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An Atomic Force Microscopical Study of the Synaptonemal Complex

CONSTANT A. J. PUTMAN,* AXEL J. J. DIETRICH,† BART G. DE GROOTH,* JAN VAN MARLE,† CHRISTA HEYTING,‡ NIEK F. VAN HULST* and JAN GREVE*

*Department of Applied Physics, University of Twente, P.O. BOX 217, NL-7500 AE Enschede, The Netherlands
†Institute of Human Genetics; Department of Electron Microscopy, University of Amsterdam, Meibergdreef 15, NL-1105 AZ Amsterdam, The Netherlands
‡Institute of Genetics, Agricultural University, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands

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Abstract—The chromosomal structure which is specific for meiosis, the synaptonemal complex (SC), plays a major role in chromosome pairing and the recombination of genetic material. The SC was studied using atomic force microscopy (AFM). The results of this study confirm the results of light and electron microscopic studies of the SC. The SC primarily shows a tripartite structure, composed of two lateral elements and a central element connected to each other by transversal elements. The lateral element of the SC shows a lateral subdivision in secondary lateral strands. In addition, a new repetitive transversal subdivision of the lateral elements was observed. Furthermore, the SC substructure which is probably responsible for recombination, the recombination nodule, could be observed ouside the confines of the SC.

Key words: Atomic force microscopy (AFM), synaptonemal complex (SC), lateral element, transversal element, central element, recombination nodule.

INTRODUCTION

During the prophase of the first meiotic division the homologous chromosomes pair and crossing-over takes place. A meiosis specific chromosomal structure plays a major role in chromosome pairing and crossing-over: the synaptonemal complex (SC). In principle all eukaryotic species demonstrate the presence of SCs during meiosis. The structure and composition of substructures is evolutionarily highly conserved. The SCs are composed of two lateral elements (LEs), a central element (CE) and transversal elements (Moses, 1968; Von Wettstein *et al.*, 1984; Westergaard and Von Wettstein, 1972). More substructures have been observed in LEs of SCs in preparations of meiocytes (Wahrman, 1981; Del Mazo and Gil-Alberdi, 1986; Pujol *et al.*, 1988; Dietrich *et al.*, 1992). In isolated SCs this subdivision of the LEs could also be observed (Heyting *et al.*, 1985; Dietrich *et al.*, 1992). However, the observed multistrandedness of the LEs could be caused, at least partially, by artefacts of the preparative and imaging techniques used. Since all techniques used in microscopical studies of biological objects will produce their own specific artefacts, it is essential to use a wide range of different techniques.

In the past decade, a whole new series of high resolution microscopes has been introduced, the so-called scanning probe microscopes. These microscopes have a common system consisting of a submicron sized probe which is scanned in close proximity to the sample surface. In the scanning tunneling microscope (STM; Binnig *et al.*, 1982)—the first scanning probe microscope—a tunneling current is measured between a sharp metal tip and a conducting surface. Although thin biological structures, such as DNA molecules on a conducting substrate, can be imaged with the STM (Beebe *et al.*, 1989; Amrein *et al.*, 1989), larger biological structures require a layer of conducting material. A direct descendant of the STM, the atomic force microscope (AFM; Binnig *et al.*, 1986), has overcome this problem. In the AFM a sharp stylus is mounted on a weak cantilever. When the stylus is brought into contact with the sample surface, interaction forces between the tip of the stylus and the surface will deflect the cantilever. Measurement of the deflection of the cantilever while the sample is raster scanned beneath the tip, gives a topographic representation of the sample surface.

A wide variety of samples has been imaged with the AFM in recent years, some of them revealing the atomic structure of the surface. DNA molecules (Bustamante *et al.*, 1992; Hansma *et al.*, 1992), cells (Butt *et al.*, 1991), chromosomes (De Grooth *et al.*, 1992; Heckl *et al.*, 1992; Putman *et al.*, 1992a; De Grooth and Putman, 1992) and gap junctions (Hoh *et al.*, 1991).These represent only a small sample of the biological specimens which have been studied with the aid of AFM. The AFM has potential advantages over electron microscopes. The AFM can be operated under ambient conditions, which does not require ultra high vacuum. For recent reviews on

* Correspondence: Constant Putman, Department of Applied Physics, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands. Phone/Fax: +31 53 893157/+31 53 309549.

biological applications of scanning probe microscopes the reader is refered to Engel (1991) and Hoh and Hansma (1992).

