standing the biology of obesity and how best to treat it. The propensity of obese persons to sit more than lean individuals has several potential explanations. Rodent studies support the concept that there are central and humoral mediators of NEAT (*10*, *11*). For example, we have shown that a neuropeptide associated with arousal, orexin (*12*), increases NEAT in rats when injected into the paraventricular nucleus (PVN) of the hypothalamus. Preliminary data suggest that PVN injections of orexin also cause dose-dependent increases in standing posture allocation in rats (*13*). Thus, there may be central and humoral mediators that drive the sedentary behavior of obese individuals. The negative relationship between fat mass and movement (Fig. 1B) raises the intriguing possibility that body fat releases a factor that slows physical activity in obesity. However, these data also demonstrate that posture allocation is not the mechanism by which NEAT is modulated with short-term overfeeding. One hypothesis is that this occurs through modulation of energy efficiency; this is an area worthy of future investigation.

These data may also have implications for obesity intervention. One could argue that obese individuals have a biologically determined posture allocation and therefore are destined to become obese. If this were true, obesity would have been as common 50 years ago as it is today. However, obesity rates have increased and continue to do so (*14*). We speculate that obese and lean individuals respond differently to the environmental cues that promote sedentary behavior. If the obese volunteers adopted the NEAT-enhanced behavior of their lean counterparts, they could expend an additional 350 kcal per day. Over a year, this alone could result in a weight loss of ~15 kg, if energy intake remained unchanged. Herein lies the rationale behind nationwide approaches to promote NEAT in small increments (*15*). For example, in Rochester, Minnesota, in 1920 before car use was commonplace the average walk to and from work was 1.6 miles (*16*). If walking this distance to work were reinstituted by our obese subjects, all of whom currently drive to work, an extra 150 kcal per day could be expended. We will need to use similar measures to promote NEAT as an impetus to create an active and dynamic environment in which, for example, dancing supersedes television as a leisure activity. Approaches that succeed in getting people out of their chairs and moving could have substantial impact on the obesity epidemic.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/307/5709/584/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 and S2
References

20 October 2004; accepted 7 December 2004
10.1126/science.1106561

Sequence-Directed DNA Translocation by Purified FtsK

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DNA translocases are molecular motors that move rapidly along DNA using adenosine triphosphate as the source of energy. We directly observed the movement of purified FtsK, an *Escherichia coli* translocase, on single DNA molecules. The protein moves at 5 kilobases per second and against forces up to 60 piconewtons, and locally reverses direction without dissociation. On three natural substrates, independent of its initial binding position, FtsK efficiently translocates over long distances to the terminal region of the *E. coli* chromosome, as it does in vivo. Our results imply that FtsK is a bidirectional motor that changes direction in response to short, asymmetric directing DNA sequences.

DNA translocases are adenosine triphosphate (ATP)–driven machines required for DNA replication, recombination, and transfer within and between cells (*1*–*6*). FtsK is a membrane-bound and septum-localized *E. coli* translocase that coordinates cell division with chromosome segregation (*7*). At times, the product of chromosome replication is a circular dimer rather than two monomers. These dimers are resolved by the XerCD site-specific recombinase at a site near the terminus of replication, termed *dif* (*8*). Recombination between the distant *dif* sites requires FtsK. In addition, at the time of cell division some DNA may remain in the septal region, and FtsK appears to act as a pump to clear this region of DNA (*9*, *10*). FtsK may also promote disentanglement of chromosome termini by means of an interaction with topoisomerase IV (*11*).

Translocases move along DNA or move the DNA if the translocase is anchored. In either case, translocases must be able to move in a specific direction relative to the DNA. Translocase directionality could be determined by strand polarity for single-strand tracking enzymes, nonrandom orientation of translocase binding through localized accessory factors or binding sites, or DNA sequences that affect the enzyme during translocation. In *E. coli*, provocative genetic experiments showed that lambda-phage DNA inserted near *dif* disrupted chromosome dimer resolution in a manner dependent on insertion orientation (*9*). Thus, DNA sequences could be directing FtsK toward *dif* so that it can activate XerCD recombination.

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Here, we measured FtsK translocation on single DNA molecules and showed that FtsK moves fast (5 kb/s) and can work against a heavy load (>60 pN) in an ATP-dependent manner. We directly observed its movement along DNA and found that purified FtsK moves toward *dif*, demonstrating that DNA sequence alone is sufficient to direct FtsK toward its target. Our results are consonant with a model of FtsK in which bidirectional motors alternate activity in response to directing DNA sequences.

