TIMELINE

Chromatin history: our view from the bridge

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Thirty years ago, our conception of chromatin structure underwent a total metamorphosis as the nucleosome era began. In Kurosawa's classic movie 'Rashomon' (1951), each participant had a different perspective of the same pivotal event. This review outlines our perception of history.

J. J. Grandville's 'Le pont des planètes' (FIG. 1) provides an artistic metaphor for our view of chromatin. The history of chromatin can be said to begin with W. Flemming, who suggested the name 'chromatin'[1–3] (see TIMELINE). At the time (~1880), Flemming's research was focused on nuclear division ('mitosis' was another term suggested by him). Cell biology had achieved a level of technical and conceptual maturity with the development of microscopes with minimal optical aberrations[4], the increased availability of fixatives and stains[4], improvements in preparative techniques[5], and with the beginning of the chemical characterization of nuclear substances[3,6,7].

During Flemming's lifetime, seminal descriptions of DNA and histones were emerging from biochemical studies (see TIMELINE). F. Miescher and A. Kossel, both students of E. Hoppe-Seyler, laid the crucial groundwork for the characterization of chromatin components. Miescher, as is well known, developed methods for the isolation of nuclei from pus leukocytes and, in 1871, described a strong phosphorus-rich acid, which he called 'nuclein'[8]. Later, he described acidic 'nuclein' and basic 'protamin' from the isolated sperm heads of the Rhine salmon.

Kossel, encouraged by Hoppe-Seyler, continued the investigations, describing, in 1884, the 'histon' in acidic extracts from avian erythocyte nuclei[9]. He developed the intriguing, although now rejected, notion that protamines arose as breakdown products from the wasting muscle mass of the migrating salmon. Flemming, influenced by H. Zacharias' microscopy studies of protease-digested isolated nuclei (1881), which showed a resistance of 'nuclein' to degradation, wrote: "…in view of its refractile nature, its reactions, and above all its affinity to dyes, is a substance which I have named chromatin. Possibly chromatin is identical with nuclein, but if not, it follows from Zacharias' work that one carries the other. The word chromatin may stand until its chemical nature is known, and meanwhile stands for that substance in the cell nucleus which is readily stained."(REFS 1,3).

And so the name 'chromatin' still stands, and is likely to remain into the future.

Chromatin — the dark ages

The first half of the twentieth century revealed great strides in the emerging field of genetics, but was largely devoid of advances in understanding the structure of chromatin. Well known are the rediscovery of Mendelian principles by H. de Vries (1900), the development of gene theory and the principle of linkage by T. H. Morgan (1910), the identification of a 'transforming principle' by F. Griffith (1928), and the demonstration that this 'principle' is DNA by O. Avery, C. MacLeod and M. McCarty (1944)[10]. However, little was accomplished in characterizing the basic proteins of chromatin, other than their extraction in strong acid[11]. The 1941 Cold Spring Harbor Laboratory Symposium — entitled 'Genes and Chromosomes: Structure and

