Partitioning the Energetics of Walking and Running: Swinging the Limbs Is Expensive

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Explaining the energetics of walking and running has been difficult because the distribution of energy use among individual muscles has not been known. We estimated energy use by measuring blood flow to the hindlimb muscles in guinea fowl. Blood flow to skeletal muscles is controlled locally and varies directly with metabolic rate. We estimate that the swing-phase muscles consume 26% of the energy used by the limbs and the stance-phase muscles consume the remaining 74%, independent of speed. Thus, contrary to some previous suggestions, swinging the limbs requires an appreciable fraction of the energy used during terrestrial legged locomotion. Models integrating the energetics and mechanics of running will benefit from more detailed information on the distribution of energy use by the muscles.

Terrestrial legged locomotion is the only major locomotor activity for which energy use by the bulk of the active muscles cannot be linked with certainty to their mechanical function. In the two other major locomotor modes, swimming and flying, the function of most of the muscle volume is to produce mechanical power in repetitive contractile cycles, which provide the needed lift and/or thrust (*1, 2*). However, during level walking and running at a steady speed, net mechanical work and power are zero (*3*). Mechanical work must be performed in each stride to lift and reaccelerate the center of mass and to accelerate the oscillating body segments, but these increases in energy are reversed as the body returns to its starting height and the segments decelerate. The cyclical fluctuations in kinetic and potential energy during the stride allow for possible energy storage and recovery in elastic structures and for exchange between gravitational potential energy and kinetic energy (*3–5*). Because the overall effectiveness of these energy-saving mechanisms is not known, the magnitude of the work that must be produced or absorbed by active muscles in each stride is not clear (*5, 6*).

Muscle activity is also needed to produce force to support the body weight during the stance phase of the stride. On the basis of a series of experiments integrating the mechanics and energetics of running, Taylor and colleagues (*6–10*) suggested that production of force on the ground is the major determinant of the energy cost of running—the "force hypothesis." Calculating the volume of active muscle and the amount of energy used for work and force production from externally measurable movements and forces is difficult because of the complexities of the locomotor system, including unknown amounts of energy storage and release, the presence of cocontraction by antagonistic muscles, uncertainties about the distribution of force among synergistic muscles, the presence of muscles acting across two or more joints, and muscles undergoing active lengthening. These various complexities, and the simple fact that a large number of muscles participate in walking and running, leave the current picture of muscle energy use and mechanical function during terrestrial locomotion clouded.

Here, we examined the division of energy use by muscles active during the stance and swing phases of walking and running. Some studies suggest that the cost of swinging the limbs is very small relative to stance-phase costs (*7*); others have emphasized the potential importance of these costs (*11, 12*). As a component of the force hypothesis, Taylor (*6*) suggested that swing-phase costs were low enough to be ignored. However, evidence about stance-phase and swing-phase costs has relied almost exclusively on experiments that attempt to deduce muscle energetics from externally measurable and alterable parameters (e.g., load-carrying experiments) (*7, 13*).

Partitioning the energetics of walking and running requires overcoming difficulties in measuring the energetic contributions of each of the active muscles. The use of arterial-venous differences in oxygen content and blood flow to derive direct measurements of energy use by individual muscles is impractical, if not impossible, because of the large number of muscles involved and the difficulties of accessing their blood supplies. Indirect measures used previously to indicate muscle recruitment have substantial limitations in estimating energy use during steady-state aerobic exercise. Measuring muscle recruitment according to electromyographic activity is valuable for indicating timing and relative recruitment, but the results are difficult to relate quantitatively to energetics when measured across many muscles and animals. Glycogen depletion, measured biochemically or visualized histochemically, has also been used to indicate muscle recruitment (*14*), but this technique probably works best under conditions of high energy demand (*15, 16*) or prolonged fatiguing contractions (*17*).