Our experiments used the C-terminal motor domain of FtsK ($FtsK_{50C}$) (*12*, *13*). We first asked whether $FtsK_{50C}$ is able to pull DNA against a load, as its role in vivo suggests. To this end, we tethered a single molecule of lambda-phage DNA between two beads, one of which was held on a micropipette (Fig. 1A). The DNA was then extended under constant tension by buffer flow. When $FtsK_{50C}$ and ATP were introduced, the DNA tether rapidly shortened against the applied force, visualized as a decrease in the bead-to-bead distance (Fig. 1A and Movie S1). We conclude that tether shortening results from the formation of an expanding DNA loop closed off by two points of contact with $FtsK_{50C}$. This loop is probably topologically constrained by the translocase, because bulk assays indicate that FtsK generates twin supercoiled regions (*13*). Topological domain formation by translocases may be widespread, as another translocase, human Rad54, has been shown by scanning force microscopy to form supercoiled DNA loops (*14*).

Activity occurred in bursts that were identified by an algorithm that recognizes continuous changes in interbead distance, thus excluding periods of enzyme inactivity (*12*). A plot of DNA extension versus time for a typical experimental run is shown in Fig. 1B. At a constant force from 10 to 15 pN, the velocity of individual bursts from four experimental runs (totaling 108 bursts) averaged 5.0 ± 0.9 kb/s at 25°C (fig. S1), a rate comparable with those of a recent study (*15*). The kinetics of $FtsK_{50C}$ are considerably faster than other DNA translocases assayed in single-molecule experiments (*16*–*20*). Not only is $FtsK_{50C}$ exceptionally fast, it is also unusually powerful. In the same assay, against high loads of 35 to 40 pN, $FtsK_{50C}$ velocity only drops by about half to 2.8 ± 0.5 kb/s.

Although decreasing bead-to-bead distance results from an expanding DNA loop, we also observed that the bead-to-bead distance could increase, as if the DNA loop were contracted. The rate was nearly the same during loop expansion as during loop contraction (Fig. 1B inset), which suggests that contraction too is an active process of $FtsK_{50C}$. This bidirectionality of $FtsK_{50C}$ suggests that it consists of more than a single unidirectional motor (*21*). Occasionally, we saw an abrupt collapse of the loop, consistent with complete release of one of the contact points between the DNA and $FtsK_{50C}$. Translocation and loop formation then immediately resumed, which indicates that $FtsK_{50C}$ remained firmly bound to the DNA at at least one contact point during these events. These rapid reversals along with the small standard deviation of the observed rates make it very unlikely that there are multiple FtsK complexes acting simultaneously.

In a complementary assay for DNA translocation, a molecule of lambda DNA was tethered between two beads as in the reeling assay, but instead of being left free, the second bead was caught in an optical trap, allowing direct measurement of the force exerted on the DNA by $FtsK_{50C}$. As $FtsK_{50C}$ translocated, it pulled the trapped bead toward the micropipette with a concomitant increase in force, indicative of loop formation. As the DNA loop enlarged, $FtsK_{50C}$ experienced an increasing load that was released by reverse translocation or, less frequently, by a dissociation of the protein from one of the DNA contact points.

Visible aggregates of the enzyme, referred to here as particles, were observed at higher protein concentrations, particularly above 100 nM. These particles bound individually to the DNA at random positions as protein was introduced to the flow cell during the optical-trap assay (Fig. 1C and Movie S2). As soon as an $FtsK_{50C}$ particle bound to the DNA, DNA looping was initiated, as evidenced by the shortening of the tether. These particles traveled along the DNA at 4.9 ± 0.9 kb/s, the same rate measured in the reeling assay, thus making it possible to track a single active $FtsK_{50C}$ complex.

