Despite the lack of eye-specific segregation of afferents after PND 9 to 25 activity blockade, the afferent projections were stereotyped in their patterning and did not represent a random diffuse projection to the entire LGN as when activity is blocked before development of segregation. Instead, the projections from both eyes were concentrated in a region similar to the normal location of lamina A (Fig. 4, C and D) and appeared to avoid lamina A1, indicating a substantial expansion and relocation of the afferent projection from the ipsilateral eye. This spatially restricted but unsegmented pattern suggests that there might be activity-independent cues in lamina A that are relatively attractive to axons from both eyes. In normal animals, activity-dependent competition occurs, and contralateral axons appear to have a competitive advantage, allowing them to take over the attractive real estate of lamina A and force the ipsilateral axons into lamina A1. Without competition, both eyes’ axons would have equal ability to arborize in lamina A and select this region preferentially over lamina A1. The preferential arborization of axons in lamina A seen in this study was not observed in previous studies in which the development of segregation was prevented or retarded by activity blockade or enucleation before establishment of eye-specific layers (3–5, 12, 13).

This difference could be explained if a preference for lamina A is established during the initial axonal segregation (PND 0–9) and requires a period of normal neuronal activity. This study indicates that activity-dependent competition is vital not only for initial establishment of specific connections in the mammalian visual system but also for maintenance of these connections at least for some time during development. The possibility of attractive molecular cues or gradients in lamina A and the interactions between such cues and activity-dependent competition in normal development remain important open questions.

Reports

All known polymerases synthesize nucleic acid in a 5’ to 3’ direction. This feature requires that the two antiparallel strands of DNA be replicated asymmetrically. The “leading” strand is made continuously at the replication fork, whereas the “lagging” strand is formed discontinuously. Because DNA polymerases are incapable of de novo initiation, cells use other mechanisms to prime DNA synthesis throughout replication. In 1971, Kornberg and co-workers proposed that DNA replication initiation required RNA transcription (1). Since then, replication-priming RNA polymerases (primases) have proven central to cellular and many viral replication mechanisms. Primases initiate leading-strand synthesis once and lagging-strand synthesis multiple times during the course of replication. Depending on the organism, primases exist either as individual proteins or as primase-helicase polypeptides; in almost all cases their activities are coupled to the replication by protein-protein interactions with other replication factors (2).

Escherichia coli primase (DnaG) interacts with the replicative DnaB helicase, single-
stranded DNA binding protein (SSB), and DNA polymerase III holoenzyme (3–6). Although DnaG is capable of synthesizing 60-nucleotide-long primers in vitro (3), this primer length is restrained to 11 (±1) nucleotides in the context of the replisome (7). During lagging-strand synthesis of the E. coli genome, DnaG proteins must transcribe about one primer per second (8). Homologs of DnaG have been identified in all prokaryotes, as well as in several bacteriophages.

On the basis of sequence analysis, these proteins appear structurally distinct from primases known to act in archaeal and eukaryotic replication.

DnaG has been shown by proteolysis to comprise three structural domains: a 12-kD NH2-terminal Zn2+-binding domain (ZBD), a 36-kD core domain containing the polymerase region, and a 15-kD COOH-terminal DnaB-interaction domain (DnaB-ID) (9, 10) (Fig. 1A). A recombinantly expressed DnaG core fragment including residues 111 to 433 (DnaG-RNAP) retains the ability to transcribe RNA in vitro, although with a reduced RNA polymerase activity (11). This domain is not expected to be functional during replication in vivo, as it lacks both the DnaB-ID and the ZBD (9).

Purified DnaG-RNAP was crystallized in two distinct forms, depending on whether YCl2 or SrCl2 was included in the crystallization conditions (12). The structure of DnaG-RNAP in the SrCl2 cell was determined by multilayer thin anomalous dispersion (MAD) phasing (Fig. 1B) and was subsequently refined to 1.6 Å resolution (13). The YCl2 form was solved by molecular replacement and refined to 1.7 Å resolution (14) (Table 1). Although a third crystal form grown in the presence of DyCl3 was also obtained, these crystals diffracted to 2.5 Å and were used only for difference-Fourier analysis.