We used blood flow measured with colored microspheres (*18*) as our proxy measure for energy use by the skeletal muscles. In this technique, 15-μm microspheres injected into the left ventricle are distributed with the blood and lodge in the systemic capillaries throughout the body. Because the distribution of spheres in a well-mixed volume of blood is proportional to the flow rate, the tissue blood flow rate can be calculated from the number of spheres trapped if the number of spheres in a specified flow is known. This calibrated value for spheres per flow is obtained by withdrawing systemic arterial blood at a known rate throughout the period when the spheres are flowing out of the heart. The microsphere technique for measuring tissue blood flow during exercise has been used and validated extensively (*19*) but has never been used to estimate total energy use by all of the individual muscles.

Measuring blood flow may be the best option for estimating relative energy use by the individual muscles that are active during aerobically supported walking and running. In mammals, blood flow to skeletal muscles is controlled locally by mechanisms linked to metabolic rate (*20, 21*). Previous studies with microspheres have shown that flow is recruited as expected from the known recruitment order of fiber types, including regionally within muscles (*19*). Although less information is available on control of flow to skeletal muscle in other vertebrate groups, the available evidence suggests that the mechanisms found in mammals are common to all vertebrate groups, including birds (*22, 23*). Blood flow to the active muscles increases directly with oxygen consumption in diverse species, including humans (*24*) and running birds (*25*). The use of microspheres to quantify blood flow, and thus (indirectly) energy use, allows the simultaneous measurement of flow

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to each of the active muscles as well as to tissues unrelated to locomotion.

The helmeted guinea fowl *Numida meleagris* offers several advantages in estimating the relative costs of stance versus swing. First, the muscles involved in locomotion are all confined to the hindlimbs and pelvis, without the involvement of trunk muscles and the division of labor between forelimbs and hindlimbs found in quadrupedal mammals. Second, the activity of the hindlimb muscles in guinea fowl can almost all be partitioned into swing phases and stance phases, according to the extensive measurements of electromyographic activity reported by Gatesy (*26*) and additional measurements presented here (Fig. 1). Third, the guinea fowl is one of only three species of birds in which the aerobic scope has been defined (*27*). Finally, these birds are excellent treadmill runners, and reproducible curves of oxygen consumption versus speed—including measures of maximal oxygen consumption ($V_{O_2,max}$)—were elicited from the individual birds used in the present study.

We collected blood flow data on each bird under four levels of metabolic effort: rest and treadmill exercise at 0.5 m s^{-1}, 1.5 m s^{-1}, and 2.3 to 2.8 m s^{-1}. Aerobic energy use under these conditions was quantified with separate measurements of organismal oxygen consumption (V_{O_2}) for each bird (Table 1). We used an open flow system with the bird wearing a clear lightweight plastic mask (*18*). Resting values were obtained after the bird had sat in a darkened box for 10 min. The highest treadmill speed was adjusted for individual birds to match the speed that elicited a V_{O_2} equal to 90% of $V_{O_2,max}$.

Our data on blood flow are consistent with our assumption that blood flow is recruited in proportion to the metabolic rate of the muscles and is highly specific to individual muscles. First, the increase in total blood flow to the legs during treadmill exercise was directly proportional to the increase in oxygen consumption by the animal (Fig. 2). Second, muscles not used in terrestrial locomotion, such as the flight muscles, showed little or no increase in flow as running speed was increased (fig. S1). Third, as running speed was increased, the increase in flow to the individual leg muscles was highly specific and likely reflected differences in recruitment (fig. S1).

Most of the thigh muscles were assigned as stance-phase or swing-phase muscles on the basis of the electromyographic data in (*26*). We confirmed these data for a number of thigh muscles and also recorded electromyograms (EMGs) for most of the major muscles in the lower leg (Fig. 1).

