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Genomic Responses to Behavioral Interactions in an African Cichlid Fish: Mechanisms and Evolutionary Implications

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Key Words

Neural plasticity · Evolution · Immediate-early gene · Epigenetic · Phenotypic plasticity · *Astatotilapia burtoni* · Social behavior

Abstract

Phenotypic plasticity in *Astatotilapia burtoni* allows individual males to alternate between dominant and subordinate status, two physiologically and behaviorally distinct phenotypes. Because these phenotypes are completely reversible, they provide an excellent model for studying the molecular mechanisms of phenotypic plasticity. The ability to express alternate phenotypes in *A. burtoni* depends on the ability to regulate gene expression on both short- and long-term time scales. Previous studies have demonstrated that dominant males, who have increased reproductive capacity, have higher expression of several genes involved in reproduction (e.g., genes for steroid receptors). These differences in gene expression and reproductive physiology are controlled by interactions among males. Recently, it was found that the same interactions that lead to stable long-term changes in gene expression also induce short-term and transient changes in expression of *egr-1*, an immediate-early gene transcription factor. This immediate-early gene response is part of a general mechanism for mediating changes in gene expression that underlie phenotypic plasticity. Longer stable changes in gene expression must involve other mechanisms, such as dynamic modifications of the epigenome. Recent data suggests a direct link between the immediate-early gene response and epigenetic modifications. These mechanisms which link behavioral interactions to changes in gene expression allow phenotypic variation to occur without corresponding changes in the genome and, as a consequence, they have implications for evolution. In the case of *A. burtoni*, phenotypic plasticity is likely to slow evolution because it produces highly adapted phenotypes under the primary niches encountered in the life-history of the species and the plasticity itself is likely to be an adaptive trait.

Introduction

For many animals, a critical feature of the environment is the presence and state of conspecifics. Thus, it is not surprising that behavioral interactions among conspecifics influence many aspects of the physiology of the participants. The phenomenon of behavioral regulation of physiology is a special case of the more general phenomenon of environmental regulation of physiology which we can think of more broadly still as environmentally-induced phenotypic plasticity. The nervous system plays an important role in mediating environmentally-induced phenotypic plasticity. Therefore, understanding the proximate and ultimate aspects of phenotypic plasticity is fundamental to understanding the role of the brain in behavior and evolution. Behavioral neurobiologists study phenotypic plasticity as a matter of course and although neuroethologists do so with an eye toward the

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evolutionary history of the study organism, they rarely consider whether and how such plasticity impacts evolution.

Phenotypic plasticity has important implications for evolution because it allows organisms to respond to changes in the environment without corresponding changes in the genome. Environmental regulation of phenotype is a particularly important mechanism in cases where generation times are long and ecological niches are complex because changes in the environment can outpace the rate of genetic evolutionary change [Colvis et al., 2005]. Phenotypic plasticity can be defined as a property of genotypes that allows the expression of different phenotypes when exposed to different environmental conditions [West-Eberhard, 1989; Pigliucci et al., 2006]. Phenotypic plasticity may affect many aspects of an organism's biology, from behavior and physiology to development, and may occur on short- or long-term time scales and be reversible or permanent. For example, learning is a behavioral adaptation that can be reversed rapidly whereas developmental plasticity, such as the development of alternative morphologies [e.g., Pfennig, 1992], might be permanent. The nervous system plays an important role in phenotypic plasticity at the behavioral and physiological levels because its function is to coordinate internal state with external events. A classic example is found in seasonal breeders where the nervous system responds to increasing day length by activating the necessary behavioral repertoires and physiological profiles for reproduction [Dawson et al., 2001].

Although diverse, all forms of phenotypic plasticity share a fundamental biological property: the expression of alternative phenotypes by a single unchanging genome [West-Eberhard, 1989; Pigliucci et al., 2006]. Although phenotypic plasticity occurs within a single generation and without genetic mutation, many aspects of phenotypic plasticity involve the regulation of which genes are expressed [Colvis et al., 2005]. Two such mechanisms for mediating phenotypic plasticity that are particularly relevant to the nervous system are the immediate-early gene response and dynamic changes in epigenetic modifications. The degree of complexity that is achieved through these mechanisms allows for the expression of a wide array of phenotypes on the basis of a single unchanging genome. To explore some of these issues within a neuroethological context, I will first review the well known example of environmental regulation of phenotype in the cichlid fish, *Astatotilapia (Haplochromis) burtoni*. I will then turn to a discussion of some of the molecular mechanisms underlying this type of phenotypic plasticity before addressing possible evolutionary implications. The central concept of this paper combines well developed theories of the evolutionary consequences of phenotypic plasticity [West-Eberhard, 1989; Price et al., 2003; Pigliucci et al., 2006] with known neurobiological mechanisms of phenotypic plasticity [Clayton, 2000; Levenson and Sweatt, 2005] and can be stated as follows: Behavior influences phenotype through changes in neural gene expression and this enables phenotypic variation in the absence of a changing genome. However, this plasticity might slow evolution if it produces highly adapted phenotypes under the primary environmental niches encountered in the life-history of an organism.

