SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE AND MOVIE LEGENDS

Figure S1. Corrections, Controls, and Models for the DNA Rotation Experiment, Related to Figure 2

(A) Correcting rotor bead motion for hydrodynamic drag to calculate DNA rotation by the φ29 packaging motor. The rotation angle for the two sample traces shown in Figure 2B is plotted as the observed rotor bead motion (blue) and the derived DNA rotation (cyan). See Extended Experimental Procedures for the detailed procedure of deriving DNA rotation.

(B) Analysis of the simulated bead-rotation signal. In the simulation, the mock DNA was rotated by the packaging motor at a filling-dependent rotation density (red line). The resulting motion of the rotor bead, accounting for biological, thermal, and instrumental noise and error, was simulated (Extended Experimental Procedures). The simulated motion was then analyzed using the same procedure as the experimental data shown in Figure 2D, illustrating that the original filling dependence of DNA rotation can be recovered (black circles; error bars are SEM), thus validating our approach.

(C) Sequence independence of the rotation density. The DNA backbone twist across each basepair was computed for the sequences used in our experiments, based on previously reported values (Wynveen et al., 2008). The results were averaged over every 100-bp window. Note that there were two possible DNA substrates of nearly identical length that could be packaged in these experiments (see Extended Experimental Procedures for details). The local helical twists of the two possible substrates are identical at 34.6 degrees per basepair along the entire sequences (blue and green). If the observed change in rotation density were simply a sequence-dependent effect, the twist per basepair would be required to drop substantially toward the end of the sequence (red), contrary to our calculations.

(D) Simultaneous DNA translocation and rotation by packaging motors on trepanated proheads. Sample packaging traces (dotted lines – complex 1; solid lines – complex 2) show that motors on trepanated proheads rotate DNA (blue) during translocation (red). Trepanated proheads were enriched by freeze-thaw cycling of pRNA-free proheads and subsequently adding pRNA onto the prohead.

(E−F) Putative scenarios in which the DNA-contacting subunit precesses around the ring in successive cycles. (E) The special subunit that makes specific contacts with DNA backbone phosphates precesses around the pentameric ring counter-clockwise by one subunit after each packaging cycle, producing a +58° DNA rotation per cycle. (F) The DNA-contacting special subunit precesses clockwise by one subunit after each cycle, yielding a -86° DNA rotation per cycle. Neither scenario is compatible with the rotation density obtained experimentally (see Extended Experimental Procedures for further discussion).

Figure S2. Sample Packaging Traces at Various ADP Concentrations, Related to Figure 3

These experiments were performed at a fixed ATP concentration of 250 μM. The DNA packaging velocity at a given capsid filling decreases as the ADP concentration increases (Figure 3G).

Figure S3. Using Simulations to Explore the Effect of Each Kinetic Rate in the Chemical Cycle of the φ29 ATPase on the Michaelis-Menten Parameters, Related to Figure 4

(A−C) Dependence of the maximum velocity V_{max}, the Michaelis constant K_M, and their ratio V_{max}/K_M on the ATP-tight-binding rate (A), the ATP-docking rate (B), and the ATP-undocking rate (C). V_{max} is sensitive to changes in $k_{ATP\_tight\_bind}$, but is not affected by changes in k_{ATP_dock} or k_{ATP_undock}.

(D) V_{max}/K_M is insensitive to changes in the rate of ADP release, P_i release, ATP hydrolysis, or condensation of ADP and P_i.

Figure S4. Characterizing the Properties of Long-Lived Pauses (LLPs), Related to Figure 4

(A−B) LLPs occur predominantly during the dwell phase. (A) Left: A sample packaging trace segment shows regular dwells and bursts (blue) and the beginning of an LLP (red). Raw 2500-Hz data are shown in gray. Downsampled 100-Hz data are shown in blue and red. Right: Residence time histogram computed from the downsampled data as previously described (Chistol et al., 2012). The largest peak (red arrowhead) corresponds to the location of the LLP and the smaller peaks correspond to the locations of regular dwells. Two regular dwells serve as anchors (blue arrowheads) for inferring the size of the burst immediately before the LLP and aligning different LLP-containing segments. To remove any filling-dependent variation in the burst size, the segments were scaled such that the separation between the two anchors is always 10 bp. (B) Residence time histograms (thin gray curves) from 159 LLP-containing segments were aligned using their anchor dwells (blue arrowheads at 0-bp and 10-bp) and averaged (thick blue curve). The averaged histogram shows that the LLP peak is located around -20 bp (red arrowhead), i.e., integer multiples of a full burst size from the anchor dwells, indicating that most of the LLPs occur during the dwell phase of the motor's packaging cycle. Inset: Distribution of pre-LLP burst sizes shows a major peak at 10 bp (after the aforementioned scaling to remove the filling dependence of the burst size), again indicating that most LLPs occur during the dwell.

