

Choices, choices, choices

Jerold Chun

Individual olfactory sensory neurons express only one of more than a thousand different odorant receptors, suggesting that DNA rearrangement may be involved. Based on a clever new technical approach, two groups now conclude that this is not the case.

Mammalian olfactory sensory neurons have a difficult decision to make. From over a thousand possible choices, each sensory neuron must pick only one type of olfactory receptor (OR) gene to express¹. How this is accomplished is still unclear. In the immune system, the diversity of immunoglobulins and T-cell receptors arises through a process called 'VDJ recombination' (Fig. 1a)². The mechanism (more generally called somatic DNA rearrangement) involves cutting segments of DNA from non-germline tissue and rejoining the segments to form composite genes, producing permanent changes in DNA and gene expression that are not passed on to future generations. The size of the OR gene family³ and its genomic organization, with over 1,000 OR genes dispersed in linear clusters and on different chromosomes^{4–6}, raised the possibility of DNA rearrangement as a mechanism for receptor gene choice in the olfactory system, too. If such a mechanism were found in mammalian neurons, it might help to explain the brain's complexity and diversity of connections and cell types. Now, in the first rigorous test of this hypothesis, two independent reports in *Nature*^{7,8} have cloned mice from single olfactory receptor neurons: neither finds evidence for DNA rearrangements.

Of course, models for explaining how ORs are expressed need not invoke DNA rearrangement (Fig. 1b,c)^{1,4}, and at least two key differences exist between OR genes and immune cell receptors. First, the entire OR is encoded by one contiguous stretch of DNA (a single exon), negating a need for combining gene segments. Second, there are no obvious rearrangement-recognition markers flanking OR genes (recombination signal sequences, heptamer/nonamer cis-elements), which are required for VDJ rearrangement. Thus, the mechanism for DNA rearrangement of OR genes would have to be

distinct from that observed in the immune system. One possible alternative mechanism might involve insertion of a transposable element with promoter/enhancer activity, which might direct expression of one OR from a basal promoter (Fig. 1d).

To examine whether DNA rearrangement occurs in OR genes, one must first devise a method for detecting the rearrangement. Historically, clonal cell lines have been used, as they can be grown indefinitely to generate large amounts of DNA with the same rearrangement that, once amplified, can be detected by standard techniques. Such an approach was used to first identify

immunoglobulin DNA rearrangements². However, no clonal cell lines expressing OR genes exist. Theoretically, single-cell PCR approaches might also work, but in the absence of defined OR genes and specific DNA sequences to target, PCR is not technically feasible. To get around this problem, Eggen *et al.*⁷ and Li *et al.*⁸ used the new—if involved—strategy of expanding a single OR neuronal genome by mouse cloning⁹. Indeed, cloning of lymphocytes that have already undergone immunological DNA rearrangements produce mice that maintain the original rearrangements in all tissues, yet also yield viable mice, validating this strategy for