DnaG-RNAP is a modular, cashew-shaped molecule of dimensions 30 Å by 35 Å by 75 Å that is composed of three subdomains (Figs. 1C and 2A). The NH2-terminal domain corresponds to NH2-terminal (residues 115 to 240), full-length DnaG-RNAP. (Fig. 1A). A recombinantly expressed DnaG core fragment including residues 111 to 433 (DnaG-RNAP) retains the ability to transcribe RNA in vitro, although with a reduced RNA polymerase activity (11). This domain is not expected to be functional during replication in vivo, as it lacks both the DnaB-ID and the ZBD (9).
subdomain has a mixed α/β fold that is unique when compared to other proteins in the structural database [DALI (15)]. The central subdomain forms a five-stranded β sheet sandwiched by six α helices. Part of this region belongs to the toprim fold family, as had been predicted by Koonin and co-workers (16). The toprim fold has been observed in a variety of metal-binding phosphotransfer proteins, including nucleases, topoisomerases, and response regulators (16, 17). The COOH-terminal subdomain comprises an antiparallel, three-helix bundle that is structurally similar to regions in a variety of unrelated proteins. The NH2- and COOH-termini of DnaG-RNAP protrude from the same side of the protein and lie ~55 Å apart.

The toprim and NH2-terminal subdomains abut each other to form a shallow, wedge-shaped cleft on the concave side of the protein (Fig. 2, B and inset). All 14 invariant, surface-exposed residues shared among bacterial primase RNAP domains cluster at this cleft surface (Fig. 3A). These residues belong to a series of conserved, primase-specific sequence motifs (18, 19). Mutagenesis experiments have demonstrated that a number of these conserved groups are important for primase activity (20, 21), implying that this region is critical for DnaG function.

In the YCl2-containing crystals, three peaks of positive difference density (>3.5σ) were observed in the DnaG-RNAP cleft (Fig. 3B). These peaks lie only 2.4 to 2.5 Å from the carboxylates of two invariant acidic amino acids (Glu-265 and Asp-309) and are not observed in SrCl2 crystals, implying that they represent Y2+ ions. The same binding surface of DnaG-RNAP also shows 7.5σ difference density in DyCl3 cocryystals, further confirming that this region can bind metal ions. The acidic metal-liganding residues are part of the toprim region of DnaG-RNAP. Superposition of the toprim regions from Mg2+-bound Methanococcus jannaschii topoisomerase VI (22) and Y2+-bound DnaG-RNAP [root mean square deviation (rmsd) = 2.0 Å for 57 Cα atoms] shows that two of the Y2+ ions seen in our DnaG-RNAP structure bind near the Mg2+ position observed in topoisomerase VI (Fig. 3B). Primase activity is known to be metal-dependent, and mutation of the Glu-265 equivalent in the homologous P4 phage primase results in loss of activity (20), suggesting that this region of the cleft serves as the active site for RNA chain elongation in DnaG.

The use of metals by DnaG is consistent with cofactor requirements in other polymerases, but metal coordination in DnaG-RNAP does not use the “palmit metal-binding fold” that is conserved among many other nucleic acid polymerases (Fig. 3C) (23). Instead, DnaG appears to use a simple phosphotransfer domain for metal coordination and thus represents a distinct structural class of polymerases. The structural differences between DnaG and “classic” polymerases may help explain the functional distinctions of primases, which include reduced processivity and lower fidelity (2).

If the cleft represents the catalytic center of DnaG-RNAP, how might this region bind DNA? In the DnaG-RNAP structure, the cleft diameter measures ~9 Å at one end and 20 Å at the other (Fig. 2, B and inset). The narrow mouth of the cleft contains several invariant basic residues (Arg-146, Arg-221, and Lys-229). The mouth opens up to a region that is lined by the metal-binding center from the toprim motif on one side and the highly conserved primase II and III motifs from the NH2-terminal subdomain on the other. Beyond the catalytic region, the cleft broadens to a shallow depression that has both basic and hydrophobic character. We propose that single-stranded DNA (ssDNA) can be threaded through the narrow mouth and that the electrostatically positive ridge of the NH2-terminal subdomain acts as an interaction surface for the template phosphodiester backbone. Synthesis would occur at the metal-binding site on the toprim side of the cleft, with the resulting RNA:DNA duplex extruded into the wide, shallow depression. Mutagenesis experiments in phage P4 (20), coupled with the observation that mutation of the invariant Lys-241 of E. coli DnaG permits transcription initiation but inhibits primer elongation (21), are together consistent with this scheme. This model for nucleic acid binding and synthesis would place the RNA:DNA hybrid proximal to the NH2-terminus of DnaG-RNAP, near the predicted position of the ZBD of the intact DnaG protein.

