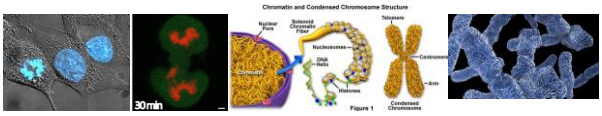
Single-cell-level measurements of transcription heterogeneity of highly mobile identical genes

Enrico Gratton and Paolo Annibale
Laboratory for Florescence Dynamics
University of California, Irvine

AAPM 57th Annual Meeting, Anaheim, 2015

Outline of the presentation

- Motivation: Spatial dynamics of proteins in the nucleus of cells
- Approach: Orbital tracking method
- System:
  - Tracking a gene on the chromatin
  - Detection and localization of gene dynamics
- Discussion/conclusions



Some open questions about chromatin

The elongation rate of Polymerase II (PolII) in eukaryotes varies largely across different cell types and genes

There is not yet a consensus whether intrinsic factors such as the position, local mobility or the engagement by an active molecular mechanism of a genetic locus could be the determinants of the observed heterogeneity

Approach

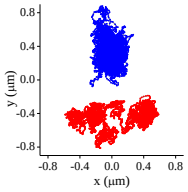
Employing high-speed 3D fluorescence nanoimaging we resolve at the single cell level multiple, distinct regions of mRNA synthesis within a labeled transgene array

This approach allows measuring the transcription kinetics as a function of time with millisecond resolution for total times of thousands of seconds

We can also determine the motion of the transcribed gene and correlate the motion with transcription and with the motion of nearby genes

3-D particle tracking in the nucleus
The orbital tracking method

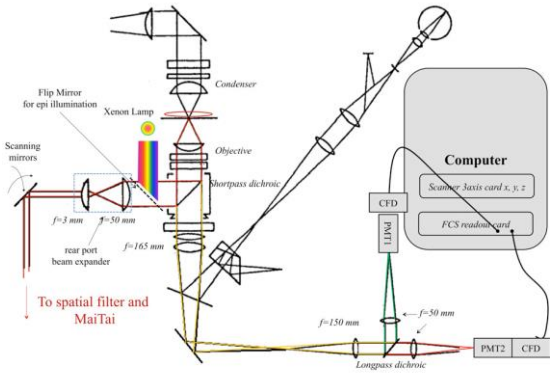
Valeria Levi



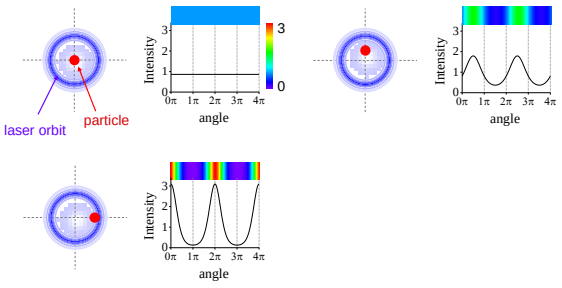
Department of Biological Chemistry, School of Sciences University of Buenos Aires (*UBA*),

Valeria Levi, Qiaoqiao Ruan, Matthew Plutz, Andrew S Belmont, and Enrico Gratton. Biophys J. 2005; 89(6): 4275-85.

Instrument setup

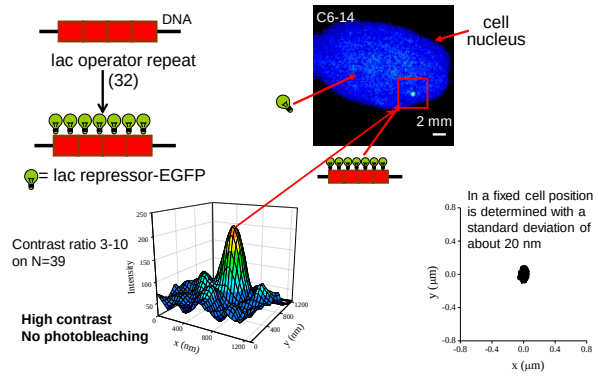


Principle of particle tracking using the orbital scanner



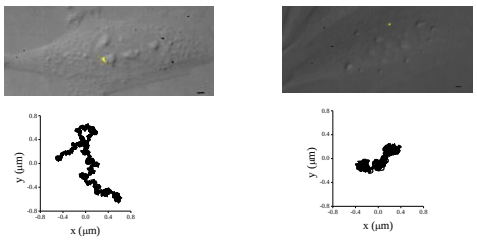
- **Localization precision depends on the photons collected along the orbit**
- **Temporal resolution depends on the sampling time of the intensity along the orbit**
- **3D orbits in space allow 3D tracking at very high speed**

Chromatin labeling *in vivo* (CHO-K1)



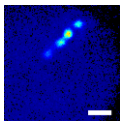
Belmont et al.

