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Evolutionary and Cellular Mechanisms Regulating Intestinal Performance of Amphibians and Reptiles[1]

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SYNOPSIS. Vertebrate intestinal tracts possess an array of structural and functional adaptations to the wide diversity of food and feeding habits. In addition to well-described differences in form and function between herbivores and carnivores, the intestine exhibits adaptive plasticity to variation in digestive demand. The capacity to which intestinal performance responds to changes in digestive demands is a product of evolutionary and cellular mechanisms. In this report, I have taken an integrative approach to exploring the mechanisms responsible for the regulation of intestinal performance with feeding and fasting among amphibians and reptiles. Intestinal performance is presented as the total small intestinal capacity to absorb nutrients, quantified as a product of small intestinal mass and mass-specific rates of nutrient uptake. For sit-and-wait foraging snakes and estivating anurans, both of which naturally experience long episodes of fasting, the dramatic downregulation of intestinal morphology and function with fasting reduces energy expenditure during extended fasts. In contrast, frequently-feeding species modestly regulate intestinal performance with fasting and feeding, trading higher basal rates of metabolism during fasting for the frequent expense of upregulating the gut with feeding. Surveying the magnitude by which intestinal uptake capacity is regulated among 26 families of amphibians and reptiles has revealed potentially five lineages that have independently evolved the capacity to widely regulate intestinal performance. The extent to which intestinal performance is downregulated with fasting among amphibians and reptiles, ranging from 0 to 90%, is largely a function of the degree by which mass-specific rates of nutrient transport are depressed, given that loss of intestinal mass with fasting is a common characteristic of vertebrates. In exploring the underlying mechanisms regulating intestinal nutrient uptake, use of the Burmese python has revealed a temporal match between microvillus surface area and intestinal nutrient transport. With feeding, pythons experience a five-fold lengthening of intestinal microvilli, with subsequent reduction after completing digestion. Identifying for the python the cellular processes responsible for the dramatic remodeling of the microvilli would assist in elucidating the mechanisms by which intestinal performance is regulated, as well as identify whether similar steps are employed by other species to regulate their intestines. In finishing, I propose three studies of digestive response: (1) investigate the responses of the ectotherm intestine to hibernation; (2) evaluate whether functional capacities of tissues are matched to digestive demands; and, (3) apply microarray technology to explore the functional genomics of intestinal adaptation.

INTRODUCTION

Intestinal tracts, functioning as the gateway between meal consumption and assimilation, are optimally designed to digest and absorb ingested food items. Thus, the diversity of vertebrate food and feeding habits is matched by an array of adaptive intestinal morphologies and physiologies (Stevens and Hume, 1995; Karasov and Hume, 1997). For example, the intestinal tracts of herbivores are longer and more complex (many include fermentation chambers) than those of carnivores, and herbivore intestines hydrolyze and transport simple sugars at greater rates than they do amino acids, whereas the opposite tends to be true for carnivore intestinal tracts (Karasov and Diamond, 1988; Stevens and Hume, 1995). The longer herbivore gut is necessary because plant material is more difficult to digest than animal material (Stevens and Hume, 1995). Differences in substrate breakdown and transport between herbivores and carnivores express an adaptive emphasis to absorb the major biomolecules of their diet (Karasov and Diamond, 1988).

The intestine of many, if not all, organisms possess an adaptive capacity to respond to changes in digestive demand. Contributing to this variation in demand are seasonal changes in nutrient and energy requirements, the amount and type of food consumed, and feeding frequency (Piersma and Lindström, 1997). The typical phenotypic response to a change in digestive demand is trophic, observed grossly as an increase or decrease in intestinal mass (Karasov and Diamond, 1983*a*). For example, a doubling in food intake by lactating mice is met by a 30% increase in small intestinal mass, whereas following a five-day fast, mass of the mouse intestinal mucosal is reduced by 50% (Hammond and Diamond, 1992; Dunel-Erb *et al.*, 2001). Less well documented are cases of phenotypic modulation of intestinal function (*i.e.*, enzyme activity, nutrient transport rates, etc.) that are independent of the trophic response (Karasov and Hume, 1997). One such study found mice to double and halve mass-specific rates of intestinal glucose and amino acid transport, respectively, following a switch from a low-carbohydrate to a high-carbohydrate diet (Karasov *et al.*, 1983).

The capacity to regulate digestive performance rep-

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resents both evolutionary and cellular mechanisms. The regulation of intestinal performance has an assumed selective benefit; by matching digestive performance to demand, energy exchange is optimized. For regulation to occur, cascades of intracellular steps alter the expression of cytoplasmic and membrane proteins resulting in a remodeling of cell structure and function. While we can envision these ultimate and proximate paths of regulation, the task remains to synthesize the evolutionary scenarios and identify the cellular mechanisms that underlie intestinal response.

I present here an integrative approach to study the regulatory mechanisms of intestinal performance. I begin by proposing a selective mechanism for the adaptive regulation of digestive performance, supported by two natural cases of long-term fasting. Next, I document the independent development of narrow and wide regulation of intestinal performance among 51 species of amphibians and reptiles. This is followed by illustrating the variation among these species in the regulation of intestinal morphology and function, both of which underscore the regulation of intestinal performance. I then present the Burmese python (*Python molurus*) as a model to study the regulation of intestinal performance, and describe its use to explore the mechanisms underlying the modulation of microvillus length. In finishing, I describe three studies of digestive physiology that take an adaptive, integrative, and mechanistic approach.

ANIMALS, METHODS, AND QUANTIFYING INTESTINAL PERFORMANCE

Animals used in digestive studies (listed in Table 1) were either purchased commercially or captured under state collecting permits. Amphibians and turtles were housed between 20 and 25°C, whereas lizards, snakes, and alligators were maintained between 25 and 30°C. Animals were fed at 2 to 14-day intervals, and water was provided ad libitum. Meals consisted of crickets and mealworms for most amphibians and lizards, fish for garter snakes and water snakes, beef for turtles, and rodents for monitor lizards, alligators, larger amphibians, and other snake species. Considering the variation in how the intestine responds morphologically and functionally to feeding and fasting, the digestive state of individuals for each species was standardized prior to experiments (Secor and Diamond, 2000). Individuals of each species were fasted for two to four weeks to ensure they were post-absorptive. Following, three individuals were fed meals equaling 5%, 10%, or 25% of body mass (Table 1). The selected meal size represented a naturally large meal for each species and thus was expected to generate full morphological and functional responses of the intestinal tract. One to two days following feeding, fed animals were sacrificed, as well as three fasted animals matched in body size to the fed individuals. Animal care and experimentation were conducted under protocols approved by the UCLA Animal Research Committee and the University of Alabama Institutional Animal Care and Use Committee.