Although the classification of muscles as being active in stance versus swing is clear in most cases, some uncertainties exist. First, some of the stance-phase muscles actually start activity during late swing phase and possibly contribute to the mechanics of late swing (*26*) (Fig. 1). The flexor cruris lateralis pars pelvica (FCLP) and the iliotrochantericus caudalis (ITC) had particularly strong activity in late swing. However, the converse pattern occurred for some swing-phase muscles, such as the iliotibialis cranialis (IC) and tibialis cranialis (TC), which started activity in late stance. For simplicity, we classified these muscles as solely stance-phase or swing-phase muscles, because after correcting for the presumed delay between the onset of electrical activity and force production, most of the activity fell into a single phase of the stride. Two thigh muscles, the femerotibialis (FT) and iliofibularis (IF), showed definite activity during both swing phase and stance phase (Fig. 1). For both of these muscles, we assumed that half of the blood flow supported stance-phase activity and half supported swing-phase activity. In the data reported here, we measured flow to the whole IF. Subsequently, we discovered that the activity of this muscle is regionalized. The anterior portion of the muscle is active primarily in swing phase and the posterior portion is active largely during stance phase (Fig. 1), a pattern also occurring in chicks of the domestic fowl (*28*). Additional measurements done at a single running speed of 1.5 m s^{-1} indicate that 65% of the total blood flow is to the anterior half of the IF, which suggests that our assignment of 50% of the metabolism of this muscle to swing phase is conservative. The division of activity between stance and swing in the FT is less certain. However, when we used the highest and lowest recorded values of

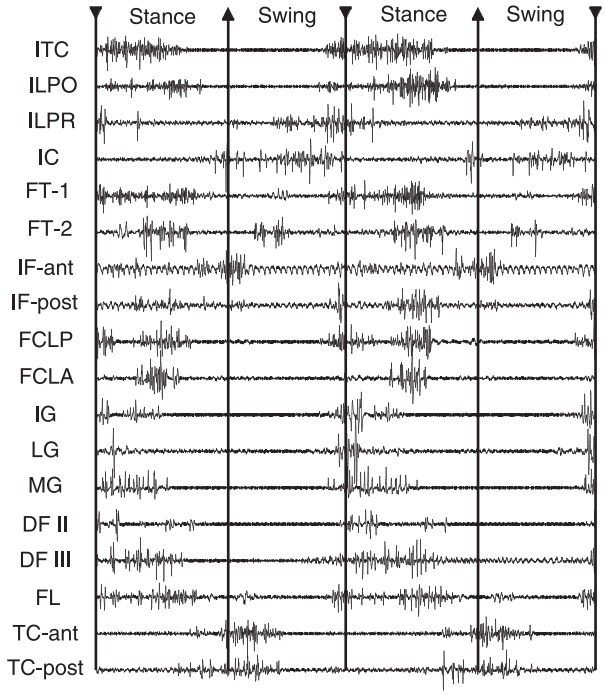

Fig. 1. Representative EMG recordings from various hindlimb muscles in guinea fowl running at 1.5 m s^{-1}. The horizontal time scale of the recording from each muscle has been adjusted to align foot-down (down arrow) and toe-off (up arrow). The actual swing and stance durations varied somewhat among the animals used, but at 1.5 m s^{-1} the stance-phase and swing-phase durations were about 210 and 180 ms, respectively. For full muscle names, see (*34*).

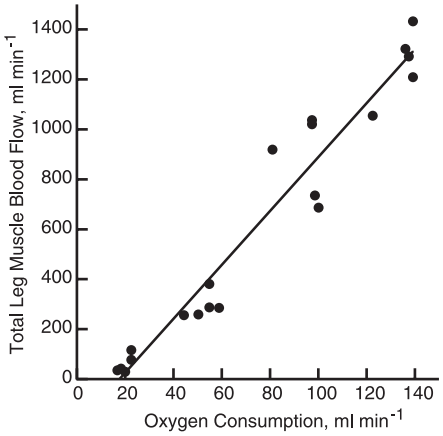

Fig. 2. Total blood flow to the hindlimb muscles of guinea fowl as a function of the oxygen consumption of the animal measured at the same treadmill speed. The highest levels of oxygen consumption represent 90% of the $V_{O_2,max}$ measured for each animal. The line is a least-squares linear regression with the equation $Q_{leg} = 10.8V_{O_2} - 192$ ($r^2 = 0.94$), where Q_{leg} is total blood flow to the leg muscles in ml min^{-1} and V_{O_2} is the oxygen consumption of the bird in ml O_2 min^{-1}.

Table 1. Mean oxygen consumption of guinea fowl at the speeds used in the blood flow experiments. $N = 5$.

