Hot nuclei at the Cold Spring

Meeting on 'Dynamic organization of nuclear function'

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of R. Palazzo). Image courtesy of R.D. Goldman and A. Goldman (Northwestern University School of Medicine).

Meeting on the 'Dynamic organization of nuclear function' held at the Cold Spring Harbor Laboratory on 13–17 Sept, 2000 (organized by Thomas Cremer, Robert Goldman, Pamela Silver, and David Spector). Image: various stages of nuclear envelope breakdown in eggs of the surf clam, *Spisula solidissima*, observed 7 min after initiation of nuclear envelope breakdown prior to meiosis I. Triple labelling: chromosomes, blue; lamins, red; tubulin, green (antibody a kind gift

review can only give a flavor of some of these presentations, due to space limitations.

Nuclear components in motion

The term 'architecture' implies a static structure, but the message of the meeting was that nuclear structures are highly dynamic. To adapt a metaphor used by A.S. Belmont (Urbana, IL) when describing the work of T. Misteli (Bethesda, MD), the structures discussed are like the city, Kyoto. Such a city maintains its overall shape and function despite the birth and death of its citizens, and the replacement of its buildings. Some quarters may be devastated by earthquakes but they soon rise again, and even the apparently-enduring temples have their beams replaced continually. Individual nuclear structures also seem to persist, even though their components are forever exchanging. For once, the term 'dynamic' was correctly used in the title of this meeting—the nucleus truly is 'hot'.

The preferred technique for monitoring this flux was fluorescence recovery after photobleaching (FRAP) of proteins tagged with green fluorescent protein (GFP). This technique has been used hitherto by a small group of experts, but it entered the mainstream at this meeting. A caveat here is that FRAP experiments generate huge amounts of descriptive data, and it was clear that there are few tools available for quantitative analysis that are accessible to most biologists. Nevertheless, R. Eils (Heidelberg, Germany) described some that enable reconstruction of surface models from image stacks collected at different times.

G. Hager (Bethesda, MD) described a particularly revealing set of experiments using FRAP. Exhaustive studies carried out *in vitro* had led to the idea that a steroid receptor–ligand complex binds stably to specific elements in chromatin to modulate gene expression. Use of a glucocorticoid receptor tagged with GFP and a cell line carrying a tandem array of binding elements

Introduction

Biologists are used to the idea that structure and function are interconnected; they can relate the gross anatomy of a limb to locomotion, the organization of a mitochondrion to energy production, and the geometry of an enzyme to a catalytic activity. Therefore, they would expect textbooks and conferences on the nucleus to relate the vital functions like replication and transcription to the underlying structure. However, those functions are usually studied after most nuclear structure has been destroyed, and then it is understandable that the nuclear interior is depicted as a featureless tangle of chromatin fibers. Of course, a functional nucleus is not like that! A recent meeting on the 'Dynamic organization of nuclear function' held at the Cold Spring Harbor Laboratory on 13–17 September, 2000 (organized by Thomas Cremer, Robert Goldman, Pamela Silver and David Spector) marked a shift from the traditional way of discussing nuclear function in terms of soluble proteins; most presentations placed function in an architectural context. Unfortunately, this

revealed apparently stable binding in living cells. However, photobleaching showed that the hormone-occupied receptor exchanged rapidly between chromatin and the nucleoplasm. Clearly, the interaction of regulatory proteins with target sites in chromatin is much more dynamic than previously believed, prompting the question: is the stable preinitiation complex believed to exist *in vitro* ever found in a living cell? Further work *in vitro* showed that promoter activation was coupled to the recruitment of an activity that remodeled nucleosomal structure around the promoter.