Following sacrifice, the small intestine was removed, weighed, flushed of contents, reweighed, and prepared for measurements of nutrient uptake by the everted sleeve technique (Karasov and Diamond, 1983*b*; Secor *et al.*, 1994). Depending on its length, the small intestine was everted intact (as for smaller amphibians) or after being divided into halves (medium-size amphibians and small lizards) or thirds (larger amphibians and most reptiles). Intestinal halves or thirds were each weighed, and the everted segments divided into 1-cm sleeves. Sleeves were individually mounted on metal or glass rods, first incubated for five minutes in Ringers solution at 30°C, and then incubated for two minutes at 30°C in Ringer's solution containing an unlabeled and radiolabeled nutrient and an adherent fluid marker labeled with a different radioisotope. From each intestinal segment, uptake rates of the amino acids L-leucine and L-proline (each at 50 mm and labeled with ^{3}H) and of the sugar D-glucose (at 20 mm and labeled with ^{14}C) were measured. To correct for the amount of radiolabeled nutrient in the fluid adherent to the intestinal sleeve, a second labeled solute (^{14}C polyethylene glycol for the amino acids and L-[^{3}H]-glucose for D-glucose) was used. L-[^{3}H]-glucose measures also correct for the passive diffusion of D-glucose. With these corrections, measurements account for total amino acid uptake (passive and carrier-mediated) and carrier-mediated D-glucose uptake. Nutrient uptake rates were calculated as nanomoles of nutrient transported per minute of incubation per milligram of intestinal sleeve wet mass. I should note that whereas the everted sleeve technique has been found to be destructive to the intestinal epithelium of several bird species (Starck *et al.*, 2000; Stein and Williams, 2003), we have not observed this method to damage the intestines of amphibians and reptiles, nor has damage been reported in other everted-sleeve studies on amphibians and reptiles (Karasov *et al.*, 1985; Toloza and Diamond, 1990)

I assigned as a measure of intestinal performance the small intestine's total capacity to transport nutrients. Intestinal uptake capacity for a nutrient is quantified as the product of small intestinal wet mass times the uptake rates of that nutrient. When the intestine was divided, uptake capacities calculated for each segment were combined to determine total intestinal uptake capacity (Secor and Diamond, 1997*a*). Uptake capacities of the three nutrients were compared statistically (ANCOVA with body mass as the covariate) between fasted and fed animals to ascertain whether they changed with feeding. The magnitude of change in nutrient uptake capacities with feeding is a reliable marker to evaluate and compare the regulation of intestinal performance (Secor, 2001).

ADAPTIVE REGULATION OF DIGESTIVE PERFORMANCE

The gastrointestinal (GI) tract, like other major organ systems, can experience a wide range of demands,

TABLE 1. *Body mass, meal type, relative meal size, and factorial postfeeding increases in the intestinal uptake capacity of L-leucine, L-proline, and D-glucose relative to uptake capacities following 2–4 wk of fasting for 25 species of amphibians and 26 species of reptiles used in this review.*

Class/ Order/ Family	Species	Mean body mass (g)	Meal type	Meal mass (% of body mass)	Factorial increase in uptake capacities of			Source[a]
					L-leucine	L-proline	D-glucose	
Amphibia								
Caudata								
Ambystomatidae	*Ambystoma jeffersonianum*	10.6	cricket	5%	1.0		2.1	1
	Ambystoma opacum	4.84	cricket	5%	1.8	1.2	1.3	1
	Ambystoma texanum	7.02	cricket	5%	1.4	1.4	1.3	1
	Ambystoma tigrinum (*larva*)	81.7	fish	10%	1.4	1.2	4.9*[c]	1
Plethodontidae	*Plethodon mississippi*	3.76	cricket	5%	1.0	2.4	2.0	1
Proteidae	*Necturus maculosus*	99.5	fish	5%	1.0	1.0	4.1	1
Salamandridae	*Taricha granulosa*	12.6	cricket	10%	1.6	2.1		2
	Taricha torosa	10.8	cricket	10%	1.0	1.0	1.3	2
Anura								
Bombinatoridae	*Bombina orientalis*	5.31	cricket	10%	2.5	2.7	1.8	3
Bufonidae	*Bufo cognatus*	61.2	cricket	10%	2.2	2.1	1.3	3
	Bufo debilis	4.37	cricket	10%	1.7	2.5	1.9	3
	Bufo marinus	142	rodent	15%	1.3	3.5	1.1	3
	Bufo punctatus	17.6	cricket	10%	1.9	1.7	2.3	3
	Bufo speciosus	51.0	cricket	10%	2.2	1.7	3.2*	3
Hylidae	*Hyla chrysoscelis*	5.57	cricket	10%	1.2	2.6	2.4	3
	Hyla cinerea	6.98	cricket	10%	1.2	2.4	2.1	3
	Hyla squirella	2.86	cricket	10%	1.9	2.0	1.6	3
Hyperoliidae	*Kassina senegalensis*	5.33	cricket	10%	1.6	1.5	1.5	3
Leptodactylidae	*Ceratophrys ornata*[Fb]	190	rodent	15%	6.2*	5.8*	10.1*	3
	Leptodactylus pentadactylus	143	rodent	15%	1.4	1.8	1.3	3
Microhylidae	*Kaloula pulchra*	26.3	cricket	10%	1.0	1.0	2.3	3
Ranidae	*Pyxicephalus adspersus*[F]	240	rodent	15%	4.6*	4.1*	9.1*	3
	Rana catesbeiana	315	rodent	15%	1.3	2.1	1.8	3
	Rana pipiens	28.9	cricket	10%	1.4	2.0	3.4*	3
Rhacophoridae	*Polypedates leucomystax*	11.5	cricket	10%	1.3	1.1	1.0	3
Reptilia								
Testudines								
Chelydridae	*Chelydra serpentina*	79.0	beef	10%	1.0	1.0	2.2	4
Emydidae	*Trachemys scripta*	325	beef	5.9%	1.2	1.3	2.0	4
Kinosternidae	*Sternotherus odoratus*	64.6	beef	5.5%	1.0	1.2	1.9	4
Squamata								
Sauria								
Cordylidae	*Gerrhosaurus flavigularis*	41.9	cricket	5%	2.0	1.3	1.8	3
Gekkonidae	*Gekko gecko*	60.2	cricket	5%	1.6	1.9	3.1	3
Iguanidae	*Gambelia wislizenii*	22.5	cricket	5%	1.4	1.0	1.3	3
	Sceloporus magister	22.8	cricket	10%	1.1	1.1	1.4	3
	Sceloporus occidentalis	8.95	cricket	5%	1.3	1.3	1.6	3
Scincidae	*Eumeces inexpectatus*	4.87	cricket	5%	1.2	1.4	1.3	3
Teiidae	*Ameiva ameiva*	53.0	cricket	5%	1.6	1.0	2.1	3
Varanidae	*Varanus exanthematicus*	270	rodent	10%	1.5	1.0	1.7	3
Serpentes								
Boidae	*Boa constrictor*[F]	375	rodent	25%	11.2*	7.9*	10.3*	5
	Lichanura trivirgata[F]	236	rodent	25%	6.2*	7.6*	10.3*	5
Colubridae	*Coluber constrictor*	170	rodent	25%	1.2	1.3	3.3	5
	Elaphe obsoleta	483	rodent	25%	1.0	1.0	1.8	3
	Lampropeltis getula	220	rodent	25%	1.5	1.7	1.6	5
	Masticophis flagellum	329	rodent	25%	1.8	1.6	1.8	5
	Nerodia rhombifer	305	fish	25%	1.8	1.6	1.4	3
	Pituophis melanoleucus	742	rodent	25%	1.4	1.6	1.0	5
	Thamnophis marcianus	52.0	fish	25%	1.6	1.8	2.7	7
	Thamnophis sirtalis	54.8	fish	25%	1.0	1.4	2.0	7
Pythonidae	*Python molurus*[F]	725	rodent	25%	12.4*	11.9*	31.6*	5
	Python reticulatus[F]	784	rodent	25%	7.2*	7.6*	21.3*	6
	Python sebae[F]	814	rodent	25%	7.3*	6.7*	31.6*	6
Viperidae	*Crotalus cerastes*[F]	112	rodent	25%	13.0*	5.0*	17.6*	5
Crocodylia								
Alligatoridae	*Alligator mississippiensis*	908	rodent	10%	1.2	1.3	2.0	3