Primer synthesis in bacteria is coupled to DNA replication by interactions between primase, the replicative helicase, and other replication factors. The precise mechanism by which these processes are coordinated at the molecular level has remained largely obscure. In E. coli, a noncovalent protein:protein interaction links the COOH-terminal domain of DnaG with the DnaB helicase (9) in a 6:1 helicase:primase complex. In T7 phage, whose helicase and primase are homologous to the equivalent eubacterial proteins (19), the COOH-terminal region of the primase is directly linked to the NH2-terminal region of the helicase, forming a single polypeptide. Electron micrographs of the phage T7 primase-helicase polypeptide have shown that the helicase region exists as a large hexameric ring, with primase domains arrayed as smaller lobes about one face of the toroid in a 6:6 helicase:primase arrangement (24) (Fig. 4, inset). ssDNA appears to be threaded through the interior hole of the ring (25), and biochemical studies of the T7 system have suggested a model in which the active site of primase faces outward from the central hole (26) (Fig. 4, left). However, a comparison of
the T7 primase-helicase electron micrograph reconstructions with the DnaG-RNAP structure, coupled with the predicted location of the DnaB-ID of DnaG, suggests that an alternative scheme is possible. The COOH-terminus of DnaG-RNAP localizes the DnaB-ID near the region of the DnaG active site that is proposed to bind RNA:DNA hybrid. A DnaB-ID–helicase interaction may therefore

Fig. 3. Catalytic region of DnaG-RNAP. (A) Proposed active-site and nucleic acid–binding region of DnaG-RNAP stained to demonstrate locations of all invariant (green) and highly conserved (yellow) surface residues. The figure was generated by GRASP (31). (B) Stereo diagram of the putative active site of DnaG-RNAP in the unliganded form (light blue) and Y2-bound form (dark blue). Three Y2 ions are shown as yellow spheres, and a Dy3 ion is shown as a magenta sphere that superposes with the lower Y2 ion. The position of a Mg2 ion bound by the active site of topoisomerase VI (22) (green) is overlaid onto DnaG-RNAP by superposition of the homologous toprim domains. One of the Y2 sites lies only 2.5 Å apart from each of the other sites, implying that the observed Y2 sites probably represent an average binding of Y2 ions about the true Mg2-binding site (or sites) of DnaG. Five invariant-residue side chains are shown: Glu-265, Asp-309 (both Y2-liganding), Asp-311, Asp-269, and Asp-345. Experimental electron-density maps indicate that these residues are in single rotamer conformations. (C) Topology diagrams of the M. jannaschii topoisomerase VI (T-VI) (22), DnaG-RNAP, and E. coli DNA polymerase I Klenow fragment (Pol I) (32) metal-binding domains.

The orders of secondary-structural elements are indicated alphabetically in lowercase (α helices) and uppercase (β strands) letters. NH2- and COOH-termini are indicated in italics, and the location of the “fingers” domain in Pol I is indicated as “F.” Hatched circles in each diagram indicate invariant metal-liganding residues (Glu-265, Asp-309 in DnaG-RNAP), while triangles indicate the positions of nearby invariant acidic residues (Asp-269, Asp-311, Asp-345). Although the spatial arrangements of metal-binding residues are similar between toprim and palm folds owing to chemical restraints on metal coordination, more detailed comparisons between the active sites await studies of primase-template complexes.

