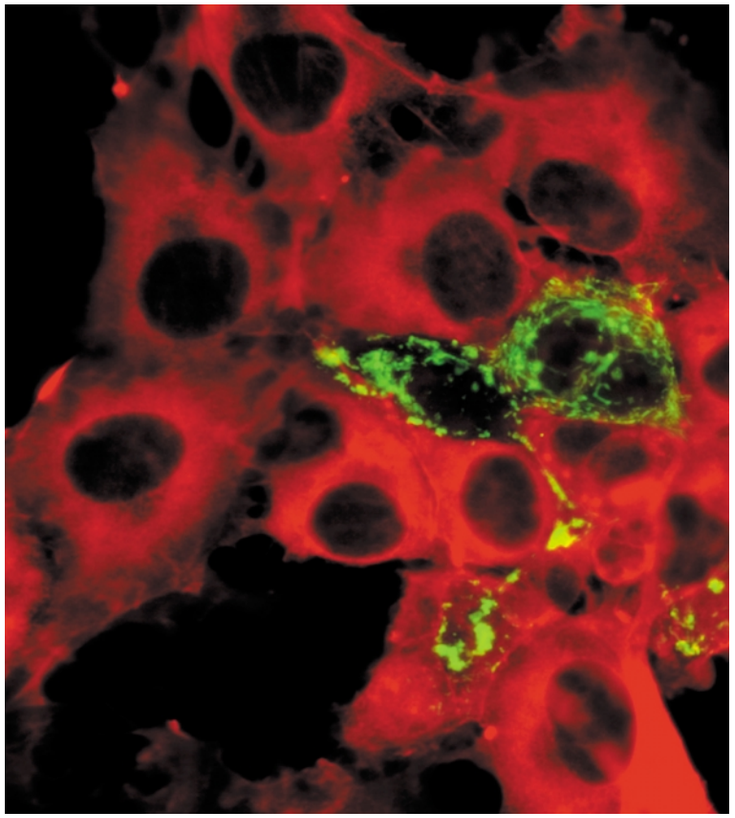


In COS-7 cells that express *FILIP–GFP* (green), the levels of endogenous Filamin A (red) is markedly reduced compared with neighbouring cells.

CYTOSKELETON

Development moves on

A key event in the development of the human embryonic brain is the migration of neural cells from the ventricular zone through the neocortex. The extracellular factor Reelin provides one cue that tells cells when to stop migrating, but the all-important trigger of when to start migrating has remained a mystery. Sato and colleagues report in *Nature Cell Biology* that a new molecule, FILIP, has a tight rein on neural cells in the ventricular zone, and prevents their migration by paralysing their motile machinery — the actin cytoskeleton.

The approach that was taken by Sato and colleagues to identify factors that mediate the 'start migrating' signal was to look at which genes are expressed differentially before and after neural migration. Of the genes pulled out, one of them showed an intriguing expression pattern that was restricted to the ventricular zone. This new gene — called *FILIP* — encodes two proteins, the shorter of which colocalizes with filamentous actin. This led the authors to ask whether FILIP might interact with F-actin. To address this, they conducted a two-hybrid screen and, reassuringly, pulled out the actin-binding factor Filamin A, which is known to be important for mediating cell motility. Consistent with this, both isoforms of FILIP colocalized with Filamin A *in vivo*.

Can FILIP affect the function of Filamin A? To get a handle on this, Sato and colleagues looked at how FILIP affects cell motility. Cultured cells that expressed *filip–gfp* showed reduced motility compared with control cells, which led the authors to reason that FILIP might inhibit Filamin A and thereby inhibit cell migration. In support of this, they showed that transfection of COS-7 cells with FILIP-GFP leads to degradation of filamin A (see figure), a

DNA REPLICATION

A time for silence

The timing of DNA replication is thought to be linked to transcriptional activity — actively transcribed genes are replicated early, whereas transcriptionally silent genes replicate later. Nuclear localization has also been implicated in timing, with late-replicating genes clustering near the nuclear periphery. But what determines which genes replicate when? Although the exact mechanisms are not known, a paper in *Current Biology* now shows a clear link between replication timing and a group of proteins that are involved in transcriptional silencing.