(C−D) Distinguishing LLPs from regular dwells. Dwell time distributions (gray) at low (C) and high (D) capsid filling were fit by Gamma distributions (blue curves). Insets display the tails of the distributions. The long events unaccounted for by the Gamma distributions were designated as LLPs. At 15−30% filling, the longest event is ~0.5 s, whereas at 95−100% filling, the longest event is ~35 s. See Extended Experimental Procedures for further discussion.

Figure S5. Sizes of the Bursts and Burst Fragments at Different Levels of Capsid Filling, Related to Figure 6

(A) Distributions of burst sizes at different levels of capsid filling. Each distribution can

be fit by a sum of Gaussian distributions representing integer 10-bp, 9-bp, and 8-bp burst sizes (dashed black, blue, and red curves, respectively). Solid green curves represent the shapes of the summed distributions. Vertical dashed lines represent the mean burst sizes.
(B) Two sample packaging traces at high capsid filling (80−100%) and low external loads (7−10 pN) exhibiting burst fragmentation (black arrows). Raw 2500-Hz data are shown in gray and downsampled 100-Hz data in black. Stepwise fit to the data highlights dwells and bursts in red and green, respectively. Under these conditions, bursts are either instantaneous or broken up into two resolvable fragments. *s* denotes the size of the first segment and *b* denotes the size of the entire burst.
(C) Normalized sizes of the first resolvable burst segments, *s*/*b*, at different levels of capsid filling. The mean value of *s*/*b* is ~0.5 at all filling levels, consistent with four steps making up each burst and two steps making up each resolvable burst segment.

Movie S1. Rotation of the DNA Concomitant with Translocation, Related to Figure 2

The rotor bead position was tracked using center-of-mass algorithms for transverse position and brightness for longitudinal position. In the Mie scattering regime relevant for imaging micron-sized beads, the bead center appears brighter when it is further away from the camera, and darker when it is closer, as confirmed by manually moving the micropipette (not shown).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Derivation of the Angular Velocity of DNA Rotation

The packaging motor twists the DNA, and the torque stored in the twisted DNA rotates the rotor bead through the viscous fluid. The hydrodynamic drag torque T_{drag} acting on the rotor bead as it rotates can be calculated from the fluid's viscosity η, the rotor bead's radius r, and its angular velocity ω: $T_{drag} = -14\pi\eta r^3\omega$, where $14\pi\eta r^3$ is the drag coefficient γ (Bryant et al., 2003; Happel and Brenner, 1965; Landau and Lifshitz, 1976). During packaging, hydrodynamic drag causes the angle of the rotor bead θ_{bead} to lag by an amount $\Delta\theta_{lag}$ behind the angular rotation of the DNA. This difference is absorbed as unwinding of the DNA, which behaves as a torsional spring and generates an elastic torque $T_{elastic} = (C/L)\Delta\theta_{lag}$ that drives the bead rotation. Here, L is the length of the DNA between the motor and the rotor bead, and C = 460 pN·nm^2 is the torsional modulus of DNA (Bryant et al., 2003). Because at steady state the drag torque must be equal and opposite to the elastic torque, it is possible to estimate the angular lag $\Delta\theta_{lag}$ from $14\pi\eta r^3\omega = (C/L)\Delta\theta_{lag}$. Then the actual DNA rotation $\theta_{DNA} = \theta_{bead} + \Delta\theta_{lag}$. Analysis of the DNA rotation without correction for the hydrodynamic drag of the rotor bead resulted in a biased estimate of the local rotation density, because the rotor bead lagged at the beginning of packaging and then caught up toward the end of packaging (Figure S1A). Note that the DNA-rotor bead system is an overdamped harmonic oscillator whose time resolution is given by the autocorrelation time of the rotor bead $\tau = \gamma L/C$, which is on the order of seconds in this experiment. In deriving the angular velocity of DNA rotation from the data, we used a boxcar-filter of 15-s width, comparable to the autocorrelation time of the system. Thus, the estimate of the DNA angle is averaged over many turnover cycles of the motor. Because the elastic torque saturates at -10 pN·nm due to a denaturing transition (Bryant et al., 2003), the error cannot be uniquely determined from such a high torque. However, such torques were observed for only a few seconds at a time (Figure 2B), and the correction remains valid once the torque again became less negative than -10 pN·nm. Any packaging complex that sustained a saturating torque for long stretches was excluded from the analysis for this reason. Simulations show that transient spikes of apparently saturating torque – of Brownian origin – do not introduce significant error into the analysis (see the next section).