[a] Sources: 1 Wooten and Secor, unpublished data; 2 Secor, Kats, and Diamond, unpublished data; 3 Secor, unpublished data; 4 Secor and Diamond, 1999; 5 Secor and Diamond, 2000; 6 Ott and Secor, unpublished data; 7 Stubblefield, Ultsch, and Secor, unpublished data.

[b] A superscript F designates species that naturally fast for extended periods either as a function of their sit-and-wait foraging tactics or because they estivate during the dry season.

[c] An asterisk (*) designates a significant postprandial increase in intestinal uptake capacity for that nutrient.

but unlike, for instance, the pulmonary or cardiovascular systems which must provide at least a minimum level of performance, GI tracts of many organisms are routinely quiescent. While episodes of digestive quiescence may be extremely short or nonexistent for a few select endotherms (*e.g.,* shrews), periods of fasting range usually from only a few hours (typical of active endotherms) to several months (characteristic of hibernation, estivation, and for sit-and-wait foraging ectotherms), to well over a year. For example, female rattlesnakes (*Crotalus viridis*) may forego feeding for the entire activity season that they give birth and thus are aphagic for two hibernating cycles and the intervening summer (Macartney and Gregory, 1988). In considering the potential variability in its performance (a function of changes in digestive demand) and the wide continuum of how often it is used (a function of feeding habits and life histories), the GI tract has been an open target of selection.

One potential selective target has been the capacity to regulate digestive performance in response to variation in digestive demand, a product of changes in meal size and/or type. Although such a capacity may be relatively subtle for animals with modest variations in their meal size (*e.g.,* most endotherms and many ectotherms), it is apparent for those whose meals can vary widely in size. For example, Burmese pythons, able to digest meals equivalent to their own body mass, respond to an increase in meal size by increasing their small intestine's capacity to transport nutrients (Secor and Diamond, 1997*a*, *b*).

A second potential outcome of selection is the differential capacity to regulate digestive performance linked to variation in fasting duration. Animals that feed relatively frequently tend to exhibit small changes in digestive performance with feeding and fasting (Secor, 2001). In contrast, those animals whose life histories include long episodes of fasting regulate digestive performance much more widely with feeding and fasting (Secor, 2001). What sets these two patterns of digestive regulation apart is that the latter group severely downregulates digestive performance with fasting and consequently must upregulate it with feeding.

An evolutionary explanation for the downregulation of digestive performance with fasting may be surmised from the following information. We know for birds and mammals at least that the intestinal epithelium possesses a high cellular turnover rate and therefore is considered to be costly to maintain (Johnson, 1987; Starck, 1996). Many animals, especially ecotherms, possess life histories that include long episodes of aphagia during which the gut is quiescent (Gregory, 1982; Pinder *et al.,* 1992). And, while fasting, animals rely solely upon stored energy to meet metabolic needs. Thus any mechanisms that can reduce energy expenditure will favor survival. Therefore, the capacity to significantly downregulate digestive performance during long episodes of fasting would be selected for, given the benefits of a reduced rate of energy expenditure. Such an adaptive response is evident for the following two cases.

Case I, infrequently-feeding snakes

Snake species that employ the sit-and-wait tactic of prey capture characteristically feed infrequently at intervals of one to several months (Pope, 1961; Secor and Nagy, 1994). For four species of infrequently-feeding snakes, the completion of digestion triggers a dramatic downregulation (by 90%) of small intestinal performance (*i.e.,* nutrient uptake capacity), a function of a 50% reduction in intestinal mass and an average 78% reduction in mass-specific rates of nutrient transport (Fig. 1A). In contrast, more modest regulatory responses are exhibited by snake species that feed more frequently in the wild (at 1 to 2 week intervals); following a 30-day fast, intestinal performance is reduced by only 40%, largely due to a 25% decrease in small intestinal mass (Fig. 1B). I theorized that the benefit to infrequently-feeding snakes in downregulating their GI tract during fasting is the lowering of their standard metabolic rate (SMR), which averages 50% less than that of frequently-feeding species (Secor and Diamond, 2000). When modeled, the lower SMR, even when offset by the occasional cost of gut upregulation, reduces the overall energetic expenditure of infrequently-feeding species compared to the alternative strategy of a more modest regulatory response and subsequently higher SMR (Secor, 2001). This is not to imply that wide regulation of gut performance is a superior energetic strategy; while it appears to be for infrequently-feeding snakes, snakes that feed more frequently benefit from a more modest, and less costly, upregulatory response to their frequent meals even though it entails a higher SMR (Secor, 2001).

Case II, estivating anurans

Many amphibians and reptiles inhabit regions that experience an annual dry season of several months characterized by high temperatures and low water and food availability. Unable to migrate to more favorable habitats, amphibians bury themselves into the soil and estivate for the duration of the dry season (Pinder *et al.,* 1992). For estivating and consequently aphagic anurans, several mechanisms have been described to reduce their metabolic expenditure and thus aid in energy conservation, including the depression of protein synthesis and enzyme activities (Fuery *et al.,* 1998; Storey, 2002). To these mechanisms, I add the downregulation of intestinal performance triggered by fasting and further enhanced by estivation. For the African bullfrog (*Pyxicephalus adspersus*; Ranidae) and the South American horned frog (*Ceratophrys ornata*; Leptodactylidae), two species that estivate for up to eight months (Loveridge, 1976), a one-month fast results in a 78% and 84% reduction in intestinal performance, respectively (Fig. 2). In contrast, a one-month fast induced only a respective 44% and 23% reduction in intestinal performance for the non-estivating American bullfrog (*Rana catesbeiana*; Ranidae) and the

