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Identification and analysis of vocal communication pathways in birds through inducible gene expression

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ABSTRACT

The immediate-early gene *zenk* is an activity-dependent gene highly induced in auditory processing or vocal motor control brain areas when birds engage in hearing or producing song, respectively. Studies of the expression of *zenk* in songbirds and other avian groups will be reviewed here briefly, with a focus on how this analysis has generated new insights on the brain pathways and mechanisms involved in perceptual and motor aspects of vocal communication and vocal learning.

Key words: zenk, songbird, auditory, learning, birdsong.

INTRODUCTION

Activity-dependent genes are genes whose expression is induced in the brain when neuronal circuits are activated by various stimuli or behaviors (Morgan and Curran 1989, Sheng and Greenberg 1990). Analysis of the expression of inducible genes has, thus, been very useful to map the brain areas associated with specific stimuli or behaviors (Kaczmarek and Robertson 2002). The immediate-early gene *zenk* (a.k.a. *zif-268, NGFI-A, egr-1* or *Krox-24*) is highly induced in discrete areas of the avian brain when birds hear song (Mello et al. 1992, Mello and Clayton 1994), or when they engage in singing behavior (Jarvis and Nottebohm 1997). Studies on the regulation of *zenk* have yielded high-resolution maps of brain activation associated with perceptual and motor aspects of vocal communication in songbirds and other avian groups. As discussed in detail elsewhere (Kaczmarek and Robertson 2002), *zenk* and some other inducible genes have been linked to synaptic plasticity and some forms of long-term memory in rodents (Guzowski et al. 2000, Jones et al. 2001). Therefore, the brain areas revealed by *zenk* expression analysis in birds are likely associated with neuronal plasticity and may be involved in long-term memory formation and/or storage (Mello 1998).

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ZENK INDUCTION BY HEARING BIRDSONG

Zenk induction by hearing song occurs in the auditory nucleus of the midbrain, as well as in several discrete areas in the caudomedial telencephalon (Mello and Clayton 1994). Tract-tracing studies have revealed that areas showing song-induced *zenk* expression are interconnected and are part of a complex circuitry that arguably constitutes the central auditory processing pathways in the avian brain (Mello et al. 1998, Vates et al. 1996). The *zenk* response to song in these areas occurs in both sexes and is present in both avian vocal learners (avian orders where song is learned, usually from a parent or tutor, i.e. songbirds, parrots and hummingbirds) and vocal non-learners such as doves and chicks (Jarvis et al. 2000, Jarvis and Mello unpubl. obs.). Electrophysiological recordings have confirmed that areas revealed by *zenk* expression respond to song auditory stimulation (Chew et al. 1995, Stripling et al. 1997). Importantly, in songbirds, the areas revealed by song-induced *zenk* expression exclude the song control system (Jarvis and Nottebohm 1997, Mello and Clayton 1994), i.e. a set of interconnected brain areas known to be involved in birdsong production and several aspects of song learning (see reviews in Brenowitz et al. 1997). Thus, a complex auditory system distinct from the song control system is activated by birdsong and appears to play an important role in birdsong auditory processing (Mello 2002).

The area that shows the most prominent induction of *zenk* is the caudomedial nidopallium (NCM), an area that is likely analogous to supragranular layers of the mammalian auditory cortex (Karten and Shimizu 1989, Mello et al. 1998). *Zenk* induction in NCM is rapid and transient, and apparently reflects a tuning of neuronal units in NCM to features present in conspecific song (Mello et al. 1992). Furthermore, the topographical distribution of the *zenk* response within NCM depends on acoustic features present in the song stimulus, as has been most clearly revealed by analyzing *zenk* expression in the Domestic Canary *Serinus canaria* (Waterslager strain) NCM in response to the presentation of various classes of canary song syllables (Ribeiro et al. 1998). Interestingly, the electrophysiological responses of NCM neurons to song decrease rapidly upon repeated presentations of the same song stimulus (Chew et al. 1995). This "habituation" is song-specific and long-lasting, and its maintenance depends on gene expression in NCM during discrete time windows that follow the song presentation period. The habituation of NCM neurons may, therefore, represent a mechanism that contributes to the laying-down of long-lasting song auditory memories. Song-induced *zenk* expression occurs during the time window of required gene expression for long-term habituation, and thus *zenk* could be involved in this long-term plasticity, but this possibility remains to be tested directly. Further evidence implicating NCM and its associated *zenk* response in the formation and/or long-term maintenance of song auditory memories derives from studies on the ontogeny of the *zenk* response to song (Jin and Clayton 1997), and from the discovery that *zenk* expression in the NCM of adults in response to a tutor song correlates with the degree to which the birds copied that song during the song learning period (Bolhuis et al. 2000). Long-lasting song auditory memories mediated by NCM and other auditory processing areas may play a prominent role in processes such as individual recognition, mate selection and vocal learning (Mello 1998).