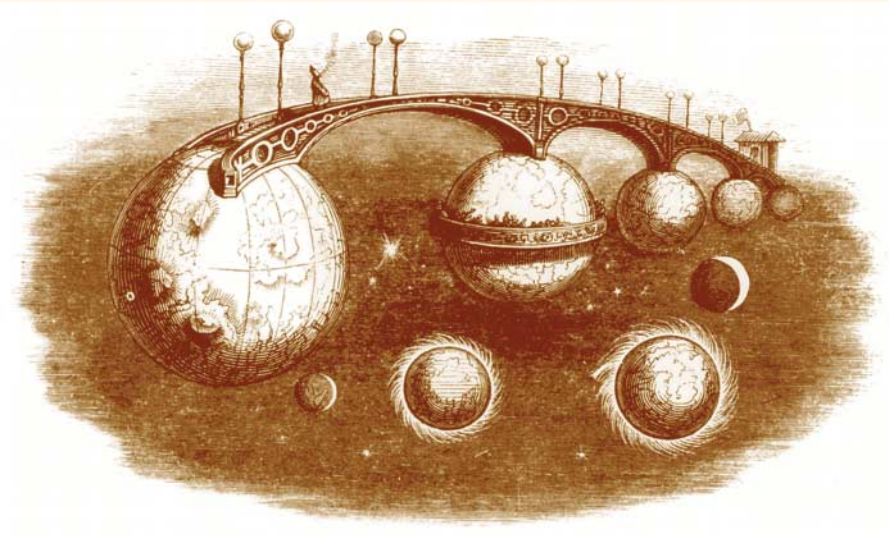
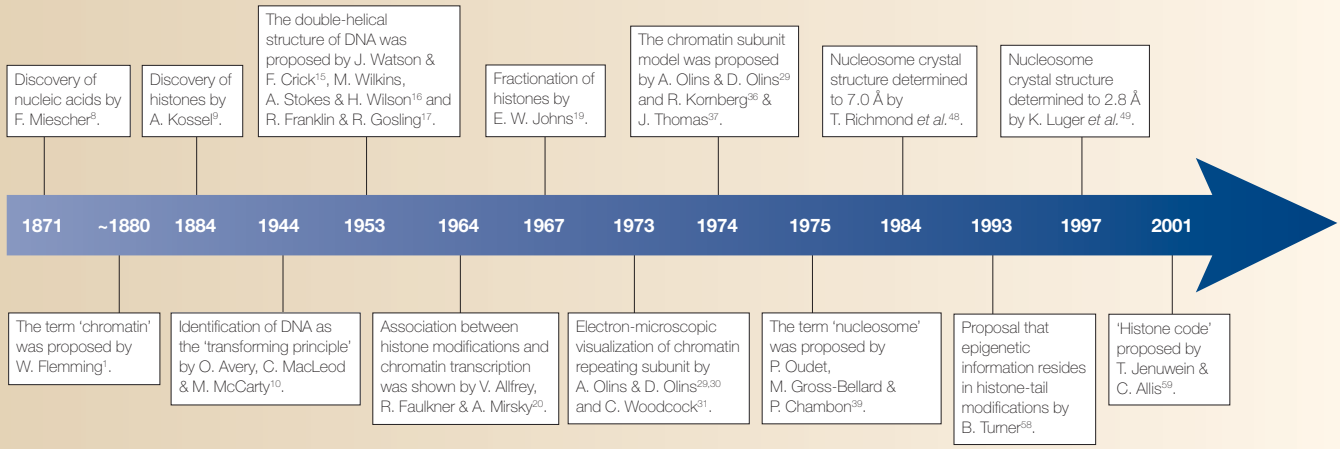


Figure 1 | **A fanciful view of chromatin structure.** 'Le pont des planètes'. Reproduced from REF. 67.

Timeline | **History of chromatin**



Organization' — was characterized by the lack of significant new information on the structure of chromatin, beyond the earlier studies of Miescher, Kossel, Zacharias and Flemming. The discovery of the polytene chromosomes in *Drosophila* and the correlation of chromosome bands with specific genes by E. Heitz and H. Bauer (1933), T. Painter (1933) and C. Bridges (1935) provided exciting experimental material for the exploration of chromatin structure. But the methods for studying chromatin were not very advanced beyond those of the late nineteenth century — that is, the combined use of enzymatic digestion, or solvent extraction, and light microscopy.

A provocative study by D. Mazia[12] illustrates the vast gulf that existed between scientists' perspectives in 1941 and today. Mazia conducted an analysis of salivary-gland polytene chromosomes and plant chromosomes with a number of proteases and spleen 'nuclease'. Some of his summarizing remarks include: "The salivary chromosome, and, very likely, the plant chromosome, seems to be composed of a continuous framework and a matrix which occupies a considerable volume…The continuous skeleton seems to be composed of a histone-like protein…Nucleic acid is attached to the protein part of the chromosome through its phosphoric residues…Removal of nucleic acid does not affect the continuity of the chromosome…The nucleic acid of the salivary chromosome is probably not in a highly polymerized form."

In a companion article, J. Schultz[13] summarizes the prevalent view of the nature of the genetic material: "What is of interest, however, in these early analyses [of the constituents of cell nuclei] is that each species examined had its own special type of protein, and that the nucleic acids were all of a uniform composition, as far as the methods went. For example, each species of fish sperm has its own typical protamine; and the histones obtained from other types of sperm have even more diversity. Moreover, it is by no means certain that the substances from one species are uniform and not mixtures. Thus even our limited knowledge of the protein carries the promise that there may be the specificities that the geneticist desires within them." It is clear that, in 1941, the scientific consensus accepted that chromosomes and chromatin formed the structural basis of the genes, with most investigators leaning towards the histones as the site of genetic information.