FRAP was also used in many other ways. T. Misteli measured the residence time of histone H1-GFP on chromatin. Like the glucocorticoid receptor, H1-GFP also bound and rebound to chromatin. Furthermore, trichostatin A (an inhibitor of histone deacetylase and an inducer of chromatin remodeling) decreased its residence time. D. Hoogstraten (Rotterdam, The Netherlands) examined the relationship between transcription and the nucleotide excision repair of lesions in DNA induced by ultraviolet light. Some of the components involved (the XPA damage-recognition factor, the XPB helicase of TFIIH and the endonuclease ERCC1/XPF) were tagged with GFP, and their mobilities monitored in chinese hamster ovary cells or human fibroblasts transformed with SV40. Each tagged component had its own characteristic distribution in unirradiated nuclei, and each seemed to diffuse freely. However, UV-irradiation induced a transient and dose-dependent immobilization, which presumably reflected targeted binding to a lesion. The results provided no evidence for the existence of a preformed repair 'holoenzyme'; rather, they were consistent with the assembly of a complex at the lesion. S. Huang (Chicago, IL) tagged nucleolar components (including UBF, TBP, nucleolin, fibrillarin) with GFP, and found that the high mobility of UBF was not reduced when transcription ceased during mitosis.

Striking movements were also monitored in other ways. For example, A.S. Belmont analyzed the changes in chromatin compaction induced by a steroid hormone. A GFP-lac repressor was fused with the wild-type estrogen receptor, and introduced into cells bearing a tandem array of lac operator elements embedded in heterochromatin. The array—now marked by GFP—unfolded, and estrogen addition partially recondensed it within 30 min. T. Pederson (Worcester, MA) tracked the movements of ribosomal RNA. Oligonucleotides complementary to rRNA and tagged with a 'caged' fluorescein were introduced into cells where they concentrated in nucleoli. On 'uncaging', the hybridized oligonucleotide could be followed as it moved out of the nucleolus.

The nuclear pore

Pores containing GFP-tagged nucleoporins (i.e. POM121 and Nup153) proved to be the most stable of the structures described (J. Ellenberg, Heidelberg, Germany). They moved little in the plane of the nuclear membrane of living cells, and—on photobleaching—the bleached region persisted for most of a cell cycle. This was in contrast to the rapid recovery of fluorescence seen when the same molecules were bleached in the endoplasmic reticulum, or in the cases described above. M.P. Rout (New York, NY) brought us up to date on other components of the nuclear pore complex. None of the ~30 proteins in the yeast complex encode motors or ATPase domains, so the complex is

unlikely to pump cargoes across the nuclear membrane, as has been suggested by biochemical studies. Instead, it might act as a 'virtual gate', without actually opening or shutting. Diffusion of macromolecules through the central channel would be entropically unfavorable, but—when complexed with a karyopherin—the binding energy of the complex to the sides might facilitate transport. Indeed, 12 of the components of the pore complex contain 'FG' repeats, so transport might be mediated by a diffusional hopping of a cargo/importin- β complex from repeat to repeat (M. Stewart, Cambridge, UK). P.R. Clarke (Dundee, UK) and M. Hetzer (Heidelberg, Germany) described cell-free systems from frog's eggs that should prove useful for analyzing pore and envelope assembly; for example, both structures spontaneously assemble around Sepharose beads coated with the GTPase Ran, and these pseudo-nuclei actively import nuclear proteins.

Specific nuclear compartments

The functions of many nuclear compartments proved to be elusive in spite of attempts to pin them down. For example, interchromatin granule clusters (ICGCs) contain many pre-mRNA splicing factors, but splicing probably occurs elsewhere. Overexpressing the SR protein kinase (Clk/Sty) disrupts the ICGCs and disperses their constituents throughout the nucleus, but transcription (measured by incorporation of Br-UTP into RNA) is unaffected (P. Sacco-Bubulya, Cold Spring Harbor, NY). Coiled bodies (recently renamed Cajal bodies) also lack a specific known function, but they seem to be intimately involved in storing and assembling much of the machinery involved in gene expression (J. Gall, Baltimore, MD).

Chromatin

Heterochromatin retains its allure. For example, the elements responsible for maintaining the activity of housekeeping genes in the presence of vast quantities of heterochromatin in a mammalian cell are unknown. M. Antoniou (London, UK) described a methylation-free CpG island encoding two back-to-back promoters that can 'open' chromatin in both transgenic mice and transfected cells in tissue culture. A. Csink (Pittsburgh, PA) went on to show that heterochromatin is not the universal silencer of gene expression that we thought it was; it can shut off expression from the *white* promoter easily, but has less effect on the *hsp26* and *hsp70* promoters. As a result, the mechanisms underlying the inactivity of heterochromatin are likely to be much more complex than we originally thought.