Dynamics of the fluorescent spot in the context of structures in the cell and nucleus



- association with nucleolus (54%) or nuclear envelope (32%) (N=59)
- corralled motion
- jumps between regions of confined motion (N=30/59)

Motion of the whole cell/nucleus ?

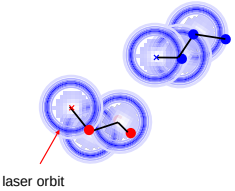


Bar, 2 μm

DHFR-BAC cell line
multiple insertions of Lac-operator repeat

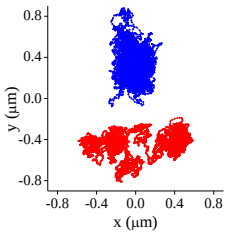
↓

simultaneous tracking of two particles



- particle 1
- particle 2

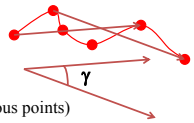
Motion of the whole cell/nucleus ?



jumps in 36% of the cells (N=80)

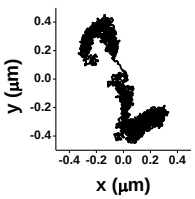
Statistical tests: Analysis of direction/velocity

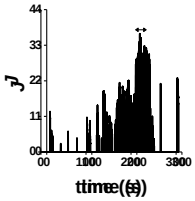
$$J = \frac{d_{vector}}{< d_{vector} >} \alpha$$



$\alpha = 1$ if $\gamma \leq 20^{o}$ (for at least 5 contiguous points)
$= 0$ if $\gamma > 20^{o}$

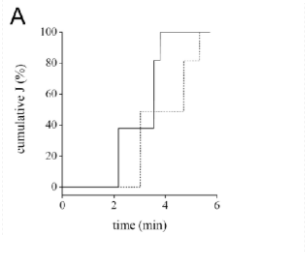
High values of $J \rightarrow$ the sequence is moving fast and in a linear path

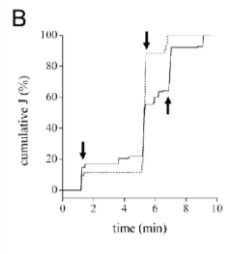




random motion
of the cell

Cumulative J probability identifies correlated movements at two locations in the same chromosome

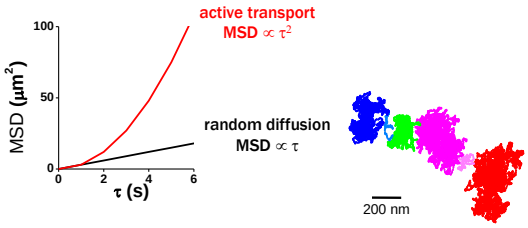




Uncorrelated jumps

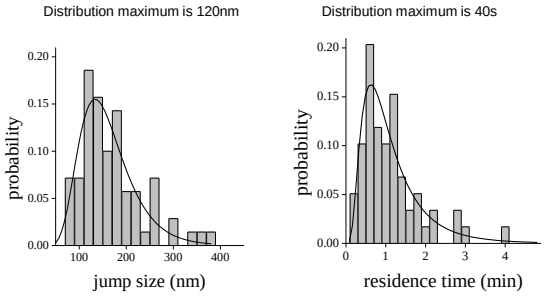
Correlated jumps

Local random diffusion and jumps

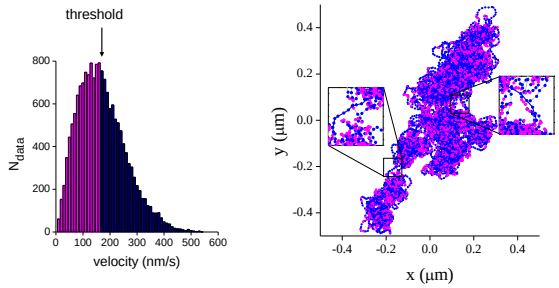


Observation: the trajectory is not due to a "pure" diffusion process. The MSD is not Gaussian (Levy's statistics)

Statistics of jump size and residence time



Statistical tests: Analysis of instantaneous velocity (not MSD!)