In the present study the morphology of isolated SCs is described using the AFM.

MATERIALS AND METHODS

Preparation of SCs

Male Wistar rats of 6—10 weeks old were sacrificed, the testes removed and SCs were isolated according to Heyting and Dietrich (1991).

Preparation of SC suspension for AFM study

The SC suspension was diluted with distilled water to obtain a suitable concentration of SCs. One drop of the suspension was placed on a glass microscope slide. The drop remained there for approximately 5 min at room temperature. Either the drop was removed and a drop of fixative was placed on the same spot or the original drop was left to dry. A fixative consisting of a 4% solution of *para*-formaldehyde was used. The fixative remained on the slide for approximately 5 min at room temperature. After fixation or air-drying the slide was rinsed carefully with distilled water. The samples were then air-dried and examined in the AFM.

Atomic force microscope

The atomic force microscope features an inverted optical microscope (Putman *et al.*, 1992a). The standard microfabricated cantilever with an integrated pyramidal tip (Albrecht *et al.*, 1990; purchased from Park Scientific Instruments, Sunnyvale, CA, U.S.A.) is positioned within the field of view of the optical microscope by translating the objective with an *xy*-stage. Biological objects on a transparant substrate, e.g. microscope slide, can be selected and positioned under the tip with a second *xy*-stage. The displacement of the cantilever is detected by the optical beam deflection technique (Meyer and Amer, 1988; Putman *et al.*, 1992b). In this technique a laser beam is focused on the the cantilever and reflected towards a 2-segment photodiode. A variation in height on the sample surface gives a difference signal due to a shift of the laser beam on the photodiode. A feedback loop keeps the difference signal at a preset value by moving the sample up and down. This is achieved by applying a varying voltage to the *z*-electrode of the piezo tube used for raster scanning the sample underneath the tip. Displaying these applied voltages or the error signal—signal due to displacements of the cantilever which are not compensated for by the feedback loop (Putman *et al.*, 1992c)—in a 2-dimensional matrix, gives a representation of the sample surface. The samples were studied in air and the images presented here were recorded in the error signal mode.

The system which uses a scanning needle carries the risk of double images due to microtips at the apex of the pyramidal tip (Hoh and Hansma, 1992). This type of artefact can be recognized by rotating the sample and imaging the same object again.

The SCs observed were classified according their morphology in different meiotic prophase stages (Dietrich and de Boer, 1983)

RESULTS

The Figs 1–5 show isolated SCs and their details at different magnifications. Figure 1 shows SCs treated with formaldehyde fixative. Lateral elements are clearly visible in Fig. 1a, as are the attachment plaques in Fig. 1a and 1b at the ends of the SCs. Unfixed SCs, as presented in Fig. 2, show a more frayed image when compared with SCs that were treated with fixative (Fig. 1). Unfixed SCs clearly