Chromatin past(a)

Thirty-two years later, in 1973, Cold Spring Harbor Laboratory held its next symposium devoted to chromatin — entitled 'Chromosome Structure and Function'. Based on the published articles and discussion from this symposium, it is apparent that none of the scientists in attendance were aware of the significant conceptual change (that is, the discovery of the fundamental chromatin subunit structure) that was evolving in a few laboratories. Indeed, we had our own data and chromatin model by then, but resolved not to discuss our results until we had a paper in press. It is useful to reconstruct perspectives about chromatin structure just before the discovery of the nucleosome (for an extensive description, see REF. 7).

Of the many significant scientific advances that occurred between the two Cold Spring Harbor Laboratory meetings, a few clearly had profound influences on workers in the field of chromatin structure: the discovery of the protein α-helix (L. Pauling and co-workers; 1951)[14]; the double-helical structure of DNA (J. Watson, F. Crick, M. Wilkins, R. Franklin and R. Gosling; 1953)[15–17]; the demonstration that the continuity of a single DNA molecule constitutes the backbone of a chromatid (J. Gall; 1963)[18]; the fractionation of histones (E. Johns and co-workers; 1960s)[19]; and the discovery of the association between histone modifications (acetylation and methylation) and chromatin transcription (V. Allfrey and co-workers; 1964)[20]. Another key advance — by G. Zubay and P. Doty (1959) — that was made in this period was the preparation of soluble chromatin molecules[21], which allowed the application of important biophysical techniques towards the determination of chromatin structure.

The success of fibre X-ray diffraction in solving the structure of the α-helix and of DNA, combined with the belief that nucleohistone (that is, the complex of DNA and histones) is a fibrous macromolecule, stimulated a number of laboratories (V. Luzzati and A. Nicolaieff; S. Bram and H. Ris; J. Pardon, B. Richards and M. Wilkins) to use low-angle X-ray scattering, or diffraction, as a means of elucidating chromatin structure. The favoured models, based on X-ray data, were superhelical in character (FIG. 2). Pardon and co-workers stated: "We must stress that although the present data is insufficient to solve the structure of DNH (nucleohistone), the Wilkins supercoil, with a pitch of 120 Å and a radius of 50 Å, is the most satisfactory model yet proposed to explain the diffraction from the regular component..." (REF. 22).

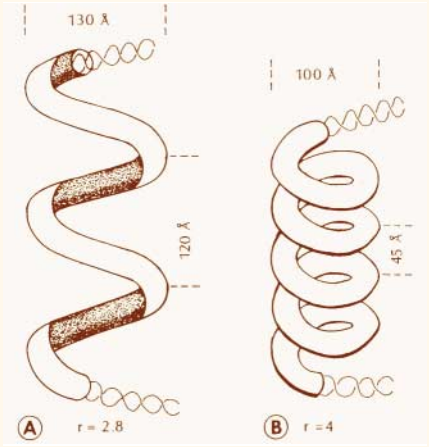


Figure 2 | **Superhelical models of chromatin.** **a** | Richards and Pardon's (1970) and **b** | Bram's (1972) interpretations of nucleohistone structure, based on low-angle X-ray diffraction (as drawn by DuPraw[68]). The Richards and Pardon model has a larger pitch (120 Å) than the more tightly coiled model of Bram (pitch: 45 Å). The various dimensions of these and other models were based on X-ray scattering intensities and the positions of 'spots' (reflections) relative to the chromatin fibre axis. Chromatin fibres never gave 'spots' as sharp as those observed with DNA fibres. Reproduced with permission from REF. 68 © (1974) Cold Spring Harbour Laboratory Archives.

