Show and tell: visualizing gene expression in living cells

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Summary

The development of non-invasive methods of visualizing proteins and nucleic acids in living cells has provided profound insight into how they move and interact with each other in vivo. It is possible to evaluate basic mechanisms of gene expression, and to define their temporal and spatial parameters by using this methodology to label endogenous genes and make reporter constructs that allow specific DNA and RNA regulatory elements to be localized. This Commentary highlights recent reports that have used these techniques to study nuclear organization, transcription factor dynamics and the kinetics of RNA synthesis. These studies show how imaging gene expression in single living cells can reveal new regulatory mechanisms. They also expand our understanding of the role of chromatin and RNA dynamics in modulating cellular responses to developmental and environmental signals.

Key words: RNA, Chromatin, Gene expression, Live cell imaging, Nuclear organization, Transcription factor

Introduction

The use of auto-fluorescent proteins cloned from jellyfish (green fluorescent protein, GFP) and reef coral (red fluorescent proteins, RFPs) as protein-labeling tools revolutionized our understanding of how factors are organized in cells, and how they interact with each other and the cellular environment (reviewed in Belmont, 2003; Misteli, 2001; Phair and Misteli, 2000; Shaner et al., 2005). Because expression of GFP fusion proteins is a non-invasive labeling technique, not only can it be used to track changes in factor dynamics over time in single cells, but artifacts caused by fixation, cell permeabilization and non-specific antibody labeling can be avoided. Mutagenesis of GFP and the RFPs has produced spectral variants and monomeric forms of these proteins that are brighter, more photostable, less toxic and can be used for multi-color labeling in single cells (reviewed in Shaner et al., 2005). Additionally, improvements in automated fluorescence imaging systems and camera sensitivity have reduced the phototoxicity that cells experience – as demonstrated by the fact that they can be imaged throughout the cell cycle and during mitosis (Fang and Spector, 2005), DNA replication (Kitamura et al., 2006) and transcriptional activation (Janicki et al., 2004).

This technology has also revealed much about the dynamic workings of the nucleus. For example, ‘snapshot’ images suggest that nuclear bodies and chromatin domains are composed of statically associated proteins. However, photobleaching of these GFP-tagged factors revealed that many constantly exchange with the diffusing population (reviewed in Belmont, 2003; Misteli, 2001; Phair and Misteli, 2000). Therefore, many nuclear structures are, in fact, in a dynamic equilibrium between assembly and disassembly. Additionally, continual diffusion of factors throughout the nucleus means that they are able to assemble rapidly into functional complexes upon encountering sites and substrates for which they have high binding affinities. For example, DNA repair factors rapidly accumulate at sites of DNA damage (Essers et al., 2006), and entry into S-phase triggers the localization of DNA replication factors to replication forks (Leonhardt et al., 2000). Therefore, investigating the temporal and spatial regulation of nuclear factor dynamics has provided important insight into how cells respond to changing stimuli.

Live cell gene expression systems

By combining GFP/RFP with techniques to label specific nucleic acid sequences in living cells, it has also been possible to examine the dynamics of interactions of regulatory factors with cis-acting sequence elements. DNA and RNA sequences, which can be visualized by expressing GFP-tagged binding proteins, can be introduced into cells adjacent to inducible genes or transgenes, and chromatin and RNA can be monitored during transitions between activation and silencing, during differentiation, and throughout the cell cycle. Repetitive arrays of bacterial and mammalian DNA sequence elements and intact mammalian promoters containing multiple transcription factor (TF)-binding sites have been used to visualize chromatin (see Fig. 1). Stable expression of these constructs produces cell lines with multi-copy transgene arrays, which can be specifically localized in live interphase cells. It has also been possible to study regulatory dynamics at certain endogenous genes – mammalian ribosomal genes and heat shock genes in *Drosophila melanogaster* polytene nuclei – because of the inherent repetitiveness of their sequences (Dundr et al., 2002; Yao et al., 2006). Generating RNAs that contain arrays of a stem loop structure from the MS2 RNA bacteriophage (the 19-nucleotide translational operator), which will bind to a GFP-tagged MS2-coat-protein construct, has allowed RNAs

