

Isolation of Full-Length cDNA and Chromosomal Localization of Human NF-κB Modulator NEMO to Xq28

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Key Words

NF-κB · IκB · IκB kinase · NEMO · HTLV-1 Tax · Human chromosome Xq28

Abstract

NEMO is an essential component of the IκB kinase complex. Others have shown that expression of mouse NEMO can complement the lack of responsiveness to NF-κB stimuli in two NEMO-deficient cell lines. Here we report the isolation of a full-length human NEMO cDNA. Virtual translation of human NEMO cDNA predicts a 48-kD coiled-coil protein which shares 87.9% identity and 90.5% similarity with the mouse homolog. By sequence alignment, we mapped the human NEMO gene to chromosome Xq28. We note that the NEMO and the G6PD (glucose-6-phosphate dehydrogenase) loci are arranged in a head-to-head orientation separated by no more than 800 bp. This map location is further supported by the sequence of an alternatively spliced variant of human NEMO mRNA. Thus, human NEMO is an X-linked gene closely adjacent to the G6PD locus.

The transcription factor NF-κB regulates the expression of various cellular and viral genes. NF-κB plays important regulatory roles in immune and inflammatory responses, and in apoptosis. NF-κB exists ambiently in the cytoplasm via association with inhibitor protein IκB. Activation of NF-κB is induced by a wide variety of stimuli including cytokines, phorbol ester, bacterial lipopolysaccharide (LPS), and virus infection [1, 9].

One pathway of NF-κB activation involves site-specific phosphorylation and subsequent ubiquitination/degradation of IκB. The dissociation of IκB results in the unmasking of the NF-κB nuclear localization signal facilitating entry of protein into the nucleus. Thus, IκB phosphorylation represents a critical mechanism for regulation of nuclear NF-κB activity [1, 9, 13]. To date, at least two IκB kinases, IKK-1 and IKK-2, have been cloned and characterized [17, 22, 25, 36]. However, it has been suggested that these factors are components of a larger multi-protein complex [17, 20].

Recently, a novel component of the IκB kinase complex in mouse cells was identified by complementation cloning [34]. This dimeric protein, termed NEMO (NF-κB essential modulator), was found to associate directly with IKK-2. Expression of NEMO was shown to complement the lack of responsiveness to NF-κB stimuli in two

