
**100 YEARS AGO**

**Snake-stones are fairly common in South Africa, and are described as white, porous stones, which, when applied to the place where the snake has bitten a person, adhere till all the poison is drawn out into them, after which they are placed in milk, which in turn draws the poison from the stones, and renders them again fit for use. The farmers firmly believe they are taken from the head of a snake. It is suggested that snake-stones are made of pumice. To the uneducated, the structure of pumice has a close resemblance to that of bone, and this may possibly explain the popular delusion that snake-stones are made of bone… The fact that the fable of the stone having been taken from the head of a snake is exactly the same in the Malay States as is prevalent in South Africa is interesting, though the Malay slaves which the early Dutch obtained from Batavia in exchange for quaggas, zebras, ivory, &c., may have carried the legend with them. It is not an uncommon custom in Germany for people to carry about with them nuggets of raw gold to draw out of their bodies all the more subtle evils, such as those produced by spirits and devils, while for the grosser evils they carry a potato. Is the snake-stone legend a derivative of these, or are they subsequent to the snake-stone?**

From *Nature* 26 July 1900.

**50 YEARS AGO**

**The mango, a favourite tropical fruit, which is widely cultivated in India, belongs to the Malaysian genus *Mangifera* Linn. (fam. Anacardiaceae). Taxonomic study shows that it contains forty-one valid species, three of which, *M. indica* L. (wild and cultivated), *M. sylvatica* Roxb. (wild in the hilly forests of north-east India), and *M. khasiana* Pierre (a species of doubtful occurrence) have been reported from India. About a thousand cultivated varieties of mango occur in India, all of which are included in the single species, *M indica* L. They differ from one another mainly in fruit characters, on the basis of which they have been classified into three groups: round-, ovate-oblong, and long-fruited… The three species investigated — *M. indica* L. (including twenty-three grafted varieties, and one wild race), *M. sylvatica* Roxb. and *M. caloneura* Kz. (a Burmese wild species) — show striking stability in their chromosome numbers, all having $n = 20$ and $2n = 40$ chromosomes.**

From *Nature* 29 July 1950.

than that for the exponential network, in which all nodes are statistically identical. To put their theory to test, Albert and co-workers analyse two of the most popular networks: the Internet and the World-Wide Web. Although many people use these terms interchangeably, for the purposes of this study they are considered as separate entities. The World-Wide Web is defined as the network of web pages (the nodes) joined together by hyperlinks, whereas the Internet is the physical communication network linked together by routers (Fig. 1). Albert and co-workers discover that the error tolerance of these two networks has exactly the same characteristics as that of the scale-free network. This result, together with earlier studies[3–5] revealing the power-law structures of these networks, confirms the connection between network structure and performance. More importantly, it provides valuable insight into the structure and weakness of these essential networks.

What can we learn from this study? The good news is that we do not have to worry about random fluctuations of these networks. The bad news is that Internet terrorists could cause great damage by targeting the most connected routers or web sites. The average performance of the Internet is reduced by a factor of two if just 1% of the most connected nodes are destroyed; and with only 4% of its most important nodes destroyed, the Internet loses its integrity, becoming fragmented into small disconnected domains. Of course, modelling the real Internet means taking into account such details as the bandwidth of different links, the varying error susceptibility of different nodes and, most importantly, different communication protocols. It remains to be seen how these detailed considerations will affect the conclusions of the current study. Nonetheless, this work represents a notable first step towards understanding the robustness of the Internet.