Janet Leatherwood, Rolf Sternglanz and colleagues first tested whether a transcriptional silencer can regulate the timing of replication initiation in budding yeast. To do this, they compared the replication of a yeast replication origin, *ARS305*, with that of the same sequence, but which also contained two copies of a transcriptional silencer called *HMR-E* that were integrated 225 bp downstream (*ARS305*:(*HMR-E*)$_2$). As expected, *ARS305* replication intermediates appeared 25 minutes after cells were released from arrest in G1. By contrast, no intermediates were detected from *ARS305*:(*HMR-E*)$_2$. Using a yeast mutant strain that lacks the intra-S-phase checkpoint — which allows late-firing origins to be detected — the authors showed that *ARS305*:(*HMR-E*)$_2$ can initiate replication, but that it fires about 30 minutes later than *ARS305*.

The *HMR-E* silencing system works by recruiting a complex of so-called Sir proteins through binding sites for the origin-recognition complex (ORC), Rap1 and Abf1. So, the authors next asked whether the effect of HMR-E on replication depends on the Sir complex. Mutation of *SIR4, SIR1* or the ORC-binding sites in (*HMR-E*)$_2$ led to the early replication of *ARS305*:(*HMR-E*)$_2$ — indeed, this sequence was replicated at the same time as *ARS305*.

It seems, then, that the Sir proteins are essential for resetting replication from early to late. But is simple targeting of a Sir protein to an early origin enough to make it fire late? To test this, the authors used a Gal4(1–147)–Sir4 hybrid protein (G_{BD}–Sir4), which can silence transcription around GAL4-binding sites. They then inserted five such sites next to *ARS305*, to create *ARS305*:(*G*)$_5$. As expected, G_{BD}–Sir4 blocked the formation of replication intermediates at *ARS305*:(*G*)$_5$. The authors then showed that G_{BD}–Sir4 did not block replication initiation — rather, it reset the origin to fire late.

Leatherwood, Sternglanz and colleagues conclude that Sir proteins are a cause of late replication. But is the structure of the chromatin a more important determinant of replication timing than subnuclear localization? Yes it is, say the authors, as simply tethering *ARS305*:(*G*)$_5$ to the nuclear periphery was not enough to confer late replication.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Zappulla, D. C., Sternglanz, R. & Leatherwood, J. Control of replication timing by a transcriptional silencer. *Curr. Biol.* **12**, 869–875 (2002)

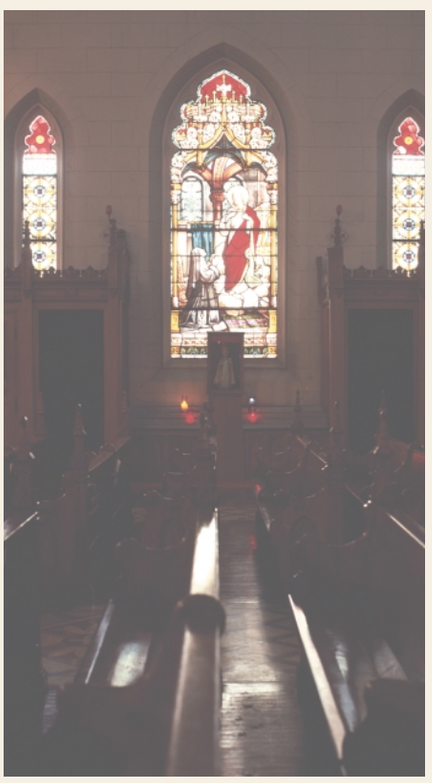
process that occurs through a calpain-dependent mechanism.

So, does FILIP regulate migration *in vivo* in the developing neocortex? Sato and colleagues tested this by introducing FILIP complementary DNAs into the rat neocortex. Consistent with the results from COS-7 cells, they found that the cells that were transfected with *filip* had altered morphology and impaired migration compared with control cells. Finally, they showed that FILIP and Filamin A interact at the right time and place *in vivo*, leading to a model in which FILIP degrades Filamin A in the ventricular zone, and prevents premature cell migration. The question now is what the developmental trigger is that mediates FILIP degradation and thereby frees Filamin A from its tethers to allow cell motility. Given the importance of this timing, it seems imperative that this is tightly regulated.

