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THE NUCLEOLAR LOCALISATION SIGNAL OF THE HTLV-I PROTEIN *p27rex* IS IMPORTANT FOR STABILISATION OF IL-2 RECEPTOR α SUBUNIT mRNA BY *p27rex*

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SUMMARY: In this study we investigated the mechanism of stabilisation of IL-2 receptor α subunit mRNA by the HTLV-I protein *p27rex*. We tested the role of the nucleolar targetting signal in *rex* by introducing mutations. Three deletion mutants could not express *rex* protein in the nucleolus and although protein was still expressed in the nucleoplasm none of the mutants could stabilise IL-2Rα mRNA. A substitution mutant could be expressed in the nucleolus and could also stabilise IL-2Rα mRNA. The data show that the nucleolar targetting signal is crucial for stabilisation of IL-2Rα mRNA by *rex* and raise the possibility that transport of mRNA from nucleus to cytoplasm can involve the nucleolus.

T-cells made leukemic by infection with human T-cell leukemic virus I (HTLV-I) have an abnormal, unregulated expression of the p55 (α) subunit of the IL-2 receptor : IL-2Rα is expressed continuously and at high numbers compared with normal T-cells in which it is expressed only transiently, after activation or by antigen (1-4). In addition, IL-2Rα mRNA of normal T-cells has a half-life of 4-6 hrs but is

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Abbreviations: IL-2R, interleukin 2 receptor; LTR, long terminal repeat.

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completely stable in leukemic T-cells (5). Two of the gene products of HTLV-I, *p40tax* and *p27rex*, regulate the expression of the viral genome. *p40tax* functions as a trans activator of the viral LTR (6) and *p27rex* stabilises incompletely spliced viral mRNAs thereby allowing their expression in the cytoplasm. In addition both viral proteins can interfere with the expression of the IL-2Rα gene - *p40tax* by activating transcription of the gene (8) and *p27rex* by stabilising IL-2Rα mRNA (5).

p27rex contains a nucleolar targetting signal (NOS) in the N-terminal 19 amino acids (9), disruption of which abrogates *rex* function of promoting expression of incompletely spliced forms of viral mRNA (10). Here we describe a study on the mechanism of stabilisation of IL-2Rα mRNA by *p27rex* in which we demonstrate that a functional NOS is also crucial for the ability of *p27rex* to stabilise IL-2Rα mRNA.

MATERIALS AND METHODS

Plasmids: the following plasmids have been described before: CDM-Tac expresses the IL-2 receptor α subunit mRNA and protein with high efficiency from the CDM8 vector (5); pKCRH2 is a eukaryotic expression vector (11); pKCR27x expresses only *p27rex* of the regulatory proteins encoded in HTLV-I (5). Mutants of pKCR27x were made in the nucleolar targetting signal of *rex* by deleting codons for amino acids 8-18 (pKCR27xdNOS1), 12-18 (pKCR27xdNOS2) and 2-7 (pKCR27xdNOS3), and by substitution of codons $arg^{9}thr^{18}$ to $thr^{9}arg^{18}$ (pKCR27xsNOS2) (10).
Cell culture: COS 7 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 2mM L-glutamine.
DNA transfection: COS 7 cells were transiently transfected with plasmid cDNAs by the procedure of Hiraki et al. (12) with chloriquine treatment for 2.5 hrs. Cells were seeded at $2\text{-}3x10^{5}$ per 10 cm dish 12-18 hrs before transfection.
Assessment of mRNA stability: the procedure of Kanamori et al. was used (5). Actinomycin D was added at 5 μg/ml 60 hrs after transfection. Cells were harvested 0, 6 and 12 hrs later and total RNA was isolated electrophoresed and blotted as described (5). Individual RNAs were detected using cRNA probes internally labelled with [^{32}P]-UTP synthesised by SP6 polymerase as described (5). Probes for IL-2Rα and actin have been described (5).
Immunochemistry: COS 7 cells transfected with cDNAs coding for *p27rex* wild-type or mutant proteins were assessed for their ability to

express *p27rex* proteins by indirect immunofluorescence. Nucleoli were stained with serum from a patient with progressive systemic sclerosis. Cells were fixed and stained 40 hr after transfection as described (10).