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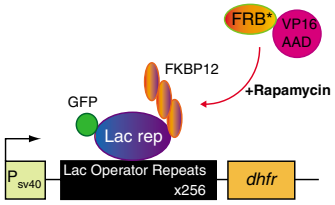
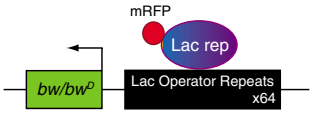
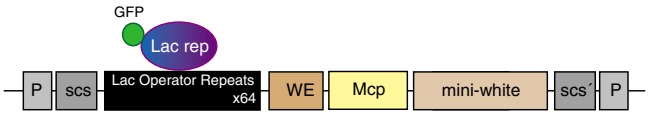
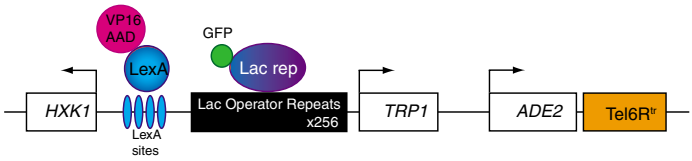
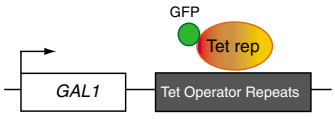
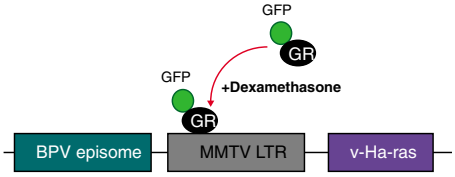
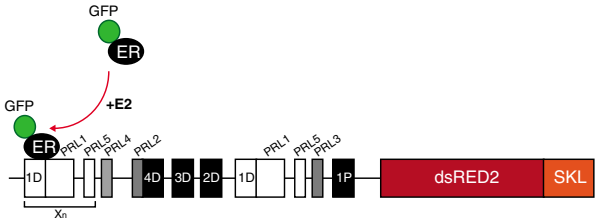
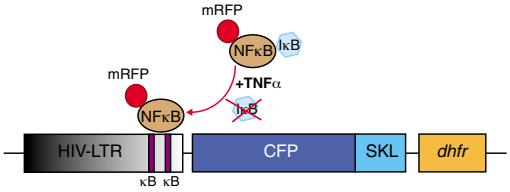
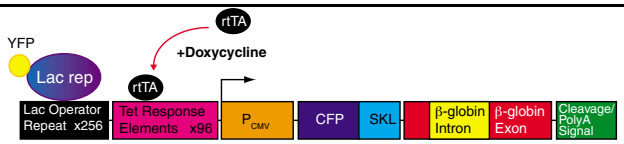
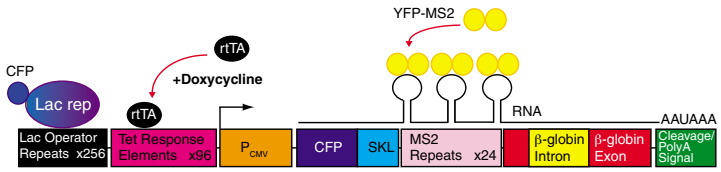
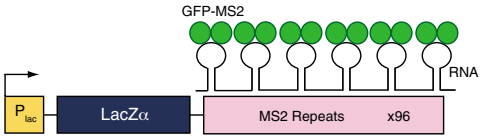
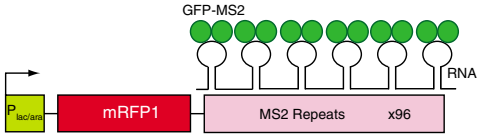
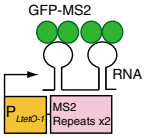
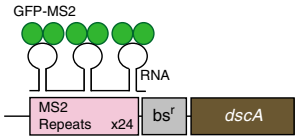
	System name and application	System details	Transcription induction method	Visualization DNA	Visualization RNA	References
A	**LacO-DHFR transgene** Nuclear positioning of chromatin during transcriptional activation		VP16 targeting through rapamycin-induced heterodimerization of FKBP12 and FRP*	Yes	No	Chuang et al., 2006
B	**LacO-tagged *brown* (*bw/bw^{D}*)** Nuclear positioning of chromatin in developing *Drosophila* tissue		Differentiation	Yes	No	Thakar et al., 2006
C	**LacO-tagged P-element-*Mcp*-mini-*white*** Long-distance chromatin interactions in *Drosophila*		Differentiation	Yes	No	Vazquez et al., 2006
D	**LacO-tagged *GAL7-GAL10*** Transcription-dependent nuclear positioning of genes in *Saccharomyces cerevisiae*		Galactose induction	Yes	No	Drubin et al., 2006
E	**LacO-tagged *HXK1*-Tel6R** Transcription-dependent nuclear positioning of genes in *Saccharomyces cerevisiae*		Galactose induction and VP16 targeting through LexA recognition sites	Yes	No	Taddei et al., 2006
F	**TetO-tagged *GAL1*** Transcription-dependent nuclear positioning of genes in *Saccharomyces cerevisiae*		Galactose induction	Yes	No	Cabal et al., 2006
G	**MMTV-LTR-ras-BPV transgene** Dynamic interactions of steroid receptors with their response elements		GR + Dexamethasone	Yes	No	McNally et al., 2000; Rayasam et al., 2005; Stavreva et al., 2006; Stavreva et al., 2004
H	**Pit-1-ERE-dsRED2-SKL transgene** Exchange dynamics of ER at its response elements		ER + 17-β Estradiol	Yes	No	Sharp et al., 2006
I	**HIV-CFP-SKL-DHFR transgene** Exchange dynamics of NFκB at its response elements		NFkB + TNFα	Yes	No	Bosisio et al., 2006