More generally, the work of Albert and co-workers provides a useful framework for qualitatively describing and analysing network performance. It will be interesting to see what we could learn about other complex network systems, such as neural networks or gene regulatory networks[6], by using similar analysis. In these biological systems, error tolerance is not just a passive property of the network structure; rather, it is part of the driving force by which evolution selects the network structure. Perhaps if we could understand why certain network topology is preferred and selected by nature, such knowledge could ultimately help us design more robust artificial networks. ■

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Cell cycle

# Conducting the mitotic symphony

David Cortez and Stephen J. Elledge

The process by which chromosomes condense and segregate during mitosis (nuclear division) can be likened to a symphony in which many instruments, working individually, are coordinated to produce a collective piece of elegance and beauty. The conductor, with a wave of the baton, ensures that each musical instrument enters the symphony at the proper time. The conductors of the mitotic symphony, called 'checkpoints', do much the same thing, and prevent errors in chromosome segregation that can lead to diseases such as Down's syndrome and cancer. On page 430 of this issue[1], Scolnick and Halazonetis identify a new conductor that monitors early movements in the mitotic symphony, and describe a possible link between cancer and a failure of this conductor to do its job.

Before mitosis in an animal cell can proceed, the chromosomes must duplicate and the cell must build a 'spindle' — a machine, based on cytoskeletal filaments called microtubules, that will transport the chromosomes into the two daughter cells (Fig. 1). The spindle forms outside the nucleus, between two microtubule-organizing centres (centrosomes). This dual origin of the spindle means that it is bipolar. As the spindle forms, the chromosomes within the nucleus begin to condense into a more compact form that allows them to move more easily; this phase of mitosis is called prophase. The nuclear 'envelope' then breaks down during prometaphase, allowing each pair of duplicated chromosomes to attach to the spindle. The chromosomes align in a plane between the spindle poles during metaphase, and await a signal that abruptly separates the

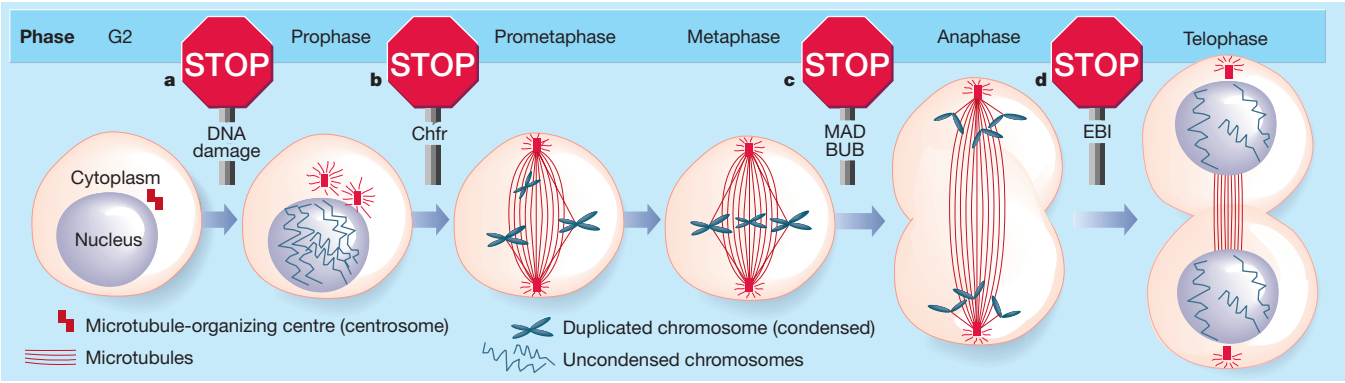


Figure 1 **The conductors of the mitotic symphony. The phases of nuclear division (mitosis) in animal cells are shown. Mitosis is regulated by at least four conductors, called checkpoints.** a, **The DNA-damage checkpoint delays entry into mitosis until any damaged DNA is repaired.** b, **Scolnick and Halazonetis[1] have shown that, in response to microtubule poisons, a checkpoint dependent on the Chfr protein delays chromosome condensation during prophase.** c, **The MAD/BUB-dependent checkpoint prevents chromosome separation and entry into anaphase until the chromosomes are attached correctly to the microtubule-based chromosome-segregation machinery (the spindle).** d, **At least in yeast, EB1 participates in a checkpoint that delays the final production of two daughter cells in response to an incorrectly orientated spindle.**

chromosomes of each pair, marking the start of anaphase.

