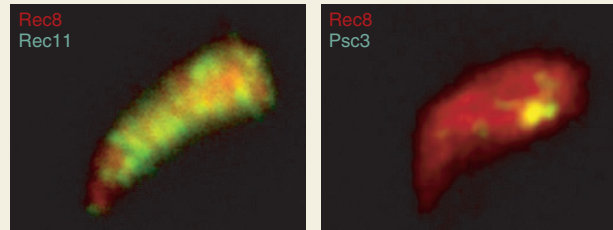


## Recasting meiotic cohesion

Chromosome segregation consists of a series of highly orchestrated events that are essential for cell division. A central aspect of this process is cleavage of cohesin, a multisubunit complex that maintains the structural integrity of chromosomes. Cohesin consists of two structural components, Smc1 and Smc3 (Psm1 and Psm3 in the fission yeast *Schizosaccharomyces pombe*, respectively), and two accessory components that are specific for mitotic or meiotic events. Meiosis poses a far more complex problem for chromosome cohesion than mitosis, as first reductional division of chromosome pairs at meiosis I must occur before a mitotic-like equational division at meiosis II. The meiotic cohesin complex is central to recombination events, and studies in fission yeast have identified Rec8 as a meiosis-specific accessory subunit that replaces Scc1, a subunit specific to the mitotic complex. During meiotic prophase, a Rec8-containing complex is localized along the entire chromosome, facilitating chromatid cohesion and recombination. Centromeric Rec8 ensures that each kinetochore of sister chromatid pairs is attached to the same spindle pole (monopolar attachment). Initially, Rec8 is only disrupted along chromatid arms during meiosis I. By meiosis II, however, Rec8 at centromeres is disrupted, allowing separation of sister chromatids. A good candidate for the second accessory meiosis-specific subunit is Rec11, which replaces the mitosis-specific Psc3 subunit. Mutation of Rec11, similarly to mutation of Rec8, reduces recombination. However, a direct demonstration of Rec11 function in the meiotic cohesin complex has been lacking.

Now, a study by Watanabe and colleagues (*Science* **300**, 1152–1155 (2003)) provides a more detailed molecular explanation of these events. Their first significant observation is that sister chromatids dissociate prematurely during meiosis I in *rec11Δ* cells. This suggests that Rec11 is central to sister chromatid cohesion. Using a similar approach, they also examine the role of Rec11 in centromere function and find that monopolar spindle attachment is



Localization of Rec8 (red) and its partners Rec11/Psc3 (green) on chromosomes during meiotic prophase. Rec8 localizes along whole chromosome regions; however, Psc3 associates mainly at the clustered centromeres and Rec11 along the arm regions.

dependent on Rec8, but not on Rec11. This raises the intriguing possibility that Rec11 is not the only binding partner of Rec8.

Enter Psc3. Analysis of *psc3* mutants demonstrate that it may have a minor role in cohesion along meiotic chromosome arms, but is central to kinetochore regulation at meiosis I and II. Here, the authors go further, demonstrating that two distinct Rec8–Psc3 complexes exist: the first complex is localized to pericentromeric regions and is dependent on the heterochromatic state of this region for its localization. In contrast, a second Rec8–Psc3 complex is localized to the central core and does not require heterochromatin for its localization. Interestingly, these differences in localization reflect distinct functions at different stages of meiosis: the pericentromeric complex is required for centromere cohesion until meiosis II, whereas the central-core complex is most probably required earlier, for monopolar attachment at meiosis I.

Thus, the authors envisage the following model of meiotic chromosome segregation: first, the Rec8–Rec11 complex is disrupted during meiosis I, allowing separation of sister chromatids along chromosome arms; second, the pericentromeric Rec8–Psc3 complex maintains chromosome cohesion until meiosis II, when its disruption results in complete dissociation of sister chromatids; third, the central-core Rec8–Psc3 complex establishes monopolar attachment of sister kinetochores.