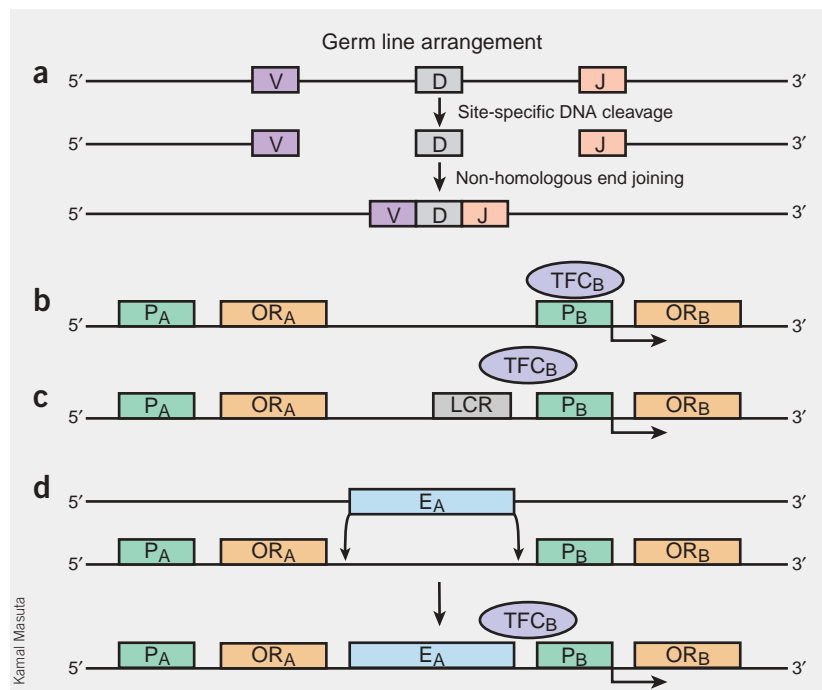
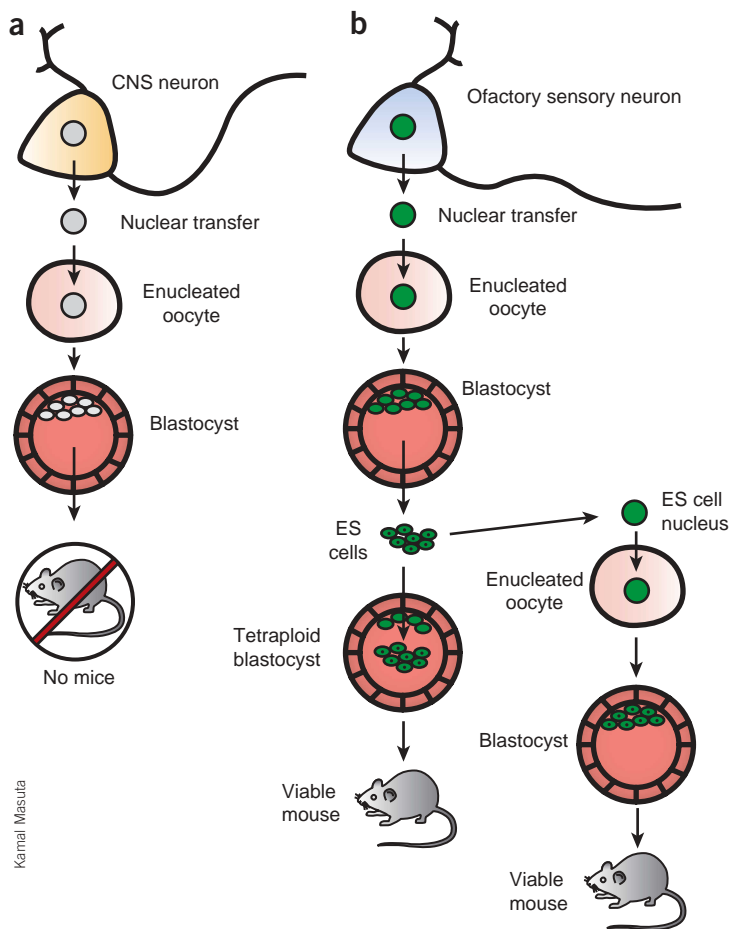


Figure 1 Models for regulating gene expression in the immune and olfactory systems. (a) Classical 'VDJ' recombination that occurs in the immune system to generate immunoglobulins. Component gene segments that do not themselves encode mature immunoglobulins are brought together to form a composite coding region that serves as the antigen recognition portion of an antibody². In the olfactory system, receptor expression controlled by short promoter (P) elements (b) or by distant loci (locus control region, LCR) (c) could provide sufficient information to allow appropriate expression of OR genes in the presence of appropriate transcription factors (TCF)⁴. (d) Olfactory receptor expression controlled by DNA rearrangement³, in which a distant segment of DNA with promoter/enhancer activities is placed, through rearrangements, in proximity to a basal promoter to provide specific expression⁴; other variations are also possible.

The author is in the Department of Molecular Biology at the Helen L. Dorris Institute for Neurological and Psychiatric Disorders, The Scripps Research Institute, ICND118, 10550 North Torrey Pines Road, La Jolla, California 92037, USA. e-mail: jchun@scripps.edu



Kamali Masutla

Figure 2 Different mouse-cloning strategies. In all approaches, a nucleus from a single neuron is isolated and transferred to a recipient enucleated egg, which further develops in culture into a blastocyst that, following intrauterine implantation, could become a mouse. (a) Nuclei from CNS neurons were unable to generate viable mice^{9,11}. (b) In the new studies^{7,8}, permanently labeled nuclei (shown in green) from olfactory sensory neurons were used in a two-step cloning approach¹⁰ in which totipotent ES cells derived from the cloned blastocyst were injected into specially modified tetraploid blastocysts. The approach generated viable cloned mice derived only from the transplanted ES cells (versus contributing to the placenta, which is of distinct embryological origin). To determine totipotentiality, Eggen *et al.* also used an ES cell nucleus that itself had been derived from an olfactory neuron. The resulting cloned mouse is a clearer indication of the totipotent state of the neuronally derived ES cell nucleus.

range of expressed ORs, despite having originated from a neuron expressing an identified OR subtype.