Phenotypic Plasticity in an African Cichlid Fish

As a model for understanding how information in the environment regulates phenotype through changes in gene expression, I will focus on social regulation of reproductive physiology in the African cichlid fish *A. burtoni* [Fernald, 2002]. *A. burtoni* males typically express one of two phenotypes: dominant (also called territorial) or subordinate (also called non-territorial). Dominant males defend spawning grottos that they construct in the substrate of ponds and they display bright body coloration to advertise their status. In contrast, subordinate males avoid dominant males, school with females and other subordinate males, and have dull body coloration [Fernald and Hirata, 1977a, b]. These morphological and behavioral differences are associated with differences in reproductive physiology: dominant males have higher reproductive capacity than subordinate males [Fraley and Fernald, 1982]. Furthermore, all known aspects of phenotypic plasticity in *A. burtoni* (morphology, behavior, physiology) are reversible and under social control [Fernald, 2002]. Furthermore, it has been shown that the critical social cue inhibiting reproduction in subordinate males is the presence of larger dominant males [Francis et al., 1993]. Social regulation of phenotype in *A. burtoni* is probably an adaptation to competition for territorial resources and, thus, understanding their natural history is essential to understanding their physiology, especially if one is ultimately interested in the evolutionary implications of such phenotypic plasticity.

A. burtoni live in the small shore pools of Lake Tanganyika in Africa. Early behavioral observations revealed that brightly colored males build and defend spawning grottos in the soft substrate of these ponds [Fernald and Hirata, 1977a, b]. Male-female pairs exchange gametes

