for single frog fibers (*16*). These are the V_{max} values that were reported here.
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23. This technique [M. A. Fedak, N. C. Heglund, C. R. Taylor, *J. Exp. Biol.* **79**, 23 (1982)] is inherently noisy, so the peak values were obtained by averaging over 25 ms. This averaging may slightly underestimate the actual maximal value, but a higher maximal value would only strengthen the conclusion that the fibers are generating maximum power during jumping.
24. The hindlimb extensors, hip abductors, and iliosacral muscles make up ~17% of the frog's mass.
25. The comparison of muscle and whole animal power generation shows that during jumping the muscles were stimulated at sufficiently high frequencies to result in maximal activation. This is consistent with quantitative EMG analysis on the SM that showed a spike frequency of 200 to 300 Hz.
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29. Although the onset of the EMG was unambiguous, the off-time was more difficult to determine precisely. Typically, the EMG consisted of a large-amplitude signal lasting about 45 ± 3 ms that was followed by a lower amplitude signal for an additional 23 ms. Over the shortening phase of the contraction, the forces were nearly identical in 45- and 68-ms duration stimulations. Specifically, the 45-ms stimulation produced 96% of the work generated by the 68-ms stimulus, which was the maximum work of which the muscle was capable. Hence, over the range of possible EMG durations, the muscle is maximally activated during the entire shortening phase.

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