Finally, the researchers used ES cell DNA derived from a tagged OR neuron to search by classical means for possible rearrangements surrounding that receptor—no rearrangements were identified. Therefore, DNA rearrangements are not necessary for OR expression. That the two research groups used different ORs also strengthens this conclusion.

The results of Eggen *et al.*⁷ and Li *et al.*⁸ refocus attention on epigenetic mechanisms of OR expression. In this active field, novel interactions relevant to OR expression are being identified, such as intracellular negative feedback by ORs themselves, which may account for the one-receptor/one-neuron rule¹². Eggen *et al.* look beyond olfaction *per se*, by providing further data on the totipotentiality of a neuron. They cloned mice using direct transfer of a neuron-derived ES cell nucleus into an oocyte (Fig. 2b). The normal-appearing mice that resulted from these apparently totipotent nuclei suggested that even a postmitotic neuronal nucleus could be reprogrammed to produce an entire organism.

As with most scientific studies, some caveats may be worth considering. Although Eggen *et al.* validly concluded that their cloned mice did not contain DNA rearrangements that interfered with development of a viable mouse, it is notable that biologically important DNA rearrangements of the immune system are maintained in, and are fully compatible with, normal cloned mice¹⁰. Therefore, 'clonability' cannot be equated with an absence of DNA rearrangement. It also remains formally possible that a subset

revealing DNA rearrangements¹⁰. To assess the neuronal genome of a single, identified OR gene, the two research groups extended the cloning approach with elegant variations that blended a range of other molecular genetic techniques including embryonic stem (ES) cells (semi-immortal cells that can reconstitute a mouse), targeted knock-out/knock-ins and lineage tracing.

One way to think about cloned mice is as a genome magnifier, as a single neuronal nucleus produces an entire mouse in which all non-immune cells are classically expected to be genomically identical. Whereas cloning of mice was reported several years ago using non-neural cumulus cells⁹, attempts to produce mice from CNS neurons (Fig. 2a) had proven unsuccessful^{9,11}. Unlike earlier cloning reports⁹, Eggen *et al.* and Li *et al.* used a two-step cloning process (Fig. 2b), whereby donor nuclei are first transferred into enucleated oocytes, which are allowed to form blastocysts from which ES cells are derived. These ES cells are then transferred to a modified recipient embryo (a tetraploid rather than diploid blastocyst) before transfer to a host mouse for *in utero* development; the end result is that cloned mice are actually

produced from the ES cells rather than directly from a neuronal nucleus itself.

Both groups first used permanently tagged olfactory sensory neuron nuclei to produce ES cells and then cloned mice, allowing identification of the tag in subsequent steps. The results showed that at least some olfactory neuronal nuclei were competent to produce cloned, apparently normal mice. If restricted expression of a single OR gene in the differentiated olfactory sensory neuron was permanent, then the researchers might have expected to see mice expressing only one OR type, along with olfactory sensory neurons showing a single, stereotyped neuroanatomical projection pattern. However, further analyses of these mice indicated normal OR gene expression, with expression of multiple receptors and normal neuroanatomy, including spatial distribution of olfactory sensory neurons.

However, this first approach could not identify which single OR subtype was being used by the neuron from which a mouse was cloned. Thus, the two groups went further by permanently tagging individual neurons of defined OR identity followed by cloning. As in the first experiment, OR neurons in the mice again showed a normal

of ORs might still use a rearrangement mechanism: although there is no evidence for this, these two studies only analyzed two of more than 1,000 expressed ORs.