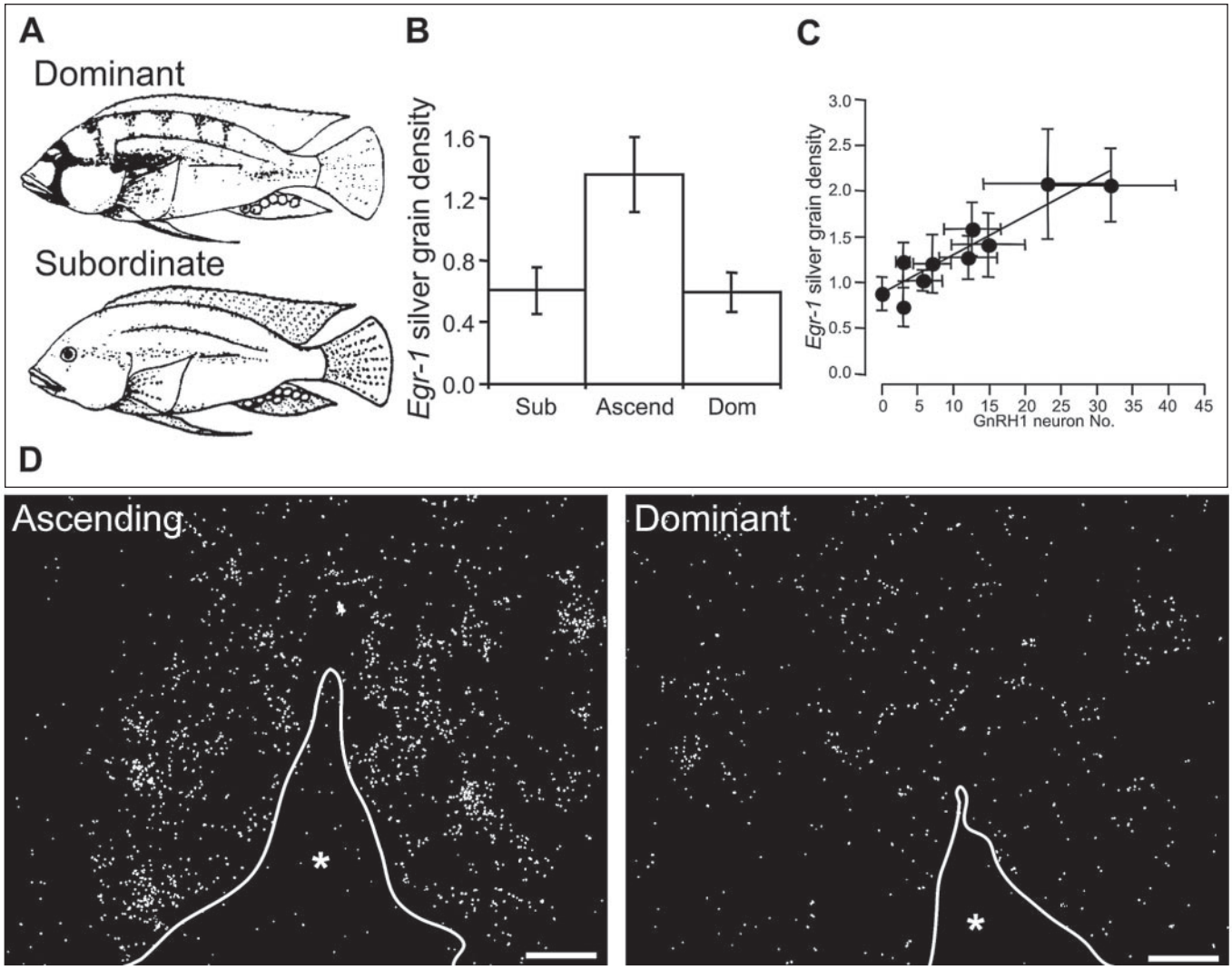

Fig. 1. Immediate-early gene responses to social opportunity in *A. burtoni*. (**A**) Stable dominant and subordinate males differ in body coloration (not shown). (**B**) Social opportunity induces rapid increases in the expression of the immediate-early gene *egr-1* in the preoptic area in males ascending to dominance (Ascend). Stable dominant (Dom) and subordinate (Sub) males have similar and low levels of *egr-1* gene expression. (**C**) Among ascending males, *egr-1* gene expression is greatest in regions with high densities of GnRH1 neurons. (**D**) Photomicrographs of *egr-1* gene expression in the preoptic area of an ascending male compared to a dominant male (bars represent 40 μm). The asterisks indicate the preoptic recess of the third ventricle.

within the spawning grotto; thus, the possession of a spawning grotto is important for the mating success of a male because females are unlikely to mate outside the confines of a spawning grotto. The substrate that is used for building the grottos is limited and, as a result, males compete for the opportunity to build grottos. The winners of these competitions, who tend to be larger than losers, display bright colors to advertise their dominant status, whereas the losers maintain a dull body coloration similar to that of females (fig. 1A). Thus, competition for substrate material results in two alternative phenotypes. Because subordinate males do not appear to mate, the two morphs are unlikely to be alternative mating strategies as seen in species with sneaker males [e.g., Brantley and Bass, 1994]. However, if they are not alternative mating strategies, why should a subordinate male suppress his reproduction? In other words, what advantage is accrued to the subordinate male by waiting to reproduce?

This important question was addressed by Hofmann et al. [1999] when they demonstrated that subordinate males tend to grow at a faster rate than dominant males, presumably enabling them to overtake established dominant males growing more slowly. It appears that subordinate males are engaged in a waiting strategy in which they invest in growth at the expense of reproduction until such time that they are larger than other males in the community.

The differences in reproductive physiology between dominant and subordinate males can be measured at every level of the brain-pituitary-gonad axis [Fernald, 2002]. Many of these differences are reflected in differences in expression of particular genes. For example, compared to subordinate males, dominant males have higher expression of the GnRH1 gene in the preoptic area [White et al., 2002], higher expression of the GnRH receptor 1 gene in the pituitary [Au et al., 2006], and increased expression of androgen and estrogen receptor genes in the brain [Burmeister et al., 2007]. Although much of the work to date has focused on candidate genes, a broader look will probably demonstrate that phenotype is associated with distinct patterns of genome-wide differences in gene expression [Aubin-Horth et al., 2007]. Because the alternative phenotypes of *A. burtoni* are completely reversible, they provide a powerful model for studying phenotypic plasticity and their underlying molecular mechanisms. In this paper, I will use social regulation of reproductive phenotypes in *A. burtoni* as a model for asking how behavioral interactions regulate phenotype through gene expression. In doing so, I will draw primarily from data collected using *A. burtoni* but I will also speculate on the possible role of mechanisms elucidated in other model systems.