Strikingly, the $FtsK_{50C}$ particles consistently translocated along the lambda DNA in the same overall direction (75 out of 75 observations), even though occasionally some would temporarily reverse direction, as in the reeling assay. To be certain that unidirectionality was in fact a property of the translocase, we inverted the DNA molecule with respect to either the optical trap or the two types of beads used. The particles always moved in the same direction relative to the lambda DNA sequence. It is notable that they move in the direction predicted by genetic experiments (*9*). The occasional reversals show that $FtsK_{50C}$ directionality is dynamic; it can change on

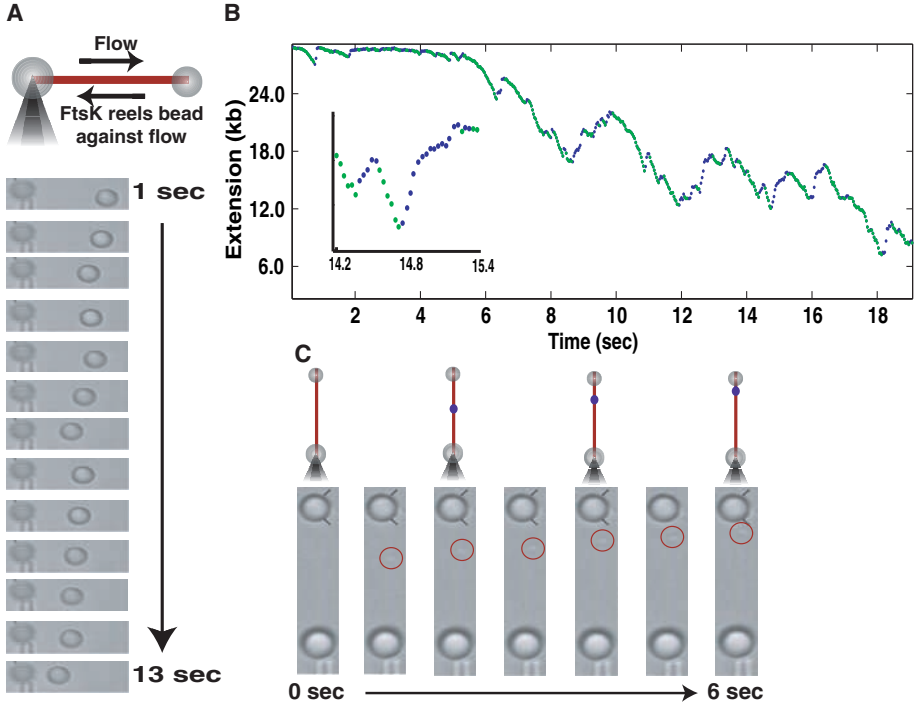


Fig. 1. Measurement of FtsK translocation with two assays. (**A**) In the reeling assay shown, each end of a lambda DNA molecule is attached to a bead. One bead is held by suction through a micropipette. Translocation by FtsK forms a loop in the DNA tether, which reels in the free bead against a flow force of 10 to 15 pN. The shortening of the tether with 100 nM $FtsK_{50C}$ and 3 mM ATP is seen in the video frames spaced at 1-s intervals. (**B**) Tracking of free-bead position as a function of time shows bursts reeling in (green) and reeling out (blue). Reeling in and reeling out velocities are often similar (insert). (**C**) $FtsK_{50C}$ translocation can be directly visualized on single lambda DNA molecules. The DNA was tethered between two beads with one held by a micropipette and the other in an optical trap. The tether was maintained at a constant tension by moving the micropipette in response to DNA looping with an automated force-feedback program. Video frames at 1-s intervals are shown with the FtsK particle surrounded by a red circle.

small scales but is ultimately unidirectional over large distances.

To test the directionality of $FtsK_{50C}$ on its natural substrate, we constructed three DNA tethers from the *dif* region of the *E. coli* chromosome (Fig. 2A). These 27- to 30-kb substrates contain the DNA immediately to the left (L) of *dif* on the chromosome, the right (R) of *dif*, or centered (C) on *dif*. If DNA sequences neighboring *dif* are sufficient to direct FtsK, then the net movement of $FtsK_{50C}$ particles should always be toward *dif*. The particle-tracking assay described above was used to test this hypothesis.

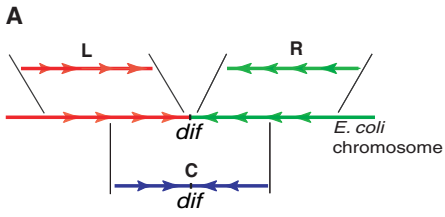
Every $FtsK_{50C}$ particle observed on the L and R substrate (15 of 15) translocated to the *dif* proximal end of the DNA tether. Traces of $FtsK_{50C}$ particles moving on L and R are shown in Fig. 2B. The rate of movement was 4.8 ± 0.9 kb/s for the bursts and 3.2 kb/s overall, the same rate as on lambda DNA. Also, as on lambda DNA, $FtsK_{50C}$ occasionally reversed direction before turning back toward the *dif* proximal end.

