Figure 1. A mechanism to explain the programme of origin firing in S phase.

Limiting firing factors (blue and purple) are first recruited to high affinity sites on a subset of licensed origins, promoting their activation at the beginning of S phase. After these origins have fired and are unlicensed, firing factors are released to bind the next highest affinity sites on other licensed origins, which in turn fire, releasing factors to bind even lower affinity sites on other licensed origins, and so on. Recycling of factors from one origin to the next in this way would proceed until the genome has been completely duplicated.

Previous work in fission yeast indicated that the recruitment of limiting initiation factors to origins is a point of control for replication initiation [19,20]. Tanaka et al. [3] and Mantiero et al. [4] have now extended this by showing that the limiting nature of initiation factors is responsible for the temporal programme of origin firing during S phase.

References

1. Zegerman, P., and Diffley, J.F.X. (2010). Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation. Nature 467, 474–478.
2. Lopez-Mosqueda, J., Maas, N.L., Jonsson, Z.O., Defazio-Eli, L.G., Wohlschlegel, J., and Toczyski, D.P. (2010). Damage-induced phosphorylation of Sld3 is important to block late origin firing. Nature 467, 479–483.
3. Tanaka, S., Nakato, R., Katou, Y., Shirahige, K., and Araki, H. (2011). Origin association of Sld3, Sld7, and Cdc45 proteins is a key step for determination of origin-firing timing. Curr. Biol. 21, 2055–2063.
4. Mantiero, D., Mackenzie, A., Donaldson, A., and Zegerman, P. (2011). Limiting replication initiation factors execute the temporal programme of origin firing in budding yeast. EMBO J. 30, 4805–4814.
5. Kamimura, Y., Tak, Y.S., Sugino, A., and Araki, H. (2001). Sld3, which interacts with Cdc45 (Sld4), functions for chromosomal DNA replication in Saccharomyces cerevisiae. EMBO J. 20, 2097–2107.
6. Aparicio, O.M., Stout, A.M., and Bell, S.P. (1999). Differential assembly of Cdc45p and DNA polymerases at early and late origins of DNA replication. Proc. Natl. Acad. Sci. USA 96, 9130–9135.
7. Moyer, S.E., Lewis, P.W., and Botchan, M.R. (2006). Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. Proc. Natl. Acad. Sci. USA 103, 10236–10241.
8. Ferguson, B.M., and Fangman, W.L. (1992). A position effect on the time of replication origin activation in yeast. Cell 68, 333–339.
9. Vogelauer, M., Rubbi, L., Lucas, I., Brewer, B.J., and Grunstein, M. (2002). Histone acetylation regulates the time of replication origin firing. Mol. Cell 10, 1223–1233.
10. Hayashi, M.T., Takahashi, T.S., Nakagawa, T., Nakayama, J.-i., and Masukata, H. (2009). The heterochromatin protein Swi6/HP1 activates replication origins at the pericentromeric region and silent mating-type locus. Nat. Cell Biol. 11, 357–362.
11. Donovan, S., Harwood, J., Drury, L.S., and Diffley, J.F. (1997). Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. Proc. Natl. Acad. Sci. USA 94, 5611–5616.
12. Blow, J.J., and Dutta, A. (2005). Preventing re-replication of chromosomal DNA. Nat. Rev. Mol. Cell Biol. 6, 476–486.
13. Bruck, I., and Kaplan, D.L. (2011). Origin single-stranded DNA releases Sld3 protein from the Mcm2-7 complex, allowing the GINS tetramer to bind the Mcm2-7 complex. J. Biol. Chem. 286, 18602–18613.
14. Bruck, I., Kanter, D.M., and Kaplan, D.L. (2011). Enabling association of the GINS tetramer with the Mcm2-7 complex by phosphorylated Sld2 protein and single-stranded origin DNA. J. Biol. Chem. 286, 36414–36426.
15. Ilves, I., Petojevic, T., Pesavento, J.J., and Botchan, M.R. (2010). Activation of the MCM2-7 helicase by association with Cdc45 and GINS proteins. Mol. Cell 37, 247–258.
16. Varrin, A.E., Prasad, A.A., Scholz, R.-P., Ramer, M.D., and Duncker, B.P. (2005). A mutation in Dbf4 motif M impairs interactions with DNA replication factors and confers increased resistance to genotoxic agents. Mol. Cell Biol. 25, 7494–7504.
17. Raghuraman, M.K., Brewer, B.J., and Fangman, W.L. (1997). Cell cycle-dependent establishment of a late replication program. Science 276, 806–809.
18. Dimitrova, D.S., and Gilbert, D.M. (1999). The spatial position and replication timing of chromosomal domains are both established in early G1 phase. Mol. Cell 4, 983–993.
19. Patel, P.K., Kommajosyula, N., Rosebrock, A., Bensimon, A., Leatherwood, J., Bechhoefer, J., and Rhind, N. (2008). The Hsk1(Cdc7) replication kinase regulates origin efficiency. Mol. Biol. Cell 19, 5550–5558.
20. Wu, P.-Y.J., and Nurse, P. (2009). Establishing the program of origin firing during S phase in fission yeast. Cell 136, 852–864.

