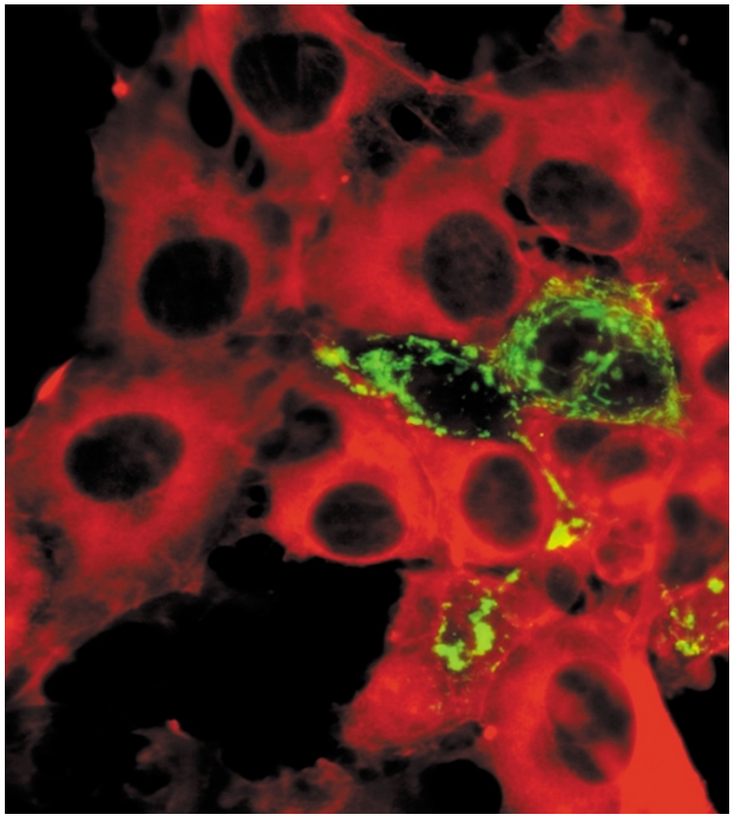

In COS-7 cells that express *FILIP–GFP* (green), the levels of endogenous Filamin A (red) is markedly reduced compared with neighbouring cells.

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.

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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)