1	CG AGC TGG ACT GTT TCT ACT CCT CCC TCC TCC ACT GCG GGG TCT GAC CCT ACT CCT TGT	62
63	GTG AGG ACT CCT CTA GTT CAG AGA CAT ATT CTG TTC ACC AAA CTT GAC TGC GCT CTA TCG AGG	125
126	TCG TTA AAT TCT TCG GAA ATG CCT CAC ATA <u>TAG</u> TTT GGC AGC <u>TAG</u> CCC TTG CCC TGT TGG ATG	188
1	M 1	
189	AAT AGG CAC CTC TGG AAG AGC CAA CTG TGT GAG ATG GTG CAG CCC AGT GGT GGC CCG GCA GCA	251
2	N R H L W K S Q L C E M V Q P S G G P A A	22
252	GAT CAG GAC GTA CTG GGC GAA GAG TCT CCT CTG GGG AAG CCA GCC ATG CTG CAC CTG CCT TCA	314
23	D Q D V L G E E S P L G K P A M L H L P S	43
315	GAA CAG GGC GCT CCT GAG ACC CTC CAG CGC TGC CTG GAG GAG AAT CAA GAG CTC CGA GAT GGC	377
44	E Q G A P E T L Q R C L E E N Q E L R D A	64
378	ATC CGG CAG AGC AAC CAG ATT CTG CGG GAG CGC TGC GAG GAG CTT CTG CAT TTC CAA GCC AGC	440
65	I R Q S N Q I L R E R C E E L L H F Q A S	85
441	CAG AGG GAG GAG AAG GAG TTC CTC ATG TGC AAG TTC CAG GAG GCC AGG AAA CTG GTG GAG AGA	503
86	Q R E E K E F L M C K F Q E A R K L V E R	106
504	CTC CGC CTG GAG AAG CTC GAT CTG AAG AGG CAG AAG GAG CAG GCT CTG CGG GAG GTG GAG CAC	566
107	L G L E K L D L K R Q K E Q A L R E V E H	127
567	CTG AAG AGA TGC CAG CAG CAG ATG GCT GAG GAC AAG GCC TCT GTG AAA GCC CAG GTG AGC TCC	629
128	L K R C Q Q Q M A E D K A S V K A Q V T S	148
630	TTG CTC GGG GAG CTG CAG GAG AGC CAG AGT CGC TTG GAG GCT GCC ACT AAC GAA TGC CAG GCT	692
149	L L G E L Q E S Q S R L E A A T K E C Q A	169
693	CTG GAG GGT CGG GCC CGG GCG GCC AGC GAG CAG CGC CGG CAG CTG GAG AGT GAG CGC GAG GCG	755
170	L E G R A R A A S E Q A R Q L E S E R E A	190
756	CTG CAG CAG CAC ACC GTG CAG GTG GAC CAG CTG CGC ATG CGC AGC GTG GAG GCG	818
191	L Q Q Q H S V Q V D Q L R M Q G Q S V E A	211
819	GCG CTC CGC ATG GAG CGC CAG GCC CGC TCG GAG GAG AAG AAC CTG GCC CAG TTG CAG GTG	881
212	A L R M E R Q A A S E E K R K L A Q L Q V	232
882	GCC TAT CAC CAG CTC TTC CAA GAA TAC GAC AAC CAC ATC AAG CGC AGC GTG GTG GGC AGT GAG	944
233	A Y H Q L F Q E Y D N H I K S S V V G S E	253
945	CGG AAG CGA GGA ATG CAG CTG GAA GAT CTC AAA CAG CAG CTC CAG GCG GAG GAG GCC CTG	1007
254	R K R G M Q L E D L K Q L R M Q G Q S V E A	274
1008	GTG GCC AAA CAG GAG GTG ATC GAT AAC CTG AAG GAG GAG GCC GAG CAG CAC AAG ATT GTG ATG	1070
275	V A K Q E V I D K L K E E A E Q H K I V M	295
1071	GAG ACC GTT CGG GTG CTG AAG GCC CAG CGC GAT ATC TAC AAC GCG GAC TTC CAG GCT GAG AGG	1133
296	E T V P V L K A Q A D I Y K A D F Q A E R	316
1134	CAG GCC CGG GAG AAG CTG GCC GAG AAG GAG CTC CTG CAG GAG CTG GAG CAG CTG GAG CAG CTG CAG	1196
317	Q A R E K L A E K K E L L Q E Q L E Q L Q	337
1197	AGG GAG TAC AGC AAA CTG AAG GCC AGC AGT CAG TCG GAG TCG GGC AGG ATC GAG GAC ATG AGG AAG	1259
338	R E Y S K L K A S C Q E S A R I E D M R K	358
1260	CGG CAT GTC GAG GTC TCC CAG GCC CCC TTG CCC CCC GCC CCT GCC TAC CTC TCC TCT CCC CTG	1322
359	R H V E V S Q A P L P P A P A Y L S S P L	379
1323	GCC CTG CCC AGC CAG AGG AGC CCC CCC GAG GAG CCA CCT GAC TTC TGC TGT CCC AAG TGC	1385
380	A L P S Q R R S P P E E P P D F C C P K C	400
1386	CAG TAT CAG GCC CCT GAT ATG GAC ACC CTG CAG ATA CAT GTC ATG GAG TGC ATT GAG TAG GGC	1448
401	Q Y Q A P D M D T L Q I H V M E C I E *	420
1449	CGG CCA GTG CAA GGC CAC TGC CTG CGG AGG ACG TGC CGG GGA CGC TGC AGT CTG CGC TTT CCT	1511
1512	CTC CGG CCT GCC TAG CCC AGG ATG AAG GGC TGG CTG GCC ACA ACT GGG ATG CCA CCT GGA GCC	1574
1575	CCA CCC AGG ACC TGG CGG CGG CAC CCT ACCT CAG CTG TTG ATC CGC TGG TCC CCT CTT TTG	1637
1638	GGG TAG ATG CGG CCC CGA TCA CGG TCG ACT CGC TGC TCT TTT TGT TCC CCT CTG TCT GCT CGA	1700
1701	ACC ACT TGC CTC GGG CTA ATC CCT CCC TCT TCC ACC CGG CAC TGG GGA AGT CAA GAA TGG	1763
1764	GGC CTG GGG CTC TCA GGG AGA ACT GCT TCC CCT GGC AGA GCT GGG TGG CAG CTC TTC CTC CCA	1826
1827	CCG GAC ACC GAC CGG CCC GCT GCT GTG CCC TGG GAG TGC TCC CCT CTT ACC ATG CAC ACG GGT	1889
1890	GCT CTC CTT TTG GGC TGC ATG CTA TTC CAT TTT GCA GCC AGA CGG ATG TGT ATT TAA CCA GTC	1952
1953	ACT ATT GAT GGA CAT TTG GGT TGT TTC CCA TCT TTT TGT TAC CAT AAA TAA TAA TGG CAT AGT AAA	2015
2016	AAT CCT TGT GCA TTA AAA AA	2035