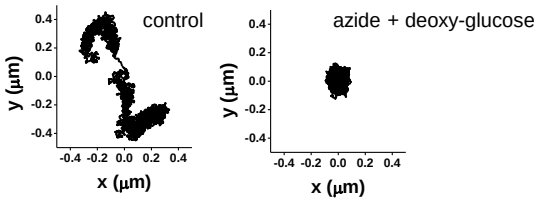


87 % of points in jumps are blue (N=23)

Jumps in the trajectories are ATP dependent

Strategy
- Statistical tests → during the steps the DNA sequence moves "linearly"
- Experimental tests → the jumps are ATP and temperature dependent

ATP depletion



➡ No jumps (N = 30)

Open questions about chromatin

Variability of elongation of Polymerase II (PolII) across different cell types and genes

Intrinsic factors such as the position, local mobility or the engagement by an active molecular mechanism of a genetic locus could be the determinants of the observed variability

Approach

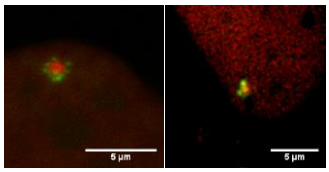
High-speed 3D fluorescence nanoimaging can resolve at the single cell level multiple, distinct regions of mRNA synthesis within a transgene array

We can measure kinetics of transcription with millisecond resolution for total times of thousands of seconds

We can also observe the motion of the transcribed gene and correlate the motion with transcription and with the motion of nearby genes

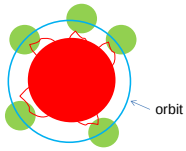
3D Tracking of a DNA Locus During Transcription

Paolo Annibale



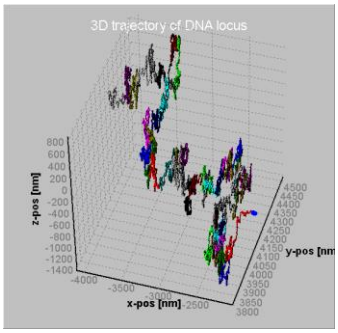
488 nm and 561 nm excitation 910 nm excitation

Adapted from Janicki et al., *Cell* 2004



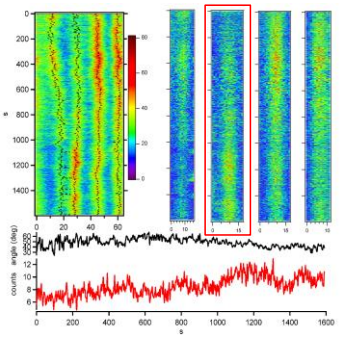
U2OS 2-6-3 Cell line is a courtesy of Dr. Robert Singer (Albert Einstein College, New York) and Dr. Xavier Darzacq (ENS,Paris)

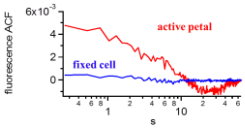
3D Motion of the center of mass of the gene



The resolution of the gene location in 3D is 10 nm, enough to establish distances among genes

Time evolution of the fluorescence intensity in the green channel (MS2) along the orbit

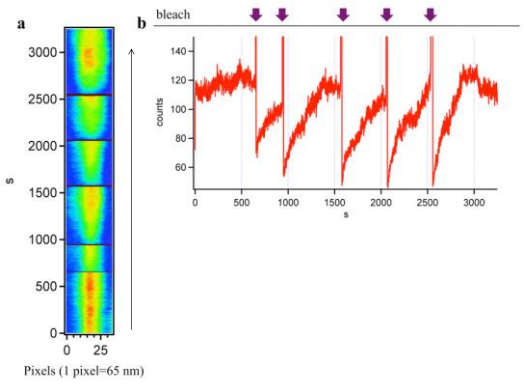




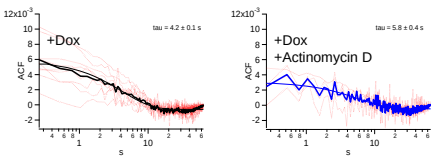
Each loci has a different dynamics even if the same transcript is being produced at the same time.

