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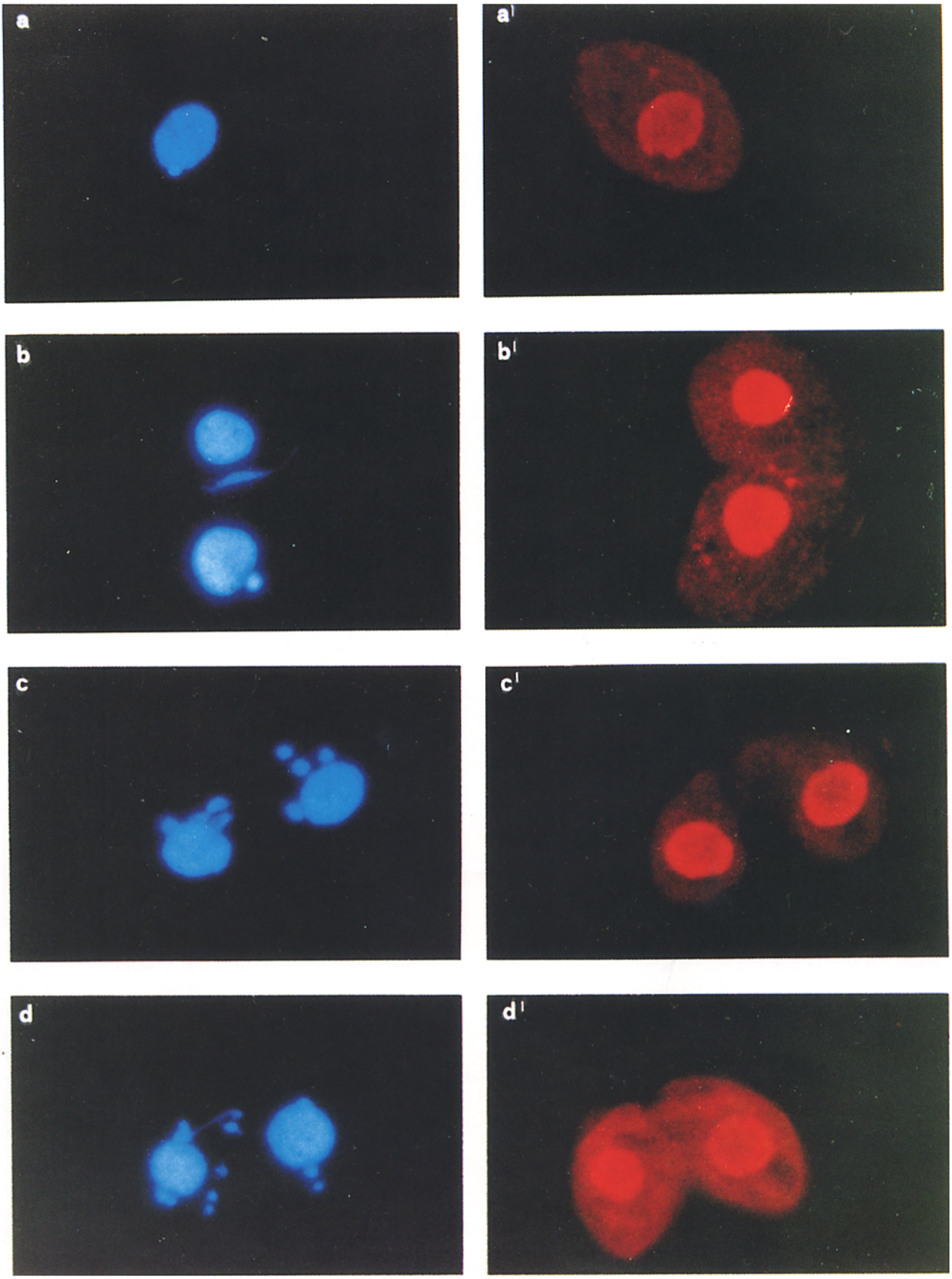
Due to an editing error, Figs. 2 and 3 were reversed and appeared with the incorrect legends. The figures and their correct legends follow.

Figure 2. Indirect immunofluorescence of conjugating *T. thermophila*, prefertilization stages. (*a* and *a'*) A single starved cell before conjugation. The micronucleus is located in an indentation of the macronucleus. (*b* and *b'*) A conjugating pair in meiotic prophase after 2 h of conjugation. Crescent formation in one partner whereas in the other partner the micronucleus has just left its original position. (*c* and *c'*) A pair after the second prezygotic division (5 h of conjugation). Four anteriorly located micronuclei are visible in both cells. (*d* and *d'*) After the third prezygotic division (6 h of conjugation) two anterior pronuclei are visible. In the posterior part of the cells remaining



micronuclei are eliminated. (*a–d*) DAPI stain revealing the nuclear stage. (*a–d'*) Indirect immunofluorescence using anti–tetracetylated H4 histone antibodies and rhodamine-conjugated second antibody. Note that no reaction occurs in micronuclei during the prefertilization stages indicating that tetraacetylation of H4 histone is not correlated to deposition of histones during replication or to the chromatin reorganizations occurring during meiosis and prezygotic divisions.

dues which are the target for postsynthetic acetylation and this pattern is conserved between *T. thermophila* and mammals (lysines at positions 4, 7, 11, 15 and 5, 8, 12, 16, respectively) (20, 24).