We already knew about the existence of two conductors of this mitotic symphony (Fig. 1). One, the spindle-assembly checkpoint, ensures that each pair of chromosomes is correctly attached to a bipolar spindle before anaphase[2]. A second conductor in yeast depends on the *BIM1*/*EB1* gene[3]; this checkpoint delays the exit from mitosis when the spindle is orientated abnormally.

Scolnick and Halazonetis[1] have now defined a third mitotic conductor, which coordinates an earlier part in the process. This conductor delays chromosome condensation in response to mitotic stress induced by microtubule poisons. The existence of this checkpoint was suggested by earlier experiments[4] in which a delay in chromosome condensation in response to a microtubule poison was seen in normal human cell lines but not in several tumour cell lines. Scolnick and Halazonetis have now identified a gene — *chfr* — that seems to be required for delaying prophase in human cells. The sequence of the *chfr* gene is similar to that of the fission yeast *DMA1* gene, which is involved in a later mitotic checkpoint that delays a cell's exit from mitosis in response to spindle damage[5].

Scolnick and Halazonetis also studied eight different human tumour cell lines, and found that the Chfr checkpoint did not function in four of them. Three of these cell lines do not express the *chfr* gene; in the fourth cell line, this gene contains a missense mutation, which results in the production of a defective copy of the Chfr protein. When the authors transfected the cell lines with normal or mutant *chfr*, they found that the wild-type — but not the mutant — gene restored the checkpoint, showing that *chfr* is essential for this checkpoint.

It is not yet known which microtubule-dependent process is coordinated by the Chfr checkpoint, but during prophase an array of microtubules extends from the centrosomes, which then migrate to opposite poles of the cell (Fig. 1). Chfr may coordinate these events with chromosome condensation, although it is unclear why this should be necessary. Perhaps it is actually the breakdown of the nuclear envelope that needs to be coordinated with centrosome separation: if the envelope is dissolved before the centrosomes migrate, the chromosomes might associate prematurely with the centrosomes, interfering with subsequent steps in mitosis.

How does this new conductor delay entrance into mitosis? Entry into mitosis requires the activation of a complex containing a protein kinase (called cdc2) and its regulatory subunit (cyclin B). Cyclin B/cdc2 activity remains high during the Chfr-dependent delay[1], so its activity is not the target of Chfr. Perhaps Chfr instead controls the localization of cyclin B to the nucleus, which is required for mitosis. In the fungus *Aspergillus nidulans*, the kinase NIMA appears to be required for mitotic entry after cdc2 is activated. NIMA is also needed for nuclear localization of *A. nidulans* cyclin B/cdc2 (ref. 6). So the human NIMA protein is one plausible target of the Chfr checkpoint.

Another possibility is that Chfr is involved in controlling the degradation of a protein that regulates chromosome condensation and nuclear-envelope breakdown. Chfr contains a domain that is found in several proteins involved in tagging other proteins with the peptide ubiquitin[7], a label that marks proteins to be degraded. Chfr also contains an FHA domain, which may bind phosphorylated peptides[8], so protein kinases might act upstream of Chfr.

What happens to the cell when this mitotic conductor does not do its job properly? Cells that lack *chfr* are more sensitive to microtubule poisons and exhibit abnormal nuclear morphology after treatment with them — an effect that might result from a failure of checkpoint function. In principle, the level of expression of *chfr* or its ability to function could be used as a diagnostic marker. Chemotherapeutic protocols that exploit this sensitivity to microtubule poisons could then be designed for use in cancer treatment. Indeed, several classes of approved anticancer drug work by disturbing microtubule function[9].

