TRANSCRIPTION

Puffing with PARP

Vincenzo Pirrotta

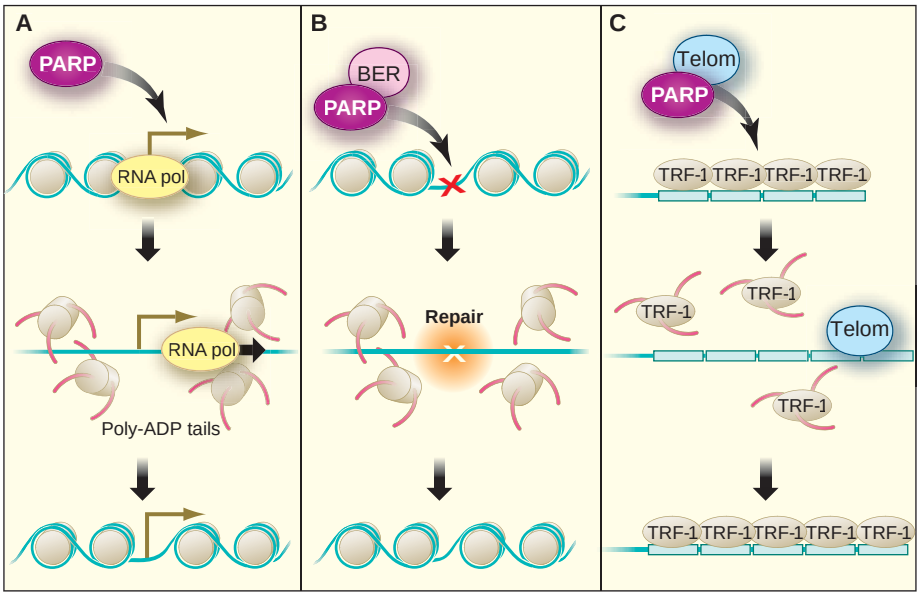
Environmental stimuli such as heat shock, hormone treatment, stress, or acute infection induce the transcription of certain target genes. The transcriptional response is rapid, producing many mRNA transcripts, but transient, fading shortly after the inducing signal disappears. In the fruit fly *Drosophila*, this rapid transcriptional response is clearly visible under the microscope as "puffs" in the giant polytene chromosomes of the salivary glands. (These giant chromosomes are produced by repeated cycles of DNA replication in the absence of cell division.) Within minutes of a heat shock stimulus, the chromosomal sites of heat shock genes become decondensed, swell to many times their original diameter, and accumulate many new mRNA transcripts. Similar puffs occur at chromosomal sites of hormone-responsive genes in response to increased production of the steroid hormone ecdysone in *Drosophila* larvae approaching pupation.

It has been debated for decades whether puffs are natural consequences of high-level transcription or whether specific puffing signals precede transcriptional activity, because puffing still occurs when transcription is blocked by actinomycin or by mutation of target gene promoters (*1*). Recent work from the Spradling laboratory (*2*), including the report on page 560 of this issue (*3*), introduces a new player in puff formation. Tulin and Spradling (*3*) show that a remarkable enzyme involved in the repair of DNA damage (*4*)—poly(ADP-ribose) polymerase (PARP)—is crucial for puff formation in *Drosophila* polytene chromosomes.

Activated by binding specifically to nicked DNA, PARP uses nicotinamide adenine dinucleotide (NAD) to add long chains of adenosine diphosphate (ADP)–ribose residues to target proteins (*5*). Many target proteins are known, including the histone proteins of nucleosomes, transcription factors (oct-1, p53, AP2, NF-κB, YY1), and DNA repair proteins (*6*). In addition, a primary target of PARP is PARP itself, which acquires multiple ADP-ribose tails up to 100 residues long. PARP stimulates transcription in vitro, but in the presence of NAD, it can also block transcription by targeting unbound transcription factors and preventing them from rebinding to the DNA.

