is more severe in flies expressing the A30P α-synuclein mutation. The significant decrease in motor activity in the A30P mutant is intriguing and may reflect a more aggressive disease, although altered transgenic expression could account for the difference. These transgenic flies recapitulate several cardinal features of human PD, including age-dependent onset, chronic progressive selective loss of dopamine neurons followed by loss of motor control, and development of Lewy body–like pathology (see the table).

Masliah and colleagues (*4*) engineered their mice to overexpress human wild-type α-synuclein driven by the platelet-derived growth factor promoter, which resulted in expression of α-synuclein in all neurons. The enzyme tyrosine hydroxylase (which is required for the synthesis of dopamine) was used as a marker for dopamine neurons and dopaminergic function. At 12 months of age, there is decreased expression of tyrosine hydroxylase protein and decreased activity of the enzyme in mice expressing the most α-synuclein. In addition, there is a decrease in the number of tyrosine hydroxylase–positive nerve terminals in the striatum, an area that receives projections from nigral dopamine neurons. Cytoplasmic inclusions containing α-synuclein and some containing ubiquitin are also observed. Additionally, the α-synuclein transgenic mice have impaired motor performance on the rotorod test (in which animals must balance on a rotating bar).

These mice demonstrate that overexpression of α-synuclein can injure dopamine neurons, induce formation of inclusions, and result in motor deficits. However, some cardinal features of PD have yet to be observed in these mice. There is no loss of dopamine neurons and the inclusions do not contain fibrils characteristic of Lewy bodies. This may be due to the age of the animals. Examining older mice may reveal loss of dopamine neurons with more characteristic Lewy body–like inclusions. Inclusions are also observed in the nuclei of neurons in the transgenic mice, which is not a feature of PD (see the table). Future studies will determine whether the decrease in motor performance is due to dopaminergic deficits or as yet unappreciated deficiencies in other motor areas. The Masliah *et al.* study is the first report of a genetically engineered mouse carrying a gene implicated in PD. Mice carrying the mutations associated with familial PD are sure to follow shortly. Building on the experience of expressing normal α-synuclein, taking cues from the fly model, and examining other lines of mice carrying the different familial mutations should enable the construction of a mouse model that recapitulates most, if not all, of the features of PD.

The initial characterization of these transgenic animals reveals that overexpression of wild-type and mutant α-synuclein induces neuropathological deficits with accompanying behavioral abnormalities. Are other features of PD also reproduced? It will be important to determine whether the neuronal injury in the mouse is confined to the dopaminergic system and whether dopamine neurons eventually die in the transgenic animals. Future work will determine whether there are decrements in the enzymes of mitochondrial complex 1 or increases in oxidative stress, which are features of the sporadic form of PD that affects the majority (>95%) of patients (*5*). However, the fly and mouse models provide us with a way to understand how overexpression of wild-type or mutant α-synuclein results in dopamine cell loss and neurological dysfunction.

Now that several genes have been linked to familial PD and the first animal models have been developed, a new era in PD research has begun. The power of fly genetics will allow identification of modifier and suppressor genes and other pharmacological and genetic manipulations that will help us to understand the signaling pathways that are critical for selective degeneration of dopamine neurons. The near future will see the generation of additional lines of mutant α-synuclein transgenic mice (in fact, α-synuclein A30P mutant transgenic mice have recently been reported); engineering mice that overexpress other PD-associated genes or that do not express them at all will soon follow. These animals will grant insight into selective dopamine neurodegeneration and should provide small-animal models to screen for new therapies that might affect the onset and progression of the more common (sporadic) form of PD.