One of the great strengths of the *A. burtoni* model is that the phenotypic differences are completely reversible and can be controlled in the laboratory. Thus, to understand how phenotypic plasticity is induced, we typically focus on the social and physiological events that underlie the transition from a non-reproductive subordinate male to a reproductive dominant male. When a male detects an opportunity to ascend in status, his body coloration and behavioral repertoire can change within minutes [Burmeister et al., 2005] whereas changes in *GnRH1* gene expression lag behind [White et al., 2002]. In these experiments, social opportunity is defined as the opportunity to ascend in status and this transition is physiologically distinct from the state of achieving dominance following social ascension. In the laboratory, social opportunity is experimentally achieved by presenting a subordinate male with the absence of larger dominant males. By manipulating social context, and therefore social opportunity, we are able to investigate the intervening molecular events that link recognition of social opportunity with the changes in *GnRH1* gene expression that are necessary for reproductive plasticity.

To begin to address the events leading from social opportunity to reproductive plasticity, Burmeister et al. [2005] focused on the role of the immediate-early gene *egr-1* during the transition from subordinate to dominant status. *egr-1* (also known as *zenk*, *NGF1-A*, *zif268*, and *krox24*) is a good candidate for linking social opportunity to changes in GnRH1 neurons for three reasons. First, *egr-1* expression is regulated by synaptic activity [Worley et al., 1991]. Second, *egr-1* is a transcription factor that can affect long-term changes in gene expression in the brain [Beckmann and Wilce, 1997; O'Donovan et al., 1999]. Third, an earlier study demonstrated that *egr-1* is inducible in the GnRH1-containing preoptic area of *A. burtoni* [Burmeister and Fernald, 2005]. Burmeister and Fernald [2005] also demonstrated that *egr-1* gene expression in *A. burtoni* brains peaks approximately 30 min after stimulation. By combining the time course of gene expression with the behavioral time course, it was possible to measure *egr-1* expression that was induced by social opportunity. Specifically, Burmeister et al. [2005] sacrificed individuals 20 min after they first expressed dominance and compared them to stable dominant males and stable subordinate males. They found that only males who were ascending to dominance showed an increase in *egr-1* expression in the region of the preoptic area that expresses *GnRH1* [Burmeister et al., 2005]. Specifically, they found that males ascending to dominance have a greater than 2-fold induction of *egr-1* expression in the preoptic area compared to subordinate and dominant males whereas stable subordinate and dominant males have similar levels of *egr-1* (fig. 1B, D). Because dominant males and ascending males have similar behavioral profiles, but different *egr-1* profiles, this indicates that the *egr-1* induction in ascending males is specific to social opportunity and is not related to dominance per se. It also suggests that the increase in *egr-1* is related to the initiation of changes associated with the acquisition of dominance, as opposed to the maintenance of dominance. Furthermore, this expression pattern does not occur throughout the preoptic area. In fact, the *egr-1* induction in ascending males only occurs in the rostral sections and is greatest in the midrostral area. This heterogeneity of *egr-1* expression in ascending males can be accounted for by the distribution of GnRH1 neurons within the preop-

tic area. The induction of *egr-1* was greatest in regions of the preoptic area that contained many GnRH1 neurons and GnRH1 neuron number accounts for 86% of the observed variation in *egr-1* expression in the preoptic area of ascending males (fig. 1C) [Burmeister et al., 2005].

Burmeister et al. [2005] further found that *egr-1* is expressed by *GnRH1*-expressing neurons and they proposed that the *GnRH1* gene mightbe a target of the egr-1 protein by identifying candidate promoter binding sites for egr-1. Although sequence analysis can only provide a putative connection between egr-1 and the *GnRH1* gene in *A. burtoni*, it points to a potential molecular switch underlying physiological plasticity in this species. A similar role for *egr-1* has been demonstrated in rat pups where *egr-1* links maternal behavior (licking and grooming) to changes in the expression of the glucocorticoid receptor in the hippocampus [Meaney et al., 2000]. More generally, *egr-1* is a critical link between experience and memory [Jones et al., 2001; Bozon et al., 2003]. Even if we assume that *egr-1* is a molecular switch that changes expression of the *GnRH1* gene from low to high, the quintessential feature of reproductive plasticity in *A. burtoni,* it does not provide a mechanism for the persistent increase in *GnRH1* gene expression in dominant males. Recall that stable dominant males have similar levels of *egr-1* expression compared to subordinate males. Another mechanism must be at play in order to account for the long-term increase in *GnRH1* gene expression.