ZENK INDUCTION BY SINGING

In contrast to hearing song, *zenk* is highly induced in the nuclei that constitute the song control system when songbirds engage in active singing behavior (Jarvis and Nottebohm 1997). This system can be subdivided into a direct motor pathway, required for singing behavior, and an anterior forebrain pathway, which has been implicated in song learning (Brenowitz et al. 1997). *Zenk* expression in song control nuclei is associated with the motor action of singing, as it occurs even in deafened birds, where auditory feedback is absent (Jarvis and Nottebohm 1997). Furthermore, *zenk* expression by singing also occurs in nuclei of the anterior pathway, which was originally thought not to participate in singing behavior in adults (Bottjer et al. 1984, Scharff and Nottebohm 1991). The *zenk* expression data, however, provides clear evidence for the activation of anterior forebrain song nuclei when birds sing, indicating that this pathway is also involved in the neural control of singing behavior. Since *zenk* expression in the anterior pathway is context dependent (Jarvis et al. 1998), this pathway may participate in coordinating song with other behaviors related to the specific behavioral context of singing.

The *zenk* induction patterns associated with hearing and producing birdsong occur in free-ranging birds within their natural habitat, as has been demonstrated in Song Sparrows *Melospiza melodia* using a song playback challenge paradigm (Jarvis et al. 1997). Thus, brain gene regulation in association with birdsong is not just a laboratory finding, but actually occurs in birds in their natural setting. *Zenk* regulation by song has also been used to identify and characterize auditory and vocal control brain areas in parrots and hummingbirds, the only vocal learning birds besides songbirds. The analysis in the Budgerigar *Melopsittacus undulatus* (Psittacidae) has revealed new details on the morphology of vocal control areas previously identified by tract-tracing methods, as well as some previously unidentified vocal nuclei (Jarvis and Mello 2000). In hummingbirds, the *zenk* expression maps constitute a first demonstration of the occurrence of telencephalic vocal control nuclei in this avian order, further substantiating the link between the occurrence of such nuclei and vocal learning (Jarvis et al. 2000). This comparative approach has revealed striking similarities in auditory and vocal control pathways in all vocal learning birds, indicating that these pathways evolved under strong evolutionary constraints in vocal learning avian orders. Most importantly, it has become clear that all vocal learning groups evolved a set of nuclei (including both pallial and striatal areas) that are dedicated to the motor control of learned vocalizations. Current studies are evaluating whether telencephalic vocal control areas might also be present in species representative of avian orders that did not evolve vocal learning, including doves and sub-oscines.

CONCLUSIONS

In summary, analysis of song-induced expression of the inducible gene *zenk* has generated novel insights on the functional organization of the brain pathways that control perceptual and motor aspects of vocal communication in birds. On-going molecular studies on the function of *zenk* and other inducible genes in songbirds and mammals should help reveal the significance of inducible gene expression for neuronal function and plasticity, and for the laying-down and maintenance of behaviorally relevant memories.

RESUMO

O gene imediato *zenk* é um gene dependente de atividade que é marcadamente induzido em áreas cerebrais de processamento auditivo ou de controle vocal motor quando pássaros ouvem ou produzem canto, respectivamente. Estudos da expressão de *zenk* em pássaros canoros e outros grupos de aves será revisto neste artigo, enfocando como esta análise tem gerado novas perspectivas no entendimento das vias e mecanismos envolvidos em aspectos perceptuais e motores da comunicação e do aprendizado vocais.

Palavras-chave: zenk, pássaros canoros, processamento auditivo, aprendizado, canto.

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