Although pleasing, this model contains several unresolved but important details. For example, is the spindle checkpoint transiently activated in every cell cycle or only in response to spindle perturbation? Does Mad2 inhibit Cdc20/Slp1 by simple binding as inferred from the arrest caused by Mad2 overproduction? If preliminary experiments suggesting that Mad2 is bound to Cdc20 constitutively are correct (*11*), then how might Mad2 inhibit Cdc20? One possibility is that in response to the checkpoint signal additional Mad2 molecules bind and inhibit Cdc20 or that Mad2 facilitates an inhibitory modification of the Cdc20 pool.

Two critical issues remain: How are Cdc20/Slp1 activation and anaphase timing normally regulated in the absence of checkpoint activation? How do Cdc20/Slp1 and Cdh1/Hct1 regulate the substrate specificity of the APC? A clue to how these proteins function comes from an unanticipated source, the SCF (Skp/Cdc53-cullin/F-box protein) complex, an E3 ubiquitin ligase unrelated to the APC that controls the G_1–to–S phase transition in *S. cerevisiae*. The SCF complex promotes the degradation of proteins such as the cyclin-dependent kinase (Cdk) inhibitor Sic1. It has been hypothesized that the substrate specificity of the SCF complex is conferred by different F-box proteins (*14*). For Sic1, this protein is Cdc4, which has both an F-box motif and WD repeats. Sic1 association with and ubiquitination by the SCF requires Sic1 phosphorylation (*15*, *16*) and the WD repeats of Cdc4 (*15*), suggesting that WD repeats recognize phosphoproteins. Because WD proteins serve as specificity factors for both the APC and SCF, they may allow the APC to be indirectly regulated by phosphorylation, as they do in the SCF.

How does the cell control APC substrate selection to ensure the proper order of mitotic events? Although all known APC substrates contain a motif called a destruction box that is required for degradation, they disappear at different times during mitosis (*17*). The substrate timing problem is now the central mystery of mitosis, for it holds the key to the order of mitotic events. As with most cell cycle events, phosphorylation is a prime candidate for regulation of APC function, perhaps directly controlling substrate selection as in the SCF pathway or controlling Cdc20 and Cdh1/Hct1 association with APC.

In addition, Cdk activation correlates with APC inactivation in late G_1 (*18*), and Cdk inactivation in G_2 is sufficient to activate the APC (*19*). How might Cdk activity negatively regulate the APC? Cdks could phosphorylate protein inhibitors of the APC, perhaps through association with the WD repeat proteins Cdc20 and Cdh1/Hct1, or by direct phosphorylation and inhibition of the specificity factors themselves. Thus, Cdks may simultaneously set up the mitotic apparatus as well as the inhibitory barriers that must be overcome for APC activation and mitotic progression. APC activation could be achieved either by inhibitor destruction or reversal of inhibitory phosphorylation. This mode of APC regulation could temporally control substrate selection. If APC substrates include inhibitors of specificity factors required for ubiquitination of subsequent APC substrates, an ordering mechanism could operate in a domino fashion to sequentially activate the destruction of APC substrates. Checkpoints could prevent inhibitor destruction and arrest the cycle. Alternatively, the order of substrate selection could be set by a clock mechanism initiated by Pds1 or Cut2 destruction. Regardless of the outcome, the frenzied pace of activity in this field should provide enough excitement to keep all but the most severe narcoleptics wide awake.

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TRANSCRIPTION

Inner Workings of a Transcription Factor Partnership

Barbara J. Graves

How do proteins that turn genes on and off recognize their sites of action within the genome? Lock-and-key type molecular complementarity between a regulatory protein and its DNA binding site provides the primary recognition. A constellation of electrostatic and hydrophobic interactions between matching surfaces of the DNA helix and the protein establish high-affinity and sequence-specific binding. The effectiveness of this macromolecular matchmaking, first elucidated by elegant experiments in prokaryotes, is challenged by the complexities of eukaryotes. There are hundreds of regulatory transcription factors that function by binding DNA sequences within their target genes. Almost all of these proteins are encoded by multigene families. Members of a family display the same structural fold for binding DNA and recognize similar DNA sequences. How can specificity be obtained within such a complex world?

Combinatorial arrays of multiple proteins add specificity and stability to DNA-protein interactions. Homodimers and heterodimers can be formed between members of the same gene family. Alternatively, partnerships can form between two proteins that belong to unrelated groupings. Wolberger and colleagues have studied one such partnership (*1*), and their report on page 1037 provides a snapshot of the molecular basis of combinatorial control of transcription. The report describes the crystal structure of the ternary complex of GABPα, a member of the *ets* gene family, with its heterotypic partner GABPβ on a DNA duplex.