In vivo, FtsK must bind to a long DNA molecule (the chromosome) and efficiently find its way to *dif*. The behavior of $FtsK_{50C}$ on our C tether mimics the in vivo situation. Here, the $FtsK_{50C}$ particles moved rapidly toward *dif* from either direction. Upon reaching *dif*, the particles oscillated for a number of minutes around *dif* (Fig. 2B and Movie S3), and their average position coincided with *dif*. These oscillations were only observed on the C substrate and indicate that $FtsK_{50C}$ constantly samples DNA sequence to ensure proper directionality.

Our findings contradict the conclusion of a recent study that FtsK is a sequence-independent translocase (*15*). That study, however, used magnetic tweezers in which DNA is viewed down its axis. Therefore, the position or movement of FtsK could not be detected. In our assay with optical tweezers, the direction of view is perpendicular to the helix axis, and we can readily see FtsK particles. The data in the other study is readily explained by the model we present below.

We tested whether $FtsK_{50C}$ pulls DNA into a loop from two directions at once, as does T antigen (*22*), by measuring simultaneously the position of the $FtsK_{50C}$ particle and the two beads. Analysis of 25 translocation events on the C substrate showed that the DNA shortening occurred only from one direction at any given time. For example, during $FtsK_{50C}$ translocation toward the micropipette, as diagrammed in Fig. 3A, the tether length below the particle decreased, whereas that above it increased slightly as a result of stretching caused by the high forces generated by $FtsK_{50C}$ translocation (Fig. 3, C and D) (*23*, *24*). We conclude that the $FtsK_{50C}$ complex is coupled in such a way that only one motor is active at any given time (*25*).

After observing that DNA enters the loop from only one side of the $FtsK_{50C}$ complex, we used the bead-particle correlation analysis to observe loop release. The DNA in the loop could be released from either side of the complex. However, there would only be net



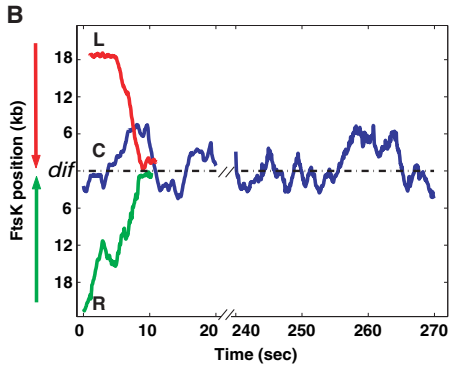


Fig. 2. FtsK translocates toward *dif* in vitro. (**A**) To test FtsK directionality on its natural substrate, the three DNA regions of the *E. coli* chromosome shown were amplified to give products spanning 29.5 kb to the left of *dif* (L), 28.7 kb to the right of *dif* (R), and 27.9 kb centered on *dif* (C). (**B**) $FtsK_{50C}$ quickly translocated toward the *dif* proximal end with both the L (red) and R (green) substrates (15 out of 15 observations). Once the FtsK particle reached the end of the tether, it was obscured by the bead and no longer visible. The particles did not translocate back in the opposite direction even after several minutes. With the C substrate (blue), $FtsK_{50C}$ oscillated about *dif* for 5 to 15 min. The location of *dif* is shown as a black horizontal dot-dash line.