Fig. 1. See next page for legend.

	System name and application	System details	Transcription induction method	Visualization DNA	Visualization RNA	References
J	**LacO-TRE-CFP-SKL-β-globin transgene** Study of spatial organization and timing of gene expression		rtTA + Doxycycline (pTet-ON system)	Yes	No	Tsukamoto et al., 2000; Wang et al., 2006
K	**LacO-TRE-CFP-SKL-MS2-β-globin transgene** Study of spatial organization and timing of gene expression		rtTA + Doxycycline (pTet-ON system)	Yes	Yes	Janicki et al., 2004; Shav-Tal et al., 2004
L	**LacZa-MS2 transgene** Transcription kinetics in *Escherichia coli*		IPTG induction	No	Yes	Golding and Cox, 2004
M	**mRFP1-MS2 transgene** Transcription kinetics in *Escherichia coli*		IPTG / arabinose induction	No	Yes	Golding et al., 2005
N	**MS2 transgene** Transcription kinetics in *Escherichia coli*		Tetracycline induction	No	Yes	Le et al., 2005
O	**MS2-tagged *dscA*** Transcription kinetics in *Dictyostelium*		Differentiation	No	Yes	Chubb et al., 2006

ara, arabinose; BPV, bovine papilloma virus; bs, blasticidin; *bw*, *brown*; bw^D, $brown^{Dominant}$; CFP, cyan fluorescent protein; DHFR, dihydrofolate reductase; *dscA*, discoidin Ia; dsRED2 – red fluorescent-protein variant; E2, 17-β estradiol; ER, estrogen receptor; ERE, estrogen-responsive element; FKBP12, FK506-binding protein, molecular mass 12 kDa; FRB*, FKBP rapamycin-binding domain of FKBP-rapamycin-associated protein; GFP, green fluorescent protein; GR, Glucocorticoid Receptor; HIV-LTR, human immunodeficiency virus 5′ long terminal repeat; *HXK1*, hexokinase isoenzyme 1; IκB, inhibitor of κB; κB, NFκB-binding site; Lac rep, Lac-repressor protein; MMTV LTR, mouse mammary tumor virus long terminal repeat; mRFP, monomeric red fluorescent protein; NFκB, nuclear factor κB; P, P-element; P_{CMV}, human cytomegalovirus promoter; Pit-1, pituitary-specific transcriptional factor 1; $P_{lac/ara}$, Lac/ara promoter; $P_{LtetO-1}$, Tet promoter; P_{SV40}, Simian virus 40 promoter; rtTA, reverse tet transactivator; scs/scs', insulator elements; SKL (Ser-Lys-Leu), peroxisome-targeting peptide; Tel6R, telomeric region on the right arm of chromosome VI; Tet rep, tetracycline-repressor protein; TNFα, tumor necrosis factor α; TRE, Tet-response element; v-Ha-ras, Harvey viral ras; VP16 AAD, VP16 acidic activation domain; WE, *white* enhancer; YFP, yellow fluorescent protein.

Fig. 1. Live cell gene expression imaging systems. The table shows schematic representations of the systems discussed in this review. It also includes information on their DNA/RNA visualization and transcriptional activation methods. Reports of other Lac repressor targeting constructs have not been included, for simplicity.

to be tracked in living cells (Bertrand et al., 1998). These DNA- and RNA-visualization methods have been used, separately and in combination with various transcriptional induction techniques (see Fig. 1), to examine gene expression in single living cells.

