

The story of quadruplex DNA – it started with a Bang!

John Lagnado
(Honorary Archivist)

When I was editing Natalya Gromak's book review of *Quadruplex Nucleic Acids* which appeared in the February 2012 issue of *The Biochemist*, I was perplexed to read "The ability of guanosine-rich DNA to form unusual structures, characterized by the ready formation of polycrystalline gels, has been observed since the 19th Century. However, the structure of G-quadruplexes was only identified in 1962." I subsequently discovered from Natalya that what she wrote was based on an introductory sentence taken from the book under review, which read:

"Self-association of guanosine at millimolar concentrations has been observed in solution since the 19th century [my italics] as characterized by the ready formation of polycrystalline gels. In the 1960s Gellert et al. determined the associated guanine bases to be in a tetrameric arrangement by crystallographic methods, described simply as a G-quartet arrangement"¹

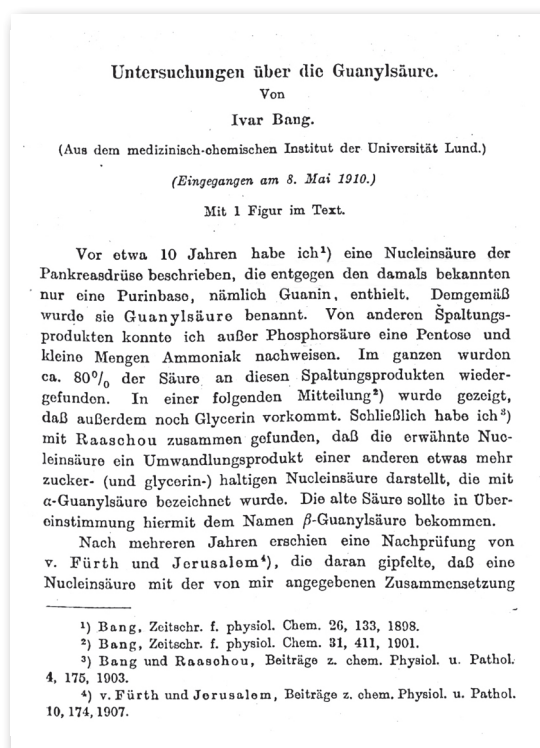
Being lazy by nature, I let this rest...until, that is, I read Mark Burgess's recent news item (see www.biochemist.org/news 21 January 2013) which revealed the exciting news that the quadruple-helix DNA structures had now been demonstrated in the human genome². No doubt younger readers of this magazine are much more aware than I was (until very recently) of these structures and of why they have now achieved such a 'star' status. Nevertheless, what I had read rekindled my curiosity and eventually led me to discover that it was the Scandinavian (bio)chemist, Ivar Bang who first described the puzzling behaviour of guanylic acid in his 1910 paper in the *Biochemische Zeitschrift*³.

So, who was Ivar Bang?

I could find no mention of Bang in Joseph Fruton's monumental history of biochemistry, but I did eventually come across a first clue about him in a short biographical paper published in *Clinical Chemistry* in 1986⁴. From

*The 'grandparent' of the journal currently known as *FEBS Journal* (since 2005) and previously as the *European Journal of Biochemistry* (since 1967).

†He was naturalized as a Swedish citizen in 1903



this I learned that Ivar Christian Bang (1869–1918) was born in the small town of Gran, Norway, and qualified as a medical doctor in 1895. After an internship in the medical-surgical departments of the local hospital, he spent a year in the Institute of Physiology, another year studying chemistry in Wiesbaden, Germany, then clinical chemistry in Berlin. But then, crucially for our story, he went on in 1897 to train in physiological chemistry (i.e. biochemistry) with Professor Olof Hammarsten (aha! that rings a nucleic acid 'history bell' ...) in Uppsala, Sweden. After a short spell practising medicine in Oslo (1900–1902), he was appointed professor of medical biochemistry at the University of Lund, Sweden†, where he spent the rest of his short life as a (clinical) laboratory scientist. In fact, it soon became clear to me that Bang was best known as the 'founder of modern clinical chemistry'⁵ and is mostly remembered for his pioneering contributions to the development of analytical microtechniques for hospital laboratories.

