Rise, fall and resurrection of chromosome territories: a historical perspective. Part I. The rise of chromosome territories

T. Cremer,[1] C. Cremer[2]

[1]LMU Biozentrum, Chair of Anthropology and Human Genetics, Department of Biology II, Martinsried, Germany; [2]Kirchhoff Institute of Physics and Biophysics of Genome Structure, IPMB, University of Heidelberg, Heidelberg, Germany

It is now generally accepted that chromosomes in the cell nucleus are organized in distinct domains, first called chromosome territories in 1909 by the great cytologist Theodor Boveri. Yet, even today chromosomes have remained enigmatic individuals, whose structures, arrangements and functions in cycling and post-mitotic cells still need to be explored in full detail. Whereas numerous recent reviews describe present evidence for a dynamic architecture of chromosome territories and discuss the potential significance within the functional compartmentalization of the nucleus, a comprehensive historical account of this important concept of nuclear organization was lacking so far. Here, we describe the early rise of chromosome territories within the context of the discovery of chromosomes and their fundamental role in heredity, covering a period from the 1870th to the early 20th century (part I, this volume). In part II (next volume) we review the abandonment of the chromosome territory concept during the 1950th to 1980th and the compelling evidence, which led to its resurrection during the 1970th to 1980th.

Key words: nucleus, chromosome territories, Rabl, Boveri, Sutton.

Correspondence: Prof. Dr. Thomas Cremer,
Chair of Anthropology and Human Genetics
LMU Biozentrum, Grosshadernerstr. 2 82152 Martinsried
E-mail: Thomas.Cremer@lrz.uni-muenchen.de

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The nucleus is a highly organized organelle, yet a consensus on basic principles of the global nuclear architecture and cell type and species specific differences has not been achieved so far. Chromosomes in both animal and plant cell nuclei occupy distinct territories, but in contrast to the general acceptance of chromosome territories, many questions on their internal structure and their interactions with neighbouring chromosome territories have not been resolved. The present plurality of models (for review see Cremer *et al.,* 2006) reflects the complexity of nuclear architecture and highlights the still unresolved role that this architecture may play in epigenetic gene regulation. While numerous recent reviews point out the state of this research (Bartova and Kozubek, 2006; Cremer *et al.,* 2006; Foster and Bridger, 2005; Kosak and Groudine, 2004; Pederson, 2004), we present here a comprehensive historical perspective. In part I (this volume) we describe the discovery of the fundamental role of chromosomes in heredity and the rise of chromosome territories as a fundamental principle of nuclear architecture during the late 19th and early 20th century. In part II, which will appear in a subsequent volume of EJH, we review the abandonment of the chromosome territory concept during the 1950th to 1980th and the compelling evidence, which led to its resurrection since then.

The opportunity to write this review arose, when one of the authors (T.C.) was awarded the Fourth Maffo Vialli International Award For Histochemistry in 2005 (Eur. J. Histochem, 2005). This honour was bestowed with the task to present an account of the scientific achievements of the price winner at the 2005 annual meeting of the Italien Society of Histochemistry, as well as the chance (and the burden) to write an article for the European Journal of Histochemistry - EJH. Whereas one of the authors (T.C.) has received his professional training as a human geneticist, the

other (C.C.) has become a physicist with a long standing interest in the development of new laser tools for cell biological investigations, in particular the development of light optical instruments with a structural resolution beyond the Abbe limit. The development of cytology and histochemistry as a tool for single cells studies has been always closely connected with advancements in microscopy (for details see part II of this review). During our careers as scientists we have developed concepts and experimental approaches together and tried to realize them together as much as possible. We hope that our readers will benefit from our complementary scientific backgrounds. We are grateful to the chief editor of EJH, Professor Manfredi Romanini for her consent to present our views in the context of a comprehensive historical perspective.

A potential reader may ask: *I have little time, what can I learn from the following historical account?* If only for necessary constraints in length, reviews of scientific matters typically mention only those hypotheses, which still appear valid having survived all kinds of experimental attacks. Why should any student bother with seemingly outdated experiments and theories instead of reading the newest review, which supposedly gets it all right? The heroic flavour with which a scientific historiography has often been presented, may foster the impression that a given field of research has developed as a continuous line of scientific successes driven by scientific heroes. Their theories finally prevail, although they are typically ignored or flatly rejected by less gifted scientific peers. Learning about the history of science in the context of this outmoded perspective of heroes, who got it (and still get it) all right from the beginnings is indeed quite boring. Yet, in fact, there are no such heroes in science and a comprehensive historical perspective demonstrates again and again that great biological problems are not solved step by step. The real development of a particular science, such as cytology and cytogenetics, is much more complex, full of mistakes and ignorance. It is also more entertaining and enjoyable than many may expect. Ignorance of conceptual failures and misinterpreted experimental evidence can hide the fact that these failures played a role as indispensable triggers for a change in scientific paradigms. We will provide a few selected examples for this view below. Readers interested to read a more extensive historical account are referred to two books describing the period from the first recognition of the cell to the development of the cell theory and chromosome theory of heredity (Cremer T 1985; Harris 1999).

Beyond any historical exercise occupation with the lively history of an important scientific subject also helps to recognize that mistakes, narrow-mindedness and blatant ignorance are not a matter of the past, but - despite all the fanfare with which the newest turns of science are accompanied by leading journals - a matter of today. Even our present technical possibilities help only little to illuminate the huge dark field of human ignorance. Throughout history scientists were always eager to piece together fragmentary and shaky pieces of evidence into an all-embracing theory. The authors of this review are - very admittedly - part of this problem. Do we – at least implicitly – mean that all efforts of enlightenment have been and still are in vein? Our answer is an emphatical no and we hope that the following historical account presents answers why we think so.

Discovery of chromosomes as bearers of a hereditary substance

During the first half of the 19th century the idea that cells arise *de novo* within a cell or in a cell free fluid carrying the right mixture of nutritients, minerals, nitrogen-, carbon- and phosphorus-sources was still commonplace among leading scientists. Matthias Schleiden (1804-1881) and Theodor Schwann (1810-1882), the recognized founders of the cell theory, firmly believed so (Schleiden, 1838; Schwann, 1839). In a textbook written for students Schleiden goes at great length to explain, why good scientific research should avoid grandiose deductions (Schleiden, 1845). He despised philosophers like Georg Friedrich Hegel (1770-1831) and Friedrich Wilhelm Schelling (1775-1854) for their grandiose development of deductive theories. In contrast Schleiden was determined to carry out his own research in an entirely inductive way free from any dogmatic standpoints. *If we proceed strictly in an inductive (in philosophy critical) manner, then each single claim is made with the immediate support of scientific evidence. Everybody then has the possibility to convince himself, if he wishes to do so, whether or not a claim is rightly supported by the immediate assurance of facts. Each error is immediately detected and thus will not have a long lasting impact on science. In this respect any dog-*

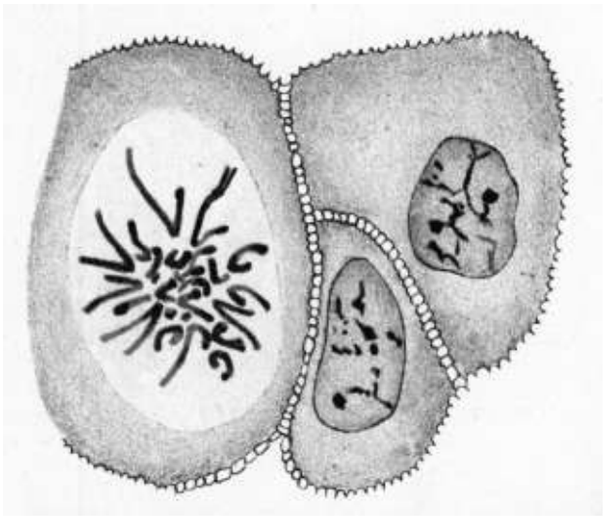

Figure 1. On the left side a cell from the human cornea is depicted in the process of indirect nuclear division. Two neighbouring cells on the right side are shown during the resting stage (interphase) (Flemming, 1882).

matic embodiment of scientific results obtained by the inductive approach is entirely wrong, because readers are deprived of the possibility to make their own judgement, which level of security and thrustworthiness they would like to attribute to results presented in such a dogmatic manner. An inductive path of research, so Schleiden believed, would yield an accumulation of firm evidence and in the end a definitive and ever lasting theory. In his own case Schleiden felt that his deeply felt security of judgement was infallible, because it was based on the *inappellable security of direct sensual perception* (*die inappelable Sicherheit der unmittelbaren sinnlichen Erkenntnis*), namely his direct microscopic observations of cells. Following this education of his students, Schleiden presents evidence that yeast cells originate de novo within three days in filtered red currant juice with some sugar added. So much in support of Schleiden's *unappeallable security* derived from direct microscopic observations. It took several more decades and the ingenuity of a Louis Pasteur (1822-1895), as well as a change of scientific opinion in general, to make the generatio spontanea theory of cells finally obsolete. While Louis Pasteur won this scientific controversy, his friend the physiologist Claude Bernard (1813-1878) wrote in his diary that even Pasteur rejected the spontaneous formation of germs and replaced it by the new germ theory of infectious diseases arguing for the propagation of pre-existing germs *more on the basis of preconception than of scientific evidence* (Waller, 2002).

