to study similarities and variations at the sub-chromosomal level among morphologically similar neurons.

In Purkinje neurons of the adult mouse, the number of centromeric kinetochore clusters and spatial distribution, as detected by immunocytochemistry [8] were shown to be routinely similar leading to the possibility that the same chromosomes contribute their centromeres to a given cluster. Accordingly, we hypothesized that a pair of centromeres has two possibilities in their spatial positions. One, they may always form part of a cluster and therefore are always associated. Second, if they are part of two separate clusters, they never cluster. Our recent work has shown routine clustering of centromeres of one homologue each of chromosomes 2 and 11 in Purkinje neurons [11]. To test whether centromere clustering between other chromosomes in Purkinje neurons has chromosome-chromosome specificity, we carried out FISH using chromosome-specific para-centromeric sequences from randomly picked pairs of chromosomes.

Results

Using immunocytochemistry to the kinetochrore proteins associated with centromeres, our results show that the number of centromere clusters in the nuclei of cortical pyramidal, cerebellar Purkinje and cerebellar granule neurons (Fig. 1) are much less than the chromosome complement for the species (40 in number). By comparing with the fibroblast cell nucleus, we have previously reported that the increased signal size of the centromeres observed in Purkinje neuronal nucleus results from clustering of multiple centromeres [8]. Using FISH experiments, simultaneous application of probes for two randomly selected centromeres resulted in four discrete signals in Purkinje neurons for all the nine pairs of chromosomes tested. Measurements of inter-signal distances showed that one homologue each of the chromosome pairs exhibited wide range of clustering (Fig. 2). We have previously reported the routine clustering of chromosome pairs 2 and 11 in Purkinje neurons [11]. In contrast to this, pairs 2&3, 2&8 and 6&8 routinely did not show clustering of centromeres. Whenever clustering was observed, it was restricted to one pair of homologous chromosomes; the second pair did not cluster in any of the cells examined. In summary, the extent of centromere clustering among pairs of chromosomes studied in Purkinje neurons is limited to only one pair of homologues and the percentage occurrence showed a wide range of variation between zero and hundred suggesting varying combinatorial association.

Discussion

Functional regulation in cells involves different structural changes in chromosomes, DNA and proteins. In neurons, plasticity as well as metabolic requirements are also controlled by mechanisms ranging from regulation of subunit assembly of multi-unit proteins [12] to generation of hybrid metabolites from limited set of genes [13]. Controls on gene expression by centromeric heterochromatin-mediated silencing was shown to relocate a gene into its proximity [14]. Centromeric repeat homology is also found in small RNAs in RNA interference (RNAi) effecter complex RITZ [15]. In these contexts, the clustered centromeric heterochromatin in Purkinje neurons [8,11,16] may have important functional roles. The size and number of centromeric clusters in adult Purkinje neurons remained almost the same with less variability [8] leading to the hypothesis that it is the same chromosomes that always contribute their centromeric domains to a given cluster. We haven't directly assessed the composition of a particular cluster. Instead our approach was an indirect one by using pairs of centromere probes to detect the spatial relationship. According to our hypothesis a pair of centromeres will either always cluster or not cluster at all. Contrary to our expectations, the results have shown a trend towards wide range of clustering. The reasons for this remain unknown. Epigenetic variations in expression profiles of genes may account for the variability observed since heterochromatin may play a major role in epigenetics [17]. Some centromeres show higher levels of clustering indicating the possibility that clustering of at least some chromosomes may be obligatory for maintaining and/or regulating housekeeping gene expression; while others are facultative depending on the varying functional demands made on these neurons or depending on the specific network connections. In addition, the structural variations may contribute to the functional demands on morphologically similar neurons representing different somatic locations of the cerebellar homunculus [18,19]. Since the present work was done using parasagital sections from the cerebellum, the results may be interpreted as that of a parasagital topographical feature, until further investigations in other planes are carried out.

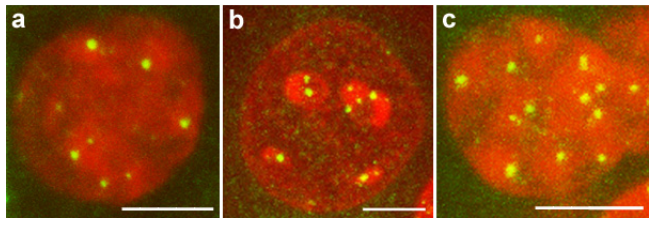

Figure 1
Superimposed stacks of confocal sections showing centromeric clusters (green signals) in representative nuclei (red) of a) cerebral cortical pyramidal neuron b) cerebellar Purkinje neuron and c) cerebellar granule neuron of mouse observed by immunocytochemical staining of the kinetochore proteins. DNA in the nuclei is stained with ethidium bromide (red). Scale bar in all the figures = 5 μm