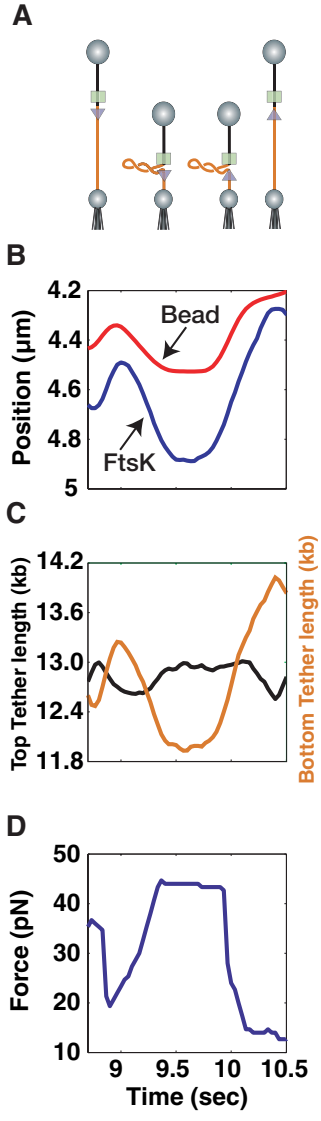


Fig. 3. Simultaneous tracking of FtsK, force on the tether, and flanking DNA lengths demonstrates that DNA is reeled from only one side at a time. (**A**) Diagram of the experiment showing a DNA molecule held between two beads. The portions of the tether above and below FtsK are colored black and orange, respectively, to illustrate that only the DNA below FtsK is reeled into the loop. The upper bead is in an optical trap (not shown), and the lower bead is on a micropipette. The FtsK complex is shown as two coupled bidirectional motors, with the active motor depicted as a blue triangle pointing toward the direction of movement and the inactive one as a green square. Translocation can generate a loop and displace the bead in the optical trap and also release the loop, returning the bead to the trap. (**B**) Correlated movement of the bead in the trap (red) and FtsK particle (blue), corresponding to the diagram in (A). The bead trace is offset to facilitate comparison. (**C**) As $FtsK_{50C}$ translocates down toward the micropipette, only the tether below the $FtsK_{50C}$ complex (orange) decreases in length (25 of 25 observations, including the opposite case in which the $FtsK_{50C}$ complex moves up and the upper tether length decreases), which demonstrates that DNA is reeled from only one side at any one time. The tether lengths are corrected for stretching because of the high forces on the tether. (**D**) The force generated by $FtsK_{50C}$ during this burst.

translocation if the loop were released from the opposite side from which it was formed. We observed all four possible movements, i.e., DNA entering the loop from either side and releasing from either side (fig. S2, A to D). The correlated movement of the bead in the optical trap and the $FtsK_{50C}$ particle provides clear evidence that the only site of looping is at the visible $FtsK_{50C}$ particle. The symmetry of movements seen here supports a model of two coordinated, bidirectional motors, which we propose are rectified by short, asymmetric DNA sequences.

We also observed translocation in the absence of detectable looping, especially at forces above 35 pN (fig. S2, E and F). This type of movement presumably occurs when there is no stable, static FtsK-DNA contact point. Therefore, even though translocation at low forces typically proceeds with two points of contact between the motors and DNA, only one may be needed.

We estimated the strength of the $FtsK_{50C}$ motor using the C tether in the optical-trap assay (fig. S3). The tension in the DNA tether increases as $FtsK_{50C}$ decreases the DNA end-to-end distance by looping and pulls the bead from the center of the trap. We measured the force at which the bead suddenly reverses direction, because the DNA loop is presumably released as a result of a slip at the contact point with the active motor. DNA loops were released at forces ranging from 15 pN to more than 60 pN, with a mean of 35 pN (fig. S3). The upper bound of this force range is close to that which causes a phase transition in DNA (*26*, *27*).

Our working model for FtsK translocation (Fig. 4) depicts the FtsK complex as two bidirectional motors coupled in such a way that only one is active at any given time. The inactive motor remains firmly bound to the DNA, thus creating the second contact point necessary for loop formation. An expanding supercoiled domain is generated between the two motors as one binds the DNA tightly while the other tracks the DNA helix. We propose that each motor is bidirectional to account for all of the patterns of movement we observed in our correlation analyses (fig. S2): The FtsK complex must be able to pull DNA through either motor and also release it through either motor. Net translocation of FtsK occurs, however, only when DNA is taken in through one motor and released through the opposite motor (Fig. 4). It is unclear how FtsK reverses direction, but if it tracks one strand of the DNA duplex, a switch to the complementary strand would cause translocation reversal. Although it is not shown explicitly in Fig. 4, we believe that the net directionality of FtsK is imprinted when it moves past a short, asymmetric sequence whose polarity inverts at *dif*. Directionality is then maintained over a distance long enough to give overall unidirectionality but with occasional switches.

The high speed at which FtsK translocates along DNA (5 kb/s at 25°C) is about the rate expected for this family of translocases in vivo (*2*, *15*). Thus, the simple purified system we studied recapitulates the rate as well as the directionality of translocation in vivo. The ability of FtsK to work against a heavy load is essential for its role in vivo. FtsK is rendered stationary by its insertion into the septum and must move a considerable section of the chromosome burdened with large transcription and translation complexes. Moreover, during translocation FtsK will encounter proteins and RNA bound to the DNA, so the ability to apply force during translocation would allow FtsK to clear these potential roadblocks without slowing down appreciably. The proposed annular structure of FtsK with DNA in the lumen would make FtsK and similar translocases effective "wire-strippers," clearing DNA (*28*) to reset epigenetic and cell-cycle–specific nucleoprotein structures at the apt time of replication termination.

