Developmental neurobiology: **Alternative ends for a familiar story?**

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Somatic DNA recombination is essential for production of functional antigen receptor genes of T and B lymphocytes, but it is thought to be unique to the immune system. Recent studies have now shown that recombination-related genes are also necessary for normal neuronal development.

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Neurons of the mammalian nervous system are remarkable for their heterogeneity and the complexity of their inter-connections. There appear to be many similarities between the neurogenic mechanisms through which these features first arise and processes that play key roles in the development of other organ systems, including the involvement of myriad genes associated with cell proliferation, differentiation and/or death. Researchers investigating these neurogenic mechanisms generally assume that the genomic information available to each developing neuron is constant, each neuron having identical DNA sequences. This assumption appears to hold true for all other somatic tissues, with one obvious exception: the T and B lymphocytes of the immune system, which undergo somatic DNA rearrangements to produce mature genes encoding T-cell receptors and immunoglobulins, respectively. This process, known as V(D)J recombination [1], is responsible for the thus-far unparalleled degree of immunological diversity that is fundamental to the immune response.

Might a similar form of somatic DNA rearrangement take place in the nervous system and contribute, in some way, to the generation of neuronal heterogeneity and complex connectivity? Recent, independent studies in the Lindahl [2] and Alt [3] laboratories have provided indirect support for this possibility, by demonstrating that the deletion of genes known to participate in non-homologous end joining of DNA ends in V(D)J recombination results in early embryonic neuronal death. Specifically, deletion of either one of the genes encoding DNA ligase IV [2,3] or its dimerization partner XRCC4 [3] was found to give the same general phenotype of early neuronal death, and consequent embryonic death. We shall attempt to place these results in the context of what is known about mammalian neuronal development.

Early neurogenesis

Neurons of the mammalian central nervous system (CNS) first arise during embryonic development from neuro-proliferative zones that surround the cavities, or ventricles, that contain cerebral spinal fluid. In the cerebral cortex, for example, this proliferative region is known as the 'ventricular zone' (Figure 1), and developmental events in this region serve as an archetypal example of neurogenesis elsewhere in the neural tube, including the spinal cord. During neurogenesis, cells can undergo one of three general fates: they can continue to proliferate, differentiate to become postmitotic, or die. Those that become postmitotic, a step referred to as the 'birth' of a neuron, migrate out of the ventricular zone, through a superficial region termed the 'intermediate zone', until they reach the most superficial portion of the cerebral wall that will give rise to the gray matter of the adult cerebral cortex, the cortical plate.
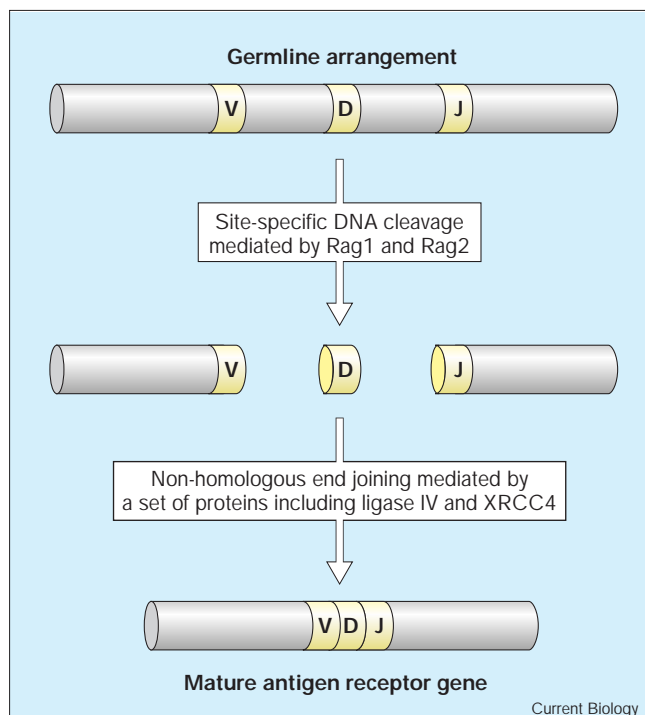
Once within the cortical plate, the immature neurons continue to differentiate, eventually attaining adult-like features during postnatal life. These embryonic zones can