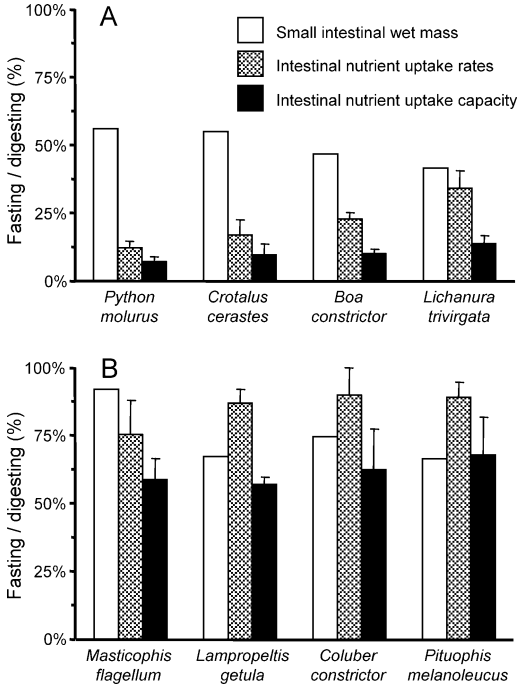

FIG. 1. Small intestinal wet mass, intestinal nutrient uptake rates, and intestinal nutrient uptake capacity of fasted snakes presented as a percentage of those variables measured from digesting individuals of four species of infrequently-feeding snakes (A) and of four species of frequently-feeding snakes (B). For nutrient uptake rates and uptake capacities, bars represent the average fasted percentages (+1 SE) for the uptake of L-leucine, L-proline, and D-glucose. Note that with fasting infrequently-feeding snakes reduce intestinal mass, nutrient uptake rates, and therefore uptake capacity by much greater magnitudes than frequently-feeding species. Source of data is Secor and Diamond (2000).

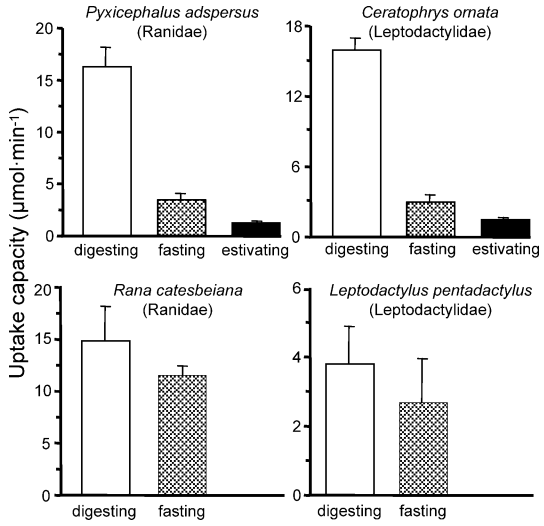

FIG. 2. Mean (+1 SE) intestinal uptake capacity of L-leucine of four anuran species during digestion and fasting, and for two of those species during estivation. For the anuran families Ranidae and Leptodactylidae, two species (*Pyxicephalus adspersus* and *Ceratophrys ornata*) that estivate and thus are aphagic during the dry season experience significant downregulation of intestinal performance with fasting, and again with estivation. In contrast for the same two families, two species that do not estivate (*Rana catesbeiana* and *Leptodactylus pentadactylus*) exhibit no significant decline with fasting in intestinal performance. Sample size for each bar is 3.

smokey jungle frog (*Leptodactylus pentadactylus*; Leptodactylidae). If denied access to water in the laboratory, fasted *P. adspersus* and *C. ornata* will enter into estivation, characterized by a hunched, head-down posture, and the build-up of multiple layers of dried epithelium that forms a covering cocoon which serves to reduce evaporative water loss (Loveridge and Withers, 1981; McClanahan *et al.*, 1976). Following one month of laboratory estivation, intestinal uptake capacities of *P. adspersus* and *C. ornata* were further reduced from fasting values by 75% and 50%, respectively. By shutting down their intestinal tracts during estivation, these anurans reduce their daily energy expenditure and therefore increase the duration that they can survive dormancy.

SURVEY OF THE REGULATION OF INTESTINAL PERFORMANCE

My proposed adaptive explanation for the regulation of intestinal performance links the wide regulation of intestinal performance with long episodes of fasting, as well as implies that modest regulation of gut performance is characteristic of animals that feed frequently and often are digesting (Secor, 2001). Support for this adaptive scenario of digestion would include the demonstration that modest and wide regulation of digestive performance have evolved independently in concert with feeding ecologies characterized by short and long periods of fasting, respectively. Investigating the adaptive interplay between feeding ecologies and

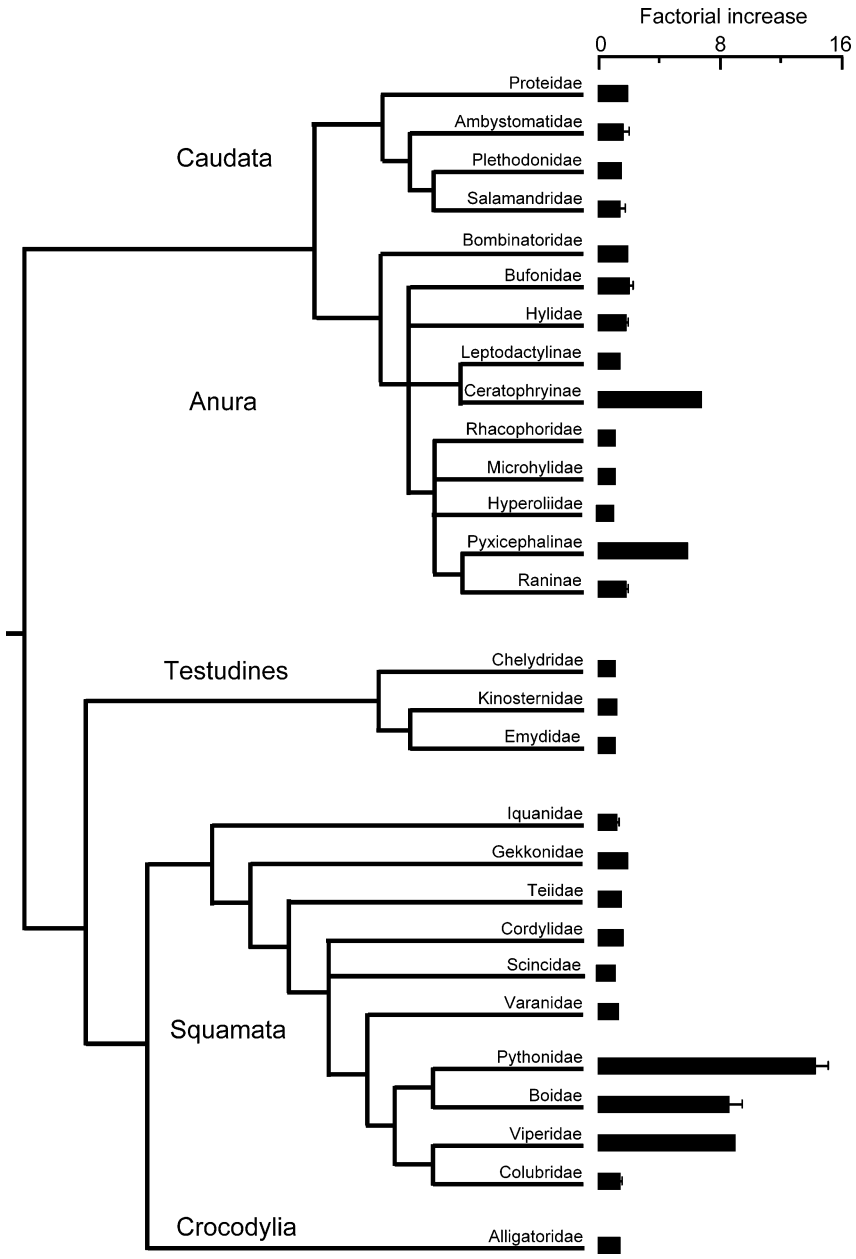

FIG. 3. Phylogenetic assessment of the postprandial increase in intestinal nutrient uptake capacity for 24 families and 4 subfamilies of amphibians and reptiles. The hypothetical phylogeny was constructed using phylogenies presented in Pough *et al.* (2001), Zug *et al.* (2001), and Pianka and Vitt (2003). Bar lengths represent the mean factorial increase for the uptake capacity of L-leucine, L-proline, and D-glucose. For families represented by multiple species (see Table 1), bar length and error bars signify mean and 1 SE of averaged factorial increase in uptake capacities among those species.

the regulation of digestive performance among amphibians and reptiles has been a major focus of my laboratory (Secor, 2001). Amphibians and reptiles are favorable for study because they exhibit distinct differences in feeding habits and digestive performances.

