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encode many parameters simultaneously, but the fidelity of encoding at the level of individual neurons is weak. However, because encoding is redundant and consistent across the population, extraction methods based on multiple neurons are capable of generating a faithful representation of intended movement. The realization that useful information is embedded in the population has spawned the current success of brain-controlled interfaces. Since multiple movement parameters are encoded simultaneously in the same population of neurons, we have been gradually increasing the degrees of freedom (DOF) that a subject can control through the interface. Our early work showed that 3-dimensions could be controlled in a virtual reality task. We then demonstrated control of an anthropomorphic physical device with 4 DOF in a self-feeding task. Currently, monkeys in our laboratory are using this interface to control a very realistic, prosthetic arm with a wrist and hand to grasp objects in different locations and orientations. Our recent data show that we can extract 10-DOF to add hand shape and dexterity to our control set. This technology has now been extended has been extended to patients who are paralyzed and cannot move their arms or hands.

Symposium: Genomics and Biophysics

1930-Symp
Sequencing the Unsequenceable: Expanded CGG Repeats in the Human FMR1 Gene
Paul J. Hagerman[1], John Eid[2], Paul Peluso[2], David Rank[2], Jun Yin[1], Luke Hickey[2], Flora Tassone[1], Erick Loomis[1].
[1]University of California, Davis, Davis, CA, USA, [2]Pacific Biosciences, Menlo Park, CA, USA.
Alleles of the FMR1 gene with more than 200 CGG repeats generally undergo methylation-coupled gene silencing, resulting in fragile X syndrome, the leading heritable form of cognitive impairment. Smaller expansions (55-200 CGG repeats) result in elevated levels of FMR1 mRNA, which is directly responsible for the late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). Despite the importance of this gene, no existing DNA sequencing method is capable of sequencing through more than ~100 CGG repeats, thus limiting the ability to precisely characterize the disease-causing alleles.
The recent development of single molecule, real-time sequencing represents a novel approach to DNA sequencing that couples the intrinsic processivity of DNA polymerase with the ability to read polymerase activity on a single-molecule basis. Further, the accuracy of the method is improved through the use of circular templates, such that each molecule can be read multiple times to produce a circular consensus sequence (CCS). We have succeeded in generating CCS reads representing multiple passes through both strands of repeat tracts exceeding 700 CGGs (>2 kb of 100 percent CG) flanked by native FMR1 sequence, with single-molecule readlengths exceeding 12 kb. This sequencing approach thus enables us to fully characterize the previously intractable CGG-repeat sequence, leading to a better understanding of the distinct associated molecular pathologies. The method will enable us to study details of allele-expansion mosaicism and repeat instability in a manner not previously possible. Real-time kinetic data also provides insight into the activity of DNA polymerase inside this unique sequence.
The methodology should be widely applicable for studies of the molecular pathogenesis of an increasing number of repeat expansion-associated neurodegenerative and neurodevelopmental disorders, and for the efficient identification of such disorders in the clinical setting.

1931-Symp
Visualizing Transcription In Vivo at Nucleotide Resolution using Nascent RNA Sequencing
Stirling Churchman.
Department of Genetics, Harvard Medical School, Boston, MA, USA.
It is now clear that transcription is more complicated than what was thought even a few years ago both in the intricate use of post-initiation control and the mass production of rapidly degraded transcripts. Dissection of these pathways requires strategies for precisely following transcripts as they are being produced. Native elongating transcript sequencing - NET-seq, accomplishes this goal by exploiting the extraordinary stability of the DNA-RNA-RNAP ternary complex to capture nascent transcripts directly from live cells without crosslinking. The identity and abundance of the 3' end of purified transcripts are revealed by deep sequencing thus providing a quantitative measure of RNAP density with single nucleotide precision. Our data reveal pervasive RNA polymerase pausing and backtracking throughout the body of transcripts. Average pause density shows prominent peaks at each of the first four nucleosomes indicating that nucleosome-induced pausing represents a major barrier to transcriptional elongation in vivo.

