Modulation of Epidermal Growth Factor Receptor Gene Transcription by a Polymorphic Dinucleotide Repeat in Intron 1*

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The influence of a highly polymorphic CA dinucleotide repeat in the epidermal growth factor receptor (EGFR) gene on transcription was examined with a quantitative nuclear run-off method. We could demonstrate that transcription of the EGFR gene is inhibited by approximately 80% in alleles with 21 CA repeats. In experiments with polymerase chain reaction products that spanned a region of more than 4,000 base pairs and contained the promoter, two enhancers, and the polymorphic region in the first intron of the gene, we found that transcription activity declines with increasing numbers of CA dinucleotides. In vivo pre-mRNA expression data from cultured cell lines support these findings, although other regulation mechanisms can outweigh this effect. In addition, we showed that under our experimental conditions RNA elongation terminates at a site closely downstream of the simple sequence repeat and that there are two separate major transcription start sites. Our results provide new insights in individually different EGFR gene expression and the role of the CA repeat in transcription of this proto-oncogene.

Epidermal growth factor receptor (EGFR) is a membrane-spanning 170-kDa glycoprotein that stimulates cell growth after binding of specific ligands (1, 2). It has been found to be expressed in many normal and malignant cell types (3). Overexpression of EGFR alone is sufficient to transform NIH 3T3 cells in an EGF-dependent manner (4, 5). The level of EGFR expression is primarily regulated by the abundance of its mRNA (6, 7). Transcription of the EGFR gene starts at multiple initiation sites within the GC-rich promoter that lacks a TATA or CAAT box (8). It has been shown that the first intron of several genes including EGFR has important regulatory function (9–12). Two enhancer elements with cooperative function in the receptor gene have been identified, upstream of the promoter and downstream in intron 1 (13), and EGFR transcription is in part prematurely terminated in intron 1 (14). A polymorphic simple sequence repeat (SSR) with 14–21 CA dinucleotides and a heterozygosity of 72% in a Caucasian reference pedigree (15) was revealed close to the downstream enhancer element. It was already demonstrated for the acetyl-CoA carboxylase gene, that a CA repeat in one of the two promoter regions can repress promoter activity (16). This repression can be released by a tissue-specific factor or by removal of the dinucleotide repeat. Therefore, we raised the question whether the CA-SSR could also play a role in epidermal growth factor receptor expression. The close proximity of the EGFR downstream enhancer to the CA repeat in intron 1 led us to the hypothesis that variations in the number of these dinucleotides could be partially responsible for individual differences of the EGFR proto-oncogene expression found in humans. To probe a potential regulatory function of this highly polymorphic region, we investigated the influence of the CA-SSR on transcription activity in vitro and characterized RNA synthesis in the EGFR 5′-region in relation to the number of dinucleotide repeats. For this purpose, we applied an in vitro method for quantification of RNA synthesis from different DNA templates in a reproducible, homogenous, and cell-free transcription factor matrix. Nuclear extract from A431, a cell line with high EGFR expression capacity, was used as a model for studying the regulation of EGFR transcription. By using this approach, it was also possible to detect all occurring RNA species simultaneously, including prematurely terminated pre-mRNAs.

EXPERIMENTAL PROCEDURES

Cell Lines and DNA Preparation—A431 (epidermoid carcinoma cell line), MDA-MB-231, MDA-MB-468, BT-474 (cultured in RPMI 1640, 10% FCS), HBL-100 (mammary cell line, cultured in RPMI 1640, 5% FCS), and MCF-7 were purchased from the American Type Culture Collection (ATCC). MKN7 (gastric carcinoma cell line, cultured in Dulbecco’s modified Eagle’s medium with 10% FCS) was a generous gift of Prof. C. C. Benz, University of California San Francisco. If not otherwise specified, the cell lines were derived from breast carcinomas and cultured in Dulbecco’s modified Eagle’s medium with 10% FCS at 5% CO2. Genomic DNA of cell lines was prepared with the QiAmp tissue kit (Qiagen), according to the manufacturer’s protocol. The DNA concentration was determined with a UV photometer.