Electron-microscopy data were only partially consistent with the supercoil models. Beautiful thin-section microscopy by H. Davies and co-workers supported the view that supercoils (called 'unit threads') were highly aligned in parallel arrays beneath the nuclear envelope. They even created a macroscopic model for the aligned chromatin fibres: "In further model experiments, threads of spaghetti in liquid gelatin have been shaken in a smooth-walled container. The crystalline patches on the surface, which can be examined after the gel is set, are similar to the ordered patches of unit threads shown in our electron micrographs…" (REF. 23). Not everyone was impressed with the contribution of electron microscopy towards unraveling the structure of chromatin. In the concluding article of the 1973 Cold Spring Harbor Laboratory Symposium, H. Swift observes: "Spread whole chromosomes under the electron microscope look even at their best something like a bad day at a macaroni factory…" (REF. 24). Surface spreading and critical-point drying obliterated the substructure of chromatin fibres, giving them a uniform gelatinous appearance, not unlike pasta.

By 1973, there was a general consensus that metaphase chromatids are uninemic (that is, consisting of a single DNA helix running from telomere to telomere), whereas salivary-gland chromosomes are polytenic (that is, consisting of many parallel and aligned chromatids). The bands (and puffs) of polytene chromosomes, the loops of lampbrush chromosomes and the chromomeres of spread mitotic chromosomes were all thought to reflect the linear ordering of chromatin structural states along the chromatids.

Serendipity

As far as we know, given the preconception of a regular helical chromatin fibre, none of the co-discoverers of the nucleosome could have predicted its existence. Certainly, we were surprised and excited, knowing that we had stumbled on a fundamental structural principle of eukaryotic chromosomes. If "chance favours the prepared mind", we had the good fortune to be prepared in a number of miscellaneous ways, which form the basis for our story of discovery.

After having worked for several years on DNA–basic-protein complexes as models of chromatin, we took a sabbatical in the academic year 1970/71 in the Department of Biophysics of Kings College in London, where we had hoped to work with Pardon, Richards and Wilkins. But Pardon had left Kings, and Wilkins was no longer interested in chromatin. As our first stroke of good luck, we rearranged our plans and worked with W. Gratzer and H. Davies — both stimulating and gracious hosts. Davies, as mentioned previously, had obtained beautiful electron micrographs of chromatin 'unit threads' in chicken erythrocyte nuclei. We were introduced to this marvellous material for studying the structure of inactive chromatin. These were sophisticated chickens — the coop was on the roof of the Department of Biophysics on Drury Lane, in the heart of London's theatre district. We spent that year isolating nuclei and examining the influence of ionic strength and cations on nuclear and chromatin ultrastructure[25].

During that year, R. Clark and G. Felsenfeld[26] and R. Itzhaki[27] independently published evidence that approximately 50% of the DNA in isolated chromatin seems to be accessible to nuclease degradation or polylysine binding. These two papers had a profound influence on our thinking — in particular, the statement "that extensive contiguous regions of the DNA helix are completely free of chromatin protein" (REF. 26). Although evidence was published which argued that the extent of nuclease digestion depended on time and enzyme concentration[28], we returned to our lab at the Oak Ridge National Laboratory in Oak Ridge, Tennessee, determined to visualize both the naked and covered regions of DNA, anticipating long stretches of 'naked' DNA interspersed with 'unit threads'. We tried critical point-drying methods, but the chromatin resembled Swift's "bad day at a macaroni factory" (REF. 24).

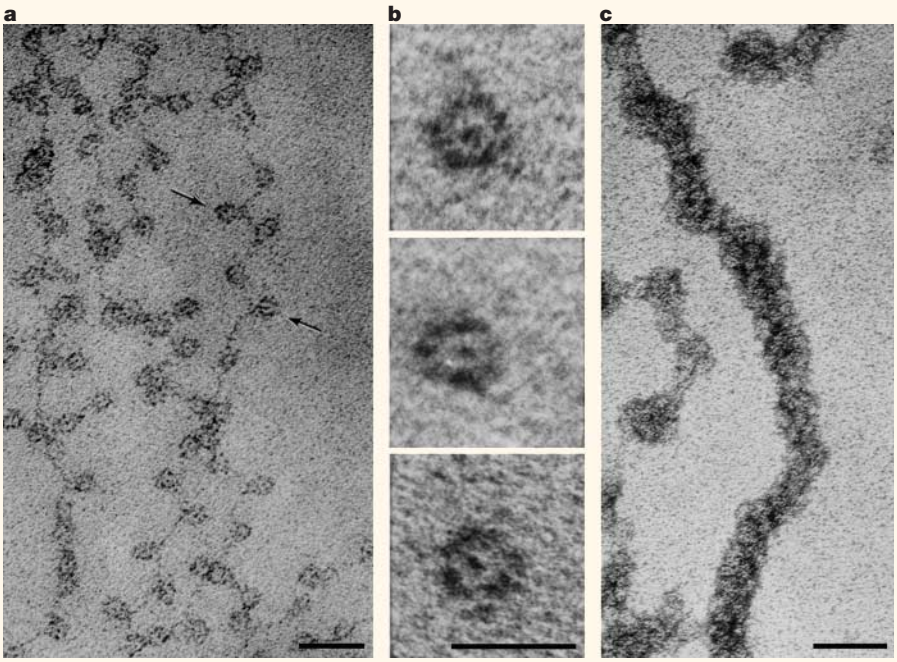
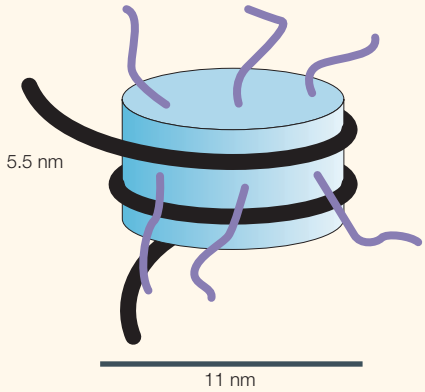


