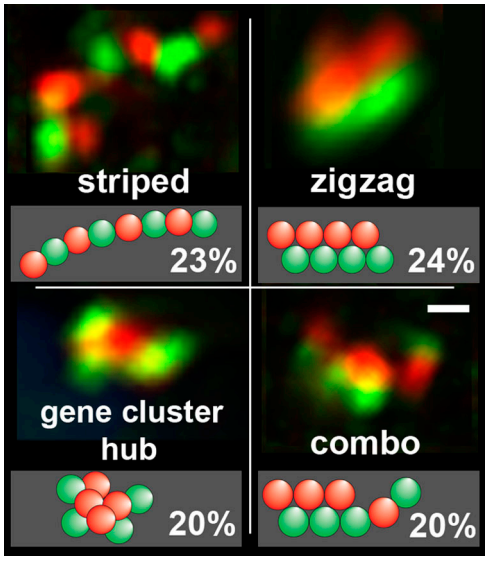
In This Issue



Gene clusters (red) and deserts (green) group together in characteristic patterns.

Chromatin zigzags

Gene-poor chromosomal regions are more often found in the nuclear periphery, and gene-rich regions are more often found in the nuclear interior. But Shopland et al. (page 27) are the first to analyze how multiple gene-poor and gene-rich regions are organized relative to each other. They find that gene-rich regions often cluster together while pushing interspersing genic deserts to the nuclear periphery, even in the absence of active transcription.

Shopland et al. studied a 4.3-Mb region of mouse chromosome 14 that has four gene-rich regions interspersed with four gene deserts. FISH probes that distinguished the genic and nongenic regions showed that the chromosome bent into three classifiable patterns: a striped pattern that resembled the linear sequence order; a zigzag pattern with the four coding regions next to one another and the gene deserts displaced to one side; and a clustered "hub" of gene-rich segments with peripherally arranged deserts. Combinations of these three patterns were also evident. The deserts often lined up at the edge of the nucleus, where they might contact the lamin meshwork.

The chromosomal arrangements did not appear to depend on transcription at a common site, nor did the gene-rich regions associate with aggregates of RNA splicing factors referred to as speckles. Moreover, the patterns persisted when transcription was blocked by drugs.

Given the limited influence that transcription appeared to have on the genome organization, it remains unclear how or why the chromosome bends into these configurations. The researchers speculate that the gene-rich regions share some regulatory proteins, as might the deserts, and thus are drawn together by cross-talk. There are genes in the region that act in the same developmental pathways, which might support this idea, but while coexpressed they have not been shown to be coregulated. Whether such associations are the result of passive chromatin wiggling or an active pulling process remains to be seen. JCB

Opposites attract

The nectin family's preference for heterophilic interactions prevents one dendrite from forming an attachment to another and leads to proper wiring in the nervous system, according to Togashi et al. (page 141).

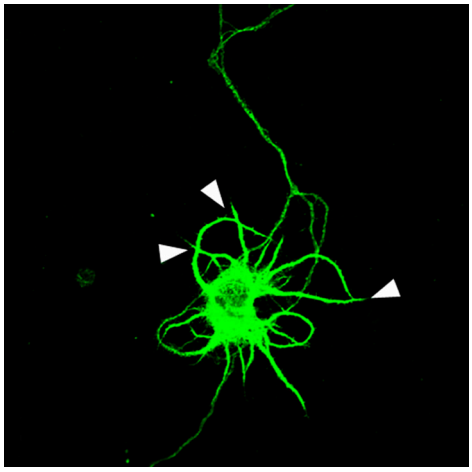
During embryonic development, neurons send out axons and dendrites. And though dendrites bump into other dendrites, only connections between axons and dendrites mature into synapses.

Cadherin and catenin proteins are found on both sides of a neuronal synapse and are required for synapse formation but do not appear to control the selective attachment between axons and dendrites. Members of the nectin subfamily of immunoglobulin proteins, however, are distributed asymmetrically at mature synapses with nectin-1 (N1) on the axonal side and nectin-3 (N3) on the dendritic side.

Looking at hippocampal neurons in culture, Togashi et al. found that N1 expression was restricted to axonal projections and the localization was dependent on the protein's cytoplasmic domain. N3 was expressed throughout the cell but was more abundant in dendrites.

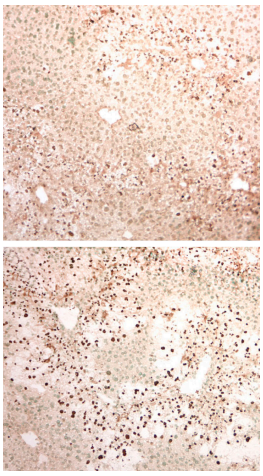
Disruption of these patterns by overexpression of N1, which drove N1 into dendrites, led to inappropriate dendro-dentritic contact. N1–N3 complexes were formed at these abnormal contacts. Additionally, linking the N3 cytoplasmic domain to the N1 ectodomain led to aberrant junctions.