Fig. 1. The nucleotide and deduced amino acid sequences of human NEMO. Two in-frame stop codons upstream the translation initiation site are underlined. Presence of poly (A) tail at the 3'-terminus is not shown. The GenBank accession number for this nucleotide sequence is AF091453.

NEMO-defective cell lines, one of which was transformed by human T-cell leukemia virus type 1 (HTLV-1) oncoprotein Tax.

Previously, we have studied aspects of Tax-mediated transcription [11, 12, 30] and transformation [26, 29] in mammalian cells. In particular, we have identified cellular targets of Tax, including GPS2 [14], Int-6 [23], cyto-keratin [32], Rb [24] and MAD1 [15, 16]. Tax has also been suggested by others to target components of the I κ B

kinase complex including MEKK-1 [35], NIK [8, 33], IKK-1 [5, 8, 33] and IKK-2 [5, 8, 33]. The characterization of mouse NEMO as a novel Tax target [34] prompted us to investigate its involvement in the interaction of Tax with the I κ B kinase complex. As a first step, here we report the isolation of a full-length human NEMO cDNA and the chromosomal localization of this gene to Xq28, in close proximity to the locus of glucose-6-phosphate dehydrogenase (G6PD).

Materials and Methods

Molecular Cloning of Human NEMO

Standard procedures of molecular cloning were followed [27]. Full-length nucleotide and protein sequences of mouse NEMO were used to search the National Center for Biotechnology Information expressed sequence tag (EST) database dbEST (World Wide Web site: <http://www.ncbi.nlm.nih.gov>). Multiple overlapping EST clones were identified. To derive and confirm the 5' sequences, rapid amplification of cDNA ends (RACE) was performed using a HeLa cell cDNA library. Genomic sequences were identified by screening the GenBank database. Double-stranded DNAs were sequenced on both strands using the dideoxy method.

Sequence Analysis

Nucleotide and peptide sequences were analyzed with the Wisconsin software package (Version 8.1, Genetics Computer Group, Inc.) and the CGG (Computational Computer Group, Sanger Center) genomic analysis tools. Similarity searching and comparison were performed by the BLAST and BESTFIT programs. COILS [19] and PAIRCOIL [2] algorithms were used to assist prediction of coiled coils in the NEMO protein. Predictions of RNA polymerase II promoter and transcriptional initiation sites were through the TSSG and TSSW server [31] (World Wide Web site: <http://dot.imgen.bcm.tmc.edu:9331/gene-finder/gf.html>).