Determination of the Number of CA Repeats in Intron 1—A 114–128-bp PCR fragment containing the polymorphic region was amplified with 50 pmol of previously described primers (15). One of the primers was labeled with fluorescein at the 5’-end. The 50-μl PCR reaction mixture contained 200 ng of DNA of cultured cells, 1.5 mM MgCl2, 7.5% dimethyl sulfoxide (Sigma), 10 μM dNTP each (Perkin Elmer), 1× PCR amplification buffer, 1.5 units of Taq polymerase (Promega), and light white mineral oil (Sigma). After PCR, 1 μl of the products plus 0.3 μl of Genescan 500 TAMRA molecular weight standard (Perkin Elmer-Applied Biosystems) were denatured in 12 μl of formamide, separated in an Applied Biosystems Prism 310 genetic analyzer with POP4 polymer, and fragment lengths were determined.

Amplification and Purification of the 4,050-bp EGFR PCR Fragment—250 ng of genomic DNA were used in a 50-μl PCR reaction with 0.55 mM MgCl2, 7.5% Me2SO, 50 μM dNTP each, 1× PCR amplification buffer (Promega), a blend of 2.5 units Taq polymerase (Promega) with 1 unit Pwo polymerase (Roche Molecular Biochemicals), and 55 pmol of the following primers: B1/U, CCT TCA GAG ACA GCA AAG GCC; B1/2, CCT GAA ACC AGA ACT CGG ACA AGC G (5′–3′). The polymerase mixture was added to the reaction after overlaying with mineral oil, 4 min of denaturation at 100 °C, and cooling to 95 °C. Cycling profile: 1st...
cycle, 95 °C for 30 s; 62 °C for 1 min 30 s; 72 °C for 5 min; 2nd to 32nd cycle: 95 °C for 2 min 30 s; 62 °C for 1 min 30 s; 72 °C for 5 min in a Robocycler Gradient 40 (Stratagene). After amplification, several 50-μl aliquots of reaction mixtures were combined, DNA was ethanol precipitated, resolved in TE buffer and electrophoresed through a 0.7% agarose gel in 1× TBE running buffer. The product band was cut from the gel and purified using QiAEx (Qiagen). Finally, the UV-quantitated DNA was again ethanol precipitated and resolved in diethyl pyrocarbonate treated water. The products were stored at −20 °C until used for further analysis.

**Heterodimer Analysis**—Equal amounts of the purified EGFR 4,050-bp PCR products from A431 and each other cell line were combined and denatured for 4 min at 100 °C under a mineral oil layer. Following the addition of one-ninth volume 10× PCR amplification buffer for efficient hybridization, the mixture was cooled for 10 min in a 42 °C heating block and kept at room temperature until loaded with Ficoll loading dye on a native 5% PAA, 1× TBE mini gel. Electrophoresis was carried out at 15 V/cm for 3 h. Homo- and heterodimers were detected by ethidium bromide staining.

**In Vitro Run-off Assay**—Transcription in vitro was carried out as described previously (17) with the following modifications. Approximately 50 ng of PCR product was combined with 6 μl of 1× transcription buffer (20 mM HEPES (pH 7.9), 100 mM KCl, 0.5 mM dithiothreitol, 0.2 mM EDTA, 20% glycerol, in diethyl pyrocarbonate-treated water), 1 μl of RNasin, 2 μl of 50 mM MgCl₂, 1 μl of 10 mM NTP mix (all Promega), 5 μl of A431 nuclear extract (25 μg of protein, Santa Cruz Biotechnology), and diethyl pyrocarbonate-treated water to a final volume of 25 μl. Following careful mixing, transcription took place for 1 h at 30 °C, before 2.9 μl of 10× restriction buffer and 10 units of the restriction enzyme PsI were added to obtain smaller DNA fragments and improve efficiency of the later transfer to a nylon membrane. The solution was incubated further for 1 h at 37 °C. Subsequently, 71 μl of 0.3 M Tris (pH 7.4), 0.3 μM sodium acetate, 0.5% SDS, 2 mM EDTA, 3 μg/ml RNAse A, and 100 μl of phenol/chloroform/isoamyl alcohol (25:24:1) before ethanol precipitation in the presence of 1 μl of GlycoBlue coprecipitant (Ambion) to increase recovery.