Fig. 4. Model for primase structure and function within the replisome. (Inset) Organization of the helicase and primase components of the replisome as observed in the bacteriophage T7 primase-helicase polyprotein (24). Primase (purple) directly abuts the helicase (gold). The lagging-strand DNA is thought to be threaded through the central channel (25, 33). (Left and right panels) Models for the orientation of DnaG with respect to DnaB. DNA is shown in blue with synthesized RNA in red. Regions in gray denote the ZBD and DnaB-ID of full-length DnaG whose positions are inferred from the location of the DnaG-RNAP NH2- and COOH-termini. (Left) The primase active site faces away from the central hole of the helicase (26). ssDNA extruded from the helicase must loop back to reach the primase active site. The direction by which the RNA:DNA hybrid is translocated and ssDNA is extruded are the same (red and blue arrows, respectively). (Right) The DnaG active site faces toward the interior hole of the helicase. Two DnaB protomers have been cut away to show the central hole, where ssDNA from DnaB is guided directly into the DnaG catalytic center for transcription of RNA. The directions of RNA:DNA hybrid translocation and incoming ssDNA are opposed (arrows). Such a model suggests that primer size preferences observed in vitro (3) and in vivo (7) could arise, in part, from steric effects between the primase, helicase, and newly synthesized primer. The directionality of nucleic acid binding to DnaG is indicated as discussed in the text; although a model where DnaG-RNAP binds primer-template in a different conformation cannot be entirely excluded, existing observations agree with the orientation shown.
place the ssDNA-binding mouth of DNA primase from DNA helicase, orienting the active site of primase inward, toward the center of the ring, where it is positioned to accept ssDNA as it is extruded from DNA helicase (Fig. 4, right). Alternatively, it is possible that mechanistic differences between the M.7 helicase-primerase systems lead to different relative orientations of the primase active sites. The true relative locations of these domains awaits high-resolution studies of the primase-helicase complexes in E. coli and phage T7.

References and Notes
12. T5-overexpression plasmids encoding residues 111 to 433 of E. coli DNA helicase-DNA primase [preparation by a hexa-histidine tag were constructed and overexpressed in SG1300/pRPe4 cells. Cells were lyzed by sonication and the extract was clarified by centrifugation. Soluble DNA helicase-DNA primase was purified by applying the lysate to a nickel-affinity column and eluting the protein with 200 mM imidazole. His-tagged DNA helicase-DNA primase was further purified by size-exclusion chromatography and concentration in 10 mM potassium acetate, 0.2 M ammonium acetate, 0.05 M sodium acetate (pH 5.0), 0.1% dioxane.

13. Selenomethionine-incorporated protein was expressed as described [G. D. Van Duyn, R. F. Standaert, A. P. Karplus, S. L. Schreiber, J. Clardy, J. Mol. Biol. 229, 105 (1993)] and was purified as per the unsubstituted protein, except that 2 mM dithiothreitol was included in all purification buffers. Concentrated His-tagged DNA helicase-DNA primase was dialyzed against 10 mM Hepes (pH 7.5), 100 mM NaCl, and diluted to a final concentration of 0.2 to 0.5 mg/ml before crystallization.

14. The structure of the YCl2-based crystal form was solved by molecular replacement with AMORE [J. Navaza, Acta Crystallogr. D 50, 1507 (1994)] and the refined SCl2 structure as an initial model. The molecular replacement solution was refined to 1.7 Å resolution with a Rwork of 20.9% and a Rfree of 26.3%. The coordinates of these metals were estimated to be 1.3 Å with using Refmac/ARP [G. N. Murshudov, A. A. Vagin, E. J. Dodson, Acta Crystallogr. D 53, 240 (1997)]. V. S. Laman and K. S. Wilson, Acta Crystallogr. D 49, 129 (1993). The final model includes residues 115 to 428, with the exception of residues 192 to 194 and 267, for which electron density was not observed. No bond angles for this model fell into either disallowed or generally allowed regions of Ramachandran space.


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Mitotic Misregulation and Human Aging

Danthi H. Ly, 1 David J. Lockhart, 1 Richard A. Lerner, 1, 2* Peter G. Schultz 1, 2*

Messenger RNA levels were measured in actively dividing fibroblasts isolated from young, middle-age, and old-age humans and humans with progeria, a rare genetic disorder characterized by accelerated aging. Genes whose expression is associated with age-related phenotypes and diseases were identified. The data also suggest that an underlying mechanism of the aging process involves increasing errors in the mitotic machinery of dividing cells in the postreproductive stage of life. We propose that this dysfunction leads to chromosomal pathologies that result in misregulation of genes involved in the aging process.

The question of why we age has intrigued mankind since the beginning of time. Extensive studies of model systems including yeast, Caenoramnoblastus elegans, Drosophila, and mice as well as studies of human progeria and cellular senescence have identified a number of processes thought to contribute to the aging phenotype (1). These include the effects of oxidative damage associated with cellular metabolism and genome instabilities such as telomere shortening, mitochondrial mutations, and chromosomal pathologies. To gain greater insights into the mechanisms that control life-span and age-related phenotypes, we have studied gene