RESULTS

Assessment of expression of *rex* proteins by immunofluorescence

COS cells transfected with pKCR27x cDNA, or mutants thereof, were examined for expression and localisation of *rex* protein (fig1). As reported before (9,10) wild-type *p27rex* stained strongly in the nucleolus (fig1A) but if deletions were introduced into the NOS (mutants dNOS1, 2 and 3) proteins were expressed within the nucleus in the nucleoplasm, but outside the nucleolus (fig1B,C,D). The

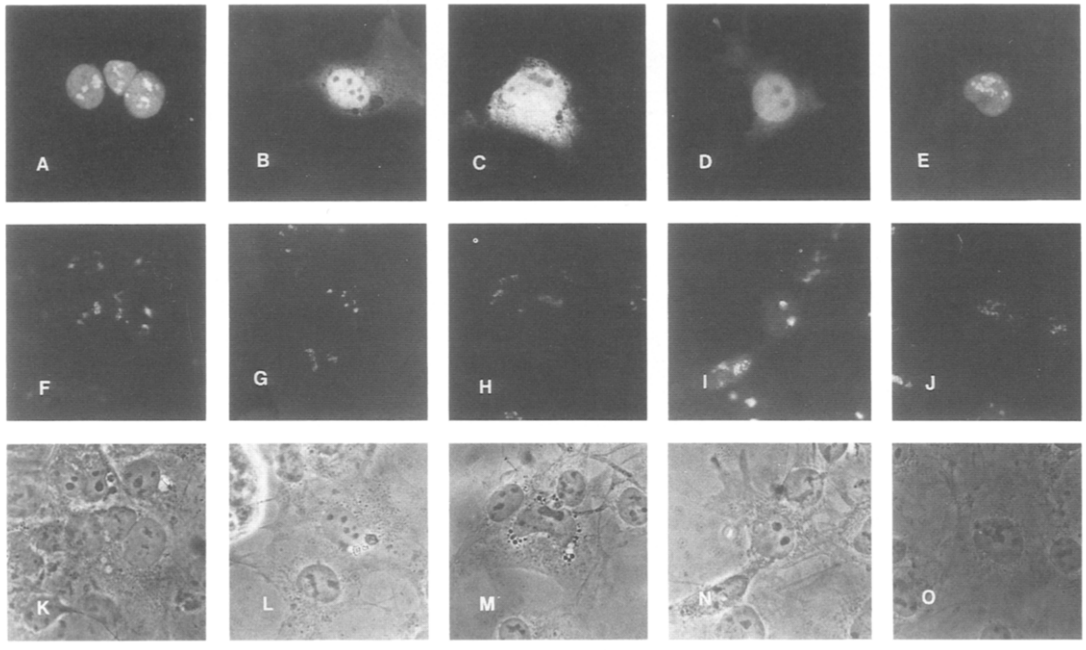

Figure 1. Expression and subcellular localisation of *p27rex* and mutants. COS-7 cells were transfected with pKCR27x (A,F,K), pKCR27xdNOS1 (B,G,L), pKCR27xdNOS2 (C,H,M), pKCR27xdNOS3 (D,I,N) and pKCR27xsNOS1 (E,J,O). A-E are photomicrographs of cells stained with antibody against the C-terminus of *rex* followed by second antibody labelled with fluorescein. F-J are photomicrographs of the same cells in A-E stained with serum from patients with progressive systemic sclerosis followed by second antibody labelled with rhodamine, to stain nucleoli. K-O are optical photomicrographs of the cells stained with antibodies shown in A-E and F-J.