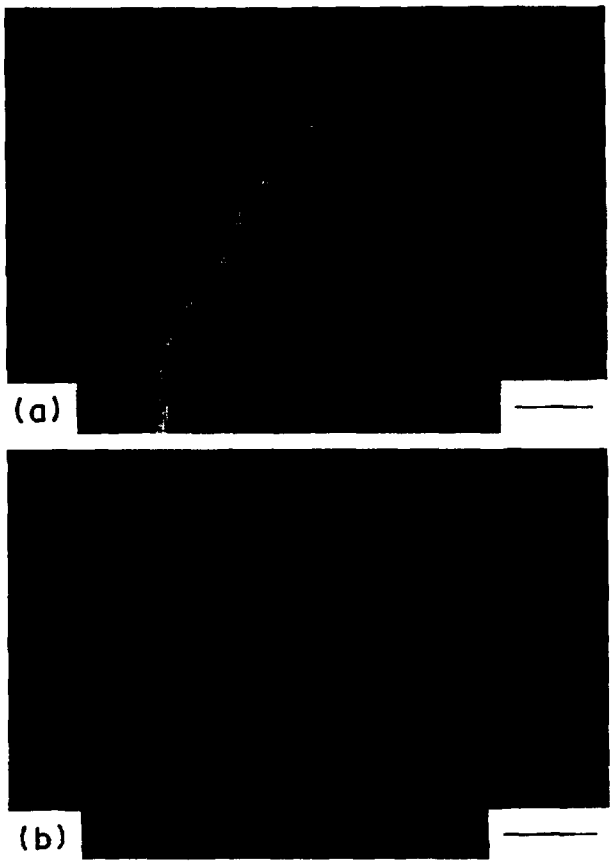

Fig. 1. Isolated SCs treated with formaldehyde fixative (a, b). Bar represents 1 μm.

show a tripartite structure, consisting of 2 lateral elements and a central element (indicated by the arrows). In addition to the division into lateral and central elements, a subdivision of the lateral elements into secondary lateral strands (Fig. 3a, upper left) and a repetitive transversal subdivision was observed (Fig. 3b). The arrows shown in Fig. 3b indicate the repetitive nature of this subdivision (Fig. 3b is a detail of Fig. 1a).

Knob-like structures, which might be ascribed to the

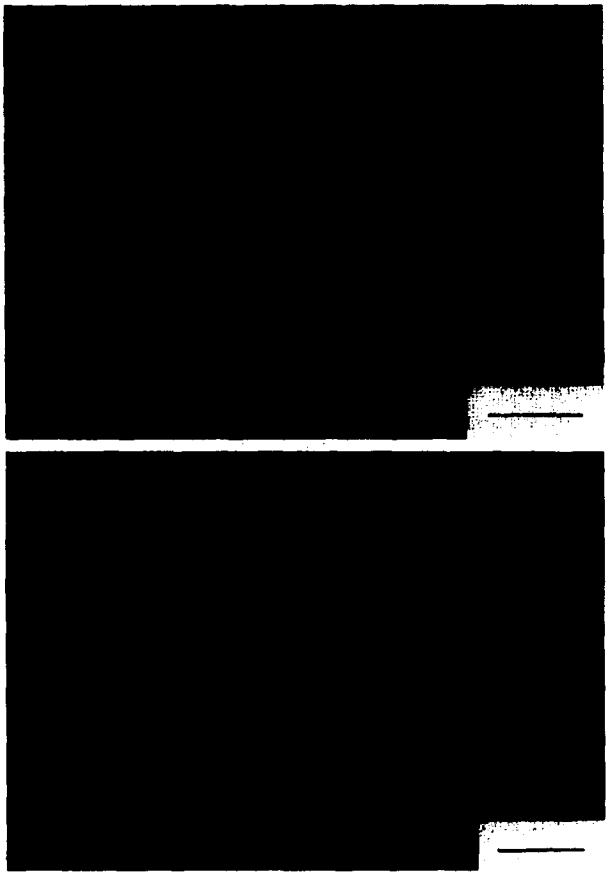

Fig. 2. Isolated, air-dried unfixed SCs. Arrows indicates the central element. Bar represents 1 μm.