**Electrophoresis, Nucleic Acid Transfer, Hybridization, and Detection**—Nucleic acid pellets were resolved and denatured at 95 °C in formamide loading dye and electrophoresed for 2.5 h at 25 V/cm through an 8-cm, 4% PAA, 8 μm urea gel in 1× TBE. For nucleic acid transfer to a Hybond N+ nylon membrane (Amersham Pharmacia Biotech), the gel was semi-dry blotted with 0.5× TBE for 45 min at 200 mA. After the transfer, the damp filter was UV irradiated at 302 nm on a transilluminator for 1 min and treated three times with boiling 1× SSC, 0.1% SDS solution to omit unspecific background in nonradioactive detection. For hybridization, the filter was pre-incubated for 1 h at 68 °C in hybridization buffer (5× SSC, 0.1% SDS, 5% dextran sulfate, 5% liquid block, Amersham Pharmacia Biotech) before 5 ng/ml fluorescent-UTP (RNA labeling mix, Roche Molecular Biochemicals) labeled RNA probe was added. The EGFR 5′-region-specific probe was generated by T7 RNA polymerase from a 4,050-bp PCR product with a corresponding promoter site incorporated in the downstream primer. Hybridization was carried out at 68 °C for 16 h. Stringency washes were 10 min at room temperature × 2 SSC, 1% SDS, and 2× 10 min at 68 °C in 0.5× SSC, 0.1% SDS. Subsequently, the bands were detected with the Gene Images detection module (Amersham Pharmacia Biotech), involving an anti-fluorescein monoclonal antibody coupled to alkaline phosphatase and CDP-Star chemiluminescence substrate.

**Quantification of Transcription Activity in Vitro**—Digital chemiluminescence imaging was carried out with a Lumimage (Roche Molecular Biochemicals), and band intensities were integrated after 60-min exposures with the LumiAnalyst (Roche Molecular Biochemicals) software. For quantification, intensities of the two RNA bands were normalized to the 1,051-bp DNA template band in the same lane.

**Ribonuclease Protection Assay**—In vitro run-off transcription was performed as described but without PsI treatment. DNA was instead digested with 10 units of RNase-free DNase I (Roche Molecular Biochemicals), phenol/chloroform/isoamyl alcohol extracted and precipitated in the presence of 50 μg of yeast RNA and a fluorescein-labeled antisense RNA, transcribed from a 572-bp PCR product plus T7 RNA polymerase promoter at the 3′-end. The probe spanned a region from nucleotide +892 to +1463 in the EGFR intron 1. RNase protection analysis was performed with the HybSpeed ribonuclease protection assay kit (Ambion) according to the included protocol. After final precipitation of protected fragments, the RNA was denatured and electrophoresed through a 6% PAA, 8 μm urea mini gel. RNA transfer and nonradioactive detection was performed as described.

**Competitive RT-PCR**—A specific competitor with an internal deletion of 20 nt of a 143-bp fragment specific for the exon 1-intron 1 boundary of the EGFR gene was constructed according to the method of Celi et al. (24). The resulting PCR product contained a T7 RNA polymerase promoter and was transcribed in vitro. The gel-purified competitor RNA was quantitated by UV absorption measurement. 500 ng of total RNA from cell lines and 10⁻¹³ or 10⁻¹⁰ mol of the competitor was subjected to reverse transcription and PCR (Titan One Tube RT-PCR system, Roche Molecular Biochemicals) with 20 pmol each of the following specific primers: primer A, GAG AGC CGG AGC GAG CTC TTC GG; primer B: GAG CGG CGA GAC CCC TTA CC. The two RT-PCR products were separated, and fluorescence was detected with a Prism 310 genetic analyzer. Peaks were integrated with the Genescan software (Perkin Elmer-Applied Biosystems) the molar amounts of the pre-mRNA template was calculated by the method reported by Roeter et al. (19). To eliminate the influence of gene amplification or loss of heterozygosity on protein expression, protein concentrations were divided by the EGFR gene dosage.