An unresolved issue, which may be technical and/or biological in nature, is that no clones have yet been reported using direct transfer of a neuronal nucleus into an oocyte (Fig. 2), despite expert attempts to do so with nuclei from other neuronal populations^{9,11}. Even with the use of an ES cell intermediate, the overall success rate of cloning with neuronal nuclei seems to be ~1%. Neuronal nuclear 'reprogramming' (might it also include some forms of DNA repair?) seems to require the ES cell intermediate step, although precisely what this step might do to the clonability of neuronal nuclei is currently unclear. The state of the remaining 99% of neuronal nuclei that cannot be cloned remains unknown. It is conceivable that DNA rearrangements exist in

some of these neurons, although the nature of such rearrangements remains purely speculative and, as noted above, might not be expected to hamper cloning. By contrast, this 99% most certainly contains nuclei with global changes in chromosome number (aneuploidy) that exist among developing and postmitotic neurons^{11,13–15}. Although the function and total extent of this aneuploidy have yet to be clarified, it could in part account for the low percentage of successful clones. It could also account for the developmental failures observed by Eggen *et al.* and Li *et al.*, as well as place limits on the percentage of totipotent neurons identified by Eggen *et al.*

That said, none of these considerations detracts from these first glimpses into a single OR neuronal genome, and these impressive technical and scientific achievements will no doubt yield further insights into both olfaction and other neural systems in the near future.

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Imaging gender differences in sexual arousal

Turhan Canli & John D E Gabrieli

Men tend to be more interested than women in visual sexually arousing stimuli. Now we learn that when they view identical stimuli, even when women report greater arousal, the amygdala and hypothalamus are much more strongly activated in men.

"A man falls in love through his eyes, a woman through her ears," wrote Woodrow Wyatt in 1918. In this issue, Hamann and colleagues¹ use functional magnetic resonance imaging to test whether males and females indeed differ in their brain responses to sexually arousing images. The authors find greater activation in males than females in the amygdala, a brain region involved in emotional arousal, and in the hypothalamus, a brain region central to reproductive functions. What distinguishes this study from a previous effort² is that the investigators went to great lengths to select stimuli and subjects that would ensure similar degrees of self-reported arousal in both sexes. Thus, the observed brain differences are less likely to reflect sex differences in arousal; instead they

reflect sex differences in the processing of sexually arousing stimuli.

Hamann and colleagues scanned 28 healthy, heterosexual volunteers, an equal number of males and females. Participants passively viewed neutral images of couples interacting in nonsexual ways (such as weddings, dancing or therapeutic massage), nude photographs of opposite-sex individuals in modeling poses (opposite-sex stimuli) and photographs of couples engaged in explicit sexual acts (couples stimuli), as well as a fixation cross condition to establish brain activation at baseline. Participants subsequently rated their sexual attraction and physical arousal in response to each image on a three-point scale. Analysis of the imaging data contrasted brain activation to the couples stimuli versus activation to neutral or fixation stimuli, thus revealing regions of significant activation for each sex separately, as well as significant differences between, and commonalities across, the sexes (Fig. 1).

Both sexes reported comparable sexual attraction and physical arousal in response to the images; both groups found the couples stimuli to be the most attractive and arousing. The most sensitive direct comparison

between males and females looked at the contrast in brain activation between the couples and neutral stimuli. Both classes of stimuli depicted couples interacting, differing only in the sexual aspect of the interaction. In this contrast, males showed significantly greater activation than females in the amygdala. This differential activation in the amygdala stands in striking contrast to many brain regions that were commonly activated for both males and females—regions associated with visual processing, attention, motor and somatosensory function, emotion and reward.

Several additional observations are noteworthy. First, brain activation data remained unchanged when the one female subject who reported low sexual arousal was excluded from the analysis. Removal of this subject caused the average arousal of the females to significantly exceed that of the males, yet it was the males who exhibited greater amygdala activation. This is perhaps the strongest indicator that amygdala activation is not related to sexual arousal *per se*.

Second, the average differences between the sexes were striking. Not only did men show greater activation than women in response to sexually explicit couple images in

Turhan Canli is at the Department of Psychology, SUNY Stony Brook, Stony Brook, New York 11794-2500, USA.

e-mail: turhan.canli@sunysb.edu

John D.E. Gabrieli is at the Department of Psychology, Stanford University, Jordan Hall, Stanford, California 94305, USA.

e-mail: gabrieli@psych.stanford.edu