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PERSPECTIVES: STRUCTURAL BIOLOGY

Light at the End of the Channel

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At the heart of bacterial and eukaryotic gene expression are the multisubunit RNA polymerases (RNAPs), industrious enzymes that catalyze the synthesis of RNA from a DNA template. Biochemical studies have provided a working model of how RNAPs move along the DNA template and catalyze the elongation of RNA, and they have revealed remarkable features of these macromolecular machines (*1, 2*). Unlike their kindred DNA polymerases, RNAPs are highly processive—that is, they are capable of synthesizing RNAs thousands and even millions of bases long without dissociating from the DNA template. The processivity of RNAPs is attributable, at least in part, to their ability to bind with exceptional tenacity to DNA and the growing RNA transcript. But despite the advantage of processivity, RNAPs synthesize RNA in fits and starts, pausing for varying lengths of time at each step of nucleotide addition and, at times, falling into an arrested state that can be reversed only by elongation factors, such as SII, GreA, or GreB. Pausing and arrest appear to result from backsliding of the polymerase along both the DNA and RNA, causing the growing end of the transcript to be displaced from the catalytic site, in a process that is either spontaneously reversible (in the case of pausing) or not (in the case of arrest).

Of the multisubunit RNAPs, eukaryotic RNA polymerase II (Pol II)—the enzyme that catalyzes synthesis of messenger RNA (mRNA)—is arguably king. With its 12 distinct protein subunits, Pol II carries out intricately regulated transcription and, in so doing, is the direct target of a diverse collection of transcription factors that control its activity at the preinitiation, initiation, and elongation stages of transcription. Despite the successful identification of many of these transcription factors, how they interact with their binding sites on the surface of Pol II and control its activity has remained a mystery, in large part because of a lack of high-resolution information about the structure of Pol II. Its large size, structural complexity, low abundance in cells, and fragility have rendered Pol II

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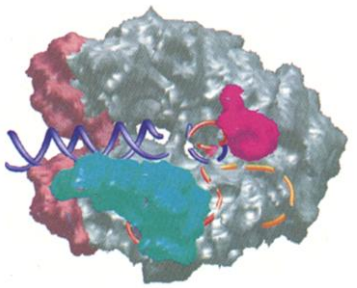
nearly inaccessible to structural biologists. But, in a research article on page 640 of this issue, Kornberg and co-workers (*3*) report the results of a structural odyssey that began nearly 20 years ago. They have achieved their long-sought goal—determining the high-resolution structures of Pol II and its complexes—and now present the ~3.5 Å resolution portrait of this giant enzyme.

Intense biochemical efforts over the past two decades have led to the purification and characterization of a large number of transcription factors that interact with Pol II. These include the general initiation factors TFIIB, IID, IIE, IIF, and IIH, which assemble together with Pol II at promoters and activate adenosine triphosphate–dependent unwinding of the DNA template and transcription initiation; the mediator complex (comprising more than 20 protein subunits), which behaves as an intermediary, enabling essential communication between Pol II and the myriad sequence-specific transcriptional activators and repressors that bind to DNA and control mRNA synthesis in response to extracellular cues; and elongation factors such as SII, ELL, and elongin, which interact directly with Pol II during transcription and expedite mRNA elongation by suppressing arrest or transient pausing of the enzyme at its many favorite "watering holes" along the genes (*4, 5*).

In their article, Kornberg and co-workers report the "backbone" structure of a catalytically active 10-subunit version of the Pol II of the yeast *Saccharomyces cerevisiae*. In light of the marked amino acid sequence similarity among eukaryotic Pol IIs, the yeast Pol II structure will likely turn out to be an excellent model for the human enzyme. The reported Pol II structure traces the peptide backbone of all 10 subunits, accounting for 83% (or 3219) of the total predicted ordered residues. It establishes unequivocally the relative positions of the 10 subunits in the enzyme and their contributions to the active site. In addition, it allows a detailed model to be proposed for the nucleic acid binding domains, first glimpsed at substantially lower resolution by two-dimensional (2D) electron crystallography (*6, 7*).