When we learn below about the evidence that chromosomes are the bearers of hereditary characters or genes, we should be aware of the breathtaking magnitude of change in thinking from the still quite generally accepted theory of generatio spontanea of whole animals like worms in the beginning of the 19th century, to a restriction of this theory to plant and animal cells, as well as germs in the mid 19th century to the first chromosome theory of heredity in the 1880th. This change – together with Charles Darwin's theory of evolution – marks one of the greatest and far-reaching revolutions ever with regard to human thinking about life.

Chromosomes and the phenomenon of indirect nuclear division

We start our journey into the history of chromosomes and cell nuclei somewhat arbitrarily in the 1870th. In 1873 Friedrich Anton Schneider (1831-1890) made an astonishing observation (Schneider, 1873). Whenever a cell divided, its nucleus was not simply tied up into two halves as proposed by Robert Remak (1815-1865) (Remak, 1855), but showed a complex sequence of unexpected events known as indirect nuclear division or mitosis. In 1882 Walther Flemming (1843-1906) published his book *Zellsubstanz, Kern und Zelltheilung* (Cellular substance, Nucleus and Cell Division), where he described the indirect mode of nuclear division with impressively detailed drawings (Figure 1) (Flemming, 1882).

Flemming, who introduced the terms chromatin and mitosis, observed that many mitotic chromosomes were clearly composed of two halves, but could not make sense of this observation. He argued that a continuous chromatin coil would become visible in the beginning of mitosis. This coil would later break up (randomly?) into pieces and these pieces would be distributed with the help of the spindle apparatus to opposite sites of the cell and taken up within the newly forming daughter nuclei (Figure 2A). In 1888 chromatin threads were baptized on the name chromosome by Wilhelm Waldeyer (1836-1921) (Waldeyer, 1888). Waldeyer was eager to choose a name, which avoided any commitment on his part to one or other theory regarding a possible function of chromosomes (for review see (Cremer and Cremer, 1988). Less meaningful names than chromatin and chromosomes emphasizing their staining properties could hardly be invented, but the names sticked forever.

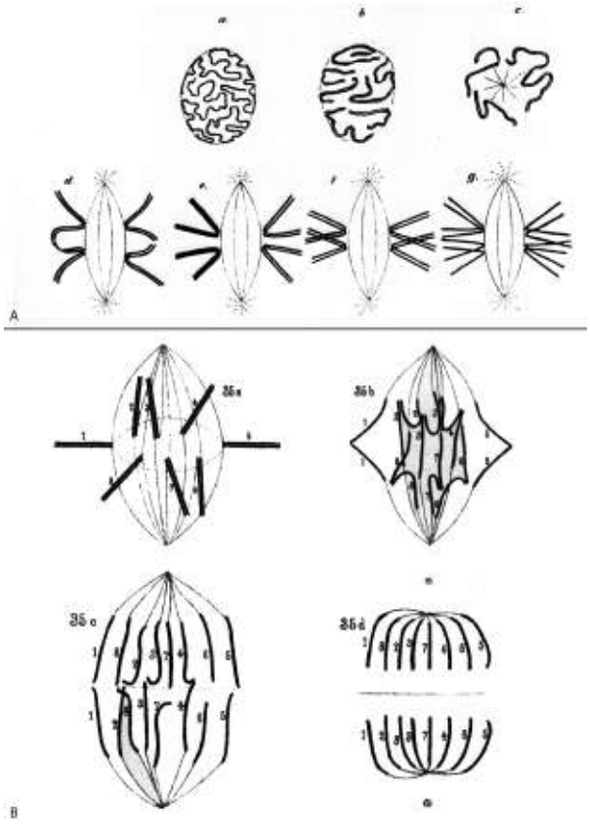

Figure 2. A. Walther Flemming's scheme of mitosis (Flemming, 1882) (for details see text). B. Emil Heuser's scheme of mitosis (Heuser, 1884). Heuser recognized for the first time that the separation of two chromatids (called *Spalthälften* by Heuser) of each mitotic chromosome (*Strahl* in Heuser's terminology) and their movements to opposite poles are a decisive feature of mitosis.

Two years later Emil Heuser made the important discovery that the chromatids (*Spalthälften*) of each chromosome were exactly separated and distributed to the two opposite spindle poles (Figure 2B) (Heuser, 1884).

Wilhelm Roux's explanation of the indirect nuclear division (mitosis)

Even before Heuser's publication appeared in 1884, Wilhelm Roux (1850-1924) had published a theory in 1883 to explain the puzzling observation of indirect nuclear division (Roux, 1883). Darwin's theory of evolution had made its impact on Roux's thinking. Such a complex process instead of a simple direct division of nuclei into two halves (Remak, 1855) could only have evolved for some important purpose. Roux asked what this purpose might be. Although now forgotten, we consider Roux's discussion of this problem as an early landmark in the development of cytogenetics (Roux, 1883). In case that the final purpose of nuclear division were only the bisection of the nuclear mass and the spatial separation of the two halves, the procedure of an indirect nuclear division would be an enormous detour, clearly without purpose to reach such a simple goal. Our judgement changes, however, when we consider that the goal of indirect nuclear division might not be just a random bisection into two halves, but a distinct distribution of qualities, which assemble the nuclear mass. Roux put forward two hypotheses: 1. *The figures of indirect nuclear division reflect a mechanism, which allows a division of the nucleus not only with respect to its mass, but also with respect to the composition of individual qualities.* 2. Roux predicted the *complex composition of chromatin..... The second hypothesis, on which our whole explanation is based upon and on which it stands or falls, is the tremendous variety of qualities.*

Roux took into account studies of Èdouard-Gerard Balbiani (1823-1899) and Wilhelm Pfitzner (1853-1903), who argued that chromosomes initially were built up from a series of small chromatin grains sticking together in a row with the help of an achromatic substance (Balbiani, 1876; Pfitzner, 1882), called nucleo-hyaloplasm by Eduard Strasburger (1844-1912) (Strasburger, 1884a; Strasburger, 1884b). In Roux's visionary view all these chromatin grains had distinctly different qualities. Chromatin grains were arranged along chromosomes and each chromosome (*Mutterfaden*, mother thread) was able to split into two daughter threads or chromatids in present terminology. *In this way the two halves of each chromatin grain could be distributed with the help of the spindle apparatus to opposite poles of the cell, irrespective of any possible brisk movements, which might disarrange parts of the filaments, if only it was secured that the filaments were not torn into pieces and dissolved from their centre*. Roux further postulated *that under normal circumstances two daughter threads resulting from a given mother-thread should always be distributed to opposites sites. Otherwise the very reason of the molecular division (Molekularteilung) [of chromatin grains] would be abolished and the latter would become superfluous.*

Roux's theory of the purpose of indirect nuclear division made only sense, if one was prepared to accept a large number of chromatin grains with different qualities and the necessity to provide each

daughter cell with an identical set of these grains. This assumption sounded very bold, if not foolish in the ears of his scientific peers. Chromatin appeared to them as a completely homogeneous mass. To this conjecture Roux answered: *The apparent homogeneity of the whole chromatin mass will not deceive those, who are aware that we view the molecular functions (Molekulargeschehen) of the cell like a big factory viewed from a balloon floating in the highest regions... It is necessary to deduce from the complex functions of the apparently homogenous organic substrate a complex structure. The fact that such a complex mechanism is needed to ensure a qualitative division for the nucleus, but not for the cytoplasm, argues that the cytoplasm is much more built up from repetitious components with equal constitution than the nucleus.* (Roux, 1883).