We observe that FtsK changes direction during translocation at several places on the DNA around *dif*, which suggests that the DNA sequences responsible for directionality are widespread. In fact, many short sequences with a skewed orientation flip abruptly at *dif* (*29*), and these have been proposed to bias the direction of FtsK translocation toward *dif* (*9*). The mechanism of FtsK movement could be analogous to the bipolar helicase RecBCD, which switches active motors when it encounters the skewed octamer called Chi in the correct orientation (*30*).

Bulk experiments with several translocases showed that these enzymes efficiently generated (+) and (–) supercoils in a circular DNA (*13*, *31*). Our finding that FtsK forms a stable DNA loop provides a ready explanation for why these opposing supercoils did not cancel: They are segregated into separate domains delimited by the protein. FtsK and other translocases could contribute to domain formation in vivo; the mobility of the contact by FtsK is consistent with recent findings of random placement of domain boundaries in the cell (*32*). The rapid formation of a large loop at the terminus could concentrate catenane links, making decatenation by Topo IV more efficient (*11*, *33*).

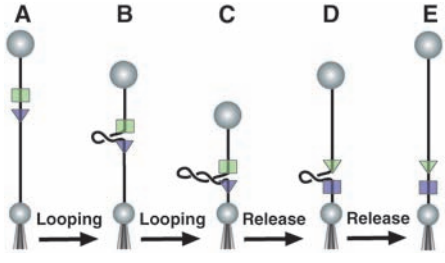


Fig. 4. Model for FtsK translocation along a single molecule of DNA. A nicked DNA molecule is held between two beads. One bead is held with a micropipette, and the other is held in an optical trap (not shown). The two coupled motors in an FtsK complex (green and blue) are shown as a triangle when active and as a rectangle when providing a static contact point. (**A**) The complex binds to the DNA, and the lower motor begins moving downward. (**B**) The DNA is bound statically to the upper motor so that translocation by the lower motor creates a supercoiled DNA loop between the two motors, decreasing the end-to-end distance of the DNA, thus displacing the bead in the optical trap. (**C**) The lower motor continues enlarging the loop, further displacing the optically trapped bead. (**D**) The motors switch activity so that the lower motor becomes static while the upper motor begins pulling out the DNA loop, relaxing the force on the trapped bead. (**E**) The upper motor finishes pulling out the loop, returning the DNA tether to its full extension. The net transfer of DNA from one side of the FtsK complex to the other results in FtsK complex translocation. No net translocation is seen when the bottom motor reverses direction. This and other alternatives are illustrated in fig. S2. The reversibility of the two motors makes it possible for asymmetric DNA sequences (not shown) to dictate the overall direction of FtsK translocation by switching bidirectional motor activity.

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26. It is difficult to measure a conventional stall force, because in almost all cases, the DNA loop releases before the motor velocity falls to zero.
27. Because processive motion is observed against a force of ~63 pN, we can place an upper limit of 1.6 nm (100 pN nm/63 pN) on the step size of the motor. This upper bound was calculated by assuming that a single ATP molecule is hydrolyzed per step and by recognizing that the efficiency must be less than 100%.
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34. We thank T. H. Massey for providing the $FtsK_{50C}$ His-tagged construct and S. B. Smith for expert assistance. This work was supported by NIH grants GM31657 (N.R.C.), GM32543 (C.B.), GM07232-27 (J.L.P.), Wellcome Trust and the Royal Society (D.S.), Ruth L. Kirschstein National Research Service Award, grant GM08295-15 (P.J.P.), Fannie and John Hertz Foundation (J.G.), Damon Runyon Cancer Research Foundation grant 1702-02 (G.J.C.), and U.S. Department of Energy grants DE-AC03-76DF00098, GTL2BN "Microscopies of MolecularMachines," and SNANOB "Design of Autonomous Nanobots" (C.B.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/307/5709/586/DC1
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7 September 2004; accepted 1 December 2004
10.1126/science.1104885

Restoration of Tolerance in Lupus by Targeted Inhibitory Receptor Expression

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Lupus, a multigenic autoimmune condition in which a breakdown of tolerance results in the development of autoantibodies, leads to a variety of pathologic outcomes. Despite the heterogeneity of factors influencing disease susceptibility, we demonstrate that the partial restoration of inhibitory Fc receptor (FcγRIIB) levels on B cells in lupus-prone mouse strains is sufficient to restore tolerance and prevent autoimmunity. FcγRIIB regulates a common B cell checkpoint in genetically diverse lupus-prone mouse strains, and modest changes in its expression can result in either tolerance or autoimmunity. Therefore, increasing FcγRIIB levels on B cells may be an effective way to treat autoimmune diseases.

