MOLECULAR BIOLOGY

Beginning at the End

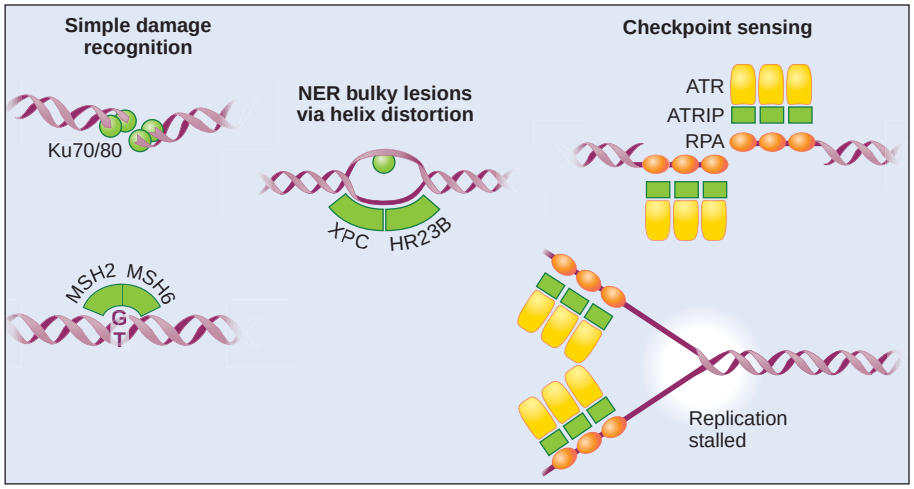
Antony M. Carr

The ability of cells to respond to DNA damage and to stalled DNA replication forks is essential for preventing genome instability, cancer, and cell death. DNA-repair pathways recognize and repair specific subsets of DNA lesions. They use defined damage-recognition proteins to sense specific lesions and subsequently to recruit the correct DNA-repair apparatus. The ATR (ATM- and Rad3-related)–dependent checkpoint pathway recognizes and signals the presence of multiple DNA damage events and stalled replication forks. The way in which the ATR-dependent checkpoint pathway detects multiple forms of DNA damage and replication problems is clarified in a paper by Zou and Elledge (*1*) on page 1542 of this issue.

Damage detection by DNA-repair pathways follows a simple pattern: Initially, one or more proteins recognize a defined DNA lesion on the basis of its topological structure. The proteins bind to the lesion, and this complex then recruits additional repair proteins to the damage site. For example, during mismatch repair or the repair of DNA double-strand breaks (DSBs) by nonhomologous end joining, the lesion is recognized by a respective protein sensor complex (Msh2-Msh6 for mismatches, Ku70/80 for DSBs). DNA repair is dependent on the subsequent association of specific repair proteins (Mlh1-Pms1 or Xrcc4-Lig4, respectively). A similar concept underlies damage recognition by more complex repair pathways such as nucleotide excision repair (NER). NER processes a range of bulky DNA adducts (damaged or cross-linked bases) but each adduct is not recognized directly by its precise geometrical signature. Instead, the recognition protein, XPC-HR23B, has an affinity for distorted helices (*2*). Because all bulky lesions distort the DNA helix, this allows recognition of a single topological signature to underpin the detection of multiple different DNA adducts.

Repair pathways recognize abnormal DNA structures through specific protein-DNA interactions, whereas the ATR pathway responds to a wide variety of damage and replication events. Do checkpoint proteins directly interact with a corresponding diversity of geometrical structures? Or, in the same way that NER recognizes multiple bulky lesions, does ATR-dependent damage recognition depend on a single topological structure? Lydall and Weinert (*3*) found that production of single-stranded DNA (ssDNA) at telomeres correlated with checkpoint signaling in yeast. They suggested that the ATR-dependent checkpoint recognizes ssDNA, a defined topological structure downstream of, and common to, most aspects of DNA repair and replication. Subsequent work supported this elegant model. Again in yeast, checkpoint signal intensity following an enzymatically induced DSB was shown to parallel the quantity of ssDNA generated by nuclease resection (*4*). Work in the frog *Xenopus* (*5*) also found that regions of ssDNA correlated with the presence of replication protein A (RPA) and ATR-dependent signaling.