Speed (m s^{-1})	V_{O_2} (ml min^{-1})	% $V_{O_2,max}$
0	20.0 ± 1.1	13.4
0.5	52.6 ± 2.5	35.2
1.5	94.9 ± 3.5	63.4
2.28 to 2.78	134.9 ± 3.2	90.1

integrated EMGs to repartition energy use by the FT, our results did not change substantially (*29*). A few muscles were not measured electromyographically, and these were assigned to swing and stance phases on the basis of their anatomical position and the activity recorded in synergistic muscles. The only muscles of any substantial size in this category were the digital flexor muscles, which were all assigned to stance phase on the basis of EMGs recorded in the superficial flexors of digits II and III (Fig. 1).

Overall, the distribution of blood flow to swing-phase and stance-phase muscles is independent of running speed. Differential recruitment of the various muscles leads to variations in the fraction of total hindlimb flow that is delivered to individual muscles (Fig. 3), but the sums of the flows to the swing-phase and stance-phase muscles represent remarkably constant fractions of the total flow (Fig. 4). At all locomotor speeds, stance-phase flow is 74% of the total flow to the leg, and swing-phase flow is 26%.

Therefore, given the reasonable assumption that blood flow is proportional to energy consumption, we reject the conclusion of the force hypothesis that swing-phase costs are not appreciable during walking and running, at least in guinea fowl. The energy use during swing phase is lower than that during stance phase, but still represents about one-fourth of the total energy used. If we assume maximum muscle efficiency, energy use during swing phase in this species is 2 to 4 times that needed for internal work as measured in (*30, 31*).

The extent to which these data can be generalized to other running animals is not known. However, the running economy of guinea fowl is typical of both bipeds and quadrupeds of similar size, and we have no reason to suspect that the locomotor mechanics of guinea fowl are atypical in any way (*30*). Complete data on the total blood flow and EMG activity of all the individual muscles used in running are not available for any other animal. A more limited comparison can be made using total blood flow calculated from mass-specific flows and muscle masses for the muscles operating around the ankle in laboratory rats (*32, 33*). In this species, the ankle flexors and digital extensors, which are expected to be active during swing phase, receive 27% of the flow to the lower leg, and this distribution is the same during walking and galloping. This division of blood flow between stance-phase and swing-phase muscles in the lower leg of rats is strikingly similar to that found for the entire limb in guinea fowl.

In proposing the force hypothesis, Taylor and colleagues (*6, 8, 9*) were looking for a solution that would unify information on mechanics and energetics and explain the effects of body size and locomotor speed on the energy cost of walking and running. The work presented here maintains this emphasis on using energetics to help understand terrestrial locomotion, but our results suggest that the force hypothesis will have to be modified, or alternative hypotheses formulated, to account for a more detailed partitioning of the energetics among the muscles used during running. The use of blood flow as a proxy measure of energy consumption of individual muscles should allow us to further test hypotheses related to changes in energy use caused by differences in body size, speed, and experimental modifications such as load carrying.

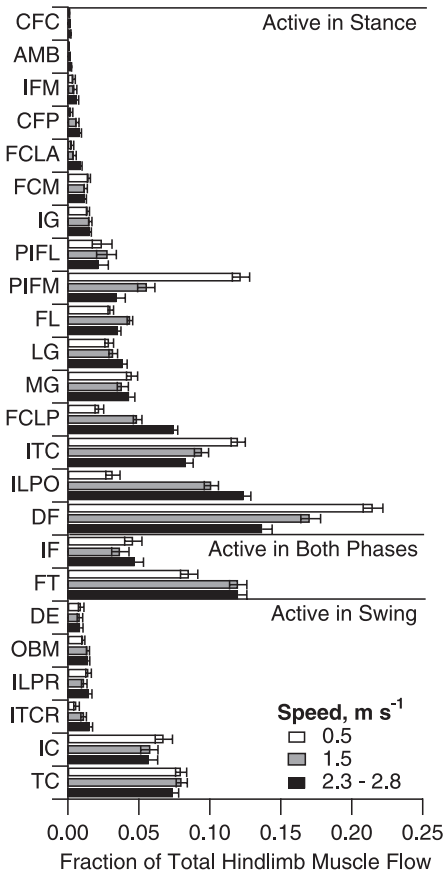

Fig. 3. Fraction of hindlimb muscle blood flow to individual muscles or muscle groups in the hindlimb of the guinea fowl. For full muscle names, see (*34*).