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## Neuronal polarization: building fences for molecular segregation

Carlos G. Dotti and Mu-ming Poo

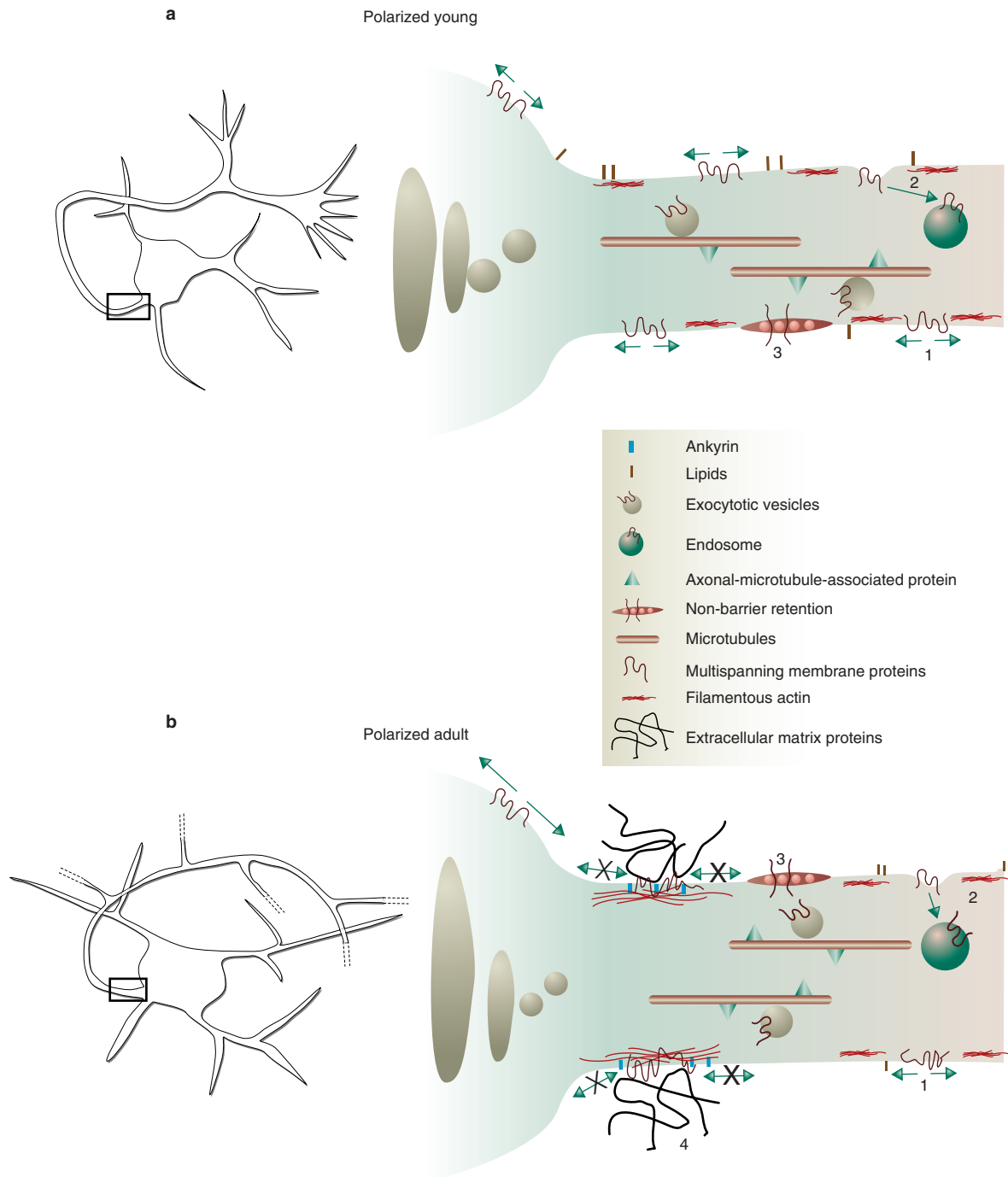
**Neurons exhibit distinct compositions in the axonal and dendrite plasma membrane, but it remains ambiguous whether or not a diffusion barrier is needed to keep the different components separated. Now, Nakada *et al.* utilize state of the art microscopy to follow the dynamics of single lipids or proteins inserted in different areas along the axonal and dendritic surface of neurons at different developmental stages. The results obtained shed new light on the mechanism underlying polarized segregation of membrane components in neurons.**

The common neuron in the central nervous system contains three easily recognizable regions — the cell body, numerous tapered and highly branched dendrites and a single long axon that ramifies near the site of contact with the target organ. As pointed out more

than a century ago by Ramon y Cajal, the neuron is polarized both structurally and functionally, with the dendrites and cell body receiving and processing information through the synaptic potential and the axon delivering information by generating and propagating

an action potential. The functional differences between axons and dendrites are direct consequences of the distinct molecular compositions of both the cytoplasm and the plasma membrane<sup>1</sup>.

