104-Plat
Load Dependence of the Degree of Cooperativity of the Unwinding of Dna Helix
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We use a dual-laser optical-tweezers (DLOT, Smith et al., *Science*, 271:795, 1996) to define the highly cooperative conformational transition in the molecule of DNA, where the natural B-DNA is converted into a new overstretched conformation called S-DNA (Bensimon et al., *Phys. Rev. Lett.* **74**, 4754, 1995; Cluzel et al., *Science* **271**, 792, 1996). Single molecules of double stranded λ-phage DNA are stretched at 27 °C with the DLOT either with ramp length changes or with force step staircases. The microchamber solution contains 150 mM NaCI, 10 mM tris-HCl, 1 mM EDTA, pH 8.0. When the DNA molecule is stretched in length clamp mode, it shows the previously described highly cooperative overstretching transition at ~60 pN, attributed to unwinding from the B-form to the 1.7 times longer S-form. Stretching the molecule in force clamp mode with a staircase of force steps (ΔF, step size1-5 pN) at 5 s intervals shows different amount of DNA elongation (ΔL) for a given clamped force F depending on ΔF The peak of the Gaussian fit to the ΔL-F relations is at ~60 pN independently of the step size, but the σ of the Gaussian is smaller at smaller ΔF. The L-F relations obtained by integrating the Gaussian curves,fitted with the Hill sigmoid equation, show a Hill coefficient n (an estimate of the order of the underlying transition) that increases with the reduction of ΔF. n is 20, 70 and 125 with $\Delta F = 5$, 2 and 1 pN respectively. These results demonstrate the importance of fine force clamp to establish the degree of bps cooperativity for the unwinding of DNA helix. Supported by MiUR, Ente Cassa di Risparmio di Firenze (2007. 1421) and ITB-CNR (Milano).

105-Plat
Unraveling the Structure of a Single DNA During Overstretching Using Multicolor Fluorescence Imaging
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Single-molecule manipulation techniques have provided a comprehensive understanding of the elastic behavior of DNA. We now know that its double-helical structure yields a significant persistence against bending on length scales of 150 base pairs and smaller. Interestingly, optical tweezers studies have also revealed, that at 65pN DNA undergoes a phase transition to another structure with significantly different elastic properties. Over a very narrow force range the polymer gains ~70% in contour length, and becomes significantly more flexible.
Until now the basic microscopic structure of overstretched DNA is under debate. Two qualitatively different models disagree on the molecular mechanism of the overstretching transition. The first one suggests that the DNA double helix unwinds to form a new structure, named S-DNA, which is usually depicted as a ladder with intact base pairing. The second model states that DNA overstretching is a force-induced melting transition, in which the hydrogen bonds between the two strands gradually break to yield single-stranded DNA, similar to thermal melting.
Using a combination of fluorescence microscopy and optical tweezers we directly visualize the DNA overstretching transition and demonstrate that it is driven by melting of the double-stranded DNA. In the experiments we use intercalating dyes and fluorescently labeled single-stranded binding proteins to specifically visualize double- and single-stranded segments in DNA molecules undergoing the transition. Our data unambiguously show that the overstretching transition comprises a gradual conversion from double-stranded to single-stranded DNA, in agreement with the force-induced melting model. Interestingly, not predicted by either model, we found that melting is nucleation-limited, typically initiating from DNA extremities and nicks.

Platform J: Calcium Fluxes, Sparks, & Waves

106-Plat
Calcium Flickers Steer Cell Migration
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Well-organized calcium signal in space, time and concentration is essential to directional movement, which is common to all cell types during development and critical to tissue remodeling and regeneration after damage. However, to date, it remains perplexing how calcium regulates the dynamics of leading lamella, which is the signalling and motility centre of a migrating cell, contains numerous effector proteins that require high levels of calcium for activation. Here we visualise, for the first time, high-calcium microdomains (''calcium flickers''), which are most active at the leading lamella of migrating fibroblasts, displaying a 4:1 front-to-rear polarisation opposite to the global calcium gradient (Fig). Calcium flicker activity is dually coupled to membrane tension (via TRPM7, a stretch-activated Ca^{2+}-permeant channel of the transient receptor potential superfamily) and chemoattractant signal transduction (via type 2 inositol 1,4,5-trisphosphate receptors). Interestingly, when exposed to a PDGF gradient perpendicular to cell movement, asymmetric calcium flicker activity develops across the lamella and promotes the turning of migrating fibroblasts. These findings illustrate how the exquisite spatiotemporal organisation of calcium microdomains can orchestrate complex cellular processes such as cell migration.

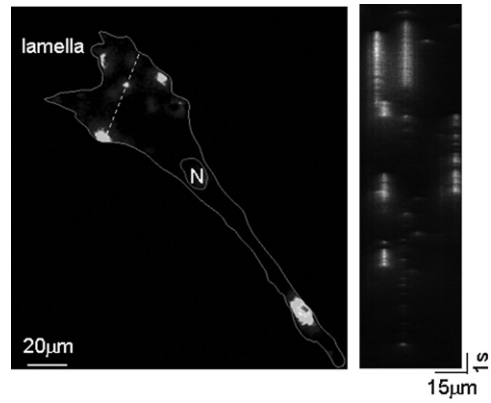

Figure: Calcium flickers in a migrating fibroblast.

107-Plat
Ca2+ Spark Restitution In Ventricular Myocytes With Modified Ryanodine Receptor Gating
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The local release of calcium (Ca^{2+}) through sarcoplasmic reticulum ryanodine receptor channels (RyR) via Ca^{2+} induced Ca^{2+} release (CICR) is called a Ca^{2+} spark. The mechanisms responsible for the termination of Ca^{2+} sparks and refractoriness after termination remain a subject of debate. While local depletion of SR Ca^{2+} is clearly important for spark termination, the roles of SR refilling versus time-dependent changes in RyR gating in refractoriness and recovery are unclear. Using our previous experimental technique, here we further our findings by altering RyR gating behavior with caffeine and tetracaine. Isolated ventricular myocytes loaded with Ca^{2+} indicator fluo-3, were rapidly perfused with 50 nM of ryanodine together with either 100 μM of caffeine, to sensitize RyRs, or 100 μM tetracaine, to inhibit RyRs. At this very low concentration of ryanodine, limited number of RyR clusters have repetitive Ca^{2+} sparks. From confocal linescan images, we analyzed consecutive spark pairs from such clusters to measure Ca^{2+} spark amplitude and the triggering probability recovery. The recovery of Ca^{2+} spark amplitude was insensitive to altered RyR gating, with a time constant of recovery of ~70 ms in all three conditions. Modified RyR gating had a great impact on the distribution of spark-to-spark delays, however. The median delay between consecutive sparks was 169 ms for caffeine, 316 ms for control, and 371 ms for tetracaine. These results indicate that changes in RyR gating affect the recovery of Ca^{2+} spark triggering probability but not the recovery of Ca^{2+} spark amplitude. This strongly suggests that Ca^{2+} spark amplitude is determined primarily by the size of the local SR [Ca^{2+}] store.

108-Plat
Quarky Calcium Sparks in Heart
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Ca^{2+} sparks are the stereotyped unit of Ca^{2+} release in the heart and Ca^{2+} blinks are the reciprocal Ca^{2+} depletion signal produced in the exquisitely