Figure 3 | **A gallery of electron micrographs of chromatin.** **a** | Low ionic-strength chromatin spread, the 'beads on a string'. Size marker: 30 nm. **b** | Isolated mononucleosomes derived from nuclease-digested chromatin. Size marker: 10 nm. **c** | Chromatin spread at a moderate ionic strength to maintain the 30-nm higher-order fibre. Size marker: 50 nm.

Box 1 | **The nucleosome**



The nucleosome is the fundamental repeating subunit of chromatin — the first level of higher-order packaging of chromosomal DNA by histones. Each nucleosome particle consists of ~200 base pairs of DNA (the actual repeat length varies among different eukaryotic species) wrapped around a histone protein core, leading to an approximate sixfold-length compaction of the DNA. The histone core consists of an octamer of pairs of four histones (H2A, H2B, H3 and H4) related by a single dyad axis. The 'core particle', derived by nuclease digestion of chromatin, is a metastable product with 146 base pairs wrapped around the histone octamer — ~90 base pairs per turn. A 'linker' region of DNA between core particles is more susceptible to nuclease degradation than the core particle DNA and is associated with histone H1. The core particle is shaped like a squat cylinder, with a diameter of ~11 nm and a height of ~5.5 nm (see figure; DNA (black) and histones (blue)). The four histones of the octamer associate by their highly α-helical globular regions. Short basic polypeptide tails extend outward from the globular regions beyond the turns of DNA, revealing sites for post-translational acetylation and methylation of lysine residues.

Our second stroke of good luck was that Oscar Miller was at Oak Ridge and had developed a detergent-based method for spreading extra-chromosomal nucleoli. We tried his method of centrifuging swollen nuclei onto carbon-coated grids during the winter of 1972/73. We accumulated many micrographs of positively- or negatively-stained spreads of chicken erythrocyte nuclei. One evening, we examined some of the micrographs under a magnifying glass and, to our complete surprise, realized that little particles were everywhere. Every chromatin strand consisted of 'beads on a string'. During the ensuing months, we focused our efforts on trying to visualize these 'ν (nu) bodies' in rat liver and calf thymus nuclei, pushing the old Siemens IA electron microscope to higher magnifications without astigmatism. We called these particles ν (nu) bodies because they were new and nucleohistone.

A third stroke of luck was that one of us was teaching graduate-level biophysics at the time, which included discussions about the haemoglobin molecule, which has a dyad axis and undergoes allosteric transition. We looked at the ν bodies and speculated that they might have similar characteristics. Based on our measurements of the size of ν bodies (70 Å) and some biophysical assumptions, we proposed a model for chromatin: "It would be conceivable, therefore, for each ν body to contain two of each type of histone molecule complexed with a double-stranded DNA with a molecular weight of about 160,000. Further packaging of the DNA might then represent a folded or helical close packing of the spherical ν bodies under the influence of metal cations and noncovalent interactions" (REF. 29). We submitted our results to *Science* and went to England during the summer of 1973, to present our observations and model to friends in the chromatin field (J. Pardon and B. Richards in High Wycombe; R. Itzhaki in Manchester; M. Bradbury in Portsmouth; and H. Davies in London). These early discussions represented an attempt to convey our excitement about this new view of chromatin structure.