RPA is an essential protein, required for DNA replication and repair. ssDNA is fragile and in vivo exists only in association with RPA. Intriguingly, cells expressing a specific mutant form of RPA exhibit attenuated checkpoint activation (*4*), suggesting that RPA participates directly in the ATR-dependent checkpoint pathway. Zou and Elledge (*1*) inhibited the expression of RPA in human and yeast cells. Partial RPA ablation correlated with reduced ATR-checkpoint signaling and with decreased binding of a checkpoint protein to damaged chromatin. Although these data underscore the requirement for RPA in correct checkpoint activation, the definitive data concerning RPA's involvement in checkpoint signaling come from a biochemical analysis that measures the binding of checkpoint proteins to RPA-coated ssDNA in vitro. ATR is always found in a complex with ATRIP (ATR-interacting protein; Mec1 and Ddc2 in yeast). In studies with purified human and yeast proteins, ATRIP and Ddc2 associate directly and specifically with RPA-ssDNA, but not with ssDNA alone, double-stranded DNA (dsDNA), or dsDNA ends. Furthermore, a fivefold reduction in Ddc2 binding is observed with RPA-ssDNA containing the mutant form of RPA. This finding provides a biochemical correlation with the previous genetic data (*4*). ATRIP association recruits ATR to RPA-ssDNA complexes. Interestingly, recruited ATR efficiently phosphorylates a subunit of a second checkpoint protein–sensing complex, Rad17, but only when Rad17 is itself independently recruited to RPA-ssDNA. Although stopping short of reconstituting a bona fide "checkosome" in vitro, these data provide a tantalizing glimpse into how the different checkpoint proteins, which are independently recruited to sites of DNA damage (*6*), may be assembled in a



ATRIP to remember. Recognition and repair of damaged DNA. (**Left**) In "simple" repair pathways, direct damage recognition depends on detection of specific damage structures. (**Middle**) NER proteins respond to multiple bulky DNA adducts by recognizing distortion of the DNA helix. (**Right**) The ATR-dependent checkpoint recognizes the RPA protein bound to ssDNA. RPA-ssDNA is exposed when damaged DNA is processed or the replication complex stalls.

CREDIT: KATHARINE SUTLIFF/*SCIENCE*

The author is at the Genome Damage and Stability Centre, University of Sussex, Falmer, Sussex BN1 9RQ, UK. E-mail: a.m.carr@sussex.ac.uk

coordinated fashion through ATR-dependent phosphorylation.

Almost all DNA-repair pathways process DNA damage through extensive RPA-ssDNA intermediates. Pathways such as base excision repair (BER) that do not generate significant RPA-ssDNA intermediates appear invisible to the checkpoint system (*7*). Stalled replication forks are known to expose extended regions of RPA-ssDNA (*8*). Thus, the ATR-dependent checkpoint can respond to multiple DNA damage and replication problems by recognizing RPA-ssDNA, a common topological intermediate. Importantly, this biochemical understanding of damage detection will help lay to rest a myriad of misunderstood observations linking specific DNA-processing enzymes (such as helicases, nucleases, repair and replication proteins) to checkpoint signaling. Any mutation influencing DNA metabolism can potentially influence the extent of RPA-ssDNA generation. This will have a corresponding, but indirect, impact on the ATR-dependent checkpoint pathway.

Is RPA-ssDNA the sole activator of the ATR-dependent checkpoint? Certainly, reports that Ddc2 (ATRIP) directly binds to DSBs (*9*) are not supported by Zou and Elledge's analysis. DSBs are the most dangerous initial lesion to a cell, and it is intriguing that the parallel ATM-dependent checkpoint pathway responds specifically to DSBs. ATM-dependent signaling requires the recombination repair protein complex Mre11-Rad50-Xrs2 (MRX), and both ATM and MRX associate with DSB-damaged chromatin. Does the ATM pathway respond directly to DSBs (before the generation of RPA-ssDNA) by directly binding to DSB-MRX complexes, or is there also a requirement for RPA-ssDNA for ATM activation? Possibly, MRX fulfills an ATRIP-like function for ATM, allowing it to respond specifically to RPA-ssDNA generated by the MRX-dependent nucleases. Recent data suggesting that ATM is activated by chromatin distortions, independently of DNA breaks (*10*), do not exclude a role for RPA-ssDNA because chromatin distortion may expose ssDNA.