The experimental systems developed using these techniques offer an unprecedented view of the inner workings of cells. Single-cell live-cell imaging makes it possible to detect transient events that might be missed when examining fixed cells at widely spaced time points or when averaging cell population data. Here, we discuss the studies that have taken advantage of this technology, focusing on the role of nuclear positioning, TF-promoter dynamics and the kinetics of RNA synthesis in the regulation of gene expression.

Nuclear real estate: location, location, location – chromatin and functional positioning

Chromatin organization and nuclear positioning are crucial regulators of gene expression and they functionally influence each other (reviewed in Misteli, 2005; Verschure, 2006). For example, in mammalian cells, chromatin at the nuclear periphery is typically gene-poor and heterochromatic; silent genes are often localized within these domains or nearby (reviewed in Spector, 2003). However, the mechanisms that

establish this organization and their temporal parameters are not well defined. Experiments designed to track specific regions of chromatin in single living cells are revealing how chromatin moves in response to transcriptional activation, how the organization of chromatin domains in undifferentiated cells influences gene expression patterns in differentiated cells and how nuclear positioning regulates transcriptional efficiency. Therefore, investigating how genes interact with the nuclear environment is providing important insight into their regulation.

Chromatin in *Drosophila* and mammalian cells moves mostly by constrained diffusion, and chromatin at the nuclear periphery is more constrained compared with that in internal regions (Chubb et al., 2002; Marshall et al., 1997; Vazquez et al., 2001). The ability of *Drosophila* and mammalian chromatin to move, albeit in a limited way, might mean that it is functionally important for it to sample the nuclear environment. Events such as somatic homolog pairing, regulation of promoters by distant enhancers, and homology searching during DNA repair and recombination allow us to predict that interphase chromatin can move long distances within the nucleus (reviewed in Bondarenko et al., 2003; McKee, 2004; Sung and Klein, 2006). Interestingly, targeting of a VP16-lac-repressor fusion protein to a 10- to 20-copy transgene array containing *lac*-operator repeats (Fig. 1A) induces its relocation from the nuclear periphery to the interior through a series of abrupt curvilinear long-range trajectories interspersed with periods of constrained motion (Chuang et al., 2006). This relocation occurs 1-2 hours after VP16 targeting and the array moves reproducibly back to the periphery at around 3 hours. Also, the movement depends directly or indirectly on actin and myosin, which may thus facilitate the relocation of genes from unfavorable to favorable transcriptional zones. Because these directed movements occur within a narrow time window after VP16-targeting, studying gene regulation in systems that allow chromatin to be observed over time in single cells is crucial for identifying and understanding dynamic mechanisms of gene regulation.