In November 1973, we presented our results at the annual meeting of the American Society of Cell Biology[30]. Viewing the abstracts of the meeting, we discovered that C. L. F. Woodcock had been able to visualize chromatin particles independently[31]. Unfortunately, his manuscript was rejected by *Nature*. A prophetic and prejudiced reviewer wrote: "A eukaryotic chromosome made out of self-assembling 70 Å units, which could perhaps be made to crystallize, would necessitate rewriting our textbooks on cytology and genetics! I have never read such a naive paper purporting to be of such fundamental significance. Definitely it should not be published anywhere!" (REF. 7). Woodcock's micrographs were not published until 1976 (REF. 32).

An important independent line of experimentation by I. Isenberg's and D. Roark's laboratories (1974) established the existence of crucial histone–histone interactions, which constitute the histone core of the chromatin subunit[33,34]. In addition, in early 1974, a careful study was published on the hydrodynamic properties of nuclease-resistant chromatin particles, revealing their compactness[35]. Several months after our *Science* paper was published, R. Kornberg, who was at the Medical Research Centre (MRC) in Cambridge at the time, presented his model of chromatin structure, postulating that ~200 base pairs of DNA formed a complex with four histone pairs[36]. This model was based in part on nuclease digestion and on histone crosslinking data, in collaboration with J. Thomas[37]. The nuclease digestion studies had been stimulated by the studies of D. Hewish and L. Burgoyne[38], which indicated a repeating structure to chromatin, but gave no estimate of DNA size or protein composition. In 1975, the chromatin subunit, which was discovered in 1973/74, received its present name 'nucleosome'[39] (FIG. 3; BOX 1).

A particular world

The discovery of the nucleosome revolutionized the perception of chromatin (FIG. 4). Higher-order packaging of chromosomal DNA and DNA-based processes, such as transcription, replication and repair, were now all viewed through a different lens[7,40,41]. DNA was no longer seen as being coated by histones (superhelical models), but conceived as being coiled on the outside of a globular histone core, which is accessible to the binding of other nuclear proteins. The nucleosome became the 'quantum' of chromatin structure, the fundamental unit for the modulation of chromatin function.

Figure 4 | **A chromatin scientist.** 'Un prestidigitateur avec les planètes'. Reproduced from REF. 67.

Box 2 | **Higher-order packaging of chromatin in the nucleus**

The eukaryotic nucleus is compartmentalized; the condensed, gene-poor heterochromatin is segregated from the more diffuse, gene-rich euchromatin. Much of the heterochromatin — in the form of fibres of 30 nm diameter (the 'unit threads'[23]) — is aligned adjacent to the nuclear envelope (NE)[23] and in association with the lamin proteins[62]. The mammalian NE consists of three genetically distinct lamins (A, B1 and B2) that are attached to the NE inner membrane via a number of integral membrane proteins. Mutations in lamin A or its integral membrane protein emerin result in Emery–Dreifuss muscular dystrophy or related diseases[62,63]. Our own recent explorations of the human Pelger–Huët anomaly, in collaboration with groups in Berlin, Heidelberg and Bar Harbor, have shown that mutations in a different NE integral membrane protein lamin B receptor (LBR) result in an altered nuclear shape and a redistribution of heterochromatin[64,65]. Furthermore, evidence has been published recently, which indicates that LBR functions in human cells as a sterol reductase in the cholesterol biosynthesis pathway[66]. It seems that the NE furnishes a framework for the attachment of inactive heterochromatin fibres and a cellular location of sterol biosynthesis. The significance of this conjunction of activities remains to be completely explored. Understanding the higher-order structure and dynamics of the compartmentalized interphase nucleus has become one of the most exciting areas in the rapidly developing chromatin field.

