structures have overcome barriers of low or anisotropic resolution to envision this interface (*11*–*13*). Previous work had identified an absolutely conserved arginine in the a subunit as essential for proton transport (*14*). This arginine has been proposed to promote rotation by electrostatic attraction of the newly deprotonated c subunit as well as to separate the aqueous channels approaching from each side of the membrane. The recent structures vary in the position of the conserved arginine relative to the closest c-ring carboxylate. Autoinhibited yeast V_o structures show a salt bridge between these residues (*12*, *13*). In the spinach chloroplast ATP synthase, the conserved arginine is ~4.5 Å from the nearest c-ring glutamate, whereas in the yeast ATP synthase, the corresponding carboxylate more closely approaches the arginine, suggesting an interaction during rotation. On the basis of oligomycin-binding results, Srivastava *et al.* suggest that the c-ring may be plastic, with conformational changes at the a-c interface propagating across the c-ring. One caveat is that all of the complexes visualized at high resolution are inhibited in some way. For example, rotation in the yeast F_1F_o structure is blocked by fusion of rotor and stator subunits; the spinach chloroplast enzyme is in the dark state, with rotation suppressed by the formation of an inhibitory disulfide bond.

The structures reported in (*1*, *2*) will drive exploration of a number of long-standing questions. All rotary ATPases have three catalytic sites in the peripheral motor, but the number of proteolipids in the membrane motor's c-ring varies from 8 to 15 and is not divisible by three in most organisms. Furthermore, in V_o c-rings, each proteolipid is twice as large as those in F_o c-rings but still carries a single proton-bearing carboxylate (*12*, *13*). Membrane motors must be able to accommodate both different ratios of protons released per ATP synthesized or hydrolyzed and different rotational step sizes.

On the basis of the three rotational states seen for the spinach chloroplast enzyme, Hahn *et al.* propose that elasticity in the peripheral stator helps to determine the step size of rotation in the membrane motor (*2*), although others have suggested that the rotor may be more elastic than the stator (*15*). Consistent with step size being mandated in part by peripheral stator structure, stators show considerably more variation between organisms than the core subunits of the peripheral or membrane motors. As their variable and conserved structural features come into focus, the underlying mechanistic principles, organism-specific differences, and regulation of these versatile and important enzymes will also emerge. ■

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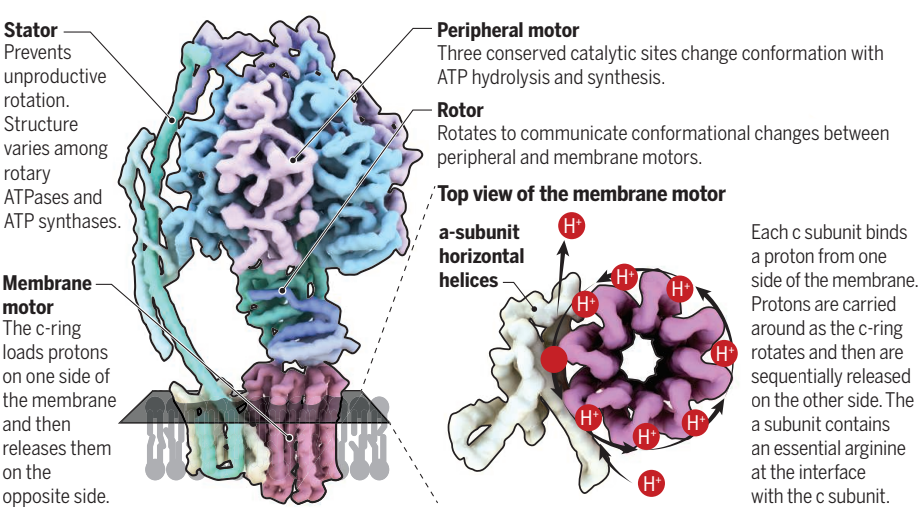
Rotary ATPases and ATP synthases

Recent structures, including two described in this issue (*1*, *2*), elucidate the mechanism by which ATPases and ATP synthases generate and release energy.



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DEVELOPMENT

How does temperature determine sex?