Genetic analyses have long predicted that whether a gene remains active or silent in a specific cell type through multiple cell divisions depends, in part, on the establishment and maintenance of physical interactions between distinct chromatin domains. For example, the interaction of a gene with a region of heterochromatin can cause position effect variegation, and the interaction of a promoter with an enhancer can maintain a gene in the active state (reviewed in Duncan, 2002; Kassis, 2002). Recent studies in developing *Drosophila* eye tissue demonstrate how random chromatin movement establishes these types of regulatory interaction (Thakar et al., 2006; Vazquez et al., 2006). The insertion of a block of heterochromatin into the eye pigmentation gene *brown* (*bw*) causes variegated eye color because it silences both the cis allele ($bw^{Dominant}$) and the wild-type allele by dragging them into an association with centromeric heterochromatin (the wild-type allele is moved as well because of somatic homolog pairing) (Csink and Henikoff, 1996; Dernburg et al., 1996; Harmon and Sedat, 2005). Tracking the *lac*-operator-tagged bw^{D} allele (Fig. 1B) showed that its dynamic movement is not affected until it moves into the vicinity of centromeric heterochromatin, which reduces its radius of confinement and prevents it from moving away (Thakar et al., 2006). Compared with that in differentiated cells, chromatin in undifferentiated cells is less constrained (Thakar and Csink, 2005). Differentiation could, therefore, establish cell-type-specific patterns of gene expression by stabilizing these types of regulatory interactions.

Interactions between gene silencing sequence elements have also been visualized in living cells. The *Mcp* element is a polycomb-response element from the *Drosophila* bithorax complex that can mediate physical interactions between remote regions of chromatin (Muller et al., 1999). When P-elements containing the *lac*-operator sequence, the *Mcp* element, the *white* minigene and the *white* enhancer (Fig. 1C) are visualized in developing eye imaginal discs by expression of GFP-lac repressor, stable pairings between inserts in homologous chromosomes and also in regions remote from one another are seen (Vazquez et al., 2006). Interestingly, the degree of pairing is highest in the eye discs, where the *white* gene is expressed. This suggests that tissue-specific factors and/or transcription facilitate pairing. Therefore, whether a gene remains active or silent in a specific cell type depends, partially, on how interactions between chromatin domains and sequence elements are established and maintained.

In *Saccharomyces cerevisiae*, interactions between active genes and the nuclear periphery have been reported to play important roles in their regulation (Brickner and Walter, 2004; Casolari et al., 2005; Casolari et al., 2004; Menon et al., 2005; Rodriguez-Navarro et al., 2004). Observations of *lac/tet*-operator-tagged galactose-inducible genes (Fig. 1D-F) showed that they become confined at the nuclear envelope upon activation and that this association enhances their transcriptional output (Cabal et al., 2006; Drubin et al., 2006; Taddei et al., 2006). Surprisingly, interactions with factors from a number of gene regulatory processes are required for this nuclear envelope targeting. These interactions are disrupted when components of the SAGA histone acetyltransferase complex, the mRNA export factor Sac3, the nuclear-pore-complex component Nup1, or the 3′ UTR sequences of these genes are deleted. This suggests that, at least in yeast, proximity to the nuclear export machinery maximizes gene expression by facilitating mRNA export and/or processing. This visual assay for gene positioning and dynamics has also been adapted to a high-throughput approach, in which mutants and small molecules can be rapidly analyzed by machine visual screening (Drubin et al., 2006). This approach, will allow us to comprehensively analyze the role of nuclear organization in gene regulation.

