mixture is added, followed immediately by digestion with DNase I (mix gently with the pipette tip) for exactly 1 min.
3. Promptly terminate the reaction by the addition of 90 μl of stop buffer.
4. Nucleic acids are recovered by phenol–chloroform (1 : 1) extraction followed by ethanol precipitation. Pellets are dissolved in 99% formamide with tracking dyes, and DNA is resolved on a 7% (w/v) sequencing gel. The gel is dried and autoradiographed. A typical example of a DNase I footprinting gel is shown in Fig. 3. Protein–DNA interactions are evidenced as areas protected from DNase I digestion or as intense hypersensitivity bands. The latter may result from changes in DNA conformation caused by the binding of a nuclear protein, which makes this particular site more susceptible to DNase I digestion.

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[42] Inhibition of Photoreceptor cGMP Phosphodiesterase by Its γ Subunit

By ALEXEY E. GRANOVSKY, KHAKIM G. MURADOV, and NIKOLAI O. ARTEMYEV

Introduction

Rod cGMP phosphodiesterase (PDE) is the effector enzyme in the visual transduction cascade. In dark-adapted rod photoreceptors, the activity of PDE catalytic α and β subunits (P$\alpha\beta$) is blocked by two identical inhibitory γ subunits (Pγ). Light stimulation of photoreceptors leads to enzyme activation by the GTP-bound transducin-α molecules (GtαGTP), which bind to Pγ subunits and displace them from the catalytic core.[1–3] Identification of the sites involved in the Pγ/P$\alpha\beta$ interface and insights into mechanisms of the PDE activity inhibition are essential for understanding

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