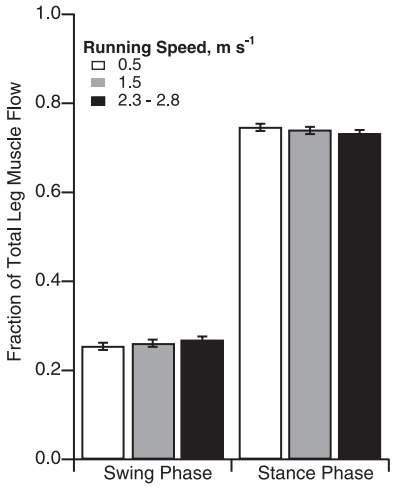

Fig. 4. Fraction of hindlimb muscle blood flow to the combined swing-phase and stance-phase muscles.

References and Notes

1. J. D. Altringham, D. J. Ellerby, *J. Exp. Biol.* **202**, 3397 (1999).
2. T. L. Hedrick, B. W. Tobalske, A. A. Biewener, *J. Exp. Biol.* **206**, 1363 (2003).
3. G. A. Cavagna, N. C. Heglund, C. R. Taylor, *Am. J. Physiol.* **233**, R243 (1977).
4. T. J. Roberts, R. L. Marsh, P. G. Weyand, C. R. Taylor, *Science* **275**, 1113 (1997).
5. F. Saibene, A. E. Minetti, *Eur. J. Appl. Physiol.* **88**, 297 (2003).
6. C. R. Taylor, in *Comparative Vertebrate Exercise Physiology: Unifying Physiological Principles*, J. H. Jones, Ed. (Academic Press, New York, 1994), pp. 181–215.
7. C. R. Taylor, N. C. Heglund, T. A. McMahon, T. R. Looney, *J. Exp. Biol.* **86**, 9 (1980).
8. R. Kram, C. R. Taylor, *Nature* **346**, 265 (1990).
9. T. J. Roberts, R. Kram, P. G. Weyand, C. R. Taylor, *J. Exp. Biol.* **201**, 2745 (1998).
10. R. Kram, *Exerc. Sport Sci. Rev.* **28**, 138 (2000).
11. M. J. Myers, K. Steudel, *J. Exp. Biol.* **116**, 363 (1985).
12. K. Steudel, *J. Exp. Biol.* **154**, 287 (1990).
13. T. M. Griffin, T. J. Roberts, R. Kram, *J. Appl. Physiol.* **95**, 172 (2003).
14. R. B. Armstrong, C. D. Ianuzzo, M. H. Laughlin, *J. Appl. Physiol.* **61**, 683 (1986).
15. J. O. Holloszy, W. M. Kohrt, P. A. Hansen, *Front. Biosci.* **3**, D1011 (1998).
16. G. A. Brooks, *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **120**, 89 (1998).
17. A. W. English, O. I. Weeks, *J. Morphol.* **191**, 161 (1987).
18. See supporting data on *Science* Online.
19. R. B. Armstrong, *Am. J. Cardiol.* **62**, 9E (1988).
20. M. H. Laughlin, D. H. Korzick, *Med. Sci. Sports Exerc.* **33**, 81 (2001).
21. C. L. Murrant, I. H. Sarelius, *Acta Physiol. Scand.* **168**, 531 (2000).
22. R. E. Klabunde, P. C. Johnson, *Am. J. Physiol.* **232**, H411 (1977).
23. K. Tyml, *Microvasc. Res.* **32**, 84 (1986).
24. P. Andersen, B. Saltin, *J. Physiol.* **366**, 233 (1985).
25. B. Grubb, D. D. Jorgensen, M. Conner, *J. Exp. Biol.* **104**, 193 (1983).
26. S. M. Gatesy, *J. Morphol.* **240**, 127 (1999).
27. D. J. Ellerby, M. Cleary, R. L. Marsh, C. I. Buchanan, *Physiol. Biochem. Zool.*, in press.
28. R. M. Johnston, A. Bekoff, *J. Comp. Physiol. A* **179**, 169 (1996).
29. Our recordings of EMGs from the FT showed variation among animals. The integrated EMG during swing phase was usually greater at higher speeds. The greatest percentage of total integrated EMG occurring during swing phase (mean of five strides) was 67% and the lowest was 20%. Using these extreme values to partition our blood flow data would change our estimate of swing-phase energy metabolism to a high of 28% and a low of 23% of total energy costs.
30. M. A. Fedak, N. C. Heglund, C. R. Taylor, *J. Exp. Biol.* **79**, 23 (1982).
31. On the basis of a net cost of locomotion in guinea fowl of 8.1 J kg^{-1} m^{-1}, the cost of swing phase is 2.0 J kg^{-1} m^{-1}. At 25% efficiency, the swing-phase muscles would thus be capable of producing a maximum mechanical power output increasing from 0.25 to 1.5 W kg^{-1} as speed is increased from 0.5 to 3.0 m s^{-1}. The actual internal mechanical power measured in (*30*) increases from 0.06 to 0.8 W kg^{-1} over this range of speeds.