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Fungal Biogeochemistry: A Central Role in the Environmental Fate of Lead

Fungi play major roles in biogeochemistry and are responsible for many metal transformations during mineral weathering. A recent finding that fungi transform lead to chloropyromorphite highlights the importance of fungi in biogeochemical processes.

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Microbes have played key roles in linking the biosphere with the geosphere, profoundly shaping the global system over the course of geological time [1]. Geomicrobiological processes have contributed greatly to the development of the current earth system through the creation of an oxygen-rich atmosphere, conversion of mineral nitrogen into biological molecules such as proteins, and the continuing biogeochemical cycling of elements essential to life, including carbon, nitrogen, phosphorous and sulphur [2]. The contribution of fungi to biogeochemical processes has until recently been seen largely through their

involvement in the carbon and nitrogen cycles in terrestrial environments, but progressively their presence and involvement in other aspects of biogeochemistry, particularly metal-related processes, is being revealed [3]. In this issue of *Current Biology*, a paper by Rhee, Hillier and Gadd [4] presents significant new information about the role of fungi in the biogeochemistry of lead through their involvement in the mycogenic transformation of lead into the phosphate-containing mineral chloropyromorphite.

Fungi are one of the most diverse organismal groups, with estimates of around 1.5 million species globally, and are ubiquitous components of terrestrial ecosystems [5]. Fungi are also present in a whole range of extreme environments, including Arctic and Antarctic soils, hot and cold deserts, hypersaline soils and lakes, rock and mineral surfaces, subaerial mineral environments and caves [6–10]. To live in such environments, fungi have developed protective adaptations to survive in response to extreme environmental conditions, including extreme temperatures, irradiation, pH, salinity, high heavy metal content and osmotic stress. In these extremophilic environments, mycologists are starting to elucidate the interactions between fungal diversity and their mineral substrata, and to appreciate the roles that fungi have in the development of mineral systems.

Rocks generally comprise several minerals and their surfaces invariably house bacteria, fungi or algae. Mineral chemistry can greatly influence microbial colonization of surfaces through effects on energy generation, nutrient acquisition, adhesion, and biofilm formation [7,11]. Microbes partially derive their energy from nutrients released by mineral surfaces, which tend to be nutritionally oligotrophic, whilst mineral microtopography may also influence microbial adhesion. As minerals weather, either via physico-chemical or biological processes, the chemistry of the derived weathering products influences the composition of bacterial or fungal species present [7]. Indeed, some weathering products such as heavy metals may be inhibitory to microbial growth and community development [7,8]. Microbes, in turn, also have profound effects on the surfaces on which they live, including

mineral surfaces. Consequently, fungi can be substantial drivers of weathering processes affecting rock/mineral surfaces, and have been found living on a very wide range of rock/mineral types [8]. Fungi can act both biomechanically and biochemically to alter rock and mineral surfaces, and can directly penetrate rocks and minerals through hyphal growth, tunneling into intact material by exploiting boundaries between crystal planes or through cracks or cleavages [12]. Fungal activity can also indirectly lead to mechanical disruption of these surfaces through production of extracellular mucilaginous substances that shrink and/or swell, applying physical pressure to disrupt mineral structure [13].

