

Is Song Special?

Akutagawa and Konishi (2001 [this issue of *Neuron*]) describe the spatial and temporal pattern of SNAg (song system nuclear antigen) expression within a subset of song-associated forebrain nuclei of grass finches. The timing and estrogen inducibility of SNAg expression suggest that it may function in establishing neural connections key to vocal learning.

The bird song system provides a feast for the systems and cognitive neuroscientist (Kuhl and Doupe, 1999). The key discovery—vocal learning—was made by Peter Marler as he traversed neighboring valleys in Wales and heard a different song dialect in each. Marler went on to show that the dialects were cultural in origin, each community acquiring a different song from its elders in a precisely staged series of learning events with considerable similarity to the stages of vocal learning in human infants. The parallel was given additional weight by Fernando Nottebohm, Marler's student, when he found that vocal motor control in canaries is lateralized to the left motor nerve. Nottebohm and colleagues went on to discover a distinctive set of brain nuclei in the telencephalon that control song (Nottebohm et al., 1976). These include HVC (the higher vocal center) and its target, RA (the robust nucleus of the archistriatum); lesions of these brain regions block production of song in adults. The song system (see Figure) also includes the HVC target, anterior forebrain nucleus X, as well as HVC afferents, Nif (nucleus interfacialis embedded in the telencephalic auditory region, Field L), and MAN (magnocellular nucleus of the anterior neostriatum just dorsal to X).

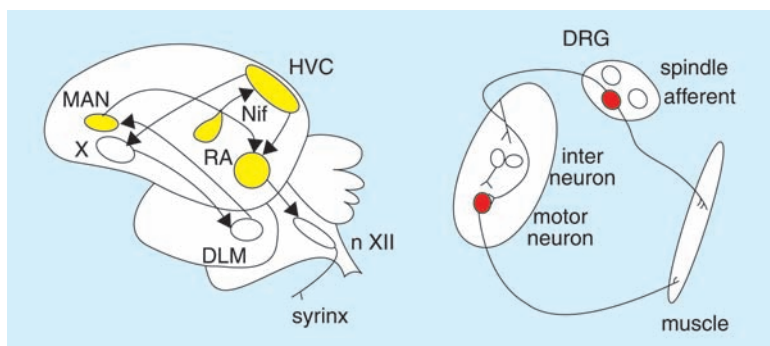
In temperate zones, song is usually a male prerogative. Nottebohm and his then student Art Arnold (1976) discovered a parallel sexual dimorphism in song nuclei with HVC, RA, and X in particular being much larger in males than in females. The difference is especially marked in another oscine song bird, the zebra finch, which is now the model organism for the song system. This finding opened the door to molecular approaches when Mark Gurney, then a student of Mark Konishi (another Marler offspring), demonstrated that song nuclei can be masculinized in females by estradiol treatment of nestlings; the females sing as adults if given testosterone (Gurney and Konishi, 1980). Konishi and Gene Akutagawa (1985) went on to show that song nuclei are also connected in a sexually differentiated way; in both sexes, developing axons travel from HVC to the archistriatum and then enter a holding pattern just above RA. In males, the axons abruptly plunge into the RA parenchyma while female axons settle in the staging area. David Clayton has recently shown that the plunge is triggered by estradiol made within the male's own brain (Holloway and Clayton, 2001). That the brain is a steroid secreting organ (a discovery made by Barney Schlinger and Arnold, 1991) is another fascinating attribute of the system.

Why is the song system so special? How did it arise

in evolution and how is it established at cellular and molecular levels? Systems, cognitive and cellular analyses of the system have made considerable progress. From a molecular standpoint, however, the bird song system has been stalled for some time. David Clayton made a brave attempt to uncover song system-specific patterns of gene expression using subtractive hybridization methods; for the most part, the patterns of gene expression he described either actively excluded song nuclei or were held in common with non-song nuclei (Clayton et al., 1988). None of the genes were expressed exclusively in song nuclei. Given the prominent role of estrogen in establishing a song-capable system, one might expect these nuclei to express the estradiol receptor (a nuclear protein that usually functions as a ligand-activated transcription factor), and HVC does. In addition, some telencephalic song nuclei can be highlighted by their expression of androgen receptors. Very little progress, however, has been made in unraveling the cascade of estrogen- or androgen-activated gene targets that control the development of specific and functional connections in the system.