Temperature-responsive epigenetic regulation clarifies a 50-year-old mystery in reptiles

By **Arthur Georges**[1] *and* **Clare E. Holleley**[2]

Sex determination in reptiles is a complex affair, because incubation temperature and genes interact in many species to regulate sexual development and decide sexual fate, male or female (*1*–*4*). A central question that has remained unanswered is, what molecular mechanism allows temperature to so profoundly influence the developmental pathways that determine sex? The means to identify a master sex-determining gene in species with genetic sex determination is well established—identify genes on the sex chromosomes, demonstrate which of these are differentially expressed in male and female embryos early in development, and manipulate their expression to demonstrate reversal of sex (*5*–*7*). Not so with identifying the mechanisms of temperature-dependent sex determination (TSD). Temperature could exert its effect on any of the many autosomal genes involved in sexual differentiation, even those peripherally involved, provided their altered expression is capable of reversing sex. Little wonder that, in the 50 years since TSD was discovered in reptiles (*8*), we have not advanced far in our understanding of the mechanisms of TSD. This is about to change. On page 645 of this issue, Ge *et al.* (*9*) report that transcription of the chromatin modifier gene *Kdm6b* (lysine-specific demethylase 6B) responds to temperature in the red-eared slider turtle *Trachemys scripta elegans*, and confers temperature sensitivity to a key sex-determining gene, *Dmrt1* (doublesex- and mab-3–related transcription factor 1).

Ge *et al.* previously showed that *Dmrt1* is differentially expressed early in embryonic development before the gonads differentiate structurally (*10*). Additionally, *Dmrt1* expression is high at male-producing tem-

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perature (MPT) and low at female-producing temperature (FPT). As such, *Dmrt1* is a strong candidate for the male sex-determining gene in this TSD species of turtle, consistent with the master sex-determining role of other DM domain–containing genes in some fish, amphibians, and birds (*7, 11, 12*). Depending on the species, these DM domain genes initiate and maintain the male sexual trajectory, and suppress genes important for female development during the critical stages of embryogenesis.

What Ge *et al*. have now discovered (*9*) is that experimental down-regulation of *Kdm6b* at 26°C (normally an MPT) shifts embryos from a male to a female developmental trajectory. This occurs because the protein KDM6B is a lysine-specific demethylase with a central role in epigenetic regulation of gene expression. Suppressing *Kdm6b* expression reduces demethylation of its target, trimethylated lysine 27 on histone 3 (H3K27), a histone modification that would otherwise repress *Dmrt1* promoter activity. Thus, high amounts of KDM6B at MPT activate *Dmrt1* gene expression and determine male sex, whereas reduced amounts of KDM6B repress *Dmrt1* expression. Trimethylated H3K27 was not found on the promoters of any other sex genes that were differentially expressed early in development. Maintaining the trimethylation of H3K27 by experimentally down-regulating *Kdm6b* suppresses expression of *Dmrt1* and leads to female development at MPT. This is convincing evidence of a role in TSD for highly conserved epigenetic modifiers including, but not necessarily limited to, KDM6B (see the figure).

Kdm6b is a member of the Jumonji gene family that is implicated in reptile and mammalian sex determination. For example, in mice, another Jumonji family member, *Kdm3a*, encodes a protein that catalyzes H3K9 demethylation of the mammalian sex-determining gene *Sry* (sex-determining region Y) to enable its expression above the required threshold for male development (*13*). In reptiles, the role of Jumonji family members appears to be more complex. Depending on the temperature, an intron is alternatively retained or excised during transcription of *Kdm6b* [and at least one other family member, *Jarid2* (Jumonji and AT-rich interaction domain containing 2)] in the red-eared slider turtle, American alligator, and the bearded dragon lizard (*14*). In red-eared slider turtles, the intron is retained in *Kdm6b* transcripts of embryos incubated at the lower MPT (26°C), but not those incubated at the higher FPT (32°C). The transcribed intron, when brought into frame, is riddled with premature stop codons, which presumably leads to altered or disrupted KDM6B function in embryos incubated at 26°C. Up-regulation of *Kdm6b* coincident with intron retention and potentially compromised function may at first seem contradictory. However, alternative splicing of Jumonji genes has the potential to alter the targets of gene silencing, gene activation, and the recruitment of chromatin remodeling complexes [for example, PRC2 (Polycomb repressive complex 2)] in ways that are not yet fully understood. Intron retention presumably interacts with the regulatory processes outlined by Ge *et al*. (*9*) to determine sex.