Half of the human tumour cell lines studied by Scolnick and Halazonetis did not express wild-type Chfr protein, so this checkpoint might be inactivated during the development of some tumours — a suggestion also made about other mitotic checkpoint proteins[10–13]. But it is not clear how inactivating Chfr might contribute to tumour formation. The Chfr-deficient cells retain the spindle-assembly checkpoint[1], and at least two of the cell lines used by Scolnick and Halazonetis exhibit low rates of chromosome loss[14]. (High rates of chromosome loss are characteristic of many tumours and may contribute to the inactivation of tumour-suppressor genes.) Although the absence of *chfr* expression in tumour lines might reflect the loss of the gene in those cells, it is also possible that *chfr* was never expressed in these types of tissue. So a firm connection between *chfr* and cancer remains to be established, but the discovery of this new mitotic conductor provides much needed insight into how the suite of mitotic checkpoints ensures the harmony of the mitotic minuet. ■

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Global change

# Deciphering methane's fingerprint

Helmut Weissert

Two hundred years ago Georges Cuvier came up with the concept of what we now call 'proxy indicators' in geology. These are biological, chemical or physical signatures preserved in the rock record that serve as fingerprints of the biosphere's evolution through time. Much of the detective work on Earth's history has depended on these fingerprints.

These days, increasingly sophisticated tracer techniques are used in combination, and their robustness is tested by simulations of past global change. This is the approach that Hesselbo *et al.*[1] have chosen in the study described on page 392 of this issue — a carbon-isotope analysis of fossil wood deposited in near-shore sediments 180 million years ago during the Toarcian Stage of the Jurassic (200–140 million years ago). The authors have identified an atmospheric carbon-isotope signal in their samples that records an anomaly in the atmospheric carbon system; moreover, it is coupled to a known perturbation of the marine carbon reservoir. Hesselbo *et al.* propose that a massive release of methane, stored in gas hydrates beneath the sea floor, caused the perturbation in the carbon-isotope record, and that the methane pulse triggered major changes in climate and the oceans.

These conclusions are based mainly on carbon-isotope geochemistry. The relative concentrations of the two stable carbon isotopes (carbon-12 and carbon-13) in organic matter and biologically generated carbonate are used as fingerprints that reflect events in the carbon system. Marine sediments deposited over the past 600 million years retain the carbon-isotope signature from the time of their deposition, and oscillations in the geological record show that marine carbon-isotope composition was not stable[2]. Differences in that composition can be related either to changes in the carbon isotopes entering the oceans, or to isotopic variations in total carbon sedimentation, which are caused mainly by varying rates of organic-carbon burial in sediments on the sea floor.

Organic carbon is enriched in the light isotope, carbon-12. So, at times of high rates of burial, carbon-13 became more abundant in the ocean water and is reflected as a positive carbon-isotope anomaly in the rock record left by marine sediments. Burial of excess organic carbon was initiated by a warm, greenhouse climate, and coincided with crises in biocalcification in reefs and in calcareous plankton[3], which were triggered by high levels of atmospheric $CO_2$ (ref. 4) and related changes in ocean chemistry and climate. Both processes contributed to a lowering of the amount of $CO_2$ in the atmosphere.

Negative carbon-isotope anomalies record episodes of oceanic carbon-12 enrichment in the past. Extraordinary carbon-12 enrichment of the marine carbon reservoir within only a few tens of thousands years coincided with known environmental catastrophes, as at the Cretaceous/Tertiary boundary[5], some 65 million years ago. A collapse of the marine ecosystem and the consequent interruption of the marine carbon flux into sediments may have caused the observed carbon-12 enrichment[5]. Other negative anomalies are evident at the base of positive carbon-isotope events[6], and have been considered as the fingerprint of rapid influx of volcanic $CO_2$ enriched in carbon-12. This volcanic $CO_2$ would have triggered the biotic changes recorded in the subsequent positive carbon-isotope anomalies.