**RESULTS**

**Sequence of the EGFR Promoter/Enhancer Region Reveals No Mutations in Cell Lines but Contains a Polymorphism in Intron 1**—To characterize sequence and function of the EGFR promoter/enhancer region, we amplified a 4,050-bp PCR product from several cell lines. It contained most of the upstream enhancer (nucleotides −1439 to −1109; +1 corresponds to the start of exon 1), the promoter region, exon 1, a polymorphic CA-SSR, and a downstream enhancer site in intron 1 (+1788 to +2318) of the EGFR gene (Fig. 1). Because of the locally very high GC content and the ability of this region to form stem loops and triplex structures (8, 9), especially stringent conditions with unusually low Mg²⁺ concentration under 1 mM, an organic solvent that reduces secondary structure formation (18) and a special blend of Taq DNA polymerase with a proof reading enzyme had to be applied to obtain this long amplicon. The exact number of CA repeats in the PCR products was determined by microsatellite PCR and fragment analysis with an internal DNA size standard. We used heterodimer analysis to check for further differences in the sequence of the EGFR promoter/enhancer region in the cell lines used for this study. A431, an epidermoid carcinoma cell line with an amplified EGFR gene, was used as a reference, because reported EGFR
sequence data were obtained from this cell line (14). After hybridization of the A431 4,050-bp PCR product with the fragment from each other cell line, we found homodimer bands of identical products and heterodimers bands formed by nonidentical fragments (Fig. 2). Heterodimers run more slowly in native PAA gels due to the presence of unpaired bases in DNA double strands. The PCR product of A431 formed no detectable heterodimer when denatured and rehybridized alone. MDA-MB-231 with the same CA-SSR (Table I) also provided no detectable heterodimers with A431. All other cell line with higher numbers of CA repeats formed a single heterodimer band running more slowly with increasing differences in their polymorphic regions. Even the 2-bp difference in A431/MDA-MB-231 heterodimers was clearly detectable. Because we were able to detect differences with an at least 2-bp resolution and heterodimer bands corresponded exactly to the previously determined allelic pattern of cell lines, we conclude that detected heterodimer bands were induced only by known polymorphic differences, and there was no evidence for mutations in these cell lines.

**EGFR Transcription Activity in Vitro Declines with Increasing Numbers of CA Repeats in Intron 1**—To examine the influence of the CA-SSR in intron 1 on EGFR gene transcription we used seven PCR products from cell lines that contained no detectable mutations within the 5′-region but different numbers of CA repeats on the right side (Fig. 2). The number of CA repeats in intron 1 of the EGFR gene was determined by microsatellite PCR, EGFR gene dosages, previously published (19), or unpublished data from our laboratory. EGFR pre-mRNA concentrations were measured by competitive RT-PCR.

| Cell line     | CA repeats | Transcription activity in vitro | Gene dosage | EGFR pre-mRNA |
|---------------|------------|---------------------------------|-------------|---------------|
| A431          | 16         | 91 ± 30                         | 33          | 1.836 ± 0.367 |
| MDA-MB-231    | 16         | 109 ± 36                        | 1           | 0.037 ± 0.007 |
| MDA-MB-468    | 17         | 58 ± 19                         | 15.5        | 0.158 ± 0.032 |
| HBL-100       | 18         | 57 ± 19                         | 1           | 0.009 ± 0.002 |
| BT-474        | 18         | 34 ± 11                         | 1           | 0.007 ± 0.001 |
| MKN7          | 20         | 18 ± 6                          | 1           | 0.027 ± 0.005 |
| MCF-7         | 21         | 21 ± 7                          | 0.5         | 0.002 ± 0.000 |

In the three experiments with accordant findings. The DNA template spanned the approximate region of transcription termination.

Transcript amounts vary 5-fold from fragments with 16–21 CA. In triple experiments, a coefficient of variation of 33% was found. A correlation of transcription activity in vitro with the number of CA repeats in intron 1 is manifested by declining transcription activities with increasing counts of dinucleotides. Approximately the same course is revealed when using the 1,650 nt instead of the 1,650 nt transcript band for quantitation. The ratio of the two run-off products (1,650:1,550 nt) fluctuates around 0.8 (data not shown), a value that reflects the use of two major in vitro transcription start sites under our conditions.