Molecular Mechanisms of Phenotypic Plasticity

Reproductive plasticity in *A. burtoni* clearly involves regulation of genes that are specific to reproduction (e.g., genes for GnRH1 and the androgen receptors). Nonetheless, the mechanisms regulating expression of these genes are likely to be general among diverse forms of phenotypic plasticity and conserved among species. Although there are many such mechanisms, I will focus on only two because they provide both short- and long-term links to changes in gene expression and because they interact with one another in the cell. These are the immediate-early gene response and the dynamic modification of epigenetic markers.

Immediate-early genes (IEGs) are a general mechanism for regulating changes in phenotype ranging from sensitivity of the cortisol response [Meaney et al., 2000] to learning [Jones et al., 2001; Bozon et al., 2003]. IEGs were initially defined by their ability to be induced independently of new protein synthesis as they were discovered by stimulating cell cultures with trophic factors in the presence of protein synthesis inhibitors [Greenberg et al., 1986a, b; Milbrandt, 1987; Christy et al., 1988]. It has been estimated that there might be as many as 100 genes that can be defined as part of the IEG response [Sheng and Greenberg, 1990]. Among the genes discovered this way was *egr-1*, which shares a number of traits with other IEGs. Later it was demonstrated that in normal adult neurons, induction of IEGs is regulated by synaptic activity [Worley et al., 1991]. The shape of induction curves might differ among species and potentially among brain regions, although in most published reports the accumulation of *egr-1* mRNA peaks approximately 30 min following stimulation [Mello and Clayton, 1994; Zangenehpour and Chaudhuri, 2002; Burmeister and Fernald, 2005]. In summary, a defining feature of IEGs is that they are expressed within minutes of membrane depolarization. How is this accomplished by the cell?

In the case of *egr-1* and similar IEGs, the following model has been developed (fig. 2). Presynaptic activity triggers at least two second messenger cascades, one involving the increase of calcium within the cell and the other involving MAP-kinase cascades [Murphy et al., 1991; Whitmarsh et al., 1995; Treisman, 1996; Harada et al., 2001; Sweatt, 2001; Buchwalter et al., 2004]. These pathways activate existing transcription factors (e.g., Elk1 and SRF) through phosphorylation which causes them to translocate to the nucleus and converge on the promoter for *egr-1* [Thiel and Cibelli, 2002]. Expression of *egr-1*, and similar IEGs, requires the cooperative binding of two different constituitively expressed transcription factors that are, themselves, regulated by distinct signaling cascades. *egr-1* itself codes for a transcription factor and, as such, the egr-1 protein exerts its effects by changing expression of target genes. egr-1 affects target gene expression either by inducing or suppressing transcription [Gashler et al., 1993]. Induction of transcription occurs when egr-1 is activated through phosphorylation [Waters et al., 1990; Gashler et al., 1993]; repression of transcription can occur when egr-1 is expressed at low levels, a condition that promotes binding by the corepressor proteins NAB1 and NAB2 [Russo et al., 1995; Svaren et al., 1996; Thiel et al., 2000]. Evolutionary conservation of the egr-1 protein suggests that its function is common among vertebrates [Burmeister and Fernald, 2005].

Genes might be regulated in the longer term through epigentic modifications, such as DNA methylation and histone acetylation. There are several definitions of epigenetics, some of which require heritable changes in gene expression that are not coded in the DNA sequence itself.