A few years ago, however, Dickens *et al.*[7] proposed a new explanation for the negative carbon-isotope anomaly at the Palaeocene/Eocene boundary 55 million years ago. They calculated that only a sudden influx of methane derived from sources under the sea floor could account for the anomaly. Methane is enriched in the light isotope, carbon-12. It is stored in sedimentary gas hydrates beneath the oceans, and consists of solid crystals of water and methane that are stable under a wide range of temperatures and pressures.

Dickens *et al.* argued that deep-water warming or a rapid pressure decrease (or both) resulted in sudden release of methane (Fig. 1). The methane was oxidized to $CO_2$, which was enriched in methane-derived carbon-12. Higher $CO_2$ concentrations in oceans and the atmosphere were a consequence of this environmental catastrophe. Plants take up $CO_2$ in photosynthesis, so a pulse of methane into the atmosphere should be evident in the terrestrial carbon-isotope records of organic matter.

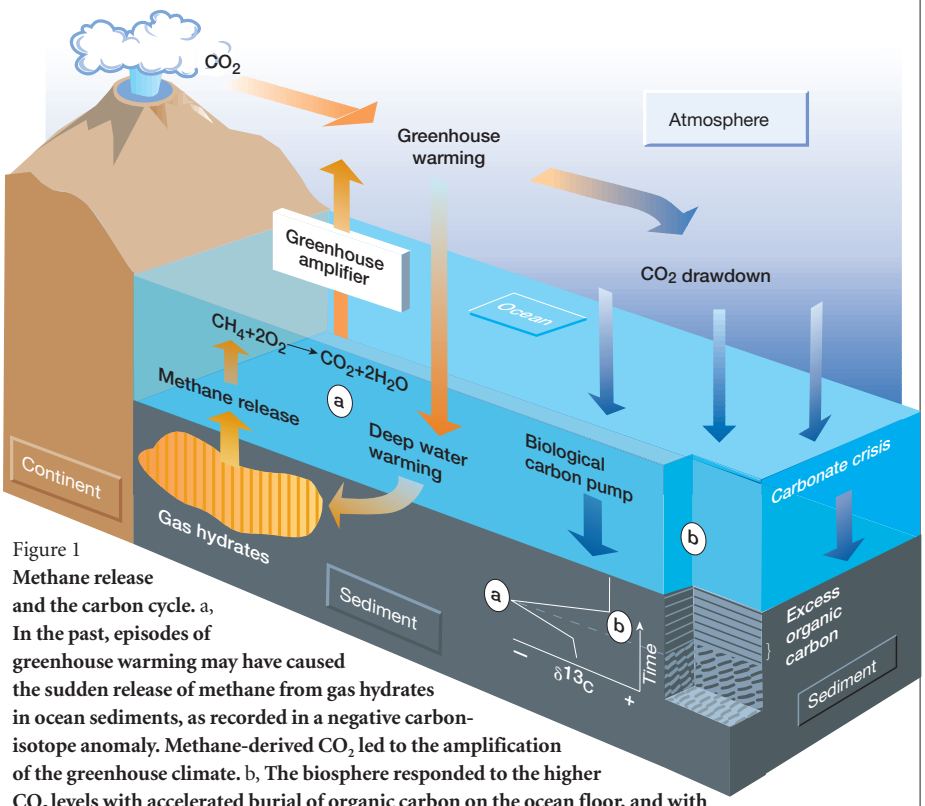


Figure 1 **Methane release and the carbon cycle.** a, **In the past, episodes of greenhouse warming may have caused the sudden release of methane from gas hydrates in ocean sediments, as recorded in a negative carbon-isotope anomaly. Methane-derived $CO_2$ led to the amplification of the greenhouse climate.** b, **The biosphere responded to the higher $CO_2$ levels with accelerated burial of organic carbon on the ocean floor, and with crises in carbonate production, as recorded in positive carbon-isotope anomalies. Both processes contributed to a lowering of atmospheric $CO_2$ levels. Hesselbo *et al.*[1] have identified a terrestrial isotopic signal that provides evidence of a massive release of methane about 180 million years ago.**