After the nectin proteins formed heterophilic connections, cadherin and catenin proteins accumulated at the nascent synapse and formed homophilic connections, allowing maturation of the junction. Togashi et al. hypothesize that such interplay of heterophilic and homophilic adhesion molecules gives cells the variety of contacts they need to sort properly. JCB



Dendrites with excess nectin 1 aberrantly touch each other (arrowheads).

Text by Rabiya S. Tuma
rabiya@nasw.org



Fas-induced death (top) is increased when mutated keratin 8 cannot absorb phosphorylations (bottom).

Soaking up stress

On page 115, Ku and Omary add one more function to keratin's expanding repertoire. In addition to acting as a structural protein in liver cells, keratin 8 (K8) apparently absorbs excess phosphorylation during stress and thus reduces the likelihood of apoptosis.

Humans who carry a G61C polymorphism in K8 are predisposed to liver disease when exposed to insults such as infection. Unlike keratin-associated disorders in skin, these K8 variants do not appear to cause disease directly by decreasing the mechanical strength of cells. In fact, Ku and Omary found that mouse hepatocytes expressing G61C had normal mechanical strength, suggesting that the G61C polymorphism was affecting another stress response process.

G61C interfered with phosphorylation at serine-73 (S73), which is one of two sites in K8 that are phosphorylated by stress-activated kinases during apoptosis. When stress kinases were activated, cells expressing G61C or S73A K8 proteins were more likely to die than those with wild-type K8. Moreover, the duration and degree of phosphorylation of other stress kinase substrates increased in the mutant cells, relative to control cells.

The researchers conclude that K8 soaks up excess phosphorylation activity under stress conditions and thus hinders cell entry into apoptosis. Because S73 is conserved in several other keratins, Ku and Omary propose that the keratin-as-sponge function may be shared by other intermediate filaments, as previously suggested for neurofilaments. JCB

Death by Cdc6

The replication initiation factor Cdc6 is cleaved during apoptosis, and expression of a cleavage product is sufficient to induce apoptosis in otherwise unstressed cells. On page 77, Yim et al. report that the cleavage products can act as dominant-negative inhibitors of replication and amplify pro-death signals.

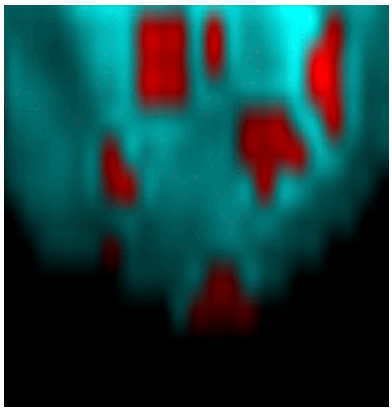
In addition to the previously identified cleavage product, Yim et al. identified a second Cdc6 fragment produced by caspase-3. Both Cdc6 fragments were sufficient to induce cell death without additional pro-apoptotic signals. Moreover, in cells exposed to apoptosis-inducing factors, ectopic expression of the Cdc6 peptides increased the rate of cell death. In contrast, expression of noncleavable Cdc6 suppressed apoptosis, indicating that fragmentation of the protein plays a causal role in the process even in the presence of known triggers.

Truncated Cdc6 interferes with loading of Mcm2 on the chromatin and thus disrupts assembly of the prereplication complex on chromosomes. That in turn induced DNA damage and activated the DNA damage response pathway, including phosphorylation of p53 and upregulation of apoptosis. Thus, even when the original apoptosis signal did not stem from a problem with DNA replication, the DNA damage pathway was subsequently activated during apoptosis.

In addition to showing how Cdc6 influences programmed cell death, the data demonstrate that apoptosis can be triggered downstream of the caspases—an observation that has largely escaped notice until now. JCB

Microclustered signaling

Inhibitory signaling in natural killer (NK) cells is restricted to microclusters of cell surface receptors rather than being evenly distributed across the immune synapse, report Treanor et al. on page 153.



Microclusters of active KIR (red) keep NK cells from killing their targets.

Natural killer (NK) cells survey the body looking for infected or damaged cells. Activation of their killing function can be achieved by any one of a number of cell surface proteins, but this is reversed if the killer Ig-like receptor (KIR) on the NK cell surface recognizes a major histocompatibility complex I protein on the surface of the cell under investigation. Then the KIR is phosphorylated and produces a strong inhibitory signal, preventing the NK cell from killing the surveyed cell.

To look at the distribution of KIR activity in the immune synapse, Treanor et al. detected FRET between GFP-tagged KIR and a fluorescently labeled antiphosphotyrosine antibody. At any given point in time, a subset of the KIR molecules were active in microclusters. Lck, which is important for phosphorylation of KIR, was also found in microclusters.

Based on these data and recent reports showing a similar punctate distribution for activated T cell receptors, Treanor et al. hypothesize that the uneven distribution has important functional consequences. For example, it could be that such localization of signal somehow allows the cell to integrate information from multiple activating and inhibitory receptors within an immune synapse. With that possibility in mind, the group is now trying to see how both activating and inhibitory signals distribute across the contact region at the same time. JCB