The *ets* gene family dramatically illustrates the specificity problem (*2*). *ets* genes are present in all metazoan phyla, with more than 20 homologs in the human genome. The ETS domain, a highly conserved 85–amino acid region, defines the family and directs DNA binding. The DNA binding sites of all *ets* proteins include the core recognition sequence 5′-GGA-3′. Additional DNA contacts that also require conserved sequences extend the binding site to include at least nine base pairs. With such a high degree of conservation, how is specificity programmed into the family? For example,

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GABP is ubiquitous in mammalian systems and regulates expression of respiratory and translational machinery genes. The widespread distribution of GABP overlaps that of many tissue-restricted *ets* gene products. What determines that GABP regulates only its target genes?

One answer comes from the versatility of the ETS domain–DNA interaction. The winged helix-turn-helix motif of the ETS domain is composed of three α helices and a four-stranded β sheet (*1*, *3*–*5*). In the GABPα structure, helix 3 of the helix-turn-helix motif binds DNA in the major groove with two invariant arginines hydrogen bonding directly to the guanine residues of the GGA core. Other structural elements, including the β sheet and helix 1, make phosphate contacts on each flank of the GGA core, and these contacts indirectly specify additional sequence preferences (see the figure). The phosphate contacts made by GABPα are similar, but not identical, to those reported in the crystal structure of PU.1 with DNA (*5*). Furthermore, the structures of PU.1- and GABP-DNA complexes display slightly different bonding networks between helix 3 and the GGA motif. Indeed, PU.1 is known to bind an alternative core, AGA, and this difference can be explained by the structural differences at the DNA-protein interface. How these and other subtle variations in DNA recognition can determine specificity within the *ets* family can now be investigated.

Specific association with GABPβ further regulates the DNA binding specificity of GABPα. GABPβ interacts with GABPα through four ankyrin repeats. Each repeat is composed of two α helices in a coiled-coil configuration with an intervening loop punctuated by a β turn at its tip (*1*, *6*). Each tip interacts with a distinct part of GABPα, including parts of the ETS domain and the carboxyl-terminal flanking region, which includes helices 4 and 5. This heterotypic interface illustrates the diversity of structural coupling between interacting proteins while emphasizing the need for multiple contacts to mediate stable and specific interactions.

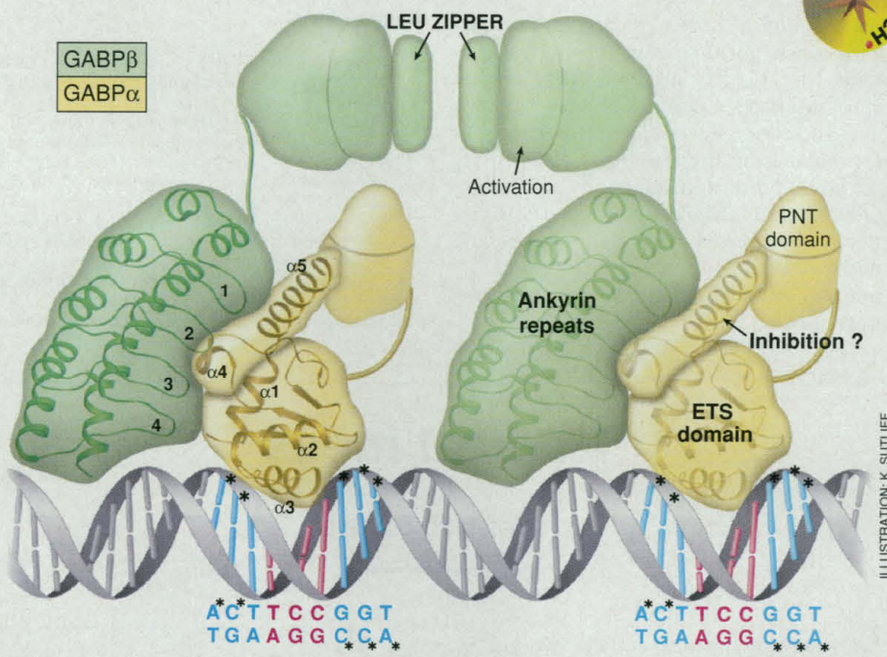
Insights from the GABP-DNA structure extend beyond the static picture of the complex. The GABPα-GABPβ ternary complex is 100 times as stable as a binary complex formed only with GABPα. However, GABPβ does not directly contact DNA. Instead, intermolecular interactions between the two subunits indirectly affect DNA binding. First, a lysine within the third ankyrin repeat hydrogen bonds with a glutamine of GABPα that directly contacts DNA. This glutamine contacts a single phosphate in concert with a backbone amide of a leucine at the amino-terminus of helix 1. Three other helix 1–ankyrin repeat interactions also are detected. This structural coupling suggests that GABPβ buttresses the helix 1–DNA interaction and that this single phosphate contact is critical for stable DNA binding.