32. R. B. Armstrong, M. H. Laughlin, *J. Physiol.* **344**, 189 (1983).
33. M. H. Laughlin, R. B. Armstrong, *Am. J. Physiol.* **244**, H814 (1983).
34. Abbreviations for muscle names: CFC, caudofemoralis pars caudalis; AMB, ambiens; IFM, ischiofemoralis; CFP, caudofemoralis pars pelvica; FCLA, flexor cruris lateralis pars accessoria; FCM, flexor cruris medialis; IG, gastrocnemius intermedias; PIFL, puboishiofemoralis pars lateralis; PIFM, puboishiofemoralis pars medialis; FL, fibularis longus; LG, gastrocnemius lateralis; MG, gastrocnemius medialis; FCLP, flexor cruris lateralis pars pelvica; ITC, iliotrochantericus caudalis; ILPO, iliotibialis lateralis pars postacetabularis; DF, combined digital flexors; DF II, flexores perferantes et pefororati digiti II; DF III, flexores perferantes et pefororati digiti III; IF, entire iliofibularis; IF-ant and IF-post, anterior and posterior regions of the iliofibularis; FT, femerotibialis (all heads); FT-1 and FT-2, two regions of the femerotibialis intermedius; DE, combined digital extensors; OBM, obturatorius medialis; ILPR, iliotibialis lateralis pars preacetabularis; ITCR, iliotrochantericus cranialis; IC, iliotibialis cranialis; TC, entire tibialis cranialis; TC-ant and TC-post, anterior and posterior regions of the tibialis cranialis. Muscle nomenclature is based on (*35*).
35. J. C. Vanden Berge, G. A. Zweers, in *Handbook of Avian Anatomy: Nomina Anatomica Avium*, J. J. Baumel, A. S. King, J. E. Breazile, H. E. Evans, J. C. Vanden Berge, Eds. (Nuttall Ornithological Club, Cambridge, MA, 1993), pp. 189–247.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5654/80/DC1
Materials and Methods
Fig. S1

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MicroRNAs Modulate Hematopoietic Lineage Differentiation

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MicroRNAs (miRNAs) are an abundant class of ~22-nucleotide regulatory RNAs found in plants and animals. Some miRNAs of plants, *Caenorhabditis elegans*, and *Drosophila* play important gene-regulatory roles during development by pairing to target mRNAs to specify posttranscriptional repression of these messages. We identify three miRNAs that are specifically expressed in hematopoietic cells and show that their expression is dynamically regulated during early hematopoiesis and lineage commitment. One of these miRNAs, miR-181, was preferentially expressed in the B-lymphoid cells of mouse bone marrow, and its ectopic expression in hematopoietic stem/progenitor cells led to an increased fraction of B-lineage cells in both tissue-culture differentiation assays and adult mice. Our results indicate that microRNAs are components of the molecular circuitry that controls mouse hematopoiesis and suggest that other microRNAs have similar regulatory roles during other facets of vertebrate development.