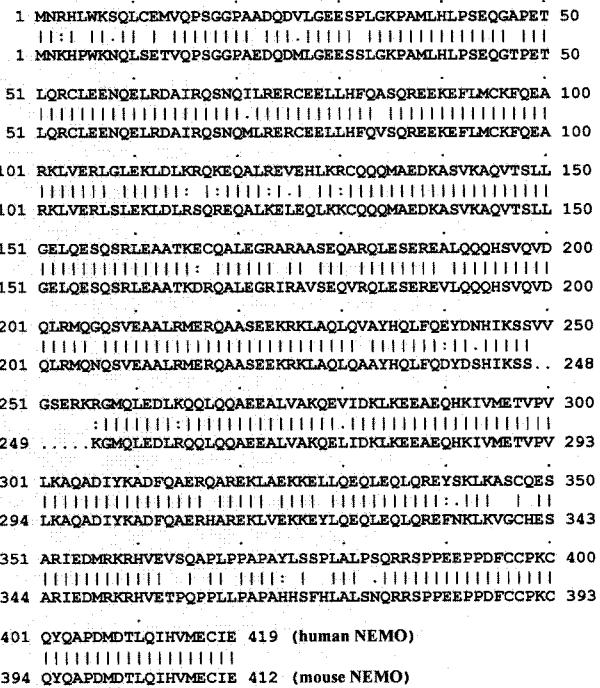
Chromosomal Mapping

The nucleotide sequences (GenBank accession numbers L44140, X55448, X53815) of the YAC and cosmid clones previously mapped to human chromosome Xq28 were used for physical alignment of human NEMO. The flanking marker is G6PD [3, 4, 21].

Results and Discussion

Molecular Cloning of Human NEMO cDNA

A 2,035-bp full-length human NEMO cDNA was cloned and sequenced (fig. 1; GenBank AF091453). The presence of two in-frame stop codons (underlined in fig. 1) immediately upstream of the putative translation initiation codon supports a conclusion that the presented NEMO open reading frame (ORF) is likely to be complete. The human NEMO ORF contains 421 amino acid residues, with a predicted molecular size of 48 kD. We performed a homology search using the BLAST program. This revealed that human NEMO shares 87.9% identity and 90.5% similarity with its mouse homolog (fig. 2). In addition, NEMO is distantly related to a subgroup of coiled-coil proteins including FIP2 that interacts with adenovirus E3 14.7-kD protein [18] (GenBank AF061034, $P(N) = 4 \times 10^{-15}$), NuMA [6] (PIR S23647, $P(N) = 6 \times 10^{-7}$), centrosome-associated protein c-NAP1 [7] (GenBank AF049105, $P(N) = 1 \times 10^{-6}$), NIK (GenBank U88984, $P(N) = 5 \times 10^{-6}$), and KIAA0445 that



The figure displays a sequence alignment between human NEMO (top) and mouse NEMO (bottom). The alignment shows high conservation of amino acids, indicated by vertical bars above the sequence. Colons represent similarity between corresponding positions. The alignment spans from residue 1 to 419. Human NEMO starts with a signal peptide (MNRHILWKSQ...), followed by a coiled-coil domain (LQRCLEENQEL...), and a kinase domain (RKLVERL...). The kinase domain includes a ATP-binding motif (GELQESQS...), a catalytic loop (RLEAAATK...), and a C-terminal tail (QIRMQNQS...). The mouse NEMO sequence is identical to the human sequence up to residue 394, followed by a stop codon (TAA).

Residue Range	Sequence Content	Identity (%)	Similarity (%)
1-419	MNRHILWKSQ... (Human) / MNKHPWK... (Mouse)	100	~90
420-421	Stop Codon (TAA)	0	0
422-419	Stop Codon (TAA) / Stop Codon (TAA)	0	0

Fig. 2. Amino acid sequence alignment of human and mouse NEMO. Bars indicate identity and colons represent similarity. The alignment was generated by the BESTFIT program in the Wisconsin package.

interacts with Tax [28] [Jin and Jeang, unpubl. data] (GenBank AB007914, $P(N) = 2 \times 10^{-4}$).

Further analysis revealed that the coiled coils in NEMO encompass residues 51 to 353 (fig. 3). This coiled-coil domain of NEMO likely facilitates its protein-protein interaction with other factors including IKK-2. Based on this reasoning it would be of interest to investigate whether NEMO can also interact directly with adenovirus E3 14.7-kD protein, centrosomal proteins, NIK and Tax. In this regard, Tax has been reported to associate with IKK-1, IKK-2, NIK and MEKK-1 [5, 8, 33, 35]. Plausibly, Tax would cooperate with NEMO in the regulation of I κ B and NF- κ B.