**Nuclear Transcription in Vitro Produces EGFR-specific Pre-mRNA**—We further characterized and confirmed the identity of the two in vitro transcribed pre-mRNA species by a ribonuclease protection assay. We hybridized nuclear transcription in vitro products in solution with an RNA probe that spanned the approximate region of transcription termination.
estimating the length of nuclear run-off transcription products (Fig. 4). The probe included the CA repeat with 5’- and 3’-flanking regions plus a 20-nt T7 RNA polymerase promoter at the 5’-end. Controls of the RNase protection assay demonstrated integrity of the RNA probe (−RNase) and the absence of self-protecting structures or unspecific hybridization (−Transcr.). Hybridization of the probe with nuclear run-off products and subsequent digestion of single-stranded RNA (+RNase) provided a single product that was slightly shorter than the undigested probe. Consequently, the point of termination can be mapped to a site immediately before the 3’-end of the probe at nucleotide +1,463. Since there is no second termination site detectable within the region of the elongation block in our assays, we deduce that the double band is due to two major start sites between nucleotides −107 and −257 (8) in transcription in vitro under our conditions.

**Level of EGFR pre-mRNA Expression in Vivo Roughly Reflects the Transcription Activity in Vitro—**After we have shown that transcription activity in vitro depends on the number of CA repeats in intron 1, we wanted to know if the data in vitro correspond to the effective EGFR RNA expression in vivo. We measured the amount of EGFR pre-mRNA specific for the end of exon 1 and start of intron 1 from the indicated cell lines by competitive RT-PCR (Table 1). Fig. 5B shows the results with respect to the CA-SSR and after normalization to an EGFR gene dosage of 1. A431 and MDA-MB-468 have EGFR genes amplified 33-fold or 15.5-fold, respectively and MCF-7 is hemizygous for EGFR as determined by competitive differential PCR (19). It is obvious that the fragments with 16 and 20 CA repeats exhibit disproportionately elevated pre-mRNA expression levels when compared with the other fragments. But in general, molar amounts of pre-mRNA normalized for gene dosages also show a tendency of declining expression with increasing numbers of CA repeats.

**DISCUSSION**

Transcription of the EGFR gene and the structure of the 5’-region of the gene has been extensively investigated. The promoter region as well as parts of intron 1 are exceptionally GC-rich and potential secondary structures like stem loops and triple helices were described (8, 9). A short palindromic sequence 2 kilobase pairs upstream from exon 1 with predicted cruciform structure has been linked to termination of transcription in nuclear run-on experiments with isolated A431 nuclei (14). To characterize the action of promoter and enhancers relative to the length of a polymorphic region in intron 1, we decided to use an approach with transcription in vitro of PCR products that contained all known regulatory elements of the EGFR gene. In seven tumor cell lines, we found no signs of mutation in the 5’-region by heterodimer analysis at a resolution of at least 2 bp (Fig. 2). Therefore we used PCR products from these cell lines as templates in our assay and determined the number of CA repeats in the EGFR intron 1 and divided by the EGFR gene dosage to avoid effects of gene amplification and loss of heterozygosity.

Transcription in vitro mimics the initiation of transcription in vivo in a constant transcription factor matrix. Influences of differentially expressed transcription factors or gene copy numbers are excluded by use of cell free extracts in excess and DNA transcription templates in any concentration. A431 nuclear extract provides elevated transcription activity. Hence, products are easy to analyze and the situation in cells with high amounts of positive transcription factors but also with transcriptional repressors (14, 20) active on the EGFR gene promoter and enhancers can be examined. Quantification of transcripts appropriately reflects the activity of promoters and enhancers in the DNA applied as long as it is normalized to the DNA content of the in vitro run-off mixture, all other components are present in the same concentrations and activities and

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**Fig. 4.** Ribonuclease protection assay of the nuclear in vitro transcribed EGFR RNA species. Run-off transcription products were hybridized in solution with a 592-nt RNA probe that spanned the region of transcription termination in intron 1 (nucleotides +882 to +1463, see also Fig. 1) before single-stranded RNA was digested by RNase treatment. The observed band (+RNase) runs slightly faster than the undigested probe (−RNase). A negative control that was not allowed for run-off transcription (−Transcr.) revealed no bands. The left lane contains an RNA size marker with the indicated fragment lengths.