1932-Symp
Monitoring Protein Synthesis One Codon at a Time through Ribosome Profiling
Jonathan Weissman[1,2].
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The ability to sequence genomes has far outstripped approaches for deciphering the information they encode. We have developed a suite of techniques based on ribosome profiling (deep sequencing of ribosome protected fragments) that dramatically expand our ability to follow translation in vivo. I will present recent applications of our ribosome profiling approach including the following: (1) Development of ribosome profiling protocols for a wide variety of eukaryotic and prokaryotic organisms. (2) Uses of ribosome profiling to globally monitor when chaperones, targeting factors or processing enzymes engage nascent chains. (3) Deciphering the driving force and biological consequences underlying the choice of synonymous codons.

1933-Symp
Development of Single Molecule Sequencing System for Direct Single Cell Analysis
Sotaro Uemura.
RIKEN Omics Science Center, Yokohama City, Japan.
The transcriptome is broadly defined as the entire RNA component including non-coding RNA of an individual cell. Recent studies have shown that gene expression is invariably heterogeneous even in evidently similar cell types. Such stochastic variations in the transcriptomes have important implications for cell-fate decisions. Also differences in transcriptomes may provide critical information on the composition of cell types in diseased tissues, including tumors that could contain a small number of cancer stem cells.
Current single-cell techniques typically require several numbers of critical steps of preparation, such as cDNA synthesis or amplification steps. However these steps introduce multiple biases and significant sample loss that hardly reflect the original molecular counts of transcripts at single cell level. To solve these issues we are developing all in one direct single cell analysis system at single molecule detection, which has basically minimized number of steps. In this symposium we report early stage of our progress on the development.

Platform: TRP Channels

1934-Plat
Optical Recording of Single-Channel TRPV1 Activity and Mobility in Isolated Dorsal Root Ganglion (DRG) Neurons and Cultured Mammalian Cells
Eric Senning, Sharona E. Gordon.
University of Washington, Seattle, WA, USA.
TRPV1 is a calcium-permeable non-selective cation channel recognized for its sensitivity to heat, low pH, oxidation and numerous endogenous agonists. To date there is very little known about the activity of the individual channel in the intact cell. In order to explore TRPV1 in this context we employed total internal reflection fluorescence (TIRF) microscopy to observe the open states of the channel as a fluorescent ''sparklet'' at the site where calcium influx occurs. Preliminary investigations into the sparklet activity elicited by capsaicin in DRG neurons indicate that TRPV1 is free to move laterally in the plasma membrane while conducting calcium into the cytosol. Importantly, two-state fluorescent sparklets indicate that channels function independently in vivo. In HEK293T/17 cells transiently transfected with TRPV1-eGFP we simultaneously imaged mobile channels by their eGFP label and their capsaicin-activated sparklets. We implemented both the eGFP photobleaching subunit counting strategy as well as sparklet intensity analysis of TRPV1 sites. Consistent with results in DRG neurons, we found that individual channels, rather than assemblies of channels, were present and functional in the plasma membrane. Further analysis of the subset of mobile sparklets revealed that the mobility of TRPV1 sparklets steadily decreased with its open duration. Activity dependence of TRPV1 mobility may represent a new form of channel regulation in which its function becomes spatially compartmentalized.

1935-Plat
Structural Insights into the Dynamics of the TRPA1 Activation Mechanism
Vera Y. Moiseenkova-Bell[1], Teresa L. Cvetkov[1], Liwen Wang[1], Kevin W. Huynh[1], Gregorio Fernandez-Ballester[2], Sudha Chakrapani[1], Antonio Ferrer-Montiel[2], Mark R. Chance[1].
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TRPA1 is a Ca^{2+}-permeable, non-selective cation channel and one of the key pain sensors in mammals. Pain sensation mediated by TRPA1 involves modification of N-terminal cysteine residues on the channel by thiol-reactive