The Pol II structure is not only a landmark achievement in x-ray crystallography, but is also a testament to the tenacity of Kornberg and co-workers. To cope with the large size and structural complexity of Pol II, they pioneered the development of general methods for preparing 2D protein crystals on lipid bilayers that could be used to determine low-resolution structures of large multiprotein complexes by electron crystallography. Because the fourth and seventh largest Pol II subunits, which are dispensable for catalytic activity, are present in substoichiometric amounts, crystallization required use of the 10-subunit "deletion enzyme" lacking these two subunits. Analysis of 2D crystals of elongating 10-subunit Pol II provided a ~16 Å view of the polymerase and offered the first fuzzy images of the enzyme's active site and nucleic acid binding domains (*7*). Their discovery that 2D Pol II crystals would efficiently seed formation of 3D crystals provided an entrée into the realm of x-ray crystallography. Unfortunately, these initial 3D Pol II crystals did not diffract x-rays efficiently. This meant that meaningful x-ray data could be obtained only after several improvements in crystal preparation. These include using an oxygen-free environment to avoid damage to Pol II during purification and development of a crystal soaking procedure that allowed preparation of crystals with homogeneous unit cell dimensions (*5*).



Staring into the jaws of Pol II. Yeast RNA polymerase II (Pol II) bound to the DNA template and the elongating mRNA transcript. About 20 base pairs of duplex DNA (dark blue) downstream of the catalytic site is gripped by a movable clamp formed by portions of Rpb1, Rpb2, and Rpb6 of Pol II (blue) and a pair of jaws formed by Rpb5 and portions of Rpb1 and Rpb9 (light pink). Orange or yellow dashed lines correspond to possible paths of ~10 bases of mRNA lying in grooves that lead away from the RNA-DNA hybrid. A "wall" or "flap" (bright pink) is located near the catalytic site in the channel between Rpb1 and Rpb2.

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The new high-resolution Pol II structure reveals the overall architecture of the enzyme. More important, taken together with the 3.3 Å structure of a bacterial RNAP recently reported by Darst and colleagues (*8*), this structure provides insights into the molecular bases of the extraordinary stability and high processivity of the elongation complex (Pol II, the DNA template, and elongating mRNA chain), as well as its propensity to pause and arrest. In Pol II, the two largest subunits, Rpb1 and Rpb2, interface to form the core of the enzyme with the smaller subunits on the surface (see the figure). The catalytic site Mg^{2+} ion lies at the end of a deep cleft formed between Rpb1 and Rpb2 and corresponds to the 25 Å "channel" identified in earlier, lower resolution structures. This cleft or channel can house ~20 base pairs of the DNA template downstream of the catalytic site. A formidable pair of jaws formed by Rpb5 and portions of Rpb1 and Rpb9 surrounds the channel near the leading edge of the elongation complex and may enhance the stability and processivity of the elongation complex by gripping the DNA template downstream of the catalytic site. Either of two "grooves" beginning near the upstream end of the transcription bubble could increase the stability of the elongation complex by binding the RNA transcript in a region ~10 to 20 nucleotides downstream of the catalytic site. A hinged domain, formed by portions of Rpb1, Rpb2, and Rpb6, could act as a "sliding clamp" that encircles the DNA template and locks it in place (*9*) while still allowing movement of the DNA within Pol II during both mRNA chain elongation and backsliding. Intriguingly, juxtaposition of one of the possible RNA grooves and the DNA clamp make it likely that a transcript occupying the groove could lock the clamp in the closed position, perhaps accounting for the marked increase in the stability of the elongation complex after transcripts ~10 to 20 nucleotides long are synthesized.

A "pore" directly below the catalytic site Mg^{2+} ion may function as a conduit to allow entry of ribonucleoside triphosphates into the catalytic site. This pore could also provide a route by which the 3′-end of the growing transcript can exit the catalytic site as a result of backward movement of Pol II on the DNA during pausing and arrest. In addition, this pore could allow the entrance of elongation factor SII, which reactivates arrested Pol II by promoting cleavage of the extruded portion of the RNA transcript.

With the high-resolution structure of Pol II, a new era of investigation of the mechanism and regulation of mRNA synthesis has begun. In addition to its obvious value for researchers investigating the catalytic mechanism of Pol II, the new Pol II structure will enable a more rational assault on the mechanisms of action of the host of transcription factors that regulate the enzyme. In the future, these transcription factors will be defined in large part by their interactions with their targets—the jaws, clamps, grooves, and pores that together constitute the flesh and bones of Pol II, now brought to life at 3.5 Å resolution.

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