Assignment¹ of erythropoietin (EPO) to blind subterranean mole rat chromosome 1q by in situ hybridization

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¹ To our knowledge this is the first time this gene has been mapped in the blind subterranean mole rat.

Rationale and significance

Erythropoietin is the main regulator of the circulating red blood cells; however, it also stimulates neovascularization (Jaquet et al., 2002). Four *Spalax* species of the *Spalax ehrenbergi* superspecies reside in Israel in different climatic regions (Nevo et al., 2001). *Spalax* is adapted to underground life and is highly tolerant to hypoxia due to respiratory and cardiovascular adaptations (Nevo, 1999). This is emphasized by sensitive angiogenic (Avivi et al., 1999) and erythropoiesis mechanisms (Shams et al., 2004) that highlight the key role of genes related to these adaptations. As part of our study of the *Spalax* hypoxia tolerance we report here on the localization of erythropoietin gene to chromosome 1q. This is the first gene to be mapped on *Spalax* chromosomes.

Materials and methods

A ≈2-kb genomic fragment of *Spalax judaei* (2n = 60) erythropoietin gene (GeneBank accession no. AJ715794, positions 1436–3419) was amplified by PCR with the following primers: Sense: 5'-GCCTAATAAGGTTCAAAGACAG-3' and antisense: 5'-GTACAGCTTCAGYTTYCCCCGGA-3'.

The PCR mix was pre-heated at 96 °C for 5 min followed by 35 cycles of: denaturation at 95 °C for 1 min, annealing at 52 °C for 50 s and extension at 72 °C for 2 min, plus a final extension step at 72 °C for 8 min. The PCR product was purified, ligated to pGEM T-easy vector (Promega), and transformed into *E. coli* XL1-blue cells. The authenticity of the PCR products was verified by colony PCR with nested primers and by DNA sequencing of the clone from both orientations.

The plasmid itself or the purified *Eco*RI-digested erythropoietin insert (1 µg) were used as probes. The probe was labeled by using Klenow enzyme (Roche) and Cy3-dUTP (Amersham) according to methods described by Feinberg and Vogelstein (1983), and Simpson et al. (1988).

Chromosome slides were prepared from bone marrow of *S. galili* (2n = 52). The standard procedure of in situ hybridization was performed after simultaneous denaturation of DNA and chromosomes at 80 °C for 2 min.

Probe name: s60EpoI2f-b1

Probe type: Cloned PCR fragment

Insert size: Approximately 2 kb

Vector: pGEM-Teasy

Proof of authenticity: Sequencing

Results

Mapping data:

Location: 1q medial region (Fig. 1)

Number of cells examined: 20

Number of cells with specific signal: 14

Most precise assignment: 1q medial region, between AT-rich bands

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Fig. 1. Metaphase spread of a male *Spalax galili* ($2n = 52$) after FISH with EPO. Arrowheads indicate hybridization signals, and their position on inverted DAPI-stained chromosomes (insert).

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