To assess the extent that intestinal performance is regulated, we compare nutrient uptake capacities of the small intestine between fasted and fed individuals. To date (Fall 2004), we have measured uptake capacities of 25 species of amphibians representing 12 families, and 26 species of reptiles representing 14 families (Table 1). The majority of these species are considered frequent digesters, whereas eight species fast for extended periods, either as sit-and-wait foragers or while estivating.

I illustrate this survey of intestinal response by linking the postfeeding increases in intestinal nutrient uptake capacities to a hypothetical phylogeny of the amphibians and reptiles used in this study (Fig. 3). This phylogeny was constructed using several separate phylogenies developed specifically for amphibians and reptiles (Pough *et al.*, 2001; Zug *et al.*, 2001; Pianka

and Vitt, 2003). For convenience and simplicity sake, I terminated the branches at the family or subfamily level. For families represented by multiple species (9 of the 26), I present the average factorial increase in uptake capacities among those species. I should note that the presentation of the phylogeny is only to illustrate the variation in digestive response in a historical context.

The wide regulation of intestinal performance is presently exhibited within the anuran subfamilies Ceratophryinae (family Leptodactylidae) and Pyxicephalinae (family Ranidae), as well as within the snake families Pythonidae, Boidae, and Viperidae. The eight species studied within those taxa habitually fast for months and collectively experience 5 to 30-fold swings in nutrient uptake capacity with fasting or feeding. In contrast, the 43 species that feed frequently averaged a 1.7-fold increase in uptake capacity among the three nutrients. For only several frequent feeders (*Ambystoma tigrinum* larva, *Bufo speciosus*, and *Rana pipiens*) did uptake capacity of a nutrient increase significantly with feeding.

From these findings we can predict other lineages that similarly possess species which widely regulate digestive performance. Among amphibians, members of the families Amphiumidae (*Amphiuma*), Sirenidae (*Siren*), Pelobatidae (*Scaphiopus*), Hylidae (*Cyclorana*), and Limnodynastidae (*Neobatrachus*) estivate for months and thus may also severely downregulate digestive performance with fasting (Pinder *et al.*, 1992). Additional examples for reptiles may include turtles (*e.g., Kinosternon sonoriense*), lizards (*e.g., Varanus albigularis*), and large crocodilians (*e.g., Crocodylus johnstoni*) that estivate during the dry season (Kennett and Christian, 1993; Peterson and Stone, 2000; Phillips, 1995). For snakes, wide regulation of gut performance is likely characteristic of other sit-and-wait foraging species that dominate the families Boidae, Pythonidae, and Viperidae. Even within the families Elapidae and Colubridae, both dominated by actively foraging, frequent feeders, wide regulation of intestinal performance may have evolved as isolated events for several long-term fasting species. The sit-and-wait foraging Australian death adder (*Acanthophis*, Elapidae) experiences long episodes between infrequent meals (Shine, 1980), while the African egg-eating snake (*Dasypeltis*, Colubridae) may only feed during the nesting season of birds (Branch, 1998).

PROXIMATE MECHANISMS MODULATING INTESTINAL PERFORMANCE

As mentioned, intestinal performance is assessed as intestinal nutrient uptake capacity, a product of small intestinal mass times mass-specific rates of nutrient transport. For the 51 species studied, fasting is associated with an 8 to 93% (mean = 42 ± 4%) reduction in intestinal nutrient uptake capacity. As a start to deciphering the mechanisms responsible for the fasting depression in intestinal performance, I determined the relative contribution of both the decrease in intestinal mass and the decrease in nutrient uptake rates that result in the decline in uptake capacity. In plotting the percent decline in small intestinal mass against the percent decline in nutrient uptake rates, three findings emerge (Fig. 4). First, clustered in the upper-left quadrant are the frequently-feeding species that exhibit smaller declines in intestinal mass and function, and thus modestly regulate intestinal performance. Grouped in the lower-right quadrant are the long-term fasters that significantly reduce both intestinal mass and function with fasting.

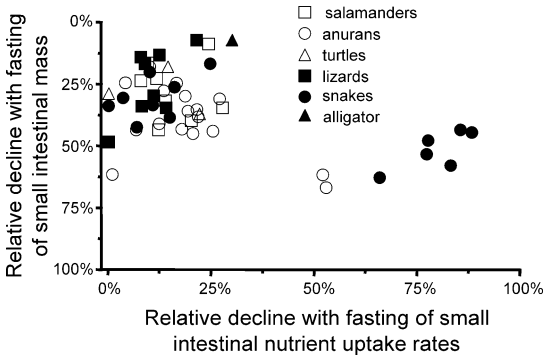

FIG. 4. Relative decline in intestinal nutrient uptake rates with fasting plotted against the relative decline in small intestinal wet mass with fasting for 51 species of amphibians and reptiles. The plotted decline in nutrient uptake rates represents the average of the relative declines experienced for L-leucine, L-proline, and D-glucose. Note the two distinct clusters, the upper left cluster contains frequently-feeding species, whereas the lower right cluster contains species that fast in the wild for extended periods.

Second, all species experienced a reduction (mean = 34 ± 2%) in intestinal wet mass with fasting. Atrophy of the intestinal mucosa is a characteristic vertebrate response to fasting, observed for fishes (McLeese and Moon, 1989), amphibians (Cramp and Franklin, 2003), reptiles (Secor and Diamond, 2000), birds (Hume and Biebach, 1996), and mammals (Dunel-Erb *et al.*, 2001). With fasting, intestinal enterocytes generally reduce their width, resulting in a shortening of the villi and a decrease in mucosal mass (Carey, 1990). Feeding rapidly reverses this response; enterocytes swell and intestinal mass and luminal surface area increases (Dunel-Erb *et al.*, 2001). Among the species thus far studied, there is noted variation in how much the small intestine is reduced in mass with fasting. While there is no observable correlation between intestinal mass loss and body size or phylogeny, there is in regards to feeding habits; frequent feeders reduce intestinal mass less so with fasting than species which fast for long periods.

The third finding is the extreme variability in the regulation of mass-specific rates of nutrient transport, ranging from literally no change to as much as a 90% reduction with fasting. Likewise, in the transition from fasting to digesting (not shown), transport rates consequently remain static or increase by as much as 10-fold. Considering the almost universal trophic response of the intestine to fasting, it appears that the

observed variation in how much digestive performance is regulated is largely a function of how much mass-specific nutrient transport rates can be altered.