Alison Schuldt, Associate Editor,
Nature Cell Biology

References and links

ORIGINAL RESEARCH PAPER Nagano, T. *et al.* Filamin A interacting protein FILIP regulates cortical cell migration out of the ventricular zone. *Nature Cell Biol.* **4**, 495–501 (2002)



UBIQUITYLATION

Dual control

Mitogen-activated protein kinases (MAPKs) transduce signals from the cell surface to the nucleus. When triggered by a mitogenic stimulus, the extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) MAPKs are phosphorylated, and hence activated, by MAPK/ERK kinase (MEK). This activated state is only transient, as ERK1/2 can be dephosphorylated by MAPK phosphatases (MKPs). However, it now seems that the control that is exerted by MEK/MKP isn't enough for ERK1/2. In *Molecular Cell*, Hunter and colleagues report that ERK1/2 protein levels can also be controlled by MEK kinase 1 (MEKK1)-mediated ubiquitylation.

The authors first investigated the effect of serum, epidermal growth factor (EGF) and sorbitol on ERK1/2 protein levels. In cells that were treated with EGF or serum, they observed a rapid and transient rise in activated ERK1/2, without a change in ERK1/2 protein levels. Using sorbitol (which produces a hyperosmotic condition), however, they found that active ERK1/2 persists for longer and that prolonged treatment results in decreased ERK1/2 protein levels. They showed — using a specific proteasome inhibitor — that the ubiquitin/proteasome pathway mediates this ERK1/2 degradation.

As neither serum nor EGF affected ERK1/2 degradation, this degradation is unlikely to be mediated by upstream components of these signalling pathways. Hunter and co-workers therefore focused on MEKK1, which has previously been implicated in ERK1/2 activation. They found that treatment of cells with sorbitol — but not serum or EGF — increased MEKK1 activity, and they showed a direct association between ERK and MEKK1.

Certain types of E3 ubiquitin ligases — which mediate the transfer of ubiquitin from E2 ubiquitin-conjugating enzymes to substrates — contain a RING-finger domain. MEKK1 contains a plant homeodomain (PHD), which has a RING finger-like domain sequence and structure, so the authors investigated whether the MEKK1 PHD has E3-ligase activity towards ERK1/2. They showed that the MEKK1 PHD can ubiquitylate both active and inactive ERK1/2 *in vitro* and *in vivo*, and they observed that both the kinase and E3-ligase activity of MEKK1 are involved in the regulation of ERK1/2 ubiquitylation *in vivo*.

Two residues in the docking motif of rat ERK2 are important for its interactions with other proteins, and Hunter and colleagues found that mutating these residues disrupted the ERK2–MEKK1 interaction and reduced ERK2 ubiquitylation, without affecting ERK2 activation (perhaps because only transient interactions are required for ERK2 activation). They also showed that the docking-site-mutant ERK2 is resistant to sorbitol-induced degradation.

Prolonged stress stimuli (for example, sorbitol treatment) can induce cells to undergo apoptosis. As active ERK1/2 functions as an anti-apoptotic signal, the authors tested whether MEKK-1-mediated degradation of ERK1/2 is needed for sorbitol-induced apoptosis. They found that cells transfected with docking-site-mutant ERK2 were mostly resistant to sorbitol-induced apoptosis — ERK1/2 was not ubiquitylated/degraded and could therefore carry out its anti-apoptotic function — whereas cells that were transfected with wild-type ERK2 were only partially resistant. They also showed that cells that were transfected with an inactive docking-site-mutant ERK2 were susceptible to sorbitol-induced apoptosis, because, although MEKK1 cannot induce the degradation of this ERK2 mutant, it is inactive and therefore cannot carry out its anti-apoptotic function.

MEKK1 therefore functions not only as an upstream activator of ERK1/2 through its kinase domain, but also as negative regulator of ERK1/2 through the E3-ligase activity of its PHD. Hunter and co-workers have found a new enzymatic function for MEKK-1, and have shown that there is dual control, at the least, for the downregulation of ERK1/2 activity.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Lu, Z. *et al.* The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2. *Mol. Cell* **9**, 945–956 (2002)

FURTHER READING Weissman, A. M. Ubiquitin and proteasomes: themes and variations on ubiquitylation. *Nature Rev. Mol. Cell Biol.* **2**, 169–178 (2001)

WEB SITES

Tony Hunter's laboratory:
http://www-biology.ucsd.edu/faculty/hunter.html

Encyclopedia of Life Sciences: http://www.els.net
Ubiquitin pathway