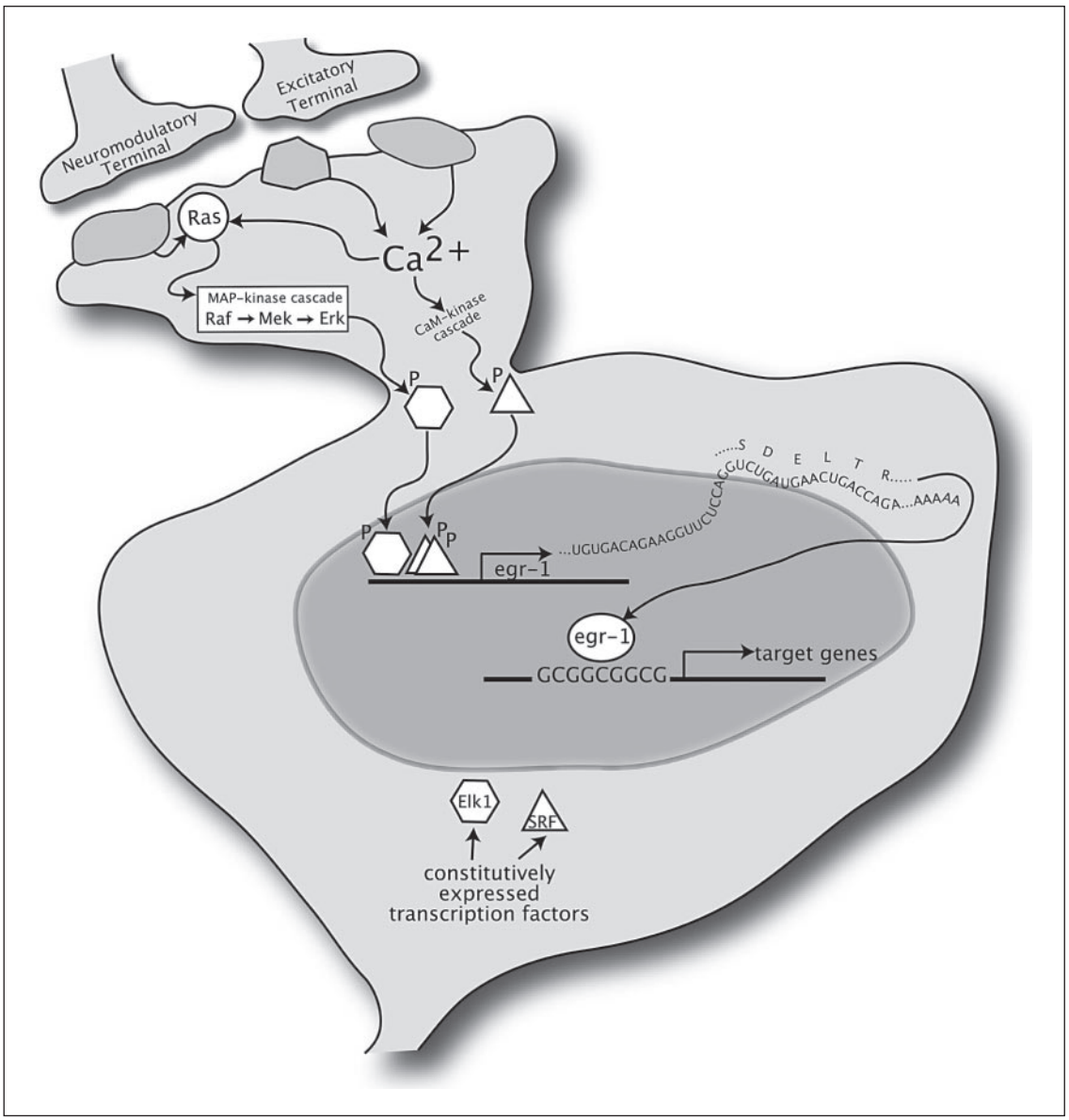

Fig. 2. A model for the molecular events linking membrane activity to expression of the immediate-early gene *egr-1*. Excitatory and neuromodulatory inputs activate postsynaptic receptors and channels that initiate second messenger cascades culminating in the activation (via phosphorylation, P) of two types of constitutively expressed transcription factors that cooperatively promote *egr-1* gene expression.

The type of epigenetic change that I will discuss has been described as a stable maintenance of gene expression that is accomplished through the physical modification of DNA or its associated proteins (e.g., histones) [Levenson and Sweatt, 2005]. These modifications regulate gene expression by regulating the availability of promoter sites to transcription factors. Epigenetic modifications have long been known to be important for the cellular memory that is acquired during development and many epigenetic modifications are thought to be permanent and life-long. However, there is increasing evidence that these modifications are plastic and that they respond to events in the external environment [Levenson and Sweatt, 2005].

Two types of epigenetic modification are DNA methylation and histone modification. Both exert strong effects on gene expression and may be dynamically changed

in adult neurons [Levenson and Sweatt, 2005]. DNA methylation involves the attachment of methyl groups to cytosines that are followed by guanines, so called CpG islands. When methyl groups are attached to CpG islands, they can effectively block the binding of transcription factors to these sites. This mechanism causes long-term changes in the binding affinity of egr-1 to the glucocorticoid receptor promoter [Weaver et al., 2004, 2007]. Histones might be modified in a number of ways, including phosphorylation, methylation, and acetylation, that change the physical relationship between DNA and histones. For example, acetylation of histone tails involves the addition of acetyl groups to lysine residues, thus neutralizing their positive charge and reducing their attraction to DNA. With less binding of the histone tail to DNA, the DNA is more accessible to transcription factors. This relationship is reflected in the observation that increased histone acetylation is associated with active transcription sites [Struhl, 1998].

