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Architectural and Molecular Studies of Nuclear Matrix Proteins

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Our understanding of the proteins which compose the internal architecture of the nuclear matrix has lagged behind recent progress defining domains of replication, transcription and prem RNA splicing associated with these structures. We are currently focusing our attention on identifying the major proteins which constitute the nuclear matrix. A limited number of interior nuclear matrix proteins, termed nuclear matrins, were previously identified by our lab. In this presentation I will report progress on the analysis of the two nuclear matrins, P-250 and SFA-Cyp. The cDNA encoding, splicing factor associated-cyclophilin (SFA-Cyp), a novel nuclear matrin, will be presented. Antibodies raised against a fusion protein of SFA-Cyp indicate that it is a 103k Da protein which is located in the nucleus and enriched in the nuclear matrix. Also, this antibody stains a series of bright foci in mammalian nuclei which colocalize with splic-

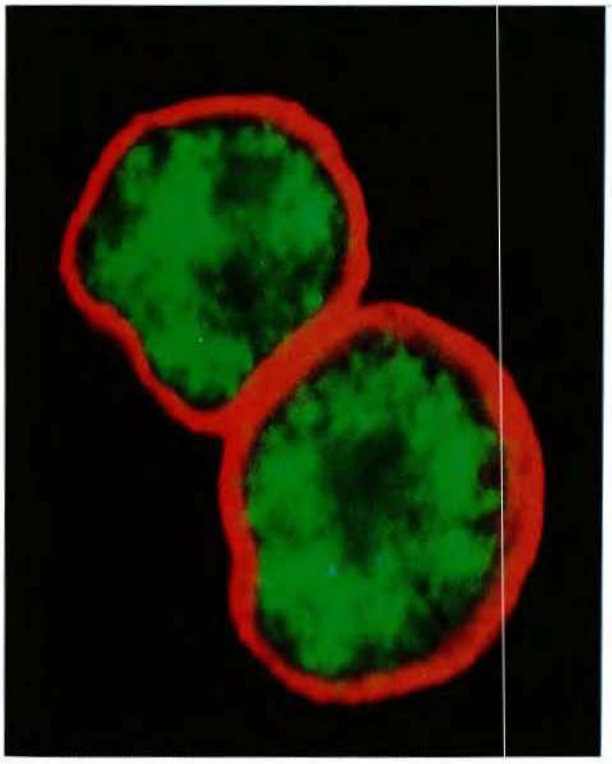

Fig. 1.

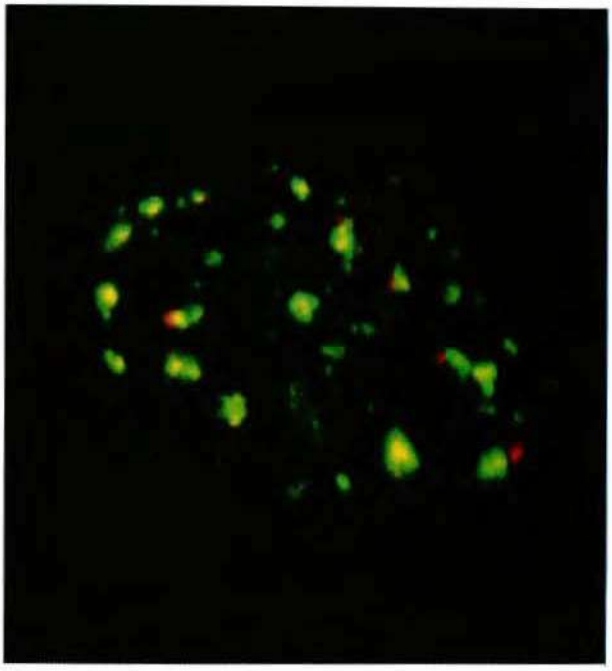

Fig. 2.

