As the LSCC-BM2 cells represent non-adherent cells growing in suspension and as they are strongly sensitive to the detergent treatment, it was necessary to develop an appropriate extraction procedure for preparation of the nuclear matrix. A very elegant method for encapsulation of free cells in 0.5% agarose was described (Jackson et al., 1988; Hozák et al., 1993). Agarose beads containing cells were used for nucleoskeleton preparation and visualization of replication sites (Hozák et al., 1994). In our experiments we have embedded the cells in 0.5% agarose, cut small blocks (maximum 0.5 mm^3) and used them for further extraction procedure. The agarose blocks did not prevent penetration of solutions into and out of the cells, but protected the cells and the extracted structure from mechanical damage. The extraction procedure used represents a gentle method of removing chromatin from the nuclear skeleton. Some previous procedures used high salt (Berezney and Buchholtz, 1981; Brasch, 1982) or strong detergent treatment (Mirkovitch et al., 1984), which removed most of the histones and other proteins before digestion of chromatin and altered the interior nuclear morphology. The modified method of Fey (Fey et al., 1986) using moderate salt concentration (0.25 M ammonium sulphate) eluted chromatin in the form of intact nucleosomes, and extraction of other proteins was also diminished. We have used this gentle method because subnuclear fractionation of the v-Myb protein showed its high variability in distribution between chromatin and nuclear matrix compartments depending on ionic strength (Boyle and Baluda, 1987). However, even using this technique we observed that the v-Myb protein was extracted from nuclei in different steps of the procedure as was apparent from Western blot analysis. To minimize this extraction we used prefixation of structures by paraformaldehyde prior to the nuclear matrix preparation procedure. Indeed, this fixation led to substantial intensification of anti-v-Myb labelling on nuclear matrix structures.

Two techniques were described for staining with collodial gold bead-coupled antibodies, preembedment and postembedment immunostaining (Nickerson et al., 1990). As LSCC-BM2 cells were found to be very fragile and sensitive to preparation conditions, preembedment immunostaing was not suitable, resulting in our hands in damaged structures and obscure morphology of the embedded material. Postembedment immunolabelling was most convenient in this case, with adequate sensitivity and no unspecific background. In conclusion, our findings presented in this paper demonstrated that the v-Myb oncoprotein interacts with the nuclear matrix of avian haematopoietic cells. This interaction is dependent on experimental conditions, and without prefixation a substantial amount of the v-Myb protein is released from structures during the extraction procedure. Experiments of co-localization of the v-Myb protein with some proteins specific for nuclear matrix are in progress.

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