In addition to either declining or not changing, nutrient uptake rates have also been found to increase with fasting. During hibernation, small-intestinal mass of the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) is reduced by 50%, while mass-specific uptake of 3-O-methylglucose doubles (Carey and Sills, 1992). In my own lab, we occasionally observe uptake rates of an individual nutrient to average higher during fasting than during digestion. Plausible explanations for the fasting-related rise in mass-specific nutrient uptake, concurrent with intestinal atrophy, include increased expression of nutrient transporter, increased electrochemical gradient for nutrient-coupled Na^+ entry, and increased density of transporters as enterocytes are reduced in width (Carey and Sills, 1992). The lack of any change in mass-specific rates of nutrient uptake with feeding or fasting implies that function is tightly matched to morphology. A fasting decrease in uptake rates suggests that the number and/or activities of transporters are actively depressed more so than can be explained by loss of gross structure.

As previously described, intestinal nutrient uptake rates can change with feeding and fasting, sometimes dramatically. There are several described specific and nonspecific mechanisms by which intestinal function is regulated (Ferraris, 1994). First, resident apical membrane transporters and enzymes can increase or decrease their activities. Changes in transporter turnover rates have been proposed to contribute to developmental shifts in nutrient transport rates that occur with ontogenetic changes in diet (Buddington and Diamond, 1992; Toloza and Diamond, 1990). Second, membrane densities of transporters and enzymes change as a function of changes in their rates of synthesis. An increased density of intestinal Na^+/glucose co-transporter (SGLT1) may explain the increase in intestinal D-glucose uptake for mice switched from a low to high-carbohydrate diet (Ferraris *et al.*, 1992). And third, the functional surface area of the luminal epithelium can be modulated by increasing or decreasing enterocyte number and/or dimensions. If transporter and enzyme densities remain unchanged, then function would be altered accordingly. For example, a compensatory response to the surgical removal of a segment of small intestine is mucosal hyperplasia of the reattached remnant intestine. The increase in villus height results in nonspecific increases in function (nutrient breakdown and absorption) per length of remnant intestine, and the partial restoration of lost performance (Fenyö *et al.*, 1976; Hanson *et al.*, 1977). Identifying the cellular and molecular mechanisms of intestinal regulation for amphibians and reptiles will aid in further developing evolutionary explanations, as well as gaining insight into the potential targets of selection.

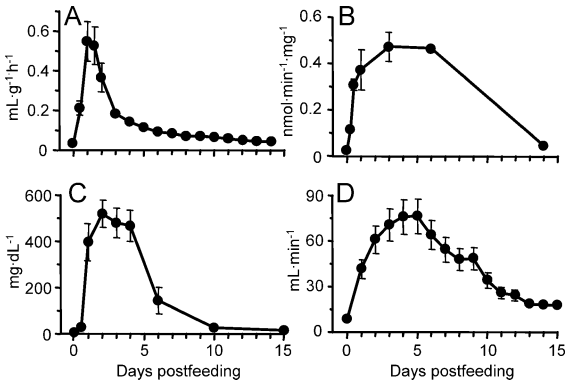

FIG. 5. Oxygen consumption rates (A), intestinal glucose uptake rates (B), plasma concentrations of triglycerides (C), and superior mesenteric blood flow (D) of Burmese pythons (*Python molurus*) plotted as a function of time following the consumption of rodent meals equaling 25% of snake body mass. Burmese pythons experience dramatic increases in these physiological variables following feeding, and reversible declines upon the completion of digestion.

PYTHON MODEL TO STUDY THE REGULATORY MECHANISMS OF INTESTINAL PERFORMANCE

Scientific studies of mechanisms underlying development, regulation, and adaptation have benefitted greatly from the discovery and use of appropriate animal models. Such models share the characteristics of possessing to an extreme degree a trait or response of interest and the ease of acquisition, care, and study. Identifying the regulatory mechanisms of intestinal performance would be best served using an animal that routinely exhibits broad swings in intestinal function. Such an animal is the Burmese python, a snake that exhibits a number of unprecedented physiological responses to feeding and fasting (Secor and Diamond, 1998). My collaborators and I have discovered that Burmese pythons experience with feeding 8-fold increases in intestinal blood flow, 20-fold increases in nutrient transport rates, 40-fold increases in metabolic rates, and 160-fold increases in plasma lipid concentrations (Fig. 5; Secor and Diamond, 1997*a*, *b*, 1998; Secor and White, 2003). Routinely experiencing wide swings in intestinal performance, pythons are an ideal model for studying the proximate mechanisms of intestinal adaptation.

Having identified the postprandial increases in SGLT1 protein and mRNA expression in the python (S. Secor and M. Martin, unpublished data), we are now focusing on the regulatory role of luminal surface area. This attention is sparked by the findings that Burmese pythons exhibit a 5-fold increase in the length of their intestinal microvilli within 24-hours after feeding (Secor *et al.*, 2000, 2002; Starck and Beese, 2001). Microvilli remain lengthened during digestion and then progressively shorten following the completion of digestion. In contrast to pythons, mammals and frequently-feeding amphibians and reptiles exhibit either no or very minor changes in microvillus length with fasting (Fig. 6). As a function of their height, width, and density, the microvilli alone are responsible for

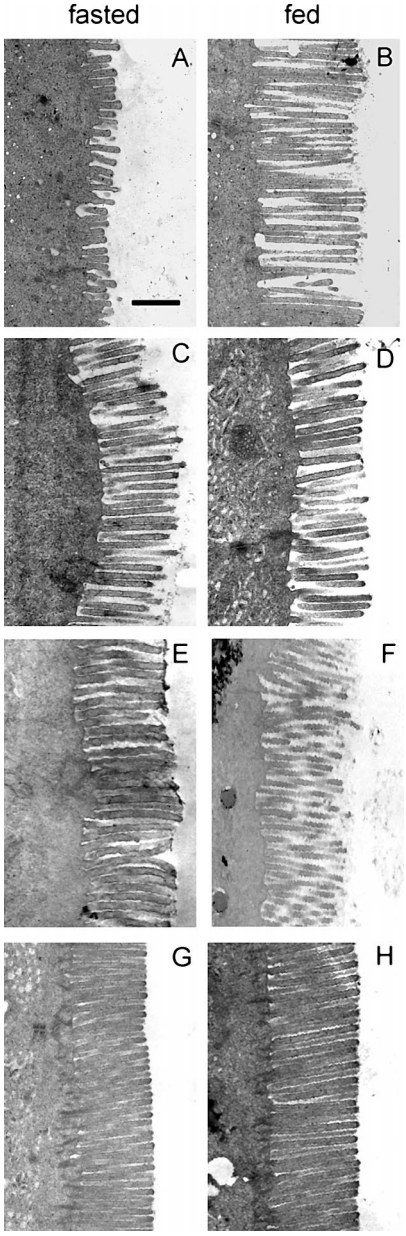

FIG. 6. Electron micrographs of intestinal microvilli from fed and fasted Burmese pythons (*Python molurus*, A, B), bullsnakes (*Pituophis melanoleucus*, C, D), smokey jungle frogs (*Leptodactylus pentadactylus*, E, F), and laboratory mice (*Mus musculus*, G, F). Note that the fasting-related decline in microvillus length for Burmese pythons is not shared by these other species. The bar in A represents 1 μm.

increasing the functional surface area of the small intestine by 30 to 50-fold (Mooseker, 1985; Karasov and Hume, 1997). Therefore, lengthening or shortening the microvilli would have dramatic consequences on intestinal performance. For the Burmese python, comparing postfeeding profiles of intestinal microvillus surface area and nutrient uptake rates illustrate how closely intestinal function tracks intestinal morphology (Fig. 7).