**Fig. 5.** Transcription activity in vitro and in vivo. A, run-off transcription product bands were quantitated in relation to the DNA content of each lane. Relative RNA quantity therefore indicates the run-off transcription activity in vitro. Results were combined and arranged in order of the number of CA repeats in DNA templates. Activities are given in percent, relative to the fragments with 16 CA dinucleotides. B, in vivo levels of EGFR exon 1/intron 1-specific pre-mRNA in cell lines determined by competitive RT-PCR. Amounts per 500 ng of total RNA are given in atomoles. Individual values for each cell line (see Table 1) were arranged in order of the number of CA repeats in the EGFR intron 1 and divided by the EGFR gene dosage to avoid effects of gene amplification and loss of heterozygosity.
nucleic acid transfer efficacy is identical. Therefore, transcription activities may be compared only on the same blot and if the same batch of nuclear extract has been used. In contrast to reporter gene assays, the necessity of cloning DNA fragments into expression vectors and limitations due to unknown plasmid transfection efficiencies and post-transcriptional regulation are omitted. Moreover, additional information can be obtained, for example about different RNA species and initiation or termination sites within the examined DNA segment. Problems connected with quantitation of signals on x-ray films like low linearity of signal/response ratios and the requirement for multiple expositions were resolved by use of a chemiluminescence imager with a high dynamic quantification range.

In a study of the acetyl-CoA carboxylase gene, it was demonstrated (16), that a nonpolymorphic sequence of 28 CA repeats within the promoter region can repress the activity of one of the two promoters by 70%. However, in this special case mediation of promoter inhibition seems to require a CAAT box. In the case of EGFR, a similar effect is observed although there is no CAAT box in the promoter region, and the CA-SSR is located more than 1,000-bp downstream of the promoter. Interestingly, differences in the number of CA repeats in the EGFR intron 1 show different levels of transcription modulation. Fig. 5A shows the association of decreasing transcriptional activity with increasing numbers of CA repeats. In addition, it could be demonstrated by ribonuclease protection assays that transcription in vitro with A431 nuclear extracts terminates at a site near the polymorphic region in intron 1 (Fig. 4). As already mentioned in a previous study, EGFR transcription was found to be in part prematurely terminated approximately 2-kilobase pairs downstream of exon 1, a site about 540-bp further downstream than our observed termination site. This discordance may be explained by the use of a 1-kilobase pair probe in the earlier reported run-on hybridization experiments, which covers our termination sites as well as the more downstream site. Therefore multiple termination sites spanned by this probe would appear as a single block. On the other hand, presence of a low abundant longer transcript that was not detected in our experiments is possible, because transfer efficiency of lengthy nucleic acids from PAA gels is significantly reduced. We conclude that our results suggest a dual function of the polymorphic region. First, an indirect effect that enhances or represses transcription in vitro up to 5-fold depending on the number of CA repeats, and second, a block of RNA elongation unaffected by the length of the SSR.

To examine whether the observed effect in vitro also has importance in vivo, we measured EGFR pre-mRNA expression levels of the cell lines used and normalized the results to the EGFR gene dosages. Fig. 5B also shows declining transcription levels with increasing numbers of CA repeats except for the 20 CA allele. The corresponding cell line MKN7 obviously uses other mechanisms to up-regulate EGFR transcription despite the CA-SSR effect. The cell lines with 16 CA fragments also appear to further enhance EGFR transcription. As seen in the in vitro experiments, the number of CA repeats can mediate an up to 5-fold transcription repression or activation in our A431 model. Up-regulation by the action of transcription factors could easily overcome this more basal effect. Taken together, these data demonstrate that allele-dependent modulation of EGFR transcription can be observed in carcinoma cell lines in vivo, but not surprisingly, there are other regulation mechanisms that can outweigh it.

To elucidate the function of the CA stretch in transcription modulation, we considered properties affecting the bendability of the EGFR downstream enhancer region. Bending of DNA in a sequence-dependent manner has an important function in many biological events like DNA replication, site-specific recombination, and transcription (21). Helical conformation analysis (22, 23) on the basis of the CA-SSR and flanking sequences indicate that the dinucleotide repeat is highly flexible. The intrinsic DNA curvature propensity of the poly-CA stretch, a measure for helical asymmetry frequently associated with a rigid conformation, is remarkably low, whereas the bendability is prominently elevated. The longer the CA stretch, the longer the highly bendable section becomes, too. This could favor a DNA secondary structure that supports or hinders binding of a factor to the neighboring enhancer element if the polymorphic segment is prolonged. In this way, a transcriptionally active protein that binds downstream of the CA-SSR could serve as a mediator of allele dependent modulation of EGFR transcription.

