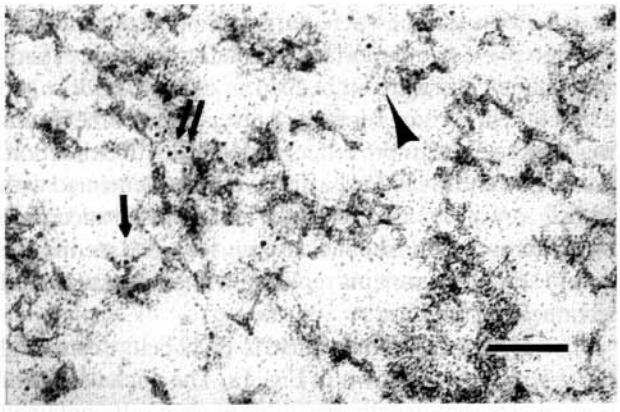

Fig. 5. As in Fig. 3, but after prefixation of cells with 4% paraformaldehyde. Matrix embedded in LR White resin. Bar: 0.1 μm

one or two compact nucleoli, but in about 50% of cells large unravelled nucleoli with typical interstices were observed. The nuclear envelope was formed by two membranes with frequent perinuclear localization of chromatin. The cytoplasm was characterized by structurally modified mitochondria and extensive vacuolization in most of the cells. The extraction of cells with Triton X-100, in physiological ionic strength and pH of CSK buffer, preserved the nuclear and partly also the cytoskeleton morphology (Fig. 1b). Ultrathin LR White sections showed preservation of the nuclear membrane and distinct regions of chromatin organization. The stained nucleolus was easily identified. The treatment of extracted structures with DNAase I and elution with 0.25 M ammonium sulphate left the nuclear matrix free of chromatin (Fig. 1c). Ribonucleoprotein (RNP)-containing matrix with some filaments and nuclear lamina were

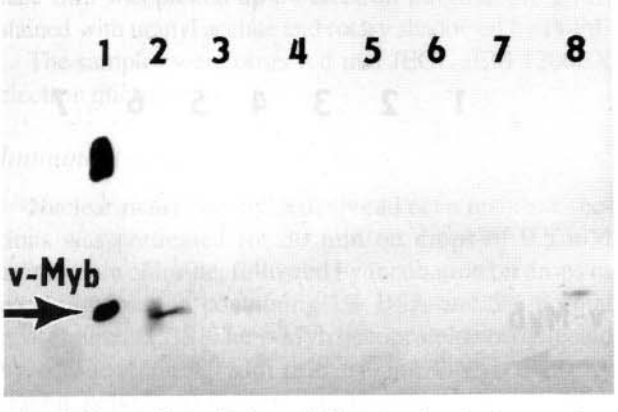

Fig. 6. Extraction of the v-Myb protein during nuclear matrix preparation with prefixation of cells with 4% paraformaldehyde as detected by Western blot analysis. Line 1: v-Myb protein marker (isolated from baculovirus expression system). Line 2: v-Myb protein marker from LSCC-BM2 cells. Line 3: extraction of LSCC-BM2 cells with 0.5% Triton X-100. Line 4: second extraction with 0.5% Triton X-100. Line 5: digestion of chromatin with DNAase I. Line 6: salt extraction after DNAase treatment. Line 7: second extraction after DNAase treatment. Line 8: v-Myb protein marker.

apparent. A more distinct filamentous structure was observed on unembedded resin-free sections (Fig. 1d), and especially when nuclear matrix was visualized by the aqueous spreading technique (Fig. 2). This spreading method revealed an overall fibrous structure (Fig. 2a, arrow), where at higher magnification details of the elaborate fibrogranular network were seen (Fig. 2b).

The immunoelectron microscopical studies performed on the nuclear matrix embedded in LR White resin revealed the cooperation of the v-Myb protein with this nuclear structure. However, this interaction was rather poor when matrix extraction was performed without appropriate prefixation (Fig. 3). As apparent from Western blot analysis, when prefixation of cells was omitted, a substantial amount of the v-Myb protein was extracted with detergent (Fig. 4, line 2 and 3) as well as by DNAase treatment (line 4) and ammonium sulphate extraction (line 5). No Myb protein was extracted by following salt extractions (line 6). When paraformaldehyde fixation was applied before detergent treatment, the collodial gold labelling of the v-Myb protein on nuclear matrix structures was more intense (Fig. 5). Western blot analysis revealed in this case only a small amount of the v-Myb protein extracted by detergent treatment (Fig. 6, line 3), whereas DNAase treatment and ammonium sulphate extraction did not release any apparent amount of this protein (lines 4, 5, 6, 7). In both cases, with or without prefixation, the micrographs showed that the anti-v-Myb label decorated mostly the fibrillar structures (Fig. 3 and 5, arrow), but some marker was found also in less dense structures (Fig. 3 and 5, arrowhead). The labelling was specific as no collodial gold marker was detected in control experiments where primary antibody was omitted (not shown). The label was frequently found in clusters (double arrow) decorating fibrillar structures (Figs. 3 and 5); however, many individual markers along the nuclear matrix were also apparent (Figs. 3 and 5). These results confirm, immunoelectron microscopically, the interaction of the v-Myb protein with components of the nuclear matrix.

Discussion

The nuclear matrix has been implicated in a wide variety of processes which involve interactions with nucleic acids, including DNA replication (Jackson et al., 1988), transcription (Thorburn et al., 1988) and RNA splicing (Harris and Smith, 1988). In addition, the association of transcriptionally active genes with the matrix was also reported (Cook and Brazell 1980; Ito and Sakaki, 1987). As protein products of the v-*myb* and c-*myb* genes possess the characteristics of transcription factors, their cooperation with nuclear matrix can be expected. In this report we present an ultrastructural study of the v-Myb protein product interaction with isolated nuclear matrix of avian haematopoietic cells expressing the v-*myb* oncogene.