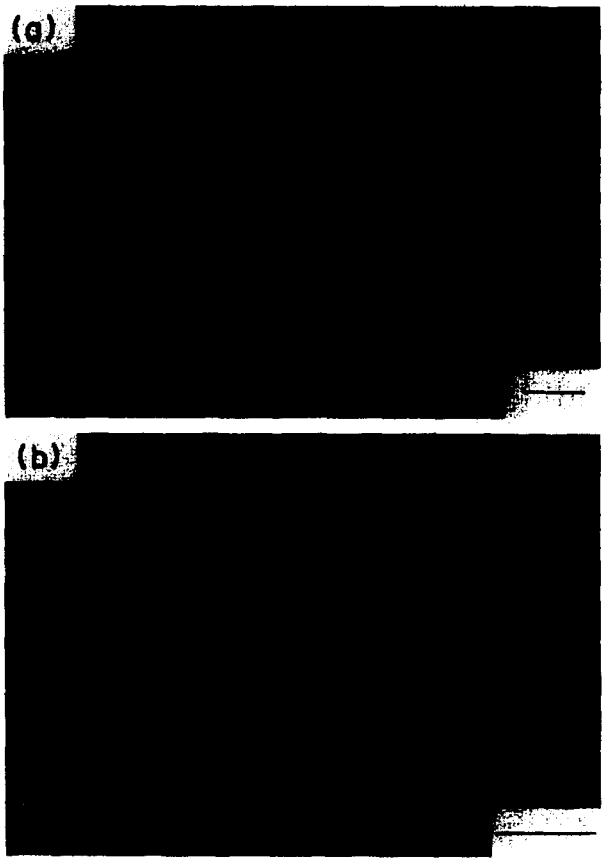

Fig. 3. Details of SCs treated with formaldehyde fixative (a, b). Arrows indicate transversal subdivision of lateral elements. Bar represents 0.25 μm.

recombination nodule (RN)—a subunit probably responsible for DNA recombination (Carpenter, 1988)—could be observed on fixed (Fig. 4a) as well as unfixed samples (Fig. 4b). Figure 4b shows a detail of a cluster of SCs. Lateral elements and attachment plaque (bottom right) are again evident. The central element is clearly visible just below the RN indicated by the arrow.

The attachment plaques of the SCs show a typically multistranded structure. This is especially clear in isolated SCs from the later stages of meiotic prophase, when the SC starts to disintegrate (Fig. 5). Furthermore, a subdivision of the lateral elements into secondary lateral strands is indicated.

DISCUSSION

In this study the synaptonemal complex (SC) is visualized using an AFM. We also engaged in several earlier attempts to image SCs with an STM. The conducting material that covered the SCs appeared to present a serious drawback for the study of SCs. The AFM does not have this disadvantage and the morphology of substructures could be studied in detail. In addition, the possibility to rapidly locate and select objects at low magnification with a light microscope proved to be essential, because in preparations of isolated SCs, the SCs are widely dispersed in a low density over the object slides. Earlier attempts to image SCs with an AFM without an integrated optical microscope, did not provide results even after several days of imaging.

The use of isolated SCs is a prerequisite for the study of SCs using an AFM because the surrounding material and cell constituents are removed. The AFM images obtained, were most satisfactory and they confirmed the results of earlier research carried out with the aid of electron microscopy, where a double and triple strand structure in the LEs of isolated SCs was observed (Heyting *et al.*, 1985; Dietrich *et al.* 1992). In this study the lateral element also appears to be subdivided in lateral structures (see Fig. 3a and 5). The observed double structure of the lateral elements (LEs) was confirmed by rotating the sample; this excludes artefacts due multiple imaging by microtips at the apex of the pyramidal tip.

The two major structures could represent the axes of the two sister chromatids of one chromosome as proposed by Wahrman (1981). These are kept together, which is neccessary for meiotic recombination, when the four chromatids should be in a paired position. The two major strands could be paired by a third strand of smaller size. The possible connecting role of the third element between the two major components of the LE has been described by Dietrich *et al.* (1992). The possibility of an AFM study of isolated SCs will facilitate the further study of this third LE strand.