Biologists working on neuronal polarity



**Figure 1** The progression of neuronal polarity. **(a)** During early polarization, membrane carriers containing axonal and dendritic components travel from the cell body to the future axon (framed) and future dendrites along microtubules. On fusion to the membrane, some components diffuse laterally (1), whereas others are retrieved by endocytosis and retrogradely transported (2). These components can thus change distribution from one domain of the neuron (for example, the axon) to the other (the dendrite). Even at these early stages, some

components can be specifically retained in the membrane to which they were initially delivered through interactions with the underlying cytoskeleton or in so-called membrane cholesterol-sphingolipid rich rafts (3). **(b)** Late in development, lateral mixing between axon and dendrites is prevented because a diffusion barrier at the axonal initial segment (framed) has formed (4). This consists of membrane lipid-protein clusters forming a tight connection with the underlying actin cytoskeleton and also with molecules of the extracellular matrix.

have long been interested in two aspects of neuronal membrane segregation: first, what orientates different membrane proteins and

lipids after synthesis in the cell body and second, how they stay in the right place after arriving at their destination at the axon or

dendrite surface. Numerous data suggest that in the *trans* face of the Golgi apparatus, membrane proteins and lipids are sorted into

different vesicle carriers and these carriers are transported out towards the axon or dendrites, in a microtubule-dependent manner. Once at the plasma membrane, distinct mechanisms operate to specify fusion and retention-retrieval<sup>2,3</sup>. In addition, as many proteins and lipids undergo relatively free lateral diffusion in the plane of the membrane, it has been hypothesized that some form of physical barrier exists to prevent the mixing of membrane components. This is now confirmed on page 626 in the current issue of *Nature Cell Biology* by Nakada *et al.*<sup>4</sup> who demonstrate that individual lipids and proteins can hardly diffuse along the plane of the membrane in the initial segment of the axon. In addition, their data suggests that diffusion is impeded because of the existence of tight pockets of membrane components anchored to the sub-adjacent cytoskeleton. Although other laboratories previously attempted to address this issue, neither the data nor the conclusions were as satisfactory.

The first attempt to directly test if there is a diffusion barrier at the axonal hillock was made by Kobayashi *et al.*<sup>5</sup> who monitored lateral diffusion of fluorescently-labelled lipids incorporated in the axonal membrane of cultured neurons. They found that incorporated lipids could diffuse in the axon, but could not access the cell body, suggesting the existence of a diffusion barrier at the axonal hillock. However, Futerman *et al.*<sup>6</sup> subsequently argued that a small amount of fluorescent lipid capable of diffusing could have been largely undetectable because of the dilution of the probe in the large mass of the cell body. In agreement, Winkler and Poo<sup>7</sup> later demonstrated that fluorescent lipid incorporated into dendrites or axons could diffuse to the cell body, thus arguing that the axonal hillock does not impose a barrier to lipid diffusion. However, there appeared to be a reduced diffusion rate between the axon and the cell body for the lipid, as compared with that between the dendrite and soma, suggesting that a diffusion barrier may indeed exist but that its function might be to slow down — rather than completely block — diffusion. More recently, Winkler *et al.*<sup>8</sup> advanced the idea of the axonal diffusion barrier by shifting its proposed location from axonal hillock to the initial segment, the short segment of axon past the hillock. Importantly, they also provided the first indication that restriction of protein mobility could be due to the underlying cytoskeleton, as protein mobility was increased after pharmacological disruption of actin filaments.

Taking advantage of a series of methodological innovations, the work of Nakada *et al.* not only confirms the previous propositions that a

diffusion barrier exists (refs 5, 8), but it also indicates how the barrier functions. Nakada and colleagues labelled and tracked the movement of individual molecules — in contrast to previous work that analysed a large number of labelled components. Nakada *et al.* also used the unsaturated phospholipid 1- $\alpha$ -dioleoylphosphatidylethanolamine (DOPE), because it is known to be highly resistant to diffusion blockade by other integral membrane proteins and cholesterol. Thus, immobility of this lipid strongly suggests that endogenous proteins or lipids will also be immobilized. This study demonstrated that the diffusion coefficient of individual DOPE molecules labelled with either a fluorophore or a gold particle is severely restricted in the axonal initial segment, with a diffusion coefficient that is two orders of magnitude lower than that found in the distal axon, the cell body or dendrites. Because of the nature of the lipid under study, it is difficult to envision that membrane components beyond the initial segment, whether in the cell body or distal parts of the axon, could cross this domain to blend with components of other territories.