Spradling and colleagues previously characterized the *PARP* gene of *Drosophila* (*2*). In the new work, they show that the PARP protein is widely distributed in *Drosophila* polytene chromosomes but is normally inactive (*3*). However, upon exposure to a heat shock stimulus, PARP accumulates rapidly at heat shock gene loci, where it develops intense poly-ADP-ribosylation activity. Similarly, PARP accumulates at sites of ecdysone-induced puffs in polytene chromosomes of fruit fly larvae shortly before pupation. Another prominent PARP target is the nucleolus, where *rRNA* genes are the sites of the major transcriptional activity of cells even though they are surrounded by condensed heterochromatin that usually inhibits transcription. In the presence of PARP mutations or an inhibitor of PARP activity, puffing does not occur and transcription of heat shock genes in response to a heat shock stimulus is reduced by a factor of 5 to 10. Fruit fly larvae with mutations in PARP die when the maternal PARP supply is exhausted, apparently because they are unable to express the ecdysone-responsive genes necessary for molting and metamorphosis.

PARP enzymes have been found in all metazoans but not in budding yeast. Mammals have four PARP homologs including tankyrase, which is involved in maintaining the ends of chromosomes (telomeres) (*7*). Mice carrying mutations in PARP-1, the major source of PARP activity, are viable (*8*)—probably because the other PARP homologs compensate—but they tend to accumulate chromosomal abnormalities and are defective in DNA damage repair. They have a deficient immune response and show abnormalities in the expression of NF-κB–dependent genes such as those encoding interferons (*9, 10*). The immune response of insects to bacterial or



PARP, a versatile polymerase. (**A**) During transient activation of gene expression in response to environmental stimuli such as heat shock, PARP is recruited (possibly by transcription factors) to condensed chromatin (green) containing the genes to be switched on. PARP induces dissociation of nucleosomes (beige) by adding long ADP-ribose tails to their histone proteins. This results in decondensation of chromatin, enabling the RNA polymerase (yellow) to transcribe target genes unhindered. PARP prevents rebinding of transcription factors to the DNA by adding ADP-ribose tails to them, and finally itself dissociates from the DNA. Later, a glycohydrolase removes the ADP-ribose tails from histones and the chromatin returns to its normal condensed state. (**B**) During DNA repair, PARP binds to single-strand nicks in the DNA (red cross) together with other components of the base excision repair (BER) complex (pink). PARP induces decondensation of chromatin by poly-ADP-ribosylating nucleosomes, and accelerates ongoing transcription (while preventing de novo transcription), thus enabling DNA repair to take place. (**C**) The PARP enzyme tankyrase targets TRF-1 (telomere repeat binding factor) bound to telomeres at the ends of chromosomes. Presumably, PARP adds ADP-ribose tails to TRF-1, preventing this factor from binding to DNA and allowing the enzyme telomerase (blue) to add DNA repeats to the chromosome ends (*6*). Later, the ADP-ribose tails are removed from TRF-1, which binds once more to the telomere repeats and represses any further elongation.

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fungal pathogens also depends on the activity of NF-κB homologs. Tulin and Spradling find that this response is defective in PARP mutant larvae, which are exceptionally sensitive to bacterial infection.

How is PARP involved in the induction of gene expression? Tulin and Spradling envision that inactive PARP is recruited, presumably by certain transcription factors, to target genes where it becomes activated. PARP then adds long ADP-ribose tails to the histone proteins of nucleosomes around which the DNA is wrapped (see the figure). Nucleosomes containing poly-ADP-ribosylated histones are unable to remain tightly packed, resulting in "loosening" or decondensation of the chromatin. RNA polymerase is now able to transcribe the target genes without hindrance. In vitro experiments suggest that transcription is initially facilitated by PARP, but as soon as transcription factors dissociate from the DNA, they too become inactivated through poly-ADP-ribosylation, thus preventing repeated cycles of transcription (*11*). In this way, PARP ensures a strong but transient transcriptional response to a heat shock or ecdysone stimulus. Ultimately, PARP poly-ADP-ribosylates itself and dissociates from the DNA, its poly-ADP tails later being removed by poly(ADP-ribose) glycohydrolase. The mechanism of PARP action seems adapted to facilitate sudden bursts of transcriptional activity in response to transient environmental signals. However, increasing evidence suggests that PARP activity may be involved in a variety of other chromatin transactions such as telomere elongation, DNA surveillance and repair, apoptosis, DNA replication, the activation of repressed chromatin, and possibly genomic remodeling during development and differentiation.