MicroRNAs (miRNAs) are ~22-nucleotide (nt) noncoding RNAs that can play important roles in development by targeting the messages of protein-coding genes for cleavage or repression of productive translation (*1–3*). Examples include the *lin-4* and *let-7* miRNAs, which control the timing of *Caenorhabditis elegans* larval development (*4–6*); *Bantam* miRNA, which regulates *Drosophila* tissue growth by stimulating cell proliferation and preventing apoptosis (*7*); and miR-14, which affects *Drosophila* fat metabolism and prevents apoptosis (*8*). Humans have between 200 and 255 genes that encode miRNAs, an abundance corresponding to almost 1% of the protein-coding genes (*9*). Based on the evolutionary conservation of many miRNAs among the different animal lineages, it is reasonable to suspect that some mammalian miRNAs might also have important functions during development (*10–14*). Moreover, genes for miR-142, miR-15, and miR-16 are at sites of translocation breakpoints or deletions linked to human leukemias (*15–18*). However, no mammalian miRNAs have established functions (*19*).

As a first step toward testing the idea that miRNAs might play roles in mammalian development, and more specifically that some might regulate mammalian hematopoiesis, we cloned ~100 unique miRNAs from mouse bone marrow, using the protocol of Lau *et al*. (*20*). Most had already been identified as vertebrate miRNAs, but their expression in bone marrow had not been examined. miR-181 (*9*, *12*, *21*), miR-223 (*9*), and miR-142s (*18*) were carried forward for further analyses, because they, unlike miR-16 and most of the other miRNAs cloned, were differentially or preferentially expressed in hematopoietic tissues (Fig. 1).

miR-181 was very strongly expressed in the thymus, the primary lymphoid organ, which mainly contains T lymphocytes. It was also strongly expressed in the brain and lung and was detectable in bone marrow and the spleen. miR-223 was nearly exclusively expressed in bone marrow, the primary hematopoietic organ, which consists of hematopoietic stem cells and myeloid, erythroid, and lymphoid cells at various differentiation stages. miR-142s, whose gene is at the site of a translocation associated with an aggressive B cell leukemia (*16*, *18*), was highly expressed in all the hematopoietic tissues tested, with little or no expression in nonhematopoietic tissues. Expression at embryonic day 13 in fetal liver, an embryonic hematopoietic organ, suggests that miR-142 might also function in early hematopoietic development.

Because the bone marrow, spleen, and thymus each have specialized functions in adult hematopoiesis and comprise largely different cell types, the differential expression of the miRNAs in these complex tissues sug-

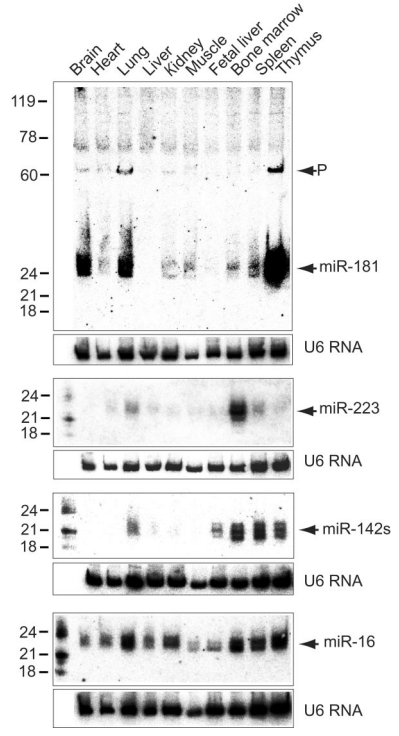

Fig. 1. Northern blots showing tissue expression of four miRNAs cloned from mouse bone marrow (*25*) (fig. S1). As loading controls, blots were also probed for U6 small nuclear RNA. The lengths (in nucleotides) of RNA markers are indicated, as are the bands that represent the mature miRNAs (miR) and presumed hairpin precursors (P).

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