Bang's earliest research work focused on nucleoproteins and nucleic acids, and our story



Ivor Bang

really begins when Bang joined Olof Hammarsten's Physiological Chemistry laboratory in Uppsala in 1897. At this time, it was (erroneously) thought that there were two or more types of nucleic acids – the so-called animal (containing thymine) and plant/yeast (containing uracil instead of thymine) nucleic acids; perhaps even guanylic, thymidylic or adenylic homopolymeric nucleic acid⁶. At the end of the 19th Century, such possibilities were invoked to account for the 'curious nucleic acid' first isolated from the pancreas by Olof Hammarsten in 1894⁷. Olof Hammarsten (1841–1932) had given the name of 'guanylic acid' to the non-protein part of the substance 'beta-nucleoprotein' he had isolated from ox pancreas. This was because "on hydrolysis it releases an excessive amount of guanine but no traces of other bases such as thymine and cytosine, thus differentiating it from other nucleic acids known at the time."⁷

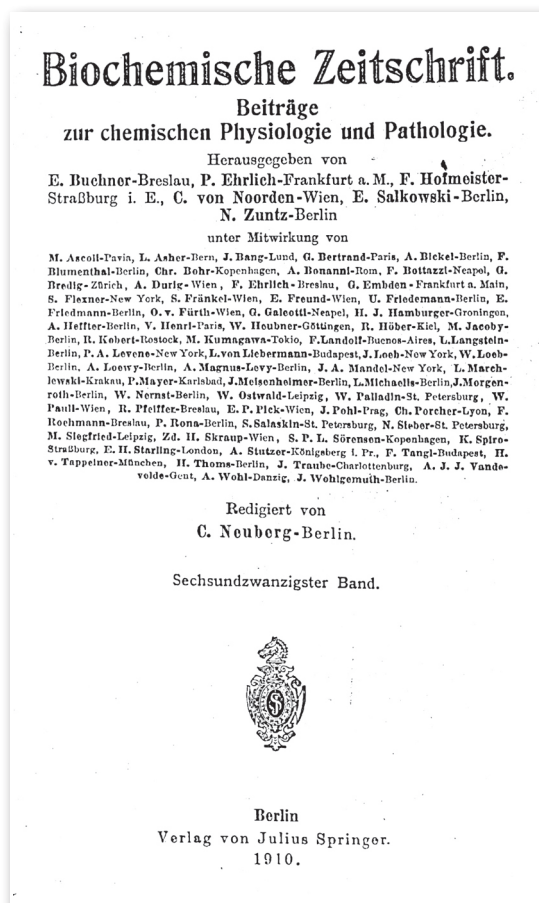
Ivar Bang spent much of the next 12 years or so coming to grips with the structure of guanylic acid, which he elucidated in his 1910 paper, demonstrating for the first time that it consisted of equimolar

amounts of guanine, pentose and phosphoric acid. However, he believed – mistakenly as it turned out – that this represented a (polymeric) nucleic acid ('guanylic acid'), rather than a simple nucleotide, mainly on the basis that concentrated solutions of the 'guanylic acid' (isolated from ox pancreas) formed gels after neutralization with KOH, when re-acidified with acetic acid³. In its historical context, this was not such an outlandish conclusion, as 'thymus nucleic acids', as they were then called, were already known to form these kinds of gel. But what surprised me most when I was struggling to translate Bang's 1910 paper³ was how difficult it was to find any mention of *gel formation*... gelling is mentioned twice, in a single paragraph, on one page in this 19-page paper[‡].

Indeed, much of the paper focuses on describing further improvements on Hammarsten's original method (1894)⁷ for isolating nucleic acid from pancreas, and on providing a thorough chemical analysis of its breakdown products. But the paper is also peppered with rebuttals and critiques of competitors who had challenged Bang's earlier publications on 'guanylic acid' (1898–1908). Bang's combative nature is already evident from some of his earlier publications which I stumbled across while browsing through volume 31 of the *Zeitschrift für Physiologische Chemie*. In particular, we find a four-page riposte (*Erwiderung*)⁸ to Albrecht Kossel's critique of Bang's 1898 paper⁹, which was immediately followed by Kossel's succinct reply (of eight lines)¹⁰ and then...yet another paper by Bang¹¹. Kossel (1853–1927) then apparently abandoned his pioneering research on nucleic acids and focused on the protein components of nucleoproteins (i.e. the 'protamines' and 'histones'), which contributed significantly to his being awarded the Nobel Prize in Physiology and Medicine in 1910. But in his Nobel lecture, 'The Constitution of the Nucleus', Kossel clearly referred to guanylic acid as a simple nucleotide (like inosinic acid), rather than as a larger more complex nucleic acid.