An interaction between the first ankyrin repeat of GABPβ and helix 5 of GABPα is a second potential effector of enhanced DNA binding. The authors speculate that helix 5 is functionally analogous to helix 4 of Ets-1, which also lies on the carboxyl-terminal side of the ETS domain (*4*). In Ets-1, helix 4 negatively regulates DNA binding (*7*). In the absence of DNA, this helix packs against helix 1 as well as against two additional helices that lie amino-terminal to the ETS domain. This helical packing is inhibitory and must be disrupted during DNA binding (*8*). In the GABPα-GABPβ complex, helix 5 does not contact helix 1. The authors propose that GABPβ alters the position of helix 5, derepressing DNA binding. This model predicts that helix 5 inhibits the DNA binding of GABPα alone and that helix 5 will be positioned differently in the absence of GABPβ, predictions that can now be tested.

Optimal DNA binding by GABP requires more than the ETS domain–ankyrin repeat interaction. A leucine zipper motif within GABPβ, which is not present in the crystal structure, directs formation of a heterotetramer (α_2-β_2). In this configuration GABP recognizes two GGA sites and displays even higher DNA binding affinity (*9*) (see the figure). Among the *ets* proteins, GABPα is the only one that binds DNA as an oligomer. Thus, GABPβ acts at several levels to add specificity and affinity to the DNA binding activity of GABPα.

Future studies should address whether other *ets* proteins are regulated by analogous partners. GABPβ apparently exhibits a high degree of specificity for GABPα, as ternary complexes with other *ets* proteins have not been detected (*10*). There are a plethora of candidate partners for other *ets* proteins. The report by Wolberger and colleagues (*1*) provides a structural and mechanistic framework for understanding these partnerships (for example, positioning helix 1 of the ETS domain as well as counteracting inhibitory sequences that lie outside of the ETS domain).

DNA binding cooperativity is frequently reported between transcription factors that



Working together to bind DNA. The specificity of DNA binding by the *ets* protein GABPα is determined by formation of a heterotetramer (α_2-β_2) that recognizes a binding site with two 5′-GGA-3′ cores. In the α subunit, the ETS domain functions in DNA binding, inhibitory sequences are proposed to negatively regulate DNA binding, and the pointed (PNT) domain is a structural domain conserved in some ETS domain proteins. In the β subunit, the leucine zipper motif (LEU ZIPPER) mediates β-subunit interaction, the transactivation domain is required for transcriptional activation, and ankyrin repeats form the interface with the α subunit. Flexibility in the linkage between the leucine zipper region and ankyrin repeats of GABPβ is proposed to accommodate recognition of direct repeats of GGA (shown) or inverted repeats of the GGA core with variable spacing. The asterisks indicate phosphate contacts detected in the crystal structure.

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function at a single promoter. Together with biochemical studies of other *ets* proteins, the GABP structure suggests that autoinhibition, conformational change and allosteric effects are potent strategies for modulating the affinity and specificity of DNA-protein interactions and derepressing autoinhibition. Just as transcription is controlled both by positive- and negative-acting proteins, transcription factors can be themselves regulated by opposing pathways.