Another important aspect of Nakada *et al.* is that the reduction in lateral mobility of DOPE at the initial segment correlates with the expression of ankyrin — an adaptor protein that links the membrane and the actin cytoskeleton — confirming the earlier suggestion made by Winkler and colleagues<sup>8</sup> that actin is a key element for proper function of the diffusion barrier. Furthermore, the voltage-dependent sodium channels, which are known to concentrate in the initial axonal segment<sup>9</sup>, were found to be similarly immobile in the axonal initial segment in an actin-dependent manner.

Finally, the work of Nakada *et al.* also demonstrates that reduced lipid mobility at the axonal initial segment occurs exclusively in mature neurons, not existing in younger neurons despite the presence of morphologically distinguishable axon and dendrites. As the appearance of the diffusion barrier coincides with the appearance of spontaneous activity in cultured neurons, Nakada *et al.* suggest that the establishment of the barrier may relate to proper neuronal function. Interestingly, an early sign of amyotrophic lateral sclerosis, a disease with axonal transmission defects, is the loss of the initial segment in the anterior horn neurons<sup>10</sup>. This indicates that the initial segment performs both diffusion barrier and action potential initiation functions through the formation and maintenance of a highly organized supramolecular structure based on sodium channel-cytoskeleton clusters.

Collectively, previous work and the gradual formation of the diffusion barrier at the initial segment, as demonstrated by Nakada and colleagues, allows us to envision the following 'roadmap' for the acquisition of membrane identity in polarized neurons (Fig. 1). During the early stages of axon/dendrite differentiation, newly synthesized membrane proteins and lipids that are destined to reach the axon or dendrite undergo an initial unspecific sorting and transport to the axon and dendrites<sup>11</sup>. Therefore, it is likely that — as in any other non-polarized cell — these newly delivered membrane components are inserted into the axonal and dendritic membrane (probably at the growth cones) and then locate to their final destination by either retrieval and retrograde transport and/or free diffusion of the inserted component (Fig. 1a). As neurons differentiate, there is gradual maturation of both the Golgi sorting machinery and the microtubule-transport system that guarantee proper polarized transport<sup>11,12</sup>. In addition, the maturation of specific retention-retrieval and lateral diffusion mechanisms, such as rafts, confer polarized distribution to some membranous components (Fig. 1a). Final axonal and dendritic membrane segregation, one of the keys of functionality, is obtained on the appearance of a diffusion barrier at the axonal initial segment through the assembly of a membrane-cytoskeletal meshwork. This not only results in the trapping or immobilization of selected groups of membrane proteins required for electrical transmission, but also prevents the intermixing of freely mobile molecules in the adjacent territories (Fig. 1b). The appearance of membrane specialization such as pre- and post-synaptic specialization and nodes of Ranvier will also be required for proper function.

Several issues remain to be addressed. It is important to dissect the molecular basis of the assembly and characteristics of the cytoplasmic and membrane complex that form the diffusion barrier. It will also be important to know whether other membrane complexes which have more widespread distribution, such as lipid rafts, contribute to the assembly of the barrier and how intrinsic or extrinsic factors during development may dictate the assembly of the complex at the initial segment. The evidence for a diffusion barrier found in non-myelinated axons of cultured neurons also needs to be corroborated by data obtained from *in vivo* preparations. The wrapping myelin sheets and development of nodes of Ranvier in myelinated axons will certainly impose additional constraints in the mixing of membrane components in the axon. Nevertheless, the work of Nakada *et al.*

has highlighted the existence of a functional barrier in the membrane of polarized neurons during development and underscores the importance of further studies on membrane heterogeneity for better understanding of neuronal functions. □