The controversy about the nature of the 'curious nucleic acid' originally isolated from pancreas was finally resolved by the clear-cut studies of Phoebus Aaron Levene (1869–1940). After demonstrating in 1909 that guanosine, a 'simple nucleoside' could, like guanylic acid, form gels, he concluded (correctly) that gel formation itself did not argue against 'guanylic acid' being a 'simple molecule'^{12,13} as Bang had repeatedly claimed. Bang did not quote Levene's 1909 paper.

‡ "...das ganze Filtrat *gelatinisiert* [my italics] beim Abkühlen..... Wir finden also auch hier eine Übereinstimmung zwischen der Guanylsaure and der Thymusnucleinsaure, die bekanntlich auch nach Kochen mit Alkali bei Gegenwart von Alkaliacetat *gelatinisiert*" (p. 300). This translates roughly as... "...the whole filtrate gelatinizes on cooling... We therefore observe here too a relationship between guanylic acid and thymonucleic acid which, as it is known, also forms a gel after boiling in alkali, in the presence of sodium acetate."



A bit of luck

At this point, having finished the first draft of this piece, I began wondering again who first picked up Bang's observations on the gelling properties of guanylic acid, and within what more modern context. My luck was to discover a very recent new book on quadruplex DNA – and even more to the point to

discover within it Gang Wu's scholarly historical account *Guanylic Acid Self-Assembly: 100 Years Later*¹⁴. What I hadn't realized before reading this was the serendipitous nature of the discovery of the G-quartet motif in 1962 (see Gellert et al.¹⁵),

"In 1910, Bang reported that concentrated solutions of guanylic acid formed a gel. We have also observed that concentrated solutions (25.0 mg/ml) of guanylic acid (GMP) at pH 5 are extremely viscous and, if cooled, form a clear gel....."

as related by Davies in his autobiography published in 2005:

"Marie (Lipsett) had originally thought that she been able to make poly G but was then disappointed to discover that what she had was unpolymerized GMP that was forming a viscous solution that looked just like DNA."¹⁶

Echoes of Bang indeed, as clearly acknowledged in the first paragraph of the paper by Lipsett and colleagues¹⁶. Davies then goes on to describes how on hearing of Mary Lipsett's observations, he immediately '...rushed over and pulled some fibres that gave diffraction patterns that could be explained by the formation of G-quartets'.

And that, dear patient reader, is the end of my story. ■

Many thanks to the librarians at the Wellcome Library and the British Library for their help in retrieving the original German papers quoted, and to Kate Bennett, Library Specialist at the Royal Society of Chemistry, for making available to me a copy of the article quoted in Wu¹⁴.

References

1. Neidle, S. and Balasubramanian, S. (eds) (2012) *Quadruplex Nucleic Acids*, Royal Society of Chemistry, London
2. Biffi, G., Tannahill, D., McCafferty, J. and Balasubramanian, S. (2013) *Nat. Chem.* **5**, 182–186
3. Bang, I. (1910) *Biochem. Z.* **26**, 293–311
4. Schmidt, V. (1986) *Clin. Chem.* **32**, 213–215
5. Van Slyke, D.D. (1957) *J. Clin. Lab. Invest.* **10** (Suppl. 31), 18–26
6. Olby, R. (1974) *The Path to the Double Helix*, (Chapter 6) Macmillan Press, London
7. Hammarsten, O. (1894) *Z. Phys. Chem.* **19**, 19–37
8. Bang, I. (1901) *Z. Physiol. Chem.* **31**, 407–410
9. Bang, I. (1898) *Z. Physiol. Chem.* **26**, 133–159
10. Kossel, A. (1901) *Z. Physiol. Chem.* **31**, 410
11. Bang, I. (1901) *Z. Physiol. Chem.* **31**, 411–427
12. Levene, P.A. and Jacobs, W.A. (1909) *Ber. Dtsch. Chem. Ges.* **42**, 2469–2473
13. Levene, P.A. and Jacobs, W.A. (1912) *J. Biol. Chem.* **12**, 411–420
14. Wu, G. (2013) In *Guanine Quartets: Structure and Application* (Spindler, L. and Fritzsche, W., eds), pp. 3–14, Royal Society of Chemistry, London
15. Gellert, M., Lipsett, M.N. and Davies, D.R. (1962) *Proc. Nat. Acad. Sci. U.S.A.* **48**, 213–218
16. Davies, D.R. (2005) *Annu. Rev. Biophys. Biochem. Struct.* **34**, 1–20