This report describes the association of decreased transcription activity with a prolonged polymorphic sequence in the EGFR intron 1 close to an enhancer region. The results are important for the understanding of EGFR proto-oncogene expression and probably other genes with comparable constellations of CA repeats and transcriptionally regulative elements. The knowledge of microsatellite function in relation to negative or positive enhancers provides new insights in individually different gene expression and the linkage of inherited polymorphisms to serious diseases like cancer.

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REFERENCES

1. Savage, C. R., Jr., and Cohen, S. (1972) J. Biol. Chem. 247, 7609–7611
2. Carpenter, G., and Cohen, S. (1987) Annu. Rev. Biochem. 56, 881–914
3. Adamson, E. D., and Rees, A. R. (1981) Mol. Cell. Biochem. 34, 129–152
4. DiFiore, P., Pierce, J., Fleming, T., Hazon, R., Ullrich, A., King, C. R., Schlessinger, J., and Aaronson, S. (1987) Cell 51, 1063–1070
5. Vein, T. J., Bequintot, L., Vass, M. C., Willingham, M. C., Merlino, G. T., Pastan, I., and Lowy, D. (1987) Science 238, 1408–1410
6. Merlino, G. T., Ishii, S., Wang-Peng, J., Knutsen, T., Xu, Y.-H., Clark, A. J. L., Stratton, R. H., Wilson, R. K., Pow Ma, D., Roe, B. A., Hunts, J. H., Shimizu, N., and Pastan, I. (1985) Mol. Cell. Biol. 5, 1722–1734
7. Xu, Y., Richert, N., Ito, S., Merlino, G. T., and Pastan, I. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7308–7312
8. Ishii, S., Xu, Y.-H., Stratton, R. H., Roe, B. A., Merlino, G. T., and Pastan, I. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4920–4924
9. Chrysochloris, S. A. (1993) Nucleic Acids Res. 21, 5736–5741
10. Franklin, G. C., Donovan, M., Adam, G. I. R., Holmgren, L., Pfeifer-Olsson, S., and Ohlsson, R. (1991) EMBO J. 10, 1365–1373
11. Bornstein, P., McKay, J., Liska, D. J., Apona, S., and Devarayaulla, S. (1988) Mol. Cell. Biol. 8, 4851–4857
12. Sica, A., Tan, T. H., Rice, N., Kretschmar, M., Gosh, P., and Young, H. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1740–1744
13. Maekawa, T., Imamoto, F., Merlino, G. T., Pastan, I., and Ishii, S. (1989) J. Biol. Chem. 264, 5488–5494
14. Hawley, J. D., and Waterfield, M. D. (1991) J. Biol. Chem. 266, 1746–1753
15. Chi, D. D., Hing, A. V., Helms, C., Steinhacker, T., Mishra, S. K., and Donis-Keller, H. (1992) Hum. Mol. Genet. 1, 135
16. Tae, H. J., Luo, X., and Kim, K. H. (1994) J. Biol. Chem. 269, 10475–10484
17. Dignam, J. D., Lebovits, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
18. Shen, W.-H., and Hahn, B. (1992) Trends Genet. 8, 227
19. Roether, A., Brandt, B., and Barneakov, A. (1997) DNA Cell Biol. 16, 443–448
20. Hou, X., Johnson, A. C., and Rosner, M. R. (1994) J. Biol. Chem. 269, 4307–4312
21. Travers, A. A., and Klug, A. (1990) in DNA Topology and Its Biological Effects (Cozzarelli, N. R., and Wang, J. C., eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
22. Gabrielian, A., and Pongor, S. (1996) FEBS Lett. 393, 65–68
23. Gabrielian, A., Simoncits, A., and Pongor, S. (1996) FEBS Lett. 393, 124–130
24. Celi, F. S., Zenilman, M. E., and Shuldiner, G. (1993) Nucleic Acids Res. 21, 1047–1052