August Weismann's chromosome theory of heredity

During the 1880th August Weismann (1834-1914) proposed the first chromosome theory of heredity. Weismann distinguished between *ideal* and *real* theories. While scientists might be content to provide an ideal theory, i.e. a formal explanation based upon assumptions, whose credibility remain unproven for the time being, it was his great ambition to develop a real theory, i.e. a theory, which provided not just a formal explanation but the correct one (Weismann, 1892b). Weismann felt that ideal theories are a first and indispensable step to postulate assumptions, which can explain the phenomenon in question, even if such assumptions are put forward on a purely speculative basis. Ideal theories *provide the basis for the later formulation of a real theory. Above all they give an impulse to test in search of the real explanation again and again the reality of phenomena in question* (Weismann, 1892b). During the 1880th the time seemed ripe to Weismann to develop a real theory of chromosome heredity.

In the early 1890th Weismann considered the evidence compelling in favour of a material substrate of heredity located in chromosomes. He called this substrate the germ plasm (*Keimplasma*) (Weismann, 1892a). Studies of Oscar Hertwig (1848-1922) in the 1870th on the fertilization of sea urchin eggs had demonstrated that following fertilization a male and a female pronucleus became visible, which later merged into a single nucleus (Hertwig, 1876; Hertwig, 1877; Hertwig, 1878). As a side note we wish to mention here that Oscar Hertwig also postulated (rephrased here in modern terminology) that sea urchin chromosomes (*Körnchen*) were generated from the nucleolus (*Keimfleck*), which he observed in the nucleus of sea urchin eggs (Hertwig, 1878). A contemporary reader of Hertwig's publications had no possibility whatsoever to distinguish between Hertwig's theory of fertilization, for which he is still considered a scientific hero, and the erroneous and totally misleading role he provided to the nucleolus. Both theories were presented with the same pretence of reliability. In favour of his theory of the nucleolus as a maker of chromosomes Hertwig claimed that he had observed such a connection *step by step*, including transitional stages (Hertwig, 1978; see also Cremer, 1985). In concluding these remarks on fertilization, Hermann Fol (1845-1892) and Eduard Strasburger (1844-1912) should be mentioned. Fol contributed evidence that indeed only a single sperm penetrated an egg cell (Fol, 1877), while Strasburger showed that fertilization in plant species also required the penetration of a single male germ cell into an egg cell (Strasburger, 1884b).

In biology the rise of major new hypotheses and theories, whose most essential parts withstand all later experimental tests, is typically connected with the discovery of a suitable biological model system. For studies of chromosome structure and function in the context of fertilization and early development the horse roundworm, known in zoological circles as *Ascaris megalocephala or Parascaris equorum* was introduced by Édouard van Beneden (1846-1910) in 1883. This animal became extremely useful for several reasons. It possesses one (*A.m. univalens*) or two pairs of large chromosomes (*A.m. bivalens*), a number which could be easily counted and allowed van Beneden to demonstrate that the size and number of chromosomes was the same in the male and female pronucleus (van Beneden, 1883) (Figure 3A). Van Beneden noted that the chromosomes contributed by the two pronuclei remained distinctly separate during the first cell division. Each chromosome split into two halves with one half being integrated into the nucleus of each daughter cell. As a result two daughter cells were formed, which both contained the same set of maternal and paternal chromosomes. *Ascaris megalocephala* shows another peculiar feature first described by Theodor Boveri

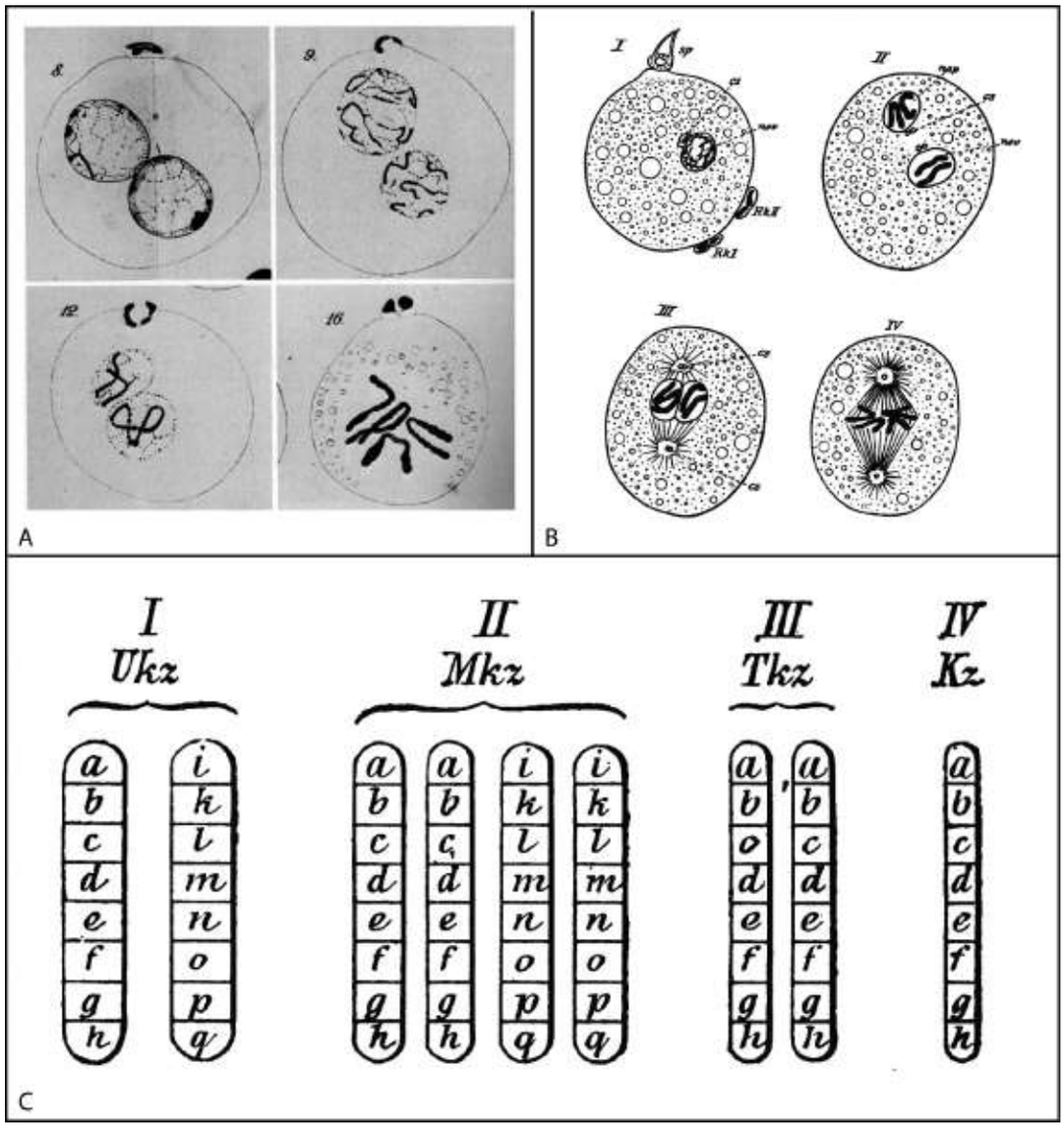

Figure 3. Fertilized eggs from *Ascaris megalocephala bivalens* (van Beneden, 1883) reveal a male and a female pronucleus. Above left: paternal and maternal pronuclei are located side by side. The male pronucleus although derived from a tiny sperm head has achieved the same size as the female pronucleus. When pronuclei enter mitosis they show two morphologically identical chromosomes. B. Scheme of fertilization in Ascaris megalocephala univalens (Weismann, 1892a) C. Schematic behaviour of the idants [Weismann's term for chromosomes] during different stages of germ cell development in *Ascaris m. univalens* (Weismann, 1892b). In the *Urkeimzelle* (Ukz, I), i.e. a stem cell of the germline, each of the two idants is built up from a linear array of ancestral plasms or ids (from the greek word eidos = stature (Gestalt); for Weismann's definition of ids see text). In the *Mutterkeimzelle* (Mkz, II), i.e. a cell during meiotic prophase, the number of idants and ids has doubled. After the first reduction division, each of the two *Tochterkeimzellen* (Tkz, III) again contains only two idants. As a result of the second reduction division *Keimzellen* (Kz IV), i.e. germ cells with a single idant, arise. Note that Weismann lacked the concept of homologous chromosomes. Accordingly, he suggested that each of the two idants in a *Urkeimzelle* carried different sets of ids.

in 1887. The large chromosomes are only retained in the germ line, while their interior parts become fragmented into a number of small chromosomes in somatic cell lines. This observation of chromosome diminuition was later put forward against Boveri's concept of chromosome individuality (for details see Cremer, 1985 and below). Whether chromosomes disintegrated in cell nuclei or retained their structural identity became one of the great controversies of cytogenetics in the early 20th century (see below). Oscar Hertwig and his brother Richard (1850-1937), for example, proposed that the substances, which build up the male and female pronucleus would completely pervade each other during the formation of the diploid cell nucleus (Hertwig and Hertwig, 1887).