Figure 1
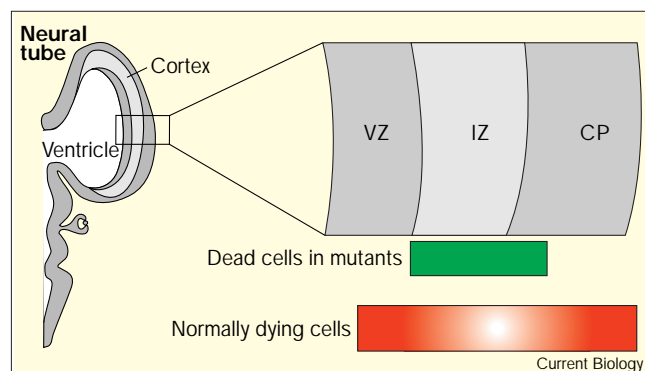
A comparison of the distribution of dead cells observed in DNA ligase IV or XRCC4 mutant mice [2,3] (green rectangle) with the distribution of dying cells observed in wild-type mice [4] (red radially filled rectangle). All neurons of the central nervous system proper arise in the neural tube, within which three embryonic zones can be identified: a neurogenic region known as the ventricular zone (VZ), and two predominantly postmitotic regions, the intermediate zone (IZ) and the future gray matter of the cerebral cortex, the cortical plate (CP). The ventricular zone gives rise to neurons of the cortex that, once becoming postmitotic, migrate superficially through the intermediate zone to locations within the cortical plate, where full neuronal maturation takes place.

Figure 2

Germline arrangement

V   D   J

↓

Site-specific DNA cleavage
mediated by Rag1 and Rag2

V   D   J

↓

Non-homologous end joining mediated by
a set of proteins including ligase IV and XRCC4

↓

V D J

Mature antigen receptor gene

Current Biology

V(D)J recombination brings together germline gene segments to produce mature genes encoding the antigen receptors of T cells and B cells – T-cell receptors and immunoglobulins, respectively. Two steps can be distinguished: site-specific DNA cleavage by Rag1 and Rag2, and joining of cleaved segments by the non-homologous end joining machinery that includes DNA ligase IV and XRCC4.

be distinguished by a variety of routine histological techniques, and homologous zones can generally be identified in other portions of the neural tube. The intermixing of cell proliferation, differentiation and death during neurogenesis has led to suggestions [4,5] that some form of cell selection might be occurring during the formation of the CNS, akin to what occurs during the development of the immune system as a result of processes in which V(D)J recombination plays a crucial part. Although the cellular events are suggestive, there is no clear evidence for genetic recombination in the nervous system. But recent advances in understanding the enzymology of V(D)J recombination in the immune system have allowed indirect approaches to this question that have produced some intriguing results.

V(D)J recombination in lymphocytes
V(D)J recombination allows the formation of functional antigen receptor genes from fragments that are separated in the germline genome. It occurs in two general steps that involve distinct proteins (Figure 2). In the first step, recombinase proteins encoded by the genes *RAG1* and *RAG2* [1] cleave DNA in a site-specific manner adjacent to the gene segments destined for recombination, resulting in double-strand breaks. In the second step, the DNA

ends are processed and joined together, thereby assembling the gene segments into a potentially functional immunoglobulin or T-cell receptor gene.

The DNA end-joining reaction is critically dependent on non-homologous end joining factors, which include the XRCC4–ligase IV heterodimer [6,7], the Ku protein — another heterodimer, consisting of subunits Ku70 and Ku80 — and DNA-dependent protein kinase. V(D)J recombination occurs only in lymphocytes, and is a fundamental driving force for the multiple selective processes that eliminate inappropriate lymphocytes. A prime example of cell selection can be seen in the thymus, where the overwhelming majority of T cells die, on the basis of the specificity of their surface T-cell receptors formed through V(D)J recombination.

Some history
In 1967, nearly a decade before genetic recombination was demonstrated experimentally in lymphocytes, Dreyer *et al.* [8] postulated that recombined genes encoding cell-surface proteins might play a part in guiding the correct re-innervation of the goldfish tectum by retinal axons, acting as a kind of molecular code to ensure the topographically correct retino–tectal mapping is achieved. Since this idea was put forward, however, no gene that undergoes the requisite recombination in the nervous system has been found. This is perhaps not too surprising, even if the idea is not simply fanciful, as the reagents that were instrumental in the discovery of lymphocyte V(D)J recombination — DNA sequences from a known candidate locus, and clonal cell lines in which the rearrangement event takes place — do not currently exist for the nervous system.