Simulation of Bead Rotation Signal

In order to assess the validity of our analysis of the local rotation density, we performed Brownian dynamics Monte Carlo simulations of the motion of the rotor bead given assumptions about the characteristics of the packaging motor, mechanical properties of the DNA, thermal fluctuation and dissipation, and sources of measurement error. The tether length decreased at a velocity that varied with capsid filling (Rickgauer et al., 2008) (Figure 4B). Random static and dynamic noise comparable to measurement noise in the optical tweezers was added to the tether length. Then we assumed that the packaging motor rotated the DNA at a filling-dependent rotation density similar in form to that displayed in Figure 2D. Next, the response of the rotor bead to the elastic torques generated in the DNA due to motor-induced rotation was added, using the known value

of DNA's torsional rigidity C, and including torque saturation. We also included a hydrodynamic drag torque proportional to the bead's angular velocity following the expression $T_{\text{drag}} = -14\pi\eta r^3\omega$ as shown above. For consideration of the effects of anomalous drag coefficient, see the next section. Finally, a fluctuating thermal torque N_f acting on the rotor bead was added. All of these contributions appear in the overdamped Langevin equation of motion for the rotor bead:

$$-\gamma\dot{\theta}_b = -\frac{C}{L}(\theta_{DNA} - \theta_{rb}) + N_f(t)$$
$$\langle N_f(t)\rangle = 0$$
$$\langle N_f(t)N_f(t')\rangle = 2\gamma k_B T\delta(t - t')$$

The timestep was set to 33 ms, matching the rate of video acquisition and much smaller than the autocorrelation time of the rotor bead in this geometry $\tau = \gamma L/C$, which is on the order of seconds. Twenty-two simulations of packaging while monitoring tether length and rotor bead angle were analyzed for local DNA rotation density using the same procedure as described in the above section. When the lag of the rotor bead was properly accounted for, the original filling dependence of DNA rotation was recovered (Figure S1B).

Consideration of Error in Drag Coefficient of a Bead in Close Proximity to Two Other Beads

The above formula for the drag of a bead rotating about an axis tangent to its surface is true in an unbounded fluid, where the boundary conditions are specified on the bead surface and at infinity. However, in our experimental geometry, there are nearby no-slip surfaces that must be considered. The drag coefficient for systems like this has not, to our knowledge, been treated before either analytically or numerically. To understand the hydrodynamics, we break the system into two smaller problems: the hydrodynamics of a bead close to another bead, and the hydrodynamics of a bead between two surfaces.

The first problem – orbital motion of a bead near another bead – can be simplified into a linear combination of two cases – pure rotations and pure translations – and the results superposed. Because of the linearity of the equations, this result should be exact (Happel and Brenner, 1965). The relevant form of motion can be decomposed into: (I) translation of a bead perpendicular to the line connecting it to another bead, (II) rotation of a bead about the axis connecting it to another bead, and (III) rotation of a bead about an axis perpendicular to the line connecting it to the other bead. Cases I and III were solved in the form of an infinite series by Davis (Davis, 1969). By weighting the values in Davis's work by the component of the orbit in each of those directions, we calculated their contributions to the drag torque. For example, let us consider a ratio $R = 0.1$ (the inter-bead surface-to-surface distance divided by the rotor bead radius), which is a lower bound for our experiment. The additional rotational drag torque is 0.5 times the unbounded ($8\pi\eta r^3$) and the additional translational drag is 0.3 times $6\pi\eta r$. Case II was treated by Jeffery (Jeffery, 1915). In this case, the drag torque for pure rotations is seen to increase by a factor of 0.1 at the same distance. Combining these three results,

$$\gamma_{total} = \gamma_{rot} + r^2\gamma_{trans} \approx 1.1 \cdot 1.5 \cdot 8\pi\eta r^3 + 1.3 \cdot 6\pi\eta r^3 \approx 21\pi\eta r^3$$

