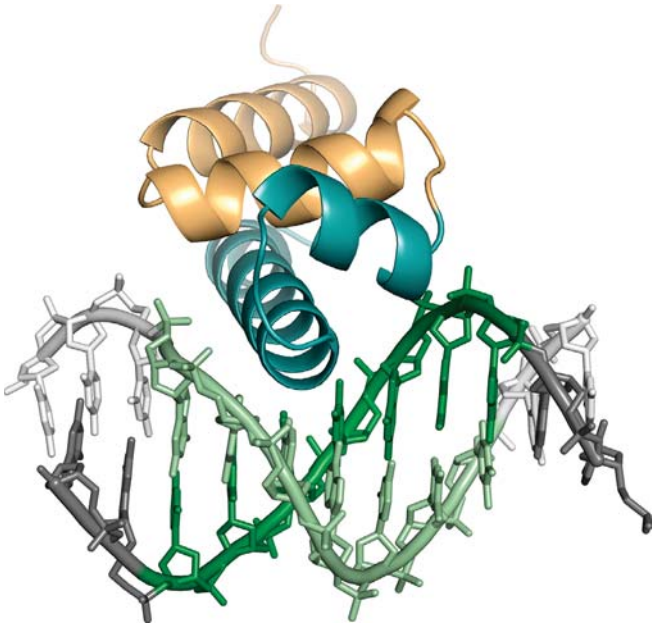

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A crystal structure of domain 4 of an *E. coli* Group IV sigma factor bound to –35 element DNA identifies a unique interaction mechanism that relies on the rigid conformation of the DNA consensus sequence.

mediate their cosmopolitan regulatory duties. Structural studies provide important clues to the nature and function of associations between sigma factors and DNA. In a new study, William Lane and Seth Darst used structural analysis techniques to determine the detailed shape of one type of sigma factor. They show that it binds to short DNA sequences using a molecular recognition method that has not been seen before in sigma factors.

Sigma factors come in two structurally unrelated families: sigma 54 and sigma 70. The sigma 54 family is associated with a diverse range of metabolic processes. The much larger sigma 70 family encompasses four groups: the Group I "primary" sigma factors facilitate metabolic and growth processes; the Group II–IV "alternative" sigma factors mediate specialized processes like sporulation and the environmental stress response. The sigma 70-type sigma factors recruit the RNA polymerase holoenzyme to bipartite promoter sequences, comprising conserved sequence elements centered about 10 and 35 base pairs upstream of the transcription start site. These so-called –10 and –35 elements are recognized by distinct structural domains of the sigma factor. Structures of one of the most studied sigma factors, a primary sigma factor called sigma-A, have been solved in previous studies. Here, Lane and Darst analyzed the –35-element-binding domain (domain 4) of an alternative Group IV sigma factor found in *Escherichia coli*, called sigma E4. Group IV sigma factors comprise the largest and most diverse set of sigma factors.

Both sigma-A4 and sigma-E4 allow RNA polymerase to bind to the –35 promoter element, but in each case the sequence is very different. In the case of sigma-E4, the sequence is GGAACTT (and others that resemble it). Previous studies showed that sigma-A4 recognizes its consensus sequence, TTGACA, through direct interactions with these six nucleotide bases. It was tempting to assume that sigma-E4 would operate in a similar manner, since the two sigma factors are similar in structure.

But, using X-ray crystallography, Lane and Darst showed that sigma-E4 binds its consensus sequence using a more subtle method. By determining the structure of the sigma factor bound to its consensus sequence, they found that sigma-E4 doesn't recognize the identity of the sequences per se but the shape of the DNA helix at those sequences. While one region of the sigma factor sits deep within a groove along the double helix's side, another region holds the promoter –35 sequence straight. The AA in the center of sigma-E4's consensus sequence, the researchers believe, is required for the DNA to assume this shape.

Because evolution has conserved the site in these proteins that sits alongside the AA of the consensus sequence, Lane and Darst propose that this method of recognizing –35 promoter sequences may be common across the Group IV sigma factors. With further studies of the structures of sigma factors and their means of recognizing specific promoters—and thus activating specific genes—researchers can better predict the full complement of genes a given promoter will regulate, and in turn gain insight into the diverse physiological responses they help mediate.

Lane WJ, Darst SA (2006) The structural basis for promoter –35 element recognition by the group IV s factors. DOI: 10.1371/journal.pbio.0040269

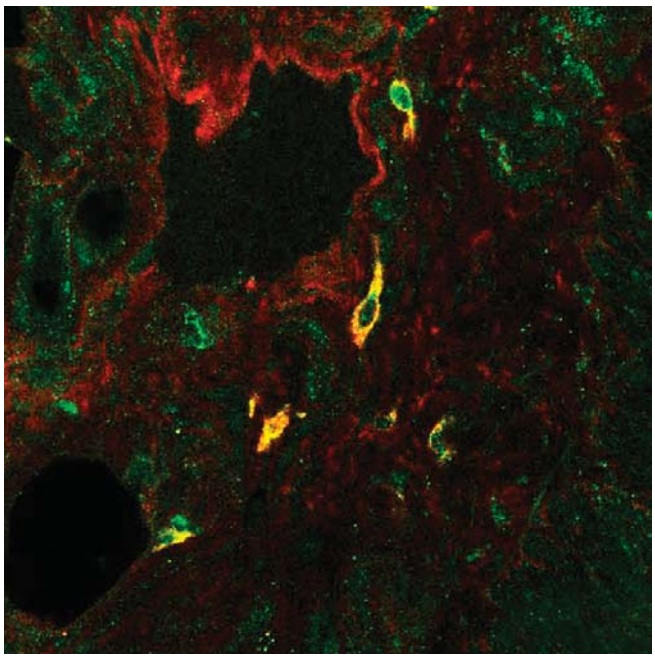
Anatomy of a Fever

Liza Gross | DOI: 10.1371/journal.pbio.0040305

Many parents experience fear and anxiety when their child comes down with a fever, unaware that fever is an ancient, often beneficial, response to infection. The fever response is conserved across all mammals and many vertebrate classes. (Even reptiles and other cold-blooded animals fare better against infection when they develop fever by soaking up the sun's heat.) Among other potential adaptive benefits, a higher temperature can inhibit the growth of bacterial strains that lack sophisticated mechanisms for coping with heat shock.