Chemical weathering of minerals by fungi is linked to fungal metabolism at a micro-environmental scale, leading to pitting or etching of mineral microtopography, displacement of minerals, or chemical dissolution [14]. The main mechanism of fungal weathering is thought to be acid production through proton efflux by ATPases, production of organic acids such as oxalic and citric acids that are not only acidic but also able to complex metals into soluble ligands, and CO_2 formation leading to carbonic acid production [8]. Fungi can also alter surface redox conditions and can act as nutrient sinks for anions and cations dissolved from mineral surfaces [8].

A consequence of weathering reactions is mycogenic mineral formation — secondary minerals formed as a result of fungal weathering reactions [15], either as precipitation of organic or inorganic secondary minerals, or the formation of crystalline material associated with fungal cell walls. Many of these secondary minerals are metal oxalates or carbonates. This is an area that has been extensively explored by Geoff Gadd and co-workers over the last few years, who report in this issue of *Current Biology* the significant observation that common soil fungi are able to convert metallic lead to the secondary lead-containing mineral chloropyromorphite [4]. This group incubated lead shot in the presence of two common soil fungi and used energy-dispersive X-ray analysis and X-ray powder diffraction analysis to reveal that chloropyromorphite (Pb5(PO4)3Cl) was the dominant lead mineral formed in the presence of fungi

on lead shot surfaces, but not in abiotic controls where other more biologically available lead minerals (minium (Pb3O4), hydrocerussite (Pb3(CO3)2(OH)2) and litharge (PbO)) were formed. Gadd and co-workers speculate that chloropyromorphite is not produced directly from lead metal by fungi, rather it is produced from other lead mineral precursors produced either by the fungi themselves or abiotically, and an elegant model for chloropyromorphite formation is presented. The authors also discuss the role of phosphate in this process and the possible remobilization of chloropyromorphite by fungal organic acid excretion [4]. Phosphate availability, together with pH, appears to be important in defining the fate of metal minerals in combination with interaction with microbial activity, and fungi are now known to mediate formation of phosphate minerals of a number of metals, including iron, aluminium and uranium. The significance of this new work is that, for the first time, fungi have been shown to be involved in the formation of pyromorphite, as well as in its dissolution.

This work is important because lead is a widespread environmental contaminant from industrial, extractive, sporting and natural sources and we know little about the biogeochemical processes determining the diversity of lead minerals in the natural environment. Lead is highly toxic to most life forms and is particularly problematic in ground or surface waters, especially at low pH or if the water is used as drinking water [16]. Soluble lead can alter ecosystem structures at all levels. Pyromorphite is the most stable lead mineral, and phosphate additions have been proposed as a route to aiding the stabilization of lead contaminated sites by production of pyromorphite. It appears from this new work that understanding fungal activity may be central to generating a complete view of the biogeochemistry of lead and for developing processes to reduce the biological availability, and hence the toxicity, of lead as a pollutant [17].

References
1. Ehrlich, H.L. (1998). Geomicrobiology: its significance for geology. Earth Sci. *45*, 45–60.
2. Gorbushina, A.A., and Broughton, W.J. (2009). Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. Annu. Rev. Microbiol. *63*, 431–450.