By immunoblotting of histones separated on Triton/acetic acid/urea gels we have previously shown that our antibody reacts only with the tetraacetylated and to a lesser extent with the triacetylated form of H4 histone (30). Here we have tested the reactivity of the antibody with histones extracted from isolated macronuclei of *T. thermophila*, separated by SDS and by Triton/urea/acetic acid gel electrophoresis and blotted onto nitrocellulose sheets. The binding of the antibody has been revealed by anti-rabbit immunoglobulins antibodies conjugated to horseradish peroxidase. The results are depicted in Fig. 1. The antibody stains specifically the band corresponding to H4 histone on blots from SDS-gels and bands corresponding to the tetra- and triacetylated form of this histone on blots from TAU gels. This indicates, as expected, that the antigenic determinant, that is the pattern of ϵ acetyl-lysines in the amino-terminal portion of the molecule, is not immunogenically distinct from the corresponding pattern in mammalian H4 histone.

A slight cross-reaction is observed with the band corresponding to H2a and H3 histones which are not separated on this SDS gel and with a band probably corresponding to H1 histone on blots from TAU gels. The different cross-reaction on blots from different gel systems indicates that only the binding to acetylated forms of H4 histones is specific.

Immunofluorescence Analysis of Conjugating T. thermophila

The antibody has been used in immunofluorescence analyses of vegetatively growing, starved, and conjugating *Tetrahymena thermophila*. The results are shown in Figs. 2 and 3. For conjugation, cells with different mating types have been mixed after initialization under starving conditions. At different times, samples have been removed and prepared for indirect immunofluorescence staining, using the anti-tetraacetylated H4 histone antibody and a rhodamine-conjugated second antibody (*red stain*, sections on right in Figs. 2 and 3). The nuclear stage of the cells has been revealed by counterstaining the same cells with DAPI, a DNA specific fluorescence dye (14) (*blue stain*, sections on left in Figs. 2 and 3).

In starved nonconjugating cells, the micronucleus is located in an indentation of the macronucleus and shows no staining with the antibody whereas the macronucleus strongly reacts (Fig. 2, *a* and *a'*), indicating that only the macronucleus contains highly acetylated forms of H4 histone. The same is observed in vegetatively growing cells (data not shown).

Upon conjugation, the micronucleus undergoes meiosis and Fig. 2 *b* shows early meiotic prophase with crescent formation in one partner whereas the other one shows a slightly earlier stage where the micronucleus has just left its pouch. Fig. 2 *c* shows the situation after the second prezygotic division and Fig. 2 *d* shows the result of the third prezygotic division where pronuclei are seen in the anterior part of the cells and micronuclei which will be eliminated are located in the posterior part of the cells. During all these nuclear events which include DNA synthesis in meiotic prophase and between nuclear divisions (7), the anti-tetraacetylated H4 histone antibody does not stain micronuclei whereas macronuclei are labeled (Fig. 2, *a'-d'*), indicating that the antibody used does not reveal histone deposition related acetylation processes which occur during DNA-synthesis. Deposition-related histone acetylation leads to the diacetylated form of H4 histone showing a different acetylation site usage (13). No diacetylated forms are recognized by the antibody used here (Fig. 1).

This situation persists up to the first postzygotic division (Fig. 3, *a* and *a'*) where none of the two micronuclei in each partner is stained by the antibody. After the second postzygotic division two micronuclei migrate to the posterior part whereas the other two micronuclei of each cell remain in the anterior part and begin to swell. Over these young macronuclear anlagen a strong label is now observed whereas the posteriorly located micronuclei show no staining with the antibody (Fig. 3, *b* and *b'*) indicating that at this stage differential acetylation occurs in different nuclei depending on their position within the cytoplasm. The importance of the position is emphasized by the fact that in the few cases where we observed an apparent migration failure of micronuclei, H4 histone is acetylated in all four micronuclei (Fig. 4), although the cause of this event remains unknown and its frequency does not allow statistical evaluation.

In different analyses it has been shown that at this cytological stage during conjugation, transcriptional activity is still carried out by the centrally located macronucleus whereas the young macronuclear anlagen are still silent (38, 39). As the onset of transcription may depend on refeeding (9), it must be pointed out that in the experiments reported here conjugating pairs have not been refed. Moreover, histone acetylation appears to peak rapidly as it is difficult to see intermediate states. As judged from the position and size of the labeled macronuclear anlagen this process clearly precedes

Figure 3. Indirect immunofluorescence of conjugating *T. thermophila*, postfertilization stages. (*a* and *a'*) Conjugating pair after the first postzygotic division (6.5 h of conjugation). Two micronuclei have formed from the fertilized micronucleus. (*b* and *b'*) Second postzygotic division (7 h of conjugation). Two anteriorly located micronuclei (also referred to as young macronuclear anlagen) begin to swell and will later on develop into new macronuclei whereas two products of the second postzygotic division have migrated to the posterior part of the cell and will become new micronuclei. The old macronucleus is still centrally located. (*c* and *c'*) Macronuclear anlagen development (8 h of conjugation). The old macronucleus has migrated to the posterior part of the cells but is only slightly pyknotic. Macronuclear anlagen have enlarged and are now centrally located whereas micronuclei have not undergone morphological changes. (*d* and *d'*) Macronuclear anlagen development after 9 h of conjugation. The old macronucleus is in an advanced stage of elimination whereas new macronuclei continue to enlarge. (*a-d*) DAPI stain revealing the nuclear stage. (*a'-d'*) Indirect immunofluorescence using anti-tetracetylated H4 histone antibodies and rhodamine conjugated second antibody. Note that up to the first postzygotic division of the fertilized nucleus no acetylation takes place in the micronuclei. After the second division, when also morphological differentiation begins, anteriorly located young macronuclear anlagen are heavily stained by the antibody indicating the onset of histone acetylation which rapidly reaches maximum levels. Acetylation of H4 histone persists during macronuclear anlagen development, whereas no reaction occurs in the old macronucleus in its final position in the posterior part of the cell. New micronuclei are never stained by the antibody.