Chromosome pairing

The mechanism underlying chromosome pairing remains one of the most intriguing and least understood. As A. Dernburg (Stanford, CA) illustrated, the worm *Caenorhabditis elegans* is particularly useful for studying this process during meiosis. The entire adult animal, as well as the gonad within it, is optically transparent, so chromosome movements can be followed in real time. A 'pairing center' towards the end of each chromosome plays some role in the process, but homologs without a center still align and pair. It seems that pairing is maintained by the center, but other mechanisms must be sought to explain how the process initiates.

Nucleoskeletons

Whether or not some kind of internal nucleoskeleton exists is controversial. For example, the lamins have been seen in the interior on various occasions, but such sightings are not usually mentioned in polite society. However, the lamins were outed by K.L. Wilson (Baltimore, MD) and P.A. Fisher (Stony Brook, NY), and T.P. Spann (Chicago, IL) even found that they might play an important structural role during transcription. Microinjecting lamin A bearing an N-terminal deletion reduced the incorporation of Br-UTP into nucleoplasmic RNA, and addition of the mutant protein inhibited transcription by isolated nuclei. Other possible skeletal components were also sighted in the nucleus. Actin is an abundant nuclear protein, and it is added cotranscriptionally to the pre-mRNP particles found in the Balbiani rings of *Chironomus* where it interacts with an hnRNP protein (P. Percipalle, Stockholm, Sweden). A possible partner (a form of myosin I β with an N-terminal extension of 16 residues) was also identified in mammalian nuclei. Significantly, this nuclear myosin was found close to RNA polymerase II in nuclei, and antibodies directed against it blocked transcription *in vitro* (P. de Lanerolle, Chicago, IL). One long-standing candidate for a skeletal component [the nuclear mitotic apparatus protein (NuMA)] seems to play a more subtle role than hitherto suspected. D. Cleveland (San Diego, CA) showed that it disappears naturally from nuclei when they differentiate, concurrently with changes in nuclear shape. Its distribution also changes dramatically during mitosis, as NuMA-GFP moves to the poles along spindle fibers when the nuclear envelope breaks down. Later, it is closely associated with an actin-related protein in the dynactin complex, and with cytoplasmic dynein. Taken together, these results make it likely, but do not prove, that the nucleus has a well-developed skeleton analogous to that in the cytoplasm, and that this skeleton plays an important role in many different nuclear functions.

Diseases

The field of nuclear organization is even mature enough for the meeting to have included a section devoted to diseases, and H.T. Orr (Minneapolis, MN) opened the conference with a discussion of spinocerebellar ataxia type 1. This autosomal dominant neurodegenerative disease is caused by the mislocalization of a mutant protein (ataxin-1) to the nucleus. Transgenic mice expressing a mutant protein that lacks a nuclear localization signal no longer develop nuclear inclusions or exhibit ataxia. Clearly, subcellular localization of the protein matters in the development of this disease. X-linked Emery-Dreifuss muscular dystrophy can also be caused by protein mislocalization. In this case, emerin (a lamin-binding protein found in the nuclear membrane) mislocalizes to the endoplasmic reticulum (K.L. Wilson, Baltimore, MD). Talks on spinal muscular atrophy (L. Pellizzoni, Philadelphia, PA), various leukemias (E. Columbo, Milan, Italy) and other syndromes, including Treacher Collins (U.T. Meier, New York, NY), Werner's and Bloom's (N. Neff,

New York, NY; H. Yan, Philadelphia, PA) all highlighted the close association of relevant proteins with specific nuclear structures. Furthermore, mislocalization of RNA was also shown to play a role in disease. Nuclear accumulation of mRNAs containing expanded tracts of CUG repeats contributes to pathogenesis of myotonic dystrophy by disrupting the function of a family of proteins that regulates alternative splicing (T.A. Cooper, Houston, TX). Taken together, these studies suggest that the study of human disease will provide further insights into nuclear structure and function—and *vice versa*.

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The organisers from left to right: Robert Goldman, Pamela Silver, David Spector and Thomas Cremer

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