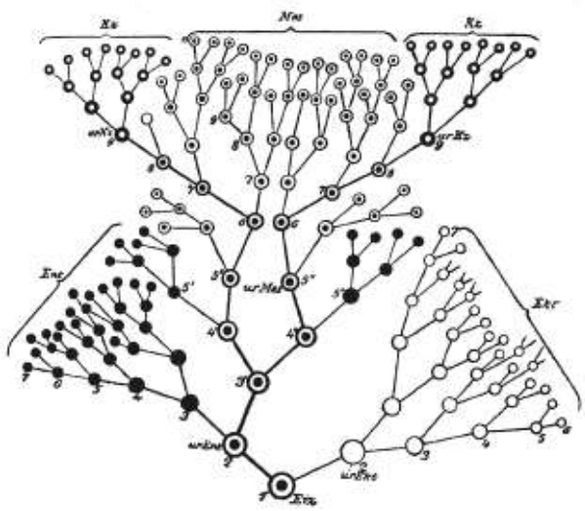

Figure 4. Scheme of the cell genealogy during the early development of *Ascaris nigrovenosa* (Weismann, 1892a). Ent: cells of the entoblasts. Mes: cells of the mesoblast. Kz: cells of the germ line. UrEnt, UrMes and UrKz are the founder cell of all entoblast, mesoblast and germ cells, respectively.

Evidence that the tiny head of a single sperm, which penetrates the egg cell, contributes chromosomes apparently identical in number and size with the chromosomes present in the pronucleus of the egg cell after two meiotic divisions (Figure 3B,C) led Oscar Hertwig, Strasburger and Weismann conclude that the nucleus was the sole bearer of a hereditary material, Weismann's germ plasm. This conclusion was not generally accepted for several decades to come – and rightly so. Opponents of the nucleus theory of heredity, such as Friedrich Meves (1868-1923), were not convinced and proposed an important role of mitochondria (Meves, 1918). Nagging doubts on the role of the nucleus as the sole bearer of a hereditary substance were sustained by a perspicuous problem. It was not enough to carry the germ plasm from one generation to the next. It was of the same importance that the hereditary substance played its role during development and differentiation of somatic cells. How should a substance locked away in the chromosomes of the cell nucleus play such a role? Any plausible chromosome theory of heredity needs to explain how the hereditary characters located in the chromosomes can influence the structure and function of a cell.

Weismann postulated that idants, as he liked to call the chromosomes, were built up from ids (Figure 3C). These ids should not be confused with later concepts of genes. According to Weismann each id contained in a most minute form the hereditary substance provided by a single ancestor. Each id, he suggested further, should contain a number of determinants, which had the capability to generate a number of biophores (Weismann, 1892a) able to migrate into the cytoplasm through very small pores in the nuclear membrane. Each differentiated cell type was dependant on the action of a single determinant and its biophores. Thus, a determinant might be considered as a kind of super gene controlling the structural and functional identity of a given cell. Weismann's next task was to explain, how it is possible that from among *hundreds of thousands of determinants* only one will become functional in a differentiated cell type. He postulated that the development of different cell types is brought about by a series of unequal division during ontogenesis.

Figure 4 shows a scheme of the cell genealogy Weismann presented for the early development of *Ascaris nigrovenosa* (Weismann, 1892a). *During each cell division on the way from a fertilized egg to an entire organism, each daughter cells obtains one half of the idioplasm according to mass, but not necessarily according to its quality. The quality of the idioplasm remains only the same in cases, where the functional relevance of the emerging daughter cells remains the same, yet it differs, wherever daughter cells emerge with a different potential for further development.* What happens is a *deterministic (gesetzmässig) dissection of the determinants into smaller and smaller groups until each cell contains only a single kind of determinant essential for its final destination.* Increasing cell differentiation according to Weismann was reflected by an increasing reduction in the complexity of a cells idioplasm. Only cells in the germ line retained the whole set of ids and determinants till reduction by the two meiotic divisions. Only cells of the germ line, but never soma cells contributed to the transmission of hereditary characters of an animal from one generation to the next. Weismann postulated the continuity of the germ plasm in an uninterrupted succession of germ line cells. From his chromosome theory of heredity Weismann inferred that the transmission of characters newly acquired during the lifetime of an individual to its descendants is impossible. In 1868 Charles Darwin had proposed his *provisional hypothesis* of pangenesis. It stated that *gemmules* formed in the soma could be carried to germ cells via the blood stream and effect them in a way that

allowed the transmission of an acquired character to the next generation. Such a hereditary transmission of acquired characters, Weismann noted, would require that Darwin's gemmules had the capability *to induce a change in the finest molecular structure of the germ plasm* to the effect that offspring generated with germ cells carrying such changes would be able to develop exactly the same molecular structure in cells of the corresponding tissue or organ in the absence of the environmental influences, which had brought about this molecular structure during the parent's lifetime (Weismann, 1892b). Weismann's argument against the hereditary transmission of acquired characters resisted all later attempts to resuscitate Jean Paul Lamarck's (1744-1829) view of evolution. Despite the recent discovery of epigenetic mechanisms, which have provided a molecular glimpse how environmental conditions may induce a hereditary change in the germ plasm without a change in the DNA sequence, there is still not the slightest hint for any cross-talk between somatic and germ line cells to the effect that phenotypic changes acquired by the soma could induce adequate changes of the DNA code carried in the germ line. Unfortunately, any cross-talk of somatic cells with germ cells, which might still be discovered, will not allow the hereditary transmission of capabilities acquired by hard work from parent to offspring, such as the capability to hunt mammoths more effectively or to become a skilled piano player. Wilhelm Roux, who fully agreed with Weismann, experienced his arguments against the hereditary transmission of acquired characters as a *salvation from a cognitive nightmare* and a liberation from problems, which appeared to Roux *more difficult to solve than the problem of the evoluton of purposeful systems without intelligent design* (*welche schwerer lösbar erscheinen als das der Entstehung des Zweckmäßigen ohne zwecktätiges Wirken*) (cited from (Hertwig and Hertwig, 1920).

At the time when Weismann published his chromosome theory of heredity (Weismann, 1892a; Weismann, 1892b), he did not know of Mendel's work. Yet, as we will see below, Mendel's theory was indispensable for the formulation of a*real* theory in Weismann's sense. This theory became only feasible in the beginning of the 20th century, when Mendel's founding contribution to the new field of genetics was recognized (see below). Looking again on Figure 3C we recognize that Weismann's

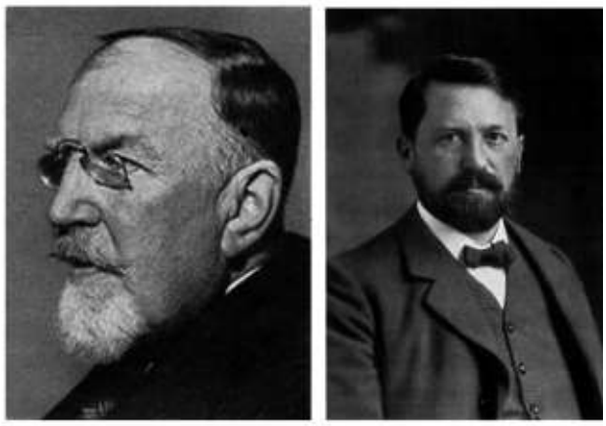

Figure 5. Carl Rabl (1853-1917) and Theodor Boveri (1862-1915).

chromosome theory of heredity was not fit to explain Mendelian segregation ratios. It also did not predict that the loss of a chromosome should have a strong impact, if not lethal consequences for the development of an organism. Since each chromosome contained, in modern terms, the genomes from a series of ancestors, why should not a single chromosome, even part of it, suffice to control the complete development of an animal?