Chromosomal Mapping of Human NEMO Gene

By sequence alignment, we identified three genomic clones that contain the 5'-noncoding sequence of the human NEMO cDNA. These are HSG6PDG (GenBank X53815), HSG6PDGEN (GenBank X55448), and HUMFLNG6PD (GenBank L44140). These three genomic fragments all co-localize to human chromosome

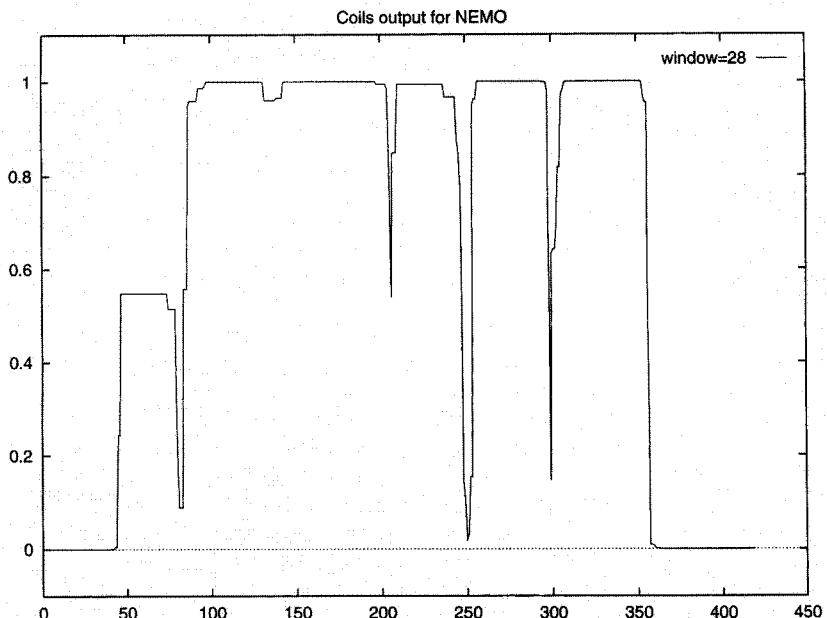


Fig. 3. Predicted coiled-coil structure in human NEMO. The x-axis represents residues in NEMO. The y-axis is the probability to form coiled coils. The plot was generated by the COILS program [19] using the MTIDK matrix. A 2.5-fold weighting was given to position *a* and *d*. Prediction by the PAIR-COIL program (2) yielded similar results.

Xq28 [3, 4, 21]. The deduced NEMO gene in the three clones was noted to be closely linked to the G6PD locus, but the orientations of the NEMO and G6PD genes are in opposing directions. Thus, NEMO maps to Xq28, in close contiguity to G6PD.

It cannot be formally excluded that our NEMO cDNA might be chimeric for two genes. While this occurrence is rare, it has been previously documented [10]. To address this concern, we searched for additional human NEMO cDNA clones. Another independent human NEMO cDNA (GenBank AI124572) was identified. The coding sequences of AF091453 and AI124572 are identical. However, the two 5'-noncoding regions were completely different in sequence. Interestingly, both 5' untranslated sequences (AF091453 and AI124572) were intactly present adjacent to each other and separated by 170 nucleotides in the G6PD genomic clones HSG6PDG, HSG6PDGEN and HUMFLNG6PD (fig. 4). Thus, we reasoned that the two cDNAs likely represent alternatively spliced variants of the same human NEMO transcript. The AI124572 sequence provides further support to the deduced map location of human NEMO, which is separated from G6PD by less than 800 bp and is predicted to be transcribed in the opposing direction towards the telomere (fig. 4, 5). A prominent CpG island exists in the

G6PD-NEMO region and the putative promoters for NEMO and G6PD are separated by 740 bp.