Although these epigenetic mechanisms have long been understood, evidence for their dynamic nature in adult neurons is more recent and the data suggests that these epigenetic modifications can be regulated by synaptic activity. Levenson and Sweatt [2005] review several examples of dynamic epigenetic modification in neurons. For example, in the suprachiasmatic nucleus, light causes an increase in acetylation of histones associated with the *per* genes, demonstrating that a natural stimulus can be conveyed to and encoded in the epigenome in a dynamic fashion and, perhaps, on a daily basis [Crosio et al., 2000]. Furthermore, acetylation of specific histones (H3 and not H4) increases following contextual fear conditioning and this can be blocked by inhibiting NMDA-receptor dependent signaling cascades [Levenson et al., 2004]. This suggests that the familiar mechanisms of synaptic plasticity that participate in the IEG response are also responsible for inducing stable and long-term changes in gene expression through the epigenome.

Szyf et al. [2005] recently proposed a mechanism for linking the IEG response with long-term changes in the epigenome. As an example, consider the case of activating a silent gene that is regulated by egr-1. In this example, the promoter for the gene is methylated which results in low binding by transcription factors such as egr-1. Szyf et al. [2005] propose that at high levels of egr-1, the probability of binding to this low-affinity site will increase. According to the model, once bound to a promoter, egr-1 recruits proteins that acetylate the associated histones. Proteins that acetylate histones are called HATs (histone acetyltransferases) and many proteins that are transcriptional cofactors are also HATs. Once the associated histones have been acetylated, this increases the accessibility of the promoter to demethylase which converts the promoter to a high-affinity binding site. The result is the long term conversion of the low-affinity binding site to a high-affinity binding site and increased transcription of the genes downstream. Szyf et al. [2005] proposed this mechanism as a way to link two observations related to the change in glucocorticoid receptor (GR) expression in rat pups in response to being licked and groomed by their mothers. The first observation is that, in response to these maternal behaviors, egr-1 protein expression is increased which in turn promotes the expression of GR [Meaney et al., 2000]. This is a familiar IEG response that links synaptic activity to immediate changes in gene expression. The second observation is that these pups show a life-long increase in GR expression, long after the maternal behaviors have ceased [Francis et al., 1999] and these long-term changes in GR expression are the result of modifications of the epigenome associated with the GR promoter [Weaver et al., 2004]. Weaver et al. [2007] recently showed that egr-1 expression can lead to epigenetic modifications of the GR promoter in transient transfection assays, providing support for the Szyf et al. [2005] model. If this, or a similar model, holds true in vivo, it will likely be a conserved mechanism for linking short- and long-term changes in gene expression.

Could such a mechanism be at work in the phenotypic plasticity observed in *A. burtoni*? Although there are no data to bear on this question, such a mechanism could explain the observation that transient changes in *egr-1* are associated with the transition from subordinate to dominant status, but expression levels of *egr-1* cannot explain the stable differences in *GnRH1* expression in dominant males compared to subordinate males. Social regulation of *GnRH1* expression in *A. burtoni* has been referred to as the 'social set point' model because activity levels of GnRH1 neurons (as assessed by neuronal soma size or *GnRH1* expression) is determined by social cues, but the 'set-point' activity level is maintained by negative feedback from androgens [Soma et al., 1996]. This explains the combination of findings that dominant males have higher androgen levels [Parikh et al., 2006] as well as higher *GnRH1* expression [White et al., 2002] in spite of negative feedback regulation of GnRH1 by androgens [Soma et al., 1996]. It is tempting to propose that the molecular mechanism for the social set point will lie in the epigenome associated with the *GnRH1* gene.

Implications for Evolution

The brain plays a central role in phenotypic plasticity, enabling animals to express varying phenotypes in response to environmental cues. Learning is an archetypical example of the role of the brain in phenotypic plasticity and, as reviewed above, the brain is important in other types of plasticity as the primary integrator of the environment with physiology. In the case of *A. burtoni*, the result is the ability to express two very different phenotypes that are completely reversible. Behavioral neurobiologists are interested primarily in the mechanisms that generate this plasticity and neuroethologists are accustomed to considering the role of evolutionary history in shaping these mechanisms. Here we will turn to the issue of whether and how such plasticity impacts evolution.

Phenotypic plasticity has provided an interesting puzzle for evolutionary biologists [West-Eberhard, 1989; Pigliucci et al., 2006]. Historically, phenotypic plasticity had been marginalized in the field of evolutionary biology because the principal focus has been on the relationship between genetic variation and variation in phenotype. Phenotypic plasticity, by definition, is a distinct mechanism for generating variation in phenotype as it is induced by the environment and operates in the absence of genetic variation. The puzzle for evolutionary biologists has been whether phenotypic plasticity influences evolution or not. And, if so, should phenotypic plasticity accelerate or slow evolution? The answers to these questions continue to generate controversy [de Jong, 2005; Pigliucci et al., 2006].