3. Sterflinger, K. (2000). Fungi as geologic agents. Geomicrobiol. J. *17*, 97–124.
4. Rhee, Y.J., Hillier, S., and Gadd, G.M. (2012). Lead transformation to pyromorphite by fungi. Curr. Biol. *22*, 237–241.
5. Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. *105*, 1422–1432.
6. Kis-Papo, T., Grishkan, I., Oren, A., Wasser, S.P., and Nevo, E. (2001). Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. Mycol. Res. *105*, 749–756.
7. Gleeson, D.B., Clipson, N., Melville, K., Gadd, G.M., and McDermott, F. (2005). Characterization of fungal community structure on a weathered pegmatitic granite. Microbial Ecol. *50*, 360–368.
8. Gadd, G.M. (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol. Res. *111*, 3–49.
9. Ruisi, S., Barreca, D., Selbmann, L., Zucconi, L., and Onofri, S. (2007). Fungi in Antarctica. Rev. Environ. Sci. Biotechnol. *6*, 127–141.
10. Rooney, D., Hutchens, E., Clipson, N., Baldini, J., and McDermott, F. (2010). Microbial community diversity of moonmilk deposits at Ballynamintra cave, Co. Waterford, Ireland. Microb. Ecol. *60*, 753–761.
11. Burford, E.P., Fomina, M., and Gadd, G.M. (2003). Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineralogical Mag. *67*, 1127–1155.
12. van Schöll, L., Kuyper, T.W., Smits, M.M., Landeweert, R., Hoffland, E., and van Breemen, N. (2008). Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. Plant. Soil *303*, 35–47.
13. Burford, E.P., Kierans, M., and Gadd, G.M. (2003). Geomycology: fungi in mineral substrata. Mycologist *17*, 98–107.
14. Smits, M.M., Herrmann, A.M., Duane, M., Duckworth, O.W., Bonneville, S., Benning, L.G., and Lundstrom, U. (2009). The fungal–mineral interface: challenges and considerations of micro-analytical developments. Fung. Biol. Rev. *23*, 122–131.
15. Fomina, M., Burford, E.P., Hillier, S., Kierans, M., and Gadd, G.M. (2010). Rock-Building Fungi. Geomicrobiol. J. *27*, 624–629.
16. Papanikolaou, N.C., Hatzidaki, E.G., Belivanis, S., Tzanakakis, G.N., and Tsatsakis, M. (2005). Lead toxicity update. A brief review. Med. Sci. Mon. *11*, 329–336.
17. Gadd, G.M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology *156*, 609–643.

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Social Neuroscience: More Friends, More Problems…More Gray Matter?

The social brain hypothesis generically posits that increasing social group size relates is associated with an increase in neocortex size. A new study identifies, within a species, the specific neural circuit that may confer the primate ability to manage social relationships as they increase in number.

Maia ten Brink and Asif A. Ghazanfar

The oil industrialist, John D. Rockefeller, once said that "The ability to deal with people is as purchasable a commodity as sugar or coffee and I will pay more for that ability than for any other under the sun". It is unclear how many people Rockefeller actually bought in his lifetime, but at the very least he recognizes that the ability to interact effectively with them would be more valuable than anything else in the world. Who could argue with that? Consider the many different relationships in your life — your parents, siblings, extended family, friends, colleagues, competitors, and so on. As for all primates, our lives are an intricate web of relationships; every relationship is unique and it seems for each we are performing an energetically costly balancing act. Naturally, adding more individuals to one's web increases the effort required to maintain all. How do we manage this? There is, of course, the banal suggestion that it is our bigger brains that confer this ability. While there is a

positive correlation between neocortex size and social group size across primate species [1], this is a rather coarse measure and not particularly illuminating — there are a number of other behavioral variables that also correlate with the size of the neocortex. A recent study by Sallet and colleagues [2] gives us greater insights into what *specific* neural circuits may mediate the cognitive balancing act required for increasing the size of one's social group.

Using magnetic resonance imaging (MRI) scans, Sallet *et al.* [2] investigated whether group size is related to brain differences in rhesus monkeys (*Macaca mulatta*). According to veterinary considerations, 23 monkeys were assigned to groups ranging in size from one to seven individuals. The authors capitalized on this restructuring of their institution's monkey colony by scanning each monkey's brain 15 months after the group assignments and then asking: is there a correlation between an individual's social group size and the neocortex? They compared differences

in an individual brain's gray matter density relative to a group-averaged brain by measuring where, and how much, the imaged brain needed to be expanded or compressed in order to match the average brain. The 'determinant' of the resulting matrix is a scalar value that can be used as a dependent variable and regions which showed a >5 mm³ difference from the average brain were identified as significant. Remarkably, the authors found that *specific* regions of the neocortex varied in the gray matter density according to social group size. The regions with increased gray matter are known to be important for processing social signals (facial expressions, eye gaze, vocalizations) in monkeys [2]: the superior temporal sulcus, the superior and inferior temporal gyri, the amygdala and the rostral prefrontal cortex (and we know that most are directly connected with each other [3]). For every additional member of a social group, density increased by about 5%.

This pattern of results is consistent with three recent human studies relating social network size (as measured by Facebook or questionnaires) to the superior temporal sulcus [4], the amygdala [5] and the prefrontal cortex [6]. Nevertheless, to lend credence to the idea that these are indeed regions involved in the task of balancing social