Many questions remain to be answered. Where is PARP parked when it is inactive? Which molecules recruit PARP to target genes and activate this unusual polymerase? What controls the glycohydrolase activity that removes poly-ADP tails from target proteins and from PARP itself? In the case of DNA damage, single-strand nicks in the DNA are the recruiting agents for PARP, and binding of PARP to the damaged DNA induces catalytic activity. Perhaps DNA nicks are also involved in recruiting PARP to target genes. The work of Tulin and Spradling will undoubtedly stimulate renewed efforts to understand this remarkable enzyme, which is an unusual addition to the arsenal of molecules that modify chromatin and control gene expression.

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GEOPHYSICS

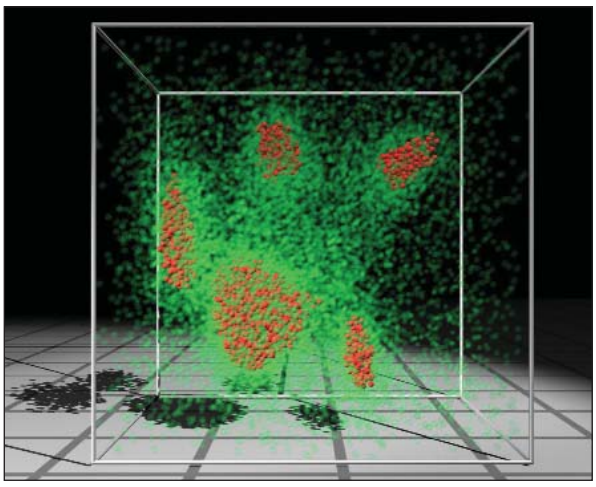
An Ensemble View of Earth's Inner Core

Malcolm Sambridge

Seismic studies show that Earth's inner core is solid. On page 552 of this issue, Beghein and Trampert (*1*) suggest that the structure of this inner core is more complex than previously thought. Their results indicate changes in the behavior of seismic properties in the inner core. The approach used by the authors also shows much promise in resolving other data-inference problems in geophysics.

More than 30 years ago, seismologists first realized that they could build models of Earth's interior by randomly generating large numbers of alternate models and retaining only those that satisfied observations to an acceptable level (*2*). Although initially appealing, this "Monte Carlo inversion" rather fell out of favor when it became clear that uniform random sampling is inefficient for large numbers of unknowns. Earth models found in this way were usually few in number, rather exotic in character, and often bore little resemblance to each other. It was therefore difficult to draw conclusions about which one (if any) resembled the real Earth (*3*).

Attention turned to linearized inversion techniques, which dealt with the "nonuniqueness" problem by deliberately restricting the allowable character of the Earth models (a procedure known as regularization). This approach has been widely used in geophysics, with numerical damping of linear systems of equations the most common way of imposing regularization.



Neighbourhood sampling in five dimensions. Only three dimensions are shown. Transparent green spheres correspond to Earth models with poor fit to data. Solid red spheres represent those with acceptable data fit. Red "islands" correspond to multiple populations of solutions. Explorative direct search methods must be used to identify all potential solutions. This process has yielded new models for Earth's inner core.

The seismic study of Earth's solid inner core reported by Beghein and Trampert (*1*) suggests that the tide may have turned yet again, with direct search techniques providing new insights where linearized procedures fail. In truth, such direct search inversion techniques never really disappeared from the geophysicist's toolbag. Instead, they became more efficient with the advent of simulated annealing in the 1980s and genetic algorithms in the 1990s. Both of these methods are widely used to solve global optimization problems in many areas of the natural sciences (*4*).

Beghein and Trampert (*1*) use a new class of direct search technique, known as "neighbourhood sampling" (*5*), to constrain inner core anisotropy from seismic observations of normal modes (see the figure). Many regions of parameter space are explored simultaneously by randomly searching in the neighborhoods of earlier models, with preference given to those that fit the data relatively better.

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