ing factors (figure 1 shows anti-lamin A/C (Texas red) and anti-SFA-Cyp (FITC) in primary rat hepatocytes, figure 4 & 5 show human HeLa S3 cells stained with the anti-snRNP antibody Y12 (Texas red) and anti-SFA-Cyp (FITC)). As suggested by its derived amino acid sequence and immunofluorescent pattern SFA-Cyp may chaperone specific proteins to sites of splicesome assembly where it may then assist these proteins to associate with forming splicesomes. A monoclonal antibody raised against nuclear matrices recognizes a protein of 250kDa that is enriched in the nuclear matrix and termed P-250. The protein detected by our antibody was shown by antibody cross reactivity studies not to be a NuMA protein. Laser scanning confocal microscopy of P- 250 visualizes a complex architectural arrangement in the nucleus consisting of several large bright foci and many small, less intensely staining granules. Some of the large bright foci are also stained by splicing factor specific antibodies (figure 2 shows anti-P-250 (Texas red) and anti-SFA-Cyp (FITC) in mouse 3T3 cells). This may indicate an association of P-250 with splicing factors.

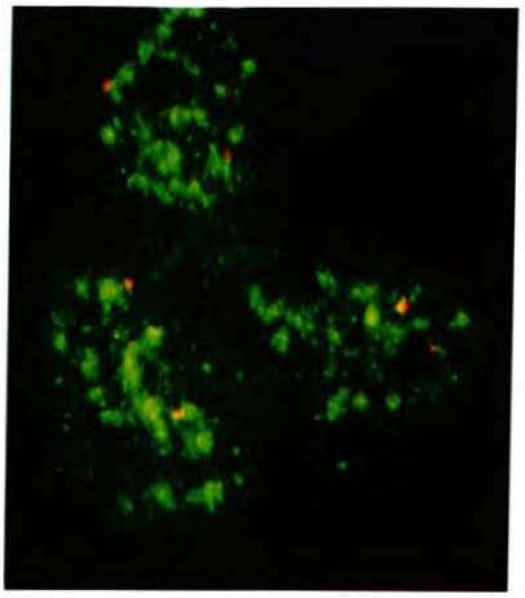

Fig. 4.

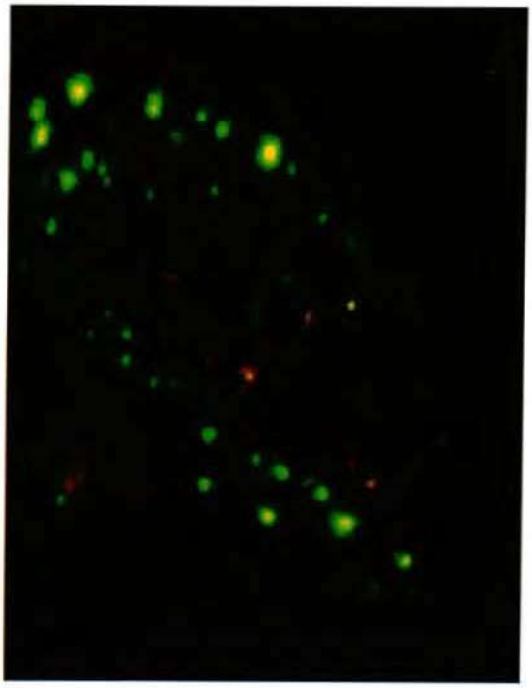

Fig. 3.

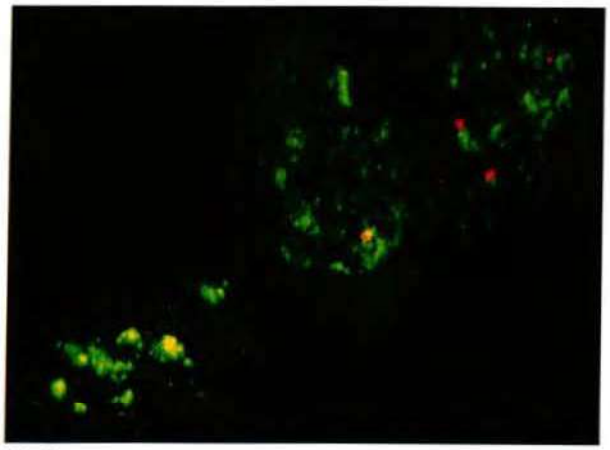

Fig. 5.