Some loci are persistently more active than others during times of 1600s

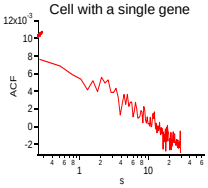
Photobleaching recovery of one individual loci of gene expression



Induction of expression with different treatments and at different time



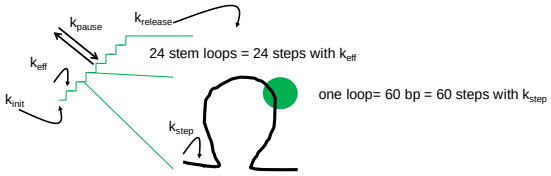
Cell with a single gene

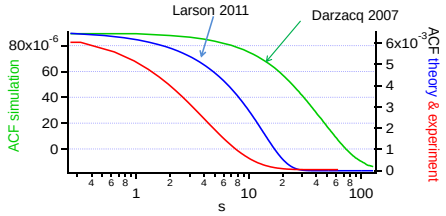


The loci respond to expression regulators

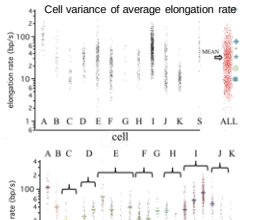
A cell line with a single copy of the gene has very similar behavior than the cell with many genes

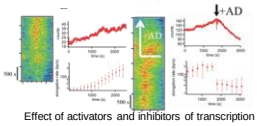
Comparison of experimental and theoretical expectations according to proposed models





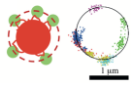
Elongation rate statistics from different loci and different cells



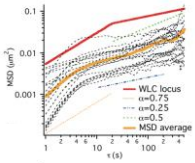


Effect of activators and inhibitors of transcription

Elongation rate is loci dependent



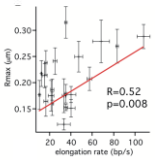
Color coded average rate of elongation for different petals



Black dashed lines: experimental MSD of the petals.

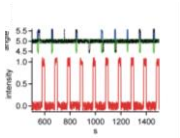
Orange line: average of experimental MSD.
Red line: simulated MSD for a wormlike chromatin fiber at equilibrium.

Most MSD traces display crossover between a $t^{0.75}$ rigid rod regime for time scales <5-10 s (dotted orange line) to $t^{0.25-0.5}$ (dashed-dotted blue and green lines) regime characteristic of entangled fibers between approximately 10-100 s.

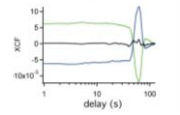


Scatter plot of confinement radius R_{max} .

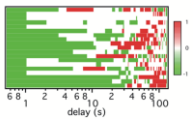
Correlation between angle and intensity



Simulated trace of intensity fluctuations and angular displacement of a petal

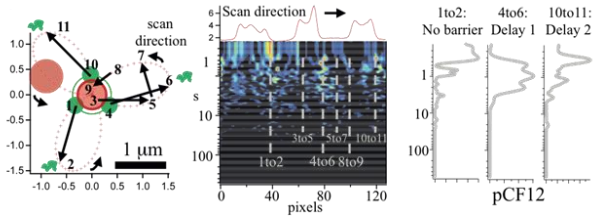


A peak in the cross-correlation function is present only if there is a predominant directional motion.

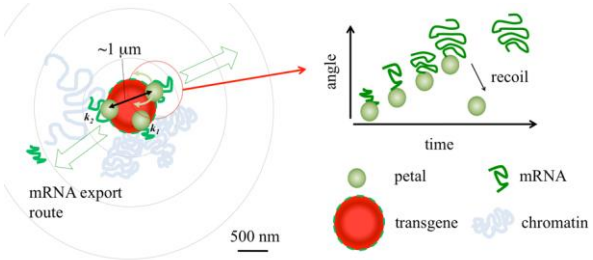


Binary map of cross-correlation of the fluorescence intensity and the angular displacement of the petals, calculated in 128 s time bins. Each line corresponds to a different experiment, and the sign of the cross-correlation amplitude is arranged in order to display negative values first.

Spatial pair cross-correlation at points along the orbit using a tri-fold trajectory



Model of transcription and mRNA export from multimer copies of the same locus within a transgene array



Conclusions

•We followed a loci of gene transcription with nanometer spatial resolution and millisecond time resolution for 1000's of seconds

•In a large transgene array only few sites of elongation occur

•A gene with active RNA synthesis has a spatial dynamics correlated to the RNA elongation and release

•The specific rate of transcription varies between identical genes in the same cell and at the same time

•RNA transcripts follow specific trajectories after release which imply the presence of topological barriers

•Our observations point out to a loci dependent regulation of gene expression

Thank you

Valeria Levi
Paolo Annibale

NIH-P41-GM103540

NIH-P50-GM076516