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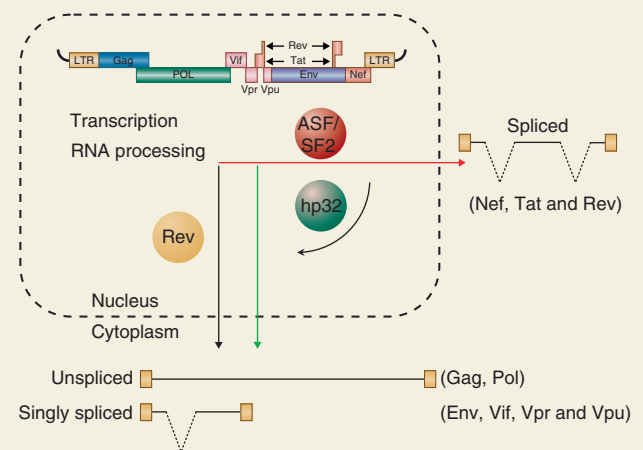
## A game of HIV and mouse

HIV has been a cumbersome object to study. This is not least because *in vivo* analysis has to be carried out in hosts that support replication of this retrovirus; namely, humans or monkeys. Macaques may breathe a sigh of relief now that Peterlin and colleagues report on page 611 of this issue that another hurdle to replication in murine cells has been overcome.

HIV replication in mouse cells has been studied for some time, and indeed this has resulted in some fundamentally important insights into its biology. Initially, the viral coreceptors CXCR4 (for T-cell tropic HIV strains) and CCR5 (for macrophage-specific strains) were identified as determinants for murine HIV entry along with the human CD4 receptor. Despite resolving the entry problem, viral replication still failed and subsequently the defect was pinpointed to murine CyclinT1 (CycT1), a subunit of the viral Tat transactivator associated factor P-TEFb: expression of human CycT1 in mouse cells was sufficient to rescue Tat-driven viral transcription. However, even expression of human receptor and coreceptor along with CycT1 was not sufficient to support full viral replication in mouse, suggesting that other post-transcriptional blocks must exist.

The single HIV transcript is subject to a complex set of splicing reactions, and a total of 46 RNA species have been reported. Three RNA species stand out as functionally important: the unspliced 9-kb transcript, forming the viral genome and encoding the viral proteins Gag and Pol; a singly spliced 4-kb species encoding the Vif, Vpr, Vpu and Env proteins; and a multiply spliced 2-kb species encoding Tat, Rev and Nef. The regulator of virion gene expression (Rev) is responsible for mediating nuclear export of the 9- and 4-kb species to protect them from splicing, which is an essential step in virus replication. It was noted over a decade ago that only multiply spliced species would accumulate in murine cells and that Rev function was compromised. Indeed, it soon became clear that this defect could be complemented with a factor expressed from human chromosome 11.

Peterlin and colleagues confirm that viral RNA is indeed spliced excessively in murine cells, relative to human cells, with a significant decrease of the 9- and 4-kb transcripts. This excessive splicing precludes nuclear export of the longer RNA species by Rev. The authors had previously identified a Rev-associated protein in mouse that is homologous to human splicing-factor-associated protein p32 (hp32). Indeed, hp32 also binds Rev and functions to



Splicing and transport of HIV transcripts in murine cells. ASF/SF2 commits HIV pre-mRNA to splicing. In murine cells, multiply spliced transcripts are observed in the cytoplasm of infected cells (red arrow). hp32 counteracts effects of ASF/SF2 and facilitates accumulation of genomic and singly spliced viral transcripts transported into the cytoplasm by Rev (green arrow), allowing for assembly of infectious virions.

inhibit HIV splicing in murine cells. Importantly, co-expressed hp32, but not mp32, could rescue Rev activity in murine cells, resulting in increased levels of unspliced HIV genomic RNA. To identify the basis of this difference, murine and human p32 hybrids were tested for inhibition of HIV splicing, narrowing down the region responsible to the amino terminus. Directed mutagenesis of divergent residues identified a single glycine-to-aspartate substitution as the culprit. The rescued HIV transcripts gave rise to Gag protein and indeed to fully infectious HIV particles in cell culture supernatants, although levels were not restored entirely to those observed in human cells. hp32 is encoded on chromosome 11, and this data therefore identifies the decade-old Rev-complementing activity. At this time, successful infection of transgenic 'humanized' mice expressing the viral receptors, transcriptional regulator, anti-splicing factor and possibly other interacting proteins remain the keenly awaited proof that HIV can successfully infect this, the favoured model organism. Alternatively, HIV may be 'muranizable' by propagation in replication-competent murine cells. Either way, a mouse susceptible to AIDS would undoubtedly be an invaluable asset to virologists.

**BERND PULVERER**