Fever, which is mediated by a lipid called prostaglandin E_2 (PGE_2), can pass through multiple temperature phases. While it's well established that PGE_2 originating in brain cells causes the second and later phases, the initial phase of fever has proven difficult to characterize. Of particular interest is whether fever onset is triggered by PGE_2 that originates inside or outside the brain—a question that has dogged researchers for nearly three decades. Now, Alexandre Steiner, Andrej Romanovsky, and colleagues provide evidence that PGE_2 synthesis doesn't begin in the brain as previously thought, but in the lungs and liver. They also describe the molecular mechanisms that produce PGE_2 in these organs.

Many of the mechanisms of fever have been established by exposing rodents to bacterial endotoxins called



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Cyclooxygenase-2 (green immunofluorescence) and the macrophage marker ED2 (red immunofluorescence) in rat lung at the onset of bacterial lipopolysaccharide-induced fever (photo: Jordi Serrats).

lipopolysaccharides (LPS). The first phase of LPS-induced fever starts within 30 minutes after exposure to the pyrogen. During the second and subsequent phases—between 90 minutes and 12 hours after LPS administration—brain cells increase production (called upregulation) of enzymes involved in PGE_2 synthesis. Thus, fever starts about an hour before the PGE_2-synthesizing enzymes—cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1)—are upregulated in the brain, suggesting that fever must be triggered by PGE_2 produced peripherally, outside the brain.

To test this hypothesis, Steiner et al. gave rats an intravenous (IV) solution of PGE_2 bound to albumin, the primary transporter of PGE_2 in the blood. Controls received an IV albumin solution. After confirming that the PGE_2 infusion induced fever, the researchers collected venous and arterial blood from LPS-treated rats. (PGE_2 synthesized in tissues amasses in venous blood; arterial blood delivers PGE_2 to the brain.) At the onset of fever, PGE_2 was significantly elevated both in the venous and arterial blood.

To investigate the origin of fever-inducing PGE_2, Steiner et al. used an antibody-based technique. (Antibodies are too large to cross the blood–brain barrier.) Rats were pretreated with IV anti-PGE_2 antibody serum (controls received normal serum) and then injected with IV LPS. The first phase of LPS fever was significantly attenuated by the antibody (but not the serum), which was found in the blood but not in the brain. These results show that LPS-induced fever is triggered by circulating PGE_2.

A previous study from the Romanovsky laboratory reported that onset of fever is accompanied by significantly increased transcription of COX-2 and mPGES-1 in the lung and liver, and a moderate increase of the COX-2 transcript in the hypothalamus (the body's "fever center"). In this study, they examined the protein distribution of these enzymes in all three tissues. After LPS exposure, the content of neither protein was increased in the hypothalamus. But the lung and liver did show increased COX-2 expression—primarily in macrophages, which play a role in the inflammatory response—along with activation of an enzyme (cytosolic phospholipase A2) involved in the early steps of PGE_2 synthesis. These organs, but not the hypothalamus, also showed signs of inflammatory signaling. Interestingly, the researchers did not find increased levels of mPGES-1, which facilitates the final step of PGE_2 synthesis, suggesting that the cell's normal supply of this enzyme manages the task.

Altogether, these results provide a cellular and molecular portrait of the first phase of fever and show that it depends, at least in part, on PGE_2 that originates in peripheral tissues.

Steiner AA, Ivanov AI, Serrats J, Hosokawa H, Phayre AN, et al. (2006) Cellular and molecular bases of the initiation of fever. DOI: 10.1371/journal.pbio.0040284

Master Proteins Dictate Retinal Differentiation Timetable

Liza Gross | DOI: 10.1371/journal.pbio.0040293

The embryonic construction of the vertebrate retina is a highly ordered affair. Following a precise timetable, six different specialized cell types emerge from a mass of identical, proliferating cells. The process of retinal cell differentiation, when so-called progenitor cells stop dividing and choose among the six fates, depends primarily on homeobox genes, major regulators of embryonic patterning. How these genes control the timing of retinal cell differentiation has remained an open question—until now.

In a new study, Sarah Decembrini, Federico Cremisi, and colleagues show that three homeobox genes work in conjunction with a cellular timepiece that determines the sequential emergence of distinct cell types. Surprisingly, the schedule of both homeobox gene expression and retinal cell differentiation is controlled by the translation, rather than by the transcription, of the genes.

Retinal cells transform light signals into visual information for further processing in the brain. After light stimulates the rod and cone photoreceptors, visual signals travel to horizontal and bipolar cells, which in turn interface with amacrine cells. Ganglion cells, which then relay these signals to the brain, are the first-born cells—that is, the first to exit the cell cycle and stop dividing. Though their birthdays vary somewhat by species, the horizontal, cone, and amacrine cells come next, then the rod and bipolar cells.

Decembrini et al. suspected that cell identity may be tied to cell cycle progression because different retinal cell types are produced when cell cycle length is manipulated. To test this hypothesis, they studied a subset of homeobox genes, including *otx5*, which supports photoreceptor differentiation, and *vsx1* and *otx2*, which promote bipolar differentiation.