Questions remain as to whether Jumonji genes such as *Kdm6b* are responding directly to temperature or, alternatively, are regulated by upstream temperature-sensitive elements yet to be discovered. One such candidate to recently emerge (*4, 14*) is the gene *Cirbp* (cold-inducible RNA binding protein), which encodes a temperature-inducible RNA binding protein with broad imputed function in messenger RNA stabilization and translational regulation (*15*). *Cirbp* is expressed early in gonadal development in the common snapping turtle *Chelydra serpentina*, and its expression influences sex determination of embryos incubated under a regime in which temperature is equivocal in its influence (*4*). Remarkably, a single point mutation in this gene is sufficient to eliminate temperature sensitivity.

These recent findings (*4, 9, 14*) have dramatically shifted the focus of inquiry from direct thermosensitivity of candidate sex-determining genes to higher-order thermosensitive epigenetic processes that differentially release influential sex genes for expression. We are on the cusp of finally understanding the mechanisms by which temperature exerts its influence on sexual fate. A central role for these highly conserved and fundamental processes of chromatin modification leaves open the possibility that different sex genes can become enlisted to function as temperature-sensitive sex-determining genes, thus explaining the astonishing diversity of sex determination in reptiles (*12*). ■

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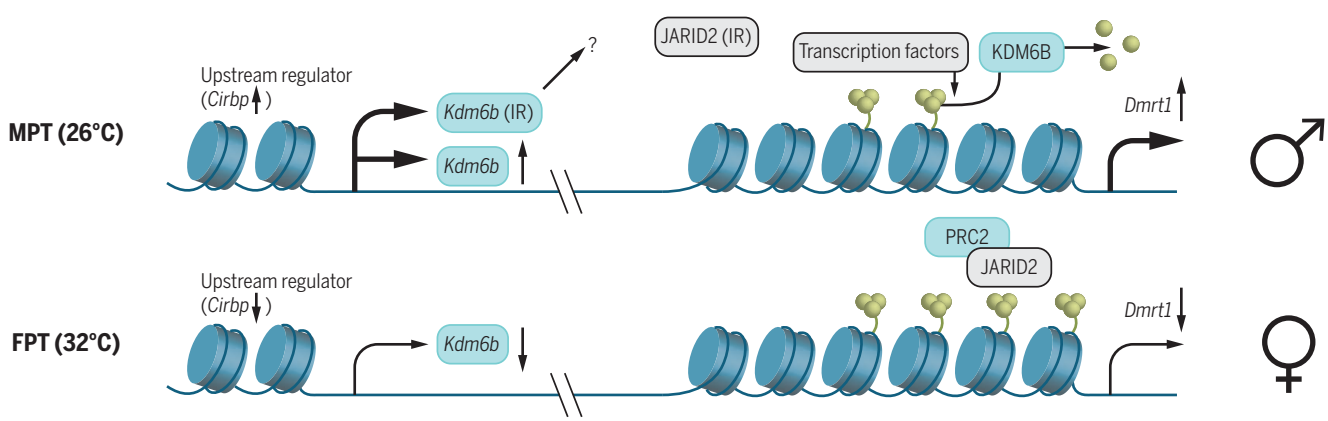
Proposed temperature-dependent epigenetic regulation

At MPT, *Kdm6b* expression is up-regulated directly or by an upstream temperature-sensitive regulator such as *Cirbp*. KDM6B then demethylates the *Dmrt1* promoter, leading to up-regulation of its expression and male development. Additionally, at MPT, transcription of *Kdm6b* and *Jarid2* with a retained intron (IR) is up-regulated; their function is unknown. At FPT, *Kdm6b* and *Jarid2* expression is down-regulated and they are transcribed without the retained intron. Presumably, *Jarid2* is sufficiently expressed to enable PRC2 to trimethylate H3K27 on the *Dmrt1* promoter and suppress its expression, leading to female development.



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