That is, the net effect of the proximity of the rotor bead to another bead at this distance (equivalent to 44 nm in our experiment) is to increase the drag coefficient to about 1.5 times the value in an unbounded fluid ($14\pi\eta r^3$). In analyzing the data presented here, we approximated this correction as a function of inter-bead distance by a fit,

$$\gamma = \gamma_0\left(1 + \frac{1.5}{1 + \exp\left(\frac{L}{100}\right)}\right)$$

Here, L is the distance between the rotor bead and the other bead, measured in nm. This fit has no physical basis other than that it reproduces to within ~10% the expected drag increase across the range of R = 10 to 0.1 (equivalent to distances of 4.4 μm to 44 nm in our experiment).

Now, consider the second problem – the hydrodynamics of a bead between two surfaces. There is no available solution for the drag coefficient at an arbitrary position between two walls, let alone at an arbitrary position between two spheres. In analyzing our data, we separately calculated the drag correction due to each nearby bead and then summed the contributions from each. This assumption of superposition was first invoked by Oseen (Happel and Brenner, 1965), although it cannot be justified based on linearity because the boundary conditions used to solve for each individual obstacle are different from the boundary conditions describing the complete system. A recent computational paper, looking at the drag between two plane walls, showed that the error in this assumption is about 5% when the bead is close to one wall, but that superposition is a 60% overestimate midway between the two walls (Swan and Brady, 2010). Given that the drag coefficient of a rotor bead between two other spheres should be less than that of a rotor bead between two walls, we expect that superposition will yield an overestimate of the drag coefficient.

Given all the uncertainties in estimating the drag in our experiments, we sought to assess how these uncertainties propagate into the data analysis. We performed additional Brownian dynamics Monte Carlo simulations of the motion of the rotor bead, including the phenomenological alteration of the drag coefficient at close bead proximities. Once the rotor bead motion had been simulated, the data were analyzed for local rotation density using several different weights for the assumed additional drag, ranging from 0 to 20 times the weight imposed during the simulation. The results did not change (data not shown). Moreover, the range over which the drag coefficient changes significantly (~250 nm) corresponds to less than 1 kbp of DNA packaged. Thus the effect of bead proximity is to bias only the last kbp of data. These simulations indicate that errors in the hydrodynamic drag – both overestimates and underestimates – do not introduce significant bias in the measurement.

Putative Salt- and Sequence-Dependence of the DNA Rotation Density

Wang measured an average DNA helical pitch of 10.4 bp/turn in physiological conditions (Wang, 1979). The helical pitch is known to vary by as much as 1.5% depending on cations present. In the standard packaging buffer containing 25 mM Tris-HCl, 5 mM $MgCl_2$, and 50 mM NaCl, the deviation from the conditions of Wang's experiments is expected to be about -0.3%, well within the error of Wang's measurement. Thus, in this

study we used an average helical pitch of 10.4 bp/turn. An error on the order of 0.3% would generate a shift of about 0.1 °/bp in the rotation density.

The helical pitch of DNA also varies within one molecule, locally correlating with base steps found in the DNA primary sequence. The helical twist per base step has been surveyed for each of the ten distinct base steps using crystallographic, NMR, and electrophoretic data (Wynveen et al., 2008). Based on the consensus solution parameters described by Wynveen et al., we computed the local helical twist along the DNA substrate packaged in these experiments. Note that there were two possible sequences for the DNA substrate, due to the fact that the viral DNA was ligated via an SpeI site to a pool of molecules generated by nearly-exactly bisecting an 11.6-kbp plasmid with SpeI and EcoRI. We computed the local twist for both possible sequences, which differ only in the final 5.8 kbp. The local twist of both possible substrates does not differ significantly from the canonical 10.4 bp/turn (34.6 °/bp) along the entire sequences (Figure S1C). Thus the observed change in local DNA rotation density (Figure 2D) is not a sequence-dependent effect, otherwise the twist per basepair would be required to drop substantially toward the end of the sequence, contrary to our calculations.