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ATMOSPHERIC CHEMISTRY

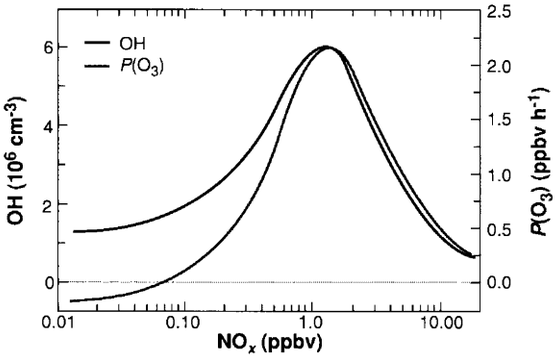
Radical Ideas

Dieter H. Ehhalt

In a recent research article in *Science*, Wennberg *et al.* (*1*) derived the production rate of ozone (O_3) in the middle and upper troposphere from measured concentrations of the hydrogen peroxy radical (HO_2), the hydroxyl radical (OH), and nitrogen oxide (NO). They were able to do so because of the unique and central role OH plays in tropospheric chemistry: OH is the main oxidizing agent in the troposphere, reacting with most trace gases, in many instances as the first and rate-determining step in a chain of reactions. Thus, OH controls the removal and, therefore, the concentrations of many manmade gaseous pollutants as well as natural trace gases. For example, of the 2800 million tons of carbon monoxide (CO) that are turned over annually in the atmosphere, 85% is removed by the reaction with OH to form carbon dioxide (CO_2) (*2*).

The hydroxyl radical is ubiquitous. It is formed primarily in the reaction of water vapor with excited oxygen atoms from the photolysis of O_3 by solar ultraviolet radiation. The required wavelength range below 320 nm nearly coincides with that causing sunburn. We should expect OH formation because O_3 is found virtually everywhere as part of a natural cycle, which in mixing, pushes it down from the stratosphere.

The reaction of OH with molecules, such as CO or hydrocarbons, returns another radical, HO_2 [see reactions 1, 2, 9, and 10 in (*1*)], which eventually is recycled to OH. Thus, the oxidation by OH is catalytic: one OH can destroy several molecules. The rate of HO_2 recycling, and therefore the OH concentration, is greatly enhanced by the presence of NO. At the same time, the other nitrogen radical, NO_2, which is always present along with NO, reacts with OH to form nitric acid (HNO_3), removing OH from the atmosphere. The opposing actions of both processes lead to a highly nonlinear dependence of the OH concentration on $NO_x = NO + NO_2$ (see figure).



Nonlinear chemistry. OH concentration and net O_3 production rate P as a function of NO_x concentration, calculated for a clean rural site (ppbv, parts per billion by volume per hour). All other parameters were held constant at the values observed at noon in Mankmoos, eastern Germany (53.8°N, 11.7°E) on 16 August 1994 (summertime). The exact position of the maximum in OH and $P(O_3)$ depends on the choice of the other parameters. In the upper troposphere, its position is shifted to considerably lower NO_x concentrations (*1*, *5*).

The nitrogen oxides NO_x have another important role. The reaction recycling HO_2 oxidizes NO to NO_2. Similar reactions take place with organic peroxy radicals formed in the oxidation of hydrocarbons. The resulting NO_2 is rapidly photolyzed in the sunlit atmosphere to produce NO and an oxygen atom that quickly recombines with molecular oxygen to form O_3. This reaction chain is the sole process of O_3 production in the troposphere. In combination with anthropogenic emission of NO, it is the cause of the regional episodes of O_3 pollution as well as of the general increase in the background O_3 concentrations at northern mid-latitudes during this century (*3*). The emission of NO into the upper troposphere by aircraft are another, relatively recently identified element of man's impact on the tropospheric NO_x and O_3 budget (*4*, *5*). Without NO, the chemical processes of trace-gas removal induced by OH lead to a destruction of O_3, a phenomenon that has been observed in pristine background air.

Therefore, NO_x not only influences the rate at which HO_x (= OH + HO_2) is cycled between OH and HO_2, it also controls how much O_3 is produced during each cycle. The resulting net production rate of O_3 depends on NO_x concentration in a highly nonlinear fashion, quite similar to the one found for the OH concentration (see figure).

This nonlinear dependence makes model predictions about changes, even the sign of the change, in local OH concentration and net O_3 production induced by the additional input of NO_x dependent on the preexisting levels of NO_x. As in the case of the upper troposphere, these concentrations are often not known. It also causes problems in the design of O_3 abatement strategies, because the lowering of NO_x emissions in areas with a large NO_x burden can lead to local increases in O_3 concentrations, at least temporarily.

Given its role as a driver of tropospheric chemistry, the measurement of OH provides a crucial test of our understanding of atmospheric chemistry. In the case of the upper troposphere, the measured OH concentrations exceed those predicted by models by up to a factor of 5 (*1*). This discrepancy requires the consideration of additional sources of HO_x. Besides the photolysis of acetone, the photolysis of hydrogen peroxide and methyl hydrogen peroxide have been put forward as plausible explanations (*1*).

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