A look back: finding chromatin's footprints

Researchers have been aware of the existence of chromatin for well over a hundred years, with Albrecht Kossel first identifying this darkly staining nuclear material in 1884 (ref. 1). Subsequent research soon had identified chromatin as a combination of various histone proteins and DNA in a roughly equal weight ratio, although its actual structural details would remain enigmatic for nearly a century to come.

Roger Kornberg first arrived at the MRC Laboratory of Molecular Biology in Cambridge in 1973, with the goal of working with Aaron Klug, an expert in X-ray crystallography and a colleague of Francis Crick. Having become acquainted with Crick's recent studies of chromosomal structure[2], Kornberg approached Klug about the possibility of applying physical techniques to the detailed analysis of chromatin. "He was very enthusiastic," recalls Kornberg, "and he knew, as it turned out, an immense amount about it—more than I ever could have imagined… [but] he warned me that it would be, in his words, 'a messy problem'."

Messy indeed—early studies of histones appeared to demonstrate that the purified proteins aggregate freely and randomly, and a major review published on the topic in 1973 asserted that the evidence strongly disfavored a unitary structure for chromatin[3]. Kornberg had noticed several problems with contemporary histone studies, however—in particular, he was concerned about the harsh conditions under which histones were purified. "My key recognition was that these were actually denatured molecules, and that's why the interactions apparently made very little sense," he says. "All of the histones stuck to one another every which way, and there was no pattern or sensible set of interactions from which one could hope to deduce anything about the structure."

Kornberg performed a new series of histone experiments, and incorporated his findings with a reinterpretation of existing data to develop a new model, wherein chromatin is composed of 'nucleosomes'—repeating units of chromosomal DNA wrapped around a core octamer of histone proteins[4,5]. It was an intriguing—and controversial—concept, and promptly caught the attention of newly arrived MRC postdoc Markus Noll. "Kornberg's model wasn't really completely supported by experimental facts—it was still quite a bit speculative," says Noll, "but he really had put together the facts that he thought were credible into a beautiful model."

Likewise, Kornberg was not fully certain of his theory, until he became aware of recent data from a pair of Australian researchers who had found that nuclear DNA, when incubated with calcium and magnesium, could be partially digested by endogenous nucleases to yield a pattern of repeating units[6], apparently resulting from the nuclease resistance of chromatin-associated DNA. Similar work with nucleases had been performed by chromatin pioneers such as Kensel van Holde and Gary Felsenfeld, but due to the design of their experiments, a repeating pattern had not been observed.

Kornberg and Noll were intrigued, but initial efforts by Noll to purify the endogenous nuclease were thwarted by low yields of enzyme, and he began investigating various commercially available nucleases. Micrococcal nuclease worked beautifully, specifically digesting the DNA between individual nucleosomes to yield clean patterns of bands separated by 205–base pair intervals[7]—the length of DNA predicted to be nucleosomally associated in Kornberg's model. The efficiency and consistency of the cleavage confirmed that most chromosomal DNA was bound up in nucleosomal units, and micrococcal nuclease digestion rapidly became the technique of choice for chromatin analysis. "Even a few months after the paper appeared in *Nature*… it was very hard to get micrococcal nuclease," recalls Noll. "There was this meeting in 1977 at Cold Spring Harbor, and half of it was this kind of work with micrococcal nuclease–digested chromatin fragments."

Noll would briefly continue his chromatin work, using nuclease digestion to reveal the chromatin shift that occurs at active promoters[8], before making the move into developmental biology. For Kornberg, however, this was only the beginning of a long and fruitful career in chromatin research, and even now, he praises the power of nuclease digestion. In fact, his group recently used the technique to demonstrate the surprising finding that nucleosomes are fully removed from sites of active transcription[9], work that Kornberg describes as "a denouement for the entire [chromatin] story"—proof that micrococcal nuclease still remains useful for clarifying chromatin controversies.

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