DNA Rotation Predicted by Other Scenarios for the DNA-Contacting Subunit

Consideration of the geometry of the motor and the DNA generates a family of scenarios for DNA rotation, and each scenario makes an explicit prediction for the amount of DNA rotation given a certain periodicity of the motor-DNA contact. It is known that both the mechanical cycle and the chemical cycle of the motor reset once every 10 bp at low filling, so we assume that the DNA is contacted in a manner that induces rotation once every 10 bp. We consider alternative scenarios for the sequence of subunits that make specific electrostatic contacts with the DNA in each cycle – i.e., does the contact take place at the same subunit each time, does it precess sequentially around the ring, or does it have some other predictable pattern?

At the beginning of a cycle, the DNA is contacted at angle 0°. If the DNA is contacted every 10 bp, the contact point at the beginning of the next cycle is 346° around the helix (Figure 2E, Left). The simplest model consistent with the -1.4 °/bp DNA rotation is one in which contacts are made every 10 bp by the same subunit located at 0° (Figure 2E, Right). The observed rotation is inconsistent with rotary contact scenarios (Figures S1E and S1F). If contacts proceeded in the counter-clockwise direction around the pentameric motor ring such that the next subunit to make rotation-inducing contacts is located at 72°, it would generate a positive (right-handed) rotation – the opposite sign from what was observed. If contacts proceeded clockwise around the ring, the DNA would be rotated by about -9 °/bp, a much greater magnitude than was observed with any of the packaging complexes we studied.

Dwell and Burst Detection

High-resolution data were selected by evaluating the pairwise distance distribution for each packaging segment as previously described (Chistol et al., 2012). Segments with clear motor stepping were used for further dwell-burst analysis. Dwells were detected using a modified Schwarz Information Criterion (mSIC) algorithm (Chistol et al., 2012).

Briefly, the SIC algorithm is an iterative procedure that fits a series of steps to the data and computes a measure of the fit quality for every round of fitting. The algorithm adds more steps to the fit until the fit quality no longer improves. In the original algorithm (Kalafut and Visscher, 2008), the quality of the fit was assessed via the formula $SIC(j_1, \ldots, j_k) = (k+2)log(n) + nlog\left(\hat{\sigma}^2_{j_1,\ldots,j_k}\right)$, where n is the number of data points, k is the number of steps, and $\hat{\sigma}^2_{j_1,\ldots,j_k}$ is the maximum likelihood estimator of variance when k steps are fitted to the data. Since the original SIC algorithm over-fits experimental data containing colored noise, we introduced an additional penalty factor (*PF*), $SIC(j_1, \ldots, j_k) = PF(k+2)log(n) + nlog\left(\hat{\sigma}^2_{j_1,\ldots,j_k}\right)$, and optimal stepwise fits were achieved using *PF* values of 3−5. The candidate dwells identified by the mSIC method were validated using a kernel density analysis as previously described (Chistol et al., 2012). Since the dwell duration is always much longer than the burst duration, bursts can be treated as instantaneous events when only dwell durations are of interest. To accurately assess the burst duration, the SIC algorithm was run in its original form, thus over-fitting the data such that mini-dwells were assigned during the burst phase, reflecting the finite duration of individual translocation events. The duration of a given burst was then computed as the sum of the durations of mini-dwells between two validated regular dwells.

Distinguishing Long-Lived Pauses from Regular Dwells

The duration of a dwell, t, is dictated by the rates of all the events occurring during the dwell phase: ADP release, ATP loose docking, ATP tight binding, etc. If each individual event is a stochastic process with an exponentially distributed lifetime, then the dwell duration distribution is a Gamma distribution, which is a convolution of several single-exponential distributions. The probability density function for the dwell duration $t > 0$ in a Gamma distribution is given by:

$$G(t; n, \tau) = \frac{t^{n-1} \cdot e^{-t/\tau}}{\tau^n \cdot \Gamma(n)}$$

This expression assumes that the dwell phase contains n exponentially distributed processes with the same mean lifetime τ. If processes occurring in the dwell phase do not have identical lifetimes, then n represents an apparent number of rate-limiting events. A strict lower limit for the number of rate-limiting processes during the dwell phase, n_{min}, can be computed from a set of dwell durations in a model-independent fashion:

$$n_{min} = \frac{\langle t \rangle^2}{\langle t^2 \rangle - \langle t \rangle^2}$$

Here brackets denote averages over the entire data set. n_{min} places a strict lower limit on the number of rate-limiting events. An n_{min} value less than one indicates that there are at least two types of dwells, one significantly longer than the other (Moffitt et al., 2010).