Hold on loosely or don't let go: TF-promoter interactions

In vitro measurements of DNA binding affinities led to the idea that RNA polymerases initiate transcription from static stable complexes of TFs assembled at promoters (reviewed in Dilworth and Chambon, 2001; McKenna and O'Malley, 2002). However, photobleaching analysis of the GFP-tagged glucocorticoid receptor (GR) at an ~200-copy mouse mammary tumor virus (MMTV) Ras tandem array (Fig. 1G) containing 800-1000 GR-binding sites revealed that it turns over rapidly (McNally et al., 2000). This suggested that TF-promoter interactions are highly dynamic. Additionally, evaluation of the mammalian RNA polymerase I machinery on ribosomal genes showed that the assembly of elongation-

competent complexes is stochastic and, therefore, inefficient and probably rate limiting (Dundr et al., 2002). These complexes are not thought to be recycled but to assemble anew at each round of transcription. Recent studies examining the dynamics of a variety of TFs at their response elements have revealed how changes in their concentrations and binding affinities can rapidly modulate gene expression in response to changing conditions.

Steroid hormone receptors bind to their DNA response elements in a hormone-dependent fashion and initiate transcription by recruiting chromatin-remodeling and TFs to promoters (reviewed in Belandia and Parker, 2003). Studies of GFP-tagged progesterone receptor (GFP-PR) (Rayasam et al., 2005), glucocorticoid receptor (GFP-GR) (Stavreva and McNally, 2006; Stavreva et al., 2004) and androgen receptor (GFP-AR) (Klokk et al., 2007) at the MMTV array (Fig. 1G) and GFP-tagged estrogen receptor (GFP-ER) at a native prolactin promoter-enhancer reporter construct array (Fig. 1H) (Sharp et al., 2006) have provided insight into how transcription is regulated by receptor-chromatin interactions. Photobleaching analyses show that they bind transiently to their response elements, where they induce a constant opening and closing of the nucleosomes. Proteasome activity facilitates rapid GR exchange, whereas the chaperone Hsp90 stabilizes binding (Stavreva et al., 2004). Evaluation of the effects of PR and AR agonists and antagonists on exchange rates have also provided insight into their regulatory mechanisms (Rayasam et al., 2005). In general, factors that lengthen residency times increase transcriptional output. Nuclear hormone receptors program the expression of a large number of genes. Therefore, modulation of their binding-site interactions provides a mechanism through which cells can rapidly adjust to changing conditions.

Members of the NF-κB family of TFs bind as homo- or hetero-dimers to response elements in numerous genes involved in immune functions, inflammation and apoptosis (reviewed in Hayden et al., 2006). It was thought that NF-κB proteins nucleate the assembly of very stable complexes at promoters (Zabel and Baeuerle, 1990). However, photobleaching analysis of RFP-tagged NF-κB proteins at a ~2000-copy transgene array containing a human HIV 5′ LTR with two canonical NF-κB-binding sites (Fig. 1I) showed that the proteins are only transiently immobilized on their response elements (Bosisio et al., 2006). If binding were stable, the thousands of κB sites present in the genome would sequester NF-κB proteins as these TFs enter the nucleus. Instead, promoter-bound NF-κB is in a dynamic equilibrium with the nucleoplasmic population and its nuclear concentration affects the probability that a transcription-competent complex will assemble. In this way, cells can regulate gene expression by changing the abundance of TFs in the nucleus.

By contrast, heat shock induces the stable association of heat shock factor (HSF) with the native *hsp70* genes on polytene chromosomes in *Drosophila* salivary gland nuclei. This suggests that constant exchange is not required for additional rounds of transcription (Yao et al., 2006). Before heat shock, HSF is present in the nucleus and exchanges rapidly with chromosomal sites (Yao et al., 2006). In response to heat shock, it trimerizes, which increases its affinity for promoter elements, and activates transcription (reviewed in Wu, 1995). Although most reported TF-promoter interactions are highly dynamic, it is possible that other stably interacting TFs will also be discovered. Perhaps under heat shock conditions, the regulatory benefits of rapid exchange are sacrificed in favor of increased transcriptional efficiency.