Although not widely appreciated until more recently [West-Eberhard, 1989], early evolutionary biologists demonstrated experimentally that phenotypic plasticity can provide raw material for directional selection [Waddington, 1953]. Theoretically, it was reasoned that plasticity would allow organisms to persist in a new environment, which would expose populations to new selective pressures that could act, over time, on genetic mutations [Schmalhausen, 1949; Waddington, 1952]. Experimentally, some of the earliest evidence of this phenomenon came from heat shock experiments in *Drosophila* [Waddington, 1953]. Specifically, Waddington [1953] found that exposing flies to an extreme temperature during development induced a novel phenotype in a small proportion of flies. When Waddington [1953] selected for this plastic response in phenotype over multiple generations, he was able to increase its frequency in the population. In addition, Waddington [1953] found that after selection the phenotype was no longer plastic, but was genetically determined; this process has been called 'genetic assimilation.' Thus, at least under some circumstances, phenotypic plasticity might result in directional selection. More recently, evolutionary biologists have explored the conditions under which this phenomenon can be expected to occur and the parameters that predict the acceleration or the slowing of evolution by phenotypic plasticity [Price et al., 2003; Badyaev, 2005].

Phenotypic plasticity is predicted to result in directional selection under at least two conditions [Price et al., 2003]. First, phenotypic plasticity is predicted to result in directional selection if plasticity is costly. In other words, if plasticity is costly then selection will favor direct genetic control over the phenotype favored by the new environment [Dewitt et al., 1998; Sultan and Spencer, 2002]. The evolutionary history of the brain suggests that under many circumstances the costs of plasticity are outweighed by their benefits. The brain, more than any other organ, enables phenotypic plasticity and, not only is it a metabolically costly organ [Aiello and Wheeler, 1995], but increased behavioral plasticity tends to be associated with increased brain size [Sol et al., 2005]. Thus, in spite of the potential costs of increased neural plasticity, evolutionary history suggests that their benefits might outweigh the costs in many circumstances [Sol et al., 2007]. Second, if phenotypic plasticity is incomplete – that is, individuals are unable to shift their phenotype to completely adapt to the new environment – then selection is predicted to favor phenotypes that are better adapted to the new environment. Under these circumstances, the plasticity itself is expected to be reduced because the prevailing conditions (i.e., the new environment) favors gene combinations that are not useful in other environments [Waddington, 1961].

In the case of *A. burtoni*, it appears that the phenotypes expressed under the two environments enable the individual to be well adapted to each niche. In other words, plasticity appears to be nearly complete, enabling an individual male to have a physiological advantage as a subordinate male in terms of increased growth rate, or as a dominant male in terms of increased reproduction. Complete plasticity is predicted to reduce the probability of directional selection and genetic assimilation [Price et al., 2003]. Furthermore, in *A. burtoni*, as in many cases of phenotypic plasticity, the two environments are temporal in nature and can be expected to be repeatedly encountered by an individual. For example, a male *A. burtoni* is likely to begin life in an environment that favors the 'subordinate niche' because he will probably be in a popula-

tion that includes larger males. The subordinate phenotype provides the advantage of investing preferentially in growth rather than reproduction. Similarly, a male *A. burtoni* is likely to encounter the 'dominant niche' as larger males die or as the subordinate male outgrows them. Hofmann et al. [1999] found that the majority of individuals in a population will become dominant at some point when living in a changing environment designed to mimic natural habitat disruptions. Although similar studies have not been done with free-living populations, the probability that an individual will acquire dominant status must be high; if it were not, males who adopt subordinate phenotype would forgo the opportunity to reproduce and be rapidly selected against. Finally, the benefits of one phenotype over another is density dependent; in other words, a male will only benefit from being subordinate in cases where there are no social opportunities to become dominant. Thus, in the case of *A. burtoni*, it is likely that phenotypic plasticity does not result in directional selection. Rather, it seems more consistent with a scenario in which selection has favored plasticity itself.

In conclusion, behavioral interactions among conspecifics regulate phenotype through changes in neural gene expression and this enables phenotypic variation in the absence of a changing genome. The immediate-early gene response and dynamic modifications of the epigenome are two general and important mechanisms by which a single genome produces variation in phenotype. In scenarios where phenotypic plasticity is complete – that is, it produces individuals well adapted to novel environments – or in which multiple environments are encountered sequentially, selection is likely to favor plasticity, even if it is costly. In other words, in these cases plasticity is unlikely to provide raw material for natural selection.

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