Foundation of the chromosome territory hypothesis

Each interphase chromosome occupies a distinct nuclear territory. This hypothesis was first put forward by Carl Rabl (1885) and Theodor Boveri (1909). (Figure 5).

The contribution of Carl Rabl: constant number and structural persistence of chromosomes during interphase

In his famous work *Über Zelltheilung* (On cell division) Carl Rabl analyzed the numbers and arrangements of chromosome in mitotic cells from *Salamandra maculata und Proteus* (Rabl, 1885). In seven favourable cases he succeeded to count 24 chromosomes in mitotic stages of epithelial and connective tissue cells. While he was not able to clearly distinguish all chromosomes from each other in a larger number of cells, he never found more than 24 chromosomes. On this still rather shaky experimental basis Rabl postulated a law of constant number of threads for each cell type (*für jede Zellenart [existiert] ein ganz bestimmtes Zahlengesetz*). He remained undecided, whether this number might differ in different cell types, because he felt that epithelial cells in the testis had a smaller chromosome number. For other animals

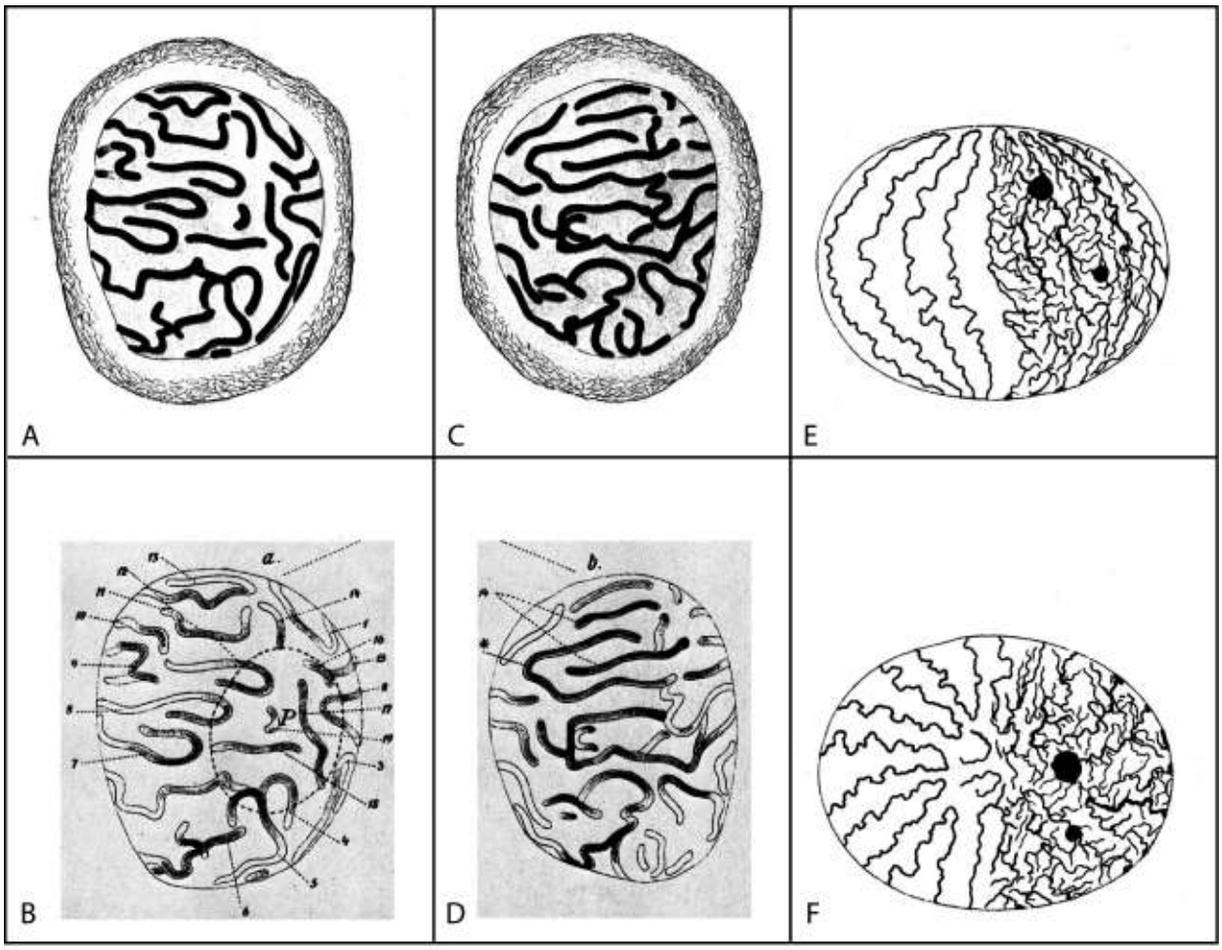

Figure 6. A-D. Drawings (A,C) and more schematic representations (B,D) of prophase chromosomes from an epidermal prophase nucleus of *Salamandra maculata* (Rabl, 1885). P: Polfeld. Rabl embedded sections from Salamandra larvae between two thin cover glasses. This approach allowed him to view individual cells from both sides. With the help of a camera lucida he documented the three-dimensional course of chromosomes and counted chromosome numbers. E and F) Rabl's model of interphase chromosome arrangements (Rabl, 1885); E. shows a lateral view, F. a view on the same model nucleus from above. In (E) Rabl's *Pol-Feld* (comprising the regions where chromosomes become attached to the spindle) is depicted at the top, the *Gegenpol-Feld* (comprising chromosome ends) at the bottom; the model nucleus shown in F. is turned around by 90° compared to (E) allowing a direct view on the Pol-Feld in the middle (for further details see text).

he postulated a constant, but likely smaller or larger number of chromosomes. To Walther Flemming, the leading authority on questions of indirect nuclear division, however, it seemed not important that each daughter cell received exactly the same number of chromosomes. Like Rabl he had counted 24 chromosomes in some epithelial cells of *Salamandra maculata* but *he had given up the time consuming search for cells suitable for such counts, because I saw from the beginning that a consistent law of chromosome numbers did not exist*. (Flemming, 1882).

Rabl's second fundamental observation concerned the order of chromosomes in mitotic cells. He noted a strikingly polarized pattern of chromosome order both at the beginning and the end of mitosis. The chromosomal regions, which become connected to the spindle (the term centromere was not known to Rabl), cluster at one side of the nucleus (*Polseite*, pole field), while the ends of chromosome extend to the opposite side of the nucleus (*Gegenpolseite*, counter-pole field) (Figure 6A-D). In addition to his *law* of constant chromosome numbers, Rabl now made a second bold hypothesis. He postulated that chromosome structure would be conserved to some extent during the formation of the nuclear scaffold (*Kerngerüst*) and thus persist throughout interphase forming distinct nuclear domains with essentially the same polarized course seen in early and late mitotic stages

(Figure 6E,F). Rabl supposed that interphase chromosome structure basically consists of primary threads (*primäre Kernfäden*). From these primary threads, secondary and tertiary threads emerge leading to a chromatin network expanding throughout the nuclear space.

Rabl's two groundbreaking hypotheses, constant number and continuity of chromosomes throughout interphase, owed their existence probably more his conviction that nature is governed by laws with no exception made for the cell nucleus compelling experimental evidence. Objections to the continuity hypothesis were dismissed by Rabl with the argument: *Nobody will likely suppose that the threads in the mother coil will form like crystals in a mother lye, or that the threads disintegrate into pieces or dissolve completely during the structural transition from the daughter coils to the quiescent stage of the daughter nuclei.* Yet in spite of differences in size all chromosomes were essentially equal to him. Rabl did not consider a given chromosome as a carrier of unique hereditary characters not shared by other chromosomes. Accordingly, he had no compelling reason to assume that the development of an animal should become disturbed by the addition or loss of a single chromosome.