Chromosome Xq28 is a region of interest in human genetics (web site: <http://www.ncbi.nlm.nih.gov/omim/>). Several disease genes and fragile sites have been localized to Xq28 [4, 21]. The rate of crossovers in this region is relatively high [21]. The chromosomal mapping of NEMO to this location provides novel opportunities to study the physiologically regulated expression of this gene.

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10 20 30 40 50 60 70 80

5' TCGTCGCCCTCCGCGCTGGCACGGCCGAAAGTGTACGACCGTTCCGGGGCTGAGCCCCCGCCGCCATTAAATCGGGG
3' AGCAGCGGGAGCGCGAGCGTGGGGCTTCACATGCTGCCAAGGCCCCGACTCGGGCGGCCGGTAAATTACGCC

66PD exon 1 <<<

90 100 110 120 130 140 150 160
GGGCGGGGGCGGGCGCCCTGGGCTGAGCGGACCCGCTCGGGCAGGGCTGGGGCGGGGCTCGGCCACCCACCCCTCGTG
CCCCCCCCCCCCCCCGGGGACCCGACTCGCCCTGGCGGAGCCGCTCCGCACGCCCGGGAGCCGGTGGGGAGGAC

170 180 190 200 210 220 230 240
CGGGCGGGGGCGGGGAGGGCAGGTGCGGCCGATCCCAAGGCCAGCCCCCTGCCCTCTGGGCACCTGGCGACTGGGCCG
GCCCCGGGGCGCCCCCTCCCGTCAAGCGGCCGAGGGCTAGGGCTCCCGTCCGGGAGGAGGAGCCGTGGAGCGGACCTCGGGCG

250 260 270 280 290 300 310 320
CCGCGGGTGGCTGCTCATACCGCTGCCGCTGCTCTGCATCCCCAATTCCGGCGGGCACGGGTGCAAGCTCCGGTAGTGT
GGCGCCACGGACGGTATGGGGGAGGGGAGAGAGCTAGGGTTAACGGCCCGGTGCCCACGTCGACGGCGCATCGAGA

330 340 350 360 370 380 390 400
CCCGCATCCCCATCGCCGCCCGCCCCGCCCCACTGTCTGGTTTCCCCGCCCTCCCGGCCGCGCTCGGCCGCTCGGGAG
GGGGCTAGGGTAGCGGCCGGCGGGGGGGAGTGAACGCCAAGGGCGAGGGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG

66PD exon 1 ???

410 420 430 440 450 460 470 480
GGCTCCAATTCCGCCGGCAGCGTGGCACGCCACGCCCTCTGGGCTGTGCTCTGGGCTCTGGGCCCCCTCTGCCGCCA
CCGAGGTGAAGGGCGGCCGTCGACCCGGTGGAGGAGACCCGAGGGAGGAGAAGCCGCCGGAGAGAGCGGCCGCGGT

490 500 510 520 530 540 550 560
CTCCCCCGGAAACCTGCTTCCGGGCTAAGCCGCCATTGGGAGCTCTCTCTGATTCCTCCCGGGAGGAGG
GAGGGGGCCCTTGGGAGGGACAAGGCCCGGAGCTTCGCCGGGATAACCCGCTCAGAGAAGAACTAAGGAGGGGCTCTCC

570 580 590 600 610 620 630 640
GCGGGGCGGGAGGCCGGAGGGCTAGACGCCGGCTCCGAGAGAGGAGGGCTGAGCGCCCTGCAAA
CGCCCCGGGGCTCGGGGCTCCGATCTGGCGGCCGAGGCTCTCTGCTCTCCCGCACATCGCCACTCGGGACGTTTC

650 660 670 680 690 700 710 720
TGGCCGGGCTGCTTATCATTAACCGAGCTTCCGGGCCCTCCAGAGCTTCCGGACTCTAGACTCTCCGGAGGGATG
ACCGGCCCGCACGAATAGTAATGGCTCGAAGGCCGGAGCTCTGGCACCCCTGAGTCTGAAGAGAGGCCCTGCCCTAC

730 740 750 760 770 780 790 800
CGCCCTACCGCGGCCCTCACACTTCTGCCGGCTTCCCGACTTCTGGGGGCCGGCTGTCTTACTTCGGGATCTT
GGGGATGCCGCCGGAGTGAAGAGCGGCCGAAGGGCTCAAGAGGCCGCCGGAAACAAAAATGAAGGCCCTAGGAA