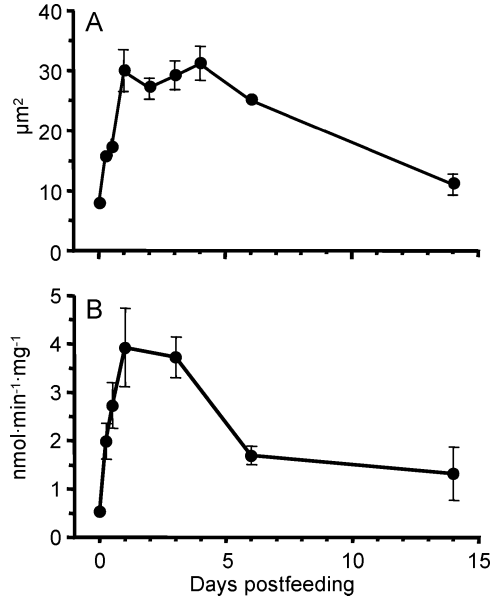

FIG. 7. Postprandial profile of intestinal microvillus surface area (per μm of flat luminal surface (A) and intestinal proline uptake rates (B). Note the temporal match between intestinal form (surface area) and function (uptake).

HOW DO PYTHONS MODULATE INTESTINAL MICROVILLI LENGTH?

If the wide regulation of intestinal performance is an adaptive process, and if function is being dictated by microvillus length, then what are the mechanisms that modulate the length of the microvilli? Addressing this question first requires familiarity with the architecture of the intestinal microvilli (Fig. 8). Each microvillus is supported internally by 15 to 30 uniform-

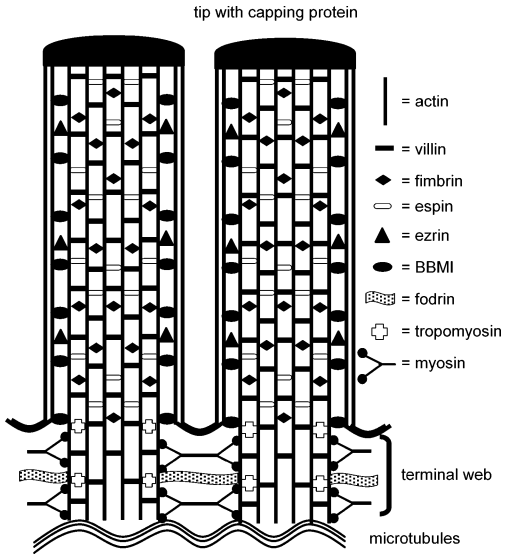

FIG. 8. Subcellular architecture of the intestinal microvilli. Each microvillus is supported internally by F-actin filaments that are cross-linked and attached to the outer membrane by an array of proteins. F-actin filaments and proteins extending into the cell contribute to the terminal web and anchor the microvilli.

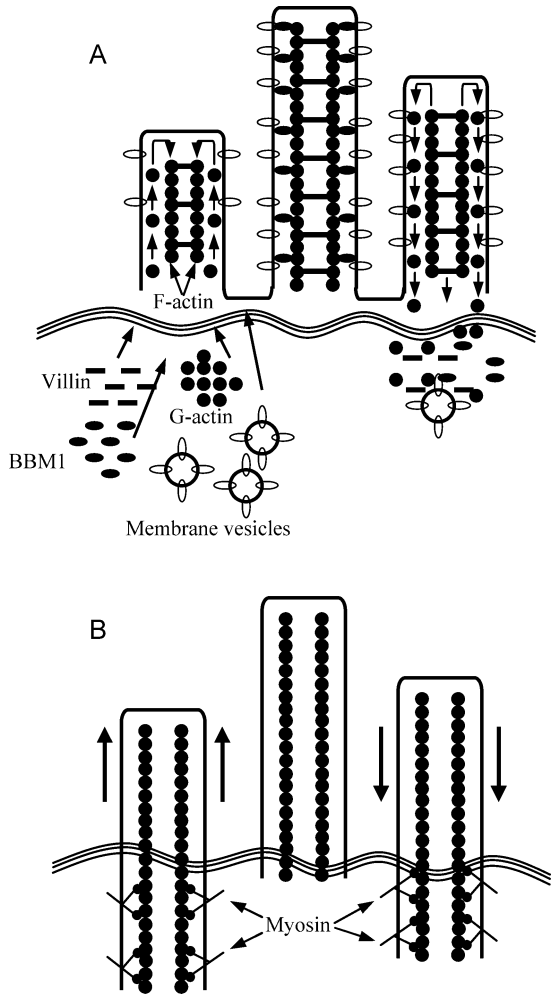

FIG. 9. Two alternative models of microvillus lengthening and retraction. Microvilli lengthen postprandially as F-actin is polymerized by the attachment of cytoplasmic G-actin, accompanied by the insertion of other cytoskeletal proteins and membrane, and then shorten during fasting as proteins and membrane are detached (A). Alternatively with feeding, microvilli elevate from the terminal web powered by myosin molecules and are then retracted back into the web with fasting (B).

length actin filaments (F-actin) that are cross-linked by binding proteins, including villin, fimbrin, and espin (Mooseker, 1985). The actin filaments are periodically tethered to the surrounding membrane by lateral bridges composed of brush-border myosin-I and ezrin (Algrain *et al.*, 1993; Mooseker, 1985). The base of the actin filaments anchor the microvilli and form with other cytoskeletal proteins (filamin, caldesmon, tropomyosin, fodrin, alpha-actinin, and several classes of unconventional myosin) the underlying terminal web (Furukawa and Fechheimer, 1997; Heintzelman *et al.*, 1994; Mooseker, 1985). The surrounding membrane is composed of a bilayer of phospholipid molecules embedded with a wide array of proteins that function largely as channels, transporters, receptors, and enzymes (Johnson, 1997). Although well described structurally, the mechanisms responsible for the assembly and disassembly of the microvillus skeleton are largely unknown (Bement and Mooseker, 1996). Equally a mystery are the mechanisms responsible for the construction and destruction of the microvillar membrane with changes in microvillus length.