The contribution of Theodor Boveri: linking chromosome individuality and chromosome territories with the Boveri-Sutton theory of chromosome heredity

Theodor Boveri employed the horse roundworm for elegant studies, which provided cytological evidence for his theory of chromosome individuality (Figure 7). His early interest in this problem is documented by two publications, which appeared in 1887 and 1888 and culminated in his grand publication from 1909 *Die Blastomerenkerne von Ascaris megalocephala und die Theorie der Chromosomenindividualität* (The blastomere nuclei of A.m. and the theory of chromosome individuality) (Boveri, 1909). In this article Boveri coined the term *chromosome territory*.

In modern terminology his basic argument in favour of chromosome territories as the interphase counterparts of mitotic chromosomes runs as follows. As long as he was able to follow individual chromosomes during the anaphase-telophase movements of the first mitosis, he noted that they maintained strikingly similar conformations and mutual positions. Boveri supposed that chromosomes were transformed into a chromosome territory at the very nuclear site, where the mitotic counterpart was last observed. In Boveri's vision a chromosome territory was composed from a network of thick chromatin bundles pervaded by an interchromatin space (Figure 7B). In his own words Boveri compared the formation of chromosome territories *with the formation of pseudopodia by a rhizopode. At all sites of the exterior layer of a chromosome appendages arise, which become longer and more numerous with time and anastomize with each other. In this way each chromosome ends up as a sponge of chromatin bundles. Each chromosome builds its own reticulum, its own chromosome territory*. Boveri also for the first time argued that chromatin architecture during interphase matters with respect to mitotic chromosome segregation. His cartoon of two neighbouring chromosome territories (Figure 7B) shows chromatin pseudopodia penetrating from one chromosome territory into its neighbour. If such pseudopodia fused within foreign chromosome territory terrain, they could become interlocked with chromatin bundles from this other chromosome territory generating a problem for the separation of the two chromosome territories at the onset of the next mitosis. A cartoon in a publication from Eduard Strasburger (1844-1912) suggested the presence of distinct chromosome territories also in nuclei of plants (Figure 8A) (Strasburger, 1905).

If chromosomes - despite substantial structural changes during M-G1 and G2-M transitions - maintained not only their individuality but also their relative arrangements throughout interphase, each mitotic chromosome should reappear at prophase in exactly the same nuclear region, where it had been transformed into a chromosome territory at the beginning of interphase. To test this hypothesis, Boveri studied fixed two- and four-cell embryos. He compared the arrangements of chromosomes just before they became invisible in daughter cell nuclei at the end of the first mitotic division with the arrangements of prophase chromosomes at the onset of the second mitotic division. He noted that chromosome arrangements in given pairs of daughter cells were indeed strikingly similar, whether fixed and stained cells were studied at the end of the first or at the beginning of the next mitosis (Figure 7A). By contrast, Boveri observed six types of chromosome arrangements in

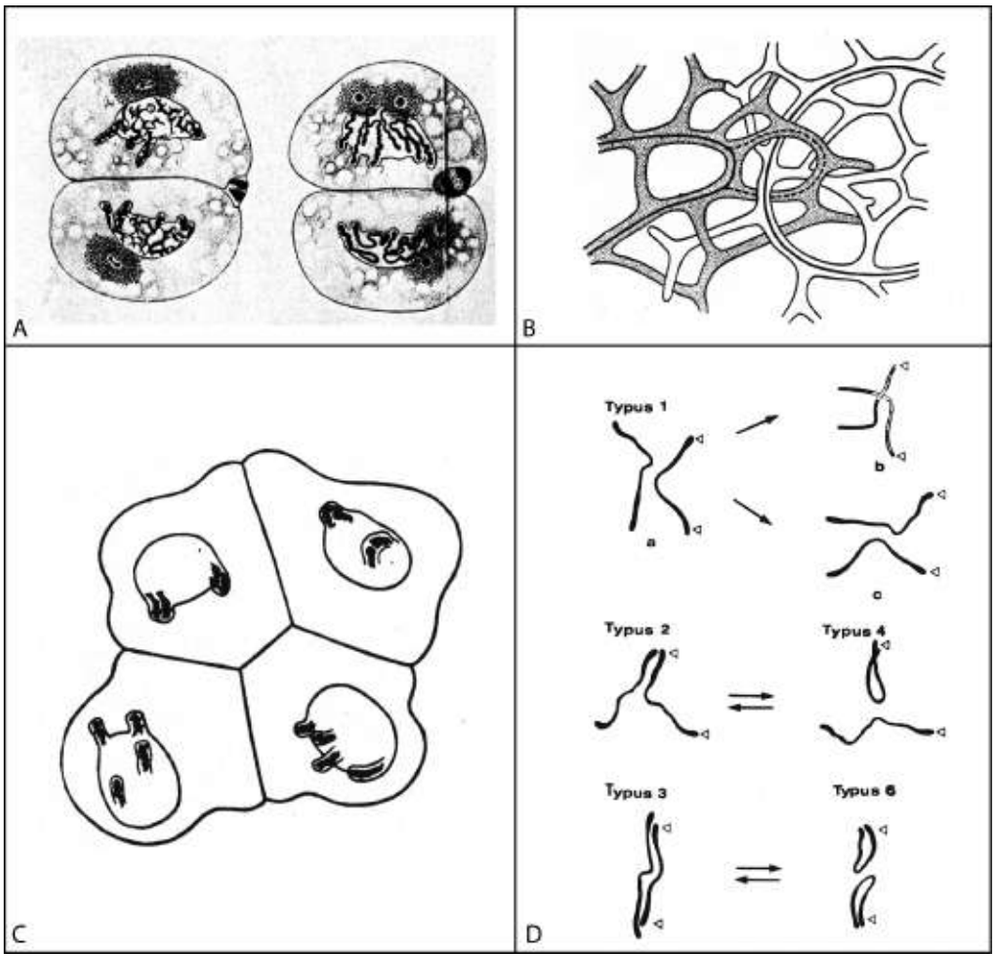

Figure 7. A. Two-cell embryos of *Ascaris megalocephala bivalens* (Boveri, 1888). The embryo on the left shows typical interphase nuclei with nuclear protrusions. The embryo on the right was studied after the two daughter cells had entered prophase of the second division and indicates that nuclear protrusion seen in interphase contain the ends of chromosomes. Note the symmetry in the chromosomal arrangements in both pairs of daughter cells. B. Boveri's model of chromosome territory structure (Boveri, 1909) (for details see text). C. Four-cell embryo of *Ascaris megalocephala univalens* (Boveri, 1909). Based on the similarities of the nuclear protrusions Boveri argued that the two upper and two lower cells, respectively, represent pairs of daughter cells. He explained the strikingly different relative locations of nuclear protrusions between the two pairs as a result of chromosome movements during prometaphase (for further details see text). D. Boveri described six types of chromosome arrangements in mitotic cells of early *Ascaris megalocephala univalens* embryos, the cartoon shows five of them. Different types were found with different frequencies, but for a given type the frequency did not change between the first and the second mitotic event. Although Boveri could not rule out the possibility of frequent chromosomal rearrangements during interphase, he interfered from his observations that such events were unlikely. In case that frequent rearrangements occurred independently in daughter cell nuclei he expected that pairs of nuclei during prophase of the second mitosis should often reveal different types of chromosome arrangements, but this was not the case. This result led Boveri conclude that chromosomes do not rearrange, but maintain their structural identity during interphase.

mitotic cells of *A.m. univalens* (for examples see Figure 7D). Pronounced differences between different types led Boveri conclude that a strict order of chromosome arrangements is not essential for normal development of *A.m. univalens*. As a special, welcome feature of the blastomere nuclei of this species chromosome ends stick into nuclear protrusions (Figure 7A,C). These protrusions served Boveri as landmarks to speculate with some substance, which chromosome territory arrangements likely existed in a given interphase nucleus. He noted that the arrangements of the protrusions were strikingly similar in daughter nuclei fixed at any time during interphase, but often strikingly dissimilar in nuclei, which were related as first cousins. Figure 7C shows a four cell embryo of *A.m. univalens*, which exemplifies this point. Boveri explained the similar positions of the protrusions in pairs of daughter nuclei and the distinctly different position in pairs of first cousin nuclei by the following assumptions: The very similar arrangements of chromosomes at the end of mitosis are the result