This type of AFM study will contribute to the insight of

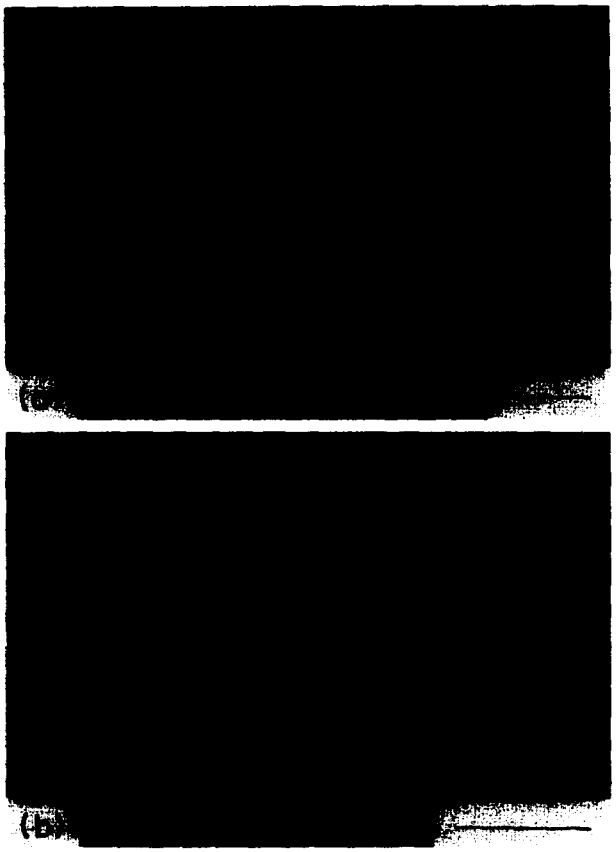

Fig. 4. Details of SCs, (a) treated and (b) untreated with fixative. Arrows indicates possible recombination nodule (RN). Bar represents 1 μm.

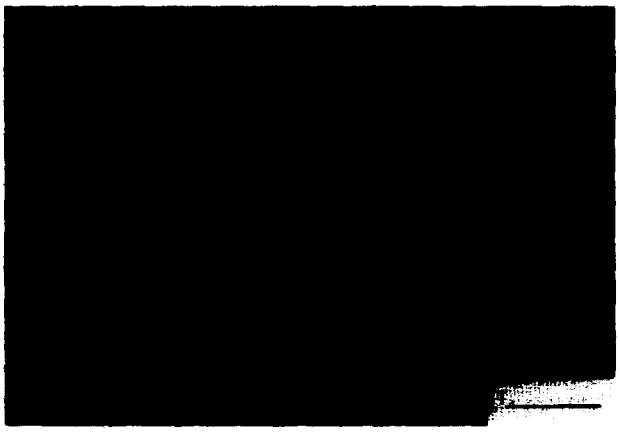

Fig. 5. Details of isolated, untreated SC. Arrow at bottom left indicates multistranded attachment plaque. Arrow right from center indicates the division of the lateral elements into secondary lateral strands. Bar represents 1 μm.

the structure and function of the SC. The solid structures imaged with the AFM are markedly different from the dispersed and relatively low electron scattering macromolecules as observed in the EM. They give some novel information concerning the overall structure of the SC since only the outside of the SCs are imaged. The most striking observation is a repetitive transversal subdivision of the LEs (Fig. 3), which is difficult to reconcile with the existing knowledge of the ultrastructure of the SCs. Tl transversal subdivision of the LEs could be a reflection the attachment of the transversal elements in the cent region, but it is also possible that it represents t attachment of the chromatin loops to the SC. T subdivision of the LEs is not easily observed in conv tional EM studies. However, we have recently initiate 3D study of the SCs using EM stereopairs and compu assisted reconstructions of sectioned SCs. From th preliminary results it appeared that LEs demonstrat helical structure with a periodicity of approximately 1 nm; a dimension which could fit in the range of t periodicity observed in this AFM study.

The substructure of the SC, which is responsible for t actual recombination of the DNA is most likely t Recombination Nodule (RN; Carpenter, 1988). Str tures, which could represent the RN, were observed in t expected position (Fig. 4b, in between the lateral e ments), but also located 'outside' the confines of t ribbon-like SC (Fig. 4a, globular structure to the ri side of the SC), as indicated by the arrow.

From this study it appeared that an AFM can be us for imaging macromolecules such as the SC. Although resolution is currently not as high as in the EM, it sufficient to be useful in order to obtain information ab the SCs quaternary structure which is difficult to obse with the EM. When studied with the EM this informati is often lost in the over-detailed projection image, wh has as an additional handicap that all the de information is lost. To retrieve this information from E images is much more laborious than using the AFM. this paper we have shown the potential of the atomic fo microscope for structural studies of meiotic structure

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