At capsid fillings above 50%, the φ29 packaging traces contain dwells that are considerably longer than would be predicted by a Gamma distribution (Figures 4F, S4C, and S4D). n_{min} values computed from the dwell duration distributions are smaller than unity at capsid fillings above 70% (data not shown), further indicating that the dwell distribution contains a mix of regular dwells and long-lived pauses (LLPs). Because these

two populations may overlap, regular dwells cannot be separated from LLPs by simply applying a duration threshold. Instead we took a probabilistic approach to identify them. The left 90th percentile of the dwell duration distribution was fit by a Gamma distribution (Figures S4C and S4D). The shape of the Gamma distribution is largely determined by the data around the peak, and the fitting result did not change significantly when the fit was performed on the left 70th, 80th, or 90th percentile of the dwells. The resulting Gamma distribution accounts for nearly all the observed dwells at low filling (Figure S4C), but cannot account for a significant portion of very long-lived events at high filling (Figure S4D). The long events unaccounted for by the Gamma distribution were designated as LLPs. As mentioned above, this assignment is probabilistic. For example, at 95−100% filling, a 1.8-s event has a 50% chance of being an LLP, whereas a 20-s event has a 99.99% chance of being an LLP. Gamma distribution fits also provide the apparent number of rate-limiting events in the regular dwell (Figure 4E), in the form of the parameter *n*.

Estimating the Magnitude of Internal Force Using Burst Duration

We found that capsid filling affects both the dwell duration and the burst duration (Figures 4D and 5C). In contrast, the external force applied with optical tweezers, F_{ext}, only affects the burst duration but has little effect on the dwell duration (Figure 5B). Therefore, we used the burst duration, τ_{burst}, as a reporter of the net force acting on the packaging motor through the DNA, which is the sum of the external force applied with optical tweezers and the resisting internal force due to DNA confinement inside the capsid, $F_{net} = F_{ext} + F_{int}$. The dependence of τ_{burst} on F_{ext} at very low capsid filling (when F_{int} is essentially zero) effectively served as the calibration for the force sensitivity of the motor: $F_{net} \approx F_{ext} = \zeta(\tau_{burst})$ (Figure 5B, dashed curve). The magnitude of F_{net} at a given capsid filling was then estimated by applying the force sensitivity calibration, ζ, to the τ_{burst} versus filling data (Figure 5C). Finally, the magnitude of the internal force was inferred by subtracting the known external force from the net force, $F_{int} = F_{net} - F_{ext}$ (Figure 5D).

Predicting DNA Rotation Density from Measured Burst Size

The dsDNA backbone winds by a full 360° across a distance of 10.4 bp – the helical pitch of DNA. During each burst, the φ29 motor packages slightly less than a full helical pitch of DNA. The predicted amount of rotation necessary to bring the same ATPase subunit into perfect register with the DNA backbone phosphates after a burst can be computed as: $\alpha = (d_{BurstSize}/d_{HelicalPitch} - 1)\cdot 360°$. The predicted DNA rotation density was then computed by dividing the rotation angle by the burst size: $\rho = \alpha/d_{BurstSize}$. At low capsid filling (15−30%), we measured an average burst size of 9.8±0.1 bp (Figure 6A). Prior to computing the predicted DNA rotation density, the measured average burst sizes were scaled by 1.02, thus bringing the low-filling burst size to an integer 10.0 bp. This procedure corrected for small systematic errors that had not been accounted for during standard calibration procedures. Note that the size of an individual burst has to be an integer basepair value, because the motor needs to make specific contacts with phosphate groups on one of the DNA strands after each cycle (Aathavan et al., 2009). A non-integer average burst size likely results from a mixture of multiple integer burst sizes. For

example, an average burst size of 9.5 bp at a given filling level suggests that the motor is equally likely to make 10.0-bp and 9.0-bp bursts at this particular filling.

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