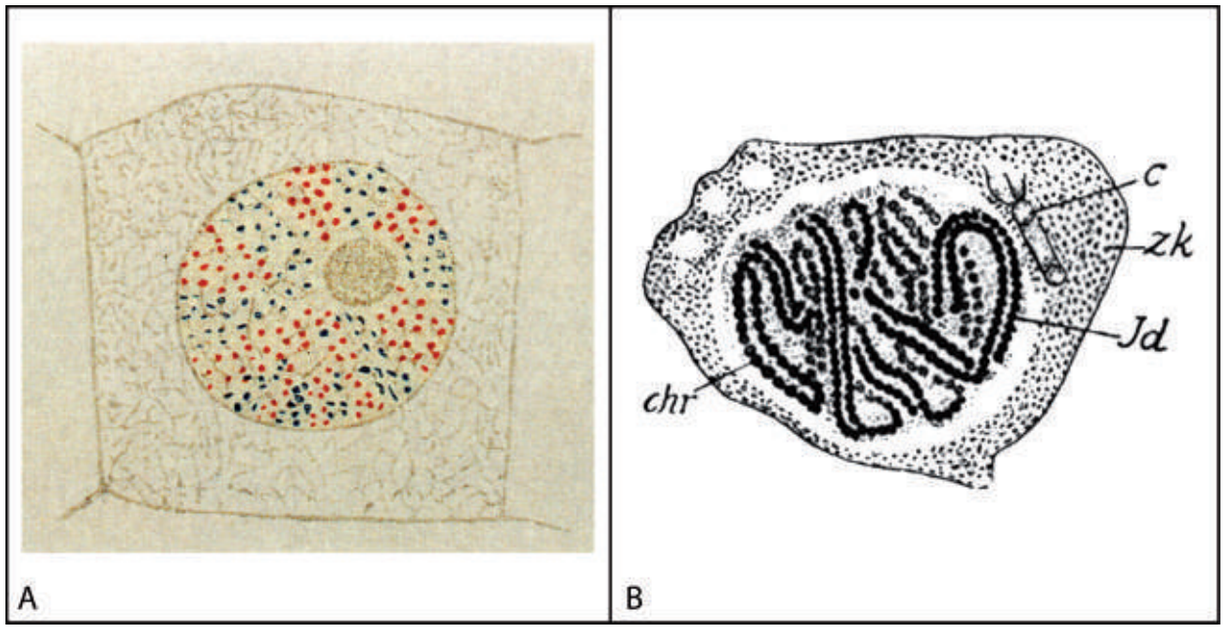

Figure 8 A. Strasburger's model of a tissue cell nucleus from *Galtonia candicans* (Strasburger, 1905). Chromosome territories (depicted in red and blue) are built up from higher order chromatin foci. B. Spermatocyte of Salamandra (Weismann, 1913). In the opinion of early cytologists like (Balbiani, 1876; Pfitzner, 1882; Strasburger, 1884a) chromatin bodies line up along chromatin threads (chromosomes) with the help of an achromatic inter-substance (called nucleo-hyaloplasma by Strasburger). Weismann originally referred to these chromatin domains as Ids and argued that each Id represents the total hereditary contribution of an individual ancestor (*Ahnenplasma*) (compare Figure 3C). Later he used the term Ids also, when referring to entire chromosomes as the largest structures of the germ plasm. c: centrosomes; zk: not explained by Weismann.

of mirror-like chromosome movements during anaphase and telophase. Chromosome territories maintain their positions throughout interphase. During mitosis chromosome movements involved in the establishment of the metaphase plate, however, often result in a change of chromosome arrangements. Accordingly, the newly formed pair of daughter nuclei may show a different type of chromosome arrangement compared with the mother nucleus. Boveri's two postulates – constancy of chromosome territory neighborhoods during interphase and changes of chromosome neighborhood as the result of chromosome movements in prometaphase - were based on shaky, indirect evidence. He lacked any possibility to visualize chromosome territories directly in the cell nucleus or even better to follow chromosome positions in single living cells through several cell generations.

Most cytologists in Boveri's time preferred the view that chromosomes would dissolve into chromatin particles during interphase and these particles would again aggregate into chromosomes at the onset of the next mitosis. Contemporary opponents of Boveri's postulates specifically argued that the observed stability of the positions of the chromosome ends sticking in the protrusions of the nuclear envelope was fully compatible with the possibility that other parts of the chromosomes disintegrated into pieces, which were rearranged into complete chromosomes only at the end of interphase or beginning of prophase. Boveri agreed that his opponents might be right, but added that they were forced to put forward an additional 'ad hoc' hypothesis to explain, why they observed always the same type of chromosome arrangement in two daughter nuclei at prophase. If chromosomes disintegrated during interphase, why did independent rearrangements in daughter nuclei not yield strikingly different arrangements in the subsequent prophase? How should one daughter cell know what the other was doing?

Boveri considered the chromosomes in his own words *as individuals, I would like to say, as the most elementary organisms.* Yet for him *individuality did not mean immutability, not a permanent identity in the mathematical sense* (Boveri, 1909). He reckoned that size, shape, structure and function of chromosomes might change dramatically during the cell cycle and development. Boveri argued that even a *complete dissolution and mix-*

ture of all chromosomes [at the beginning of interphase] was compatible with the theory of chromosome individuality, if only all particles belonging to a given chromosome possess an affinity towards each other of such a kind that they would come together again in one chromosome [at the end of interphase] (Boveri, 1909).

Given his liberal views with respect to a structural persistence of chromosome territories during interphase and all the concession he made to his scientific critics, why did Boveri care so much about chromosome individuality? To some extent he preferred the hypothesis that chromosomes retain their structural identity throughout interphase, simply because he considered it as the most straightforward explanation of the evidence that all chromosomes, which entered the nucleus at the end of one mitosis, also reappeared in the beginning of the next, including egg development with an abnormal chromosome number. But this was not all. Boveri's stubborn defence of chromosome individuality was triggered by his conviction that the new chromosome theory of heredity proposed by him and by Walter Sutton (1877-1916) shortly after the beginning of the 20th century (Boveri, 1902b; Boveri, 1903; Boveri, 1904; Sutton, 1903) stood with the evidence for chromosome individuality or fell with the lack of it.

The new field of cytogenetics started with the Boveri-Sutton theory as its founding theory. It was Boveri's and Sutton's great merit that they merged Gregor Mendel's (1822-1884) theory of heredity with all the evidence then available for chromosomes as bearers of a hereditary molecular architecture. This theory, as Boveri proudly proclaimed, explains *all the facts about certain numbers, sizes, forms and arrangements of chromosomes, which we know from normal and abnormal cases, including the fact of the reduction of chromosome number in germ cells.* (Boveri, 1909). Mendel's experimental work with peas and his theory of heredity had already been published in 1866, but remained in relative obscurity until it was rediscovered and experimentally confirmed in 1900 by Carl Correns (1864-1933), Hugo de Vries (1848-1935) and Erich Tschermak (1871-1962). In order to explain Mendelian ratios the Boveri-Sutton theory argued: 1. Each chromosome is an individual and carries a unique combination of genes. This combination is present also in its homolog, but differs from the combination found in any other non-homologous chromosome. 2. Mitosis - in concordance with Roux's visionary arguments of 1883 - evolved as a mechanism, which safeguards that both daughter cells receive the same number and combination of Mendel's hereditary characters (or genes as they were called in 1909 by Wilhelm Johannsen (1857-1927)). This requirement is fulfilled by the faithful chromatid segregation to the new daughter cells. Dissolution of chromosomes during interphase and random aggregation of genes into new chromosomes at the next prophase would lead to random increases and losses of individual genes in cycling cell populations.

The Boveri-Sutton theory explained not only Mendelian ratios in cytological terms, it made important, experimentally testable predictions, it triggered, for example, the search for linkage groups along chromosomes. *If ... it turns out that the number of combinations, which may connect single hereditary characters, is larger than the possibilities of combinations given by the number of chromosomes, one must conclude that the hereditary elements localized in a given chromosome are able to move into different cells during the meiotic divisions and this would argue for an exchange of segments between homologous chromosomes* (Boveri, 1904).