Making the message: nuclear mRNA dynamics revealed

Mechanisms that regulate the synthesis, processing, transport, translation and stability of messenger RNAs (mRNAs) are also critical control points in gene expression (reviewed in Orphanides and Reinberg, 2002). By labeling RNAs with stem loop repeats from the MS2 bacteriophage, one can examine the dynamics of specific RNAs in single cells. Recent studies using this technique have addressed how chromatin organization is coordinately regulated with RNA synthesis, how RNA moves in the nucleus, and how long and how frequently a gene is transcribed. They also demonstrate particularly well how single cell analyses can provide insight into mechanisms of gene regulation that could not be obtained by averaging effects in cell populations.

Using a system that allows all of the components involved in gene expression (DNA, RNA and protein) to be visualized in single living cells, it is possible to observe, in real time, the changes that occur at a region of chromatin during transcriptional activation (Janicki et al., 2004). This system combines lac-repressor–operator and MS2-coat-protein–translational-operator interaction units and tetracycline response elements into an inducible reporter construct (Fig. 1K). Stable integration of the transgene produces a cell line that has a highly condensed heterochromatic ~200-copy transgene array. Upon transcriptional induction, the array decondenses; RNA accumulates at the transcription site; heterochromatin protein 1α (HP1α) is depleted; and histone H3.3 is integrated. As the conversion of chromatin between the silent and active states can be observed in this cell line, it is an important tool for studying the regulation of these mechanisms (Janicki et al., 2004). Interestingly, knocking down the RNA interference (RNAi) factor Dicer (Tsukamoto et al., 2000), does not affect the organization of the chromatin in an earlier version of this system (Fig. 1J) (Wang et al., 2006). Therefore, it offers a unique opportunity to investigate the interplay between RNA dynamics and chromatin regulation and could be an important tool for identifying new mechanisms of gene regulation.

Evaluation of RNA mobility in *Escherichia coli* (Fig. 1L-M) (Golding and Cox, 2004) and in the cell line containing the human lac/tet/MS2 operator transgene array described above (Fig. 1K) (Shav-Tal et al., 2004) showed that RNA moves by simple diffusion in an energy-independent fashion. In the human cell line, messenger ribonucleoproteins (mRNPs) diffuse throughout the nucleoplasm without following any defined paths. Interestingly, a number of them also become corralled, which suggests that there are barriers in the nucleus that hinder their movement. A similar range of diffusion coefficients is found using three different techniques: single-particle tracking, fluorescence recovery after photobleaching (FRAP) and visualization of photoactivatable GFP. There have been conflicting reports as to whether nuclear mRNP movement is energy dependent or independent (reviewed in Politz and Pederson, 2000; Swedlow and Lamond, 2001). However, this study shows, by tracking single mRNPs, that it is energy independent. Understanding how RNA moves in the

nucleus has important implications for the timing of nuclear export and translation.

The ability to label specific RNAs in living cells is also providing better insight into the kinetics of transcription in individual cells. Evaluations of the 'noise' (cell-to-cell variability) in gene expression previously relied on measurements of protein levels (reviewed in Kaern et al., 2005; Paulsson, 2004). However, because protein synthesis lags behind transcription, and protein is typically made in larger quantities than RNA, it was not known whether these studies accurately reflected the contribution of RNA to the variability in gene expression. Additionally, on the basis of northern blotting and RT-PCR, it had been assumed that genes are steadily transcribed in the presence of a constant source of inducer. Interestingly, single-cell evaluations of MS2-repeat-containing RNAs detected by the GFP-tagged MS2 coat protein in *E. coli* (Fig. 1N) (Golding et al., 2005; Le et al., 2005) and *Dictyostelium discoideum* (Fig. 1O) (Chubb et al., 2006) showed that, as predicted by studies of protein synthesis kinetics, RNA is transcribed in pulses. In *Dictyostelium*, the RNA pulses from the endogenous developmentally regulated gene, *dscA* are irregular in length and spacing throughout development and a dscA-expressing cell is more likely to re-express than is a non-expressing cell to begin expressing. This suggests that genes have 'transcriptional memory'. Expressing cells are also found in clusters, which indicates that an intercellular signaling mechanism synchronizes transcription within cell populations. It has been suggested that chromatin remodeling contributes to transcriptional pulsing in eukaryotes (Blake et al., 2003; Raser and O'Shea, 2004). Given that bacterial DNA is not packaged into chromatin, it is surprising that transcription in bacteria is also discontinuous. Therefore, transcriptional pulsing may function universally to provide flexibility in the regulation of gene expression.