From decades of research on microvillar structure and dynamics, including studies on insect malpighian tubules (Bradley and Satir, 1981), echinoderm sperm (Tilney *et al.*, 1973); developing chicken intestine (Stidwell and Burgess, 1986), and cultured cell lines (Peterson and Mooseker, 1992), two models have emerged to explain the modulation of microvillus length. The more popular of the two is that microvilli lengthen as actin filaments are polymerized by the attachment at their tips of monomeric G-actin that has migrated from cytoplasmic stores (Fig. 9A). As the core filaments polymerize, other microvillar proteins (villin, fimbrin, etc.) are mobilized from the cytoplasm and inserted. Simultaneously, vesicles of membrane that contain the full complement of membrane-bound proteins are directed from the cytoplasm to fuse with the existing membrane in an exocytotic fashion (Bradbury and Bridges, 1994; Lange, 2002). The post-digestive shortening of the microvilli would occur as F-actin depolymerizes and proteins and membrane are sequestered back into the cytoplasm or are degraded (White *et al.*, 2000).

The alternative model proposes that the microvilli maintain their full length and are simply raised and lowered with feeding and fasting (Fig. 9B). The machinery for this mechanism exists within the terminal web as unconventional myosin fibers (myosin I, II, V, VI [Heintzelman *et al.*, 1994]) that could act as intracellular motors propelling the python actin filaments upward with feeding and retract them following digestion. As the core filaments are raised new membrane is added or the existing membrane stretches. With filament retraction, membranes are detached, or if stretched simply rebound.

The first step in determining which of these models best describes the cellular mechanisms underlying microvillar growth for the python is to identify the position and quantify the expression of intestinal microvillar proteins (*i.e.*, F- and G-actin, villin, brush border myosin I) for fasting and digesting snakes. The challenges one faces in using standard immuno-binding techniques (immunohistochemistry and Western blotting) to detect protein position and expression of python tissue is finding whether the available mammalian-source antibodies bind effectively to the homologous python protein. Finding a postprandial decline in cytoplasmic pools of G-actin and other cytoskeletal proteins, and concurrent lengthening of F-actin filaments would support the first model (Fig. 9A), whereas observing actin filaments extending deep into the cytoplasm during fasting and minimal postfeeding change in G-actin would promote the second model (Fig. 9B).

PERSPECTIVE STUDIES OF INTESTINAL PERFORMANCE

There is tremendous opportunity to explore further the regulatory patterns of digestive physiology. Although this field has been heavily dominated by studies on several traditional laboratory models (mice, rats, and rabbits), all of which exhibit modest GI responses, the recent attention to estivating amphibians, sit-and-wait foraging snakes, migrating birds, and hibernating mammals have demonstrated the diversity of the adaptive interplay between digestive physiology and life history traits (Carey, 1995; Karasov and Hume, 1997; Secor, 2001). In addition to developing adaptive hypotheses of the digestive system, the door is wide open for investigating the integrative response of tissue performance and their cellular and molecular mechanisms. In the spirt of these remarks, the following are three studies that would continue the investigation of the plasticity, integration, and mechanisms of intestinal response.

Intestinal response to ectotherm hibernation

Intestinal response to long-term aphagia has been studied for infrequently-feeding snakes (Secor and Diamond, 2000), estivating amphibians (Secor and Diamond, 1996), and hibernating mammals (Carey, 1995). In each case, the intestine downregulates function and/or morphology, thereby depressing overall performance. Amphibians and reptiles that inhabit temperate regions of the world likewise hibernate, and thus are aphagic for 5–8 months of the year (Gregory, 1982; Pinder *et al.*, 1992). Given that many of these species feed frequently during the summer they would be expected to narrowly regulate digestive performance during that time. But to what extent is their intestinal performance regulated during hibernation? One potential response is that they simply reduce intestinal mass, similar to what they may experience in response to fasting, and which is the characteristic response of hibernating mammals. An alternatively response is a combined reduction of intestinal mass and intestinal function, mimicking the fasting responses of infrequently-feeding snakes and estivating amphibians. The advantage of this response is that metabolic rate is lowered due to the depression of gut maintenance, thus increasing the duration of survival on a fixed amount of stored energy. Tracking intestinal form and function of hibernating amphibians and reptiles would determine which of these responses is employed during hibernation.

Integration of digestive responses

Meal digestion involves not only the components of the GI tract, but also the interactions of other visceral organs. Thus, the regulation of tissue performance during digestion would be expected to include other GI and associated organs. Evidence to support an integrative response to fasting and digestion is scant, but does include: (1) significant reductions in the activities of intestinal brushborder and pancreatic enzymes for fasting Burmese pythons (Secor and Diamond, 1998); (2) significant decreases in the masses of the liver, pancreas, stomach, and kidneys during fasting for infrequently-feeding snakes, but no such decreases experienced by fasting frequently-feeding species (Secor and Diamond, 2000); and, (3) cessation of gastric acid production following digestion for the Burmese python (Secor, 2003). An integrative approach could demonstrate the extent that the regulation of tissue performance is matched in response to varying digestive demands. Studies may also reveal that the greatest metabolic and functional demands on an organism occurs during digestion. Hence, maximum digestive demand may set the upper limits to the performance of supportive organ systems such as the respiratory and cardiovascular systems.

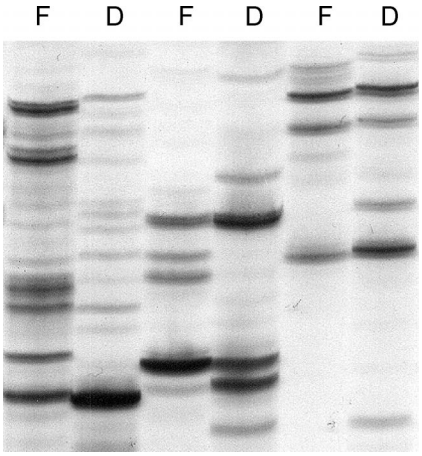

FIG. 10. A partial segment of a sequencing gel illustrating differentially expressed mRNA extracted from the intestinal mucosa of a fasting (F) and a digesting (D) python. Fasting and digesting lanes are paired to illustrate potential postprandial changes in gene expression.

Functional genomics of intestinal adaptation

Each intestinal response to feeding or fasting is the product of a cascade of cellular and molecular events triggered by chemical, hormone, paracrine, and/or neural signals. If we could backtrack the cellular events to their origin, we might pass through (in reverse order of their occurrence) the steps of protein expression, translation (protein synthesis), transcription, gene expression, and the cellular pathways that signal gene activation. Although we could envision such pathways, understanding the actual mechanisms of intestinal adaptation requires identifying the triggering signals and the genes that are differentially expressed with fasting and feeding. Undoubtedly, many genes are turned on and off with the regulation of digestive performance, as visualized by a differential display of mRNA extracted from the intestinal mucosa of fasted and fed Burmese pythons (Fig. 10). The ability to identifying differentially expressed genes has been enhanced by the recent advent of DNA microarray technology which allows the expression of thousands of genes to be simultaneously quantified (Brown and Botstein,

1999). cDNAs of targeted genes can then be sequenced to identify their products, which then can be studied to determine their role. Whereas this would certainly be a fruitful approach for the python, it nevertheless would be a very difficult undertaking considering the current lack of genomic information for reptiles (Freire *et al.*, 2003). A first step would be to identify sets of genes that are overly expressed with the regulation of intestinal performance for more widely used animal models and then focus on the expression of those genes for the python and potentially for other amphibians and reptiles.

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