Boveri and Sutton claimed that chromosomes in a haploid set differed from each other in their content of hereditary elements. In support of this claim Sutton pointed out differences in the morphology of chromosomes and their behaviour during meiosis. First hints for morphological and functional differences between individual chromosomes that might play a role in the determination of a male or female development had already been described by Henking (Henking, 1891). Boveri provided functional evidence for different roles of individual chromosomes in a series of elegant experiments using the sea urchin as a model for early development (Boveri, 1902a; Boveri, 1902b; Boveri, 1903; Boveri, 1904). He found that exposure of sea urchin eggs to particularly high sperm concentrations often yielded eggs fertilized by two sperms. The fertilized egg cell now contained two active centrosomes able to divide. As a consequence during the first mitosis of the fertilized egg four centrosomes instead of two typically participated in chromosome segregation. As a result chromosome segregation and division became abnormal and four and sometimes three blas-

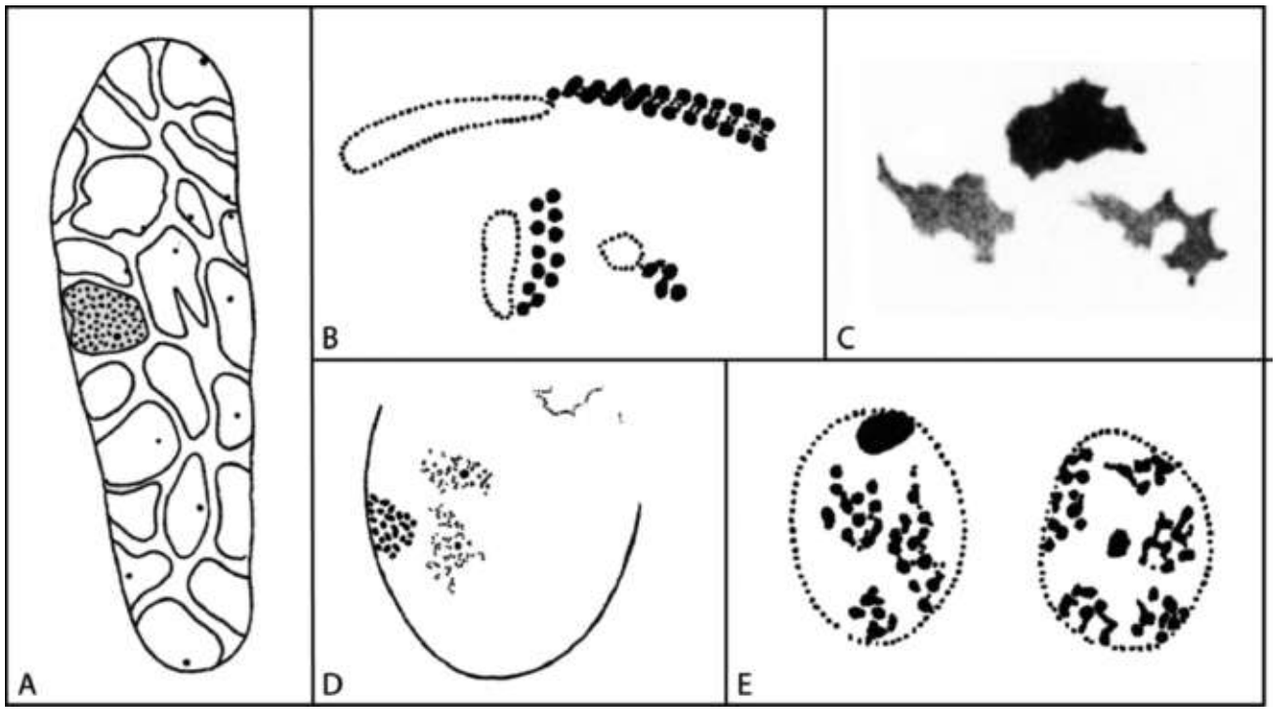

Figure 9. Drawings of chromosome structure during interphase and metaphase of the locust *Psophus stridulus* (Geitler, 1943). A) Diploid nucleus of an epithelial cell studied in the seminoferous tubule reveals *dense, nearly homogeneous chromatin clods (in one clod the chromatin fine structure is depicted*. Note that these clods (or chromosome territories in Boveri's terminology) are separated by an interchromosomal domain as proposed 50 years later by (Zirbel *et al.,* 1993). B) Chromosomes from a meiotic metaphase II show a spiral chromatin organization. C, D) Two autosome territories and the more intensely stained X-chromosome territory reveal a variety of shapes and a focal pattern of chromatin. E) Late telophase stages of a female and male meiosis II from the bed bug *Lygaeus saxatilis* show a compact X-territory (left) and Y-territory (right) together with autosome territories. The latter are apparently built up from higher order chromatin foci with an interchromatin space expanding between them.

tomeres were formed simultaneously, called *Simultanvierer and Simultandreier*, respectively. To Boveri's excitement Simultanvierer and Simultandreier were able to develop to abnormal larvae. He was not able to determine the number of chromosomes in normal and abnormal blastomeres, but deduced abnormal numbers from a pronounced variation of nuclear sizes in the abnormal larvae. By incubation of Simultanvierer embryos in Ca^{++} free seawater Boveri succeeded to disaggregate them into four single blastomeres. When he isolated these blastomeres and followed their individual development, Boveri found that the abnormal blastomeres had strikingly different developmental potentials, even when the nuclei had apparently the same size and by inference likely the same number of chromosomes. From these results Boveri concluded that not only the number of chromosome counted for a normal development but that the correct composition of individual chromosomes was indispensable. These results were neither predicted by Rabl's hypotheses nor by Weismann's chromosome theory of heredity (see above), but fit very nicely Boveri's concept of chromosome individuality.

Despite all his ingenuous experimentation and reasoning, Boveri was never able to provide unequivocal evidence for a territorial organization of interphase chromosomes. The lack of methods to visualize chromosome territories directly in the cell nucleus put a limit to his efforts. While Boveri's concept of chromosome territories is now widely accepted (see part II of this review in the following EJH issue), his steadfast support of this concept was based on wrong reasons. He considered the concept of chromosome territories as a cornerstone of his theory of chromosome individuality and consequently of the Boveri-Sutton theory of chromosome heredity. Looking again on Boveri's cartoon of the hypothetical architecture of chromosome territories (Figure 7B), we recognize that he depicts chromatin bundles with a compaction clearly beyond the level of 10 and 30 nm chromatin fibres. He even considered the possibility of

an achromatic substance to maintain chromosome territories during interphase (Boveri, 1908).

Although in Boveri's time an astonishing lot was already known about nucleic acids in the cell nucleus (Kossel, 1882; Kossel, 1911; Miescher, 1871; Miescher, 1897) the importance of these molecules for the transmission of hereditary characters through the germ line and their expression in somatic cells was not known. Boveri was, of course, also not aware of the extraordinary length of DNA compared with the diameter of cell nucleus. Today it is common knowledge found in every undergraduate textbook of cell biology that a spherically shaped diploid human cell nucleus has a diameter of roughly 5-10 micrometer, yet contains 2-4 meter DNA depending on the stage of the cell cycle. For Boveri these commonplace numbers would have been a source of great surprise. He did not know that compaction of the DNA strand with nucleosomes yields a beads-on- a-string conformation, called a thin chromatin fibre with a diameter of 10 nm. Neither was he aware of the next level of compaction into a thick chromatin fibre with a diameter of about 30 nm. One meter DNA yields thin and thick chromatin fibres with a total length of about 100.000 and 25.000 micrometer, respectively. If mitotic chromosomes would totally decondense to the level of thick and thin chromatin fibres, a single interphase chromosome could expand backwards and forwards throughout the whole nucleus without violating Boveri's postulate of chromosome individuality. It is therefore no surprise that the concept of chromosome territories was generally abandoned during the 1950th to 1980th by researchers, which had no doubts whatsoever about the correctness of the Sutton-Boveri theory (see part II, next volume). Even today no consensus has been reached concerning the possible hierarchical levels of chromatin order within the nucleus. The higher the level of compaction, the more dubious becomes the evidence for and against certain models.

Still in the 1940th chromosome territories were apparently considered as a common feature of the nuclear architecture. Figure 9 shows drawings from Lothar Geitler, who studied the nuclear fine structure of locusts and bed bugs (Geitler, 1943). Geitler neither cites Boveri nor uses his term chromosome territory, but describes a clod-like formation of chromosomes in interphase nuclei (*die Ausbildung der schollenförmig entwickelten Chromosomen in den Ruhekernen*). As we will see, however, in part II of our historical account the concept of chromosome territories fell in disgrace during the 1950th to the 1970th, when electron microscopic studies failed to distinguish them.

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