An indirect approach to this issue is to ask whether the genes of immunological V(D)J recombination are expressed in the nervous system. In fact, *RAG1* does show low levels of expression in neurogenic regions [9], although *RAG2* is not clearly expressed, and *RAG1* null knockout mice have no obvious brain phenotype [10]. These results are compatible with the view that *RAG1* has a non-essential role in neurogenesis, and it is notable that DNA ligase IV and XRCC4 deficiencies affect cells in the same general anatomical region. As mentioned above, one interesting parallel between neurogenesis and lymphocyte development is the association of substantial apoptosis during normal development and it is notable that, for neuroblasts, this apoptosis commences in the ventricular zone, one of the neurogenic regions where *RAG1* is expressed.

Phenotypic consistency
A compelling aspect of the new studies is the consistency of the phenotype of mice deficient in DNA ligase IV [2,3] or its dimerization partner XRCC4 [3]. One notable feature of the mutant phenotype is the abnormal pattern of dead cells in the cortex. This is illustrated in Figure 1,

which shows how the mutant distribution of dead cells, as judged by assays that measure the terminal stages of the cell death process, compares to the wild-type distribution of embryonic cells in the process of dying, as detected by more sensitive techniques [4,5]. A comparison of these distributions indicates that, in the brains of mutant mice, newly postmitotic neurons start to undergo apoptosis as they leave the ventricular zone; these cells start to migrate but generally die before reaching their normal destination in the cortical plate — hence the increased numbers of dead cells in the intermediate zone in particular. This general appearance occurs in all examined regions of the neural tube, including the spinal cord [2,3].

Of particular note is the developmental manifestation of the mutant phenotype [2,3]. Aberrant death is not observed at the earliest embryonic ages, and it is not until entering the third trimester at around embryonic day 13 — the period when most postmitotic neurons are generated — that dead cells are clearly observed. The increasing burden of cell death with increasing embryonic age complicates determination of direct versus indirect effects of *lig4* or *XRCC4* deletion. In each case, however, it is clear that early embryonic neurons are affected in significant numbers. An additional consideration is the presence of histologically normal neurons among the dead cells, suggesting that some neurons may not be susceptible to the deleterious effects of defective non-homologous end joining — effects that Barnes *et al.* [2] note are reminiscent of the non-lethal phenotype exhibited by yeast cells with the equivalent *lig4* mutation (see below).

Suitable ends

Although there is ample reason to expect that defective non-homologous end joining is at the root of the ligase IV and XRCC4 mutant phenotypes, there are alternative explanations for these results that have little mechanistic similarity to V(D)J recombination in the immune system. It is possible that ligase IV and XRCC4 have roles in processes distinct from non-homologous end joining, and that disruption of these other processes makes the most important contribution to the mutant phenotype. But this alternative would be unlikely if mice deficient in other components of the non-homologous end joining machinery were found to exhibit a similar neuronal phenotype. Another formal possibility invoked a non-cell-autonomous mechanism, in which the observed phenotype is quite removed from the initial cellular defect, occurring many steps downstream as a result of some disrupted cell–cell interaction. Barnes *et al.* [2] note that *lig4* mutant yeast show abnormal responses to signals controlling cell growth, and raise this as yet another possible explanation for the neuronal death phenotype seen in the mutant mice.

Even if non-homologous end joining is essential for the survival of postmitotic neurons, which is the simplest explanation for the observed phenotype, there are still biologically less interesting explanations than ones based on analogy with V(D)J recombination in lymphocytes. The cell death might simply result from an excess of non-specific double-strand DNA breaks, produced for example by reactive oxygen species, as suggested by Barnes *et al.* [2], or by a developmental sensitivity to random double-strand DNA breaks, as suggested by Gao *et al.* [3]. Each of these possibilities is mechanistically far removed from V(D)J recombination, and deserve consideration. It is, however, unclear how such non-specific mechanisms could result in the precise developmentally and anatomically restricted effects seen in the mutant mice.

Regardless of the actual mechanism, these studies have provide the first clear evidence that genes required for completion of V(D)J recombination in lymphocytes are required for early neuronal survival. This adds tangibly to an emerging view of instructive similarities between immunological and neurobiological mechanisms in normal development [11]. We offer the speculation that the requirement for non-homologous end joining function in the embryonic brain reflects the operation of a novel form of cell selection that distinguishes neurons that make advantageous DNA rearrangements from those that do not.

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