>>> NEMO AF091453

810 820 830 840 850 860 870 880
TACAGCTATGACACCGGAAGCGGAGCGCTGGTAGGGAAAGGGGAGCCGAAACTGGGACTTCTCGGAGCGCCGGGCC
ATGTCGATACTGTGGCCCTCGGCCCTCGCACCATCCCCCTCCCGTCCGCTTACCCCTGAAAGAGCCCTCGGGGCCCG

890 900 910 920 930 940 950 960
CTACCAAGCGGTTCACAGTCCGGCCTCCACCCCTCTCACGCTGACGGACTCTGCTGACAGGTGTTGGGACTTTCCCAA
GATGTCGCCAACGACTGTCAAGGCCGGAGGGTGGAGAGACTCCGAGACTGCTGAGACGACTGTCCACACCGGAAAGGGTTT

970 980 990 1000 1010 1020 1030 1040
GACGGGTCAACCGTGGCGCTCCGCCCTCGAACCTCCCGGCTTCAAGGGGAGGAGCTGAGGGAGGAGAAGAGGCCCTGCGCA
CTGTCCCAAGGGTGGCAACCGGCCAGGCCGGAGGGCTTAAGGGGAGGAGAAGAGGCCCTGCGCA

>>> NEMO AI124572

1050 1060 1070 1080 1090 1100 1110 1120
TCCTGCTCCGCCCTTGTGGAGCAGTGGCAAGCGGGCCGATCAGGACCCATGGTTACTTGGGGCCGAGCTGGACTGTT
AGGACGAGGGCGGAAGACCTCGTGAACGGGTTCCGCCGGCTAGTCCTGGTACCAATGAACCCGCCGCTCGACCTGACAA

1130 1140 1150 1160 1170 1180 1190 1200
TCTACTCTCCCTCCCTCCCTCCGCTGCGGGCTCTGACCCCTACTCTCTGAGGACTCTCTAGTTCAGAGACATATTCT
AGATGAGGGAGGGAGGGAGGAGGGAGCAGCCCAAGACTGGGATGAGGAACRCACTCTGAGGAGATCAAGTCTCTGTATAAGA

1210 1220 1230 1240 1250 1260 1270 1280
GTTCAACAAACTTGTGACTGGCTCTATGAGGTGTTAAATTCTCGTGAATGCTCACACATAGTTGGCAAGCTAGGTGA
CAACTGGTTGACTGACGCCAGATGCTCACCAATTAGGAGCACTTACGGACTGTGTATCAAACCGTCGATCCAT

1290 1300 1310 1320
TCTGATTTCATATGCTGTTGCTGTTGCAAGAACAC 3'
AGACTAAAGTATACGGACAAACGAGCAAAACGTTGTTG 5'

Fig. 4. Partial genomic sequences from the 5' upstream region of the human NEMO and G6PD genes. Putative TATA boxes (positions 70 and 810) for G6PD and NEMO are underlined. Exon sequences are doubly underlined. The major transcription initiation site of G6PD was experimentally determined [21]. An alternative first exon for G6PD was separately predicted [3, 4] and is highlighted by dots.

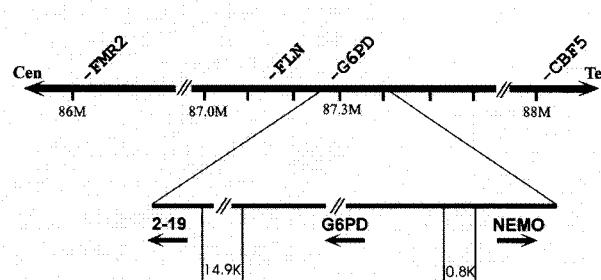


Fig. 5. Schematic delineation of human NEMO at Xq28. Positional numbering (in Mb) is anchored at the p telomere. Arrows underlying the genes indicate the orientation of transcription. Cen = Centromeric; Tel = Telomeric.

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