Questions of nucleosome positioning and nucleosome conformations began to dominate the field, and during the next 5 years, our laboratory focused on nucleosome structure and conformational states. For example, we showed that the core of the nucleosome consists of close-packed globular regions with a high α-helical content[42], and that mononucleosomes can bind the nonhistones HMG 14/17 (now called HMGN1/2) cooperatively at two sites that are symmetrically arranged around the dyad axis[43]; the same observation was made simultaneously by Felsenfeld and co-workers[44]. However, the call of microscopy and cell biology became too attractive to us, and we spent the next 17 years exploring the three-dimensional structure of the Balbiani Ring (the 'puff' site of giant mRNA synthesis in a polytene chromosome)[45,46] and the chemistry of the replication band in ciliated protozoa[47].

During this period, a race took place — between T. Richmond and co-workers at the MRC in Cambridge[48], later at the Swiss Federal Institute of Technology (ETH) in Zurich[49], and G. Bunick's group[50,51] at the Oak Ridge National Laboratory — to solve the crystal structure of the nucleosome. A description of the history of this race — which resembles the race between Scott and Amundsen to the South Pole — and a current view of mononucleosome molecular structure can be found in a recent review[52]. Simultaneous with the race to solve the nucleosome, crystallographic data was published on histone–histone interactions — the so-called 'histone fold'[53]. High-resolution (1.9 Å) information about the structure of nucleosomal DNA has recently been published[54].

Despite the obvious appeal of a helical model of nucleosomes for the 30-nm chromatin fibre, the present conception is that the arrangement of nucleosomes is not so regular and might involve zig-zag paths and regions of local disorder[55]. Indeed, the apparent complexity of 30-nm fibres probably reflects various conformational states that are involved in the transitions from inactive heterochromatin fibres to euchromatin that is active in transcription[56] or replication[57]. Underlying these chromatin conformations and functions are the post-translational modifications of the histone basic tails, which are established sites of functional information[58,59]. Chromatin structure is dynamic at all levels — from the energy-dependent remodelling that occurs during the activation of gene expression[60], to the movement of chromosomal domains within an interphase nucleus[61] (see also BOX 2). The discovery of the nucleosome represented a 'quantum jump' in the understanding of chromatin structure, but it is abundantly clear that even greater leaps lie ahead.

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DATABASES

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OPINION

Can transcription factors function as cell–cell signalling molecules?

Alain Prochiantz and Alain Joliot

Recent data support the view that transcription factors — in particular, homeoproteins — can be transferred from cell to cell and have direct non-cell-autonomous (and therefore paracrine) activities. This intercellular transfer, based on atypical internalization and secretion, has important biotechnological consequences. But the real excitement stems from the physiological and developmental implications of this mode of signal transduction.

Transcription factors are present in the nucleus, and sometimes in the cytoplasm, but on the whole they are not thought to travel between cells. This is because of their hydrophilic properties and the absence of a signal peptide. But there are exceptions and, in fact, some transcription factors travel between cells because they contain protein domains that allow them to do so. This is the case for the HIV transcription factor TAT[1] and for several homeoproteins, such as Engrailed[2,3], Hoxa5, Hoxb4, Hoxc8, Emx1, Emx2, Otx2 and Pax6 (G. Mainguy, A. Maizel, A.P. and A.J., unpublished observations). On the basis of the conservation of the internalization and secretion signals that have been identified in Engrailed (see below), it is anticipated that this property is shared by most homeoproteins.

Homeoproteins are known to contribute to cellular positioning. They were actually discovered in the fly on the basis of mutations that affect the spatial identity of segments and appendages (for example, antennae can be transformed into legs). Within a single structure, such as the spinal cord, specific combinations and concentrations of homeoproteins define the anterior–posterior and dorso–ventral positions of cells. Furthermore, the homeoprotein Engrailed can define the midbrain and the position of cells within the anterior–posterior axis of the midbrain. It is widely thought that homeoprotein function involves the regulation of genes that encode signalling molecules such as surface receptors or growth factors. By contrast, direct paracrine homeoprotein activity is not generally envisaged, although in theory it represents a parsimonious way for neighbouring cells to coordinate positional information. So the ability of homeoproteins to transfer between cells is extremely exciting. There are more than 400 of these proteins in mice and humans, and they are involved in all the main developmental decisions. Many of them also function in the control of adult physiology. For example, Engrailed 1 and Engrailed 2 (EN1 and EN2; collectively known as Engrailed) are expressed in adult aminergic nuclei that control motor behaviour, mood and addiction[4].

Because the transfer of positional information is a general phenomenon that occurs during development and throughout adulthood, because homeoproteins contribute to