All of which brings us to SNAg (song system nuclear antigen). Akutagawa and Konishi (2001) approached the song system using a method that could be viewed as old-fashioned in the era of PCR, but that had proved fruitful in outlining the wiring diagram of leech and grasshopper CNS in the hands of Birgit Zipser and Ron McKay (Zipser and McKay, 1981) and then of Corey Goodman. Akutagawa and Konishi prepared a panel of monoclonal antibodies using lightly fixed male RA as a source of antigens and found one, SNAg, that displays a striking pattern of localization to song nuclei. SNAg recognizes a core peptide (antibody-antigen binding is preserved after potential side chains are removed) within cell nuclei in HVC, RA, MAN, and Nif. Ironically (considering it was the source of antigen), the fewest number of labeled cells occur in RA. The developmental sequence of immunolabeling is dramatic: no staining in male finches at 20 days post-hatch, intense staining at 35–50 days, and a diminution in staining in HVC and Nif in adulthood. Perhaps not surprisingly given the extreme sexual dimorphism of the zebra finch song system, SNAg expression is highly sexually differentiated; no SNAg immunolabeling was detected at any stage in female zebra finch brain. Treatment with estradiol during the critical period, however, induces SNAg expression in females. The only other nucleus with SNAg labeling is an interstitial nucleus of the lateral lemniscus; staining is equivalent in the sexes.

So what is SNAg? One intriguing possibility is that SNAg, like the ETS transcription factor ER81 described by Tom Jessell and colleagues in developing spinal circuits (Figure; Arber et al., 2000), sets up the connectivity of the developing song system. ER81 is expressed both in developing motor neurons and in their proprioceptive afferents; in mutants, connections between spindle afferents and the motor neurons are deranged. Some neurons in the circuit (spinal interneurons) do not express ER81. Their connectivity must be established in another way, but be ultimately coordinated with that of ER81-expressing cells. Like ER81, SNAg is not expressed in



A Comparison of SNAG Expression to ER81

A comparison of SNAG expression in developing song system of a finch (left hand panel) to ER81 (an ETS family member) expression in developing spinal cord of mice (Arber et al., 2000). The bird brain is shown in side view (anterior to the left) while the spinal cord is a cutaway view (dorsal is up). ER81 mutant mice fail to form appropriate connections between spindle afferents and the motor neurons. As is the case for SNAG in song control nuclei, some neurons in the circuit (spinal interneurons) do not express ER81.

all synaptically connected song nuclei. The hindbrain syringeal motor neurons, for example, are SNAG negative. HVC contains two populations of forebrain projection neurons, one to RA and another to X. Only RA, however, expresses SNAG. If SNAG is involved in wiring at all (pure speculation at this point), overall connectivity must also involve other, coordinating factors.

So perhaps instead SNAG expression is key to initiating the special connections that underlie the ability to *learn* vocal behaviors as opposed to producing unlearned song. In this scenario, Nif and HVC (the instigators) control requisite connectivity during a critical period using timed onset of SNAG expression; SNAG expression in MAN and RA permits modification of vocal output according to auditory experience. Sarah Bottjer has shown that MAN plays a key role in song learning (Bottjer et al., 1984). Estradiol treatment, which can evoke SNAG expression in otherwise nonexpressing females with a time course apparently identical to the male pattern (Akutagawa and Konishi, 2001), is also permissive for masculinization of MAN and RA and for vocal learning in females (Simpson and Vicario, 1991).

Many birds sing but few are learners. Could SNAG be a key to the evolution of vocal learning? The pattern of labeling seen in zebra finches is replicated in other grass finches including Bengalese, spice, and strawberry finches (and is not sexually differentiated in the latter where both sexes sing). However, canaries (another oscine song bird learner) do not evince immunolabeling with SNAG. Thus, either the antigen is restricted to the estrildidae (grass finches) or perhaps the canary epitope of a shared nuclear protein cannot be recognized by the finicky SNAG antibody (this seems more likely).

Will it be possible to go directly from the pattern of SNAG expression to finding out what SNAG is and what it does? The finickiness of the antibody could severely hinder tracking down the identity of SNAG even in grass finches; the antigen was derived from fixed tissue (RA), and thus far Westerns only work on fixed material. Given the dearth of SNAG expression in female brains, one approach would be to subtractively hybridize female MAN and male MAN samples (MAN has the most robust SNAG expression at all ages after 35 days, but is never expressed in females) or to repeat the immunosuppression procedure using unfixed male MAN as an antigen. Another, far less daunting, approach is to look for SNAG candidates. Could it be, given its clear nuclear localization, a nuclear receptor? The pattern of androgen and estrogen receptors does not overlap precisely with

SNAG; it could, however, have another ligand such as retinoic acid, be a heterodimeric partner for a nuclear receptor, or be an “orphan receptor.” Since the Nottebohm group has shown that HVC manufactures retinoic acid, the retinoic acid receptors are hot prospects. Other prospects include ETS and DRG11-related transcription factors (see work from Jessell and Anderson laboratories). Time, and cloning, will tell. Even a small bit of peptide sequence would suffice to unleash a torrent of PCR-based searches for this song system-specific gene using the expression pattern as a bioassay.

Vocal learning is rare. In birds, it shows up in oscines, parrots, and hummingbirds, and in mammals, thus far, only in ourselves. Human speech is a highly specialized system that is culturally transmitted. How our presumably speechless primate progenitors acquired the capacity for language remains mysterious. The SNAG antibody of another group of vocal learners could be a key to the kinds of changes in gene expression that permit the translation of what is heard into what is uttered.

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