Conclusions and perspectives

Genes are expressed through a series of stochastic events that includes chromatin movement, TF dynamics, and transcriptional kinetics. By combining inducible reporter constructs with DNA/RNA sequence elements that can be visualized in living cells through interactions with GFP-tagged binding proteins, it is possible to test hypotheses about gene regulation in a cellular context. The ability to experimentally control transcription also makes it possible to examine gene activation and silencing immediately after their initiation when transient regulatory events might be occurring. Although seeing a dynamic event in a living cell does not always offer an immediate explanation for its mechanistic role in the process, such observations do offer the opportunity to consider new regulatory paradigms. In fact, observations made in living cells have dramatically changed ideas, derived from in vitro experiments, about how TFs interact with their promoter elements (McNally et al., 2000). Therefore, because there are regulatory mechanisms that cannot even be imagined outside the in vivo environment, it is essential that methods be developed to examine gene expression in living cells.

Concerns have been raised, however, as to whether mechanisms deduced by studying gene regulation using transgene array systems are relevant to the regulation of single-copy genes and endogenous regions of repetitive DNA. In general, the questions that can be asked, in biological research, are limited by the availability of investigative tools. Using GFP-tagged binding proteins to visualize exogenous insertions of DNA and RNA has been one of the best ways of studying gene expression in living cells. Because the large number of binding sites at a multi-copy transgene array serve to concentrate the GFP-tagged binding factors at the site, the fluorescent signal from these arrays can be imaged with a high signal-to-noise ratio and the arrays can, therefore, be easily tracked. Nuclease assays and comparisons of data obtained in fixed cells also suggest that the structure and function of the adjacent chromatin is not substantially disrupted by these insertions (Heun et al., 2001; Thrower and Bloom, 2001). Additionally, the turnover rates of nuclear hormone receptors (Becker et al., 2002; Sharp et al., 2006) and TFs (Bosisio et al., 2006) at multi-copy transgene arrays containing natural promoters and the repetitive rRNA genes (Dundr et al., 2002) are very similar, which suggests that results obtained in these transgene array systems are relevant to endogenous genes.

Transgene silencing is known to increase proportionally with transgene copy number and many transgene arrays have been characterized as heterochromatic (reviewed in Henikoff, 1998) – including some of the arrays described in this review (Janicki et al., 2004; Wang et al., 2006). Therefore, what is being evaluated in these systems are dynamics at repetitive DNA that was packaged into heterochromatin before activation. Because the regulation and packaging of integrated foreign DNA is also crucial for cell function, these systems provide a good opportunity to study these processes. They might also be informative about viral defense mechanisms. Finally, these experiments are done in single living intact cells and, therefore, are a good method of examining and confirming interactions initially detected by disruptive techniques, such as biochemistry and fluorescence in situ hybridization (FISH).

There are many outstanding questions regarding how gene expression is temporally and spatially organized in cells. How and when is decondensed repetitive DNA repackaged into heterochromatin? How are transcription and chromatin organization regulated throughout the cell cycle? How does mRNA efficiently leave the nucleus? In the future, targeting tags to specific genes and improving our ability to see single-copy genes will allow us to address questions about how they are dynamically regulated. As new systems and methodologies (such as minimal tags) are developed, we will continue to move closer to visualizing endogenous conditions and we will gain new insights into gene regulatory mechanisms.

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