The ability of the immune system to distinguish self from nonself is central to its ability to protect against pathogens and, at the same time, maintain nonresponsiveness to self. This property is established at discrete checkpoints both during development and in the adult. To date, several early developmental checkpoint mechanisms have been identified. These include the deletion of autoreactive lymphocytes during early development of the immune system (*1*–*3*); anergy, which converts autoreactive cells to a state that precludes them from becoming activated (*4*, *5*); and editing, a mechanism for modifying autoantibodies that renders them nonautoreactive (*6*, *7*). Although these developmental checkpoints purge the immune repertoire of autoreactive cells, the processes of central tolerance remain incomplete, allowing self-reactive cells that express antigen receptors to escape into the periphery (*8*, *9*). In addition, mechanisms that enhance antibody diversity, such as somatic mutation, can generate potentially autoreactive antigen receptors in the adult (*10*). Thus, checkpoints that operate in the periphery of mature individuals are critical for maintaining tolerance and for establishing tolerance to self-antigens that only appear after maturity. Less is known about these peripheral checkpoints, although a principal element has emerged whereby the balance between stimulatory and inhibitory signals regulates the activation and expansion of lymphoid cells. Inhibitory signaling, in particular, is a critical feature of peripheral tolerance, providing a means for establishing thresholds for stimulation and for active deletion of autoreactive cells from the peripheral repertoire. Perturbations in inhibitory signaling pathways have been shown to be genetically associated with autoimmunity (*11*, *12*).

Genetic studies have associated a large number of loci and candidate genes, in addition to inhibitory signaling pathways, with susceptibility to the development of autoimmune diseases (*11*, *13*). In the context of multifactorial and multigenic diseases such as lupus, it is possible that single overriding factors may ultimately dictate whether the disease progresses or not. The selection and proliferation of immunoglobulin G (IgG)–producing B cells represents one such overriding peripheral checkpoint that is under the potential control of inhibitory signaling pathways.

Our previous work demonstrated that the expression of the inhibitory Fc receptor FcγRIIB was required for the maintenance of tolerance (*14*). C57BL/6 mice that are deficient in this receptor develop spontaneous lupus-like autoimmunity and progress to fulminate glomerulonephritis and premature mortality (*14*). Studies of bone marrow transfer into recombinase-activating gene (RAG)–deficient mice suggested that FcγRIIB deficiency in the B cell compartment is most likely responsible for the loss of tolerance seen in these mice. In support of this idea, several strains of mice that develop spontaneous autoimmune disease, such as NZB, NOD, BXSB, and MRL/lpr, have also been shown to express reduced levels of FcγRIIB on activated or germinal-center B cells. This reduced expression results from a polymorphism in the promoter of this gene (*15*–*18*). These results suggest that the absolute level of FcγRIIB expressed on some B cells may regulate the ability of these cells to maintain tolerance and that relatively small changes in the expression of this inhibitory receptor may permit the survival and expansion of autoreactive cells. To test this hypothesis, we developed retroviral vectors that are capable of expressing FcγRIIB upon transduction of bone marrow cells, which can restore the wild-type level of FcγRIIB to B cells derived from autoimmune-prone strains. Bone marrow was derived from the autoimmune-susceptible strains NZM 2410, BXSB, and B6.*Fcgr2b*$^{-/-}$ and transduced with either FcγRIIB-expressing retrovirus or parental (mock) virus lacking FcγRIIB. The bone marrow of irradiated recipients was reconstituted with autologous retroviral-transduced bone marrow, and the mice were followed for the development of autoimmunity and autoimmune disease. Mice that received autologous bone marrow transduced with the parent virus developed autoimmune disease and had reduced viability comparable to that of unmanipulated autoimmune-prone strains (Fig. 1A). In contrast, mice that received autologous bone marrow transduced with FcγRIIB-expressing retrovirus showed improved survival.

The basis for this protection was investigated by examination of the immune status of

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