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Characterization of the KRAB-zinc finger protein Kid-1 and its adaptor protein KRIP-1

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In this study it could be demonstrated that Kid-1 is distributed in various patterns (patches or speckles) in transfected cells, in addition Kid-1 also is tightly associated with the nuclear matrix.

The patchy distribution of Kid-1 in the nucleus could be detected early after DMSO shock treatment, but the speckles appeared late and seemed to emanate from disintegrating patches. The colocalization studies showed that Kid-1 colocalizes with the nucleolar transcription factor UBF, so that the patchy distribution of Kid-1 corresponds to intact nucleoli, while the speckles represent disintegrated nucleoli. A mutagenesis study indicated that the large zinc finger cluster comprising zinc fingers 5 through 13 is required for the sorting of Kid-1 into the nucleolus, whereas the smaller zinc finger cluster of Kid-1 comprising zinc finger 1 through 4 was distributed homogeneously in the nucleoplasm and was easily extracted from the nucleus. Interestingly, nuclear run-on assays revealed that rRNA synthesis in disintegrated nucleoli containing Kid-1 was repressed, whereas it was still going on in intact nucleoli containing Kid-1. The finding that KRIP-1 was redistributed from the nucleoplasm into the nucleolus, when Kid-1 was coexpressed, supports the hypothesis that transcriptional repression of the rDNA genes causes the disintegration of the nucleolus. Such a model would argue against the

previous finding that KRAB-zinc finger proteins can only repress transcription mediated by polymerase II and III (Moosmann *et al.*, 1997).

In order to learn more about the function of Kid-1, a yeast two-hybrid approach was employed to search for proteins interacting with the KRAB-B domain and the region containing the PEST and Raf homology domains. It could be shown that the dynein light chain (DLC) interacts with the region containing the PEST and Raf homology domains of Kid-1. The role of the KRAB-B domain remains elusive, since we did not identify any KRAB-B interacting proteins. However, it is possible that the protein interacting with the KRAB-B domain is not represented in this embryomic cDNA library.

DLC was the first cytoplasmic dynein light chain identified. It is widely expressed in many cell types, with both nuclear and cytoplasmic locations. So far little is known about the function of dynein in the nucleus. DLC may function as a transporter, shuttling Kid-1 from the cytoplasm to the nucleus and finally into the nucleolus. Alternatively, DLC may play a role in the process of transcriptional regulation through Kid-1. In that context it is interesting that also a strong interaction of Kid-1 with IκBα was found, but only a poor interaction with IκBβ. The IκB family members function as inhibitors of NF-κB transcription factors. IκBα not only acts in the cytoplasm by binding to NF-κB proteins, but it can also function in the nucleus where it releases NF-κB from the preinitiation complexes (Tran *et al.*, 1997). It is tempting to speculate that newly synthesized IκBα translocates into the nucleus and forms a complex with Kid-1 and DLC. Through the adaptor protein KRIP-1, Kid-1 might help IκBα to remove NF-κB proteins from the preinitiation complexes, thus shutting off NF-κB target genes.

The connection between the nucleolar distribution of Kid-1 and its interaction with IκBα presents a puzzle so far. In light of reports that both the nucleolus and NF-κB are involved in the apoptotic process (Shaw, 1995; Baeuerle and Baltimore, 1996; Steph *et al.*, 1998), it is possible that Kid-1 also plays a role in progammed cell death. DEDD (death effector domain-containing DNA-binding protein), another nucleolar protein, translocates from the cytoplasm

into the nucleolus, where it can inhibit rDNA transcription and induce apoptosis (Steph et al., 1998). Kid-1 therefore may lead to apoptotic cell death both by inhibiting the anti-apoptotic action of NF-κB and by repressing rDNA transcription in the nucleolus. Future experiments will have to focus on this hypothesis in order to learn more about the biological role of Kid-1.