Zou and Elledge demonstrate that a simple paradigm for DNA-damage signaling is conserved from bacteria to humans. Prokaryotes sense RecA-ssDNA, whereas eukaryotes sense RPA-ssDNA. Detecting multiple DNA perturbations, particularly those caused by replication stress, is vital to coordinate DNA repair with cell cycle progression and apoptosis. Such coordination is essential for survival of cells and the whole organism. That ssDNA underlies damage detection shows that, in the end, the beginning of signaling has a simple explanation.

References

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GEOCHEMISTRY

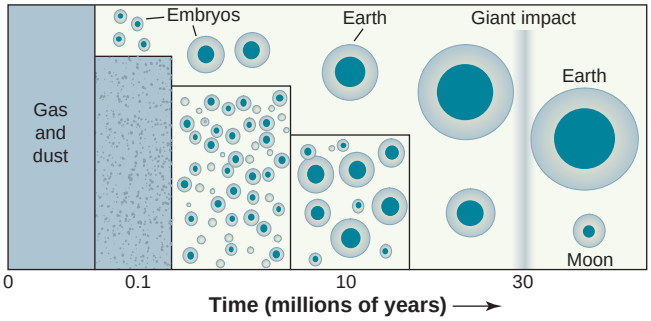
How Old Is Planet Earth?

Stein B. Jacobsen

Recent reports (*1*–*4*) on the tungsten (W) isotope composition of meteorites have led to a completely revised time scale for the formation of the terrestrial planets. The results show that most of planet Earth had formed within ~10 million years (*1*, *2*) after the formation of the solar system some 4567 million years ago (when the first solid grains formed in the solar nebula) (*5*). The Moon-forming event happened ~30 million years after solar system formation, when Earth was fully grown (*2*, *3*).

The decay of the hafnium isotope ^{182}Hf (with a half-life of 9 million years) into ^{182}W is the best "clock" we have for tracing the formation of terrestrial planets during the first 50 million years of solar system history. The behavior of these elements during metal-silicate separation, which occurs during the formation of planetary cores, is well understood.

Hafnium is a lithophile element (it has a strong affinity for silicate liquid) and stays entirely in the silicate mantle (and crust) of the planet. Hence, the mantle is where radioactive decay of ^{182}Hf to ^{182}W occurs. In contrast, tungsten is siderophilic (it has a strong affinity for iron melt), and about 90 to 95% of it is partitioned into the metal when metal and silicate separate in the core-forming process. After 50 million years, the Hf-W chronometer is a dead clock because almost all ^{182}Hf has decayed, but for the first 50 million years of solar system history, it is ideal for tracking a planet's growth.



The formation of Earth. The first new solid grains formed from the gas and dust cloud called the Solar Nebula some 4567 million years ago. Within 100,000 years, the first embryos of the terrestrial planets had formed. Some grew more rapidly than others, and within 10 million years, ~64% of Earth had formed; by that time, proto-Earth must have been the dominant planet at 1 astronomical unit (the distance between Earth and the Sun). Accretion was effectively complete at 30 million years, when a Mars-sized impactor led to the formation of the Moon. The figure is not to scale.

In the earliest work on this chronometer (*6*, *7*), we found that the solar system's initial ^{182}W/^{183}W value was about 3 to 4 parts in 10,000 lower than the present terrestrial value, and inferred a relatively short time scale for the formation of Earth. This short time scale was challenged by Lee and Halliday (*8*), who reported that Earth and chondritic meteorites have essentially identical ^{182}W/^{183}W values to within 20 parts per million—indicating that Earth formed relatively late, after the decay of ^{182}Hf (when the Hf-W clock was dead). They reported an age of core formation within Earth corresponding to 60 ± 10 million years after solar system formation. This age has been widely cited. However, because the clock was dead by this time, it should have been reported as any time between 50 million years after solar system formation and the present.

Last year, three groups reported that ^{182}W/^{183}W in chondrites is lower than that of Earth by 2 parts in 10,000, and thus intermediate between the initial solar value and that of Earth today (*1*–*4*). These new results have fundamentally changed the way in which the

The author is in the Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138, USA. E-mail: jacobsen@neodymium.harvard.edu