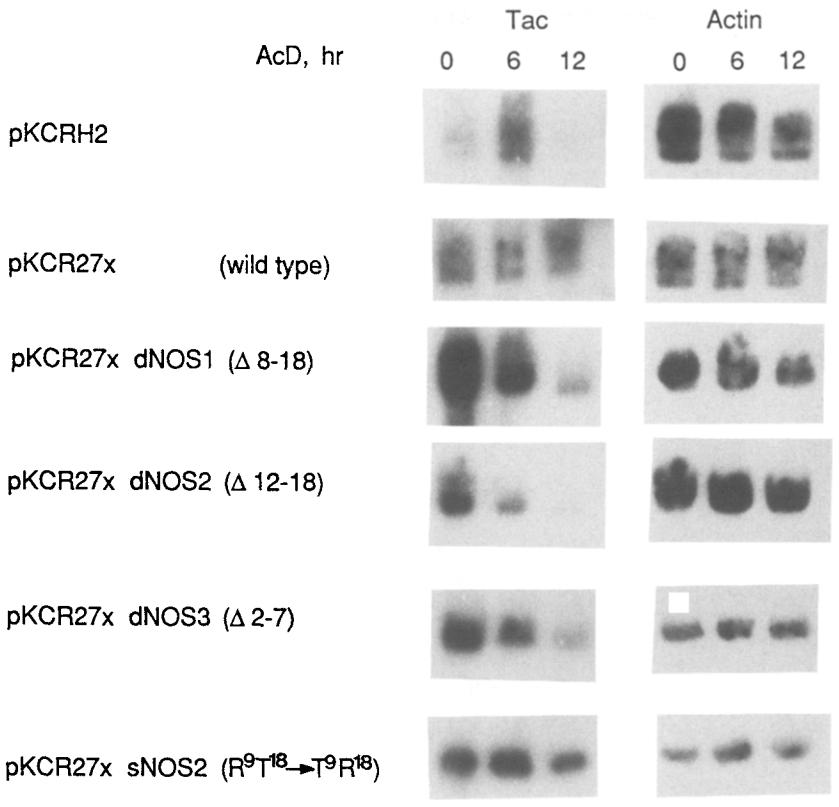

Figure 2. Correlation between nucleolar localisation of *rex* and ability of *rex* to stabilise IL-2Rα mRNA. COS -7 cells were transfected with 5μg of CDM-Tac per 10 cm plate plus 5 μg of test plasmid. pKCRH2 was used as a negative control. Data are autoradiographs of Northern blots of total RNA from cells treated with actinomycin D for 0, 6 and 12 hrs. Data are taken from a single experiment. Blots were probed twice, first with either IL-2Rα or actin and secondly vice versa so that each blot has its own internal control for quantification of mRNA.

substitution mutant sNOS2 was expressed in the nucleolus, similar to wild-type *p27rex* (fig1E).

Role of NOS in stabilisation of IL-2Rα mRNA

It has been shown previously that when cotransfected with *rex* in COS cells IL-2Rα mRNA expressed from the CDM-Tac plasmid was stabilised (5). In this study we used the same system to compare wild-type *rex* (fig2 2nd row) with proteins mutated in NOS for their ability to stabilise IL-2Rα mRNA (fig2). The data show that there is a correlation between the ability of *rex* to localise to the nucleolus and ability to stabilise IL-2Rα mRNA. All three mutants with deletions in

NOS could not stabilise IL-2Rα mRNA (fig2 3rd,4th and 5th rows compared with pKCRH2 negative control in the 1st row) nor showed nucleolar staining (fig1 B,C,D). The substitution mutant sNOS2 could stabilise IL-2Rα mRNA (fig2 6th row), consistent with its expression in the nucleolus.

DISCUSSION

Consistent with the requirement that *rex* must have a viable NOS to maintain viral function of the protein (10) the data from this study show that a functional NOS is also necessary for *rex* to stabilise IL-2Rα mRNA. To interpret the data more fully it is necessary to know whether the sequence of *rex* labelled as NOS, the N-terminal 19 amino acids, has functional activity in addition to its role as a signal for targetting to the nucleolus. Data from Greene's group support the idea that NOS has only a targetting function (13). In their study mutations were introduced throughout the length of *rex* and the effects on localisation and biological activity assessed. Mutations outside NOS which lead to loss of activity still allowed the *rex* protein to be expressed in the nucleolus (13), whereas mutations within NOS which lead to loss of activity also caused lack of expression in the nucleolus. The data suggest that domains of *rex* responsible for nucleolar localisation and for biological activity are quite distinct.

If nucleolar localisation is crucial for proper expression of *rex* activity then by inference IL-2Rα mRNA, and HTLV-I mRNA (10), must at some stage in the passage from nucleus to cytoplasm make contact with the nucleolus. The contact may be direct in that the mRNA itself passes through the nucleolus, or it may be indirect - *rex* may release a factor from the nucleolus which can interact with the mRNA in the nucleoplasm. Whatever the explanation the data presented here and elsewhere (10) raise the intriguing possibility of the involvement of the nucleolus in mRNA transport and stabilisation, something which was first suggested 20 years ago by the work of Harris and coworkers (14,15).

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